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Triton X-100 Enhances Ion-Pair-Driven Molecular Recognition in Aqueous Media. Further Work on a Chemosensor for Inositol Trisphosphate

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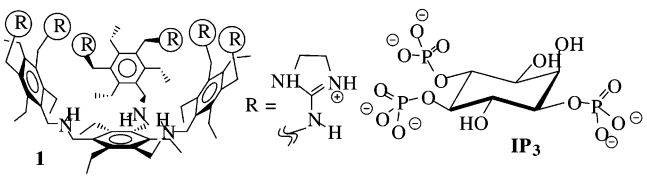
Abstract: Molecular recognition in water based on ion-pairing is hampered by competition from the solvent. We have reported that synthetic receptor **1** recognizes inositol trisphosphate (IP_3) with a binding constant of 10^8 M^{-1} in methanol. In this paper, instead of working in methanol, we report that the surfactant Triton-X 100 also yields a large absorbance or fluorescence change and a binding constant on the order of 10^6 M^{-1}.

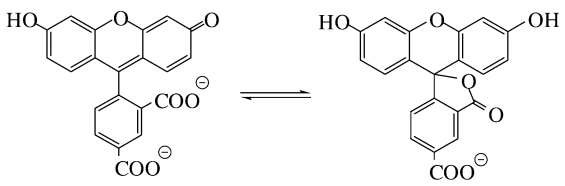
Many researchers in the field of molecular recognition using synthetic receptors are currently striving to create receptors that are selective and bind with good affinities in water.[1] If one does not exploit the hydrophobic effect, one is left to hydrogen bonding and ion pairing as the dominant forces for molecular recognition. However, these forces are weaker in water than lower dielectric media due to the competition from this solvent.[2] Techniques to enhance the affinities between synthetic hosts and guests in water can potentially expand the usefulness of synthetic receptors.[3]

We reported a few years ago that receptor **1** has a high affinity toward IP_3,[4] an important second messenger in living cells.[5] Compound **1** possesses guanidinium groups for hydrogen-bonding and charge-pairing interactions that are preorganized to complement the three phosphate groups of IP_3. A competition assay was employed to sense IP_3 using 5-carboxyfluorescein (**2**) as the indicator. In this previous study, we found that **2** preferentially adopts a cyclized form (colorless and nonfluorescent, eq 1) in 100% methanol. Upon addition of **1** to **2** in methanol, the yellow color reappears, as does the fluorescence, because **1** induces ring opening of **2**. Subsequent addition of IP_3 causes the release of **2**, which results in a ring closing to give the colorless/nonfluorescent form of **2**. Therefore, a large absorbance and fluorescence decrease is observed upon addition of IP_3 in methanol. Moreover, a high binding constant, 10^8 M^{-1}, was achieved in methanol.



The affinity is reduce to below 10^5 in water, and the cyclized from of the indicator is not present in water. To overcome the problems related to solvent competition in aqueous media, and to utilize the cyclized from of fluorescein, we turned our attention to surfactants.

The surfactants, Triton X-100, dodecyl sulfate sodium salt (SDS), and cetyltrimethylammonium bromide (CTAB), were mixed with **2** at pH 7.4 (10 mM hepes buffer). However, at neutral pH, these surfactants did not cause the change in absorbance or fluorescence of **2** as observed in methanol. However, 5-carboxyfluorescein (**2**) is known to cyclize to the closed form under moderately acidic conditions (eq 1).[6]



When the pH was dropped from 7.4 to the moderately acidic pH of 4.0, we found that the fluorescent form still largely remained. Yet, at this pH, addition of 2% Triton X-100 (formate buffer) led completely to the colorless/nonfluorescent form of **2.** At pH 4, the equilibrium between the open and closed form of **2** can be shifted completely to the closed form upon addition of the surfactant.

Upon addition of **1** to **2** in 2% Triton, the yellow color reappears (Figure 1a), as does the fluorescence (Figure 2a). Subsequent addition of IP_3 results in a large absorbance and fluorescence decrease (Figures 1b and 2b). This modulation of the absorbance and fluorescence spectra measured for Triton X-100/aqueous solutions is very similar to that previously found for pure methanol.[4] SDS and CTAB did not induce the colorless form of **2**, even at pH 4.0.

Clearly, Triton X-100 has a dramatic influence on the spectroscopy of **2**. This nonionic surfactant has an average number of 9.4 oxyethylene units per molecule, and

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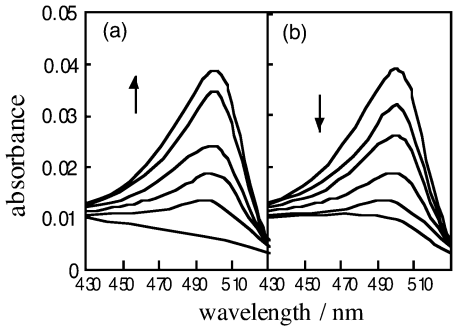

FIGURE 1. UV−visible absorption spectra of **2** upon addition of (a) **1** (0, 5, 10, 15, 21, 35 μM) and (b) IP_3 (1, 1.5, 3.7, 5.6, 8 mM) in the presence of **1** (7 μM) and **2** (2 μM) in 2% Triton X-100 in 1 μM format buffer (pH = 4.0).

