Optical sensing of inorganic anions employing a synthetic receptor and ionic colorimetric dyes

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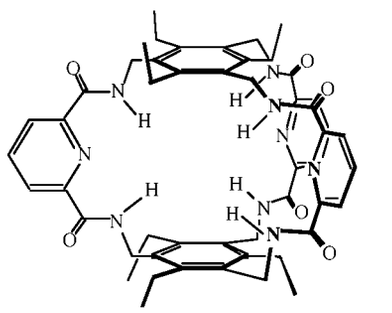
A novel optical sensing methodology for nitrate ions has been developed using an ensemble of a synthetic receptor and colorimetric dyes as indicators. Neutral receptor **1** forms one to one complexes with anionic dyes such as Resorufin or Methyl Red in organic solvents. The equilibrium between receptor and dye is perturbed by added nitrate ion, resulting in a receptor–nitrate complex. The displacement of the indicator by nitrate causes large absorbance changes, because of alternation of the microenvironment of the chromophore. The association constants between receptor **1** and various anions were determined by analyzing the intensity changes.

Introduction

The chemosensing of anions is a rapidly emerging field of chemistry.[1–4] Chemosensing can occur when a chromophore attached to a receptor molecule modulates its luminescence or absorbance upon binding of specific analytes. For example, anthracene based receptors have been reported to give fluorescence modulations upon the binding of anions due to photoinduced electron transfer.[1–4] Cyclodextrins (CD) with attached fluorophores also have been reported to change fluorescence intensity upon the binding of anions in the hydrophobic cavity.[5] In this paper, we demonstrate that even if a synthetic receptor does not have any covalently attached chromophores or fluorophores, anions can be quantified from a modulation of an absorbance or emission of indicators, using a competition assay with colorimetric or fluorescent molecules as indicators. We have previously shown this approach is useful for sensing small molecules such as citrate[6] and inositol trisphosphate.[7] The advantages of this method are: (a) it can be applied to a receptor without covalent attachment of the chromophore, (b) it is more sensitive than NMR or pH titrations, and (c) it is applicable to both aqueous and organic solvents.

We recently reported the design and synthesis of receptor **1** and its selectivity for the recognition of nitrate anion in organic media over phosphate, sulfate, and halides.[8] The receptor is a C_3-symmetric bicyclic cyclophane capable of recognizing anions exclusively through the use of neutral hydrogen bond donors (Fig. 1). In this paper, we report the optical sensing of nitrate using a competition assay with receptor **1**. As the indicators, Methyl Red (**2**) and resorufin (**3**) were employed (Fig. 1). The indicators bind to the cavity of **1** and are displaced by added nitrate, resulting in a change of the UV/VIS spectroscopy of the indicators.



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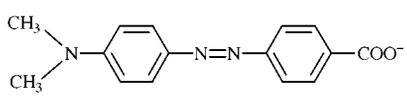

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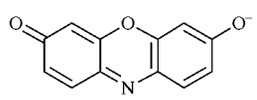

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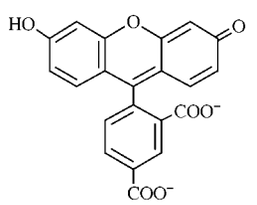

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Fig. 1 Molecular structures of receptor and indicators.

Results and discussion

A, Design of receptor 1

Modeling of receptor **1** suggests little flexibility and a cavity size corresponding to that complementary to nitrate. Further, we predicted that **1** would have a higher affinity for C_3-symmetric anions such as nitrate because the receptor itself possesses C_3-symmetry. X-Ray structural analysis of several **1**–anion complexes revealed that this receptor recognizes anions using neutral hydrogen bond donors. The hydrogen bond donors are from the amide hydrogens that connect the 2,6-diacylpyridine linker to the 1,3,5-tris(aminomethyl)-2,4,6-triethylbenzene spacers. These six hydrogens face into the interior of the cavity.

B, Synthesis of receptor 1

1,3,5-Tris(aminomethyl)-2,4,6-triethylbenzene was synthesized according to a reported procedure.[9] The reaction of 1,3,5-tris(aminomethyl)-2,4,6-triethylbenzene with 1.5 equivalents of pyridine-2,6-dicarbonyl dichloride in the presence of triethylamine gave a crude solid of receptor **1**. The purification proceeded by silica gel chromatography giving **1** as a white powder in 40% yield.

