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Enhancing Binding Affinity by the Cooperativity between Host Conformation and Host−Guest Interactions

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Supporting Information

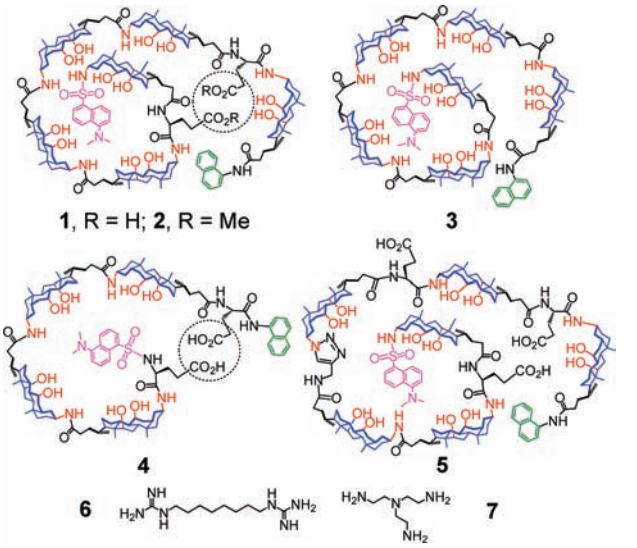
ABSTRACT: Glutamate-functionalized oligocholate foldamers bound $Zn(OAc)_2$, guanidine, and even amine compounds with surprisingly high affinities. The conformational change of the hosts during binding was crucial to the enhanced binding affinity. The strongest cooperativity between the conformation and guest-binding occurred when the hosts were unfolded but near the folding−unfolding transition. These results suggest that high binding affinity in molecular recognition may be more easily obtained from large hosts capable of strong cooperative conformational changes instead of those with rigid, preorganized structures.

Rigid supramolecular hosts have been favored traditionally by chemists because of their perceived benefits in binding affinity. The tradition traces back to Fischer's lock−key theory and was reinforced by the principle of preorganization articulated by Cram.[1] The idea of preorganization brought great advancement in supramolecular chemistry in recent decades. More recently, however, there is increasing appreciation that most biomolecular hosts (e.g., proteins) are folded linear molecules[2−5] and not nearly as rigid as preorganized macrocyclic compounds commonly employed by supramolecular chemists. It is perplexing that, if conformational mobility really represents a disadvantage to high-affinity binding, decades of efforts by chemists yielded mostly (rigid) synthetic supramolecular hosts that are no match for their (less rigid) biological counterparts.[6] The answer to this question is significant for a number of important fields, including enzymatic catalysis and drug development, in addition to supramolecular chemistry.[6] Some researchers, including Williams[7] and Otto,[8] have already started to question whether chemists, in our efforts to rigidify synthetic hosts, have wandered away from certain critical elements for success.

Here we report usual binding behavior of oligocholate foldamers. High-affinity binding was obtained with conformationally mobile hosts, using even relatively weak noncovalent forces, suggesting that cooperativity or synergism between guest-binding and the conformational change of a supramolecular host can be a powerful strategy to enhance host−guest interactions.

Oligocholates are amphiphilic foldamers capable of cooperative conformational changes.[9−11] Folding in solution is driven by the preferential solvation of the cholate hydrophilic faces in a largely nonpolar solvent mixture (Figure 1). By microphase-separating some polar solvent molecules and placing them in its internal nanocavity, the folded oligocholate efficiently satisfies the needs of both the polar solvent to be located in a polar environment and its hydrophilic faces to be solvated by polar solvent. Because folding requires phase separation of the polar solvent, folding is most favorable in a solvent mixture with marginal miscibility—e.g., 2:1 hexane/ethyl acetate (EA) with a few percent methanol (MeOH).[12]

Oligocholates **1**−**5** were synthesized by methods similar to those reported previously.[9−11] The carboxyl groups were introduced through L-glutamic acids in the foldamer sequence. The dansyl and naphthyl groups enable us to use fluorescence resonance energy transfer (FRET) to study the conformation of the molecules. Folding shortens the distance between the two fluorophores and allows the excited naphthyl donor to transfer its energy to the dansyl acceptor. The energy transfer is typically monitored by the enhanced acceptor emission when the donor is preferentially excited at 287 nm.[9−11]



