A synthetic host-guest system achieves avidin-biotin affinity by overcoming enthalpy–entropy compensation

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Edited by Ronald Braslow, Columbia University, New York, NY, and approved November 8, 2007 (received for review July 9, 2007)

The molecular host cucurbit[7]uril forms an extremely stable inclusion complex with the dicaticonic ferrocene derivative bis(trimethylammoniomethyl)ferrocene in aqueous solution. The equilibrium association constant for this host-guest pair is $3 \times 10^{10} \text{M}^{-1}$ ($K_a = 3 \times 10^{-36} \text{M}$), equivalent to that exhibited by the avidin-biotin pair. Although purely synthetic systems with larger association constants have been reported, the present one is unique because it does not rely on polyvalency. Instead, it achieves its extreme affinity by overcoming the compensatory enthalpy-entropy relationship usually observed in supramolecular complexes. Its disproportionately low entropic cost is traced to extensive host desolvation and to the rigidity of both the host and the guest.

cucurbit[7]uril | control | ferrocene derivatives | host-guest complexation | thermodynamics

The design and characterization of synthetic, monovalent host–guest molecular recognition pairs still constitutes an open challenge in supramolecular chemistry, and it is of particular interest to inquire into the limits of the affinity that can be achieved with relatively simple systems of low molecular weight. The affinities routinely displayed by protein–ligand systems represent a tantalizing target for supramolecular chemists. Can small receptors reach these affinities, or is there something special about proteins that cannot be matched by small host molecules? Honk’s emphasis on the importance of buried surface area as a determinant of affinity (1) would seem to suggest that low molecular weight hosts cannot rival receptors that are proteins.

The avidin-biotin complex is a clear inspiration, as it is one of the tightest binding biomolecular systems, achieving an extraordinarily high affinity of $10^{15} \text{M}^{-1}$ through noncovalent interactions (2). The crystal structure of the avidin-biotin complex provides some clues on how to achieve such ultra-high stability (3). Cooperative, multiple, noncovalent interactions are essential for realizing such strong complexation and, indeed, the binding sites of avidin is composed of an array of polar and aromatic residues, all of which cooperatively contribute to optimize both recognition and binding. Several aromatic amino acid residues (Trp and Phe) form a rigid “hydrophobic box” around the binding site and a number of polar residues (Thr, Ser, Asn, and Tyr) stabilize the complex through a network of multiple hydrogen bonds. This complex structure induces a large negative (favorable) enthalpy change ($\Delta H$) resulting from the formation of multiple hydrogen bonds, as well as a large negative (unfavorable) entropy change ($\Delta S$) is expected due to the severe conformational restriction of the biotin molecule upon complexation with avidin. This effect is, however, cancelled by a large, positive entropy of desolvation, eventually making the overall entropy of complexation nearly zero (4).

In our quest to design host–guest systems that reach high levels of binding affinity in aqueous media, we took inspiration from Nature and targeted molecular partners with a high degree of site/shape complementarity and chemical functionalities that can develop considerable noncovalent attractive forces between them. The cucurbit[7]uril hosts (CB[7], $n = 5-10$) (5, 6) include a number of very symmetric molecular containers, readily synthesized by the condensation of glycoluril with formaldehyde in acidic media. We have recently shown (7) that cucurbit[7]uril (CB[7], Fig. 1) forms a very stable complex ($K = 3 \times 10^{12} \text{M}^{-1}$) with hydroxymethylferrocene (guest 1). The introduction of a positive charge on the guest, positioned to interact with one of the host’s rings of carboxyl oxygens, leads to a sizable increase in the corresponding equilibrium association constant, which reaches $K = 3 \times 10^{12} \text{M}^{-1}$ for guest 2 (7, 8). We conjecture that it would be possible to further boost the affinity by appropriately positioning a second positive charge that would form similar interactions with the host’s other rings of carboxyls.

Here we report the success of this strategy, and consequently the first example of a fully synthetic, monovalent host–guest system that matches the affinity of avidin and biotin. The present study thus places synthetic hosts squarely in the same arena as proteins, and leads to a revision of our expectations for what is achievable with low-molecular-weight receptors. Further analysis, both experimental and computational, provides insights into the basic physical chemistry of molecular recognition, and especially the tradeoff between energy and entropy.