C, Binding behavior of indicators to receptor 1

In order to create a sensitive sensing strategy, the indicators must give a large absorption change upon addition of receptor **1**, since the subsequent addition of anions must lead to a large

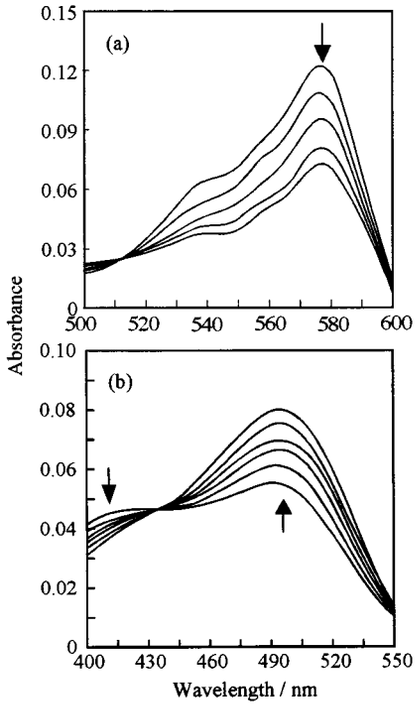
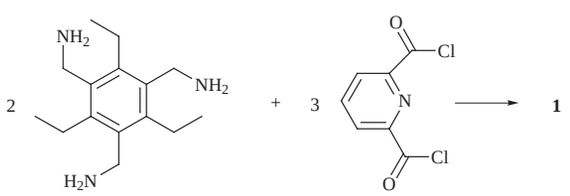


Fig. 2 UV/VIS absorption spectra of (a) Resorufin (**3**) and (b) Methyl Red (**2**) upon addition of receptor **1** in 50% methanol–50% CH_2Cl_2 (v/v). [Indicator] = 2 μM, [**1**] = 0–20 mM.



change in the opposite direction. Fig. 2 shows the absorbance spectra of **2** and **3** upon addition of receptor **1** in 50% MeOH–50% CH_2Cl_2. Isosbestic points at 513 nm for the ensemble of **3**–**1** and 492 nm for **2**–**1** were observed. This suggests 1 : 1 receptor–indicator complexes. The spectra did not change with the addition of neutral Methyl Red (protonated carboxylate), which shows that negative charges are required to form a stable complex. Addition of 5-carboxyfluorescein (**4**), the indicator we have used in several of our previous studies,[6,7] resulted in a minimal absorption change, therefore we did not further pursue this indicator. Using the Benesi–Hildebrand method,[10] the association constants (K_a) for **3**–**1** and **2**–**1** were found to be 600 M^{-1} and 1200 M^{-1} respectively in 50% MeOH–50% CH_2Cl_2. In 75% acetonitrile–25% CH_2Cl_2, K_a values for **3**–**1** and **2**–**1** were found to be 800 M^{-1} and 2000 M^{-1} respectively.

D, Selectivity for anions. A UV/VIS competition assay

To sense nitrate, we added an aliquot of a solution of **1** to nitrate ions mixed with indicator **3** in 50% MeOH–50% CH_2Cl_2.[11] We monitored the competition of nitrate and the indicator for binding to the receptor as a function of different concentrations of the receptor. The indicator is increasingly bound as **1** is added, but the amount of nitrate in solution modulates the extent to which the indicator is bound. Fig. 3 shows the results of six different experiments, each with a different concentration of nitrate and the same concentration of Resorufin (**3**). In the presence of 40 mM nitrate, the absorbance spectrum of **3** was not affected by addition of **1** because the nitrate prohibited the binding of **3** to **1**. This means that a 40 mM concentration is enough to inhibit formation of a **3**–**1** complex. Similar responses in absorption for NaBr and $NaClO_4$ were generated.

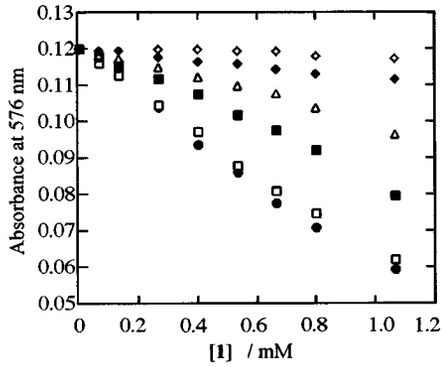


Fig. 3 Change in the intensity of absorption of Resorufin upon addition of **1** in the presence of sodium nitrate in 50% methanol–50% CH_2Cl_2 (v/v). [$NaNO_3$] = 0 (●); 1 (□); 4 (■); 10 (△); 20 (◆); 40 (◇) mM.

