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Supramolecular Probe for Bicarbonate Exhibiting Anomalous Pyrene Fluorescence in Aqueous Media

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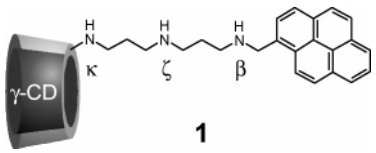
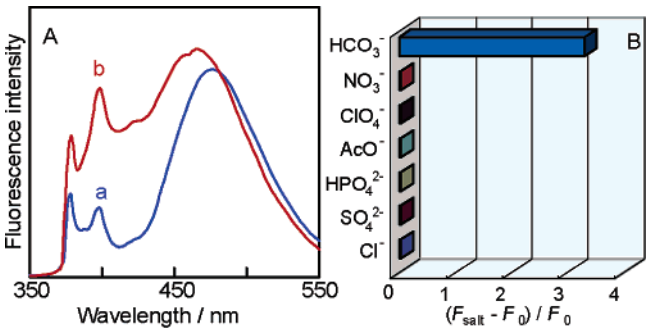
In the last two decades, noteworthy developments in supramolecular chemistry have resulted in molecular assemblies capable of recognizing both neutral and ionic species.[1] Whereas the recognition of ionic species in organic media is well documented, that in aqueous media remains a challenging task because the highly polar nature of an aqueous solution weakens the driving forces for capturing ionic species, except metal coordination. Several excellent metal-free receptors for ionic species that function well in aqueous media have appeared.[2] For instance, cationic polyamines are well-established receptors for anionic phosphates and carboxylates.[3] However, the examples of receptors that bind other inorganic anions in aqueous media are limited.[4] In particular, studies of the recognition and sensing of bicarbonate (HCO_3^-) in aqueous media are few and far between,[5] despite the fact that HCO_3^- is a physiologically important anion that plays vital roles in not only maintaining the pH of biological fluids but also signal transduction in intracellular events.[6] In this communication, we report a novel fluorescent receptor for HCO_3^- based on a cyclodextrin (CD) dimer. Our approach involves the complexation of HCO_3^- with an association dimer of the γ-CD derivative (**1**) in which a triamine linker connects the pyrene residue to the γ-CD. The association dimer formed from monocationic **1** ($\mathbf{1}_2^{2+}$) at pH 7−9 is an excellent receptor for HCO_3^-, emitting anomalous pyrene fluorescence that is not induced by other anions.

Chart 1. Structure of **1**



1

The fluorescence spectra of **1** in borate buffer (pH 8.6) are shown in Figure 1A. **1** alone exhibited typical pyrene fluorescence around 370−400 nm together with strong excimer-like fluorescence centering at 475 nm (spectra a). The latter fluorescence resulted from the formation of an association dimer, as observed in previously reported pyrene-appended γ-CDs.[7] When $NaHCO_3$ was added, a new fluorescence band appeared around 390−460 nm (spectra b). The changes in fluorescence intensity at 425 nm in the presence of several anions are shown in Figure 1B. Of note is that, except for HCO_3^-, none of the anions investigated induced the new fluorescence band. The buffer component $H_2BO_3^-$ did not affect the fluorescence of **1**, as confirmed by comparison of the fluorescence of **1** in borate buffer with that in an unbuffered solution at the same pH. As a result, we conclude that the new fluorescence band is exclusively induced by HCO_3^-. Although the triamine linker of **1** may function as a tridentate ligand for transition metal cations, Zn^{2+} (used as sulfate) alone or in the presence of 10 mM HCO_3^- had no effect on the fluorescence of **1**. Moreover, the presence of a large excess of KCl or NaCl alone or with HCO_3^- had no effect



F_0: Fluorescence intensity at 425 nm of **1** alone
F_{salt}: Fluorescence intensity at 425 nm in the presence of 10 mM salt

Figure 1. (A) Fluorescence spectra of **1** (3.0×10^{-5} M) alone (blue) and in the presence of 10 mM $NaHCO_3$ (red) in borate buffer (pH 8.6). (B) Fluorescence intensity changes of **1** (3.0×10^{-5} M) induced by several anions (10 mM) at pH 8.6.

