9-2006

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Disciplines
Chemistry

Comments
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ORGANIC LETTERS

2006
Vol. 8, No. 21
4715−4717

Detection of Hg^{2+} in Aqueous Solutions with a Foldamer-Based Fluorescent Sensor Modulated by Surfactant Micelles

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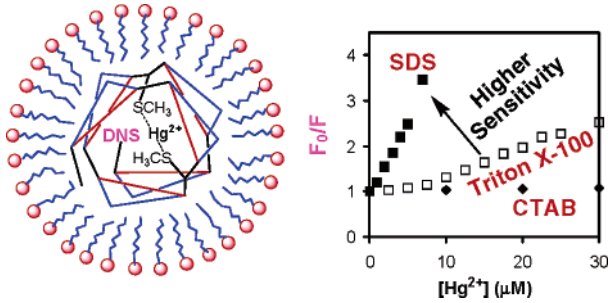
Received July 14, 2006

ABSTRACT



A hybrid foldamer constructed from six cholate units and two methionines was labeled with a DANSYL (DNS) group. The foldamer was solubilized by surfactant micelles to allow its usage as a fluorescent sensor for mercury ions present in the micromolar range in aqueous solutions. Its sensitivity was largely independent of the concentration of nonionic surfactants but was strongly influenced by both the nature and the concentration of ionic surfactants.

Foldamers are synthetic analogues of biomolecules that can adopt well-defined, compact conformations.[1] Because the conformation of a molecule can be influenced by environmental conditions such as the solvent polarity, pH, light, and the presence of specific ions or molecules, foldamers have potential applications as sensors. The biological world abounds with such sensors in which binding between a signal molecule and a protein causes a conformational change in the latter and alters its catalytic activity or binding toward another ligand or protein.[2]

We have been interested in using cholic acid as a building block to construct conformationally controllable, amphiphilic foldamers[3] and nonfoldamers.[4] Cholate foldamers can fold into helical structures in nonpolar solvents mixed with a small amount of a polar solvent.[3] The folded helix forms a nanometer-sized hydrophilic cavity, where the polar solvent is concentrated from the mostly nonpolar environment, and solvates the hydrophilic faces of the cholates to contract the otherwise extended chain. Recently, we reported **1**, which

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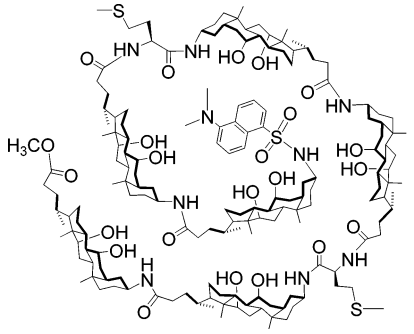
10.1021/ol061735x CCC: $33.50
Published on Web 09/14/2006

has two methionine units included in the sequence and a fluorescent Dansyl group at the chain end.[3b] As a fluorescent sensor, this hybrid foldamer has an unusual tunability in its sensitivity to mercury ions.[5] In the folded state, it is preorganized as a bidentate ligand and can bind Hg^{2+} with a binding constant (K_a) > 10^7 M^{-1}. In the unfolded state, on the other hand, binding has to overcome an unfavorable folding equilibrium and can be extremely weak (K_a < 100 M^{-1} in the worst cases). Moreover, **1** is highly selective toward Hg^{2+}, showing negligible interference from other divalent cations, such as Mg^{2+}, Zn^{2+}, Cu^{2+}, Co^{2+}, Ni^{2+}, and even Pb^{2+}.[3b]

However, being mostly nonpolar, **1** is insoluble in water and thus cannot be employed to detect mercury ions in aqueous solutions. Herein, we report the incorporation of **1** into surfactant micelles to overcome this deficiency. In addition, the micelles not only provide a hydrophobic local environment to solubilize the foldamer sensor but also allow the modulation of its sensitivity by the ionic characteristics of the surfactant.

