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Preferential Solvation within Hydrophilic Nanocavities and Its Effect on the Folding of Cholate Foldamers

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Abstract: The conformations of three cholate foldamers and one molecular basket were studied by fluorescence and NMR spectroscopy. In nonpolar solvents (e.g., hexane/ethyl acetate or ethyl acetate) mixed with a small amount of a polar solvent (e.g., alcohol or DMSO), the cholate oligomer folded into a helix with the hydrophilic faces of the cholates turned inward. Folding created a hydrophilic nanocavity preferentially solvated by the entrapped polar solvent concentrated from the bulk. This microphase separation of the polar solvent was critical to the folding process. Folding was favored by larger-sized polar solvent molecules, as fewer such molecules could occupy and solvate the nanocavity, thus requiring a smaller extent of phase separation during folding. Folding was also favored by smaller/acyclic nonpolar solvent molecules, probably because they could avoid contact with the OH/NH groups within the nanocavity better than larger/cyclic nonpolar solvent molecules.

Introduction

Biomolecules such as proteins frequently utilize controlled conformational changes to sense the presence of signal molecules, regulate binding or catalytic activities, and respond to environmental stimuli. These remarkable features have prompted chemists to investigate synthetic analogues (i.e., foldamers) of biomolecules capable of adopting well-defined, compact conformations.[1] Just as nature employs hydrogen bonds for the secondary structures of proteins, many researchers choose to use hydrogen bonds (or other strongly directional forces such as metal−ligand complexation) to fold synthetic foldamers.[1] An alternative approach, pioneered by Iverson[2] and Moore,[3] is to utilize nondirectional forces such as solvophobic interactions for conformational control. In order to approach nature's ability to transform "simple" one-dimensional peptide chains into complex three-dimensional structures by folding, chemists have to master both types of noncovalent forces (directional and nondirectional) and integrate them in synthetic foldamers. However, using solvent-derived effects for conformational control is still a major challenge in modern physical organic chemistry.

With our interest in using cholic acid as a building block to construct responsive amphiphiles,[4] we recently reported amide-linked cholate oligomers.[5] When dissolved in nonpolar solvents, such as a mixture of hexane and ethyl acetate (EA), together with a small amount of a polar solvent, such as dimethylsulfoxide (DMSO) or methanol, the oligocholate folds into a helix by curving toward the α faces (Scheme 1). Folding creates a hydrophilic nanocavity where the polar solvent is concentrated from the bulk, a mostly nonpolar mixture. This pool of polar solvent[6] was hypothesized to act as a "solvophobic glue"[7] to contract the otherwise extended chain. In this paper, we describe a further study aiming at understanding the preferential solvation[8,9] of the hydrophilic nanocavity of the folded oligocholate by the entrapped polar solvent molecules. The most significant finding in this work is that such preferential solvation depends critically on the size and the structure of the solvents. Considering that many important processes take place in nanometer-sized domains, such as in enzyme active sites or reactive sites of a high-surface-area heterogeneous catalyst, our finding may be useful in understanding related solvent effects in biology and chemistry, particularly in nanospace, where the dimension

(1) For several recent reviews, see: (a) Gellman, S. H. *Acc. Chem. Res.* **1998**, *31*, 173−180. (b) Kirshenbaum, K.; Zuckermann, R. N.; Dill, K. A. *Curr. Opin. Struct. Biol.* **1999**, *9*, 530−535. (c) Stigers, K. D.; Soth, M. J.; Nowick, J. S. *Curr. Opin. Chem. Biol.* **1999**, *3*, 714−723. (d) Hill, D. J.; Mio, M. J.; Prince, R. B.; Hughes, T. S.; Moore, J. S. *Chem. Rev.* **2001**, *101*, 3893−4012. (e) Cubberley, M. S.; Iverson, B. L. *Curr. Opin. Chem. Biol.* **2001**, *5*, 650−653. (f) Sanford, A. R.; Gong, B. *Curr. Org. Chem.* **2003**, *7*, 1649−1659. (g) Martinek, T. A.; Fulop, F. *Eur. J. Biochem.* **2003**, *270*, 3657−3666. (h) Cheng, R. P. *Curr. Opin. Struct. Biol.* **2004**, *14*, 512−520. (i) Huc, I. *Eur. J. Org. Chem.* **2004**, 17−29. (j) Licini, G.; Prins, L. J.; Scrimin, P. *Eur. J. Org. Chem.* **2005**, 969−977.

(2) Lokey, R. S.; Iverson, B. L. *Nature* **1995**, *375*, 303−305.

(3) Stone, M. T.; Heemstra, J. M.; Moore, J. S. *Acc. Chem. Res.* **2006**, *39*, 11−20.

(4) (a) Ryu, E.-H.; Zhao, Y. *Org. Lett.* **2004**, *6*, 3187−3189. (b) Zhao, Y.; Ryu, E.-H. *J. Org. Chem.* **2005**, *70*, 7585−7591. (c) Ryu, E.-H.; Jie, Y.; Zhong, Z.; Zhao, Y. *J. Org. Chem.* **2006**, *71*, 7205−7213.

(5) (a) Zhao, Y.; Zhong, Z. *J. Am. Chem. Soc.* **2005**, *127*, 17894−17901. (b) Zhao, Y.; Zhong, Z. *J. Am. Chem. Soc.* **2006**, *128*, 9988−9989. (c) Zhao, Y.; Zhong, Z. *Org. Lett.* **2006**, *8*, 4715−4717.

(6) The folded foldamer is a unimolecular mimic of a reversed micelle. A pool of polar solvent (e.g., water) often is also needed to stabilize reversed micelles formed by conventional surfactants, see: Fendler, J. H. *Membrane Mimetic Chemistry*; Wiley: New York, 1982; Chapter 3.

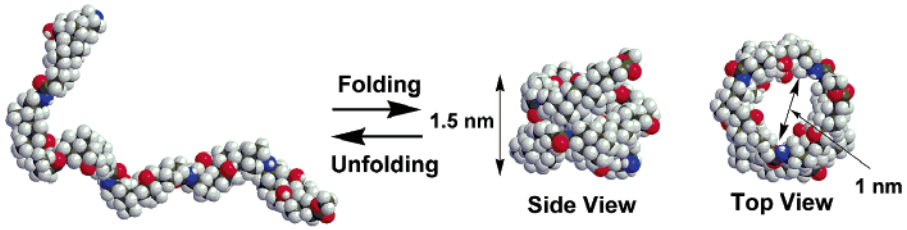
(7) Solvophobic effects typically are used to describe *direct* association of poorly solvated molecular surfaces. Folding of the oligocholates is mediated by the entrapped polar solvents, thus making them different from most other solvophobic foldamers. Nevertheless, folding is still driven by the avoidance of the hydrophilic faces from the bulk solvent (a mostly nonpolar mixture). Thus, it is reasonable to refer to the folding as "solvophobically driven".

