Habits, costly investment, and current account dynamics

Shinsuke Ikeda, Ichiro Gomi

Abstract

We analyze the current account dynamics for a small country model with habit-forming consumers and costly investment. The model has several empirical and policy implications. (i) It is consistent with stylized facts regarding the effects of productivity shocks and increases in fiscal spending and regarding the savings–investment co-movement. (ii) The long-run effect of a temporary shock on net foreign assets is often opposite to that of a permanent shock. (iii) Under strong habit persistence, the welfare dynamics are sluggish, so that a beneficial tax-financed fiscal policy may have a harmful hangover effect on welfare.

Keywords: Habit; Current account; Investment; Savings; Temporary shock

JEL classification: F32; F41; E21

1. Introduction

Being stimulated by recent developments in current account experience, many empirical studies have been conducted to report important stylized facts. They include the following: (i) adverse productivity shocks improve the current account (e.g., Glick and Rogoff, 1995; Elliot and Patás, 1996); (ii) savings and investment display a positive correlation in the short- and long-run (e.g., Feldstein and Horioka, 1980; Tesar, 1991); and (iii) temporary increases in fiscal spending
A Current Account Model with Consumption Habits and Capital-Adjustment Costs

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How the current account of the external balance of payments is determined is still one of the hottest issues in international macroeconomics. We wrote the paper (Ikeda and Gombi (1999)) to explain theoretically the actual behavior of the current account by constructing a tractable mathematical model, and to derive policy implications. In what follows, we shall explore roughly the contribution of the paper to readers who may not be economics majors.

1. Problem: Three stylized facts

With recent developments in current account experience, economic researchers have found many empirical properties in actual current account behavior. They reported important stylized facts including Facts 1 through 3 below:

- **Fact 1.** A permanent adverse productivity shock leads to an improvement in the current account (e.g., Glick and Rogoff (1995)),
- **Fact 2.** Savings and investment display a positive correlation in the short- and long-run (e.g., Coakley, Kulasi, and Smith (1996)),
- **Fact 3.** Temporary increases in fiscal spending deteriorate the current account whereas permanent ones have at most weaker detrimental effects on it (e.g., Obstfeld and Rogoff (1995)).

To explain these findings, much attention in theoretical literature has been paid to the intertemporal aspects of savings, investment, and the current account (see, e.g., Obstfeld and Rogoff (1995)). However, there are still non-negligible gaps between the empirical findings and the theory. Our main interests in this article are to construct a model which can explore these facts consistently and to derive policy implications from it.

2. Contribution

In the paper, we analyze the current account dynamics for a small country model characterized by habit-forming consumers and costly investment. As main contributions, the model is shown to have the following empirical and policy implications:

- **(C1)** The current account dynamics obtained are consistent with Facts 1 through 3.
- **(C2)** The long-run effect of a temporary shock on net foreign assets is often opposite to that of a permanent shock.
- **(C3)** When consumption habits are very persistent, the welfare dynamics are sluggish, so that a beneficial tax-financed fiscal policy may have a harmful hangover effect on future welfare. Regarding **(C2)**, it can easily be conjectured that the effect of a permanent shock is often stronger than that of a temporary shock since in many cases a permanent shock can be regarded as the limiting case of a temporary shock with persistence of the shock approaching infinity. In this sense the effect of the two shocks are usually quantitatively different. In contrast, **(C2)** suggests that the current account effects of the temporary and permanent shocks can take different signs, i.e., be qualitatively different. When using some economic policy instruments such as fiscal policy to affect the current account, therefore, it is critically important to announce clearly whether it is permanent or temporary.

3. Modeling the current account

The current account of one country’s external balance of payments equals national savings net of national investment. Both savings and investment are determined from intertemporal economic consideration. Consumers save for future consumption. With a given wealth stock, they decide how much to save jointly with how much to consume now to maximize their lifetime utilities. Investment is present payments for future production. Present production and investment are determined to maximize the discounted sum of the profit cash flow. In sum, to model the current account determination is to model two dynamic optimization problems: one is a consumption/saving choice problem for consumers and the other a production/investment choice problem for firms.

To get realistic behavior of the current account, we incorporate two kinds of adjustment costs into the choice problems: consumption habits, a kind of subjective adjustment costs of consumption, and adjustment costs of capital. Empirically, these costs are fairly consistent with macroeconomic data. Particularly, many studies support habits as a statistically significant factor to explain consumers’ behavior. Theoretically, incorporating habit formation and costly investment enable us to describe plausible transition dynamics of the current account. Habits are defined as the discounted sum of past consumptions. An increase in habits enhances preferences for future consumption. Incorporating the consumption habits allows us to describe the long-run effect of temporary shocks. When some adverse income shock temporarily occurs, a transitional decrease in consumption habits lowers the long-run consumption propensity even after the shock disappears. As a key result, temporary adverse

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1 Note that with perfect international financial markets, investors can finance their funds freely in international markets, implying that investment and savings do not have to co-move with each other. Fact 2, i.e., positive correlations between savings and investment, is called the Feldstein-Horioka puzzle.

2 In Fact 3, we use "at most weaker detrimental effects" because in many cases the current account effect of a permanent increase in fiscal spending is statistically insignificant or, even if it is significant, it is usually smaller in magnitude that the effect of a temporary increase in fiscal spending.

3 A "small country" is jargon in economics. It represents a country which is too small for its economic activities to affect foreign countries’ activities. The small-country assumption is often used for simplicity in modeling open economies.

4 In a continuous time model, investment is defined as the rate of an increase in capital stock in each instant. Without adjustment costs, investment will become infinite in response to shocks since investors can change capital stocks discretely at the moment.

5 See Ryder and Heal (1970) for the modeling of habit formation.
shocks, such as temporary deteriorations in productivity and temporary tax increases, definitely deteriorate the long-run position of net foreign assets, although the same is not true in the case of permanent adverse shocks. This property is used to explain Fact 3 and to derive policy implication (C3). Facts 1 and 2 can also be accommodated by choosing appropriately the relative magnitudes of the adjustment costs.

4. Framework

Our model, mathematically expressed in the article, can be summarized as follows:

1. This is a small country populated with infinitely-lived, identical agents. There is a composite traded good that can be used for consumption and investment.
2. The economy consists of four kinds of agents: consumers, firms, the governments, and foreign countries.
   (a) Consumers determine the time profiles of consumption and savings to maximize their utility. Consumption is habit-forming.
   (b) Firms choose production and investment flows to maximize the present value of the profit flows. Capital adjustment needs costs.
   (c) The government follows the balanced budget principle. It spends exactly the same amount as it gets through lump-sum and capital taxes.
   (d) The countries can trade assets with foreign countries at the world interest rate.

5. Analysis

From the mathematical model, we can depict the determination of net foreign asset holding and other variables as in Figure 1, where $b$ represents net foreign assets; $k$ capital stock; $c$ consumption; $z$ consumption habits defined as the weighted sum of past consumptions; and a dot denotes increments in the variable during a period. The long-run steady state is determined at points $E$, $K$, and $C$. Economic policies or shocks will shift the schedules depicted in the figure and affect the steady-state points. Transition dynamics follow the phase diagrams given in the $(k, b)$ and $(z, c)$ planes. Using the figure, we can depict the effects of various shocks.

Instead of demonstrating the detailed discussions, we sketch roughly our analytical results in Table 1.

We can see the following properties in the table:
1. Consistently with Fact 1, adverse productivity shocks likely improve the current account.
2. The effect of adverse productivity shocks are similar to that of capital tax increases.
3. As mentioned as Fact 2, savings and investment are likely to be positively correlated to each other.

The long-run effect of a permanent increase in fiscal spending on wealth and net foreign assets depends on how strong intertemporal complementarities are induced by habits. When future consumption is tightly tied to present habits, the initial consumption reduction is not so large as the lump-sum tax increases to finance fiscal spending, which reduces savings and hence the long-run net foreign assets. In contrast, consumption habits are not so strong, consumption may initially decrease so much as to increase savings and the long-run wealth.

However, when the increase in fiscal spending is temporary, it always reduces long-run net foreign assets. The temporary increase in lump-sum tax reduces permanent income and hence consumption initially. The resulting lower consumption habits reduce the long-run consumption even after lump-sum tax returns to the initial level. It follows that the net foreign asset holding required for the long-run consumption must decrease. As a result, temporary increases in fiscal spending definitely deteriorate the current account in the long run, as reported as Fact 3. This result comes from the property of the model that the short-run consumption experiences are memorized in consumers’ preferences in the form of habits and affect the long-run asset positions.

6. Welfare implication

From the model, it is shown that when consumption habits are very persistent, short-run responses in economic welfare are always smaller than its long-run responses. This short-run sluggishness of

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<th>savings ($S$)</th>
<th>investment ($I$)</th>
<th>current account ($S-I$)</th>
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<tr>
<td>adverse productivity shock</td>
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<td>capital tax</td>
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<td>fiscal spending</td>
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Table 1: The typical effects of permanent shocks
welfare is caused by a habitual propensity by consumers to give priority to short range needs. As a result, even if a sound fiscal policy is conducted, such that the initial welfare level is enhanced, it may be accompanied by "hangover" effects on future welfare, i.e., delayed negative effects on future welfare.

6 In Figure 1, "adjacent" and "distant complementarities" are also economics jargon. Roughly speaking, in the case of adjacent (distant) complementarities, an increase in habits enhances propensity to consume so much (not so much). Cigarette consumption is a typical example of adjacent complementarities. The figure shows that the dynamics differ in the two cases.

References
Cosmos and life*
(According to Henry and Bergson)

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In his essay on Kandinsky, Voir l'invisible, Henry assigns to cosmos another manner of existence than that of the visible world. The cosmos will no longer be more objective or exterior or visible: it will receive, as characteristics, the opposing qualifications of "subjective, internal and invisible." To further this elucidation of Henry's concept of cosmos, we will profit from the inherent connection that Bergson has indicated between life and matter in his magna opera, Matière et Mémoire and L'évolution créatrice.

1. Kandinsky's Abstraction and Cosmos

What signifies abstraction in the abstract painting of Kandinsky? What does this kind of painting express? It appears painters were always engaged with the visible, with the visible things that are found in the perceptible world. One comprehends the sense of their paintings by references to the external world where their reality resides. Briefly, they imitate nature. The essence of a painting, like that of all art given the Greek definition, lies in "mimesis." One could object that this Greek definition holds for the typical figurative painter, but it is not certain that it would still be valued in the case of cubism, for example. A cubist rendering no longer faithfully represents the visible elements with which we are familiar. If a cubist composes his objects of planes, of triangles and of cubes, it is because he conceives that the external world decomposes into these geometric elements. "It is always external reality, it is an interpretation of this reality which serves as the premise for these attempts at

*Translated by A.C. Elrod.
Philosophy of Life and Kandinsky’s Abstraction

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French philosopher Michel Henry

In 1999, I published a paper entitled, “Life and Cosmos (According to Henry and Bergson)” in a special issue of the Continental Philosophy Review (vol.32 no.3. The Netherlands, 1999), dedicated to Michel Henry (1922-). This contribution, an explanation of which is presented here, was edited by Professor Anthony J. Steinbock at Southern Illinois University in an effort to make this outstanding French philosopher much more accessible to English-speaking people.

In his first great work, L’essence de la manifestation (1963), Henry developed his idea of ontology of immanence. Building on this foundation, he has written many important books on fundamental problems of philosophy such as ego, body, feeling and emotion, the unconscious, and life. Theses have been translated into Japanese, English, German, and other European languages. He is also the author of novels among which L’amour les yeux fermée won the prix Renaudot in 1976.

In the fall of 1983, from October to December the Japanese Government invited Henry to join the Faculty of Letters at Osaka University where he gave impressive seminars on the unconscious, beginning with an original interpretation of Descartes’ cogito. He published the texts of these seminars in the Généalogie de la psychanalyse (1985).

Henry’s ontology

The objective of my paper was to clarify the concept of cosmos that Henry presents in his work on Kandinsky, Voir l’invisible—Sur Kandinsky, with the intent to show that Henry’s philosophy of life rejects the reality of the perceptive world, but embraces an affective world or universe. For Henry, there are two realms of existence: life and the objective, perceivable world. What is the basis of the latter? Time supplies it light for its visibility. Kant taught us this fact and Heidegger, following him, confirmed that the perceptibility of the world consists of transcendence, namely, in the movement of time going beyond itself. Time is ex-static, and objective (that is, put or throw itself forward or out) so that the visibility or perceptivity can only mean externality.

However, not all things find their existence in the visibility of time. The invisible also exist in another fashion than in time. For example, the transcendental movement of time cannot be in time, but must exist in another form just to constitute time and all of the things that are visible within it. In general, the movement of life—in so far as it is a living force—is invisible, but appears in the form of feeling or emotion, in affectivity. Life presents itself immediately by receiving itself in the form of radical passivity, without seeking its appearance in time, and without the mediation of time. Life is invisible because it accomplishes its revelation in affectivity such as pleasure or pain, and moreover it lays the foundations of the external visible world. Here is the essence of Henry’s ontology.

Abstraction of Kandinsky

What does the abstraction in Kandinsky’s painting mean? Henry claims that the painter carries out this operation to return from the outside to the inside, to reduce the visible world to the invisible life. Therefore, his abstract painting does not express the perceptive world, but the interior movement of life, which distinguishes Kandinsky sharply from a cubist and from Mondrian or Malevitch. Even if a cubist picture is composed of planes, triangles and cubes, it always represents the outside world conceived as an assemblage of these geometric elements. In a Mondrian or a Malevitch, you will indeed perceive no object, but they represent the objective world no less than any figurative painting: They intend to depict the pure visibility of the objective world, just the “Out” of its outside.

The ancient Greeks defined art as the imitation of Nature: “mimesis”. For them, art imitated the outside universe in some way. This definition of art remained valid until Kandinsky’s abstraction discovered the invisible realm of life as a substance of artistic activity. By his abstraction, Kandinsky transferred pictorial reality from the visible world to the invisible sphere of life.

It is still true that you will find in an abstract painting visible elements like colors, lines, and planes. However, the presence of these elements does not imply that abstract content must be transformed into a perceivable object to be concrete. On the contrary, colors, lines, and planes, named “pictorial forms” by Kandinsky, which are separated in the abstraction from the objective world, change their quality so that they no longer remain visible but emerge as sensible, or rather as affective. You do not see them any more, but you feel them, or more exactly, experience them much as you feel an emotion. Pulled away from the substance that determines them as its attributes or qualities in the external world, the colors and lines take on their own original meanings or values. They no longer refer to the objective world where they belong to the object and serve it—usually as practical indications for indicating how the object should be manipulated. With the abstraction, they change their reference to express the lively motion of the internal life.
Abstract line and color

Let us consider an abstract line for example. It expresses a movement, but not because it represents the trajectory of an object traversing a space or, more directly, indicates its own movement. We say with Kandinsky that the abstract line manifests a movement of the hand sketching it, or more precisely, a living force producing it through the hand. However, note that the line does not represent the living force as long as you substitute the perceptible line for the invisible force. The abstract line does not re-present, but presents the force in such a way that it provokes in us the same feeling as the one we would experience in making an effort to sketch it. You could object then that the abstract line does not express the force, but a feeling. Henry would reply with his ontology that a living force manifests itself necessarily as a feeling in affectivity because it exists to the degree that it receives itself in radical passivity to reveal itself in the quality of feeling or emotion.

As for an abstract color, it is hard imagine that red can express anything other than its visible quality of red color. Nevertheless, in Kandinsky’s abstraction, red loses its perceptive property and is no longer present as the color red per se. In the presence of an abstract red, we no longer see it, but we feel it, we experience it as sentiment. The color impresses us: it affects us.

Vermeer

In opposition to Merleau-Ponty, Henry argues that painting is not founded on the visible world but on invisible and affective life; moreover, he claims that all arts are constituted there. How should we think about a figurative picture, a Vermeer for example? Obviously, it is not abstract. Is it not a genuine painting or piece of art? Absolutely yes. The same is true for great classic painting. It seems that, in a picture of this category, colors and lines represent exclusively the quality and the form of objects perceived in the outer world. However, in reality, the colors and lines are adopted to compose the picture because they are not attributes of the objects but possess independent and affective value. A vase, placed in a pictorial space, takes on a different meaning from one in the utilitarian perceptive external world where it functions as a means or tool to realize an intention. Everything in the outside world has pictorial, affective significance so that, if you simply stop classifying it in the system of tools by doing Kandinsky’s abstraction, you will experience it in affectivity, as a feeling or emotion. This is because life organizes the objectively regulated tool system whose use is determined by the way the world is perceived. For Henry, cosmos means the universe that throws off its objectivity to appear in the affectivity of life.