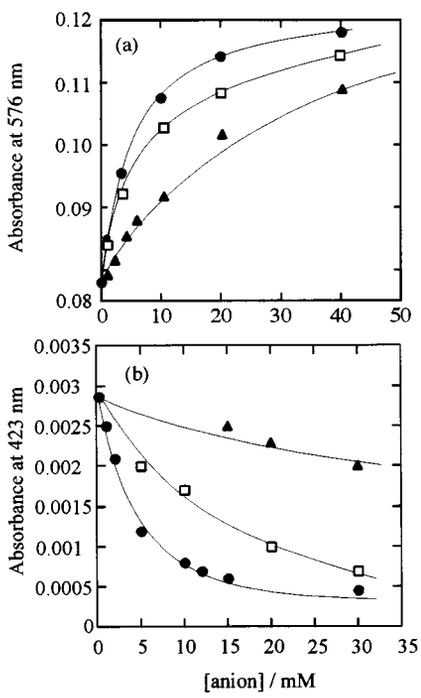


Fig. 4 Effect of addition of ions on absorption spectra of (a) **1**–**3** complex in 50% methanol–50% CH_2Cl_2; (b) **1**–**2** complex in 25% CH_3CN–75% CH_2Cl_2. (●), NO_3^-; (□), Br^-; (▲), ClO_4^-. Counter cations for these anions are (a) sodium, (b) tetrabutylammonium. [Indicator] = 2 μM.

The competitive inhibition given by the anions against the formation of a **3**–**1** complex can be used to quantitate the association constants for the anions. The absorbance changes for a 0.67 mM solution of **1** were plotted as a function of concentration of nitrate, bromide, and perchlorate [Fig. 4(a)].

We also investigated the binding behavior of anions in another solvent, 75% CH_3CN–25% CH_2Cl_2. In this solvent, higher binding constants and anion selectivity were anticipated due to increased hydrogen bond strength. When the system was changed from 50% MeOH–50% CH_2Cl_2 to 75% CH_3CN–25% CH_2Cl_2, the spectroscopic response of **3** was significantly decreased upon the binding to receptor **1**. Therefore, instead of Resorufin (**3**), Methyl Red (**2**) was used in this solvent system as the indicator. This dye gave large spectral changes at a λ_{max} of 423 nm upon addition of **1**. Fig. 4(b) shows intensity changes (423 nm) as a function of anion concentrations using Methyl Red as the indicator in 75% CH_3CN–25% CH_2Cl_2. Both graphs show that nitrate is the best anion for competing with the indicator for **1**, hence receptor **1** is most complementary to nitrate, as previously reported.[8]

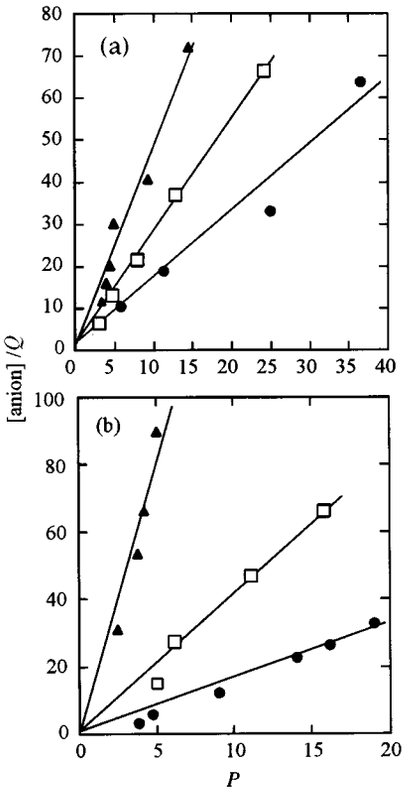


Fig. 5 Linear plots of Fig. 4 according to eqn. (2). (a) **1**–**3** complex in 50% methanol–50% CH_2Cl_2; (b) **1**–**2** complex in 25% CH_3CN–75% CH_2Cl_2. (●), NO_3^-; (□), Br^-; (▲), ClO_4^-. [**1**] = 0.67 mM.

