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Fluorescence detection of ATP based on the ATP-mediated aggregation of pyrene-appended boronic acid on a polycation†

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A novel fluorescent sensing system for ATP has been created utilizing the ATP-mediated aggregation process of pyrene-appended boronic acid on a polycation.

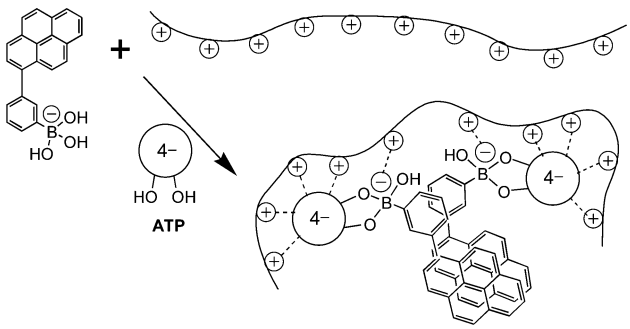
Sensing systems for the detection of biologically important anionic species have been extensively investigated.[1] ATP (adenosine 5′-triphosphate) is the energy source for biological reactions, and the development of a sensitive, selective, and readily applicable detection system is a significant target. Several fluorescent ATP sensors have been reported[2] in which electrostatic, hydrogen bonding, stacking, and metal–ligand interactions are employed as main attractive forces.

Boronic acids are known to readily and reversibly form cyclic boronate esters with diols in aqueous media.[3] For the molecular recognition of sugar derivatives, using the boronate–diol interaction seems to be the most useful way at present.[4] Since ATP possesses a diol unit, the boronate–diol interaction could be a useful tool for the selective molecular recognition of ATP. For the selective detection of ribonucleoside 5′-triphosphate, a fluorescent boronic acid was conjugated with a cationic polyamine by Patterson *et al*.[5] The compound showed high affinity for UTP (uridine 5′-triphosphate) through both the diol–boronate and electrostatic interactions. However, the selectivity seems to be insufficient since the compound also responds to non-charged saccharides (*e.g.* fructose) having high affinity with the boronate group.

We here report a novel fluorescent detection system for ATP based on the excimer formation process using a pyrene-appended boronic acid **1**[6] together with a polycation **2** (Scheme 1).[7] Caruso *et al*.[8] reported that pyrenetetrasulfonate is electrostatically bound by a polycation, and then hydrophobic association between the bound pyrene units results in an excimer emission in the fluorescence spectra. Nishizawa *et al.*[9] demonstrated a related system in which two molecules of pyrene-functionalized guanidinium receptor are assembled onto one molecule of pyrophosphate through electrostatic and hydrogen bonding interactions, and then give an excimer emission. These studies inspired us to design a novel ATP sensing system as illustrated in Scheme 2. Pyrene-appended boronic acid **1** is assembled onto polycation **2** mediated by ATP through the electrostatic interaction between ATP and **2** in addition to the ester formation between the boronate group in **1** and the diol unit in ATP. The aggregate formation brings about a change in the fluorescence emission from monomer to excimer, and thus enables us to fluorometrically detect ATP.

Figure 1 shows the fluorescence emission spectra of **1** in aqueous solutions containing **2** at various ATP concentrations. In the absence of ATP, **1** shows monomer emission[10] in the range of 360–430 nm (bottom spectrum). With increasing ATP concentration, a broad fluorescence band with an emission maximum at 482 nm, which is attributable to an excimer emission, is intensified. The excitation spectrum (see Supplementary Information) monitored at the excimer emission (482 nm) is red-shifted and broadened compared to that monitored at monomer emission (377 nm). This indicates that a ground state association of pyrene units takes place.[11] Therefore, the increase in the excimer emission intensity in Fig. 1 indicates that the amount of aggregated **1** increases with increasing ATP concentration.