(8) Marcus, Y. *Solvent Mixtures: Properties and Selective Solvation*; Marcel Dekker: New York, 2002.

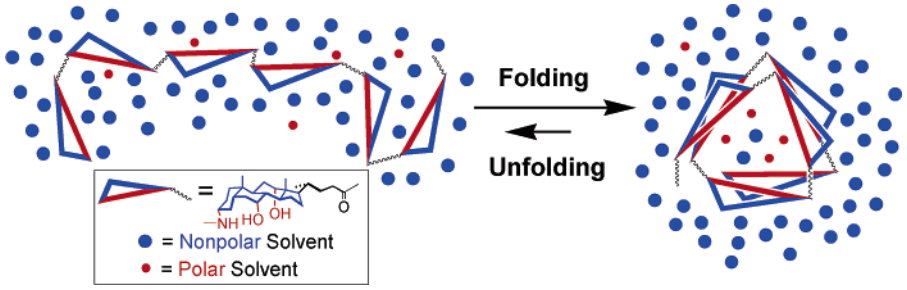
(9) Reichardt, C. *Solvents and Solvent Effects in Organic Chemistry*; Wiley: Weinheim, 2003; pp 38−42.

10.1021/ja0671159 CCC: $37.00

Scheme 1. Molecular Models of an Unfolded and Folded Cholate Hexamer



Scheme 2



of individual molecules becomes significant compared to that of the environment.[10]

Results and Discussion

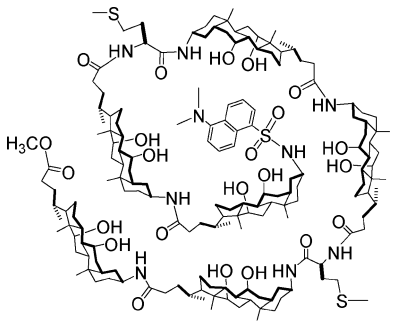
A Folding Model for the Oligocholates. Our model for the folding reaction is shown in Scheme 2. A "folding-friendly" solvent mixture is represented by five polar solvent molecules and 50 nonpolar ones. The cholate foldamer is depicted as triangles with two blue (nonpolar) sides and one red (polar) side. The preference for the folded state is understandable in such a solvent mixture. By forming the structure on the right, not only are the polar solvent molecules (at least some of them) relocated from a nonpolar medium, a less preferred environment, to a more preferred polar microenvironment, but the oligocholate itself is able to minimize unfavorable exposure of its hydrophilic faces.

The essence of the folding model is the microphase separation of the polar solvent that occurs within the nanocavity. The model apparently focuses on what is happening around and within the oligocholate and ignores the rest of the solvent molecules. It is a reasonable approach because the oligocholate is used at micromolar concentrations in most of the experiments described in this paper and, as a result, the majority of the solvent molecules will remain unperturbed during the folding/unfolding process.

If phase separation of the solvents provides the preferential solvation needed for the folding, the folded state should be more stable in partially miscible solvents than in completely miscible ones, due to easier phase separation in the former mixture. This prediction was confirmed in our previous study. DMSO is completely miscible with hexane/EA (1/1) but is only miscible up to 5−6% with hexane/EA (2/1) at room temperature. In our hands, all the oligocholates studied folded better in hexane/EA (2/1) than in hexane/EA (1/1) blended with 1−5% DMSO.[5a]

Despite its success in predicting many "folding-friendly" and "folding-unfriendly" solvents since we started our investigation, the folding model occasionally was thwarted by totally unexpected results. For example, less than 8% water can be dissolved in a 1:2 mixture of THF and 2-methyl-THF (MTHF). Thus, due to the easy phase separation of water, the oligocholates should fold quite well in this ternary mixture but, in reality, did not fold at all.[5b] Does the model in Scheme 2 overlook any important factors that control the folding process? We will try to answer this question in the following sections.

Solvent Effects in a Mercury-Binding Foldamer. The cooperative folding/unfolding of the cholate foldamers was recently utilized to create a highly tunable fluorescent sensor (**1**) for mercury ions.[5b] Interestingly, **1** was found to bind Hg^{2+} particularly weakly in water/THF mixtures, suggesting poor folding in this mixture.[11] This observation, together with the above-mentioned, unexpected, poor folding of the oligocholates in water/THF/MTHF, made us suspect that there was something special about aqueous THF.



1

To understand the role played by each solvent in aqueous THF, we first replaced THF with propanol and measured the binding constant (K_a) between **1** and Hg^{2+} in several water/propanol mixtures. THF and propanol differ in at least two important aspects. First, water is completely miscible with

(10) Strong solvent effects, which resulted from poor solvation of the interior of a host by large solvent molecules, have been reported in the literature. These effects are different from what is described in this paper. For examples, see: (a) Chapman, K. T.; Still, W. C. *J. Am. Chem. Soc.* **1989**, *111*, 3075−3077. (b) Hof, F.; Craig, S. L.; Nuckolls, C.; Rebek, J., Jr. *Angew. Chem., Int. Ed.* **2002**, *41*, 1488−1508. (c) Roncucci, P.; Pirondini, L.; Paderni, G.; Massera, C.; Dalcanale, E.; Azov, V. A.; Diederich, F. *Chem.−Eur. J.* **2006**, *12*, 4775−4784.

(11) Each monomer has a head-to-tail distance of 1.4 nm. Folding should not take place in the unfolded state. Weak binding of Hg^{2+} is therefore a direct indication for poor folding.