Bergson’s concept of matter

Henry’s concept of cosmos reminds us of Bergson. This philosopher conceived of the Universe as Consciousness in general in his famous L’évolution créatrice. Earlier, in his second book Matière et Mémoire, he described the nature of matter, opposing Descartes, as qualitatives. Just like the mind or life, matter is also a duration, but it is a duration that proceeds in infinitely weaker tension and in a much slower rhythm than life. Matter has relaxation, not because it is divisibly extended, but because its indivisible extension is a duration. If so, what relation is there between the extensive and indivisible matter and the perceived world that is constituted of individual solid bodies? In the perceptive field, things have clear-cut contours by which they are distinguished from one another. Bergson states that this division of matter into bodies arose as an invention: life invented the cutting out of bodies so that it could get enough of a grip to be able to manipulate matter. Life charged intellect with this task. The same is true for the color of bodies, except that life only condenses the extremely faint colors that matter possesses by nature, into deeper colors. In any case, it is the effort of life that creates bodies in their form and color. As a result, when a artist paints an object in Nature, he does not duplicate or re-present it, but expresses or re-produces the activity of life in creating the object with its form and color. He does not imitate the object existing in the independent outside world, he expresses the living power fabricating it. An abstract painter does the same thing; he presents the creative power of life by inventing and composing lines and colors free from the objects in the causal world.

Life works in Nature just as the painter on canvas. Why do we have art, then? Why are we not content with Nature? Could human beings get along without art? No. We need art because we want to see things that are more beautiful and to hear sounds that are sweeter than those given by Nature; thus, artists are born. Life is eager to create more beauty so that it may exploit its power more actively with a view to grow and experience more richness. Bergson talks about a mystic who resumes the evolution of life at just the point where it stopped advancing in Nature because of the resistance of matter. In achieving it, the mystic turns produced Nature into productive Nature. A artist plays the same part as the mystic in Nature.

We will now return to Bergson’s concept of matter and try to determine its more accurate connection with life or Consciousness. You will remember that for him matter is not extended but extensive. In other words, it is not quantitative but qualitative. He said there is no difference in the natures of life and matter but in the degrees between them. Life has a very strong tension while matter has an extremely weak tension. In L’évolution créatrice (chapter III), he gives a more elaborate definition of them: life has a tendency or movement of tension while matter, of relaxation. Life is an action that makes or integrates itself while matter is that which undoes or disintegrates itself. Nevertheless, it does not mean that these are two real actions opposing each other. What is real, is life’s action of making itself, and any lack of this action brings about materialization automatically. If only you interrupt life’s movement of integration, will you find that the disintegration, the undoing of matter emerges immediately.

Life contains materiality in so far as this is its detente. Les deux sources de la morale et de la religion talks about the Creation. God is love itself and He created the universe as an object of love, as life. Because it was a created life, it had to receive materiality at the same time. With a famous Bergsonian metaphor, we can imagine that life is the embers still blazing inside the cinders of continuously falling fireworks. Consequently, it is not possible that matter exists in itself, indifferently to life, and, in the case of the perceptive outside world, this is much less conceivable.

Life cannot completely stop the undoing or disintegration out
of the materiality, against the principle of Carnot, but it can slow
the speed of this action to retain its tight tension. In practice, if mat-
ter exists, life works to maintain the intensity of its tension where-
as matter is the resistance opposing this effort and is a necessary
element of the effort. The visible exterior world is constituted by
the effort that life makes to overcome matter. Life is identified by
Bergson with consciousness, and consciousness with pure dura-
tion free from any materiality. Pure duration appears in the form
of feeling or emotion. For Bergson, like for Henry, the universe,
conceived as Consciousness, is experienced in affectivity.

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Numerical renormalization approach to two-dimensional quantum antiferromagnets with valence-bond-solid type ground state

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Abstract. We study the ground-state properties of two-dimensional quantum spin systems having the valence-bond-solid (VBS) type ground states. The ‘product-of-tensors’ form of the ground-state wavefunction of the system is utilized to associate it with an equivalent classical lattice statistical model which can be treated by the transfer-matrix method. For diagonalization of the transfer matrix, we employ the product-wavefunction renormalization group method, which is a variant of the density-matrix renormalization group method. We obtain the correlation length and the sublattice magnetization accurately. For the anisotropically ‘deformed’ \( S = 3/2 \) VBS model on the honeycomb lattice, we find that the correlation length as a function of the deformation parameter behaves very much as that in the \( S = 3/2 \) VBS chain.

1. Introduction

There are many applications of the density matrix renormalization group (DMRG) \([1, 2]\) which was originally applied to one-dimensional (1D) quantum systems \([1, 2]\). Due to its remarkable success, the DMRG has now become one of the standard methods for studying 1D quantum models, two-dimensional (2D) classical models \([3]\) and \((1+1)\)-dimensional classical non-equilibrium models \([4, 5]\).

As was pointed out in \([6, 7]\), DMRG is a variational method under the matrix-product-form ansatz (MPFA) for trial wavefunctions whose usage dates back to the work of Kramers and Wannier \([8, 9, 10]\). This point of view leads to some non-trivial reformulations of the DMRG: the direct variational approach \([6, 7, 11, 12]\), the product-wavefunction renormalization group (PWFRG) \([13]\), the corner-transfer-matrix renormalization group (CTMRG) \([9, 10]\).
A Numerical Renormalization Approach to Two-Dimensional Quantum Systems

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Quantum many-body problems

There exist various materials which have non-trivial structures and interesting macroscopic properties. Microscopically, however, they are made up of atoms or molecules which are rather structureless and have simple properties. What make the variety of the materials made up of components are (a) a huge number ($\sim 10^{20}$) of components and (b) the interaction between the components. The main subject of the condensed-matter theory is to understand the macroscopic properties of matter from a microscopic point of view, by properly taking the above two points into account. The statistical mechanics gives us a framework to deal with the problem of highly many-component systems with non-negligible interactions, which has been termed the many-body problem.

Owing to the remarkable development of computer technology, roles of “direct" numerical methods in statistical mechanics have grown very much, leading to a creation of new field of discipline known as computational physics. For instance, we have (1) molecular dynamics method (MD), (2) Monte Carlo method (MC), and (3) exact diagonalization method, as standard numerical methods. The MD is the one which directly integrates the Newton's equation for many particle systems. The MC is the one which performs direct statistical sampling following the principle of the statistical mechanics. For classical statistical systems, MD and MC have achieved remarkable success. For quantum systems, we have the quantum Monte Carlo (QMC) method, which has successfully applied to various systems. Unfortunately, as compared with its classical counterpart, the usefulness of QMC has been rather limited due to the notorious “negative sign problem" which invalidates the reliability of the method at low temperatures. Since quantum phase transitions generally take place at low temperatures, the negative sign problem is a crucial defect of the method; overcoming the sign-problem-related difficulty has been one of the important subjects of study in the quantum many-body problem.

Large $N$ difficulty

In the exact diagonalization method (ED), we directly diagonalize the Hamiltonian (for quantum cases) or the transfer matrix (for classical cases) numerically. This method is free from the negative sign problem, and it is most reliable and accurate for a given system size. However, it also has a weak point that the system size it can handle is very small. Consider an $N$ site system where $S=1/2$ spin is situated at each site. Since the dimension of quantum state space at each site is 2, the total number of bases needed to describe the wavefunction is $2^N$; we should diagonalize the Hamiltonian matrix with $2^N \times 2^N$ size. This exponential explosion of dimension severely limits the feasible size $N$ because of the limitation of the available memory of the computer ($N \sim 30$, for giga-byte machines). The smallness of feasible $N$ ("large-$N$ difficulty") makes the method less powerful for studies of critical phenomena where divergent correlation length requires large $N$ calculations.

Density matrix algorithm and matrix product ansatz

In 1992, a novel numerical algorithm, density matrix renormalization group (DMRG, for short), was invented by S.R. White [1, 2]. There have been many applications of DMRG which was originally applied to one-dimensional (1D) quantum systems. Due to its remarkable success, the DMRG has now become one of the standard methods for studying 1D quantum systems, two-dimensional (2D) classical systems [3] and (1+1)-dimensional classical non-equilibrium systems [4, 5].

The DMRG is basically a numerical diagonalization method, hence, it is free from the sign problem in QMC; it is different from the ED in that we perform only approximate diagonalization within a truncated basis set. The essential point is that, given a number $m$ of retained bases, we optimize the choice of the bases by changing the representation basis. In the optimization process, we make a density matrix from a wavefunction and diagonalize it to have a set of eigenvectors from which we select $m$ bases; this is the reason why the term "density matrix" appears in the name of the method.

As was pointed out in Refs. [6, 7], DMRG is a variational method under the matrix-product-form ansatz (MPFA) for trial wavefunctions whose usage dates back to the work of Kramers and Wannier [8,9]. In the MPFA, a wavefunction $\psi(\sigma_1, \sigma_2, \sigma_3, \ldots)$ of a spin chain ($\sigma_i$; eigenvalue of $\sigma_i$), is written in terms of a product of matrices $\{A(\sigma_i)\}$, as

$$\psi(\sigma_1, \sigma_2, \sigma_3, \ldots) = Tr A(\sigma_1) A(\sigma_2) A(\sigma_3)^{-1},$$

where $Tr$ corresponds to the periodic boundary condition.

For a spin-$S$ problem, we should only store the matrix $A_{\sigma,\sigma}(\sigma)$ with $S^2 \times m^2$ elements ($m$: number of retained bases), which allows us to avoid the large-$N$ difficulty met in the ED. The

![Fig 1. The matrix element $A_{\sigma,\sigma}(\sigma)$ which composes a MPFA wavefunction.](image)

![Fig 2. Wavefunction $\psi(\sigma_1, \sigma_2, \sigma_3, \ldots)$ as a matrix product.](image)
MPFA-point of view has also lead to non-trivial reformulations of the DMRG: the direct variational approach, [6, 7, 11, 12] the product-wavefunction renormalization group (PWFGRG), [13] the corner-transfer-matrix renormalization group (CTMRG). [9, 10] Further, in this view, the success of the DMRG implies the unexpected accuracy of the MPFA wavefunctions.

**Higher-dimensional extension - tensor-product ansatz**

Unfortunately, the DMRG can only be applied to 1D systems. Having seen the success of the DMRG, it is therefore important to explore its higher-dimensional generalizations. For 2D quantum case, the ladder approach, [14-16] can be regarded as such, but it is essentially the one-dimensional algorithm. Since the DMRG is a MPFA-variational method, a promising approach in generalizing the DMRG to higher dimensions is one which is based on a generalization of the MPFA.

A natural generalization of the MPFA is, then, the tensors-product-form ansatz (TPFA) where we express the wavefunction as a product of local “tensors” (generalized objects of matrices). Accordingly, we can formulate the TPFA-variational method which can be thought of a “higher-dimensional DMRG”. What we have done in our paper is a first step in this TPFA-variational approach to higher-dimensional quantum systems, giving both the conceptual and the methodological bases. Take the spin-exchange solid (VBS) state [17, 18] for 2D quantum antiferromagnets, where the MPFA is exact for the wavefunction. We discuss the physical properties of the VBS-type state, by developing a reliable numerical method.

Let $|\text{VBS}\rangle$ be the unnormalized ground-state vector whose wavefunction is exactly given by a product of local tensors. A main part of our problem is to evaluate the expectation value of a given observable $\sigma$

$$
\langle \sigma \rangle = \langle \text{VBS}|\sigma|\text{VBS}\rangle / \langle \text{VBS}|\text{VBS}\rangle.
$$

For 1D VBS-type models, due to the matrix-product-form structure of $|\text{VBS}\rangle$, the expectation can be interpreted [17, 18] as a thermal average in a 1D classical statistical-mechanical model; the transfer-matrix method allows us to evaluate the expectation exactly. For 2D VBS-type models, a similar interpretation as a 2D classical statistical-mechanical problem is straightforward due to the TPF structure of $|\text{VBS}\rangle$. The approach we take in our work is a direct generalization of the 1D case; we treat the associated 2D classical statistical-mechanical problem by the transfer-matrix method. What is essential in this approach is that, for diagonalization of the transfer matrix, we employ the DMRG allowing us to make highly reliable, close-to-exact evaluation of the expectation value. The use of the DMRG also has an important implication, in the light of the TPFA-variational method: 2D TPFA-variational calculation is reduced to 1D TPFA-variational method, namely the DMRG. Accordingly, we can, in principle, formulate the “nested” TPFA-variational approach where $D$-dimensional TPFA-variational calculation is reduced to $(D-1)$-dimensional one, which in turn is reduced to $(D-2)$-dimensional one, and so on. In our work, based on the approach described above, we have analysed an anisotropically generalized $S=3/2$ VBS model on the honeycomb lattice. [19] We have determined the universality class of the anisotropy-induced ground-state phase transition and have discovered a crossover between the transverse and longitudinal correlation lengths.

**Recent developments and outlook**

In our paper, we have shown that given a state with TPFA-form wavefunction, we can actually obtain physical quantities in the state. It is therefore straightforward to formulate a “parametric” variational approach where we parametrize the local tensor to form a trial TPFA wavefunction and minimize the energy expectation with respect the parameters. This line of approach has been taken in Ref. [20] where maximization of the transfer-matrix expecta-
tion is performed for the 3D Ising model under the 2D-generalized Kramers-Wannier wavefunction. Recently, a “non-parametric” variational approach has been discovered where we can determine the optimal local tensor as a solution of a generalized eigenvalue problem. [21] Although the number $m$ which corresponds to the number of retained bases in the DMRG is 2 (very small) in these calculations, we have seen unexpected accuracy of the results, showing a promising future of our approach.

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High-resolution scanning tunneling microscopy imaging of DNA molecules on Cu(111) surfaces

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Abstract

Using the pulse injection method, single-stranded DNA and double-stranded plasmid DNA have been deposited on well-defined Cu(111) surfaces under ultrahigh vacuum (UHV) conditions to obtain high-resolution scanning tunneling microscopy (STM) images. These particular UHV-STM images have revealed that DNA molecules are adsorbed directly onto a clean Cu(111) surface and exhibited the detailed structures of DNA, which has not been resolved previously. The single-stranded DNA oligomers have exhibited the images of individual internal base molecules and the helix structures made of complementary base sequences. For the double-stranded plasmid DNA, the images have shown the Watson–Crick double-helix structure. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Biological molecules; DNA; Copper; Single crystal surfaces; Low index single crystal surfaces; Scanning tunneling microscopy (STM)

1. Introduction

Since the discovery of the double helix structure of DNA by Watson and Crick in 1953 [1], direct observation of DNA with real-space atomic/molecular resolution using microscopy has been extremely intriguing. After the advent of the scanning tunneling microscope (STM), many reports aiming at the observation of DNA appeared [2,3]. Among them, the highest resolution STM images were reported in 1989–1990 on DNA adsorbed on highly oriented pyrolytic graphite substrates prepared by dropping DNA solution in air [4–7]. However, later reports have identified these 'DNA images' as grain boundaries of graphite [8,9]. After these reports, almost no high-resolution STM images have appeared in scientific journals. The problem is considered to lie in the lack of a sophisticated sample preparation technique by which DNA molecules can be deposited on an atomically flat well-defined substrate surface without contamination. Since an ultrahigh vacuum (UHV) condition is one of the most well-defined conditions, we have performed observation of DNA by UHV-STM using a clean single crystal Cu(111) surface as an artifact-free well-defined substrate. We have developed a pulse injection method as a new sample preparation technique...
The First Successful Imaging of DNA Nanostructure by High-Resolution STM

KAWAI Tomoji
(Institute of Scientific and Industrial Research)

Abstract

Reproducible and reliable high resolution images of DNA have been obtained for the first time using low temperature ultrahigh vacuum (UHV) scanning tunneling microscopy (STM). Single-stranded DNA and double-stranded plasmid DNA have been deposited on well-defined Cu(111) surfaces under UHV conditions using the pulse injection method. The STM images have revealed that DNA molecules are adsorbed directly onto a clean Cu(111) surface, and exhibited the detailed structures of DNA which have not been resolved earlier. The single-stranded DNA oligomers have exhibited the images of individual internal base molecules and helix structures made of complementary base sequences. For the double-stranded plasmid DNA, the images have visualized double-helix structures. This letter offers a steady method to study DNA or DNA-protein complexes with a resolution of a single molecule level in a real space, aiming at molecular surgery as well as elucidation of variety of bio processes.

