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A calixpyridinium–pyranine complex as a selective anion sensing assembly via the indicator displacement strategy

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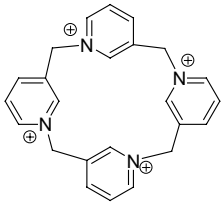
Abstract—The recently reported calixpyridinium tetracation is shown to be very well-suited for anion sensing via the indicator displacement strategy. A very strong binding interaction between the positively charged receptor and the fluorescent pH indicator pyranine (8-hydroxy-1,3,6-pyrenetrisulfonate) requires an anionic guest of comparable negative charge for effective indicator displacement. Thus, selective and sensitive signalling of ATP is achieved in aqueous solutions.

Fluorogenic and chromogenic chemosensors of anions are of considerable interest[1] due to the biological and environmental relevance of anionic species. Considering the structural variety and high solvation energies of potential anionic analytes, the design of selective chemosensors for anions is considerably more challenging than that of chemosensors for metal cations, especially if they are to work in aqueous solutions. However, a number of different approaches[2] produced satisfactory chemosensors working in aqueous media. Indicator displacement strategy proved itself to be very useful in this regard.[3] Many fluorescent or coloured indicators (dyes) form complexes with cationic receptors with concomitant changes in absorption and/or emission, and the original spectral characteristics are regenerated when the dye is displaced with the anionic guest molecule. The strength of this approach is in its broad applicability to virtually unlimited combinations of dyes and receptors. Thus with proper choice of the dyes and receptor, most anionic species can be targeted for sensing.

As an anion receptor, our choice was calixpyridinium tetracation $\mathbf{1}^{4+}$.[4] This macrocyclic compound, which resembles calixarenes to some extent, carries four positive charges due to four quaternized pyridine units. The receptor can be readily synthesized by the oligomerization of 3-bromomethylpyridine.[4] In order to raise the displacement barrier so that selectivity can be achieved, we wanted to form a strong complex with an indicator, which carries a high negative charge at neutral pH. Thus,



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pyranine which is a well-known fluorescent pH indicator (HPTS, 8-hydroxy-1,3,6-pyrenetrisulfonate) appeared to be a good choice. Titration of pyranine fluorescence in the presence and in the absence of of calixpyridinium $\mathbf{1}^{4+}$ showed that the pK_a for the deprotonation of pyranine (10 μM) changes from 7.4 to 6.5 in the presence of 0.25 mM calixpyridinium $\mathbf{1}^{4+}$. Further inspection of the titration data revealed that the dye displacement should have a maximal effect at pH 7.5 which happens to be very close to the physiological pH. In order to evaluate the strength of binding interactions at this pH, we carried out a titration of pyranine (10 μM) with increasing concentrations (0–200 μM) of $\mathbf{1}^{4+}$ (Fig.1) and Bensesi–Hildebrand treatment[5] of the titration data resulted in an association constant of $3.9 \times 10^5\,M^{-1}$ at pH 7.5. Such a strong complex naturally requires anionic species of multiple negative charges for the indicator displacement

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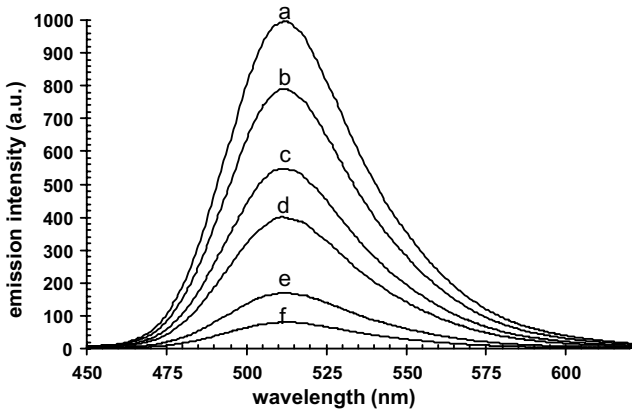
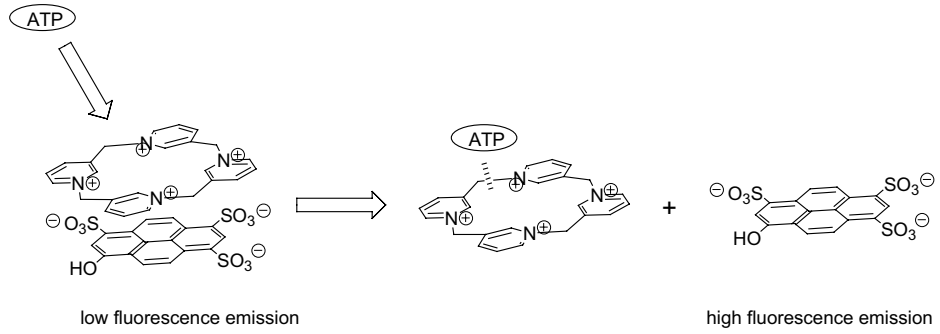


