Fluorescence Sensing of Anions via Intramolecular Excimer Formation in a Pyrophosphate-Induced Self-Assembly of a Pyrene-Functionalized Guanidinium Receptor

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In mimicry of biological systems such as DNA, the fabrication of molecular assemblies and supramolecular arrays is one of the current research topics in supramolecular chemistry.[1] A wide variety of abiotic self-assembling systems such as catenanes and double helices have been described.[1] Additionally, artificial self-assembled receptors, where their substrate binding sites or cavities are organized by metal templation[2] or by self-association of monomeric ligands,[3] have been proposed to bind target substrates. The self-assembly of a receptor monolayer with a biomolecule such as ATP at the air−water interface has also been reported.[4] As a novel example of self-assembly for optical ion sensing, a K^+ ion-induced self-assembly has been described which consists of pyrene-tethered benzo-15-crown-5, γ-cyclodextrin, and K^+ ion.[5] This ternary complex, formed in water with high selectivity and sensitivity for K^+, can be probed by pyrene dimer emission.[5] Thus, it is expected that the self-assembly of a chromophore-tethered receptor[6] by specific substrate binding offers a novel approach to the sensing of substrates in solution.

Here we report a particularly simple self-assembling system for sensing of anions, with a pyrene-functionalized monoguanidinium receptor **1**. The assembled system was quantitatively analyzed by means of ^{1}H NMR and fluorescence measurements. Although little attention has been paid to the structural analysis of such molecular assemblies,[7] determination of stoichiometry and structure is essential for understanding the photophysical origin of fluorescence and for making a rational design of a sensing system to distinguish structurally related substrates. The receptor **1** was found to self-assemble to form a 2:1 (host:guest) complex with high selectivity for biologically relevant pyrophosphate[8] ($P_2O_7^{4-}$, PPi) in MeOH. From the complexation-induced changes in chemical shifts of the pyrenyl protons of **1**, it is concluded that a sandwich-like ground-state pyrene dimer is present in the self-assembly. Formation of the self-assembly results in a remarkable change in the ratio of emission intensities of excimer to monomer due to the pyrenyl moiety of **1**. It should be noted that only when the self-assembly is formed does **1** show a change in fluorescence spectrum. The present system, therefore, shows high selectivity for PPi that can promote formation of the self-assembly. In addition, calibration via ratiometry becomes possible by exploiting both monomer and excimer emissions. For anionic species, such emission ratio sensing has been known in only a few instances,[8b,9a] despite its importance from a practical viewpoint.

1 was synthesized by reacting 1-pyrenemethylamine hydrochloride with 3,5-dimethylpyrazole-1-carboxamidine nitrate in THF. Recrystallization from THF/MeOH gave **1** as nitrate salt.[10]

The effect of anions on the fluorescence spectrum of **1** (8.0×10^{-4} M) was examined in MeOH,[11] and the results are shown in Figure 1. Spectrum a was measured in the absence of anions, where **1** showed a structured emission band at 370−450 nm which was assigned to a pyrene monomer emission.[12] As shown in spectrum b, **1** did not show any obvious spectral change upon addition of $H_2PO_4^-$ as well as other monovalent anions such as $CH_3CO_2^-$, SCN^-, Cl^-, and Br^-. However, in the presence of PPi, a structureless band with an emission maximum at 476 nm appeared, and there was quenching of the monomer emission, as shown in spectrum d. Similarly, a much less effective response was observed upon addition of HPO_4^{2-} (spectrum c). These results suggested that **1** has high selectivity for PPi over various anions ($PPi > HPO_4^{2-} \gg H_2PO_4^-$, $CH_3CO_2^-$, SCN^-, Cl^-, Br^-).

The dependence of fluorescence spectra of **1** in MeOH on the PPi concentration is shown in Figure 2. Increasing the PPi concentration up to approximately 0.5 equiv relative to the host concentration resulted in an increase in the intensity of the long-wavelength emission, while the monomer fluorescence intensity decreased. The reverse change was observed upon further addition of PPi. As shown in inset a in Figure 2, the dependence of the intensity ratio at 476 nm to that at 376 nm (I_{476}/I_{376}) on the concentration of PPi strongly suggested that two kinds of complexes are formed, both a 2:1 and a 1:1 host-to-guest complex. It seems likely that **1** forms a 2:1 complex with PPi, as shown in Scheme 1, when considering that guanidinium groups are known to bind phosphates via two-point hydrogen bonding.[14] In fact, the data in inset a of Figure 2 are fitted well with an equation assuming that the fluorescence change is only induced by the formation of a 2:1 complex between **1** and PPi,[15] and the association constants of 2:1 and 1:1 complexes are calculated as 1.2×10^8 M^{-2} (β_{21}) and 1.0×10^4 M^{-1} (K_{11}), respectively.