1. Introduction

Since the discovery of the double helix structure of DNA by J.D. Watson and F.H.C. Crick in 1953, direct observation of DNA with real-space atomic/molecular resolution using microscopy has been extremely intriguing. After the advent of the scanning tunneling microscopy (STM), many reports aiming at the observation of DNA have appeared. Among them, the highest resolution STM images were reported in 1989-1990 on the DNA adsorbed on highly oriented pyrolytic graphite substrates prepared by dropping DNA solution in air. However, later reports have identified these “DNA images” as grain boundaries of graphite [1]. After these reports, almost no high resolution STM images have appeared in scientific journals. The problem is considered to lie in a lack of sophisticated sample preparation techniques by which DNA molecules should be deposited on an atomically flat well-defined substrate surface without contamination. Since the ultra-high vacuum (UHV) condition is one of the best well-defined conditions, we have performed observation of DNA by UHV-STM on a clean single crystal Cu(111) surface as an artifacts-free well-defined substrate. We have developed a pulse injection method as a new sample preparation technique applicable to bio-molecules such as DNA to avoid decomposition. Here we report reproducible and reliable high resolution STM images of DNA deposited on a well-defined Cu(111) substrate under ultra-high vacuum condition using the pulse injection method. The STM images clearly exhibit the sub-molecular structures, i.e., double helix and individual bases, for the first time.

2. Experimental procedure

Three kinds of DNA molecules have been observed: single-stranded DNA oligomers, i) pAAAAA (containing five adenines), ii) pAAAAAATTTTTTT (containing seven adenines and seven thymines), and double-stranded plasmid DNA of 3k base-pair. The UHV apparatus consists of an STM chamber and a preparation chamber as shown in Fig.1a [2]. After repeated sputtering and annealing cycles in UHV in the preparation chamber, we obtained a clean flat Cu(111) surface. The DNA molecules were deposited using the pulse injection method (see Fig.1a). The temperature of the Cu(111) substrate was room temperature. Upon the injection of the DNA solution, the injected water is instantly pumped out, while involatile DNA stays on the substrate surface. The Cu(111) sample is transferred to the STM chamber and the STM measurements were performed using USM-401U and USM-602S2 (Unisoku, Japan), at room temperature for the pAAAAA and at liquid nitrogen temperature (−80K) for the pAAAAAATTTTTTT and the plasmid DNA in order to suppress thermal disturbance for the improvement of the resolution of STM images.

Fig. 1. Schematic diagram of the pulse injection and STM apparatus used for this study (a), comparison of STM images of Cu(111) substrate surfaces obtained before (b) and after (c) the injection of the DNA, pAAAAA. Imaging parameters: (a) V = −2 V, I = 100 pA, (b) V = −3 V, I = 100 pA. The x,y,z scales of both images are 100 x 40 x 0.4 nm. Both images were observed at room temperature. Bright objects that have not been observed before the injection are seen on an atomically flat terrace (b).
common in an aqueous environment where water molecules are involved. However, in our UHV deposition system where water molecules present around adsorbed DNA molecules are pumped out, single-stranded DNA molecule may be adsorbed on the substrate surface with as many parts of its constituent elements (sugar-phosphate chain and lying-flat bases) are adsorbed as possible in order to reduce the total energy. As a result, the circular structure of single-stranded DNA is formed.

4. High resolution image of double helical structure

A longer single-stranded DNA containing 7 adenine and 7 thymine bases are of interest, because this DNA has a longer and a complementary base-sequence, and it may have an adsorbed structure different from that of pAAAAA. Figure 3 shows a typical STM image of a pAAAAAATTTTTTTT deposited on a Cu(111) surface by the pulse injection method. We found a Z-shaped adsorbed structure (Fig. 3a) and several other types of adsorbed structures such as circle and clustered shapes as well. As can be seen in the STM image (Fig. 3a) and corresponding structure model (Fig. 3b), the single-stranded DNA, pAAAAAATTTTTTTT molecule, does not form a simple circular structure as the pAAAAA does. This result itself may not be surprising, because a DNA molecule with a longer sequence should posses a larger number of conformations or adsorbed structures. A common result is that both pAAAAA and pAAAAAATTTTTTTT oligomers take flat conformation as the adsorbed structures.

Besides single oligomer adsorbates, we have observed characteristic helical structures of pAAAAAATTTTTTTT, as shown in

![Fig. 3. High magnification STM images (a), (c) and a proposed model of single-stranded DNA oligomer, pAAAAAATTTTTTTT molecule, obtained after the injection of the sample on a clean Cu(111) substrate and observed at liquid nitrogen temperature (~80K). (a) Single pAAAAAATTTTTTTT appears to take a Z-shaped adsorbed structure. (b) A proposed structure model for the observed image (a). (c) Paired pAAAAAATTTTTTT, showing double-helix structures. Among paired or clustered DNA molecules, the presence of a paired double-helical DNA has been found. Imaging parameters: (a) $V_s = -3 V$, $I_s = 100 pA$, $6x6x0.2 \text{ nm}^3$, (c) $V_s = -2 V$, $I_s = 100 pA$, $13x13x0.2 \text{ nm}^3$.](image-url)
Fig. 3c. Apparently, a pair of mono-oligomers is forming a double helix with one and a half turns. This structure has a pitch whose periodicity of the helix is ~7nm and height of 0.16-0.36 nm. The corresponding values of Watson-Crick DNA in biological conditions are ~3.4 nm and ~2 nm, respectively. It is known that the single turn of the Watson-Crick DNA consists of approximately ten base-pairs. Since the single-stranded DNA used here is a 14mer, this DNA should take double helical turns of nearly one and a half. The observed periodicity of the helix is approximately two times longer than that of Watson-Crick DNA. We believe the interaction with substrate must have relaxed double helical structure formed by a pair of relatively short DNA oligomers. We should emphasize the fact that the single-stranded DNA, 14mer, can form a double helical structure and that structure is clearly visualized by STM.

5. High resolution images of plasmid DNA

For the direct observation of a double-helix structure, double-stranded plasmid DNA has been deposited on the clean Cu(111) by pulse injection method. Figures 4a-d show a series of STM images of 2739 base-paired double-stranded plasmid DNA deposited on Cu(111) surfaces. The steps and dislocations native to the Cu(111) substrate surface can be seen as background, which adds validity to the images. White dots dispersed on the surfaces would come from buffer species, such as EDTA. Although the plasmid DNA in the images are partly entangled, they appear as circled whole plasmid DNA molecules. Moreover, no open-circled or fragmented plasmid DNA were found even after searching hundreds of sites of the sample surface. Thus, by using this particular pulse injection method, we have successfully deposited 2739 base-paired plasmid DNA on clean well defined Cu(111) surfaces under UHV without breaking the DNA strand. The observed plasmid DNA molecules have a topographic height of 0.2-0.5 nm and a diameter of the strand is about 2-4 nm, depending strongly on STM tip conditions. In high resolution images, internal structures with periodicity of 2.6-3.7 nm along the strand are resolved as shown in Fig. 5a and its magnified image in 5b. A histogram for the periodicities along the chain is also indicated in Fig. 5a. A histogram for the periodicities along the chain is shown below the image in Fig. 5a, which shows a broad peak in the range of 2.6-3.7 nm. A magnified image (~19.5x10.4 x 1.2 nm²) of a region indicated by a dotted white rectangle frame in (a), showing an internal structure with periodicity of 2.6-3.6 nm along the strand. (c) For direct comparison between the magnified image (b) and the illustration (c), they are fitted to a same scale.

![ STM images of 2739 base-paired double-stranded plasmid DNA on Cu(111) surface ]

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High-performance resin-bonded magnets produced from zinc metal-coated Sm\(_2\)(Fe\(_{17}\)Co\(_{0.01}\))\(_2\)N\(_x\) fine powders

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(Received 14 May 1999; accepted for publication 17 July 1999)

Fine powders of Sm\(_2\)(Fe\(_{17}\)Co\(_{0.01}\))\(_2\)N\(_x\) (\(x \approx 3\)) with particle sizes (d) around 1 μm as coated with zinc metal produced via the photodecomposition of diethylzinc [Zn(C\(_2\)H\(_5\))\(_2\)], which still provided high remanence (B\(_r\)), coercivity (H\(_c\)), and energy product (B\(_r\)H\(_c\)) values of ~1.43 T and ~0.85 MAm\(^{-3}\), were molded to composite resin-bonded Sm\(_2\)(Fe\(_{17}\)Co\(_{0.01}\))\(_2\)N\(_x\) magnets with density values of ~0.83 g cm\(^{-3}\). By optimizing the preparation conditions such as grinding, surface coating, and molding for them, the highest maximum energy product of \((BH)_{max} = 186\) kJ m\(^{-3}\) for \(H_{c} = 0.73\) MAm\(^{-1}\) was recorded among all kinds of the Sm–Fe–N based magnets reported to date. Furthermore, the excellent aging behavior of the bonded ZnSm\(_2\)(Fe\(_{17}\)Co\(_{0.01}\))\(_2\)N\(_x\) magnets was observed after standing in air at ~303 K and the thermal coefficient for permanent magnets was evaluated to be \(\alpha(B) = -0.04\%\ K^{-1}\). © 1999 American Institute of Physics. [S0003-6951(99)04737-3]

Nitrogen-induced Sm\(_2\)Fe\(_{17}\) intermetallic compound, Sm\(_2\)Fe\(_{20}\)N\(_x\) (\(x \approx 3\)), with good intrinsic magnetic properties, i.e., high saturation magnetization (\(M_s = 1.52\) T), strong uniaxial magnetocrystalline anisotropy (\(H_a = 21.0\) MAm\(^{-1}\)), and high Curie temperature (\(T_c = 746\) K), has been noted to be a promising material for high-performance bonded magnets,\(^{12}\) whereas it is handicapped by a series of the defects: (1) Sm\(_2\)Fe\(_{20}\)N\(_x\) is a metastable phase and decomposed at temperatures above 873 K, (2) the coercivity mechanism is controlled by a nucleation process and thus Sm\(_2\)Fe\(_{20}\)N\(_x\) has to be ground to fine powders with particle sizes (d) below ~1 μm in diameter,\(^{8,3}\) and (3) the resulting fine powders are too active for oxygen and water to handle them even in conventional glove boxes charged by commercially available inert gas, leading to decrease the hard magnetic property owing to the oxidation of them. Recently, Sm\(_2\)Fe\(_{20}\)N\(_x\) powders have been commercialized as materials of the injection-type anisotropic bonded magnets with \((BH)_{max} = 103\) kJ m\(^{-3}\) for \(H_{c} = 0.65\) MAm\(^{-1}\), for which large amounts of polyamide-resin (~10 wt %) are used to depress the oxidation of them in air.\(^{9}\)

On the other hand, the partial substitution of Fe to other elements such as Co, Al, Ti, V, or Mn have been made in order to improve the intrinsic magnetic properties of Sm\(_2\)Fe\(_{20}\)N\(_x\) itself.\(^{6,10}\) Among the above elements, the most significant improvement of the intrinsic property is attained for the Sm\(_2\)(Fe\(_{17}\)Co\(_{0.01}\))\(_2\)N\(_x\) compounds substituted with small amounts of Co (\(x < 0.5\)) and, particularly, the hard magnetic specifications are maximized at \(x = 0.2\) reaching excellent improved magnetic parameters of \(T_c = 842\) K, \(H_a = 23.7\) MAm\(^{-1}\), and \(M_s = 1.55\) T compared with the Co-free Sm\(_2\)Fe\(_{20}\)N\(_x\).\(^{4-8}\) On the basis of the results of these results, the present authors have reported that surface-coated fine powders with Zn metal produced via photodecomposition of ethylzinc [Zn(C\(_2\)H\(_5\))\(_2\)] under UV light irradiation, ZnSm\(_2\)(Fe\(_{17}\)Co\(_{0.01}\))\(_2\)N\(_x\), provides excellent magnetic parameters of \(B_r = 1.51\) T, \(H_c = 0.88\) MAm\(^{-1}\), and

\((BH)_{max} = 371\) kJ m\(^{-3}\), of which the \(B_r\) and \(B(H)_{max}\) values are particularly the highest ones among the 2:17 nitrides reported.\(^{1,2}\) Furthermore, the compression-type resin-bonded magnets molded from the above ZnSm\(_2\)(Fe\(_{17}\)Co\(_{0.01}\))\(_2\)N\(_x\) fine powders produced the common \((BH)_{max}\) values (~154 kJ m\(^{-3}\)) which were even lower than the conventional resin-bonded Sm\(_2\)Fe\(_{20}\)N\(_x\) magnets (~176 kJ m\(^{-3}\)). This is mainly due to the magnetic squareness \((H_c/H_m)\) as evaluated in the demagnetization curves of the ZnSm\(_2\)(Fe\(_{17}\)Co\(_{0.01}\))\(_2\)N\(_x\) fine powders as the raw materials for the bonded magnets (where \(H_c\) is the H value at 0.9 \(H_m\)) are inferior to that of the ZnSm\(_2\)Fe\(_{20}\)N\(_x\) fine powders since the aggregation among the fine particles with various sizes as distributed widely prevents a rotation of the individual primary particles along the direction of the applied magnetic field during the molding process. Therefore, if the uniformly ground ZnSm\(_2\)(Fe\(_{17}\)Co\(_{0.01}\))\(_2\)N\(_x\) fine powders are obtained without any additional oxidation, one should produce bonded magnets with improved performance.

The purpose of this study is to demonstrate such high-performance permanent magnetic material based on the excellent intrinsic magnetic property of ZnSm\(_2\)(Fe\(_{17}\)Co\(_{0.01}\))\(_2\)N\(_x\) by preparing the resin-bonded magnets from the stabilized ZnSm\(_2\)(Fe\(_{17}\)Co\(_{0.01}\))\(_2\)N\(_x\) fine powders under optimized conditions and more precisely measuring their aging behavior at 303 K in air.

The Zn metal-coated ZnSm\(_2\)(Fe\(_{17}\)Co\(_{0.01}\))\(_2\)N\(_x\) fine powders were prepared partly according to the procedure described elsewhere.\(^{11}\) All treatments for them were made in a vacuum glove box charged by the deoxidized and dehydrated Ar gas (oxygen and moisture contents < 5 ppm). The compression-type resin-bonded magnets were produced from intimate mixtures of the ZnSm\(_2\)(Fe\(_{17}\)Co\(_{0.01}\))\(_2\)N\(_x\) fine powders with 2.5 wt % of an epoxy-resin under conditions of 1.4 GPa (pressure) and 1.4 MAm\(^{-1}\) (magnetic field). In addition, they were successively cured at 303 K for 10 min under the above conditions as applied. The oxygen content of the resulting powder samples was checked by a nitrogen and oxygen analyzer (Horiba, EMGA-550). The Zn metal content of...
The Bonded Magnets Made from the Surface-Coated Sm$_2$(Fe$_{0.6}$Co$_{0.4}$)$_{17}$N$_x$ Materials in This Study Offered the Highest Magnetic Properties of Any of the Sm$_2$Fe$_{17}$N$_x$-Types.

ADACHI Gin-ya
(Graduate School of Engineering)

Abstract

Rare-earth permanent magnets have superior magnetic properties when compared to conventional ceramic magnets such as ferrites. These magnets, particularly bonded magnets, are used to make electronic equipment for computers and small-sized, light handy-phones. Among the rare-earth magnet materials, the Sm$_2$Fe$_{17}$N$_x$-type materials have been promising candidates as high-performance bonded magnet materials. Partially Co-substituted materials, Sm$_2$(Fe$_{0.6}$Co$_{0.4}$)$_{17}$N$_{x}$, with high magnetic values and good oxidation resistance are being manufactured by an improved grinding and novel protection coating method without any damage caused by oxidation. The bonded magnets made from the surface-coated Sm$_2$(Fe$_{0.6}$Co$_{0.4}$)$_{17}$N$_{x}$ materials in this study offered the highest magnetic properties of any of the Sm$_2$Fe$_{17}$N$_x$-types. In addition, it allowed us to improve the corrosion resistance of the Sm$_2$(Fe$_{0.6}$Co$_{0.4}$)$_{17}$N$_{x}$ bonded magnets.

Introduction

Progress of permanent magnets

Permanent magnets play a vital role in modern human life as a component in a wide range of devices for application in electric vehicles, computers, handy-phones, etc. Among the permanent magnets, the rare-earth magnets made from rare-earth intermetallic compounds such as SmCo$_5$, SmCo$_{17}$, Nd$_2$Fe$_{14}$B, and Sm$_2$Fe$_{17}$N$_x$ ($x$=3) have been developed in the last three decades (see Figure 1). These magnets have a stronger power than the conventional ceramic or non-rare-earth alloy-type magnets, such as ferrites and alnicos. In recent years, a Japanese group succeeded in producing Nd$_2$Fe$_{14}$B-type sintered magnets with the highest maximum energy product ($BH_{\text{max}}$) of 444 kJ/m$^3$ (55.8 MGoe) for permanent magnets produced to date in the world, where the ($BH_{\text{max}}$) value indicates the magnitude of the magnetic field produced by them. The greater the ($BH_{\text{max}}$) value of the permanent magnet is, the stronger their attractive force. The rare-earth sintered magnets with high ($BH_{\text{max}}$) values have been employed as motors for electric vehicles and large-sized magnets for MRI instruments with a rather large size and heavy weight. On the other hand, the rare-earth bonded magnets made by binding magnet powders with a resin as illustrated in Figure 2 are especially suitable for use as micromotors and so on for devices with a small size and complex shape, for instance, computers and handy-phones. This is due to their high dimensional accuracy of the bonded magnets compared with the sintered ones, although the power of the bonded magnets is somewhat less than that of the sintered ones due to a dilution effect by the non-magnetic resin.

