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Efficient fluorescent ATP-sensing based on coordination chemistry under aqueous neutral conditions

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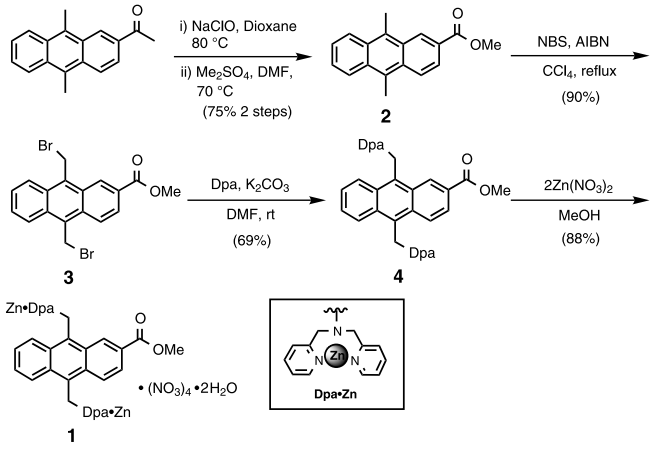
Abstract—A new fluorescent chemosensor consisting of zinc-dipicolylamine appended anthracene for adenosine triphosphate (ATP), which can efficiently act in neutral aqueous solution, was developed.

Chemosensors capable of detecting anionic species of biological importance have been actively developed for last decades.[1] Among anions, adenosine triphosphate (ATP) is known to be the universal energy currency in all of the biological systems, and thus it is a significant target to be conventionally monitored.[2] Though several fluorescent ATP sensors were reported so far, most are polyammonium type of receptors that employ electrostatic, hydrogen bonding, and stacking interactions as main attractive forces. The metal–ligand interaction is another useful interaction of choice for anion recognition event in aqueous medium.[3] In this communication, we describe a new fluorescent chemosensor consisting of zinc-dipicolylamine-appended anthracene, which can efficiently bind and sense ATP based on coordination chemistry under aqueous neutral conditions.

During our research on the artificial small molecules toward protein surfaces, we discovered that a fluorophore-appended dipicolylamine (Dpa) zinc(II) complex is the first artificial receptor for phosphorylated peptides.[4] Based on this finding, we decided to test an anthracene derivative (**1**) bearing two sets of Zn(Dpa) for ATP sensing. Synthesis of **1** is shown in Scheme 1. 2-Acetyl-9,10-dimethylanthracene[5] is converted to the ester **2** followed by the bromination with NBS to give the bis-bromomethyl derivative **3**. The nucleophilic substitution of **3** with 2,2′-dipicolylamine yields the corresponding ligand **4** followed by treatment with 2 equiv. of zinc nitrate to afford the fluorescent chemosensor **1**.[6–8]

Prior to investigation of sensing ability of **1** for ATP, we initially examined its anion selectivity using fluorometric titration experiments in a neutral aqueous solution. Fig. 1 shows the fluorescence change by various anions such as phosphate, acetate, bicarbonate, sulfate, nitrate, chloride, and azide. Upon addition of phosphate, the emission at 460 nm due to the anthracene unit clearly intensified and the change is saturated up to 10 equiv. In contrast, the large amount of azide (more than 100 equiv.) lessened the emission because of its quenching property. The other anions did not cause the fluorescence change except for bicarbonate, by which the fluorescence gradually increased in the 10^{-3} range of



Scheme 1.

Keywords: fluorescent chemosensor; ATP sensor; zinc complex; coordination chemistry.

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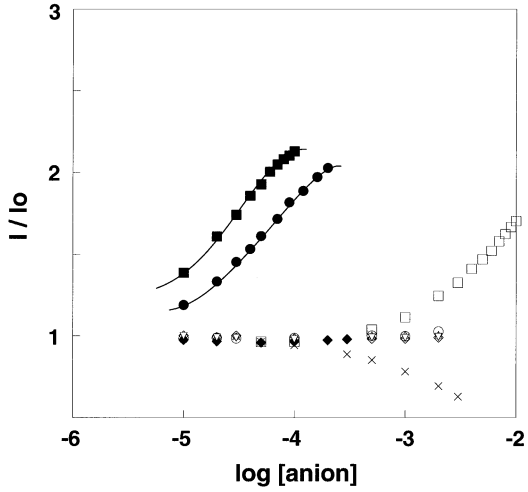