^{1}H NMR binding studies of **1** with PPi support the above conclusion that both 2:1 and 1:1 complexes are present. Figure

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(10) Compound **1** was characterized by elemental analysis and ^{1}H and ^{13}C NMR spectra.

(11) To reduce the inner filter effect, the front surface emission observation method was employed for fluorescence measurements of solutions containing a high concentration of **1**.

(12) The absorption spectrum of **1** (1.0×10^{-5} M) in MeOH agreed with a simple sum of the spectra of 1-methylpyrene (MePy) and guanidine hydrochloride, and the fluorescence spectrum of **1** (1.0×10^{-6} M) substantially coincided in shape with that of MePy, although the intensity was somewhat weak relative to that of MePy. These results showed that there was no significant interaction between pyrene and guanidinium moieties either in the ground or excited states; i.e., there was neither charge-transfer interaction[13] nor exciplex formation.[9]

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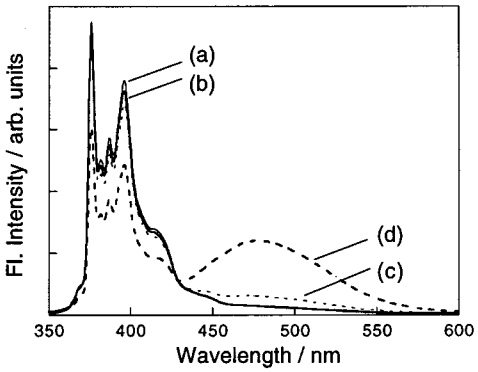


Figure 1. Fluorescence spectra of **1** in non-degassed MeOH (a) in the absence of anions and in the presence of (b) KH_2PO_4, (c) K_2HPO_4, and (d) K_4PPi ($K_4P_2O_7$). 18-Crown-6 (4.0×10^{-3} M) was added to dissolve K^+ salts. [**1**] = 8.0×10^{-4} M. [Anion] = 4.0×10^{-4} M. Excitation wavelength, 312 nm.

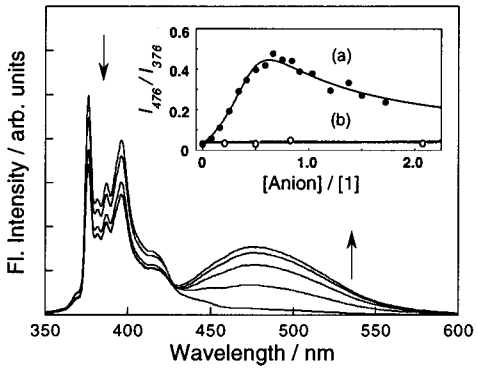
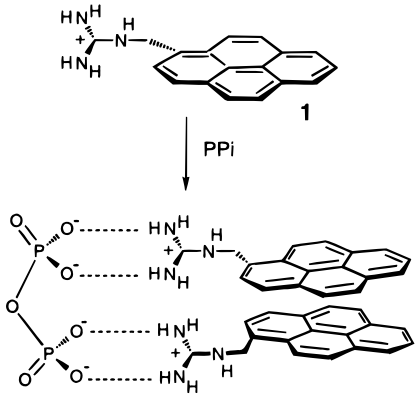


Figure 2. Fluorescence spectra of **1** upon addition of PPi (0, 140, 270, 410, 540 μM) as [K^+[18]crown-6] salt. [**1**] = 8.0×10^{-4} M in non-degassed MeOH. Excitation wavelength, 312 nm. Inset: Dependence of I_{476}/I_{376} on the concentrations of (a) PPi and (b) $H_2PO_4^-$.