Development of Sm$_2$Fe$_{17}$N$_x$-type magnet

The Nd$_2$Fe$_{14}$B magnets are the main products in the current market for the resin-bonded and sintered rare-earth magnets. However, the Nd$_2$Fe$_{14}$B-type magnets are easily demagnetized when used at high temperature. This is due to their low Curie temperature of around 586 K. Japanese and Irish groups have found that Sm$_2$Fe$_{17}$N$_x$ ($x$=3) possesses a higher Curie temperature of 746 K.
than the Nd:Fe₁₄B-type magnets, along with its excellent magnetic anisotropy. This and the related compounds have been developed as attractive candidates for high-performance magnet materials instead of the Nd:Fe₁₄B-types. However, the structure of the Sm₂Fe₁₇N₅-type compounds is the metastable phase which decomposes to SmN and α-Fe upon heating to 873 K. Therefore, the conventional sintered magnets molded by heating at the high temperature of 1000-1100°C can never be produced from the Sm₂Fe₁₇N₅-type materials, and hence these materials have been developed as bonded magnets. It is necessary for Sm₂Fe₁₇N₅-type compounds to be ground into fine powders with a particle size below 3μm in order for use as permanent magnet materials. However, since the magnetic properties of such Sm₂Fe₁₇N₅ powders are easily deteriorated by oxidation, the processes such as grinding and surface coating for protection are very important for preparing the fine powders for high-performance magnets with good oxidation resistance, as well as improving their oxidation resistance by adding extra metal components. The partial substitution of Co to Fe has been reported to be effective for improving not only the intrinsic magnetic properties, but also good oxidation resistance. 4

**Purpose of this study**

The oxidation of rare-earth intermetallic compounds results in a serious deterioration of the magnetic properties. Many groups have studied the protection of them from oxidation during the grinding and the molding processes. For the grinding process, a jet-milling method under a dry condition has been employed as a convenient one, but the resulting fine powders are inevitably oxidized because the atmosphere used contains oxygen for avoiding the aggregation among such fine particles. For protection against oxidation, conventional vacuum evacuation and electroplating methods have been applied to prepare the surface coating film for the ground fine powders. However, the magnetization values of the surface-coated fine powders were significantly reduced by the dilution effect caused by a large amount of non-magnetic coating material and by the oxidation which inevitably takes place with oxygen in the vacuum chamber and water in the aqueous solution used. In this study, our final purpose is to prepare high-performance resin-bonded Sm₂Fe₁₇N₅-type magnets with good oxidation resistance for practical uses. The present work developed the grinding method under wet conditions and the surface coating technique with a small amount of metal that was established for the partially Co-substituted Sm₂(Fe₆₀Co₄₀)₁₋ₓN₅ₓ material.

**Results of this study**

1. The magnetic properties of the as-ground and Zn-coated Sm₂(Fe₆₀Co₄₀)₁₋ₓN₅ₓ fine powders

For the grinding process, we employed the ball-milling method in an organic solution containing a surfactant instead of the conventional dry-process, and optimized the milling conditions. The highest \((BH)_{max}\) value of the as-ground Sm₂(Fe₆₀Co₄₀)₁₋ₓN₅ₓ fine powders in a high dispersion state was obtained for the milling time of 2.5 h, that provided a mean particle size of 1.1 μm in a narrow distribution range. However, although the coercivity was increased by decreasing the particle size, the \((BH)_{max}\) value gradually decreased for times greater than 2.5 h due to the damage of the fine powders by oxidation during the grinding process. Surface coating of the Sm₂(Fe₆₀Co₄₀)₁₋ₓN₅ₓ fine powders with Zn metal were performed by using the Zn metal produced via the photodecomposition of Zn(C₆H₅)₂ under UV light irradiation as shown in Figure 3. The fine powders were scarcely damaged because any further oxidation was effectively retarded due to the coating process performed in the hexane solution free from oxygen or water. The magnetic properties of the as-ground and Zn metal-coated Sm₂(Fe₆₀Co₄₀)₁₋ₓN₅ₓ fine powders prepared under the optimized conditions are summarized in Table 1, together with those of the Co-free Sm₂Fe₁₇N₅ₓ powders previously reported.6, 7, 9 The Zn/Sm₂(Fe₆₀Co₄₀)₁₋ₓN₅ₓ fine powders possessed greater \(B_r\) and \((BH)_{max}\) values than Zn/Sm₂Fe₁₇N₅ₓ and were maintained at a high level even after exposure to 323 K for 24 h in air. The reason why the deterioration by oxidation is depressed is because the

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**Table 1 The magnetic properties of the Sm₂Fe₁₇N₅ₓ-based powders**

<table>
<thead>
<tr>
<th>Material</th>
<th>((BH)_{max}) ([kJ/m²])</th>
<th>Coercivity (H_{c}[\text{MA/m}])</th>
<th>Residual magnetization (B_r[T])</th>
<th>Ref.</th>
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<tbody>
<tr>
<td>Sm₂(Fe₆₀Co₄₀)₁₋ₓN₅ₓ</td>
<td>344</td>
<td>0.84</td>
<td>1.43</td>
<td>This work</td>
</tr>
<tr>
<td>Zn/Sm₂(Fe₆₀Co₄₀)₁₋ₓN₅ₓ</td>
<td>343</td>
<td>0.85</td>
<td>1.43</td>
<td>6</td>
</tr>
<tr>
<td>Sm₂Fe₁₇N₅ₓ</td>
<td>313</td>
<td>0.86</td>
<td>1.37</td>
<td>7</td>
</tr>
<tr>
<td>Zn/Sm₂Fe₁₇N₅ₓ</td>
<td>300</td>
<td>0.84</td>
<td>1.35</td>
<td>10</td>
</tr>
<tr>
<td>Sm₂Fe₁₇N₅ₓ</td>
<td>323</td>
<td>0.9</td>
<td>1.41</td>
<td>11</td>
</tr>
<tr>
<td>Sm₂Fe₁₇N₅ₓ</td>
<td>271</td>
<td>0.72</td>
<td>1.41</td>
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Table 2 Magnetic performances of the resin-bonded magnets produced from Sm\textsubscript{2}Fe\textsubscript{17}Nd\textsubscript{1}\textsubscript{5}, based powders

<table>
<thead>
<tr>
<th>Material</th>
<th>Magnetic properties</th>
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<tr>
<td></td>
<td>(BH)\textsubscript{max} [kJ/m\textsuperscript{3}]</td>
</tr>
<tr>
<td>Zn/Sm\textsubscript{2}(Fe\textsubscript{17}Co\textsubscript{1}$<em>{1}$)$</em>{17}$Nd\textsubscript{1}\textsubscript{5}</td>
<td>186</td>
</tr>
<tr>
<td>Zn/Sm\textsubscript{2}Fe$_5$Nd\textsubscript{1}\textsubscript{5}</td>
<td>176</td>
</tr>
<tr>
<td>Sm\textsubscript{2}Fe$_5$Nd\textsubscript{1}\textsubscript{5}</td>
<td>164</td>
</tr>
</tbody>
</table>

Sm\textsubscript{2}(Fe\textsubscript{17}Co\textsubscript{1}$_{1}$)$_{17}$Nd\textsubscript{1}\textsubscript{5} fine powders are uniformly covered by a thin film of Zn metal and consequently, the dilution effect is minimized. (2) The magnetic performance of the resin-bonded magnets produced from the Zn/Sm\textsubscript{2}(Fe\textsubscript{17}Co\textsubscript{1}$_{1}$)$_{17}$Nd\textsubscript{1}\textsubscript{5} fine powders

The bonded magnets were manufactured by compacting the mixtures of the Zn/Sm\textsubscript{2}(Fe\textsubscript{17}Co\textsubscript{1}$_{1}$)$_{17}$Nd\textsubscript{1}\textsubscript{5} fine powders with an epoxy-resin as the binder in a magnetic field under pressure in the press cavity. In addition, these compacted samples were continuously cured by heat treatment combined with the above conditions. The demagnetization curves of the resulting bonded Zn/Sm\textsubscript{2}(Fe\textsubscript{17}Co\textsubscript{1}$_{1}$)$_{17}$Nd\textsubscript{1}\textsubscript{5} magnets are shown in Figure 4 together with those of the bonded Zn/Sm\textsubscript{2}Fe$_5$Nd\textsubscript{1}\textsubscript{5} and sintered ferrite ones. The resulting bonded magnets prepared in this study gave high magnetic values due to the easy orientation of the isolated fine particles with the Zn coating. In addition, the curing treatment performed in the magnetic field under a pressure of 1.4GPa was found to depress the thermal relaxation of the aligned particles. Consequently, the

(BH)\textsubscript{max} values (~186 kJ/m\textsuperscript{3}) recorded for the resin-bonded Zn/Sm\textsubscript{2}(Fe\textsubscript{17}Co\textsubscript{1}$_{1}$)$_{17}$Nd\textsubscript{1}\textsubscript{5} magnets were higher than those of the Sm\textsubscript{2}Fe$_5$Nd\textsubscript{1}\textsubscript{5}-based bonded ones (see Table 2).

(3) The behavior of the bonded Zn/Sm\textsubscript{2}(Fe\textsubscript{17}Co\textsubscript{1}$_{1}$)$_{17}$Nd\textsubscript{1}\textsubscript{5} magnets in air

The magnetic flux values of the bonded rare-earth magnets deteriorate by exposure in air at 393 K due to oxidation of the components. Figure 5 shows the deterioration behavior of the magnetic flux of the resin-bonded Zn/Sm\textsubscript{2}(Fe\textsubscript{17}Co\textsubscript{1}$_{1}$)$_{17}$Nd\textsubscript{1}\textsubscript{5} magnet at 393 K in air, together with that of the resin-bonded one made from the Co-free Zn/Sm\textsubscript{2}Fe$_5$Nd\textsubscript{1}\textsubscript{5} powders under the same conditions. The flux loss of the resin-bonded Zn/Sm\textsubscript{2}Fe$_5$Nd\textsubscript{1}\textsubscript{5} magnet was ~31.6 % for 300 h. In contrast, the resin-bonded Zn/Sm\textsubscript{2}(Fe\textsubscript{17}Co\textsubscript{1}$_{1}$)$_{17}$Nd\textsubscript{1}\textsubscript{5} magnet showed a smaller flux loss of ~5.8 % even for 300 h compared with that of the Zn/Sm\textsubscript{2}Fe$_5$Nd\textsubscript{1}\textsubscript{5} bonded ones. The high oxidation resistance of the resin-bonded Zn/Sm\textsubscript{2}(Fe\textsubscript{17}Co\textsubscript{1}$_{1}$)$_{17}$Nd\textsubscript{1}\textsubscript{5} magnets was due to improvement of the surface and bulk conditions by the uniform protection coating with Zn and the effective addition of Co, respectively.

Figure 4. Demagnetization curves of magnets.

Figure 5. Time dependence of flux loss of Sm\textsubscript{2}Fe$_5$Nd\textsubscript{1}\textsubscript{5}-type bonded magnets in air at 393 K.

Conclusion

Zn/Sm\textsubscript{2}(Fe\textsubscript{17}Co\textsubscript{1}$_{1}$)$_{17}$Nd\textsubscript{1}\textsubscript{5} fine powders with good oxidation resistance have superior magnetic properties compared to those of the uncoated or Co-free Sm\textsubscript{2}Fe$_5$Nd\textsubscript{1}\textsubscript{5} powder samples. In addition, the Zn metal coating employed in this study is effective for improving the corrosion resistance of the Sm\textsubscript{2}(Fe\textsubscript{17}Co\textsubscript{1}$_{1}$)$_{17}$Nd\textsubscript{1}\textsubscript{5} fine powders without any decrease in their magnetic properties. Moreover, one can produce the resin-bonded Zn/Sm\textsubscript{2}(Fe\textsubscript{17}Co\textsubscript{1}$_{1}$)$_{17}$Nd\textsubscript{1}\textsubscript{5} magnets with the highest (BH)\textsubscript{max} values and good corrosion resistance among a series of bonded Sm\textsubscript{2}Fe$_5$Nd\textsubscript{1}\textsubscript{5} magnets reported. Further optimizations of Zn/Sm\textsubscript{2}(Fe\textsubscript{17}Co\textsubscript{1}$_{1}$)$_{17}$Nd\textsubscript{1}\textsubscript{5} are now in progress for the practical uses such as high-performance magnets for micro-mechtronics and so on.

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International telemicroscopy with a 3 MV ultrahigh voltage electron microscope

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Abstract

The ability for remote microscope operation via a network connection was added recently to the ultrahigh voltage electron microscope (UHTEM) in Osaka University, and used successfully for the observation of thick biological samples across the Pacific Ocean by researchers at the National Center for Microscopy and Imaging Research (NCMIR) at the University of California San Diego. High-quality images at video rate were transferred by a satellite link and control signals were transmitted by an ISDN connecting the workstations at both sites. Most microscope functions operated from the console of the UHTEM were replicated on the graphical user interface of the remote workstation. By clicking on icons or in boxes in the display window with a mouse, the researcher could operate the UHTEM from the remote site. The total delay time for sending images and returning control signals was about 0.7 s, which did not interfere significantly with the smooth operation of the instrument. Researchers at the remote site were able to record images on film in the microscope which were later sent to San Diego. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Ultrahigh voltage EM; Remote operation; Telemicroscopy; Satellite channel

1. Introduction

The ultrahigh voltage electron microscope (UHTEM) [Hitachi H-3000] in Osaka University has the world’s highest acceleration voltage at 3 MV which results in superb penetrating power for specimens both in the material and biological sciences. This feature is invaluable for the observation of thick samples, for research on electron radiation effects and for various kinds of in situ experiments [1]. Researchers around the world have been eager to make use of the UHTEM for their studies but are often unable to afford the expense and time of travel to Japan. To circumvent this inconvenience, the development of a remote operation system is desirable.

Remote operation systems for transmission electron microscopes (TEMs) via network connections have been developed by some groups and the practice termed “Telemicroscopy” [2–8], “Telepresence microscopy” [9–23] or “emsScope” [24]. Remote
International Telemicroscopy with a 3MV EM

TAKAOKA Akio
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1. Introduction

The ultrahigh voltage electron microscope (UHVEM)[Hitachi H-3000][1] in Osaka University has the world's highest acceleration voltage at 3 MV which results in superb penetrating power for specimens both in the material and biological sciences. Because of the invaluable abilities in the observation of thick samples, in the research on electron radiation effects and in various kinds of in-situ experiments, researchers around the world have been eager to make use of the UHVEM for their studies. To release from troublesome travel to Japan and also to connect immediately with their data processing systems, the development of a remote operation system is desirable.

Recently, the basic technology for remote operation, computer control of scientific apparatuses, digitized compression of TV image and high speed networks, has been developed. In USA, attempts to remotely operate transmission electron microscopes (TEMs) via networks have been made in the 1990s by some groups [2]. In Japan, a remote operation system for UHVEM with communication lines has been developed and tested between Osaka University and Hitachi Ltd [3].

The group of Prof. M. Ellisman in the National Center for Microscopy and Imaging Research (NCMIR) at the University of California San Diego (UCSD) has investigated three dimensional morphometry of biological fine structures and they sometimes visited Japan to observe thick samples with the UHVEM. In July 1998, we carried out the international remote observation between the Osaka University and UCSD across the Pacific. This paper introduces the outline of the remote system and its performance.

2. Keynotes of remote operation

There are some approaches in the design of remote operation tools. We regarded the operability and flexibility as important in the remote observation. We designed a remote system which has almost the same functions as those of the intramural console in the center. We also paid attention to the quality of live EM-image and the response of remote observation.

3. Remote control of the UHVEM

As shown in Fig.1, we operate the UHVEM from a remote operation room in our center to protect the operator from the strong X-ray generated by the 3 MV electron beam. The operating signal from the intramural console supervised by the host computer [workstation] is digitized and transmitted to the interface computer in EM house. Then, the control data are distributed to the local CPU units embedded in the driving sources of the UHVEM. The console panel acts as an interface between the operator and the computer to improve the operability. The functions of the console and the host computer are complementary and compatible with one another.

The EM image on the fluorescent screen is observed on the TV monitor of console. Based on the intended purpose of each phase of specimen observation, the suitable camera is selected among the HARP-tube camera of NTSC mode (for the video image transmission and video tape recording in popular mode), the HARP-tube camera of HDTV mode (for the operation of EM by the higher resolution and larger view field), the cooled slow-scan CCD camera (for the digital recording with high sensitivity) and a high sensitivity camera with an image intensifier (for the focus adjustment with the higher contrast and sensitive image but with a narrower field).