We first studied the conformation of **1**−**3** in MeOH/EA by fluorescence spectroscopy. This binary mixture represents a more challenging environment for folding than the ternary MeOH/(2:1 hexane/EA) mixture.[13] According to Figure 2a, **1** was the only compound among the three that could fold in the binary mixture, evident from its FRET-enhanced dansyl emission in <15% MeOH. Clearly, the two carboxyl groups were crucial to the folding. There are two possible ways for the carboxylic acid groups to help folding. First, they could be involved in dimer-like hydrogen bonds and stabilize the folded helix directly. Second, because the folded helix creates a pool of MeOH in its interior (Figure 1), the polar carboxyl groups are solvated better in the folded helical conformer than in the unfolded form. The latter

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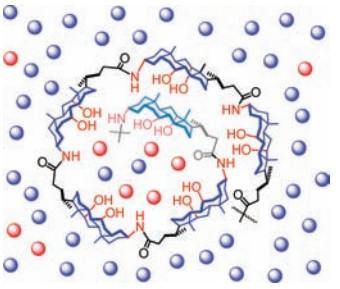

Figure 1. Preferential solvation (blue spheres, nonpolar solvent; red spheres, polar solvent) of a folded oligocholate.

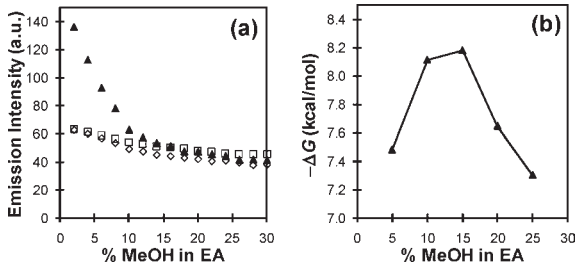

Figure 2. (a) Emission intensity of dansyl at 492 nm for **1** (▲), **2** (◇), and **3** (□) in MeOH/EA mixtures. λ_{ex} = 287 nm; [oligomers] = 2.0 μM. Corresponding fluorescence spectra are shown in the Supporting Information. (b) Binding free energy between **1** and $Zn(OAc)_2$ in MeOH/EA.

indirectly favors the folded state and was found to assist the folding of guanidinium−carboxylate-functionalized oligocholates even when the guanidinium was not involved in the salt bridge.[11]

Figure 2a shows that complete unfolding of **1** occurred at about 15% MeOH, beyond which all three oligocholates gave similar emission (i.e., no FRET).[14] A large amount of MeOH is known to diminish the need for the preferential solvation (Figure 1) and unfolds the oligocholates.[9−11] The potential hydrogen bonds between the carboxyl groups are also weakened by the polar solvent.

Table 1 shows the binding data between **1** and several guests, determined by fluorescence titration. We studied the binding of $Zn(OAc)_2$ because a similar dicarboxylated oligocholate was found to bind the metal in 1:1 stoichiometry.[15] In the case of **1**, binding affinities up to 10^6 M^{-1} were obtained; interestingly, they were higher in 10−15% MeOH than in 5 or 20% MeOH (Figure 2b).[16]

The zinc-binding data suggest that the conformation and guest-binding of the oligocholate are intimately related. The strongest synergism between the host conformation and the host−guest interactions occurred near the unfolding−folding transition. Initially, it was unclear to us whether the strong binding in 15% MeOH was coincidental, but the data suggest that cooperative conformational change of a host can enhance its binding affinity for the guest. This is an extremely exciting prospect because, if such synergism can be rationally engineered, one should be able to achieve *high binding affinity from weak noncovalent forces* as long as the binding helps the host in its conformational change. The strategy is equivalent to "magnifying" the host−guest interactions by the positive cooperativity with the host conformation.

To test the hypothesis, we turned our attention to several diamines, $H_2N(CH_2)_nNH_2$, which could only form weak carboxylate−ammonium ion pairs with **1** in the polar MeOH/EA mixtures. Fluorescence titration revealed that the short diamines (n = 2 or 4) hardly affected the emission of **1**, but the longer ones