Table 1. Binding Constants (K_a) for Foldamer **1** and $Hg(OAc)_2$ at 25 °C in Aqueous Solutions

entry	solvent composition	K_a (M^{-1})[a]	$-\Delta G$ (kcal/mol)
1	5% H_2O in THF[b]	$(2.4 \pm 0.1) \times 10^4$	6.0
2	10% H_2O in THF[b]	$(1.9 \pm 0.2) \times 10^4$	5.9
3	20% H_2O in THF[b]	$(5.5 \pm 0.6) \times 10^3$	5.1
4	30% H_2O in THF	$(4.0 \pm 0.3) \times 10^3$	4.9
5	40% H_2O in THF	$(2.6 \pm 0.1) \times 10^3$	4.7
6	50% H_2O in THF	$(1.3 \pm 0.1) \times 10^3$	4.3
7	5% H_2O in PrOH	$(3.4 \pm 0.4) \times 10^4$	6.2
8	10% H_2O in PrOH	$(1.5 \pm 0.2) \times 10^4$	5.9
9	20% H_2O in PrOH	$(1.0 \pm 0.1) \times 10^4$	5.5
10	30% H_2O in PrOH	$(3.2 \pm 0.2) \times 10^3$	4.8
11	40% H_2O in PrOH	$(2.8 \pm 0.2) \times 10^3$	4.7
12	50% H_2O in PrOH	$(2.4 \pm 0.1) \times 10^3$	4.6

[a] The association constants were determined by nonlinear least-squares fitting to a 1:1 binding isotherm. The stoichiometry of binding was previously confirmed to be 1:1 by the Job plot (ref 5b). [b] Data from ref 5b.

propanol but only partially miscible with THF. Second, propanol, similar to water, can participate as both a donor (D) and an acceptor (A) in hydrogen-bonding, whereas THF can only act as an acceptor. Therefore, if any of these properties are important to the folding of the oligocholates, they should be reflected in the binding data.[11] As shown in Table 1, however, binding of Hg^{2+} is nearly identical in both series (entries 1−6 vs 7−12). The binding free energy ($-\Delta G$) not only starts at a similar value but follows a similar trend, a gradual decrease with increasing water percentages.

Therefore, the culprit for the poor folding is not THF but water. (We will come back to this point later.) Water is certainly a unique solvent, but which property (or properties) of water makes it difficult for the oligocholate to fold? To understand this better, we studied mercury binding in a series of alcohols, both in the neat form and as a mixture with 10 vol % water. The advantage of using an alcohol instead of THF as the cosolvent is that its size, hydrophobicity, and miscibility may be systematically tuned by its alkyl group. As the alkyl group increases, the size and hydrophobicity of the alcohol increase but the miscibility with water decreases.

Figure 1a shows the normalized titration curves for the binding of **1** in neat alcohols. Clearly, mercury binding (and thus folding of the oligocholate chain) is independent of the alcohol when there is only one solvent present. Binding in the 10% aqueous mixtures, on the other hand, is highly sensitive to the nature of the alcohol (Figure 1b). Two trends are immediately noticeable upon comparing the two solvent series. First, binding is weaker in aqueous mixtures than in neat alcohols. Note that the range of $[Hg^{2+}]$ is about 120 μM in Figure 1b but only 15 μM in Figure 1a. Second, binding/folding clearly depends on the size or hydrophobicity of the alcohol in the aqueous mixture. Whereas binding in aqueous methanol (black +) is reasonably strong, it cannot even be detected in aqueous butanol (red ◇). According to Figure 1b, binding/folding follows the order of methanol > ethanol > propanol ≈ isopropanol ≈ *tert*-butanol > butanol in the corresponding aqueous mixture. These trends are also reflected in K_a obtained from the titration curves (Table 2). Whereas K_a remains nearly constant in neat alcohols, it decreases from 5.4×10^4 M^{-1} in 10% water/methanol (entry 7) to <300 M^{-1} in 10% water/butanol (entry 12). Aqueous butanol, however, is not a unique mixture, because the binding in 10% water in 2-methoxyethanol (entry 13) is equally weak (if not weaker).

Foldamer **1** by itself obviously cannot fold in neat alcohol, as no solvophobic driving force depicted in Scheme 2 exists.

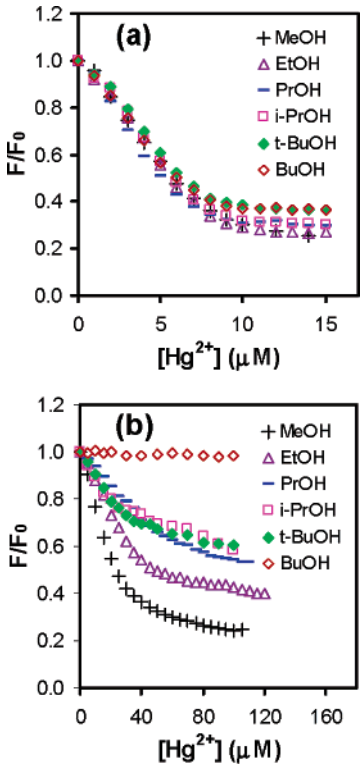


Figure 1. Normalized maximum emission intensity of the dansyl group of foldamer **1** (a) in different alcohols and (b) in 10% water in different alcohols as a function of $[Hg^{2+}]$. **[1]** = 2.0 μM.

Table 2. Binding Constants (K_a) for Foldamer **1** and $Hg(OAc)_2$ at 25 °C in Pure and Mixed Alcohols

entry	solvent composition	K_a (M^{-1})[a]	$-\Delta G$ (kcal/mol)
1	MeOH	$(2.6 \pm 0.2) \times 10^5$	7.4
2	EtOH	$(2.7 \pm 0.3) \times 10^5$	7.4
3	PrOH	$(2.5 \pm 0.2) \times 10^5$	7.4
4	*i*-PrOH	$(2.3 \pm 0.2) \times 10^5$	7.3
5	*t*-BuOH	$(1.9 \pm 0.2) \times 10^5$	7.2
6	BuOH	$(2.6 \pm 0.2) \times 10^5$	7.4
7	10% H_2O/MeOH	$(5.4 \pm 0.6) \times 10^4$	6.5
8	10% H_2O/EtOH	$(3.7 \pm 0.5) \times 10^4$	6.2
9	10% H_2O/PrOH	$(1.5 \pm 0.2) \times 10^4$	5.7
10	10% H_2O/*i*-PrOH	$(1.8 \pm 0.2) \times 10^4$	5.8
11	10% H_2O/*t*-BuOH	$(1.5 \pm 0.1) \times 10^4$	5.7
12	10% H_2O/BuOH	<300[b]	
13	10% H_2O/$MeOCH_2CH_2OH$	<100[b]	
14	10% MeOH/BuOH	$(1.3 \pm 0.2) \times 10^5$	7.0
15	10% EtOH/BuOH	$(1.8 \pm 0.5) \times 10^5$	7.2
16	10% PrOH/BuOH	$(2.3 \pm 0.7) \times 10^5$	7.3

[a] The association constants were determined by nonlinear least-squares fitting to a 1:1 binding isotherm. [b] Binding was too weak to be measured by fluorescence titration. K_a was estimated from the titration curves.