Fig.1 Intramural remote operation system of UHVEM H-3000

Network

Host computer

Operator

Image processor

Interface computer

Specimen

Aperture

Vacuum

Film exposure

TV camera

Local CPU units

HV

Lens

EM house

10 Selections : Osaka University 100 Treatises
The remote operation with communication lines is essentially an extension of this intramural remote operation system. The remote-site computer (workstation) is connected to the host computer through the ISDN (integrated services digital network). We developed a GUI (Graphical User Interface) for the monitor of the remote-computer which can be handled only by a mouse. The icons boxed in each smaller windows in Fig.2 correspond to the knobs or buttons on the console panel. Most of functions can be operated from the remote site except for the change of acceleration voltage.

4. Transmission of live image

Although the TV image of HDTV mode has an excellent quality in resolution, it is not practical for the transmission via communication lines at the present because of the requirement of large channel capacity. We tested the remote operation between Osaka University and Hitachi Ltd. [3] or between Osaka University and Atsugi Laboratory of NTT[4] with the TV image in NTSC mode which is compressed to 6 Mbps by a MPEG2 encoder board. The data were transferred via an exclusive high-speed channel.

In the summer of 1998, a high-speed international network available for us has not been fully established. Therefore, we chose broadcast quality analog video image transmission via satellite as the method for the experiments in remote transpacific operation of the UHVEM.

5. International telemicroscopy

Fig.3 shows a schematic diagram of the international telemicroscopy experiment conducted between the Research Center for UHVEM and the NCMIR (UCSD) on June 26, 1998, 1:00 - 6:00
CUT (Coordinated Universal Time) [5]. Time difference between both sites is 16 hours. The EM image was transmitted with a satellite channel. Although the Intelsat IS-802 at 174E can just cover the western coast of the USA directly, the angle of elevation becomes very low. Because of the geographical and structural restriction on the UCSD side, we used an additional domestic satellite in the USA. The image degradation by the satellite channel was negligible because the quality is guaranteed for the use on broadcasting relay services. The delay time of image by the double hops between the satellites and the earth stations is 0.5 ~ 0.6 s.

The computer was connected with an Ethernet LAN to a router used in each country, because the format of DSU (Digital service unit) is slightly different in each telephone service. Both routers were connected with an ISDN line. The ping test between the computers measured the delay time of 0.3 s for going and returning. The total time for the transmission of an image signal and the returning of the control signal was about 0.7 s. This delay is within an acceptable limit for operation of the UHVEM.

For the transfer of high quality still pictures of EM images, we used a standard Internet connected between personal computers. The image files of 1k × 1k pixels captured by the cooled slow-scan CCD camera were transmitted immediately. Additional high quality electron micrographs were recorded on film, digitized on a scanner as files of 2k × 2k and then transferred via Internet in the same day. The transfer rate of Internet link used during these experiments was about 100 kbps and required about 6 minutes to transmit a file of 4 Mbytes.

The communication between operators in both sides was carried out either by a telephone and a TV meeting software package “Cu-see me”. The “Cu-see me” via Internet is useful for the pretest of operation, the monitoring of circumstances and the chat window communications, and its low cost. It was observed that the Internet was not suitable for a conversation between USA and Japan because the connectionless-mode transmission does not work for real time voice communication. For voice communication we found telephone service is much better and quite reliable.

Fig.4 is a stereo-pair image of a selectively stained preparation from frog spinal ganglion neurons in which the cis-face of the Golgi apparatus is contrasted by osmium impregnation. Images of this 3 μm section were recorded in Japan by operation of the microscope from UCSD and transferred as image files to UCSD from Japan. Used windows for this remote operation were mainly Beam ON, Beam Position, Beam Brightness, Focus of Lens, Magnification, Shift of Stage, Tilt of Holder, Selection of TV Camera and Exposure Time. The operability was excellent as it was as if we operated the microscope from the control room of the UHVEM together with the high quality live image. The operability of diffraction mode observation under the adjustment of crystal orientation was also tested with a cross-sectional slice of an LSI device. It is confirmed that the remote operation is very smooth owing to the functional arrangement of operation windows and the excellence of the original control system of the UHVEM.

For the future aspect of international telemicroscopy, we aim to establish some remote sites of the UHVEM in America, Europe and Asia. The remote consoles will be linked with a high-speed (>100 Mbps) network such as the Asia Pacific Advanced Network (APAN) which has been rapidly extended in the world. The key points of remote operation are the transfer of high grade live image and the excellent operability at remote site. The latter can be realized by developing a portable remote console panel which has a similar arrangement to the intramural console. After realizing two key factors, it is expected that researchers practically use the UHVEM from remote sites for the research on 3-dimensional morphometry, electron radiation effects and in-situ experiments.

6. Conclusion

The capability for remote operation was added to the UHVEM of Osaka University and successful international telemicroscopy was demonstrated across the Pacific Ocean from the USA (San Diego, California) to Japan. This instrument is a precious international resource as currently it is the only instrument in the world capable of operation at ultrahigh accelerating voltage (3 MV). This success shows the possibility that the UHVEM can be used from every corner of the world if a high-speed network connection is available. Further development of such capabilities will free the researcher from the requirement of traveling long distances to use such instruments and thereby will improve the efficiency of research and enhance international collaborations.

References
A Novel Subtype of Type 1 Diabetes Mellitus Characterized by a Rapid Onset and an Absence of Diabetes-Related Antibodies


A NOVEL SUBTYPE OF TYPE 1 DIABETES MELLITUS CHARACTERIZED BY A RAPID ONSET AND AN ABSENCE OF DIABETES-RELATED ANTIBODIES

Akihisa Imagawa, Toshiaki Hanafusa, Jun-ichiro Miyagawa, and Yuji Matsuzawa,
for the Osaka IDDM Study Group

Type 1 diabetes mellitus is now classified as autoimmune (type 1A) or idiopathic (type 1B), but little is known about the latter. We classified 56 consecutive Japanese adults with type 1 diabetes according to the presence or absence of glutamic acid decarboxylase antibodies (their presence is a marker of autoimmunity) and compared their clinical, serologic, and pathological characteristics.

We divided the patients into three groups: 36 patients with positive tests for serum glutamic acid decarboxylase antibodies, 9 with negative tests for serum glutamic acid decarboxylase antibodies and glycosylated hemoglobin values higher than 11.5 percent, and 11 with negative tests for serum glutamic acid decarboxylase antibodies and glycosylated hemoglobin values lower than 8.5 percent. In comparison with the first two groups, the third group had a shorter mean duration of symptoms of hyperglycemia (4.0 days), a higher mean plasma glucose concentration (773 mg per deciliter [43 mmol per liter]) in spite of lower glycosylated hemoglobin values, diminished urinary excretion of C peptide, a more severe metabolic disorder (with ketoacidosis), higher serum pancreatic enzyme concentrations, and an absence of islet-cell, IA-2, and insulin antibodies. Immunohistologic studies of pancreatic-biopsy specimens from three patients with negative tests for glutamic acid decarboxylase antibodies and low glycosylated hemoglobin values revealed T-lymphocyte-predominant infiltrates in the exocrine pancreas but no insulitis and no evidence of acute or chronic pancreatitis.

Some patients with idiopathic type 1 diabetes have a nonautoimmune, fulminant disorder characterized by the absence of insulitis and of diabetes-related antibodies, a remarkably abrupt onset, and high serum pancreatic enzyme concentrations. (N Engl J Med 2000; 342:301-7.)

This page is made by IMAGAWA Akihisa as the reprinting of the original treatise was not permitted.
Fulminant Diabetes—A Novel Clinical Entity
IMAGAWA Akihisa
(Graduate School of Medicine)

Type 1 diabetes - a chronic autoimmune disease?
Diabetes mellitus is one of the most common diseases in the world and there are approximately seven million patients in Japan. This common disease is defined as a group of metabolic disease characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both (1-2). As only insulin is able to decrease plasma glucose level, these defects cause hyperglycemia. The vast majority of cases of diabetes fall into two broad etiopathogenetic categories, i.e. type 1 and type 2. The former one is caused by an absolute deficiency of insulin secretion. Thirst or polyuria is a typical symptom of hyperglycemia and patients suffer from life-threatening ketoadosis in type 1 diabetes, if not treated. In addition, the chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels.

Until today, loss of insulin-secreting capacity in type 1 diabetes mellitus has been considered to result from selective autoimmune destruction of pancreatic B cells (3). Autoimmune mechanism is suspected mainly for the following three reasons. The first one is a serological finding. Several autoantibodies to the cytoplasm of islet cells (islet cell antibodies), glutamic acid decarboxylase (GAD), insulin or protein tyrosine phosphatase IA-2/IA-2 B, all of which appear before the clinical onset of diabetes, are good markers for the autoimmune process. The second reason comes from a histological finding. Insulitis, i.e. mononuclear cell infiltration to the pancreatic islets, is observed in the pancreatic autopsy or biopsy specimens of the patient with type 1 diabetes. The third one is a genetic finding. Several specific subtypes of human leukocyte antigen (HLA), which are proven to contribute to immune response, are associated (resistant or susceptible) with type 1 diabetes.

However, we have reported several patients who presented with the abrupt onset of symptoms of hyperglycemia and were ketosis prone, as is typical of patients with type 1 diabetes, but nevertheless did not have insulin (4). At least 10% of newly diagnosed patients with type 1 diabetes do not have any diabetes-related autoantibodies. These findings suggest that autoimmune mechanism is not exclusive in the destruction of B cells in type 1 diabetes. The American Diabetes Association and the World Health Organization have also proposed the presence of idiopathic B cell destruction (type 1B) in type 1 diabetes besides autoimmune (immune-mediated) diabetes (type 1A) (1-2). However, the detailed characteristics of the idiopathic subtype are largely unknown. Therefore, we started the study of patients with idiopathic type 1 diabetes with reference to both clinical and histological features.

Study of 56 type 1 diabetic patients
We studied 56 consecutive recent-onset patients with type 1 diabetes during 1994-1998. First of all, these patients were divided into two groups according to the presence or absence of glutamic acid decarboxylase antibody (GADAb) as a marker of autoimmunity. This autoantibody was positive in 36 patients (63.4%) and negative in 20 patients (35.7%). The initial glycosylated hemoglobin (HbA1c) values (which showed the mean concentration of plasma glucose for a couple of past months) of the GADAb (+) patients were clearly divided into two subgroups, one with HbA1c <8.5% and another with HbA1c >11.5%. So, we compared clinical and histological features in the following three groups; GADAb (+) group, GADAb (-) and high (>11.5%) HbA1c group, and GADAb (-) and low (<8.5%) HbA1c group. No other diabetes-related autoantibodies (islet cell antibodies, IA-2 antibody, and insulin autoantibody) were detected in the serum of any patients of GADAb (+) and low HbA1c group.

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**Table 1. Characteristics of 56 Patients with Type 1 Diabetes, According to Whether the Test for Glutamic Acid Decarboxylase Antibodies (GAD) Was Positive or Negative.**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>GAD-Positive (N=36)</th>
<th>GAD-Negative, Low HbA1c (N=11)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>GAD-Negative, Low HbA1c, vs. GAD-Positive</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>11.7±2.8</td>
<td>12.9±1.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>32</td>
<td>27</td>
<td>0.06</td>
</tr>
<tr>
<td>Range</td>
<td>14-75</td>
<td>14-52</td>
<td></td>
</tr>
<tr>
<td>Male sex (m/f)</td>
<td>14/6</td>
<td>7/4</td>
<td></td>
</tr>
<tr>
<td>Body mass index</td>
<td>19.2±2.6</td>
<td>20.8±2.9</td>
<td>0.016</td>
</tr>
<tr>
<td>First-degree relative with diabetes (n)</td>
<td>7</td>
<td>1</td>
<td>0.005</td>
</tr>
<tr>
<td>Duration of hyperglycemic symptoms</td>
<td>52.4±54.1</td>
<td>45.9±36.2</td>
<td>0.001</td>
</tr>
<tr>
<td>before diagnosis (days)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abdominal pain (n)</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Abnormal findings on pancreatic ultrasound (m)</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Plasma glucose (mg/dl)</td>
<td>398±198</td>
<td>439±179</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Uric acid (mg/dl)</td>
<td>21.0±11.2</td>
<td>19.7±10.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Arterial pH (m)</td>
<td>7.36±0.07</td>
<td>7.34±0.11</td>
<td>0.001</td>
</tr>
<tr>
<td>Serum bicarbonate (mM/liter)</td>
<td>20.6±6.3</td>
<td>19.5±7.3</td>
<td>0.04</td>
</tr>
<tr>
<td>Serum amylase (mg/liter)</td>
<td>0.39±0.16</td>
<td>0.62±0.74</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Serum elastase (ng)</td>
<td>0.45±0.15</td>
<td>0.14±0.02</td>
<td>0.006</td>
</tr>
<tr>
<td>Insulin dose during first yr (U/kg of body weight)</td>
<td>0.43±0.21</td>
<td>0.34±0.24</td>
<td>0.02</td>
</tr>
</tbody>
</table>

*Data were obtained at the time of diagnosis. Plus-minus values are mean ±SD. To convert the values for glucose to mmolites per liter, multiply by 0.056. To convert the values for methionine to milligrams per liter, multiply by 0.88. HbA1c denotes glycosylated hemoglobin.
†The body mass index was calculated as the weight in kilograms divided by the square of the height in meters.
‡All affected relatives had type 2 diabetes except one relative of each of two patients in the antibody-positive group had type 1 diabetes.
§Values for amylase and elastase 1 are expressed as multiples of the upper limit of the normal range.

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33
As shown in Table 1, GADAb(-) and low HbA1c group (right column) indicated several characteristic findings. One, the duration of hyperglycemic symptoms in this group was only four days. The plasma glucose concentration was significantly higher in this subgroup, despite their lower HbA1c values. Two, their urinary C-peptide excretion (which represents endogenous insulin secreting capacity) was significantly lower. Three, all the patients had diabetic ketoadiposis. Four, serum pancreatic enzyme concentrations were high in all patients in GADAb(-) and low HbA1c group, but not in the other two groups.

The individual characteristics including HLA class II haplotype of each patient in GADAb(-) and low HbA1c group are shown in Table 2. Two haplotypes, which were resistant to type 1 diabetes, DRB1*1501/1502-DQA1*0102/0103-DQB1*0602/0601 were found in 30% in this group.

In a part of the patients, pancreatic histology was investigated. Immunohistochemical examination of the biopsy specimens revealed markedly reduced β-cell mass in all patients, as expected in patients with type 1 diabetes. In all patients in GADAb(-) and low HbA1c group, T-lymphocyte-predominant cellular infiltration to exocrine pancreas but no insulitis was seen. On the other hand, insulitis but no cellular infiltration to exocrine pancreas was seen in a GADAb(+) patient (Fig. 1).

**Proposal of a novel subtype of diabetes -nonautoimmune fulminant type 1 diabetes**

Based on these findings, we think the patients in GADAb(-) and low HbA1c group should be categorized into a novel subtype, “non-autoimmune fulminant” type 1 diabetes, within idiopathic (type 1B) diabetes. Eleven patients we identified with “non-autoimmune

patients’ near-normal HbA1c values. Insulin-secretory capacity estimated by urinary C-peptide excretion was low, and the metabolic derangement at the onset was severe.

Third, the patients had markedly elevated serum pancreatic enzyme concentrations, which accords well with the lymphocytic infiltration to the exocrine pancreas seen in the biopsies. Clinical examination excluded the diagnosis of acute pancreatitis.

This is the first report of the detailed feature of idiopathic type 1 (type 1B) diabetes, that suggests other mechanism for pancreatic β cell destruction than autoimmune one.

**Toward the cure for type 1 diabetes**

This novel subtype of diabetes is important both clinically and etiologically. From the clinical point of view, every physician should know the existence of such type of diabetes, because this subtype of diabetes is always life-threatening. The clinical course of diabetes before the diagnosis is usually mild in type 2 diabetes and even in autoimmune type 1 diabetes. However, that of this new subtype is markedly rapid and the metabolic disorder is very severe. Without the precise diagnosis and appropriate treatment, the patients would not survive.

From the etiological point of view, this study revealed that type 1 diabetes, in a part, would result from nonautoimmune β cell destruction, which is different from usual autoimmune one. The identification of this subtype is the first step for the cure. In addition, the precise recognition of this fulminant type of diabetes is necessary for the success of immune therapy in autoimmune type 1 diabetes. Immune therapy is one of the hopeful candidates to the cure for type 1A diabetes, and several protocols have been tried, but its adverse effect is widely recognized. The accurate classification based
on the etiopathogenesis would help the success of a new therapeutic trial for the cure for type 1 diabetes.

