Introduction

Although developments in science and technology have resulted in higher standards of living, people continue to strive for a safer society with access to personal medicine. Investigations aimed at the development of a high-speed, cheap, single-molecule-based sequencer have been performed worldwide using advanced technologies such as nanotechnology and biotechnology. Since the development of scanning tunneling microscopy (STM), a typical technological tool that allows for the visualization and manipulation of individual molecules and atoms, researchers have attempted to sequence individual nucleotides in DNA using STM. Although several STM images showing the apparent macromolecular structure of DNA were reported by researchers, it was concluded at about 1990 that the majority of the images represented nothing but artifacts. Notwithstanding the continued efforts of researchers devoted to the STM imaging of DNA or DNA-related molecules, the general consensus seemed to suggest that it is impossible to realize high resolution STM imaging and sequencing of macromolecules such as DNA.

It became clear that one of the difficulties concerned the sample preparation method. STM achieves highest resolution under vacuum conditions. Under other conditions such as in air or liquid, the resolution of the image is easily lost by contamination. DNA molecules require pH-controlling buffer solutions, the chemical components of which (salts and buffer molecules) can lead to disturbances in high-resolution STM observations in the following ways: degrading DNA molecules (salts and buffer molecules) can adsorb onto the sample, leading to disturbances in high-resolution STM observations; degrading DNA molecules (salts and buffer molecules) can adsorb onto the sample, leading to disturbances in high-resolution STM observations; degrading DNA molecules (salts and buffer molecules) can adsorb onto the sample, leading to disturbances in high-resolution STM observations; degrading DNA molecules (salts and buffer molecules) can adsorb onto the sample, leading to disturbances in high-resolution STM observations; degrading DNA molecules (salts and buffer molecules) can adsorb onto the sample, leading to disturbances in high-resolution STM observations; degrading DNA molecules (salts and buffer molecules) can adsorb onto the sample, leading to disturbances in high-resolution STM observations; degrading DNA molecules (salts and buffer molecules) can adsorb onto the sample, leading to disturbances in high-resolution STM observations. Indeed, DNA solution is very sensitive to disturbance. This characteristic in unique to quanine. By imaging a stretched, single-stranded M13mp18 DNA molecule, with the sample biased at this voltage, we obtained an image in which the guanine bases appeared larger and brighter, and therefore could readily be identified. By matching the relative position of the guanine bases with the known sequence, we were able to sequence a segment within the known sequence of a real long-chain DNA molecule.

Experimental procedure

We used two low-temperature STM systems, one (LT–STM, Omicron GmbH) and the other (USM-1200S2N1, Unisoku). Both STM chambers were cooled by liquid nitrogen, and the observation temperature was 80 K. The substrate used was a Cu(111) surface, cleaned to be atomically flat by argon ion sputtering at 773 K in ultrahigh vacuum. M13mp18 ssDNA was purchased from Bayou Biobals and was subjected to dialysis (Slide-A-Lyzer MINI Dialysis Unit, 10,000-molecular-weight-cut-off type; Rockford) against water to remove excess buffer solution and salt. M13mp18 DNA was dissolved at water at concentrations of 0.5 mol l-1 and was deposited on clean Cu(111) surfaces using the oblique pulse injection method at room temperature (see Fig. 1a).

The distance from the pulse valve to the substrate was 50 mm.

Paper in journals: this is the first page of a paper published in Nature Nanotechnology.

Reprinted with permission from Nature Nanotechnology.

The solution was injected towards the substrate when the valve was opened for 1.5 ms. When the DNA solution was injected perpendicularly onto the substrate, no extended DNA was observed. The image of an ss M13mp18 extended and fixed by oblique injection on a Cu(1 1 1) surface. Owing to the height differences at steps in the Cu(1 1 1) substrate, the contrast is not ideal for recognizing DNA molecules, which is introduced into the Cu(1 1 1) surface from a substrate. In this image, sections of the DNA run from the top left to bottom right. A, An extended view of the rectangular region enclosed by the white dashed line in b, and C, an image of the same region as in A, but in a pulse valve. To minimize the detection of the density of states of guanine, the measurements were made under slight lower bias conditions than in the detection of the a.c. tunnelling current by modulating the sample bias voltage (Vs=0.1 V) while keeping the feedback loop active. The undulating pattern surrounding the DNA chain in Fig. 2a is observed at low bias conditions (Vt=-2 V), and thus is a standing wave resulting from the scattering of surface electrons. Compared with the image obtained with a low bias voltage, some of the nucleotides in the high bias image (Vt=2 V) are brighter and thus correspond to guanine. To verify this, we compared the results with the sequence extracted from a databank (extensions 2911–3011) as shown in Fig. 2c, which can confirm that the guanine base molecules are completely matched either individually or in groups (g, gg, ggg). It can thus be seen that it is possible to recognize the guanine pattern with few errors simply by obtaining a pair of STM topographic images in this way (preferably dual bias mode). By closely examining the sections of the STM images of Fig. 2, where the nucleotides are neatly arranged, it appears as though the cytosine molecules are smaller than the thymine units (white arrows).

Conclusions

In conclusion, by developing a method for extending and fixing DNA strands, we have taken a step towards the realization of electronic-based single-molecule DNA sequencing. Of the four bases, we were able to precisely identify guanine because the STM is able to pick up on the characteristics of its electronic state, which is largely independent of the adsorption structure. If vibrational spectroscopy is performed using inelastic electron tunnelling spectroscopy, it should be possible to identify all of the base molecules. Furthermore, because STM can select a specific position of interest along a DNA strand, as shown in Fig.3, the technique could have a unique advantage in analysing, for example, single-nucleotide polymorphisms.