The only reason for its folding is the strong Hg−S complexation. Therefore, the observed binding energy for **1**−Hg^{2+} in a given solvent can be viewed roughly as the binding energy for a hypothetical, perfectly folded **1** minus the energy needed to fold **1** in the same solvent. This treatment assumes other factors, such as the change in solvent composition, have a negligible effect on the Hg−S interaction. This assumption is reasonable because the D_s values (= 17−19, the Lewis basicity toward soft metal ions measured with $HgBr_2$) of water, THF, and the alcohols are very similar.[12]

When only one solvent is present, both the hydrophilic and hydrophobic faces of the oligocholates are exposed to the same solvent, regardless of the folded state. As long as the internal

cavity of the folded conformer is sufficiently large to be easily accessed by the solvent, the solvent can solvate the folded state to the same extent as it does the unfolded state. Different alcohols certainly have different solvation for the foldamer. However, as long as they solvate the mercury-free (unfolded) and mercury-bound (folded) hosts similarly, the difference in solvation cancels out when the mercury binding is considered. This is probably the reason for the insensitivity of binding/folding toward the alcohol when neat alcohol is used. After all, all the alcohols studied are quite small compared to the nanometer-sized hydrophilic cavity (see later sections for more discussions on molecular sizes). It should be mentioned that this behavior would be impossible if the mercury-binding conformer is a "collapsed" structure formed by random intramolecular aggregation of the cholate hydrophilic faces. In such a case, the mercury-bound host would have a much smaller solvent-exposed surface than the free host, making the binding event highly sensitive to solvation and thus to the structure of the alcohol.

In aqueous alcohol, water is the more hydrophilic component. It is reasonable to assume that water is preferred over the alcohol by the hydrophilic (i.e., water-loving) nanocavity formed during folding, especially if the alcohol is fairly hydrophobic. In other words, folding, made possible by mercury complexation, will force microphase separation of water from alcohol when both solvents are present. This phase separation, however, costs energy and certainly will not happen spontaneously in the absence of the cholate foldamer. Therefore, the energy paid to phase-separate water from the bulk into the interior of the foldamer is a necessary cost for the folding. Other costs, such as those associated with the loss of conformational entropy during formation of an ordered, compact structure, do exist but may be more a property of the foldamer chain itself and may not depend as much on the solvent composition as the phase separation does. This is probably why binding is always weaker in the aqueous mixture than in the neat alcohol, as no such penalty will occur during folding when there is only one solvent present.

At this point, it becomes a little easier to understand why the structure of the alcohol makes such a large difference in the aqueous mixtures. The preference for water over alcohol—we still assume water is preferred by the hydrophilic cavity—should be small in aqueous methanol, as both solvents are polar and can effectively solvate the hydrophilic wall of the cavity. As a result, minimal microphase separation is needed, and the penalty for folding is small. Hence, $-\Delta G$ for **1**$-Hg^{2+}$ only decreases by 0.9 kcal/mol on going from pure methanol to 10% water/methanol (Table 2, entries 1 and 7).

In aqueous butanol, the situation is different. As a result of the higher hydrophobicity of butanol, the preference for water over alcohol by the hydrophilic nanocavity is much higher. Even though phase separation of water from butanol is easier than that from methanol, a *larger extent* of phase separation is involved in the folding in aqueous butanol as a result of this higher selectivity, meaning that more water molecules need to be phase-separated from the bulk to the nanocavity during folding.

Conceptually, there should also be a size effect. When a large alcohol is disfavored or "rejected" by the cavity, more water molecules need to come in to take its place. This effect is particularly significant because water is the smallest of common solvents. Even if the selectivity (the preference for water over alcohol caused by different hydrophilicity/hydrophobicity of the solvent) is the same, a larger alcohol still requires a *larger extent* of phase separation. Therefore, an increase in size of the alcohol or, more accurately, an increase in the *size difference* between the more polar and the less polar solvents always translates to a higher cost for the folding.

In reality, the size and the hydrophobicity of the alcohol cannot be varied independently. Thus, one cannot know whether the size difference or the hydrophobicity of the alcohol was chiefly responsible for the different behaviors of aqueous methanol and butanol. For this reason, it is interesting to compare the binding/folding of **1** in 2-methoxyethanol vs butanol. 2-Methoxyethanol is comparable in size to and more hydrophilic than butanol.[13] Yet, the binding of **1** is similar in both aqueous mixtures (Table 2, entries 12 and 13). Therefore, at least for these two mixtures, the size effect seems to dominate.

What about the branched alcohols? Mercury-binding of foldamer **1** is essentially the same in aqueous propanol, isopropanol, and *tert*-butanol (Table 2, entries 9−11, also Figure 1b). Similar behaviors in the cases of propanol and isopropanol are not surprising, given the similarity in their structures and properties, such as solubility in water. The behavior of *tert*-butanol is quite strange, but, one has to remember, among all the (isomeric) butanols, it is the only one that is completely miscible with water.[14] This unusual miscibility at least is directionally consistent with the folding model. Better miscibility of *tert*-butanol suggests a lower selectivity for water by the hydrophilic nanocavity, which is equivalent to a smaller extent of phase separation during folding. Of course, this effect is counterbalanced by the higher energetic cost (per water molecule) to separate water from *tert*-butanol than from butanol. With two opposing effects present, one cannot predict *a priori* which solvent mixture is better for the folding of **1**. Our data suggest that the extent of phase separation plays the dominant role in the *tert*-butanol/butanol comparison. Other factors may be also important here but are unclear to us at the moment. For example, if better miscibility is the only reason for the better folding in aqueous *tert*-butanol than in aqueous butanol, good folding should also be expected for aqueous 2-methoxyethanol, but that was clearly not the case (Table 2, entry 13).

Therefore, the whole picture is rather complex, partly because multiple solvent effects are involved and some of them are opposing one another, maybe partly because folding is inferred from the binding data—a reasonable but approximate treatment. At this point, the extent of phase separation seems to be the controlling factor, e.g., in the cases of water/methanol and water/butanol. This conclusion should not be generalized, however. It is certainly conceivable that miscibility can play a more important role in other mixtures. For example, the partly miscible DMSO/(hexane/EA = 2/1) mixture was clearly better for the folding than the completely miscible DMSO/(hexane/EA = 1/1),[5a] as mentioned earlier in this paper.

The above reasoning derives from general properties of solvents and hence should not be limited to aqueous mixtures.