Figure 1. Relative fluorescence emission response of **1** to the anion concentration (log[anion]): phosphate (■), carbonate (□), acetate (○), nitrate (◇), sulfate (△), azide (×), chloride (▽), methylphosphate (●), dimethylphosphate (◆). The spectra were measured in 10 mM HEPES buffer (pH 7.2) at 20°C. $\lambda_{ex}=380$ nm.

concentration. It is clear that the bis-Zn(Dpa)-appended anthracene **1** can operate as a phosphate-selective fluorescent chemosensor among many sorts of anions under the neutral aqueous solution. Among phosphate species, the chemosensor **1** can fluorometrically sense monomethyl phosphate with the slightly weak binding affinity relative to phosphate anion, whereas no fluorescence change occurs by dimethyl phosphate under the same range of concentration. Thus, the order of phosphate>monoalkyl phosphate> dialkyl phosphate is observed in the binding affinity of **1**. A plausible interpretation for the observed fluorescence enhancement could be a conformational rigidification of **1** induced by the cooperative coordination of two sets of Zn(Dpa) to the phosphate species, which can attribute to the increase of fluorescent quantum yield.[9]

On the basis of these results, we subsequently conducted the ATP sensing. Fig. 2 displays a fluorescence spectral change of **1** by addition of ATP. With increasing the ATP concentration, the emission at 460 nm increases to 3-fold in the intensity. The fluorescent titration curve (inset of Fig. 2) shows a typical saturation with 1:1 stoichiometry and gives the binding constants of 2.2×10^6 M^{-1} by the nonlinear curve fitting analysis. Binding constants for ADP, AMP, and cyclic AMP obtained by the similar titration experiments are summarized in Table 1. It is clear that the affinity of **1** for ATP is 10-fold greater than that for ADP and 30-fold greater than that for AMP. Cyclic AMP is not sensed fluorometrically. These results indicate the high selectivity of the chemosensor **1** toward ATP. Since the electrostatic interaction is an important factor to the effective metal–ligand coordination, the observed selectivity (ATP>ADP>AMP) may ascribe to the difference of the quantity of their anionic charges. Thus polyanionic adenosine oligo-phosphate (ADP and ATP)

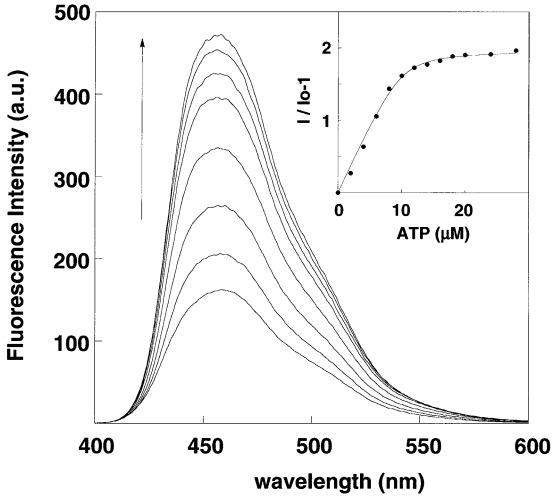

Figure 2. Fluorescence spectral change of **1** (10 μM) upon the addition of ATP: [ATP]=0, 2, 4, 6, 8, 10, 14, 18 μM from the lowest to the top trace. The spectra were measured in 10 mM HEPES buffer (pH 7.2) at 20°C. $\lambda_{ex}=380$ nm. (Inset) Fluorescent titration curve of **1** with ATP.

Table 1. Apparent association constant (M^{-1}) of **1** to AXP determined by fluorescence change

ATP	ADP	AMP	cAMP
2.2×10^6	2.2×10^5	6.6×10^4	–[a]

[a] Since the fluorescence change did not take place, we cannot evaluate the association constant.

would bind preferably with the cationic Zn complex **1** rather than AMP, and most negatively charged ATP shows the highest binding affinity for **1** among the adenosine phosphates. The binding structure of **1** with ATP is evaluated by ^{31}P NMR study; the signals of β- and γ-phosphate of ATP clearly shift to the downfield (2.5 and 2.1 ppm, respectively) upon the addition of 1 equiv. of **1** (Fig. 3).[10] On the other hand, the chemical shift of α-phosphate scarcely changes during addition

