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Tuning the Sensitivity of a Foldamer-Based Mercury Sensor by Its Folding Energy

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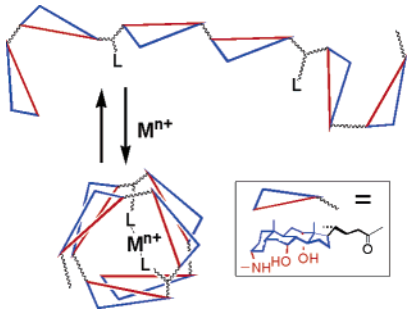
Foldamers are synthetic analogues of biopolymers capable of adopting well-defined conformations.[1] With reversible conformational changes, foldamers hold great promise as responsive materials and sensors. We recently prepared several environmentally sensitive molecules based on cholic acid, taking advantage of its facial amphiphilicity and natural curvature.[2−5] We discovered that cholate oligomers could fold into helical structures with nanometer-sized hydrophilic cavities.[5] These oligocholates have no intrinsic intrachain interactions (e.g., hydrogen bonds or $\pi-\pi$ stacking). Folding is driven completely by solvophobic interactions and is extremely sensitive to solvent changes—minute changes (<0.5%) in solvent composition can be easily detected. We reasoned that highly sensitive conformational changes could be useful for sensor designs so long as the conformational changes can be made to respond to specific analytes and be expressed in readable signals.

The general idea of employing foldamers to bind metal ions is illustrated in Scheme 1.[6] Unlike a preorganized bidentate ligand, a foldamer-based ligand requires a large conformational change to bind the metal. Because folding may be highly favorable or unfavorable under different conditions, binding affinity of the foldamer to the metal (and its sensitivity as a sensor) can be regulated accordingly. This tunability represents a distinctive advantage of a foldamer-based multidentate compared to a preorganized one, such as a macrocycle, and is crucial if reversible binding and release are desired. Also, tunability is expected as a general feature of a cooperatively folded structure and, thus, should not be limited to a particular system. In this communication, we report a highly tunable mercury sensor[7] based on this principle. Indeed, its binding affinity can be tuned over at least 5 orders of magnitude by simple solvent changes.

Connected by amide groups, cholate foldamers can be easily functionalized by incorporation of amino acids. We prepared a hybrid oligomer **1**, which contained a fluorescent donor (naphthyl) and an acceptor (Dansyl) at the chain ends, allowing the use of fluorescence resonance energy transfer (FRET) to study its conformational behavior. FRET indicated that this hybrid oligomer showed similar cooperative folding/unfolding transitions[5] as the methionine-free hexamer (Figures 4S and 5S).[8] In fact, insertion of methionine even seemed to enhance the folded conformer slightly (Figure 6S).[8] The folded state of the original oligocholates were known to be highly strained, as addition of a few percent of a polar solvent could cause unfolding. It is possible that inclusion of methionine units gives the folded structure some flexibility, which may be advantageous to a strained system.

Foldamer **2** was used in the mercury sensing because its quenching (vide infra) was not complicated by FRET. As shown in Figure 1, this foldamer could easily detect 20 nM of [Hg^{2+}] in a folding-friendly solvent mixture, 5% methanol/(hexane/ethyl acetate = 2/1).[9] During titration, the emission band blue-shifted by about 10 nm, consistent with an electron-transfer quenching mechanism found in other Dansyl-based mercury sensors.[7f] Binding

Scheme 1. Schematic Representation of a Metal-Binding Foldamer



stoichiometry was 1:1, as confirmed by the Job plot (Figure 7S).[8] Nonlinear least-squares fitting gave an association constant (K_a) of 1.5×10^7 M^{-1}, which translates to $-\Delta G = 9.8$ kcal/mol. Previously, dimethyl sulfoxide (DMSO) was found to be better at promoting folding of the cholate foldamers than methanol.[5] When 5% DMSO was used in place of methanol in the above mixture, however, a similar affinity ($K_a = 1.2 \times 10^7$ M^{-1}) was obtained.

If binding affinities did not change much in folding-friendly solvents, they could be tuned over broad ranges in folding-unfriendly ones. Table 1 summarizes binding data determined by fluorescence titrations. Several trends are immediately noticeable. First, when hexane is removed from the ternary solvents (entries 1 and 3), $-\Delta G$ decreases by 0.4 kcal/mol. Weaker binding is consistent with earlier finding that folding is promoted by limited miscibility of solvents.[5,9] Second, in the binary mixture of methanol and ethyl acetate (EA), $-\Delta G$ decreases further by 2 kcal/mol (entries 3−9) as methanol content increases from 5 to 100%. Each cholate is about 1.4 nm from head to tail. Separated by two cholate units, the sulfur groups probably cannot chelate mercury in the unfolded state. If the assumption is correct, the data can be easily explained because the folded, mercury-binding conformer has a hydrophobic exterior and is disfavored by highly polar solvents.

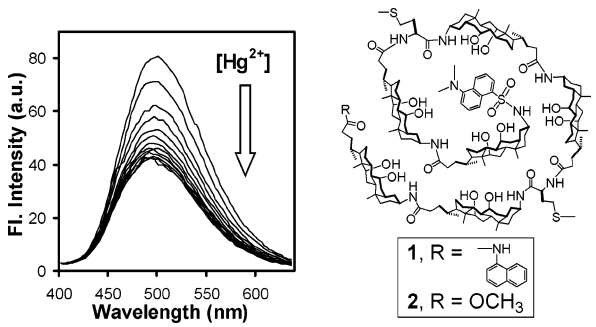


Figure 1. Fluorescence spectra of **2** with different [Hg^{2+}] ([Hg^{2+}] = 0, 0.02, 0.04, 0.06, 0.08, 0.10, 0.12, 0.14, 0.16, 0.18, 0.20, 0.22, and 0.24 μM from top to bottom) in 5% MeOH in hexane/ethyl acetate (2/1). [**2**] = 0.2 μM.