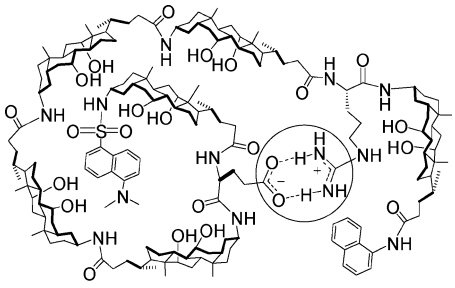
(12) (a) Sandström, M.; Persson, I.; Persson, P. *Acta Chem. Scand.* **1990**, *44*, 653−675. (b) Chen, T.; Hefter, G.; Marcus, Y. *J. Solution Chem.* **2000**, *29*, 201−216.

(13) With two adjacent oxygens, 2-methoxyethanol potentially can act as a chelating ligand and weaken the mercury binding of **1**. The D_S value for 2-methoxyethanol is not available, but that for ethylene glycol, which similarly has two adjacent oxygen donors, is 20, slightly higher than that (D_S = 19 or 18) for butanol (ref 12). Therefore, Lewis basicity may contribute but should not be the major factor.

(14) Marcus, Y. *The Properties of Solvents*; Wiley: New York, 1999; p 176.

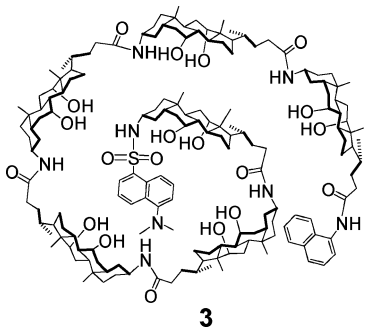
Indeed, when butanol is used as the larger solvent and methanol, ethanol, or propanol as the smaller solvent, a similar, albeit weaker, effect is observed (Table 2, compare entries 14−16 with entries 6 and 12). The weaker effect is anticipated because the size difference between methanol/ethanol/propanol and butanol is smaller than that between water and butanol. In addition, because water is far more hydrophilic (of course!) than any of the alcohols, the selectivity for the smaller alcohol over butanol by the nanocavity should be lower than that for water over butanol. Lower selectivity also means that the observed effect resulted from microphase separation of solvents will be weaker in the mixed alcohol series.

Solvent Effects in a Foldamer Stabilized by an Internal Salt Bridge. The folding of **1** is inferred from its binding toward mercury ions. The benefit of having strong Hg−S complexation is that folding can be studied in solvents totally impossible for the parent oligocholates. Nevertheless, it is desirable to confirm these conclusions in foldamers that do not involve any Hg−S interactions.



2

Studying folding in water-containing solvents is not possible with the parent oligocholates because they can only fold in the most folding-friendly solvent mixtures (e.g., 1−5% DMSO in hexane/EA = 2/1).[5a] One strategy to stabilize the folded state is through incorporation of a salt bridge, as in **2**, which has an arginine and a glutamate in the sequence. It is worth mentioning that, when the folding of **1** was studied *in the absence* of Hg^{2+}, insertion of the amino acids was found to enhance the folding slightly.[5b] This was probably not because the side chains contributed in any significant way to the folding, but because the two additional amino acids introduced a small degree of flexibility to the chain.[15] Foldamer **2** was synthesized via standard procedures (details given in the Supporting Information). The foldamer is labeled with naphthyl−dansyl, the same FRET D−A pair used to characterize the parent oligocholates (e.g., **3**).[5a,16]



3

The salt bridge undoubtedly is beneficial to the folding. When the naphthyl donor of **2** is excited in 1% DMSO/EA, the donor

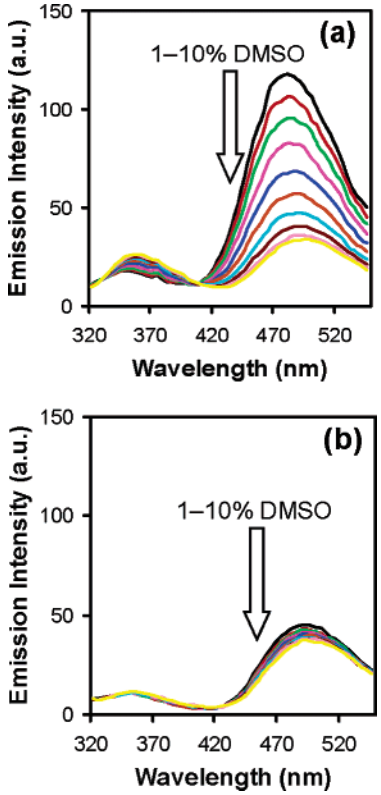


Figure 2. Fluorescence spectra of (a) **2** and (b) **3** in EA with different percentages of DMSO. **[2]** = [**3**] = 2.0 μM. Intermolecular energy transfer was previously shown to be absent under similar conditions.[5a]

emission near 350 nm is weak but the acceptor emission from the dansyl near 490 nm is extremely strong (Figure 2a), indicating efficient FRET. With more DMSO added, the donor emission becomes stronger while the acceptor emission gets weaker. The change corresponds to a transition from a folded, more compact structure to an unfolded, less compact structure. Over 1−10% DMSO, the parent foldamer **3**, on the other hand, shows a weak emission band for the acceptor and remains nearly constant, indicating a mostly unfolded structure throughout the solvent titration (Figure 2b).

Being confident of its higher folding stability, we studied the FRET of **2** in THF/MTHF (1/2) with 1−8% water. As mentioned before, the ratio of THF/MTHF was chosen to minimize the energetic cost to phase-separate water. To our amazement, the salt bridge does not help at all; FRET is completely absent in either **2** or **3** (Figure 1S, Supporting Information). Apparently, the penalty for folding is so large in H_2O/THF/MTHF that the salt bridge makes no difference. This result, however, should not be a surprise because **1** seems to fold particularly poorly in aqueous THF, as mentioned earlier. A change of solvent from 5% MeOH/EA to 5% H_2O/THF reduces the binding energy of **1** and Hg^{2+} by 3.4 kcal/mol.[5b]

(15) A certain level of flexibility is beneficial because the folded state will not be overly strained; too much flexibility, however, is detrimental to the folded conformer because the loss of entropy will be very large during folding.