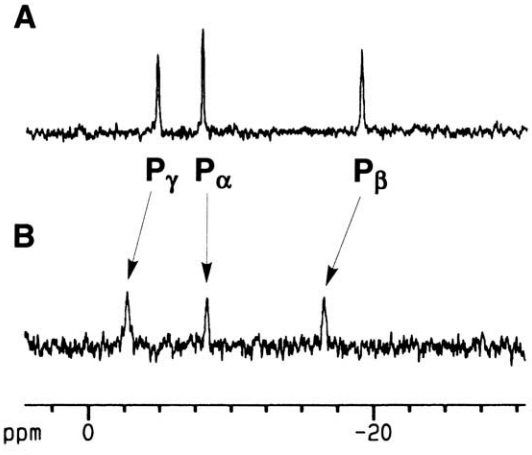

Figure 3. ^{31}P NMR spectra of ATP (A) and the complex of ATP with 1 equiv. of **1** (B). Condition: 0.25 mM ATP, pH 7.2, 50 mM HEPES/D_2O (9/1), 40°C.

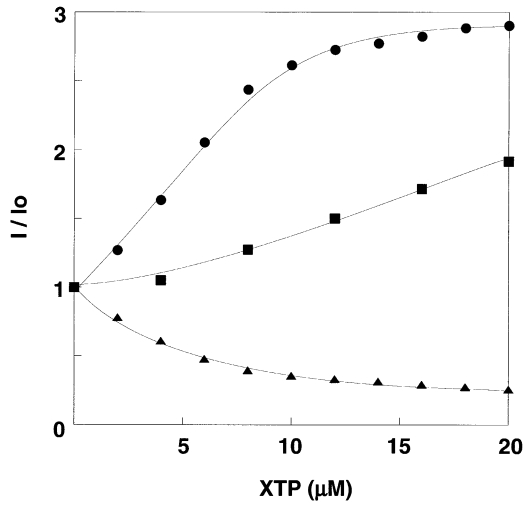

Figure 4. Relative fluorescence emission response of **1** to the nucleoside triphosphate (XTP) concentration: ATP (●), CTP (■), GTP (▲). The spectra were measured in 10 mM HEPES buffer (pH 7.2) at 20°C. $\lambda_{ex}=380$ nm.

of **1**. These data suggest that the chemosensor **1** predominantly binds to the β- and γ-phosphate of ATP but not to the α-phosphate. Based on the results of Fig. 1 that **1** can bind to phosphomonoester but not to phosphodiester, the preferential coordination of a Zn(Dpa) to γ-phosphate is the major driving force. Coordination of another Zn(Dpa) to β-phosphate may be an additional factor.

Fig. 4 displays the titration curves with three kinds of nucleoside triphosphate (ATP, GTP, CTP). The curve for CTP is rather gentle relative to that for ATP. In contrast, the addition of GTP gradually lessens the fluorescence intensity of **1**, probably due to the electron transfer from the guanine group.[11] These indicate that the chemosensor **1** has good selectivity among the nucleoside triphosphates in fluorescence sensing.

In conclusion, we developed a novel fluorescent chemosensor for ATP that can operate under aqueous neutral conditions with the pronounced emission intensity change. Clearly, the two sets of Zn(Dpa) are a useful binding motif for phosphate unit based on metal–ligand coordination chemistry. Further functionalization at the ester side chain is anticipated to provide a more sophisticated chemosensor toward phosphate species of biological importance. We are now underway along this line.

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6. Spectral data of **4**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.86 (4H, s), 3.88 (4H, s), 4.05 (3H, s), 4.64 (2H, s), 4.70 (2H, s), 7.01–7.07 (4H, m), 7.28 (2H, d, $J=7.6$ Hz), 7.40 (2H, d, $J=7.6$ Hz), 7.45–7.55 (6H, m), 7.95 (1H, dd, $J=1.6$, 9.6 Hz), 8.40–8.48 (7H, m), 9.45 (1H, d, $J=1.2$ Hz). FAB-MS; *m/e* $C_{42}H_{38}N_6O_2$ found 658 $[M+H]^+$. Spectral data of **1**: FAB-MS; *m/e* $C_{42}H_{38}N_6O_2{\cdot}2Zn{\cdot}3NO_3$ found 976 $[M+H]^+$. Anal calcd for $C_{42}H_{38}N_6O_2{\cdot}2Zn(NO_3)_2{\cdot}2H_2O$: C, 46.99; H, 3.94; N, 13.05. Found: C, 47.23; H, 3.62; N, 13.36.
7. The X-ray crystallographic analysis of an analogue of **1** that does not possess methoxycarbonyl group indicates that both zinc ions have square pyramidal five-coordinate structure with the coordination of nitrate and H_2O. (Ojida, A., Hamachi, I., unpublished result).
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