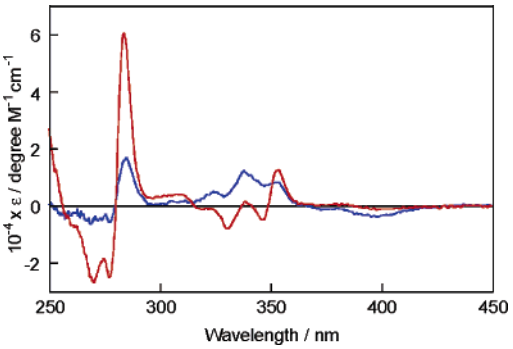


Figure 2. Circular dichroism spectra of **1** alone (3.0×10^{-5} M, blue) or in the presence of HCO_3^- (10 mM, red) in borate buffer (pH 8.6).

on the fluorescence of **1**, although either of the two salts at high concentrations (>0.5 M) slightly increased the total fluorescence of **1**, presumably due to the ionic strength effect. This indicates that the countercation effect is limited. The high selectivity of **1** for HCO_3^- was further examined in tolerance experiments; none of the anions investigated (10 mM) altered the fluorescence of **1** in the presence of HCO_3^-. This indicates that other anions did not bind to **1**. In terms of sensitivity, **1** could detect 1 mM HCO_3^- in water, when the LOD (limit of detection) is defined as $(F_{salt} - F_0)/F_0 > 0.3$ at 425 nm.

The potential of the association dimer of **1** as a fluorescent receptor for HCO_3^- is also supported by circular dichroism (cd) experiments. Figure 2 shows the cd spectra of **1** in borate buffer (pH 8.6). **1** alone showed weak positive cd bands due to the ^1B_b (250−300 nm) and ^1L_a (300−350 nm) transitions of pyrene, whereas the addition of HCO_3^- changed the cd spectrum drastically, increasing the intensity of the ^1B_b band and splitting both the ^1B_b and ^1L_a bands. The large change in the cd spectrum of **1** was observed only in the pH range of 7−10 (Figure S12). As H_2CO_3

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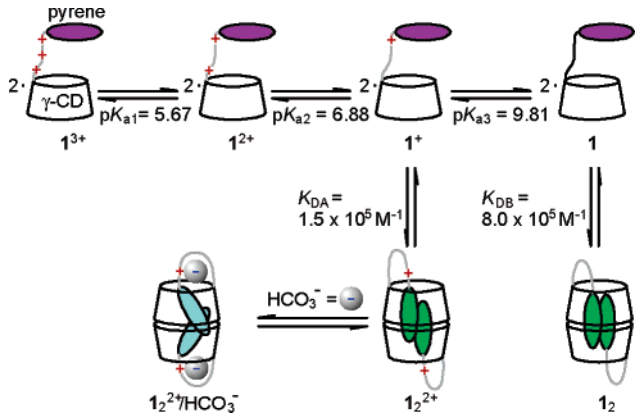

Figure 3. Proposed association behavior between **1** and HCO_3^-.

and HCO_3^- have pK_a values of 6.4 and 10.4, respectively, H_2CO_3 and CO_3^{2-} are not responsible for the large change in the cd spectrum of **1**. A similar dependence on pH was observed for the fluorescence spectra. Together, the results strongly indicate that the association dimer of **1** is an excellent fluorescent receptor for HCO_3^- in water.