Table 1 Binding constants (K_a)[a] between **1** and anions

	K_a/M^{-1}		
Solvent	NO_3^-	Br^-	ClO_4^-
CH_3OH–CH_2Cl_2 (1 : 1)	380	220	130
CH_3CN–CH_2Cl_2 (3 : 1)	500	190	70

[a] Errors <20%.

E, Binding constants

The binding constants for receptor **1** and the three anions were determined by eqn. (2).[10] The competition equilibrium is given in eqn. (1) and the binding constants of receptor for anions and

$$[\mathbf{1}\text{–indicator}] + [\text{anion}] \rightleftharpoons [\mathbf{1}\text{–anion}] + [\text{indicator}] \quad (1)$$

indicators are defined as $K_a = [\mathbf{1}\text{–anion}]/([\mathbf{1}][\text{anion}])$ and $K_i = [\mathbf{1}\text{–indicator}]/([\mathbf{1}][\text{indicator}])$.

These equations can be combined to give eqn. (2), where

$$[A]_t/P = (K_i/K_a)Q + 1 \quad (2)$$

P and Q are defined as shown in eqns. (3) and (4). A is the

$$Q = (A - A_{com})/(A_{ind} - A) \quad (3)$$

$$P = [R]_t - 1/(Q\,K_i) - [I]_t/(Q + 1) \quad (4)$$

absorbance determined at each different concentration of anion (576 nm for Resorufin, 473 nm for Methyl Red), whereas the A_{com} is the absorbance when **1** is saturated with indicator. A_{ind} is the absorbance of the indicator alone. $[A]_t$, $[R]_t$, $[I]_t$ are total concentrations of anions, receptor, and indicator respectively. K_i was obtained by independent measurements by the Benesi–Hildebrand method (see section C). The association constants of the anions were determined by plotting $[A]_t/P$ against Q (Fig. 5). These constants are listed in Table 1. Receptor **1** has binding constants for nitrate of 380 and 500 M^{-1} in 50% methanol–50% CH_2Cl_2 and 75% CH_3CN–25% CH_2Cl_2 respectively. Selectivity for nitrate is slightly increased in 75% CH_3CN–25% CH_2Cl_2.

A binding constant of 300 M^{-1} for nitrate in 75% CH_3CN–25% CH_2Cl_2 was determined by NMR spectroscopy.[8] Therefore, the binding constant determined here is in good agreement with the value obtained by ^1H NMR. Nitrate was bound to **1** even in a methanol containing solvent, but the selectivity for nitrate was low. In CH_3CN containing solution, the selectivity for nitrate was slightly increased.

In contrast, poor agreement is found between the optical method and ^1H NMR spectroscopy for the binding of bromide to **1** in 75% CH_3CN–25% CH_2Cl_2. *Via* NMR we found an affinity constant of only near 15 M^{-1}, but herein we report an affinity constant of 190 M^{-1}. We predict that the optical method is more accurate due to the ability to follow a larger change in signal than observed in the NMR, but this is still under investigation.

Conclusion

A method for determining the affinity of anions to a receptor using external indicators has been employed. Due to the change in the UV/VIS spectra, this method represents an optical sensing assay. Resorufin and Methyl Red were found to be good indicators to quantify the concentration of anions in the presence of receptor **1**. The spectroscopy of the indicators is quite sensitive to their microenvironment. Added target anions such as nitrate caused dissociation of the indicators from the receptor, resulting in a change of the UV/VIS spectra. Monitoring the intensity in absorbance gave binding constants for the anions with the receptor. The results reported herein, taken with those we have published for citrate and inositol trisphosphate,[6,7] lead us to conclude that the use of a competitive assay employing dyes and synthetic hosts is quite general and useful for the quantitative and sensitive sensing of anions.

Experimental

A, General

5-Carboxyfluorescein and Resorufin were purchased from Molecular Probes Corp. Other compounds were purchased from Aldrich Chemical Corp. and used without purification. The synthesis of 1,3,5-tris(aminomethyl)-2,4,6-triethylbenzene followed a literature method.[9] The sodium salt of Methyl Red was synthesized by the addition of 1 equivalent NaOH in aqueous solution. The tetrabutylammonium salt of Methyl Red was synthesized by the addition of 1 equivalent tetrabutylammonium hydroxide in aqueous solution. These solutions were stirred for 1 h and the water removed by lyophilization.