The basis for using surfactant micelles to solubilize **1** comes from the fact that, as a metabolite of cholesterol, cholic acid has a lipid-compatible hydrophobic backbone. In fact, bile salts, including sodium cholate, form mixed micelles readily with surfactants or phospholipids.[6] An X-ray diffraction study by Small et al. demonstrated that sodium cholate can aggregate within the hydrophobic domain of lipids to form reversed-micelle-like structures.[7] In the literature, lipid-compatible cholate derivatives have been frequently reported.[8]



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The three surfactants chosen in our study are CTAB (cetyltrimethylammonium bromide), SDS (sodium dodecyl sulfate), and Triton X-100. They are representative examples of cationic, anionic, and nonionic surfactants used previously by other researchers.[9] Their critical micelle concentrations (CMCs) are 1, 8, and about 0.3 mM in water, respectively.[9,10] The first indication for internalization of **1** within the micelles comes from its fluorescence intensity. In general, Dansyl derivatives emit more strongly in organic solvents and are nearly nonfluorescent in water.[11] In our hands, the emission intensity of **1** is stronger in less polar solvents, such as 5% MeOH/ethyl acetate, THF, and *t*-BuOH, than in more polar solvents, such as MeOH and EtOH (Supporting Information, Figure 1S).[12] In water/THF mixtures, the emission of **1** clearly decreases with a higher percentage of water. Because the intensity of **1** in the three surfactant solutions above the CMC is comparable to those in organic solvents (Supporting Information, Figure 1S), it is reasonable to assume that the Dansyl group of **1** is in a relatively hydrophobic environment. Among the three surfactants, Triton X-100 gives the strongest fluorescence for **1**, suggesting that its micelle is the most hydrophobic, in agreement with previous literature studies.[9]

The maximum emission wavelength (λ_{max}) of Dansyl is sensitive to the polarity of its local environment and typically shifts to the blue as the environment becomes less polar.[11] Compound **1** is not soluble in nonpolar solvents (e.g., hexane or pure ethyl acetate). Among the solvents studied, 5% MeOH/ethyl acetate and THF give the lowest λ_{max} at 497 and 487 nm, respectively; polar solvents afford a higher λ_{max}, up to 522 nm for 30% H_2O in THF. With surfactants, the λ_{max} of **1** ranges from 487 to 497 nm, also suggesting that **1** is in a fairly nonpolar environment (Supporting Information, Figure 2S).[12] Among the three surfactants, Triton X-100 affords the lowest λ_{max} (487 nm) for the Dansyl group, consistent with a most hydrophobic micelle.

Being confident of the micellar incorporation of **1**, we then titrated it with $Hg(NO_3)_2$ in the presence of surfactants at or above their CMC. Immediately, we noticed a large effect on its binding with Hg^{2+} caused by the type of surfactants. The fluorescence of **1** is nearly unquenched by Hg^{2+} in CTAB solutions but quenched easily in Triton X-100 and most efficiently in SDS micelles (Figure 1a). The binding strength clearly follows the order of cationic micelle ≪ nonionic micelle < anionic micelle. Such an order is not a

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(12) The Supporting Information.

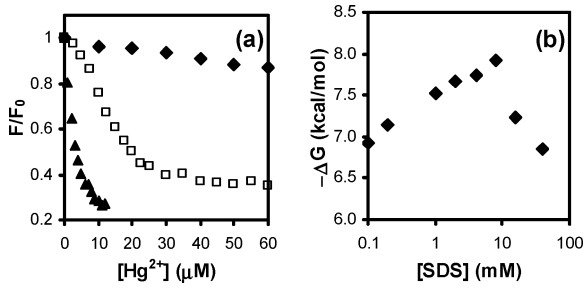

Figure 1. (a) Normalized maximum fluorescence intensity of **1** in 5 mM CTAB (◆), 1 mM Triton X-100 (□), and 8 mM SDS (▲) as a function of [Hg^{2+}]. (b) Binding energy ($-\Delta G$) between **1** and Hg^{2+} as a function of [SDS].

surprise, as Hg^{2+} is positively charged and should be repelled by CTAB headgroups but attracted by those of SDS. Nonionic micelles give intermediate affinity because neither favorable nor unfavorable electrostatic interactions are involved. In the most "folding-friendly" solvents, such as 5% MeOH in hexane/ethyl acetate (2:1), **1** was shown to detect 20 nM concentrations of Hg^{2+}.[3b] Binding is noticeably weaker when **1** is in surfactant micelles but still allows easy detection of Hg^{2+} at 1 μM (Figure 1a, ▲).