Table 1. Binding Data for Oligocholate 1 at 25 °C

entry	guest	% MeOH[a]	K_a (M^{-1})[b]	−ΔG (kcal/mol)
1	$Zn(OAc)_2$	5	$(3.1 \pm 0.6) \times 10^5$	7.5
2	$Zn(OAc)_2$	10	$(8.9 \pm 1.0) \times 10^5$	8.1
3	$Zn(OAc)_2$	15	$(1.0 \pm 0.2) \times 10^6$	8.2
4	$Zn(OAc)_2$	20	$(4.1 \pm 0.4) \times 10^5$	7.7
5	$Zn(OAc)_2$	25	$(2.3 \pm 0.4) \times 10^5$	7.3
6	$H_2N(CH_2)_2NH_2$	15	–[c]	–[c]
7	$H_2N(CH_2)_4NH_2$	15	–[c]	–[c]
8	$H_2N(CH_2)_6NH_2$	5	$(8.0 \pm 1.0) \times 10^3$	5.3
9	$H_2N(CH_2)_6NH_2$	10	$(1.5 \pm 0.1) \times 10^4$	5.7
10	$H_2N(CH_2)_6NH_2$	15	$(1.9 \pm 0.2) \times 10^4$	5.8
11	$H_2N(CH_2)_6NH_2$	20	$(0.5 \pm 0.1) \times 10^4$	5.1
12	$H_2N(CH_2)_6NH_2$	25	–[d]	–[d]
13	$H_2N(CH_2)_8NH_2$	5	$(1.0 \pm 0.1) \times 10^4$	5.5
14	$H_2N(CH_2)_8NH_2$	10	$(1.0 \pm 0.1) \times 10^4$	5.5
15	$H_2N(CH_2)_8NH_2$	15	$(2.0 \pm 0.2) \times 10^4$	5.9
16	$H_2N(CH_2)_8NH_2$	20	$(1.0 \pm 0.2) \times 10^4$	5.5
17	$H_2N(CH_2)_8NH_2$	25	–[d]	–[d]
18	$H_2N(CH_2)_{10}NH_2$	15	$(1.9 \pm 0.3) \times 10^4$	5.8
19	$H_2N(CH_2)_{12}NH_2$	15	$(1.5 \pm 0.3) \times 10^4$	5.7
20	**6**	5	$(1.9 \pm 0.2) \times 10^5$	7.2
21	**6**	10	$(3.6 \pm 0.4) \times 10^5$	7.6
22	**6**	15	$(4.2 \pm 0.5) \times 10^5$	7.7
23	**6**	20	$(2.7 \pm 0.4) \times 10^5$	7.4
24	**6**	25	$(1.1 \pm 0.2) \times 10^5$	6.8
25	**6**[e]	15	$(1.9 \pm 0.3) \times 10^3$	4.5

[a] Volume percentage of MeOH in EA. [b] Association constants determined by nonlinear least-squares fitting to a 1:1 binding isotherm. λ_{ex} = 350 nm. [c] Binding constant could not be obtained because weak (5−10%) and random quenching of the dansyl was observed. [d] Binding constant could not be obtained because extremely weak quenching (<5%) was observed. [e] Host was tetracholate **4**.

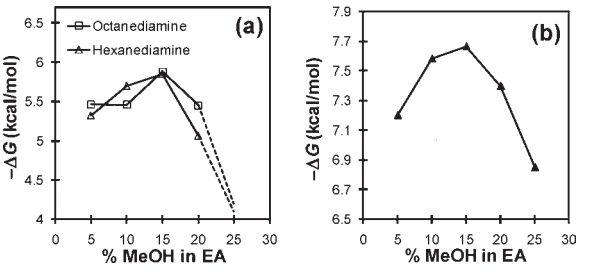

Figure 3. Binding free energy (a) between **1** and diamines and (b) between **1** and **6** in MeOH/EA mixtures.

(n = 6−12) caused significant quenching in 15% MeOH.[17] Because K_a was quite similar for the longer diamines, we chose to study hexanediamine and octanediamine in more detail.

The binding constants were surprisingly strong for these flexible guests (Table 1, entries 8−19). Although lower than those for $Zn(OAc)_2$, $K_a = 10^4$ M^{-1} is quite remarkable for binding driven by two ammonium−carboxylate ion pairs in up to 20% MeOH. The binding affinity peaked again at 15% MeOH (Figure 3a). The effect of MeOH on the ion pairs was unexpected for a polar solvent. Because of its high polarity and hydrogen-bonding ability, MeOH normally should weaken the ion pairs continuously with increasing concentrations.

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A major difference between zinc- and diamine-binding was their response to higher amounts of MeOH. In the zinc-binding, although >15% MeOH lowered the binding affinity and no binding occurred in neat MeOH, there was only about a 2-fold decrease in K_a from 20 to 25% MeOH (Table 1, entries 4 and 5). In the diamine-binding, quenching in 25% MeOH was so weak (<5%) that the binding constant could not be determined.

