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A new perylene diimide-based fluorescent chemosensor for selective detection of ATP in aqueous solution

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ABSTRACT

A new perylene diimide (PDI) ligand (1) functionalized with a Zn^{2+} -dipicolylethylenediamine (Zn^{2+} -DPEN) moiety was synthesized and first used as a fluorometric chemosensor to specifically detect the presence of ATP over other phosphate anions in aqueous solution. The solution of 1 upon addition of ATP displayed a remarkable absorption decrease compared with other anions, indicating the selectivity of 1 to ATP. Moreover, among these anions investigated, only ATP showed a considerable enhancement of fluorescent intensity of 1. The fluorescence molar ratio plot combined with the ESI-TOF mass measurement determined that binding stoichiometry between 1 and ATP is 1:1.

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1. Introduction

In recent years, much effort has been devoted to the development of artificial sensitive fluorescent chemosensors capable of selective recognition of biologically fundamental small molecules and anions due to fluorescent technique's sensitivity, speediness, and easy performance.^{1,2} Among many phosphate anions, adenosine triphosphate (ATP) has received considerable attention because of its pivotal role in energy transduction in bioenergetics, such as photosynthesis, enzyme catalysis, and cellular respiration etc.^{3,4} Therefore, the design and construction of versatile fluorescent chemosensors for selective detection of ATP has received a great deal of attention.⁵⁻⁸ Despite significant progress of these chemosenors have been made in this area. however, a majority of them can not detect ATP over other biological phosphate anions.^{1b,7a,c,9} Especially, only few of them have a remarkable fluorescence enhancement to ATP in absolute aqueous solution.^{7b} Consequently, the bioanalytical application of these sensors in aqueous medium is still limited. Thus, the creation of new fluorescent indicators with high efficiency in aqueous medium continues to be a challenging and fascinating field of research.

This work is aimed at the design and construction of a new type of fluorescent sensors **1** based on Zn^{2+} -dipicolylethylenediamine (Zn²⁺–DPEN) complex with the strong light-harvestor perylene diimide (PDI) for highly effective detection of ATP anions. First, PDIs possess a unique combination of chemical stability, redox properties, excited state reactivity, luminescence emission, and excited state lifetime and have been utilized in various optical devices.^{10,11} Second, Zn²⁺–DPEN moiety have been widely used as variable biological molecules and anions recognition reagents.^{7b,c,12} However, to the best of our knowledge, there are no examples on the combination of Zn²⁺–DPEN moiety and PDI segment as fluorescent sensors for selective detection of ATP anions. Quite recently, we described a new PDI (perylene diimide) and DPEN (dipicolylethylenediamine) derivatives as a colorimetric and fluorometric dualchannel sensor to specifically detect the presence of Cu²⁺ over a wide range of other cations.¹⁰ Herein we present the synthesis of the first such PDI functionalized with Zn²⁺-DPEN unit and the spectroscopic investigations of ATP detecting processes in aqueous solution (Scheme 1).



Scheme 1. Synthesis of chemosensor 1.





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2. Results and discussion

2.1. Synthesis of chemosensor 1

Ligand 2 was synthesized according to our previously reported methods.¹⁰ The treatment of ligand **2** with ZnCl₂ afforded the desired sensor **1** in 78% yield in CHCl₃–MeOH. The ¹H NMR spectrum of a mixture of **2** and ZnCl₂ in a 1:2 ratio shows one set of new signals in a simple highly symmetrical pattern, and the signals for the ligand 2 disappears completely (Fig. 1b). These results could approximately indicate the quantitative formation of a single selfassembled architecture.¹³ In addition, the self-assembled structure could be further demonstrated in combination of electrospray ionization time-of-flight (ESI-TOF) mass measurement and elemental analysis. The ESI-TOF mass spectrum of a mixture of 2 and ZnCl₂ in a 1:2 ratio shows the signal at m/z 1076.2 assigned to the species $[Zn_2(2)Cl_3]^+$ with the loss of one Cl^- anion, which is in good consistent with the convergence ratio of 1:2 for the $2/Zn^{2+}$ observed in the ¹H NMR study. Thus, these results strongly suggest that a metal-ligand complex can be exclusively formed through the metal coordination interaction.



Fig. 1. ¹H NMR spectra for **2** with ZnCl₂ at 293 K: (a) the ligand **2** in CDCl₃, and (b) **2**/ZnCl₂=1:2 in D₂O (the ligand **2** is insoluble in D₂O, and the complex **1** is insoluble in CDCl₃).

2.2. Absorption and fluorescence spectra study of chemosensor 1 to ATP

Fig. 2 shows the UV absorption changes of chemosensor **1** upon addition of 20 equiv of a variety of species including ATP, ADP, AMP, CMP, Na₅P₃O₁₀, Na₂HPO₄, Na₃PO₄, Na₄P₂O₇, NaH₂PO₄, Na₂SO₄, and NaCl in aqueous solution at pH 7.4 (0.01 M HEPES). As depicted in Fig. 2, a broad absorption band of chemosensor **1** (10 μ M) from 435 to 580 nm with two peaks at 500, 545 nm were observed. Among the various species, only the addition of ATP showed a remarkable absorption decrease, other biological anions, such as ADP, CMP, and AMP induced unobvious changes in the same condition, which



Fig. 2. Absorption spectra of $1\,(10\,\mu\text{M})$ upon addition of 20 equiv of different species in aqueous solution at pH 7.4 (0.01 M HEPES).

probably attributed to their low affinity with the receptor **1**, other inorganic anions also induced negligible changes. In addition, a relatively small red shift about 10 nm was observed for the addition of ATP. The λ_{max} was shifted from 500 nm to 510 nm, another peak was shifted from 545 nm to 555 nm. Thus, the experimental results demonstrated that chemosensor **1** exhibited a good selectivity toward ATP over the other species in aqueous solution.

