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A Sensitive Colorimetric and Fluorescent Probe Based on a Polythiophene Derivative for the Detection of ATP

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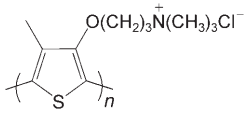
The design and construction of chemo- and biosensors for recognizing biologically important small molecules and anions has received considerable attention in recent years.[1] The anion adenosine triphosphate (ATP) is known to be a key player in bioenergetics in all biological systems, and thus it is important to be able to monitor easily the concentration of ATP in aqueous solution. Although several intriguing strategies have been developed to detect ATP, such as synthetic host–guest receptors,[2] peptides,[3] and RNA aptamers,[4] it remains a challenge to find new approaches that could improve the simplicity, selectivity, and sensitivity of ATP detection.

Recently, a new sensory technology based on conjugated polymers has been developed in view of their signal-amplification effect, and thus is sensitive to very minor perturbations.[5] In particular, water-soluble conjugated polymers provide a unique platform for the development of chemosensors for biologically relevant targets.[6] For example, we and others have successfully prepared interpolymer complexes between water-soluble polythiophene (PT) derivatives and biomacromolecules such as DNA,[7] polypeptides,[8] and polysaccharides.[9] The induced conformational changes of PTs can be followed by absorption, emission, and circular dichroism (CD) spectroscopic methods, which provides the means for implementation of these systems as sensors. Moreover, nucleobases and ATP have been demonstrated to be versatile building blocks for the construction of supramolecular aggregates.[10] Thus, we thought that if ATP can form some supramolecular complexes with conjugated polymers and induce changes in their conformation and mode of aggregation, the perceived structural changes of conjugated polymers would be useful not only for spectroscopic detection but also for rapid visual sensing. Herein, we report a water-soluble cationic polythiophene derivative that displays colorimetric and fluorescent responses to ATP through electrostatic and hydrophobic cooperative interactions. To the best of our knowledge, water-soluble conjugated polymers have not been applied to ATP sensing previously; this is the first observation of a colorimetric probe based on a conjugated polymer for ATP that operates in aqueous solution at physiological pH.

Poly(3-alkoxy-4-methylthiophene) was chosen as a model conjugated polymer to study the viability of this approach because its conformation is sensitive to external stimuli as a result of the presence of sterically demanding side chains (i.e. the 4-methyl group).[11] Therefore, the changes in the π–π^* transitions that arise from conformational alterations in the PT backbone upon the formation of a complex are convenient to monitor by using absorption and fluorescence spectroscopy. Water-soluble PT derivative, **1**, was synthesized as



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reported previously.[9] Titration of **1** with ATP in water at 20 °C was monitored by absorption spectroscopy. As shown in Figure 1, the absorption maximum of **1** in water appears at

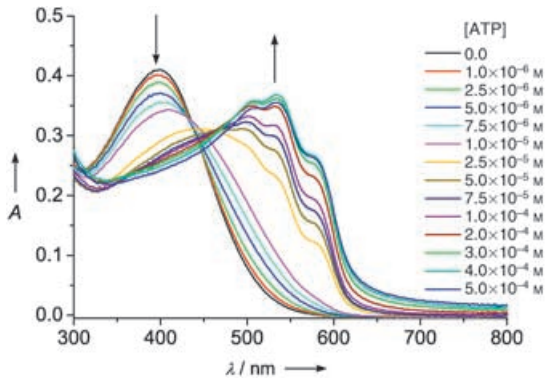

Figure 1. Variation in the absorption spectra of **1** (1.0×10^{-4} M) in water with increasing concentrations of ATP as indicated.

around 400 nm and is associated with a random-coil conformation of the PT derivatives.[11] Upon adding increasing amounts of ATP, the absorption maximum is gradually red-shifted to 538 nm with an observed dramatic color change from yellow to pink-red. This distinct shift and the appearance of two vibronic bands are characteristic of the aggregation of the PT backbone.[11,12] These results indicated promise that water-soluble **1** could be used as a colorimetric sensor for ATP.