These data demonstrate positive cooperativity between the host conformation and host−guest interactions in **1**. The synergism is the strongest near the conformational transition and falls off when the host slips deep into the unfolded region. The results are reasonable. When the host is folded, strong interactions already exist between different segments of the host and with solvents, so guest binding does little to help the intrahost interactions. When the host is too far in the unfolded region, binding has to overcome a highly unfavorable conformational change and is weakened.[13] Only when the host is near the unfolding−folding transition can the host conformation and the host−guest interactions readily help each other.

The above explanation is supported by the discussion of cooperativity in the literature. "Cooperativity" has a number of meanings[18] and frequently is used when the free energy components in a process are not additive. The process is said to have positive (or negative) cooperativity when the observed free energy is greater (or smaller) than the sum of the individual contributions. After studying the "tightening" and "loosening" effects of various ligands on their biological hosts, Williams and others raised the interesting postulation that the driving force for guest-binding does not have to come entirely from direct host−guest interactions.[7] Instead, a major part may derive from the strengthening of the existing interactions *within* the host. Indeed, few biological systems follow the rule of additivity.[19,20] Since conformational changes frequently accompany guest-binding in protein hosts,[21] it is likely that the strong binding found in protein hosts has major contributions from the hosts themselves. The notion is supported by the well-known tight binding between streptavidin and biotion. The small-molecule ligand raises the protein's melting point by 37 °C,[22] and a large number of backbone amide protons become resistant to H/D exchange.[23,24] Undoubtedly, the binding has greatly strengthened the interactions within the host. The enhanced intrahost interactions very well may be the reason why a small ligand can produce an astounding $K_a = 10^{13.4}$ M^{-1}.

Our study sheds some important light on the conformation−binding cooperativity. For example, the window for the cooperativity depends on the strength of the host−guest binding interactions. When strong interactions such as Zn−O complexation are involved, cooperativity happens over a broad range of conditions and can tolerate at least 25% MeOH. When weaker noncovalent forces are involved, the synergism occurs in a much narrower window, optimal when the host is near the unfolding−folding transition. This was probably why the 5% increase of MeOH from 20 to 25% caused little change to the Zn-binding but a precipitous drop in K_a for the diamines. Essentially, to benefit from the conformation−binding cooperativity, the guest needs to fold the unfolded host,[25] and such a transition is difficult when either the host is too far into the unfolded region or the host−guest interactions are too weak.

The above conclusion was confirmed with diguanidine **6**, which forms stronger salt bridges with carboxylates than the diamines. With stronger host−guest interactions, binding once again became detectable in 25% MeOH, and changing the solvent from 20 to 25% MeOH reduced the K_a by only 2.5-fold (Table 1, entries 23 and 24). Not surprisingly, the binding affinity peaked again in 15% MeOH (Figure 3b).

If strengthened intrahost interactions are the main reason for enhanced binding affinity, a smaller host with fewer potential intrahost interactions should bind less strongly when everything else is equal; i.e., the size of the host should matter greatly in cooperative binding. The parent oligocholates cannot fold with fewer than five cholate units.[9] Tetracholate **4** has two carboxyl groups separated by four cholates, the same as in **1**. It is unable to fold in MeOH/EA, evident from the absence of FRET in the solvent titration (Figure 4S). Indeed, binding between **4** and **6** was much weaker; K_a was at least 2 orders of magnitude lower than that between **1** and **6** (Table 1, entries 22 and 25).

To further confirm the conformation−binding cooperativity, we synthesized oligocholate **5**. We used the alkyne−azide click reaction,[26] partly because the synthesis of oligocholates always leaves an azido group at the chain end.[9] Our previous work has demonstrated that "clicked" oligocholates fold at least as well as the parent compounds.[27]

The extra carboxylic acid group did not help **5**, which was found to unfold in the MeOH/EA mixture[28] but to fold well in <8−10% MeOH in 2:1 hexane/EA (Figure 5S). It was extremely important that **5** and **1** had different conformational transitions, or we would not know whether the strongest cooperativity at the transition point was general. To our delight, when the binding between **5** and **7** was studied in the ternary solvent mixtures, the strongest binding once again occurred at the unfolding−folding transition, this time at ∼8% MeOH (Figure 6S, Table 1S). In the binary MeOH/EA mixtures, in which no cooperative conformational change could occur, the binding not only was weaker but also displayed a slight, monotonous decrease of K_a with increasing MeOH (Figure 7S). Thus, in the absence of conformational cooperativity, the normal solvent effect on the ions pairs dominated.