B, Synthesis of 2,16,18,32,45,47-hexaethyl-5,13,21,29,34,42,44,46,48-nonaazaheptacyclo[15.15.11.1^{3,31}.1^{7,11}.1^{15,19}.1^{23,27}.1^{36,40}]-octatetraconta-1,3(45),7(48),8,10,15(47),16,18,23(46),26,31,36,37,39-pentadecaene-6,12,22,28,35,41-hexone (1)

1,3,5-Tris(aminomethyl)-2,4,6-triethylbenzene (890 mg, 3.6 mmol) and triethylamine (1.3 ml) were dissolved in dry CH_2Cl_2. Pyridine-2,6-dicarbonyl dichloride (1.1 g, 5.4 mmol) in 50 ml of dry CH_2Cl_2 containing 0.3 ml of triethylamine was added dropwise. The mixture was stirred overnight. The solvent was removed by a rotary evaporation to give a crude solid. Compound **1** was isolated using flash column chromatography (R_f = 0.4) with 1% methanol in ethyl acetate as eluent (yield 40%). ^1H NMR ($CDCl_3$, 300 MHz): δ 8.50 (d, *H*-Ar, 6H), 8.05 (t, H-Ar, 3H), 4.7 (d, NH-C*H*$_2$, 12H), 2.7 (q, C*H*$_2$-CH$_3$, 12H), 1.2 (t, CH$_2$C*H*$_3$, 18H). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 38.6 (Ar-*C*H$_2$-NH), 23.45 (*C*H$_2$CH$_3$), 15.7 (CH$_2$*C*H$_3$), 164.05

(*C*=O), 126.5, 131.14, 138.72, 146.13, 148.96 (Ar); FAB-MS *m*/*z* 892.449 ($C_{51}H_{58}N_9O_6$, calc. 892.451).

C, Competitive assay using a UV/VIS measurement

Determination of indicator binding constants. Solutions of sodium Resorufin and tetrabutylammonium Methyl Red were stored in methanol at a concentration of 50 μM in the refrigerator, and diluted to 2 μM prior to any measurements. A solution of **1** was prepared in CH_2Cl_2 at a concentration of 25 μM. In order to determine the binding constants between **1** and the indicators, aliquots of the receptor were added to a 2 μM solution of Resorufin in 2 ml of 50% methanol–50% CH_2Cl_2, or a 2 μM of solution of Methyl Red in 2 ml of 75% CH_3CN–25% CH_2Cl_2, all at 25 °C.

Determination of anion binding constants. Six solutions (50% methanol–50% CH_2Cl_2, 2 ml each) of $NaNO_3$ (0, 1, 4, 10, 20, 40 mM) were prepared. To create 2 μM solution, Resorufin was added to each sample. Receptor **1** was titrated into each solution. Similar titrations were performed for NaBr and $NaClO_4$ ions. The mixture (anion, indicator, and receptor) was incubated for 10 minutes prior to any UV/VIS measurements. The absorbances at 576 and 423 nm were monitored for Resorufin and Methyl Red respectively to determine the binding constants. In the 75% CH_3CN–25% CH_2Cl_2 system, these same procedures were repeated. However, instead of the sodium salt, the tetrabutylammonium salts were used for each anion (NO_3^-, Br^-, ClO_4^-) to increase the solubility in the organic solvent system.

Acknowledgements

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References and notes

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11 Addition of an aqueous solution of nitrate to a solution of **1**–Resorufin complex resulted in a decrease of the intensity of absorption in 50% MeOH–50% CH_2Cl_2. This change in absorbance was in the opposite direction to that expected, since displacement of the indicator from the receptor should lead to an increase in absorption. Similarly addition of an aqueous solution of nitrate to an ensemble of **1** and Methyl Red resulted in a UV/VIS intensity opposite to that expected. Control experiments were performed in which just the solution of the $NaNO_3$ was titrated into solution of Resorufin, and again there was a decrease in absorbance. Similarly, when just water was titrated into the same solution, a decrease of absorbance was also found. Thus we attributed this change in absorbance to a solvent effect around the indicator. Therefore, we discontinued this procedure of adding anions to the receptor, and instead used the method reported herein.

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