The selectivity of **1** to ATP was further investigated by fluorometric detection in H_2O at pH 7.4 (0.01 M HEPES). chemosensor **1** exhibited a relative strong fluorescence emission at 559 nm upon excitation at 500 nm. Upon addition of the same amount of various anions, only ATP displayed a remarkable enhancement of fluorescent intensity of **1**, and no new emission emerged (Fig. 3). Relative to ATP, other anions did not respond distinctly to receptor **1**, although they also induced enhancement of fluorescent intensity of **1**. Besides, a relatively small decreased fluorescence intensity of sensor 1 was observed for the SO_4^{2-} and Cl⁻. Furthermore, we also investigated the selectivity of chemosensor **1** to GTP, CTP, and UTP. Among these anions, CTP lead to a less enhancement than ATP made, GTP and UTP produced a relatively greater increment than ADP and AMP made (Fig. 3).



Fig. 3. Fluorescence spectra of $1 (10 \,\mu\text{M})$ upon addition of 20 equiv of different species in H₂O. Excitation was at 500 nm, and emission was at 559 nm.

The obvious selectivity of **1** toward ATP suggests that **1** has a potential application in sensing ATP in aqueous solution. As shown in Fig. 4, when the ATP concentration increased, the fluorescence maximum at 559 and 600 nm gradually increased in intensity. A turning point appeared at the 1:1 ratio of the ATP and **1** as shown in the fluorescence titration molar ratio plot for the inset of Fig. 4, which undoubted determined that the binding stoichiometry between **1** and ATP is 1:1.¹⁴ This results is in good accordance with the previous studies of sensing of ATP using Zn(II) complex.^{7a,8a,15}



Fig. 4. Fluorescence titration spectra of 1 (10 μ M) in H₂O upon addition of various amounts of ATP (0–10 equiv). Excitation was at 500 nm, and emission was at 559 nm. Inset: molar ratio plot of the titration spectra.

Moreover, the ESI-TOF spectrum of the complex of **1** and ATP in a 1:1 ratio shows the peaks at m/z 1499.6, 1636.4, which correspond to the species $[Zn_2(2) \cdot ATP \cdot Na]^{2+}$ and $[Zn_2(2)Cl_4 \cdot ATP \cdot H_2O]^{3-}$, respectively (Fig. S4).

In all probability, the electrostatic interaction between ATP and **1** is crucial for efficient $Zn^{2+}(1)-O$ (phosphate) binding (Fig. 5).^{7b} In addition, the rigid PDI framework might be not conducive to facilitate $Zn^{2+}-DPEN$ group binding with ADP, AMP, and CMP, because they only have one or two phosphate species while ATP has three. Hence, the observed detecting preference might be attributed to the difference in the length of the phosphate species and the number of the anionic charges.



Fig. 5. Schematic illustration of the sensing of ATP with the chemosensor 1.

3. Conclusion

In conclusion, we have developed a highly sensitive and selective fluorometric sensor for ATP in 100% aqueous solution. Although the detailed mechanism remains to be explained, the results presented here may contribute to the development of new fluorescent indicators with improved properties for biologically fundamental small molecules and anions.

4. Experimental section

4.1. General remarks

¹H NMR spectra were obtained with a Varian Inova-400 (400 MHz) or Bruker AV-400 (400 MHz) spectrometer, while ¹³C NMR spectra were recorded with a Bruker AV-600 (150 MHz). The ESI-TOF mass spectra were recorded with Waters Q-Tof premier instrument. Elemental analysis was performed with a CARLO ERBA1106 instrument. Melting points were determined and are uncorrected. Absorption spectra were obtained with a UV–vis TU-1901 spectrophotometer. Fluorescence spectra were obtained with a Horiba Jobin Yvon Fluoromax-4 spectrophotometer. Unless otherwise noted, all reagents were obtained from commercial suppliers and used without further purification. Unless otherwise indicated, all syntheses and manipulations were carried out under dry N₂ atmosphere. Anhydrous solvents were dried by standard procedures.

4.2. Preparation of chemsensor 1

(*N*-bis-pyridin-2-ylmethylethane-1,2-diamino)perylene-3,4,9,10-tetracarboxdiimide (**2**) (200 mg, 0.24 mmol) in CHCl₃ (20 mL) was added dropwise slowly to a solution of ZnCl₂ (98 mg, 0.72 mmol) in 10 mL of MeOH. The reaction mixture was refluxed for 2 h. The reaction product was separated by filtration and then washed with CHCl₃ and MeOH to afford **1** as a solid (225.7 mg, 85%). Mp: >300 °C; ¹H NMR (400 MHz, D₂O): δ 2.79 (br, 4H), 4.20 (br, 8H), 4.51 (br, 4H), 7.51 (br, 16H), 7.98 (br, 4H), 8.59 (br, 4H) ppm. ¹³C NMR (100 MHz, D₂O): δ 50.4, 60.7, 66.9, 122.3, 123.1, 123.9, 125.4, 128.5, 130.7, 135.4, 136.8, 147.2, 158.4, 160.6, 165.6 ppm. MS (ESI-TOF) *m*/*z*=1076.2 [Zn₂(**2**)Cl₃]⁺. Anal. Calcd for C₅₂H₄₀N₈O₄Cl₄Zn₂: C, 56.09; H, 3.62; N, 10.06; found: C, 55.96; H, 3.79; N, 9.84.

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Supplementary data

Supplementary data related to this article can be found online at doi:10.1016/j.tet.2012.01.028. This data include MOL files and InChiKeys of the most important compounds described in this article.

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