Results and Discussion

We designed and synthesized 1,1’-bis(trimethylammoniomethyl) ferrocene (3) as a monovalent guest complementary to the host.
An Absolutely Entropy-Controlled Synthetic Host-Guest System that Achieves Avidin-Biotin Affinity

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Introduction

The design and characterization of synthetic host-guest pairs still represents an open challenge in supramolecular chemistry. As a clear inspiration, the avidin-biotin complex is one of the biological systems with strongest binding, achieving an extraordinarily high affinity of ca. $10^{15}$ M$^{-1}$ through non-covalent interactions.\(^1\) The crystal structure of the avidin-biotin complex gives us some clues on how to achieve such ultrahigh stability.\(^2\) Cooperative, multiple, non-covalent interactions are essential for realizing such strong complexation and, indeed, the binding site of avidin is composed of an array of polar and aromatic residues, all of which cooperatively contribute to optimize biotin recognition and binding. Several aromatic amino acid residues form a rigid “hydrophobic box” around the binding site and a number of polar residues stabilize the complex through a network of multiple hydrogen bonds. This complex structure induces a large negative (favorable) enthalpy change ($\Delta H^\circ$) resulting from the formation of multiple hydrogen bonds, as well as robust van der Waals contacts inside the “hydrophobic box”.\(^3\) At the same time, a large negative (unfavorable) entropy change ($\Delta S^\circ$) is expected due to the severe conformational restriction of the biotin molecule upon complexation with avidin. This effect is, however, cancelled by a large, positive entropy of desolvation, eventually making the overall entropy of complexation nearly zero.\(^3\)

![Fig.1 Chemical structures of the host cucurbit[7]uril and ferrocene guests: 1-hydroxymethylferrocene (1), 1-trimethylammoniomethylferrocene (2) and 1,1'-bis(trimethylammoniomethyl)ferrocene (3). Reproduced with permission: copyright 2007 National Academy of Sciences, U.S.A.]()

Cucurbituril-Ferrocene System

In our quest to reach high levels of binding affinity in aqueous media, we took inspiration from nature and targeted molecular partners with a high degree of size/shape complementarity and chemical functionalities that can develop considerable non-covalent attractive forces between them. The host family of the cucurbit[n]urils\(^4,5\) includes a number of very symmetric molecular containers, readily synthesized by the condensation of glycoluril with formaldehyde in acidic media. We have recently shown\(^6\) that cucurbit[7]uril (CB[7], Fig. 1) forms a very stable complex ($K = 3 \times 10^9$ M$^{-1}$) with hydroxymethylferrocene (guest 1). The introduction of a terminal positive charge on the ferrocene residue (guest 2) leads to a sizable increase in the corresponding equilibrium association constant, and we measured $K = 3 \times 10^{12}$ M$^{-1}$ for the CB[7]•2 pair.\(^6\) Interestingly, both complexation processes exhibit virtually the same enthalpy changes (Table 1). From an entropic standpoint, complexation of 1 with CB[7] is less favorable ($T\Delta S^\circ = –36$ kJ mol$^{-1}$) than that of 2 ($T\Delta S^\circ = –18$ kJ mol$^{-1}$). It is to note that the presence of a cationic residue on the side chain of guest 2 does not change the $\Delta H^\circ$ value (Table 1), but decreases by 18 kJ mol$^{-1}$ the negative entropic contribution, resulting in a substantially more stable inclusion complex.

**Table 1** Complexation Thermodynamics for Cucurbit[7]uril (CB[7]) with Selected Ferrocene Guests (Fig. 1) in Aqueous Solution at $T = 298.15K$