(16) Fluorescence resonance energy transfer (FRET) measures distance in the range of 1−10 nm, depending on the specific D−A pair utilized. Because of its nanometer-sized range, FRET has been used extensively in the characterization of conformational changes in biomolecules, such as peptides and proteins. In general, FRET is better used for measuring relative instead of absolute distances, see: (a) Stryer, L. *Annu. Rev. Biochem.* **1978**, *47*, 819−846. (b) Selvin, P. R. *Methods Enzymol.* **1995**, *246*, 300−334. (c) Lakowicz, J. R. *Principles of Fluorescence Spectroscopy*, 2nd ed.; Kluwer: New York, 1999; Chapter 13.

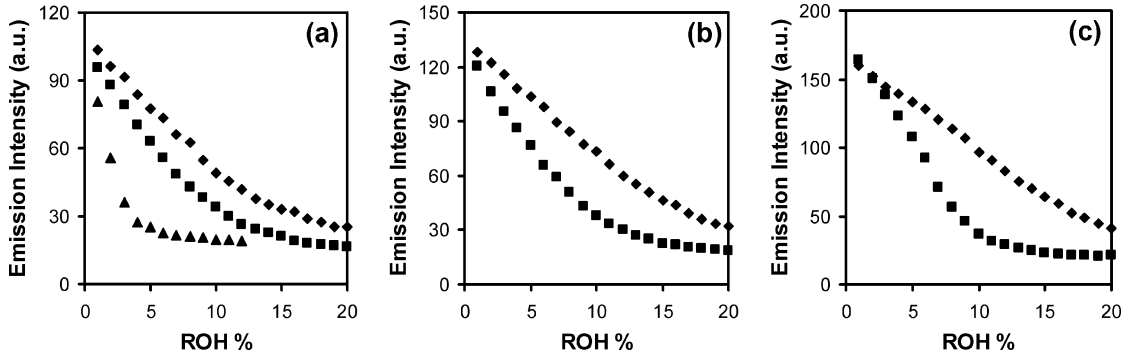


Figure 3. Maximum fluorescence intensity of the dansyl group of foldamer **2** in (a) CH_3CN/EA (1/2), (b) EA, and (c) hexane/EA (2/1) as a function of different percentages of H_2O (▲), MeOH (■), and EtOH (◆) added to the solvent mixture. **[2]** = 2.0 μM.

This energy is enough to shift a system from 95% folded to 95% unfolded.[17]

By now, we already know that folding depends not only on the size/structure of the polar solvent but also on that of the less polar solvent. Otherwise, folding in water/butanol and water/*tert*-butanol would not be so different. Since cyclic solvents such as THF/MTHF are not good for folding, we decided to try water in acetonitrile/EA (1/2). EA was an obvious choice because it worked many times as the nonpolar component in the folding-friendly solvents (e.g., DMSO in hexane/EA = 2/1 for **3** and DMSO/EA for **2**). Acetonitrile is added simply to assist dissolution of water.

Folding indeed happens in this mixture (Figure 2S, Supporting Information). In these solvent-titration experiments, the acceptor emission as a function of the solvent composition can be used to judge the folding ability of the foldamer.[5a,b] Provided the conformational change follows a two-state transition,[5a,18] a stronger acceptor band at the beginning of the titration (i.e., in low-polarity region) corresponds to a higher population of the folded conformer, as efficient FRET is only possible in the folded state. Upon addition of polar solvent, the acceptor emission will decrease when the unfolded state (with weak acceptor emission) becomes increasingly populated.[19] The curve eventually reaches a low plateau when all the foldamers become fully unfolded with a sufficient amount of the polar solvent added.

On the basis of the solvent-denaturation curve, **2** is nearly completely unfolded with 5% water in acetonitrile/EA =1/2 (Figure 3a, ▲). Since the size of the polar solvent makes a large difference, we also studied folding in acetonitrile/EA (1/2) with MeOH (Figure 3a, ■) or EtOH (Figure 3a, ◆) as the polar additive. All the curves start out with similar initial acceptor emission, which is reasonable because the initial solvents differ by only 1% in composition. Upon addition of the polar solvents, the folded conformer begins to unfold. The steeper the solvent-titration curves, the more susceptible the folded conformer is to the polar solvent and the lower its stability in that solvent mixture. According to Figure 3a, the stability of the folded conformer follows the order of water < methanol < ethanol. Therefore, once again, the oligocholate folds better as the polar solvent gets larger. This trend has nothing to do with acetonitrile because it is also observed in other mixtures, including in ROH/EA (Figure 3b) and in ROH/hexane/EA (Figure 3c). Water obviously cannot be employed in the latter two solvent systems because of its immiscibility with EA and/or hexane.

There could be two reasons for the increased folding ability of **2** in the order of water < methanol < ethanol. First, the salt bridge becomes more stable as the hydrogen-bonding ability of the solvent decreases. In other words, a more stable salt bridge in the ethanol mixture should make folding easier than in the aqueous mixture. The second possible reason comes from the microphase separation shown in Scheme 2. As the polar additive becomes larger and less hydrophilic, a smaller extent of phase separation is needed in the nanocavity, and this should impose a less costly burden on the folding process.

In order to determine which of the above two effects is more important, we studied the conformation of **2** in methanol/MTHF and compared it with that in methanol/EA. Methanol is completely miscible with either MTHF or EA (as well as with ether mentioned below). MTHF and EA are both nonpolar. Neither has appreciable solubility in water. Their E_T30 values, which are indicators for their polarity, are 36.5 for MTHF and 38.1 for EA.[20] Hence, if the strength of the salt bridge is the controlling factor in the folding process, MeOH/MTHF should be slightly better than the somewhat more polar MeOH/EA mixture. Foldamer **2** folds well in MeOH/EA (Figure 3b, ■; also see Figure 3Sa, Supporting Information) but is completely unfolded in all MeOH/THF mixtures (Figure 3Sb). Since good folding is also obtained in MeOH/Et_2O (Figure 3Sc), the difference in EA and MTHF cannot be caused by the ester/ether difference. If the strength of the salt bridge is not important, once again we are left with the microphase separation being the controlling factor.

Solvent Effects in the Parent Cholate Hexamer. When we began our investigation of the oligocholates, we thought folding should be facilitated by a small amount of polar solvent that strongly solvated the cholate α faces. That was why DMSO/CCl_4 and DMSO/hexane/EA were the main solvent systems studied in our first paper on the cholate foldamers.[5a] DMSO is a strong hydrogen-bond acceptor and should interact strongly with the OH groups on the cholate α faces. The work in the previous two sections reveals some neglected details in our folding model, i.e., the size and the structure of the polar and

(17) The difference in free energy between a 95% folded system (K_{eq} = 19, $-\Delta G_{folding}$ = 1.7 kcal/mol) and a 95% unfolded system ($-\Delta G_{folding}$ = −1.7 kcal/mol) is 3.4 kcal/mol at room temperature.