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Table 1. Thermodynamic Data for Binding between **2** and Hg^{2+} at 25 °C, Determined by Fluorescence Titration

entry	solvent composition[a]	K_a[b] (M^{-1})	$-\Delta G$ (kcal/mol)
1	5% MeOH in HX/EA (2/1)	$(1.5 \pm 0.3) \times 10^7$	9.8
2	5% DMSO in HX/EA (2/1)	$(1.2 \pm 0.4) \times 10^7$	9.7
3	5% MeOH in EA	$(7.3 \pm 1.7) \times 10^6$	9.4
4	10% MeOH in EA	$(3.8 \pm 0.8) \times 10^6$	9.0
5	20% MeOH in EA	$(1.6 \pm 0.2) \times 10^6$	8.5
6	40% MeOH in EA	$(1.1 \pm 0.1) \times 10^6$	8.2
7	60% MeOH in EA	$(7.6 \pm 0.4) \times 10^5$	8.0
8	80% MeOH in EA	$(3.9 \pm 0.6) \times 10^5$	7.6
9	100% MeOH	$(2.6 \pm 0.2) \times 10^5$	7.4
10	5% H_2O in THF	$(2.4 \pm 0.1) \times 10^4$	6.0
11	10% H_2O in THF	$(1.9 \pm 0.2) \times 10^4$	5.9
12	20% H_2O in THF	$(5.5 \pm 0.6) \times 10^3$	5.1

[a] HX = hexane; EA = ethyl acetate. [b] The association constants were determined by nonlinear least-squares fitting to a 1:1 binding isotherm.

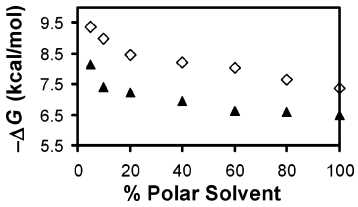

Figure 2. Binding free energy for **2**·Hg^{2+} as a function of volume percentage of methanol (◊) and DMSO (▲) in EA. See Table 1S in the Supporting Information for binding data in DMSO/EA. Fluorescence of **2** gradually decreases with higher methanol and increases with higher DMSO (Figure 8S).[8] This is a general solvent effect in the absence of Hg^{2+} and not related to binding constants determined by Hg^{2+} titration.

Third, K_a in water/THF is several orders of magnitude lower than those in the folding-friendly solvents (compare entries 10−12 with 1) and even weaker (K_a < 100 M^{-1}) in some other mixtures such as water/butanol or water/2-methoxyethanol. Because water, THF, and butanol have very similar D_s values,[10] variation in binding cannot be caused by different Lewis basicity but, instead, most likely by poor folding in these mixtures. The conclusion is in agreement with previous observations that the parent oligocholates remain unfolded in water/THF even when nonpolar solvents such as 2-methyl−THF (MTHF) was added to facilitate demixing of water.[5,9,11]

When $-\Delta G$ is plotted against percentages of the polar solvent, both methanol/EA and DMSO/EA mixtures give curves consisting of a more-sensitive region and a less-sensitive region (Figure 2). The overall solvent effect undoubtedly has contributions from conformational sensitivity, differential solvation (on the hydrophilic interior of the folded structure), and Lewis basicity. It is possible that some of them (e.g., differential solvation) are more sensitive than others at the low-polarity end.

Binding is generally weaker in DMSO mixtures than in methanol mixtures (Figure 2). Although the difference may be due to higher Lewis basicity of DMSO,[10] it may also be caused by stronger solvation of the hydrophilic faces of cholates by DMSO. Previously, it was found that displacement of internal solvent molecules in cholate foldamers[5] or cholate-based molecular containers[4] was more difficult for DMSO than for methanol. Binding with $Hg(OAc)_2$ requires partial desolvation of hydrophilic faces of cholates and should have a higher energetic cost for the more strongly solvating DMSO. It is unclear whether different solvation or Lewis basicity is mainly responsible for the observed weaker binding in DMSO mixtures, as both effects predict the same trend. However, it is quite clear that Lewis basicity is *not* the controlling factor in other cases. For instance, binding is stronger in 100% DMSO ($-\Delta G$ = 6.5 kcal/mol,[8] Table 1S) than in either H_2O/THF or H_2O/BuOH, even though DMSO is the strongest Lewis base among all the solvents tested.[10]

Interestingly, Foldamer **2** was highly selective for mercury in comparison to other divalent cations such as Mg^{2+}, Zn^{2+}, Cu^{2+}, Co^{2+}, Ni^{2+}, and even Pb^{2+} (Figure 9S).[8] The only cation that showed slight (4%) response was Ag^+. We believe that binding affinity is only part of the reason for specificity—Hg^{2+} is known to be a better quencher for Dansyl than most of the other metal ions.[7f]

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Supporting Information Available: Experimental details for the synthesis and UV and fluorescence data of the cholate foldamers (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(8) See Supporting Information.
(9) The folded oligocholate resembles a unimolecular reversed micelle. The most "folding-friendly" solvents are nonpolar ones mixed with a small amount of a polar solvent. Because the interior of a folded conformer prefers polar molecules, microphase separation of solvents happens during folding. MeOH is completely miscible with EA but barely miscible with hexane. Therefore, demixing should be easier in MeOH/hexane/EA than in MeOH/EA.
(10) D_S values (Lewis basicity toward $HgBr_2$) are 14 (EA), 17 (water and THF), 18 (methanol and butanol), and 28 (DMSO). See: (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.
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