The rapid progression of molecular biology in this decade has clarified the etiology of various diseases. Molecular biology technique would be helpful for physicians and patients both as the diagnostic means and therapeutic ones. However, in this study, we have used rather old-fashioned methods in the medicine, such as hearing the accurate clinical course, and the analysis of basic laboratory data and histology, but could find a new clinical entity. Lesson: It is still important to see a patient carefully.

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A single myosin head moves along an actin filament with regular steps of 5.3 nanometres

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Actomyosin, a complex of actin filaments and myosin motor proteins, is responsible for force generation during muscle contraction. To resolve the individual mechanical events of force generation by actomyosin, we have developed a new instrument with which we can capture and directly manipulate individual myosin subfragment-1 molecules using a scanning probe. Single subfragment-1 molecules can be visualized by using a fluorescent label. The data that we obtain using this technique are consistent with myosin moving along an actin filament with single mechanical steps of approximately 5.3 nanometres; groups of two to five rapid steps in succession often produce displacements of 51 to 30 nanometres. This multiple stepping is produced by a single myosin head during just one biochemically driven ATP hydrolysis.

Studies of the actomyosin motor have entered a new phase in the past few years. Structures of the actin monomer and the myosin head, determined by X-ray crystallography, have provided a framework for understanding the interaction between the myosin head and the actin filament. The "tilting crossbridge" model of force generation has been refined, on the basis of the myosin crystal structure, into the "lever-arm" hypothesis. In this model, small structural changes that are coupled to ATP hydrolysis in the catalytic domain of the myosin head are magnified by pivoting of the ~10-nm-long myosin light-chain domain, which acts as a lever arm. The translation caused by this pivoting of the lever arm would be ~6 nm (refs 8, 9).

New techniques for manipulating single actin filaments using microneedles or optical traps and optical sensors that can resolve objects at nanometre scales have allowed the displacement of single molecules of myosin or its subfragments to be measured directly in vitro. The size of displacements reported, however, has varied considerably. Some investigators have found myosin displacements of 4 to 10 nm (refs 11, 14–17), which are consistent with the lever-arm model. On the other hand, we have reported values of 10 to 25 nm (refs 18–20), which is significantly larger than the values expected from the lever-arm model. Large displacement values indicate that myosin heads may interact several times with an actin filament for each ATP used. It is essential to measure the displacement unambiguously to determine how conformational changes in the myosin head lead to force generation, and how mechanical cycles are coupled to ATP hydrolysis.

We have developed a new method for directly manipulating single myosin subfragment-1 (S1) molecules. S1 is the head region of myosin and contains the ATP- and actin-binding sites. The technique uses a scanning probe to measure the mechanical events that occur during generation of actomyosin displacements at high temporal and spatial resolution.

Nano-manipulation of single S1 molecules

The experimental arrangement is shown in Fig. 1a. S1 was biotinylated and fluorescently labelled on the regulatory light chain (RLC) at a molar ratio of >0.9:1 (fluorescent label:RLC). The RLC is a subunit of relative molecular mass 20,000 (20k) that is located near the carboxy terminus of S1, away from the ATP- and actin-binding sites which are located on the S1 heavy chain. The scanning probe, a 5–7-μm-long ZnO crystal whisker, with a very sharp tip (~15 nm radius of curvature), was attached to a fine glass needle mounted on a three-dimensional piezo-electric scanner. The probe was coated with streptavidin so that single myosin heads could be captured specifically at the biotinylated site on the RLC (see Methods). The stiffness of the needles was low (0.1–0.003 pN nm⁻¹), and thus the mechanical load exerted on an S1 molecule was small (<1 pN).

Single S1 molecules captured on the tip of the scanning probe were visualized by objective-type total internal reflection fluorescence microscopy (TIRFM), which produced clear images of single fluorophores at a high fluorescence-to-background ratio (Fig. 1b; an individual S1 molecule captured by the probe is identified by an arrowhead). The fluorescence was characterized by a single, approximately gaussian, intensity distribution and single-step photobleaching (data not shown); strongly indicating that the fluorescent spots were indeed due to single fluorophores. The stiffness of the S1 molecule was brought into contact with an actin bundle in the presence of ATP by manipulating the probe (Fig. 1a, right). We detected the displacements resulting from S1–actin interactions by measuring deflections of the needle, with subnanometre accuracy, using a differential pair of photodiodes.

Individual mechanical events

When S1 was not associated with an actin filament, large thermal fluctuations of the probe with an r.m.s. amplitude of ~15 nm were apparent. Upon binding of S1 to actin, the fluctuations decreased suddenly to an r.m.s. amplitude of <4.5 nm (Fig. 2a, upper trace). Motions of the probe caused by S1–actin interactions could be clearly distinguished from thermal noise by monitoring the increase in stiffness, calculated as the reciprocal of the variance of the fluctuating probe position (Fig. 2a, lower trace). The concentration of ATP was low (0.1 or 1 μM), leading to prolonged interactions between actin and myosin and thus enabling us to identify individual mechanical events. During S1–actin attachments, the stiffness of the probe-S1–actin linkage increased by more than tenfold to 0.2–1.5 pN nm⁻¹ at 20°C, and by more than fivefold to...
Probing the Flexibility of Molecular Machine

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Introduction

Actomyosin, a complex of actin filaments and myosin motor proteins, is responsible for muscle contraction. The sliding movement between actin filaments and myosin molecules is propelled by the chemical energy of ATP hydrolysis. A long-held model for this process hypothesizes that one ATP molecule is hydrolyzed by a myosin head, causing the myosin head to change its structure and pull the actin filament by one step. This model is analogous to the principle of man-made machines that operate deterministically at energies much higher than the thermal noise. However, the myosin molecule is nanometer in size and has a flexible structure, and operates at energy as small as the average energy of thermal noise, so it is very prone to thermal agitation. Actomyosin motors can thus operate under the strong influence of thermal noise, with high chemo-mechanical energy conversion (40% maximum). Therefore, the underlying mechanism of the actomyosin motor must be essentially different from that one would predict from analogy of man-made machines. To elucidate the working principle of the actomyosin motor, it is essential to resolve the intrinsic characteristics of the molecular machines.

Nano-manipulation of single myosin heads by a scanning probe

We first spied on the process of the generation of movement of single actomyosin molecules using new technologies for imaging and nano-manipulation of single biomolecules. The tiny force and displacement — just several piconewtons and nanometers, respectively — that an individual myosin head exerts on actin were measured by scanning probe-based technology (Fig.1). Single myosin head molecules, which had been fluorescently labeled at its tail end and spread on a glass surface, were visualized by an evanescent field (total internal reflection fluorescence microscope, TIRFM). A single myosin head was captured and manipulated by a very fine scanning probe under a TIRFM and brought into contact with an actin filament bound to a glass surface in the presence of ATP. The displacements due to interactions between myosin head and actin were detected by measuring deflections of the needle with sub-nanometer accuracy using a differential pair of photodiodes.

Figure 1 Direct capture and manipulation of a single S1 molecule by a scanning probe. a, Schematic drawing of the experiment. A single S1 molecule, biotinylated and fluorescently labelled >0.35:1 with Cy3 at its regulatory light chain, was specifically attached at its tail end through the biotin-streptavidin system to a scanning probe and observed by objective-type TIRFM. The displacement produced when the S1 molecule was brought into contact with an actin bundle bound to a glass surface was determined by measuring the position of the needle with nanometre accuracy. b, Fluorescence images of single S1 molecules. The micrograph shows superimposed images of single S1 molecules either captured by the probe (arrowhead) or bound to actin bundles on the surface of the coverslip. The red and yellow spots represent, respectively, images before and after movement of the stage by a piezoelectric actuator. The captured S1 molecule (arrowhead) did not move with the stage, but could be moved independently by piezoelectric scanners holding the needle. Bar, 5 μm.
Stepping motion

Displacements of 5 to 30 nm in size took place abruptly with low temporal resolution, which appeared to be consistent with the conventional model (Fig. 2a). However, we found that displacements did not take place abruptly but instead developed by multiple distinct steps on an expanded timescale (Fig. 2b and c). The size of steps was regular and coincident with the periodicity of adjacent actin molecules in an actin filament, 5.5 nm (Fig. 3a). The number of steps per displacement event varied stochastically from one to five (Fig. 3b). Steps were not always forward but backward sometimes (10% of the total number of steps). The size of backward steps was also 5.5 nm. These behaviors of myosin heads agree that the myosin head moves along actin molecules in an actin filament by Brownian motion rather than by changing shape (Fig. 3c).

Figure 2  Displacement caused by single S1 molecules. a, Upper, typical recording of the displacements made by an S1 molecule. Lower, stiffness calculated from the variance of the probe position. b, A record of the rising phase of a displacement on an expanded time scale. c, Records of the rising phase of displacements at various conditions: (i) 1 μM ATP, 20°C; (ii) 0.1 μM ATP, 20°C; (iii) 1 μM ATP, 27°C. Horizontal gridlines have been drawn at a spacing of 5.5 nm.

Chemo-mechanical coupling

How are the 5.5 nm steps coupled to the biochemical cycle of ATP hydrolysis? To answer this question, we measured the ATP hydrolysis reaction and the generation of displacement simultaneously (Fig. 4). Individual ATP hydrolysis reactions of a single myosin head were measured by monitoring association and dissociation of single fluorescently labeled ATP molecules with the head using TIRFM. Each displacement corresponded to one biochemical cycle of ATP hydrolysis, i.e., each 5.5 nm step was not coupled directly to a single ATP hydrolysis reaction.

Model of the movement of the myosin head

The unitary step size coincides approximately with the distance between adjacent actin monomers in one strand of an actin filament (5.5 nm). Each step takes place stochastically and some of the steps are backwards. Multiple steps are produced during a single biochemical cycle of ATP hydrolysis and the number of steps in each displacement is variable.

All these results indicate that myosin moves on actin monomers in an actin filament by biased Brownian motion (Fig. 3c). Myosin heads are known to bind two adjacent actin subunits in rigor. A myosin head may walk along an actin filament using these two binding sites without detaching from the filament. Each step may be produced by a mechanism such as the thermal ratchet. It is also possible that conformational changes within the actin filament play a major role in generation of force.

In order to produce multiple steps for each molecule of ATP split, chemical energy from ATP hydrolysis might be stored in the myosin head or in the actin filament, and released gradually during successive actomyosin interactions. This idea challenges the widely accepted view that force generation is directly coupled to
release of bound ligands. We have recently shown that the myosin head can attach to actin and generate force for a considerable time (>100 ms) after the release of bound nucleotide\(^1\). The results presented here suggest that the myosin head can store energy from ATP hydrolysis and release it productively later in several packets of work.

If the movement of a myosin head is due to Brownian motion, some skillful mechanism is required to bias random Brownian motion into the directional movement. The chemical energy from the ATP hydrolysis may not be directly used to generate the movement of the myosin but may be used to select unidirectional motions from Brownian motions. The mechanism that myosin does not overcome but rather use Brownian motion to effectively gain a good distance with minute energy could explain how myosin can walk under the strong influence of thermal agitation, with high efficiency of energy conversion.

References

Figure 4. Simultaneous measurement of individual ATP hydrolysis reaction and generation of displacements of single myosin heads. \(a\). Schematic of the experiment. A single actin filament with beads attached to both ends was suspended in solution by optical tweezers. The suspended actin filament was brought into contact with a single myosin head molecule bound to the surface of a coverslip. Displacements due to actomyosin interactions were determined by measuring bead displacements with nanometer accuracy. Using a TIRFM, individual ATP hydrolysis reactions were monitored as changes in fluorescence intensity due to association-hydrolysis-dissociation events of a fluorescently labeled ATP molecules with the myosin head. \(b\). Fluorescence image of an association-hydrolysis-dissociation event of fluorescent ATP molecules with a myosin head during generation of displacements. \(c\). Time course for the generation of displacements (upper trace) and changes in fluorescence intensity from fluorescent ATP bound to the myosin head (lower trace). Each displacement event corresponds to one biochemical cycle of ATP hydrolysis.
Mechanical Rotation of the c Subunit Oligomer in ATP Synthase (F₀F₁): Direct Observation

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F₀F₁, found in mitochondria or bacterial membranes, synthesizes adenosine 5'-triphosphate (ATP) coupling with an electrochemical proton gradient and also reversibly hydrolyzes ATP to form the gradient. An actin filament connected to a c subunit oligomer of F₀ was able to rotate by using the energy of ATP hydrolysis. The rotary torque produced by the c subunit oligomer reached about 40 piconewton-nanometers, which is similar to that generated by the γ subunit in the F₁ motor. These results suggest that the γ and c subunits rotate together during ATP hydrolysis and synthesis. Thus, coupled rotation may be essential for energy coupling between proton transport through F₀ and ATP hydrolysis or synthesis in F₁.

The proton-transporting ATP synthase, F₀F₁, consists of a catalytic sector, F₁, or F₀-adenosine triphosphatase (ATPase) (αβγδε), and a proton pathway, F₀ (a,b,c,d). The crystal structure of the bovine αβγ complex indicates that the α and β subunits are arranged alternately around the NH₂-terminal and COOH-terminal α helices of the γ subunit (3). The isolated F₁ hydrolyzes ATP, followed by γ subunit rotation, which is driven by conformational changes of the catalytic subunit (4). The γ subunit rotation in F₁ has been suggested by biochemical experiments (5) and has been observed directly as counterclockwise rotation of an actin filament connected to the γ subunit (6, 7).

The γ subunit rotation in F₁ should be transmitted to the membrane sector, F₀, in order to complete the ATP hydrolysis–dependent proton transport. The detailed underlying mechanism of the energy transmission between F₀ and the γ subunit remains unknown. If the c subunit oligomer rotates counterclockwise (the same direction as γ) in the membrane, the ATP hydrolysis–dependent γ subunit rotation could be connected mechanically to the F₁ sector. In this regard, c subunit rotation has been proposed (2, 8). However, to the best of our knowledge, this possibility of energy coupling has not been studied.

We designed several experimental systems to examine this possibility. The γ and c complex is shown to be a rotor (6–8) and the α, β, δ, ε, α', and b complex is proposed to be a stator in F₀F₁ (8). Therefore, we fixed F₁ α (or β) subunits on a glass surface to demonstrate the rotation of an actin filament connected to the F₀ c subunit, or conversely, the c subunits were fixed and the rotation of α (or β) was examined. ATP-dependent rotation was only successfully observed with the system described below (10). *Escherichia coli* F₀F₁ was immobilized on a coverslip through a His tag linked to the NH₂-terminal of each α subunit (Fig. 1). A c subunit GSH was replaced by cysteine and then biotinylated to bind streptavidin and a fluorescently labeled actin filament. The γ subunit cysteine residues were replaced with alanine (11) in order to avoid direct binding of the actin filament to this subunit. Thus, cysteine is present only in the c subunit of the presumed rotor complex of the
A Biological Molecular Motor, ATP Synthase

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Adenosine triphosphate (ATP), an energy currency of life

An animal or plant requires a constant input of free energy for its living processes such as cellular movement, muscle contraction, uptake of molecules and ions, and synthesis of biomolecules and macromolecules. Animals obtain this energy through the oxidation of foodstuffs, whereas plants obtain it from light. The free energy derived from foodstuffs and light is transformed into a special form before it is used. This special carrier (currency) of free energy is adenosine triphosphate (ATP). The large amount of free energy liberated through ATP hydrolysis is used for a variety of biological reactions. Most ATP is synthesized from ADP (adenosine diphosphate) and orthophosphate by ATP synthase (FoF1) in the membranes of mitochondria, chloroplasts, or bacteria (1-3). During respiration, protons are pumped outside mitochondria or bacteria, where they form an electrochemical proton gradient. The gradient drives ATP synthesis by FoF1. ATP is also synthesized in chloroplasts through the same mechanism. In this paper, we have shown that the membrane-spanning c subunit ring could rotate in FoF1 during ATP hydrolysis (reverse reaction of ATP synthesis). Our finding greatly contributed to the understanding of the biological energy conversion between the electrochemical gradient and the chemistry of ATP synthesis.

ATP synthase FoF1: from structure to subunit rotation

FoF1, a complicated membrane enzyme, consists of two functional units, a catalytic F1 sector formed from three pairs (αβγ) of α and β subunits, and one γ, one δ, and one ε subunit, and a proton pathway, Fo (one a, two b, and 10-12 c subunits) (Fig. 1). The three
catalytic sites, formed mostly from the β subunit residues, participate alternately in ATP synthesis. The crystal structure indicates that the α and β subunits are arranged around the amino and carboxyl terminal α-helices of the γ subunit (4). The isolated F₁ hydrolyzes ATP, followed by γ subunit rotation relative to the αβ₁ hexamer. The γ rotation is driven by successive conformational changes of the three β subunits. This mechanical rotation should be transmitted to the F₀ sector in order to couple the chemistry at the catalytic sites and the proton transport through F₀.