<table>
<thead>
<tr>
<th>Guest</th>
<th>$K$/M$^{-1}$</th>
<th>$\Delta H^\circ$/kJ mol$^{-1}$</th>
<th>$T\Delta S^\circ$/kJ mol$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>$(3.2 \pm 0.5) \times 10^9$</td>
<td>$-90 \pm 2$</td>
<td>$-36 \pm 2$</td>
</tr>
<tr>
<td>2</td>
<td>$(4.1 \pm 1.0) \times 10^{12}$</td>
<td>$-89 \pm 2$</td>
<td>$-17 \pm 3$</td>
</tr>
<tr>
<td>3*</td>
<td>$(3.0 \pm 1.0) \times 10^{15}$</td>
<td>$-92 \pm 1$</td>
<td>$-4 \pm 4$</td>
</tr>
<tr>
<td>3†</td>
<td>$(3.3 \pm 1.0) \times 10^{15}$</td>
<td>$-87 \pm 1$</td>
<td>$-2 \pm 4$</td>
</tr>
</tbody>
</table>

*† Obtained by different series of competition experiments.

The extrapolation of these data to dicatonic ferrocene derivatives, in which each cyclopentadienyl ring is connected to a positively charged sidearm, suggests that complexes with $K$ values in the range $10^{15}$ M$^{-1}$ should be accessible. Therefore, we prepared 1,1’-bis(trimethylammoniomethyl)ferrocene 3 and investigated its thermodynamic parameters for complexation with the host CB[7]. The results follow the anticipated trend and $K$ values around $3 \times 10^{15}$ M$^{-1}$ were determined in the case of guest 3. The enthalpic component was again ca. $-90$ kJ mol$^{-1}$ and the entropic component was found to be close to zero. Therefore, the addition of each positively charged sidearm to the guest has basically no effect on the enthalpic component.
for the complexation process ($\Delta H^o = -90$ kJ mol$^{-1}$) and causes an increase in the entropic component ($T\Delta S^o$) of 16-18 kJ mol$^{-1}$, which results in a 1000-fold increase in the corresponding binding constant. Thus, the enthalpy-entropy compensation effect which is commonly observed in supramolecular recognition systems$^{8-12}$ does not seem to operate in this case. The data points for guests 1, 2 and 3 are greatly deviated from enthalpy-entropy compensation plot for cyclodextrin-guest complexation reactions (Fig. 2).

Single crystals of the complex formed between 3 and CB[7] were obtained by slow evaporation of the solvent. The crystal structure of the complex was solved by X-ray diffraction methods and is shown in Figure 3. The structure shows the complete inclusion of the ferrocenyl residue in the CB[7] cavity, with its main axis (passing through the centers of both cyclopentadienyl rings) tilted (43.7° and 41.9° for two independent positions) in relation to the main 7-fold symmetry axis of the host. The tilting of the ferrocenyl residue allows the almost ideal positioning of each of the trimethylammonium groups to maximize ion-dipole interactions with the carbonyl rings on each of the host portals. The most stable computed conformation of this complex from the second-generation Mining Minima algorithm matches the crystal structure closely.

**Conclusion**

In this study, we have revealed that the supramolecular complexation of cationic ferrocene derivatives by the host CB[7] achieves an ultrahigh stability similar to that of the avidin-biotin complex. The extremely large binding affinity of the complexes is driven by the huge enthalpic gain originating from the tight fit of the ferrocene core to the rigid CB cavity, but is controlled absolutely by the entropic gain arising from the dehydration of the CB portals. This ultrahigh-affinity host-guest pair could serve as a strong but reversible fastener in self-assembling systems. Importantly, both the avidin-biotin and CB[7]•3 systems fail to obey the enthalpy-entropy compensation (which has been demonstrated to prevail in almost all supramolecular systems)$^{8-11}$ due to the rigid host cavity and the extensive entropic dehydration effects. We believe therefore that the failure to obey the enthalpy-entropy compensation is one of the most promising features of the CB[7]-ferrocene system and may establish a guiding principle to design extremely strong supramolecular complexes in the future.

**References**


![Fig.2](image2)

**Fig.2** The thermodynamic data obtained for complexation of guests 1, 2, and 3 with CB[7] (green dots), which are significantly deviated from the enthalpy-entropy compensation plot for cyclodextrin-guest complexation (black circles). Reproduced with permission: copyright 2007 National Academy of Sciences, U.S.A.

![Fig.3](image3)

**Fig.3** X-ray crystal structure of the CB[7]•3 complex and structure obtained by molecular modeling computations using the second-generation Mining Minima algorithm are essentially the same.