References


Zc3h12a is an RNase Essential for Controlling Immune Responses by Regulating mRNA Decay

In conclusion, by developing a method for extending and fixing DNA strands, we have taken a step towards the realization of electronic-based single-molecule DNA sequencing. Of the four bases, we were able to precisely identify guanine because the STM is able to pick up on the characteristics of its electronic state, which is largely independent of the adsorption structure. If vibrational spectroscopy is performed using inelastic electron tunnelling spectroscopy, it should be possible to identify all of the base molecules. Furthermore, because STM can select a specific position of interest along a DNA strand, as shown in Fig.3, the technique could have a unique advantage in analysing, for example, single-nucleotide polymorphisms.

References


Zc3h12a is an RNase Essential for Controlling Immune Responses by Regulating mRNA Decay

Kazumori Matsushita,1,2 Osamu Takanishi,1,2,4 Darren M. Standley,1 Yutaro Kunagai,3 Tatsukatsu Kanegae,2,4,5 Tohru Miyatake,1,2 Takashi Seko,1,2 Hioki Kesho,1,2 Haruki Hakemura1,2,7 Gisazo Akiba2,4

Table-like receptors (TRKs) recognize structural components, and evoke inflammation and immune responses.1 TRR interaction activates complex gene expression networks that regulate the inflammatory and immune mechanisms. We identified a novel TRR in non-canonical immune responses, and successfully established a method for identifying TRRs in vivo. This method is based on the premise that TRRs target the lower bias conditions than in the detection of the a.c. tunnelling current by modulating the sample bias voltage (Vs=0.1 V) while keeping the feedback loop active. The undulating pattern surrounding the DNA chain in Fig. 2a is observed at low bias conditions (Vt=-2 V), and thus is a standing wave resulting from the scattering of surface electrons. Compared with the image obtained with a low bias voltage, some of the nucleotides in the high bias image (Vt=2 V) are brighter and thus correspond to guanine. To verify this, we compared the results with the sequence extracted from a databank (extensions 2911–3011) as shown in Fig. 2c, which can confirm that the guanine base molecules are completely matched either individually or in groups (g, gg, ggg). It can thus be seen that it is possible to recognize the guanine pattern with few errors simply by obtaining a pair of STM topographic images in this way (preferably dual bias mode). By closely examining the sections of the STM images of Fig. 2, where the nucleotides are neatly arranged, it appears as though the cytosine molecules are smaller than the thymine units (white arrows).

Biology

Zc3h12a is an RNase Essential for Controlling Immune Responses by Regulating mRNA Decay

Kazumori Matsushita,1,2 Osamu Takanishi,1,2,4 Darren M. Standley,1 Yutaro Kunagai,3 Tatsukatsu Kanegae,2,4,5 Tohru Miyatake,1,2 Takashi Seko,1,2 Hioki Kesho,1,2 Haruki Hakemura1,2,7 Gisazo Akiba2,4

Zc3h12a is an RNase Essential for Controlling Immune Responses by Regulating mRNA Decay

Kazumori Matsushita,1,2 Osamu Takanishi,1,2,4 Darren M. Standley,1 Yutaro Kunagai,3 Tatsukatsu Kanegae,2,4,5 Tohru Miyatake,1,2 Takashi Seko,1,2 Hioki Kesho,1,2 Haruki Hakemura1,2,7 Gisazo Akiba2,4

Zc3h12a is an RNase Essential for Controlling Immune Responses by Regulating mRNA Decay

Kazumori Matsushita,1,2 Osamu Takanishi,1,2,4 Darren M. Standley,1 Yutaro Kunagai,3 Tatsukatsu Kanegae,2,4,5 Tohru Miyatake,1,2 Takashi Seko,1,2 Hioki Kesho,1,2 Haruki Hakemura1,2,7 Gisazo Akiba2,4

Zc3h12a is an RNase Essential for Controlling Immune Responses by Regulating mRNA Decay

Kazumori Matsushita,1,2 Osamu Takanishi,1,2,4 Darren M. Standley,1 Yutaro Kunagai,3 Tatsukatsu Kanegae,2,4,5 Tohru Miyatake,1,2 Takashi Seko,1,2 Hioki Kesho,1,2 Haruki Hakemura1,2,7 Gisazo Akiba2,4

In conclusion, by developing a method for extending and fixing DNA strands, we have taken a step towards the realization of electronic-based single-molecule DNA sequencing. Of the four bases, we were able to precisely identify guanine because the STM is able to pick up on the characteristics of its electronic state, which is largely independent of the adsorption structure. If vibrational spectroscopy is performed using inelastic electron tunnelling spectroscopy, it should be possible to identify all of the base molecules. Furthermore, because STM can select a specific position of interest along a DNA strand, as shown in Fig.3, the technique could have a unique advantage in analysing, for example, single-nucleotide polymorphisms.

Zc3h12a is an RNase Essential for Controlling Immune Responses by Regulating mRNA Decay

Kazumori Matsushita,1,2 Osamu Takanishi,1,2,4 Darren M. Standley,1 Yutaro Kunagai,3 Tatsukatsu Kanegae,2,4,5 Tohru Miyatake,1,2 Takashi Seko,1,2 Hioki Kesho,1,2 Haruki Hakemura1,2,7 Gisazo Akiba2,4

In conclusion, by developing a method for extending and fixing DNA strands, we have taken a step towards the realization of electronic-based single-molecule DNA sequencing. Of the four bases, we were able to precisely identify guanine because the STM is able to pick up on the characteristics of its electronic state, which is largely independent of the adsorption structure. If vibrational spectroscopy is performed using inelastic electron tunnelling spectroscopy, it should be possible to identify all of the base molecules. Furthermore, because STM can select a specific position of interest along a DNA strand, as shown in Fig.3, the technique could have a unique advantage in analysing, for example, single-nucleotide polymorphisms.