New aminonaphthalimide imidazolium podands, which worked as luminescence chemosensors for selectively sensing nucleoside polyphosphates through a “turn-on” manner, were prepared for fluorescent imaging of ADP and ATP in living cells.

Recently, a great deal of interest has been focused on the molecular recognition of nucleotide polyphosphates because they play a major role in understanding and evaluating several key biological processes. While considerable efforts have been devoted to developing fluorescent chemosensors for various nucleotides like ATP, GTP, UTP/UDP in the past decades. It is still not easy to find an example in which all of the twelve ribonucleoside polyphosphates were examined to evaluate the selectivity. The distinguishability of ATP from adenosine di-phosphate (ADP) and adenosine mono-phosphate (AMP) is also urgently desired for an improved sensor, since ATP is made from ADP and AMP, and converted back into these precursors in metabolism. In particular, ATP and ADP are basic and important components in bioenergetic conversion processes of living organisms, with their polyphosphate chains being the center for chemical energy storage and transfer. Thus a fluorescent sensor, which can signal the concentration of ADP/ATP from the hindrance of other nucleoside polyphosphates is needed.

As a continuance of our research work on a tripodal receptor with arms comprising benzoimidazolium hydrogen bonding moieties, herein, we report a new approach to the preparation of chemosensors that have the potential to distinguish ADP and ATP from other ribonucleoside polyphosphates, by incorporating 1,8-naphthalimide and imidazolium moieties into the preorganized tripodal receptors. Since amino-naphthalimide is a promising signaling subunit emitting in the green region (λ = 540–550 nm) with high quantum yields (Φs), the chemosensors are also successfully applied to cells imaging for ADP and ATP, respectively.

Compound TIA1 was synthesized by reaction of N-(2-(1H-imidazol-1-yl)ethyl)-4-piperidine-1,8-naphthalimide with 1,3,5-tris(bromomethyl)-2,4-dimethylbenzene in CHCl3, and characterized by EA, 1H NMR and MS (Scheme 1). TIA1 exhibited a 1,8-naphthalimide characteristic absorption band at 425 nm (log ε = 4.40) in acetonitrile solution. The addition of all the twelve ribonucleotides did not cause any significant spectra variation. Free receptor TIA1 exhibited a strong green emission at 548 nm (Fig. 1), assignable to the 1,8-naphthalimide (Φs = 0.04) upon excitation at 465 nm. The addition of ADP, caused fluorescence enhancement, and showed a steady and smooth increase until a plateau was reached (Φs = 0.12). The Hill-plot profile of the titration curve suggested a 1 : 3 stoichiometry of the host–guest complexation species with the association constant (log Kass) calculated as 10.44 (Fig. 2S5). Under the same conditions no significant fluorescence enhancements of TIA1 (20 µM) were observed in the presence of other ribonucleotide di- and triphosphates. These results suggest that TIA1 is a useful probe for the fluorescence detection of ADP with high selectivity and strong binding affinity.

Since the aminonaphthalimide absorbance band did not change upon the addition of increasing amounts of ADP, the enhancement of the fluorescence was attributed to the PET motif. It seemed that the enhancement of the fluorescence was due to a reduced electron-charge density at the imidazole site after the binding with diphosphate of the ADP, which reduced the “push–pull” nature of the ICT excited state of TIA1 (caused by the electron-donating amine and the electron-withdrawing imidazole). The selectivity
of the response with ADP over other adenosine polyphosphates was ascribed the suitable length of the linked group for the diphosphate group. The discrimination of ADP from other nucleotide diphosphates seems to come from the possible hydrogen bonding interactions between the adenine and the receptor.