To assess the sensing behaviors of this system, a series of fluorescence studies was performed against ATP, ADP, AMP and dATP (deoxyadenosine 5′-triphosphate). Fig. 2 shows the dependence of the intensity ratio at 482 nm to 377 nm (I_{482}/I_{377}) on nucleotide concentration. Large spectral changes were observed upon addition of ATP and ADP, whereas a very small response was observed upon addition of AMP. No response was observed when dATP, which has no diol unit, was added. We also confirmed that non-charged species such as adenosine and fructose induce no response at all (data not shown). This system enabled us to detect the concentrations of ATP in the range from 0.4 to 5 μM. Further increase in ATP concentration resulted in the saturation of the



Scheme 2 ATP-mediated aggregation of **1** on **2**.

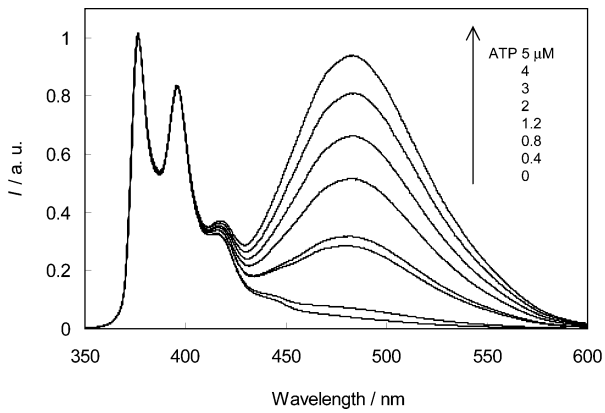
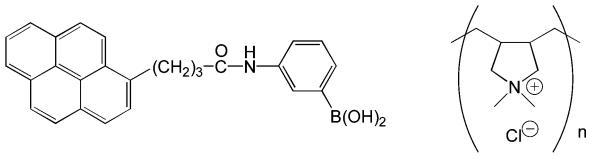


Fig. 1 Fluorescence spectra of **1** ([**1**] = 1 μM) in aqueous polycation solution ([**2**] = 31 μM)[12] buffered with Na_2CO_3 and $NaHCO_3$ (0.5 mM each, pH = 10.2) at various ATP concentrations. The spectra are normalized at 377 nm. Excitation: 342 nm.



1 **2**

Scheme 1 Structures of pyrene-appended boronic acid and polycation.

† Electronic supplementary information (ESI) available: experimental details. See http://www.rsc.org/suppdata/cc/b4/b400644e/

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excimer emission intensity. The detection limit could be decreased to 0.1 μM by employing 5-fold lower concentrations of all the components (see Supplementary Information).

The observed response selectivity seemed to be derived from the difference in the strength of electrostatic interactions between **2** and nucleotides: ATP and ADP (tetravalent and trivalent anions, respectively) are able to bind with **2** by overcoming the competition with excess carbonate ions (divalent) in buffer, however, AMP (divalent anion) loses the competition. To verify the importance of the electrostatic interaction, we changed the buffer concentration. A ten-fold increase in buffer concentration brought about a drastic alteration in the sensing selectivity as shown in Fig. 3. The response against ADP was largely suppressed, while that against ATP was preserved. Obviously, this is because the electrostatic interaction between ADP and **2** is suppressed by the higher concentration of carbonate ion. The response behavior clearly supports the view that ATP mediates the aggregation of **1** on **2** through: 1) electrostatic interaction between the triphosphate group in ATP and cationic units in **2**, and 2) ester formation between the diol unit in ATP and the boronate group in **1**.