To validate the specificity of the PT derivative toward anionic guests, the changes in the absorption spectra of **1** in water upon addition of biologically important anions such as adenosine monophosphate (AMP), adenosine diphosphate (ADP), and uridine triphosphate (UTP), as well as chloride, carboxylate, phosphate, and triphosphate ions (as sodium salts) were studied (see Supporting Information). After addition of an equimolar amount of these anions to aqueous

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solutions of **1**, most of the solutions remained yellow with $\lambda_{max} < 435$ nm except for those that contained ADP and UTP, which gave orange solutions with shifts of the absorption maxima to 435 and 461 nm, respectively. The most remarkable effect was noted, however, upon addition of ATP, which gave a pink-red solution (Figure 2). Note, the dramatic color

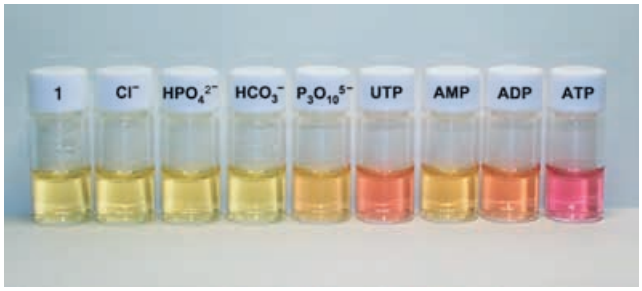

Figure 2. Changes in the color of solutions of **1** (1.0×10^{-4} M) in water induced by the addition of equimolar amounts of various anions.

change of **1** upon addition of ATP provides a very simple means for naked-eye detection of ATP in aqueous solution. Figure 3 shows the dependence of A_{535}/A_{400}, the ratio of the

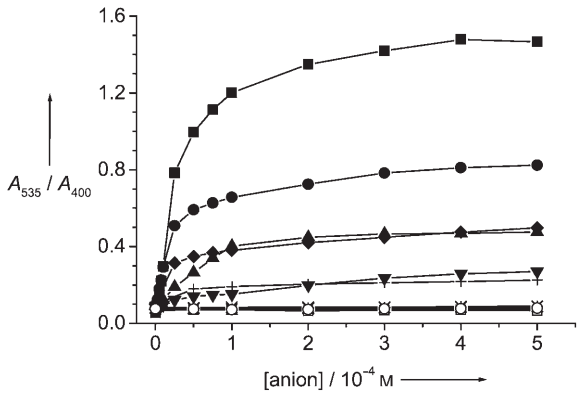

Figure 3. The dependence of the relative absorbance of **1** (1.0×10^{-4} M) at 535 and 400 nm (A_{535}/A_{400}) on the different anions at various concentrations in pure water. ATP (■); UTP (●); $P_3O_{10}^{5-}$ (▲); $P_2O_7^{4-}$ (▼); ADP (◆); AMP (+); Cl^-, $H_2PO_4^-$, HPO_4^{2-}, PO_4^{3-}, HCO_3^-, and CO_3^{2-} ions all have a negligible effect (see lowest line with overlapping unfilled symbols).

absorbance of **1** at 535 nm (π-stacked aggregates) and 400 nm (random coil), on the addition of different amount of various anions. It is clear from these results that the most striking effects are observed for ATP, and hence they confirm that the water-soluble derivative **1** is selectively responsive to ATP.

The analysis of ATP in biological systems is commonly carried out in the presence of alkali and alkali-earth metal cations. Therefore, we also studied the influence of the metal ions Na^+, K^+, Ca^{2+}, and Mg^{2+} (as their chloride salts) on the properties of **1** in solution (see Supporting Information). It was found that the absorption bands of **1** do not show any significant shift upon addition of these cations even when they are present in 20-fold excess. It is known that ATP has an intrinsic metal-binding affinity with respective binding constants of 9554 M^{-1} (Mg^{2+}), 3722 M^{-1} (Ca^{2+}), 13 M^{-1} (Na^+), and 8 M^{-1} (K^+).[13] To study the possibility of analytical interference from these cations, the influence of these cations (20-fold excess with respect to the repeating unit **1**) on the binding of ATP to **1** was also investigated (see Supporting Information). The introduction of monovalent cations (Na^+, K^+) revealed little influence on the binding of ATP with **1**, whereas the presence of Mg^{2+} and Ca^{2+} suppressed to some extent the formation of the supramolecular complex as a result of their stronger binding (larger binding constants) with ATP relative to the monovalent cations. However, this does not interfere with the analytical determination of ATP by optical and visual detection.