Scheme 1



3A shows changes in chemical shifts for the methylene protons of **1** in MeOH-d_4 as a function of PPi or $H_2PO_4^-$ concentrations. As shown in Figure 3A(b), addition of $H_2PO_4^-$ resulted in no obvious changes in chemical shifts of the methylene protons as well as the aromatic protons of **1** (cf. Figure 3B). In contrast, as shown in Figure 3A(a), upfield shifts for the methylene protons

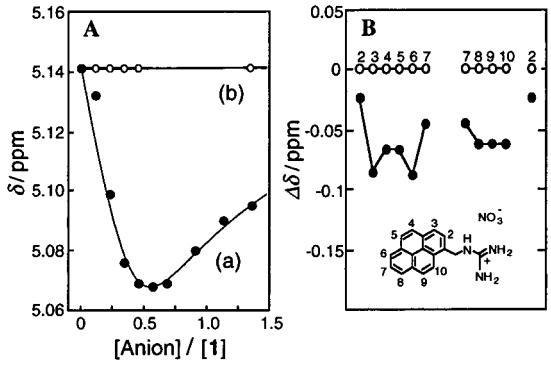


Figure 3. (A) ^{1}H NMR chemical shifts of the methylene protons of **1** in a titration with (a) K_4PPi or (b) KH_2PO_4. [**1**] = 8.0×10^{-4} M in MeOH-d_4 at 293 ± 3 K. [18-Crown-6] = 7.5×10^{-3} M. (B) Changes in chemical shift (in ppm) of the pyrenyl protons of **1** upon addition of (●) K_4PPi or (○) KH_2PO_4 in MeOH-d_4 at 293 ± 3 K. Negative values show high field shifts. [**1**] = 8.0×10^{-4} M. [K_4PPi] = 3.6×10^{-4} M. [KH_2PO_4] = 3.3×10^{-4} M. [18-Crown-6] = 7.5×10^{-3} M.

of **1** were observed until a 0.5 equiv amount of PPi was added, after which these protons showed downfield shifts. Similar changes were also observed for all the pyrenyl protons when **1** was titrated with PPi (cf. Figure 3B). The observed shielding effects can be ascribed to ring current effects,[16] suggesting a complexation-induced aggregation of host molecules. In addition, the shape of the titration curve versus the PPi/**1** ratio, coinciding with that obtained by fluorescent titration studies (cf. Figure 2), suggested that both 2:1 and 1:1 host-to-guest complexes are formed. Indeed, nonlinear fitting showed that the observed shifts can be explained as due to the formation of 2:1 and 1:1 complexes,[15,17] and the stabilities are calculated as 9.8×10^7 M^{-2} (β_{21}) and 1.3×10^4 M^{-1} (K_{11}), respectively. The binding constants obtained by the two different methods are consistent with each other. Based on these results, it is concluded that the fluorescence response results from the formation of 2:1 complexes with PPi; i.e., the structureless broad band observed at 476 nm can be assigned to the emission of intramolecular excimer in the self-assembly of **1**, where PPi works as a spacer linking two host molecules. It is of interest to note that, in the self-assembly, a sandwich-like ground-state pyrene dimer, such as found in *meso*-2,4-di(2-pyrenyl)pentane[16b] and 1,*n*-bis(2-pyrenylcarboxy)alkanes with $n = 3-8$,[16a] is present, as schematically illustrated in Scheme 1, since the shieldings affecting all pyrenyl protons were observed as shown in Figure 3B. For partial overlap dimers, the two pyrenyls should exert a shielding on the partial aromatic H atoms, as has been discussed for 1,3-di(1-pyrenyl)propane.[16a]

In summary, we have shown that the complexation-induced self-assembly of **1** can be utilized for sensing of PPi. Although the stability of the present self-assembling system should be improved, the fluorescent signaling is strikingly selective for PPi, which can act as a template of the self-assembly.

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(15) We assumed the system contained both 2:1 (H_2G) and 1:1 (HG) complexes between **1** (H) and PPi (G), where the binding constants were defined as follows: $\beta_{21} = K_{11}K_{21}$, $K_{11} = [HG]/[H][G]$, $K_{21} = [H_2G]/[H][HG]$. Assuming that the fluorescence change was only induced by the formation of a 2:1 complex between **1** and PPi, the intensity ratio (I_{476}/I_{376}) could be expressed by the following equation: $I_{476}/I_{376} = \{\Phi_{\text{free }476}[H]_0 + (\Phi_{\text{complex }476} - 2\Phi_{\text{free }476})[H_2G]\}/\{\Phi_{\text{free }376}[H]_0 + (\Phi_{\text{complex }376} - 2\Phi_{\text{free }376})[H_2G]\}$, where $[H]_0$ is the initial concentration of **1**, $\Phi_{\text{free }476}$ and $\Phi_{\text{free }376}$ are the fluorescence quantum yields for **1** at 476 and 376 nm, respectively, and $\Phi_{\text{complex }476}$ and $\Phi_{\text{complex }376}$ are those for the 2:1 complex at 476 and 376 nm, respectively.

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