It is difficult to know exactly where the guests were bound by the hosts. Because similar solvent effects were found in the binding of both hydrophilic (zinc acetate) and hydrophobic (1,8-octanediamine and 1,12-dodecanediamine) guests, the binding location could not be a determining factor. Our previous studies[9] showed that hydrophilic guests prefer to go in the hydrophilic cavity of the folded helix.[29] If displacement of solvent molecules by the guest was important, one would expect hydrophilic guests (i.e., ethylenediamine and butylenediamine) to be better guests and their binding to be stronger in less polar solvents.[30]

The selectivity in the binding between **1** and the diamines warrants some discussion. As far as size is concerned, ethylenediamine is more similar to $Zn(OAc)_2$ than octanediamine or dodecanediamine, and yet it was not bound by **1**. Also, why did the longer diamines ($n = 6-12$) bind similarly? The answers probably lie in the nature of the folded oligocholate. Although **1** appears flexible, much of the cholate is rigid due to ring fusion. The preferential solvation demands that the hydrophilic faces of the cholates point inward (Figure 1). These constraints will limit the movement of the foldamer chain. When strong binding interactions are involved (e.g., Zn−O complexation), significant strain in the host can be tolerated during binding, as the major contribution of the overall binding free energy probably comes from the host−guest binding interactions. When the binding interactions are weak (e.g., ammonium−carboxylate ion pairing), although significant binding affinity could be obtained, much of the binding free energy likely derives from the enhanced intrahost interactions. In the latter case, the strain introduced by the binding undermines the intrahost interactions and eliminates the cooperativity. For the C2−C4 dimaines, binding requires the two carboxylic acid groups

to be quite close and probably causes significant strain to the foldamer. For the C6−C12 diamines, the distance between the two amine groups may match reasonably well with the average distance between the two carboxylic groups in the folded helix. Similar binding affinity would be expected as long as the main contributors to the binding (i.e., the ammonium−carboxylate ion pairs and the enhanced intrahost interactions) are the same.

The principle of preorganization predicts that organizing a host around its guest through conformational change is detrimental because the cost of conformational change is assumed to come from the binding. This prediction is true for supramolecular hosts incapable of cooperative conformational transition. However, for large hosts capable of cooperative conformational changes (e.g., proteins), our study suggests that the conformation of the host can be exploited to "magnify" weak binding interactions. Because K_a is determined by the overall change of free energy during the binding, one must take into account *all the processes that affect free energy*. Guest-induced conformational changes of the host and solvation/desolvation, whether near or far from the binding site, will affect the binding affinity. In general, although strong binding interactions are helpful to establish high binding affinity, they are not necessary. As long as the guest can trigger a large number of cooperative intrahost interactions during the binding, high binding affinity will result, as found for biotin−streptavidin.[7,31] For this reason, the large size of biological hosts is not coincidental but critical to their functions. Another important implication is that chemists and biologists need to look beyond the targeted binding site when searching for a drug candidate or strong ligand for a biological host. The best results likely will come from combined usage of lock−key-based techniques (e.g., molecular docking) and those revealing protein dynamics for the entire structure.

■ ASSOCIATED CONTENT

Supporting Information. Experimental details; fluorescence, and binding data. This material is available free of charge via the Internet at http://pubs.acs.org.

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(29) It is difficult to imagine that the long diamines have to stay within the cavity of the folded helix during binding. Besides, such a binding motif almost certainly will have a strong dependence on the chain length of the amine guest, unlike the similar binding affinities found with the C6−C12 diamines.
(30) Beyond the unfolding−folding transition, the preferential solvation shown in Figure 1 is no longer helpful to the host. Before the transition, the cavity has a concentrated pool of polar solvent. The unfolding−folding transition, therefore, is where preferential solvation begins to disappear. From this perspective, release of the solvents should be less important at the conformational transition than in low-MeOH mixtures, as the release of phase-separated polar solvent molecules to the environment is entropically favorable, and this effect will disappear when the solvent composition in and out of the cavity becomes the same. In our cholate-based molecular basket, solvent displacement-based binding was found to weaken with an increasing amount of polar solvent in the bulk. Zhao, Y.; Ryu, E. H. *J. Org. Chem.* **2005**, *70*, 7585–7591.
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