One of the most important questions is how the proton transport through F₀ drives the γ subunit rotation, or in the reverse direction, how ATP hydrolysis-dependent γ rotation is transmitted to the proton transport. Mechanical coupling between the γ and F₀ sector and rotation of the c subunit ring (10-12 monomers forming a ring in the membrane) has been a fascinating possibility. However, nobody has seriously tested this model of energy coupling experimentally.

An experimental system for observing the c ring rotation
The γ and ε subunits were shown to form a rotor (5, 6), and the α, β, δ, a and b complex was proposed to be a stator. We have extensively characterized F₀F₁ from *Escherichia coli* through a combined approach of biochemistry, genetics and structural biology (1-3). Taking advantage of the information obtained in these studies, we engineered *E. coli F₀F₁* and immobilized a whole complex on a glass surface, as shown in Fig. 1. As F₀F₁ is too small (~10 nanometers diameter) for direct observation under a light microscope, we attached a fluorescently labeled actin filament (~1 micrometer length) to the c subunit. Thus, the c ring rotation could be followed continuously by observing the fluorescent actin filament. F₀F₁ is a reversible enzyme, and can hydrolyze ATP to form an electrochemical proton gradient. Thus, the ATP hydrolysis-dependent c subunit ring rotation was examined in this study.

Rotation of the c subunit ring
Upon the addition of ATP, we observed continuous counter-clockwise rotation (viewed from the top of Fig. 1) of an actin filament connected to the c subunit ring (Fig. 2). The rotational rate became slower with an increase in the filament length, because the longer the filament, the greater the viscous drag due to the medium became.

We then plotted the rotational rates for single molecules against the filament lengths (Fig. 3B). The data points well fitted a curve of the rotational rate calculated with a constant torque of 40 pN nm. Our previous work on the γ subunit rotation showed that the γ sub-
unit generated the same torque as that of the c subunit ring (5). These results suggest that the mechanical energy transmission from F$_i$ γ to the Fo c ring occurs with nearly 100% efficiency, consistent with the rotation of γ and c as an ensemble.

Further evidence of the γ and c complex rotation
In the issue of Science in which our paper appeared, Walker and his colleagues published a paper on the crystal structure of FoF$_1$ (7). The structure clearly shows that the γ and c subunits are tightly bound, supporting our observation of the c ring rotation. More recently, a German group also observed the c ring rotation during ATP hydrolysis using a similar system and obtained essentially the same results as ours (8).

Conclusion
Our results indicate that the c subunit ring rotates together with the γ subunit during ATP hydrolysis by FoF$_1$. In the reverse direction, proton flow should drive the c ring rotation, which drives the rotation of the γ to promote ATP synthesis. Our study clearly demonstrated that the mechanical rotation of the γ and c subunit complex is an essential feature for the energy coupling between the proton flow through Fo and ATP hydrolysis/synthesis in F$_i$. Thus, FoF$_1$ can be regarded as a nano motor functioning in biological membranes. It would be worthwhile exploring the possibility of application of this nano motor (or what has been learned from the motor) to engineering.

Fig. 3. Rotation of a filament connected to the c subunit ring. (A) The rotation of actin filaments (1.5, 2.2, 2.9, and 3.6 μm) was followed in the presence of Mg ATP. (B) Rotational rate versus length of the actin filament. The average values for the rotational rates (-30 data points) are plotted with standard deviations against filament length (closed circles). Frictional torque, N, was calculated as N=-(4πη/3)vωL$^2$ / (π(L/2)-0.447), where ω is angular velocity, v (10$^{-4}$ N-sec/m$^2$) the viscosity of the medium, L the length of the actin filament, and r (5 nm) the radius of the actin filament. The dotted line represents the calculated rotational rates for the filaments with a constant torque value of 40 pN-nm. For comparison, the rotational rates for the γ subunit in F$_i$ are plotted (open circles). Reprinted with permission from Science, Volume 286, No. 5445, pp. 1722-1724, 26 November 1999. Copyright 1999 American Association for the Advancement of Science.
Limb and Skin Abnormalities in Mice Lacking IKKα

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The gene encoding inhibitor of kappa B (IκB) kinase α (IKKα; also called IKK1) was disrupted by gene targeting. IKKα-deficient mice died perinatally. In IKKα-deficient fetuses, limb outgrowth was severely impaired despite unaffected skeletal development. The epithelial cells in IKKα-deficient fetuses were highly proliferative with dysregulated epidemal differentiation. In the basal layer, degradation of IκB and nuclear localization of nuclear factor kappa B (NF-κB) were not observed. Thus, IKKα is essential for NF-κB activation in the limb and skin during embryogenesis. In contrast, there was no impairment of NF-κB activation induced by either interleukin-1 or tumor necrosis factor-α in IKKα-deficient embryonic fibroblasts and thymocytes, indicating that IKKα is not essential for cytokine-induced activation of NF-κB.

The IκB kinase, a large protein complex, phosphorylates two serine residues of the IκB proteins. This leads to degradation of IκB and activation of NF-κB transcription factors. IKKα was identified as a subunit of the IκB kinase complex that directly phosphorylates IκB (2, 3). IKKβ was subsequently identified as a second subunit of the IκB kinase complex that forms a heterodimer with IKKα (3, 4). In vitro studies have indicated that both IKKα and IKKβ (also called IKK2) may contribute to tumor necrosis factor-α (TNF-α)- and interleukin-1 (IL-1)-induced activation of NF-κB (2–4).

To assess the in vivo role of IKKα, we disrupted the IKKα gene by homologous recombination in E14.1 embryonic stem (ES) cells (5). A targeting vector was constructed to replace an exon encoding subdomain VI of the kinase catalytic portion with a neomycin resistance gene. Two correctly targeted ES clones successfully transmitted the disrupted allele through the germ line (Fig. 1A). The heterozygous (IKKα−/−) mice were phenotypically normal and healthy. To generate IKKα−/− mice, the heterozygotes were crossed. IKKα−/− progeny were born with normal appearance and died within 4 hours after birth. Newborn IKKα−/− pups had defective development of limbs and tails (Fig. 1D), and their skin was abnormally shiny. Northern (RNA) and protein immunoblot analysis of embryonic fibroblast (EF) cells confirmed that the disruption of the IKKα gene abolished the expression of IKKα mRNA and protein (Fig. 1, B and C). Expression of mRNA and protein for IKKβ was slightly increased in IKKα−/− EF cells.

Examination of stained skeletal preparations from the fetus at 18.5 days postcoitum (dpc) revealed that IKKα−/− mice had no defect in development of bone or cartilage, although the lengths of limb, tail, and craniofacial bones and cartilage were shorter than those for wild-type animals (Fig. 1D). Leg bones were compactly and tightly folded and tail cartilage was rolled up in IKKα−/− pups. These findings indicate that skeletal development was normal; however, limb mesenchyme outgrowth was impaired in IKKα−/− fetuses. Activation of NF-κB is essential for limb development in chickens (6).

Therefore, we analyzed whether IKKα was expressed in the developing limb by whole-mount in situ hybridization (7). IKKα was expressed predominantly in the limb buds of wild-type fetuses at 12.5 dpc (Fig. 2A). In IKKα−/− fetuses, IKKα was not expressed, and the limb bud showed a slightly abnormal phenotype relative to that of wild type (Fig. 2B). IKKβ was also expressed in the limb buds, particularly the flexion of the wild-type as well as IKKα−/− fetuses at 12.5 dpc (Fig. 2, C and D). A Drosophila melanogaster homolog of NF-κB, dorsal, positively and negatively regulates expression of twist and decapentaplegic (dpp), respectively (6). The murine twist homolog is expressed in limb bud mesenchyme (9), and mutations in Twist lead to craniofacial and limb anomalies in humans (10). In the wild-type fetuses, Twist was expressed in the limb bud at 12.5 dpc (Fig. 2E). However, expression of Twist was reduced in the limb buds of IKKα−/− fetuses at 12.5 dpc (Fig. 2F). Expression of the bone morphogenic protein-4 gene (BMP4), the vertebrate dpp homolog, was not altered in the limb buds of IKKα−/− fetuses at 12.5 dpc (Fig. 2, G and H). Reduced Twist expression in IKKα−/− fetuses was also observed at 13.5 dpc (Fig. 2J). Taken together, these results indicate that IKKα regulates gene expression required for limb development, possibly through activation of NF-κB.

Tissue sections of skin at 18.5 dpc were stained with hematoxylin and eosin and examined by light microscopy. At this developmental stage, the full program of epidermal differentiation was nearly complete in wild-type mice (Fig. 3A). In contrast with the ridged and laminated normal stratum corneum of wild-type mice, IKKα−/− mice exhibited prominent parakeratosis without a visible stratum granulosum (Fig. 3B). The stratum spinosum of IKKα−/− epidermis was hyperplastic. The development
IKKα is Involved in Development of Skin and Limb

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NF-κB is a key mediator in the immune response

The NF-κB family of transcription factors plays an important role in many aspects of inflammatory and immune responses (1, 2). NF-κB is activated by a variety of stimuli, including inflammatory cytokines such as interleukin-1 (IL-1) and tumor necrosis factor-α (TNF-α), bacterial components such as lipopolysaccharide (LPS), and by viral infection. In resting cells, NF-κB proteins are maintained in the inactive state in the cytoplasm through interaction with the inhibitory IkB proteins. Upon stimulation, IkB proteins are rapidly phosphorylated on two conserved serine residues, leading to their ubiquitination and consequent degradation by the 26S proteasome. This is followed by the translocation of the NF-κB proteins into the nucleus where they induce expression of their target genes (Fig. 1). The signaling pathway leading to NF-κB activation is phylogenetically conserved. In Drosophila, three NF-κB homologues have been described: Dorsal, Dif, and Relish. Analysis of mutant flies has indicated that Dif is required for the expression of anti-fungal peptides, and Relish for the expression of anti-bacterial peptides. Dorsal was first identified as a transcription factor responsible for establishing dorso-ventral patterning during embryogenesis, but subsequently it was shown to also be involved in controlling the expression of anti-fungal peptides in the adult fly. Thus, in Drosophila, NF-κB proteins play important yet distinct roles in embryonic development and inflammatory responses (2). In mammals, five NF-κB family proteins have been identified, and mice with targeted deletions of each have been generated. All these knockout mice display severe alterations in thier immune responses, confirming the idea that NF-κB proteins play important roles in immune and inflammatory responses in vivo. However, the key molecule(s) that directly regulate NF-κB activation had been unknown for a long time.

Identification of kinases essential for NF-κB activation

Many investigators have tried to identify the kinase responsible for phosphorylation of IκB, a key step leading to NF-κB activation. In 1996, Maniatis’ group identified a high molecular weight IκB kinase complex that specifically phosphorylates the serine residues of IκBα (3). In 1997, intensive efforts of investigators at last led to success in the molecular cloning of two critical components of the IκB kinase complex (4-8). These two components, named IKKα and IKKβ (or IKK1 and IKK2), show significant sequence similarity, including conserved regions such as helix-loop-helix, leucine zipper, and kinase domains (Fig. 2). In vitro studies indicated that IKKα and IKKβ form hetero- and homo-dimers through the interaction of their leucine zipper domains. Several in vitro experiments indicated that both IKKα and IKKβ possess kinase activities and can directly phosphorylate the serine residues of IκB proteins. These studies further showed that IKKα and IKKβ are activated in response to inflammatory cytokines such as IL-1 and TNF-α. Thus, IKKα and IKKβ were shown to be essential catalytic components of the IκB kinase complex. However, the physiological role of each kinase was not clear.

IKKα is involved in embryonic development

To assess the in vivo role of IKKα, three independent groups including ours, generated IKKα−/− mice by gene targeting (9-11). IKKα−/− mice had an abnormal appearance at birth and died within 4 hours after birth. Newborn IKKα−/− pups had defective development of limbs and tails, and their skin was abnormally shiny (Fig. 3A).

![Fig. 1 Signaling pathway leading to activation of NF-κB](image)

See text for details.

![Fig. 2 Structure of IKKα and IKKβ](image)

IKKα and IKKβ are closely related in their structure. Both proteins have several conserved domains, such as a catalytic kinase domain (KD), leucine zipper domain (LZ), and helix-loop-helix domain (HLH).

![Fig. 3 IKKα-deficient mice die shortly after birth.](image)

(A) Gross appearance of IKKα wild-type (+/+ ) and mutant (−/−) newborn mice.
(B) The skeleton of IKKα wild-type (+/+ ) and mutant (−/−) embryos at 18.5dpc. Embryos were double stained with Alizarin red and A2dian blue. Bone is stained red and cartilage blue.
Examination of stained skeletal preparations from the fetuses at 18.5 days postcoitum (dpc) revealed that IKKα−/− mice had no defect in the development of bone or cartilage, but the lengths of their limbs, tails, and craniofacial bones and cartilage were shorter than those of wild-type animals (Fig. 3B). The leg bones of IKKα−/− pups were compactly and tightly folded and their tail cartilage was rolled up. These findings indicated that in IKKα−/− fetuses skeletal development was normal, but that limb mesenchyme outgrowth was impaired.

Histological analysis of the skin of IKKα−/− embryos at 18.5 dpc revealed prominent parakeratosis without a visible stratum granulosum. Immunohistological analysis using antibodies to proteins expressed at defined stages of epidermal differentiation demonstrated that epidermal development was severely compromised. Expression of IκB and NF-κB proteins in the epidermis of wild-type and IKKα−/− mice was compared by immunohistological methods. Whereas no expression of IκBα or IκBβ was observed in the basal layer of wild-type mice (Fig. 4, A and C), expression was observed in the IKKα−/− mice (Fig. 4, B and D). Furthermore, in wild-type mice, cytoplasmic expression of RelA, a p65 component of NF-κB, was lower in the basal layer than the stratum spinosum (Fig. 4E), and RelA was expressed in the nucleus in some basal layer cells (Fig. 4, E, G, I). These findings suggest that IκBαB is degraded in the basal epidermal layer cells of wild-type mice, resulting in the translocation of NF-κB to the nucleus. In contrast, RelA was expressed in the cytoplasm but not in the nucleus of all cells in the basal layer in IKKα−/− epidermis (Fig. 4, F, H, J). Thus, NF-κB was activated in the basal cell layer of wild-type, but not IKKα−/− epidermis. These results indicate that IKKα−/− mice, induced NF-κB activation in basal layer cells may be required for terminal differentiation of the epidermis at this developmental stage.

IKKα was originally identified as a kinase responsible for IL-1 and TNF-α-induced activation of NF-κB (16, 46). However, IKKα−/− embryonic fibroblast (EF) cells were shown to produce normal amounts of IL-6 in response to IL-1 and TNF-α. IKKα−/− EF cells displayed IL-1− and TNF-α–induced degradation of IκBα and IκBβ, accompanied by NF-κB activation. These results indicate that IKKα is not critically involved in NF-κB activation in response to inflammatory cytokines such as IL-1 and TNF-α.

**IKKβ is essential for NF-κB activation by inflammatory cytokines**

IKKβ-deficient mice were also generated by gene targeting by three independent groups (12–14). IKKβ−/− mice died before 14.5 dpc with severe liver degeneration and apoptosis. This phenotype of IKKβ-deficient mice is quite similar to that observed in mice lacking RelA, the p65 subunit of NF-κB (15). Lethality could be rescued by inactivation of the TNF-α signaling pathway (12). Additionally, IKKβ−/− EF cells were very susceptible to TNF-α-induced apoptosis (12). This indicates that TNF-α activates two distinct signaling pathways; one involves NF-κB and is essential for prevention of apoptosis, and the other is NF-κB-independent and responsible for induction of apoptosis. In IKKβ−/− cells, degradation of IκBαB and activation of NF-κB in response to IL-1 and TNF-α is markedly impaired. Taken together, these results suggest that IKKβ is essential for activation of NF-κB induced by inflammatory cytokines.

**Distinct physiological roles of IKKα and IKKβ**

Studies with knockout mice have revealed that the two highly conserved kinases IKKα and IKKβ have distinct and non-compensatory roles in vivo. IKKα and IKKβ have both been shown to exist as heterodimers, but are activated in response to different signals. IKKα is essential for the development of limbs and skin, whereas IKKβ is essential for the responses to inflammatory cytokines (Fig. 5). These findings raise new challenges for us. Can either IKKs function as a homodimer in vivo? Is there any significance to the heterodimerization of IKKα and IKKβ? How is each IKK activated by its specific stimuli? Elucidation of these and other questions will provide us with a more precise mechanism for NF-κB activation in the future.