Figure 1. Decrease in the emission intensity of pyranine (10 μM) with the increasing concentration of calixpyridinium receptor **1**. The concentrations are a: 0, b: 10, c: 25, d: 50, e: 100, f: 200 μM. The excitation wavelength was 440 nm.

strategy to work. The obvious target analyte was ATP. ATP recognition and sensing is of prime importance[6] as it is involved in many enzymatic processes.

studied to assess the selectivity of ATP sensing over ADP, AMP and other biologically relevant anionic species. In the titration of the complex with the anionic species, the displacement of pyranine is incomplete, therefore the emission intensity at (520 nm) of the free dye at the same concentration and pH was used for the 100% displacement point. The binding constants were obtained following the Anslyn procedure[7] for competition assays: two parameters Q and P are defined as follows. $Q = (F - F_{com})/(F_{dye} - F)$ and $P = [R] - (1/(QK_{com})) - [D]/(Q + 1)$, where F_{com} and K_{com} are the emission intensity of the complex and the stability (association) constant of the complex, respectively; and $[D]$ is the total concentration of the dye. R is the total receptor concentration and F is the emission intensity. As expected the ATP binding constant to the receptor is nearly an order of magnitude larger than that of ADP (Table 1). The dissociation free energy is a remarkable 6.1 Kcal/mol for the ATP-$\mathbf{1}^{4+}$ complex. It is clear that multiple complementary charges are highly effective as recognition elements and when coupled to the microenvironment sensitive pyranine fluorescence, a satisfactory chemosensor system for ATP has been obtained. The



The displacement assays were carried out in pH 7.5 MOPS buffer (0.1 M). Addition of ATP up to 1.0 mM resulted in significant displacement of the dye with a concomitant increase in the emission intensity of the pyranine (Fig. 2). Other anionic substrates were also

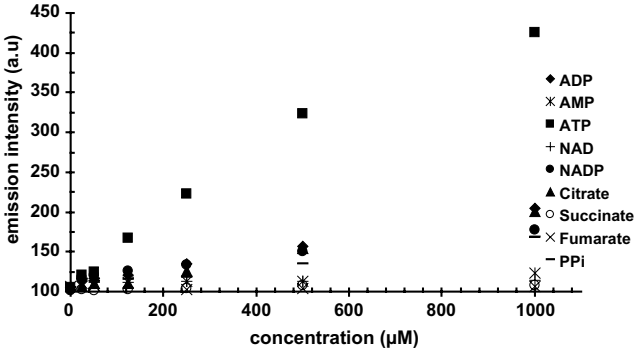


Figure 2. Recovery of pyranine (10 μM) fluorescence emission by the titration of the complex $\mathbf{1}^{4+}$-pyranine with various anionic species in aqueous medium at pH 7.5.

affinities for other anions follow the expected trend based on total negative charges.

In conclusion, we have presented the first example of the use of a calixpyridinium receptor in fluorescence sensing of anionic species. Further modification of the receptor, which is possible by functionalization at the acidic methylene bridges is likely to improve the selectivity and the nature of the signal. Work along these lines is in progress.

Table 1. Affinity constants for anion binding by the receptor $\mathbf{1}^{4+}$ obtained by indicator displacement assays

Anion	K_a (10^4 M^{-1})	K_d (mM)	$-\Delta G$ (kcal/mol, @ 298 K)
ATP	2.87	0.035	6.1
ADP	0.43	0.23	5.0
AMP	0.05	2.0	3.7
NADP	0.23	0.43	4.6
NAD	0.03	3.3	3.4
PPi	0.28	0.36	4.7
Citrate	0.51	0.20	5.1
Succinate	0.03	3.3	3.4
Fumarate	0.04	2.5	3.5

Acknowledgements

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