The monomer of **1** is not responsible for the anomalous fluorescence because, under diluted conditions ([**1**] = 3×10^{-7} M, pH 8.6) where **1** existed predominantly as a monomer, no appreciable spectral change was observed on adding HCO_3^-, except for a 1.5-fold increase in the monomer fluorescence intensity.[8] Thus, the formation of the association dimer is critical to the anomalous fluorescence induced by HCO_3^-. Compound **1** has pK_{a1} = 5.67, pK_{a2} = 6.88, and pK_{a3} = 9.81 and forms two association dimers ($\mathbf{1}_2^{2+}$ between pK_{a2} and pK_{a3}, and $\mathbf{1}_2$ between pK_{a3} and pH 12 where the dissociation of the association dimer occurs). Moreover, the response of **1** to HCO_3^- was observed at pH 8.6. Accordingly, $\mathbf{1}_2^{2+}$ is the association dimer that interacts with HCO_3^-.[9] The formation of $\mathbf{1}_2^{2+}$ forces the triamine linker to form a pseudo azacrown ring with one charged ammonium group (β-NH_2^+) and two neutral amino groups (ζ-NH and κ-NH). The numbers of the charges and hydrogen bonding sites of the triamine linker may be suitable for binding HCO_3^- and not other anions.

With regard to the anomalous fluorescence induced by HCO_3^-, the split cd bands observed in the presence of HCO_3^- are regarded as exciton coupling patterns, indicating that the two pyrene rings of $\mathbf{1}_2^{2+}$ assume a twisted conformation.[10] By contrast, the lack of exciton coupling patterns in the absence of HCO_3^- indicates that the two pyrene rings of $\mathbf{1}_2^{2+}$ assume a completely parallel conformation. Thus, HCO_3^- binding to the triamine linker of $\mathbf{1}_2^{2+}$ changes the conformation of the pyrene rings from parallel to twisted. This conformational change of the two pyrene rings of $\mathbf{1}_2^{2+}$ decreases the overlap of the π surfaces, resulting in the shift of the excimer fluorescence band to the shorter wavelength region. It is noteworthy that *trans*-1,8-bis(1-pyrenyl)naphthalene, in which the two pyrene residues assume an imperfectly stacked conformation, emits fluorescence at 400 and 425 nm.[11] These peak positions are similar to those of the HCO_3^--induced fluorescence band of $\mathbf{1}_2^{2+}$. The HCO_3^--induced conformational change of the pyrene residues, as seen in the cd spectra, was also supported by the UV−visible absorption, fluorescence excitation, and ^{1}H NMR spectra (Figures S10, S11, and S14, respectively).

On the basis of the above discussion, we proposed the association behavior between **1** and HCO_3^-, as illustrated in Figure 3. It should be noted that neither the stoichiometry (1:1 or 1:2) nor the association constant of the $\mathbf{1}_2^{2+}/HCO_3^-$ complex could be determined owing to the complicated equilibrium. Although we showed 1:2 complexation for $\mathbf{1}_2^{2+}/HCO_3^-$, the possibility of 1:1 complexation should not be excluded.

In conclusion, we demonstrated herein that the association dimer of **1** showed excellent characteristics for selective recognition and sensing of HCO_3^-, thereby inducing the new fluorescence band. Our fluorescent receptor is advantageous for determining HCO_3^- in physiological fluids because it exhibits HCO_3^- sensing ability even at pH 7.4, although the stronger fluorescence of the monomer than that at pH 8.6 slightly obscures the HCO_3^--induced anomalous fluorescence.

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Supporting Information Available: Syntheses, spectroscopic analyses of the association dimer formation, pK_a determination, and further results on the effect of HCO_3^- (pdf). This material is available free of charge via the Internet at http://pubs.acs.org.

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(8) The observed increase in monomer fluorescence under diluted conditions implies that $\mathbf{1}^+$ (monomer) may bind HCO_3^-. However, the weak and noisy spectra under these conditions prevented us from determining the binding constant of $\mathbf{1}^+$ with HCO_3^-. None of the anions investigated increased the fluorescence of $\mathbf{1}^+$.

(9) See Supporting Information for complete details of the association dimer formation of **1**.

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