To elucidate the mechanism behind the remarkable color change observed for **1** in the presence of ATP, the absorption spectra of **1** in the absence of anionic guest but in the presence of an equimolar amount of AMP, ADP, or ATP, or the presence of phosphate, pyrophosphate, triphosphate, UTP, or ATP were compared in Figure 4. As shown in Figure 4a, the absorption maximum at 400 nm of **1** is red-shifted to 416, 435, and 504 nm upon the stepwise addition of an equimolar amount of AMP, ADP, and ATP, which indicates that the number of negative charges on the anion plays a crucial role in promoting the formation of a supramolecular aggregate of **1**. However, only the presence of inorganic oligoanionic triphosphate ion does not induce a distinct change in the color of the solution of **1** and no supramolecular aggregate is

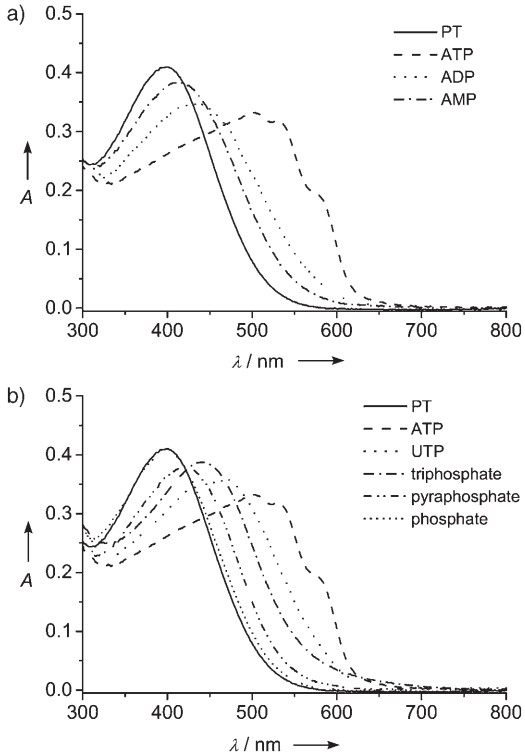

Figure 4. Absorption spectra of **1** (1.0×10^{-4} M) in water in the absence of anionic guest (——) and a) in the presence of an equimolar amount of AMP, ADP, or ATP, or b) in the presence of an equimolar amount of phosphate, pyrophosphate, triphosphate, UTP, or ATP. All spectra were extracted from the titration curves.

formed in this mixture (Figure 4b). It is also important to consider the effect of different nucleotides on the aggregation structure of **1**. As can be seen in Figure 4b, the presence of nucleotides uridine and adenosine is indispensable for accelerating the formation of a π-stacked supramolecular complex. In particular, the presence of the more hydrophobic nucleobase adenine leads to more efficient formation of supramolecular aggregates,[14] and thus displays the most pronounced color change which is noticeable even to the naked eye. Although further studies will be needed to address the molecular mechanism behind this event, some preliminary considerations concerning the origin of the color are discussed next. The electrostatic interaction between the negative charges in the triphosphate group and the positive charge on the ammonium group in **1** promotes planarization of the PT backbone upon addition of increasing amounts of ATP, and above a critical concentration efficient π–π-stacking interaction between **1** backbones is induced by the synergistic effect that arises from the hydrophobic interaction between adenine units (see Supporting Information).[15,16] Simultaneously, these interactions shift the π–π* transition to longer wavelengths and lead to a color change from yellow to pink-red. To further confirm that the sensory response is due to interpolymer π-stacking aggregation, the absorption spectrum of **1** in the aggregated state (solid film cast from aqueous solution of **1**) was also measured (see Supporting Information). The resultant pink-red solid film exhibited characteristic absorption bands that are similar to those observed for **1** in aqueous solution in the presence of ATP, which supports the viewpoint that the addition of ATP promotes the aggregation of **1**.