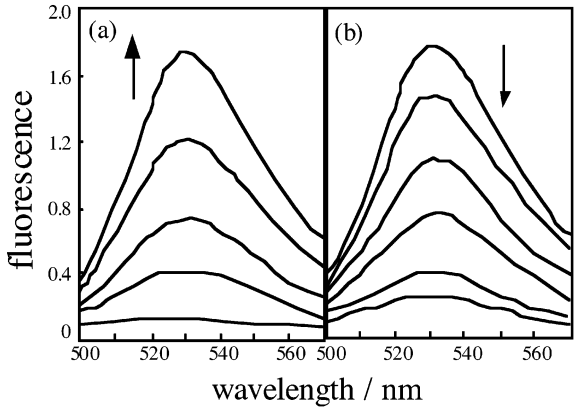

FIGURE 2. (a) Fluorescence change of **2** upon addition of **1** (0, 1.7, 3.4, 5, 6.7 μM from bottom to top) and (b) IP_3 (0, 0.25, 0.75, 1.2, 2.2, 4.1 from top to bottom) in the presence of **1** (6.7 μM) and **2**. [**2**] = 2 μM, 2% Triton X-100 in 1 μM format buffer (pH = 4.0). Excitation at 450 nm.

an average of 75 molecules undergo association to form micelles in water (CMC = 0.6 g/L, 0.6%).[6,7] We assume that **2** by itself is incorporated into the interior of the micelles through the hydrophobic effect, leading to the nonfluorescent form. However, the addition of receptor **1** shifts the equilibria of eq 1 to the open more anionic form of **2**, due to binding between **1** and **2**. Addition of IP_3 in turn displaces **2** back into the surfactant microenvironment. Although receptor **1** has a hydrophobic exterior surface, the interior of the cavity is highly charged. Therefore, it is not apparant how **1** partitions between the solvent and the micelles. Hence, the binding of **2** and IP_3 with **1** could be occurring predominantly in solution, in the micelles, or both. To study where the binding occurs in solution, and to make comparisons to methanol, we also studied methanol/water mixtures.

At pH 4.0, the phosphates in IP_3 will be in a greater state of protonation than at neutral pH and therefore IP_3 will have a lower affinity for **1** than at neutral pH. Hence, to make a fair comparison between the binding constants obtained in mixtures of methanol/water to those obtained in the 2% Triton solution, pH 4.0 formate buffer was used with the methanol/water mixtures also. Various mixtures of methanol and water at pH 4.0 were tested to find the minimum amount of methanol that gives the same optical response as 2% Triton X-100 in water. To induce the colorless form of **2** without Triton X-100 at pH 4, 40% methanol was required. Monitoring the absorption at 502 nm afforded the binding constant between **1** and **2** using the standard 1:1 algorithm (Table 1).[8]

TABLE 1. Effect of Solvents on the Affinities of IP_3 and 2 with 1. K_a Values Reported as 10^6 M^{-1}

solvent	K_a (IP_3)	K_a (**2**)
10 mM hepes (pH 7.4)	0.045[4]	0.021
2% Triton in 1 mM formate (pH 4.0)	1.2	0.036
4/6 MeOH/1 mM formate (pH 4.0)	5.0	0.076

The binding constant of **1** for IP_3 was found to be 1.2 $\times$ 10^6 M^{-1} in the Triton X-100 containing buffer via a competition analysis.[8] This value is high enough for sensing μM concentrations of IP_3. This binding constant is nearly identical to that obtained in 40% methanol but approximately 26 times greater than that for pure water. This supports binding between **1** and IP_3 occurring to a significant extent in the lower dielectric environment of the micelles. The complex between **1** and IP_3 is charge neutral and would partition nicely into the micelles. In contrast, the complex between **1** and **2** is still highly charged and would prefer the aqueous environment.

In summary, we have shown that 2% Triton X-100 in water creates a solution that is nearly identical to that of 40% methanol in water with regards to the ion-pair-driven binding event between **1** and IP_3. The surfactant leads to both a large intensity change in the absorbance or fluorescence spectra of the indicator and gives binding constants similar to the lower dielectric media. This system may prove to be useful for monitoring IP_3 directly in living cells using capillary electrophoresis.[9] Further, the use of surfactants to increase ion-pairing molecular recognition in water may prove to be a general phenomenon, one that we are exploring with other systems.

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