We also examined the effect of polycation concentration on the excimer formation behavior (Fig. 4). In this measurement, the concentration of **2** was varied, while those of **1** and ATP were kept constant. In the absence of **2**, the excimer emission was scarcely observed. With increasing concentration of **2**, the excimer emission was intensified; taking the maximal value when [**2**] = 31 μM, then further addition of **2** weakened the excimer emission. This observation demonstrates the indispensable role of the polycation

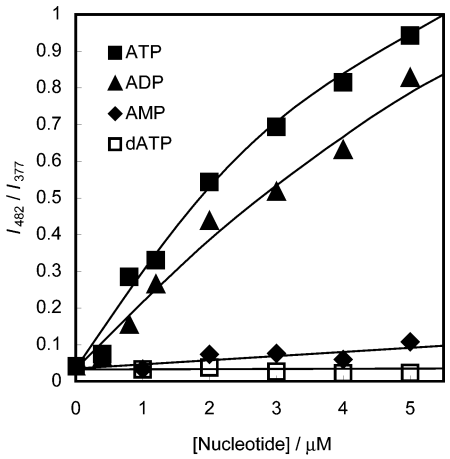

Fig. 2 Dependence of I_{482}/I_{377} on the concentration of nucleotides. Concentrations of **1**, **2**, and buffer are the same as those in Fig. 1.

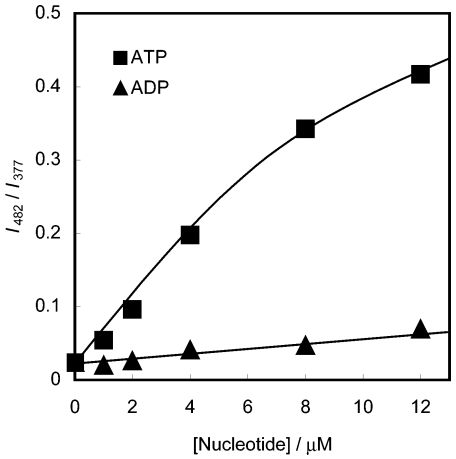

Fig. 3 Dependence of I_{482}/I_{377} on the concentration of nucleotides. The aqueous solutions are buffered with Na_2CO_3 and $NaHCO_3$ (5 mM each, pH = 10.2). Concentrations of **1**, **2** are the same as those in Fig. 1.

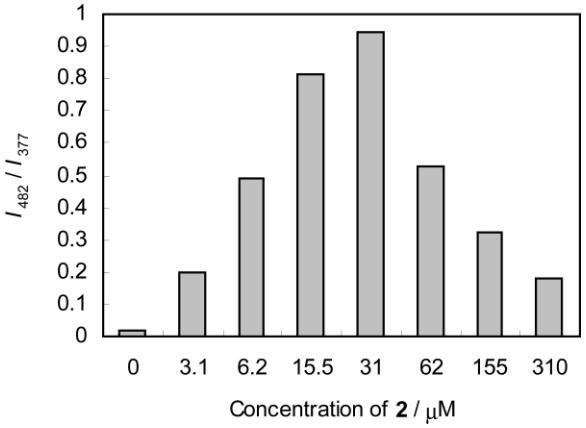

Fig. 4 Dependence of I_{482}/I_{377} on the concentration of polycation: [**1**] = 1 μM, [ATP] = 5 μM, [Na_2CO_3] = [$NaHCO_3$] = 0.5 mM, pH = 10.2.

in the present system. The amount of ATP bound by **2** should increase with adding **2**, so that the amount of **1** that is aggregated onto **2** would also increase. When [**2**] = 31 μM, the concentration of the cationic units exceeds that of the phosphate anionic charges (total 20 μM). Hence, the further addition of **2** would cause the dilution of bound ATP and **1** on the polymer chain, reducing the amount of associated pyrene units and thus the excimer strength.

In conclusion, we have developed a novel fluorescent sensing system for ATP, utilizing the ATP-mediated aggregation process of pyrene-appended boronic acid on a polycation. The present system selectively detects ATP among related compounds with the detection limit of 0.1 μM.[13]

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6 **1** was synthesized by reacting 3-aminophenylboronic acid (Tokyo Kasei) with 1-pyrenebutyric acid *N*-hydroxysuccinimide ester (Aldrich) in DMF at 80 °C for 24 h.

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13 We confirmed that the presence of 5 mM glucose does not affect the sensing behaviour towards ATP.