To emphasize the biological importance and to assess the viability of this approach for the detection of ATP at physiological pH values, we subsequently examined the sensing ability of water-soluble **1** toward ATP in an aqueous solution containing HEPES (2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid) buffer (10 mM, pH 7.4; see Supporting Information). In the absence of anionic guest, the absorption maximum of **1** in HEPES buffered solution shows a slight red shift (12 nm) relative to that observed in unbuffered aqueous solution and appears at 412 nm. This difference may be due to the size-dependent effects of counterions as reported previously.[17] Upon further addition of ATP, the absorption maximum is red-shifted gradually to 476 nm and is accompanied by the appearance of two shoulders at 536 and 583 nm. Unlike the large shift of 138 nm toward the red region as observed in aqueous solution, a relatively small red shift (64 nm) is detected in solution of **1** in HEPES buffer due to the competitive binding of HEPES anions with **1**. Despite this, it does not prevent the analytical determination of ATP with **1** (see Supporting Information) which indicates that **1** can be applied as a satisfactory ATP sensor in aqueous solution at physiological pH values.

It is known that conformational changes and interchain interactions play decisive roles in controlling the emissive properties of conjugated polymers that have been applied in some fluorescence sensory systems.[18] Given the high sensitivity of fluorometric methods relative to absorption spectroscopy, it is expected that fluorometry will extend the detection limit of ATP. The yellow, random-coiled form of **1** is fluorescent and exhibits an emission band around 536 nm upon excitation at 445 nm (see Supporting Information). Upon addition of increasing amounts of ATP, the emission intensity decreases gradually and a slight red shift and broadening of the band is observed which indicates that fluorometric detection of ATP binding is possible. Of the analytes studied here, the fluorescence quenching of **1** (5.0×10^{-6} M in 10 mM HEPES buffer at pH 7.4) is most sensitive to binding with ATP; that is, an 84% decrease in intensity at the emission maximum was observed in the presence of an equimolar amount of ATP, whereas only 12% quenching of the fluorescence was detected upon addition of an equimolar amount of AMP (Figure 5). These results indicate that the quenching of fluorescence is much more effective in the presence of ATP than with the use of AMP or ADP and that the detection limit can be extended to the order of 10^{-8} M.

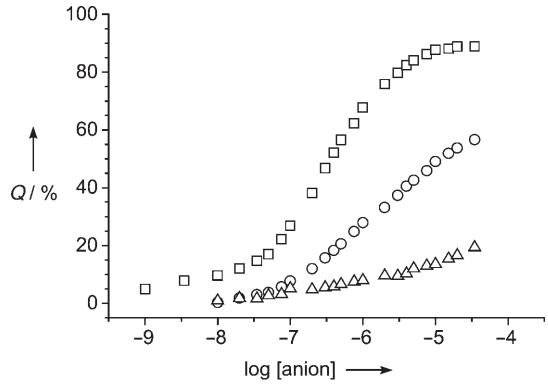

Figure 5. Fluorescence quenching of **1** (5.0×10^{-6} M) by AMP, ADP, and ATP at various concentrations in 10 mM HEPES buffered solution at pH 7.4. The fluorescence quenching $Q=[(I_0-I)/I_0]\times100\%$; I_0 is the fluorescence intensity at 529 nm of a solution of **1** (5.0×10^{-6} M); I is the fluorescence intensity at 529 nm of a solution of **1** (5.0×10^{-6} M) in the presence of different amounts of the analytes ATP (□: $\lambda_{ex}=445$ nm), ADP (○; $\lambda_{ex}=435$ nm), and AMP (△; $\lambda_{ex}=435$ nm).

In conclusion, we have developed a novel colorimetric and fluorescent sensor for ATP that operates in aqueous solution. The present findings will not only extend the application of water-soluble conjugated polymers but also provide a new approach for facile monitoring of ATP based on the different mechanisms discussed above. Therefore, we expect that the present system will be applicable to routine cellular assays after the rational design of the substituted groups (e.g. the introduction of zwitterionic groups or poly(ethylene glycol) groups) in polythiophenes to improve the membrane permeability of the probe, and further research along this line is currently in progress.

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[14] Guanosine triphosphate (GTP), which bears a purine base, can also form a supramolecular complex with **1**, which results in a similar change in color of the solution from yellow to pink-red. However, the presence of ATP can be efficiently distinguished from GTP by a chiroptical method as the two supramolecular complexes with **1** give opposite induced circular dichroism pattern (see Supporting Information).

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