(18) The two-state model seems to be reasonable for foldamers with relatively rigid repeating units, see: Prince R. B.; Saven, J. G.; Wolynes, P. G.; Moore, J. S. *J. Am. Chem. Soc.* **1999**, *121*, 3114−3121 and references therein.

(19) For detailed procedures for analyzing solvent-titration curves, see: (a) Pace, C. N. *Methods in Enzymology*; Hirs, C. H. W., Timasheff, S. N., Eds.; Academic Press: New York, 1986; Vol. 131, pp 266−280. (b) Pace, C. N. Shirley, B. A.; Thomson, J. A. In *Protein Structure: A Practical Approach*; Creighton, T. E., Ed.; IRL Press: New York, 1989; pp 311−330.

(20) Marcus, Y. *The Properties of Solvents*; Wiley: New York, 1999; pp 142−154.

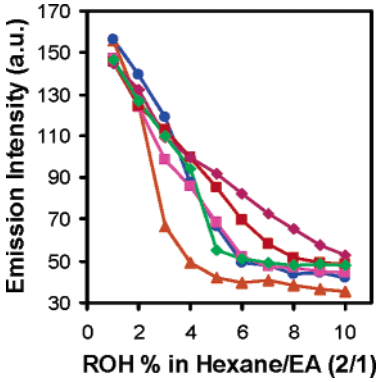


Figure 4. Maximum fluorescence intensity of the dansyl group of foldamer **3** in hexane/EA = 2/1 as a function of different percentages of MeOH (orange ▲), EtOH (blue ●), PrOH (pink ■), *i*-PrOH (red ■), *t*-BuOH (brown ◆), and BuOH (green ◆) added to the solvent mixture. **[3]** = 2.0 μM. The data points are connected to guide the eye.

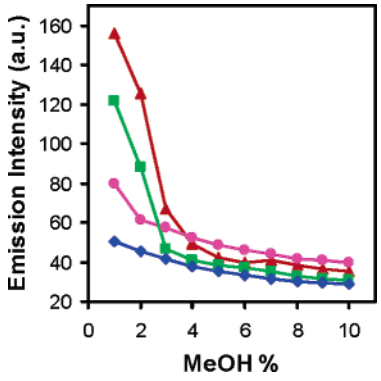


Figure 5. Maximum fluorescence intensity of the dansyl group of foldamer **3** as a function of MeOH% in hexane/EA = 2/1 (red ▲), hexane/MTHF = 2/1 (green ■), cyclohexane/EA = 2/1 (pink ●), and cyclohexane/MTHF = 2/1 (blue ◆). **[3]** = 2.0 μM. The data points are connected to guide the eye.

the nonpolar solvents. Although the conclusions are drawn from the externally stabilized (e.g., **1**, stabilized by the Hg−S complexation) or internally stabilized (e.g., **2**) foldamers, they should apply to the parent oligocholates as well.

The folding of the D−A-labeled hexamer **3** was thus studied further. With only solvophobic interactions to stabilize the folded state, it could only fold in highly nonpolar mixtures. Previously, this foldamer was found to fold reasonably well in 1−5% DMSO in hexane/EA (2/1).[5a] To verify the size effect of the polar solvent, we performed similar solvent titrations in hexane/EA (2/1) with methanol (orange ▲), ethanol (blue ●), propanol (pink ■), isopropanol (red ■), *tert*-butanol (brown ◆), and butanol (green ◆) as the polar solvents. On the basis of the titration curves in Figure 4, the folding ability increases roughly in the order of methanol < ethanol ≈ propanol < isopropanol < *tert*-butanol, if butanol is ignored for the moment.

An immediate concern is that this order is almost opposite to what is observed previously, i.e., methanol > ethanol > propanol ≈ isopropanol ≈ *tert*-butanol > butanol in the mercury-binding foldamer **1**. There is, however, no contradiction here, because the alcohol is the most polar component in ROH/hexane/EA for the folding of **3** but is the less polar component in H_2O/ROH for the folding of **1**. Once this difference is clarified, it is clear that the two orders are quite consistent with each other. In the ROH/hexane/EA mixture, as the alcohol gets larger, a smaller extent of phase separation (from hexane/EA into the hydrophilic nanocavity) is needed and is less costly—this is exactly the same size effect observed in both **1** and **2**. Why is butanol so much worse than *tert*-butanol for the folding of **3**? It is probably still due to its increased hydrophobicity, which makes butanol less able to solvate the cholate α faces than *tert*-butanol. The result is weaker preferential solvation and lower driving force for the folding.

Another conclusion from the study of **1** and **2** is that the size/structure of the nonpolar solvents is also critical. To verify this in the parent oligocholates, we studied **3** in three additional pairs of nonpolar solvents: hexane/MTHF (2/1), cyclohexane/EA (2/1), and cyclohexane/MTHF (2/1), with methanol as the common polar solvent. These comparisons are meaningful, especially because hexane (MW 86, d = 0.65 g/mL, E_T30 = 31.0) and cyclohexane (MW 84, d = 0.77 g/mL, E_T30 = 30.9) are comparable in molecular weight, density, and polarity, as are EA (MW 88, d = 0.89 g/mL, E_T30 = 38.1) and MTHF (MW 86, d = 0.85 g/mL, E_T30 = 36.5).[20] Therefore, when the four pairs of solvents, including hexane/EA, are compared, the main difference should come from the size and/or the (cyclic/acyclic) structure of the nonpolar solvent.

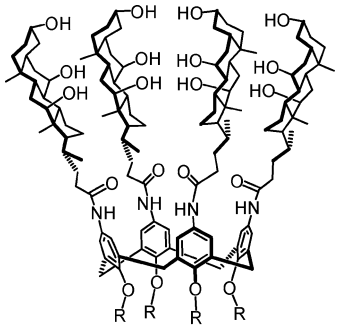
Folding once again is strongly disfavored in cyclic solvents. When either EA or hexane is replaced by the cyclic counterpart—namely, by MTHF (Figure 5, green ■) or cyclohexane (pink ●)—folding is weakened. In the mixture of methanol in cyclohexane/MTHF (blue ◆), when both nonpolar solvents are changed to cyclic ones, **3** is unable to fold at all, as shown by the weak acceptor fluorescence throughout the solvent titration. This dramatic effect of cyclic solvents is totally in line with the inability of **3** to fold in MeOH/MTHF, despite a potentially strong intramolecular salt bridge.