The binding of Hg^{2+} was previously confirmed to be 1:1 by the Job plot.[3b] The association constants determined by nonlinear least-squares fitting are summarized in Table 1. Examination of the data quickly reveals that the type of surfactants not only has a direct impact on K_a but also influences how K_a responds to the concentration of the surfactant. For example, K_a is nearly the same when the concentration of Triton X-100 is varied between 1 and 10 mM. This insensitivity also can be seen from the nearly identical titration curves in Triton solutions with different concentrations (Supporting Information, Figure 3S).[12] On the other hand, K_a is clearly dependent upon the concentration of SDS (entries 5−12, Table 1; also see Figure 4S in the Supporting Information). Furthermore, fluorescence titrations could be obtained with SDS well below its CMC of 8 mM, even down to 0.1 mM. However, when the concentration of Triton X-100 drops below the CMC, the fluorescence of **1** becomes unstable. Thus, premicellization is not required for the solubilization of **1** by SDS but seems to be important with the nonionic Triton X-100.

Table 1. Binding Data for **1** and $Hg(NO_3)_2$ at 25 °C

entry	surfactant[a]	K_a (M^{-1})	$-\Delta G$ (kcal/mol)
1	Triton X-100 (10 mM)	$(6.3 \pm 0.9) \times 10^4$	6.5
2	Triton X-100 (5 mM)	$(7.6 \pm 1.4) \times 10^4$	6.7
3	Triton X-100 (2 mM)	$(6.6 \pm 1.6) \times 10^4$	6.6
4	Triton X-100 (1 mM)	$(6.4 \pm 1.3) \times 10^4$	6.5
5	SDS (40 mM)	$(1.1 \pm 0.2) \times 10^5$	6.9
6	SDS (16 mM)	$(2.0 \pm 0.3) \times 10^5$	7.2
7	SDS (8 mM)	$(6.5 \pm 0.5) \times 10^5$	7.9
8	SDS (4 mM)	$(4.8 \pm 1.0) \times 10^5$	7.7
9	SDS (2 mM)	$(4.3 \pm 0.4) \times 10^5$	7.7
10	SDS (1 mM)	$(3.3 \pm 0.6) \times 10^5$	7.5
11	SDS (0.2 mM)	$(1.7 \pm 0.3) \times 10^5$	7.1
12	SDS (0.1 mM)	$(1.2 \pm 0.1) \times 10^5$	6.9

[a] K_a (<400 M^{-1}) could not be determined accurately in 5 mM CTAB. The solution turned cloudy with >90 μM Hg^{2+}, probably due to the low solubility of the $HgBr_2$ formed from $Hg(NO_3)_2$ and CTAB.

During aggregation, SDS molecules have to overcome substantial electrostatic repulsion that is not present during the aggregation of Triton.[13] Because of the neutrality of **1**, co-aggregation of SDS and **1** should be more favorable than aggregation of SDS with other SDS molecules. In contrast, co-aggregation of Triton and **1** does not have any particular advantage over the homoaggregation of Triton. In essence, a hydrophobic molecule such as **1** can induce the aggregation of SDS[14] but cannot do so (at least not as effectively) for the nonionic Triton. This is probably the reason pre-micellization is needed for Triton to solubilize **1** but is unnecessary for SDS.

Interestingly, the binding energy ($-\Delta G$) in SDS solutions shows a distinctive maximum at the CMC of the surfactant (Figure 1b). Negatively charged surfactants undoubtedly can enhance the effective concentration of positively charged Hg^{2+} on the surface of the micelles. This "mercury-concentrating" effect is very likely to be most effective at the CMC, at which the surfactants start to work *cooperatively* to attract the metal ion. With a further increase in the concentration of SDS, more micelles are created that are only sensor free. (Note that the surfactant is used at a much higher concentration than the sensor.) These additional micelles are expected to compete with the sensor-containing micelles for Hg^{2+} and thus reduce the binding strength.

Acknowledgment. We thank Dr. Basudeb Saha for the use of the spectroscopy facility.

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

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(13) This is the fundamental reason nonionic surfactants tend to have lower CMCs than ionic ones with comparable hydrophobes.

(14) Hydrophobic polymers are known to be highly effective at inducing the aggregation of ionic surfactants below the CMC; see: Rosen, M. J. *Surfactants and Interfacial Phenomena*, 2nd ed.; Wiley: New York, 1989; p 181.