Another intriguing point in these studies is that IKKα has an important role in embryonic development. This is apparently in contrast to results showing that mice lacking individual members of the NF-κB family of proteins are developmentally normal. However, a number of results support the role of NF-κB in development. In Drosophila, the NF-κB homologue Dorsal is essential for dorso-ventral patterning in the embryo and regulates expression of several genes important for embryonic development such as twist and decapentaplegic (dpp) (16, 17). Mutations in the human homologue of the twist gene have been shown to cause craniofacial, limb and skeletal anomalies similar to those observed in IKKα−/− mice (18, 19). When a mutant form of IκBα that suppresses NF-κB activation was expressed in the developing limb buds of chicken,
limb development was found to be affected (20, 21). A similar epidermal abnormality was observed in transgenic mice with skin-specific expression of dominant negative IκB proteins (22). Thus, numerous studies imply that NF-κB activation is involved in embryonic development, and support the notion that NF-κB activation mediated by IKKα-dependent IκB phosphorylation, is essential for outgrowth in vertebrate limb development and terminal differentiation of skin. To date, knockout mice lacking individual NF-κB family members have not shown any developmental abnormalities. This result may be because individual NF-κB proteins can complement one another. Immunohistological analysis indicated that NF-κB activation did not occur in the epidermis of IKKα−/− mice. From these findings, it can be speculated that IKKα-induced NF-κB activation is important for skin development. Alternatively, there is a possibility that IKKα regulates activation of signaling pathways in skin development that do not involve NF-κB. At present, the ligand(s) that activates IKKα during embryonic development remains unclear. Identification of ligands or upstream signaling pathways that activate IKKα will shed light on the field of skin and limb development.

Fig. 5 IKKα and IKKβ have distinct functions in vivo
IKKα and IKKβ form heterodimers, however each kinase is activated by distinct stimuli. IKKα responds to unknown morphogenic stimuli, and is essential for development of skin and limb. IKKβ responds to inflammatory cytokines, and is responsible for inflammatory responses and anti-apoptotic responses.

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Crystal Structure Determinations of Oxidized and Reduced Pseudoazurins from Achromobacter Cycloclastes: Concerted Movement of Copper Site in Redox Forms with the Rearrangement of Hydrogen Bond at a Remote Histidine

SUZUKI Shinnichiro and KAI Yasushi
(Graduate School of Science; Graduate School of Engineering)

The crystal structures of oxidized and reduced pseudoazurins from a denitrifying bacterium, Achromobacter cycloclastes IAM 1013, have been determined. The distorted tetrahedral copper sites in both forms exhibit a small change in the copper geometry, while a unique peptide bond flip is identified. In the oxidized form (light blue ball), the imidazole ring of His6 has a hydrogen bonding distance of 2.73 Å between N-5 (His6) and O-γ' (Thr36). In the reduced form (yellow and red balls), the imidazole ring rotates by 30.3° and moves 1.00 Å away from the position of the oxidized form. A new hydrogen bond between N+2 (His6) and O-1 (Glu4) is formed with a distance of 3.07 Å, while the hydrogen bond between N-5 (His6) and O-γ' (Thr36) is maintained with an internuclear distance of 2.61 Å. A concomitant peptide bond flip occurs in the main chain (His34-Pro35-Thr36). These findings might be associated with the electron transfer mechanism.

An Electric Trap: A New Method for Entrapping Cyclodextrin in a Rotaxane Structure

HARADA Akira
(Graduate School of Science)

Rotaxane, a molecule comprised of a rotor and an axle, has attracted much attention recently as a molecular machine and as a molecular device. It is usually prepared by threading a ring onto an axle and closing the end groups with bulky stoppers. More recently, it has been prepared by slipping a ring into a dumbbell structure. In either case, bulky stoppers are required for the impropment of a ring molecule in a dumbbell structure. This report introduces a new method of preparing a rotaxane by using repulsive forces between end groups and a ring molecule, even without bulky stoppers.
Formation of Linearily Arrayed Gold Nanoparticles on Gold Single-Crystal Surfaces

MURAKOSHI Kei and NAKATO Yoshihiro
(Graduate School of Engineering Science)

Advanced Materials, 12, 791-795 (2000)

The effect of illumination on the agglomeration of surface-modified gold nanoparticles (diameter ~ ca. 6 nm) both in solution and on gold (111) surfaces was investigated. With the use of wavelength-selective surface plasmon vibration excitation, a close-packed and linearily arrayed super-structure of gold nanoparticles has been formed successfully for the first time. The results imply that photo-illumination can be applied to achieve simultaneous large-volume, high-density, and low-energy formation of super-structures in the nano-size range, which was previously unexplored.

A Mandala-Patterned Bandanna-Shaped Porphyrin Oligomer, C_{1244} H_{1350} N_{84} Ni_{20} O_{38}, Having a Unique Size and Geometry

SUGIURA Ken-ichi and SAKATA Yoshiteru
(Institute of Scientific and Industrial Research)

Chemistry Letters, 1193-1194 (1999)

Inspired by the structure of plant photosynthesized protein, an attempt was made to construct a molecular structure of 21 covalently-bonded porphyrin oligomers. It has a huge, flat configuration, measuring 6.6 nano-meters. Furthermore, a successful direct observation was achieved of one molecule using scanning tunneling microscopy (STM).

Left: Molecular structure of 21 porphyrin oligomers
Right: STM image of the molecule (observation conducted by Prof. T. Kawai and Dr. H. Tanaka, ISIR)
Amorphization in Silicon by Electron Irradiation

TAKEDA Seiji
(Graduate School of Science)
*Physical Review Letters, 83, 2, 1999, 320-323, TAKEDA Seiji, YAMASAKI Jun, Copyright 1999 by the American Physical Society. Color added to the figure of the original publication.

This paper reports an experimental finding that crystalline Si transforms to amorphous Si under electron irradiation. Crystalline Si in the central area in (b) has undergone electron irradiation; as a result, Bragg spots disappear and a halo comes out due to amorphization, as seen in electron diffraction patterns in (a). Amorphization induced by the primary charged particles provides a useful insight into the mechanism of amorphization in silicon, which has been debated for a long time. In fact, we have succeeded in estimating the principal physical parameter, i.e. the threshold particle energy necessary for amorphization. Furthermore, our finding is expected to be applied to fabricating artificial nano-structures with two Si structures with different dielectric and transport properties, since an electron beam can be focused and scanned easily.

Studies of Ultra-Intense Laser Plasma Interactions for Fast Ignition

TANAKA Kazuo
(Graduate School of Engineering and Institute of Laser Engineering)

The fundamental experiment of a revolutionary new technique of laser inertial confinement fusion, "fast ignition," is reported. When laser intensity in plasmas exceeds $10^{19}$ W/cm², electrons oscillating in the laser's electric field are accelerated almost to the level of the speed of light, with their motion becoming relativistic. The diagram shows X-ray pinholes pictures of ultra-intense laser shots emitted into plasma pre-produced on a planar plastic target (viewed from the side). The focal point of the ultra-intense laser was set at (a) 100 µm, (b) 170 µm, and (c) 500 µm away from the original planar target surface. A clear local emission seen on the target surface of (b) is from the laser pulse and indicates abnormal passage through plasmas surpassing critical density. Such passage is possible with a relativistic effect.

A Wireless Near-Infrared Energy System for Medical Implants

KA WATA Satoshi
(Graduate School of Engineering)


A laser-powered cardiac pacemaker has been developed to eliminate the need for the replacement of the presently used non-rechargeable batteries. The power for recharging the pacemaker battery is delivered by near-infrared laser which transmits well through human skin and is photoelectrically converted by the solar cell in the pacemaker. The irradiation power of the laser is one-tenth that of sunlight. Running the implants by light power is a noninvasive, noninfectious, and painless method.

High-Pressure Phase Equilibrium and Raman Microprobe Spectroscopic Studies on the Methane Hydrate System

OHGAKI Kazunari
(Graduate School of Engineering Science)


Methane hydrates, which have attracted great interest recently as a key substance for the application in global environmental problems, are ice-like crystals composed of a methane molecule trapped in a hydrogen-bonded water cage. A single crystal of methane hydrate was prepared through a temperature-swing method over a few days at 321K and 463MPa. Under conditions of constant temperature and pressure, the single crystal was analyzed with the use of a laser Raman microprobe spectrometer.
Elastic Properties of a Unidirectional SiC/Ti Composite: Acoustic-Resonance Measurements and Micromechanics Predictions

OGI Hirotsugu
Graduate School of Engineering Science

Silicon-carbide-fiber reinforced Ti-alloy composite (left) is a promising material for jet-engine and aerospace structure components because it retains high strength and toughness at elevated temperatures. Nine elastic stiffnesses have been known to exist in this material and, for designing a structure, we must measure them – never trivial and often impossible task for such a fiber-reinforced composite. A new electromagnetic-acoustic-resonance technique we developed allows us to measure the complete set of elastic stiffnesses: a solenoid coil surrounding the specimen and a pair of permanent magnets (right) vibrate the specimen via the Lorentz-force mechanism and detect resonance frequencies of free vibration, which indicate all of the stiffnesses of the composite. This technique also derives the complete set of the single SiC-fiber elastic stiffnesses.

Observation of Supercurrent Distribution in YBa$_2$Cu$_3$O$_{x}$ Thin Films Using THz Radiation Excited with Femtosecond Laser Pulses

HANGYO Masanori
Research Center for Superconducting Materials and Electronics

In 1995, we found that electromagnetic wave pulses are radiated into free space from high-temperature superconducting thin films excited with ultrashort laser pulses. Their frequency spectrum extends from 0.01 to 2 THz. Based on this result, we have devised a new system of visualizing supercurrent flow without contact by using this phenomenon and successfully applied it to visualizing the supercurrent flow in high temperature superconducting thin films, as seen in the figure. The supercurrent is believed to flow mainly along the edge of the film.
Photon Pressure-Induced Association of Nanometer-Sized Polymer Chains in Solution

MASUHARA Hiroshi
(Graduate School of Engineering)

Microscopic images show trapping and the accompanying single micro-particle formation process of carbazolyl-containing copolymers in water by photon pressure of a focused 1064 nm laser beam. Dark and bright images are due to back-scattering of HeNe laser and transmission respectively. Formation time (A and B) depends on molecular structure, and the minimum size trapped by photon pressure is determined to be 12 nm.

Laser-Hole Boring into Overdense Plasmas Measured with Soft X-Ray Laser Probing

KODAMA Ryosuke
(Institute of Laser Engineering)

By using an x-ray laser probe system, we have successfully observed for the first time high-power laser-hole boring into over-density plasmas, pushing away the critical density and expelling high-temperature, high-density plasmas with intense photon pressure. Shown are the 2-dimensional electron density map (right) and the 1-D density profile (left). As the intensity of interaction laser light is increased, the enormous photon pressure overcomes the plasma pressure and creates the light wave guide in the over-density plasmas. In the experiments, the photon pressure was near GBar. This result is not only an important milestone for the laser fusion research but also useful discovery for the neutrino-driven hydrodynamic instability under the acceleration with no mass material such as light.
Control of the Cell Death Pathway by Dapaf-1, a Drosophila Apaf-1/CED-4-Related Caspase Activator

MIURA Masayuki
(Graduate School of Medicine)
Molecular Cell, 4, 757-769 (1999)

The size of the larval brain in homozygous mutant of Drosophila caspase activator Dapaf-1, believed to be the common mediator of cell death, is larger than that of the wild type (upper panel). The occurrence of brain cell death is reduced in this mutant (lower panel). This suggests that the brain tissue growth results from an excess in the existence of cells that should normally die. Dead cells can be identified by acridine orange, which turns them dark green.

Presenilin-1 Mutations Downregulate the Signalling Pathway of the Unfolded-Protein Response

KATAYAMA Taiichi
(Graduate School of Medicine)

To confirm that vulnerability of cells expressing PS1 mutants to ER stress is due to the attenuated induction of GRP78 mRNA by inhibition of the stress sensor protein IRE1 function, we infected SK-N-SH cells bearing mutant PS1 with recombinant GRP78 using Semiliki-Forest virus (SFV) and studied the response to ER stress caused by treatment with tunicamycin (Tm). Sensitivity to ER stress in SK-N-SH cell lines expressing PS1 mutants was reversed by infection with recombinant GRP78. Cells expressing PSW (wild PS1) and A246E (mutant PS1) respectively were studied after 40 hours of treatment with 0.5 μg/ml-Tm to determine neuronal death by Live/Dead assay staining. Green cells are alive, while red ones have undergone neuronal death (arrowheads). Note that the death of SK-N-SH bearing A246E was attenuated by infection with recombinant SFV-GRP78.
Actomyosin Contraction of the Posterior Hemisphere is Required for Inversion of the Volvox Embryo

OGIHARA Satoshi

(Graduate School of Science)

Development, 126, 2117-2127 (1999)

The fresh water green algae Volvox carries their embryos inside. The embryos (<100 μm) are in the shape of a hollow ball, consisting of 2,000 cells in a single layer sheet, and already have “future embryos” outside the surface. In a developmental process called inversion, Volvox embryos become inside-out within 45 minutes. Through inversion, “future embryos” become encapsulated inside the “present embryos”, thus establishing the mother-embryo relationship in spatial terms. When inversion starts, the cell sheet of an embryo begins to bend outward from an opening called phialopore, and the bend propagates to the opposite pole until the embryo becomes “inverted” (from left to right row). The bend is caused by a series of microtubule-dependent cell shape changes. Another force-generating protein actin filaments (white in the top, red in the middle/bottom) cause, together with myosin, a compaction of the pre-inverted region. This compaction reduces the equatorial diameter of the embryo and hence facilitates the passage of the posterior hemisphere through the phialopore. Without the actomyosin-dependent force, the entire part of the embryo can not pass through the narrow opening defined by the already-inverted anterior hemisphere. Nuclei (blue) and Nomarski images (green) are superimposed.

Improvement in Castleman’s Disease by Humanized Anti-Interleukin-6 Receptor Antibody Therapy

NISHIMOTO Norihiro

(School of Health and Sport Sciences)


Castleman’s disease is an atypical lymphoproliferative disease refractory to corticosteroids and chemotherapy, and the prognosis is poor. We have determined that interleukin-6 (IL-6) overproduction from affected lymph nodes is responsible for the systemic manifestations of this disease, and constituted a new therapeutic strategy to block IL-6 signal by humanized anti-IL-6 receptor antibody. The figure shows serial histopathological change of lymph nodes effectuated by this therapy. Note the reduction in both the sizes of lymph follicles and in the vascularity of germinal centers. (Hematoxylin-eosin stain; A and C before therapy; B and D after therapy; A and B, original magnification ×100; C and D, ×400)
Sign Language "Heard" in the Auditory Cortex
*NISHIMURA Hiroshi*  
(Graduate School of Medicine)  

The upper regions of the brain's temporal lobe are important both for hearing and for comprehending spoken language. We have discovered that these regions can be activated by sign language in congenitally deaf subjects, even though the temporal lobe normally functions as an auditory area. This finding indicates that, in deaf people, the region of the brain usually reserved for hearing may be activated by other sensory modalities, providing striking evidence of neural plasticity.

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Dissection of Signaling Cascades through gp130 in Vivo: Reciprocal Roles for STAT3- and SHP2-Mediated Signals in Immune Responses
*HIRANO Toshiy*  
(Graduate School of Medicine)  

Upon the binding of cytokine to its receptor, a variety of signal pathways are activated through distinct cytoplasmic regions of the receptor, leading to the expression of various biological functions. Gp130 is a common receptor sub-unit shared among the IL-6 cytokine family receptors. Two major signal pathways, SHP-2-mediated and STAT3-mediated, are activated in a manner dependent on different tyrosine residues of gp 130. We utilized knock-in technology to create mice deficient only in the gp 130-mediated SHP-2 pathway or STAT3 pathway to identify the functions of these two pathways.
Keratinocyte-Specific Ablation of Stat3 Exhibits Impaired Skin Remodeling, but Does Not Affect Skin Morphogenesis

SANOG Shigetoshi
(Graduate School of Medicine)
The EMBO Journal, 18, 4657-4668 (1999)

To elucidate the biological role of Stat3 in skin, we established Stat3 knockout mice selectively in keratinocytes using the Cre-loxP system. These mice were born normal, and their skin developed normally. However, the hair cycle and wound healing processes of the mice were severely impaired. In vitro migration of Stat3+/keratinocytes by growth factor stimulation such as EGF was impaired, indicating that Stat3 plays a critical role in transmitting a signal for cell migration. Aged mice developed spontaneous skin ulcers and hair loss, suggesting that Stat3 is required for the maintenance of skin architecture.

Activation of Neuronal Caspase-3 by Intracellular Accumulation of Wild-Type Alzheimer Amyloid Precursor Protein

Yoshikawa Kazuaki
(Institute for Protein Research)
The Journal of Neuroscience, 19, 6955-6964 (1999)

A dorsolateral-dopamine-mediated over-expression of APP, a protein associated with the brain pathology of Alzheimer's disease, induces apoptosis of neurons in cultures (A-E) and in the rat brain (F, G). Neurons accumulating APP (stained red in A, C, F) have shrunk cell bodies, small nuclei (arrowheads in C-E), and activated caspase-3 (stained green or yellow in B, D, G), which are typical of apoptosis.


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Sign Language “Heard” in the Auditory Cortex