What could be a possible reason for the poor folding of the cholate foldamers in cyclic nonpolar solvents? Recently, Sansom and co-workers, by molecular simulations, showed that water is unable to enter a hydrophobic pore of 4.5 Å diameter, despite the fact that the pore is apparently large enough to accommodate three water molecules.[21] Cyclohexane is about 6−7 Å across. It is unclear how it will enter the hydrophilic nanocavity formed by folding. However, if its behavior in a *hydrophilic* nanocavity can be mirrored at all by how water behaves in a *hydrophobic* one, one would expect cyclohexane (or MTHF) will have difficulty entering the 1 nm cavity of the folded oligocholate. On the other hand, a linear molecule such as hexane (or EA) should enter the hydrophilic nanocavity much more easily, as it can better avoid unfavorable hydrophilic/hydrophobic contact with the wall. In this sense, poor folding in cyclic nonpolar solvents is in total agreement with the folding model. When the nonpolar solvent is rejected by the nanocavity, a larger extent of phase separation of the polar solvent is needed and represents a higher cost for the folding, just as in the case of small polar solvent.

Solvent Effects in an Amphiphilic Molecular Basket. The conclusions about preferential solvation in hydrophilic nanocavities should not be limited to externally or internally stabilized cholate foldamers. Indeed, they should not be limited to foldamers at all. Basket **4** represents a perfect system to further confirm these solvent effects. This molecule has a completely different mode of conformational change. Instead

(21) (a) Beckstein, O.; Biggin, P. C.; Sansom, M. S. P. *J. Phys. Chem. B* **2001**, *105*, 12902−12905. (b) Beckstein, O.; Biggin, P. C.; Bond, P.; Bright, J. N.; Domene, C.; Grottesi, A.; Holyoake, J.; Sansom, M. S. P. *FEBS Lett.* **2003**, *555*, 85−90.

of folding and unfolding, it simply turns the cholate α faces in and out, depending on the solvent polarity.[4] Nevertheless, the fundamental driving force, at least for the reversed micelle-like conformer with inwardly facing hydrophilic faces, should be the same as in the cholate foldamers.



4, R = C_6H_{13}

The aromatic protons of **4** appear as a single peak when the solvent mixture has intermediate polarity (e.g., CD_3OD/CCl_4 = 60/40) but split into two peaks in either highly polar (e.g., neat CD_3OD) or nonpolar mixtures (e.g., CD_3OD/CCl_4 = 10/90).[4] The splitting ($\Delta\delta$) between the two peaks in several related systems was consistently found to correlate with the stability of the reversed (or normal) micelle-like conformer.[4]

Because deuterated solvents have to be used in the ^{1}H NMR experiments, we performed only a limited number of solvent titrations for basket **4**. Figure 6 shows the splitting of the aromatic protons in three solvents: D_2O/THF-d_8, CD_3OD/THF-d_8, and CD_3OD/CCl_4. When THF-d_8 is used as the nonpolar solvent, an increase in size for the polar solvent from D_2O (◇) to CD_3OD (□) enlarges the splitting, suggesting that the reversed micelle-like conformer becomes more stable. This is in complete agreement with the finding made in the foldamers. When THF-d_8 (□) and CCl_4 (△) are used as the nonpolar solvent and CD_3OD as the common polar solvent, the aromatic peaks split above and below 60% CD_3OD, corresponding to a transition to the normal and to the reversed micelle-like conformer, respectively.[4] The stabilities of the normal micelle-like conformer are similar in the two solvents, as shown by the nearly overlapping curves above 60% CD_3OD. This is not surprising, as this conformer is formed via direct contact of the cholate hydrophobic β faces[4b,c] and may not depend too much on the nonpolar solvent. For the reversed micelle-like conformer formed below 60% CD_3OD, a change from CCl_4 to THF makes it less stable, as shown by the smaller splitting of the aromatic protons. This is also in complete agreement with what is found in the foldamers.

Conclusions

The four cholate-derived molecules **1**−**4** unanimously support the folding model that describes microphase separation of solvents within a nanometer-sized hydrophilic cavity. Ironically, poor folding of the oligocholates in water/THF/MTHF, initially thought to be inconsistent with the folding model, only confirmed the model after a more thorough investigation. All the new results reported in this paper essentially had been hidden in the original model. We were just not insightful enough to recognize them.

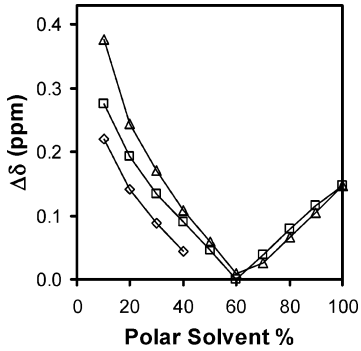


Figure 6. Difference ($\Delta\delta$) in the chemical shifts of the ortho aromatic protons of **4** as a function of the percentage of D_2O in THF-d_8 (◇), CD_3OD in THF-d_8 (□), and CD_3OD in CCl_4 (△). Basket **4** is only soluble in THF-d_8 with up to 40% D_2O. The data points are connected to guide the eye.

In summary of the peculiar solvent effects observed in the cholate foldamers, when a hydrophilic nanocavity is exposed to a mixture of polar and nonpolar solvents, the polar solvent is preferentially retained by the cavity, as a result of favorable hydrophilic/hydrophilic contact. If the cavity is rigid, as in molecular sieves, it can be used to absorb the polar solvent selectively from the mixture. When the cavity is formed as a result of conformational organization, this microphase separation of solvents strongly influences the conformational change. It takes fewer larger polar solvent molecules to occupy the cavity and provide solvation to the hydrophilic wall. Thus, a smaller extent and a less costly phase separation will occur during the conformational organization. Linear/small nonpolar solvents are more favorable than cyclic/large solvents. This is probably because they can better avoid hydrophobic/hydrophilic contact with the wall, especially when the nanocavity is only partly covered with hydrophilic groups.

Our discussion is largely limited to trends and qualitative descriptions in this work. We believe that molecular simulation and modeling may reveal additional details in the preferential solvation within nanocavities, and the information obtained can be useful in understanding related chemical and biological phenomena.

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Supporting Information Available: Experimental Section, including general experimental details, synthesis and characterization of foldamer **2**, fluorescence data, and NMR data. This material is available free of charge via the Internet at http://pubs.acs.org.

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