$^1$H NMR spectra of the receptor TIA1 (1 mM) in the presence of ADP (1 mM) exhibited a significant downfield shift (ca. 0.15 ppm) of the (C–H)$^+$ proton H$_1$ on the imidazolium ring, and a chemical shift of the H$_3$ (0.04 ppm) compared to the free TIA1 in the same experimental conditions, suggesting the formation of (C–H)$^+$ · · · O charged hydrogen bonds between the imidazolium of TIA1 and phosphate groups in ADP (Fig. 2). While most flexible di- or tripod complexes contain rigid planar fluorescent groups an excimer effect may occur after the addition of guest, however, our experiments did not support the possibility of the formation of the corresponding excimer. The small but significant upfield shifts of the signals corresponding to the aromatic protons within the 1,8-naphthalimide (ca. 0.03 ppm), demonstrated the possible π · · · π stacking interaction between the adenine and 1,8-naphthalimide groups. The downfield shifts of the H$_{13}$ (0.02 ppm) and H$_{12}$ from the adenine group during the host–guest interactions should be attributed to the additional hydrogen bonding interaction between the adenine in ADP and TIA1 receptor. It is thought that these complementary interactions are paramount for TIA1 exhibiting selectivity toward ADP over other ribonucleotide diphosphate.

![TIA1 and ADP](image)

**Fig. 2** Partial $^1$H-NMR spectra for (a) TIA1, (b) TIA1 + ADP (3 eq) and (c) pure ADP in d$_6$-DMSO, respectively.

Taking advantage of the off/on fluorescence sensing specifically of ADP by TIA1, this chemosensor was successfully applied to fluorescence imaging studies of intracellular ADP stores in living cells. HeLa cells incubated with TIA1 (10 μM) for 30 min at room temperature showed a weak green intracellular fluorescence, which suggested that TIA1 was cell permeable (Fig. S8). The cells remained viable and no apparent toxicity and side effects were observed throughout the imaging experiments. When cells stained with compound TIA1 were further incubated with ADP (0.4 mM) in phosphate-buffered saline (PBS) for 30 min and washed, a remarkable enhancing of the green fluorescence intensity (Fig. S9) was observed, suggesting the successful application in the ADP stain experiments. When cells stained with compound TIA1 were further incubated with ATP (0.4 mM) under the same experimental conditions, no remarkable fluorescence change was observed, suggesting the possibly distinguishability of ADP from ATP in the living cells.

To further investigate the selectivity of the different ribonucleotide polyphosphates and the effect of the linked groups on the sensitivity of the chemosensor TIA1 toward nucleotide triphosphates, TIA2 was designed and synthesized in multis几步 to enlarge the chain of each arm through the well-known “click” reaction with the aim to recognize the longer adenosine triphosphate (Scheme 2). The absorbance spectrum of TIA2 showed a band about 415 nm (λex = 4.41), assignable to the ICT band of aminonaphthalimide. Free receptor TIA2 exhibited an emission band at 550 nm (Fig. 3), assignable to the 1,8-naphthalimide ($\Phi_i = 0.004$) upon excitation at 395 nm. The addition of ATP caused a substantial fluorescence enhancement, which showed a steady and smooth decrease until a plateau was reached ($\Phi_i = 0.03$). The Hill-plot profile of the titration curve confirmed the presence of 1 : 2 stoichiometry of the TIA2-ATP complexation species with the association constant (log $k_{ass}$) for ATP calculated as 8.75 (Fig. S6). Under the same conditions no significant fluorescence changes of TIA2 were observed in the presence of adenosine polyphosphate. However, by addition of GTP and UTP, a fluorescence enhancement was observed with varying degree. The profile of the fluorescence titration also suggested a 1 : 2 stoichiometry for the complexation species.

![Scheme 2](image)

**Scheme 2** Synthetic procedure for TIA2.

$^1$H NMR titration of TIA2 (1 mM) in a 8 : 2 d$_6$-DMSO/D$_2$O solution upon addition of ribonucleotide polyphosphates (0.4 mM) (excitation at 396 nm).

![Emission spectra of TIA2](image)

**Fig. 3** Emission spectra of TIA2 (20 μM) in aqueous solution upon addition of ribonucleotide polyphosphates (0.4 mM) (excitation at 396 nm).
and the triphosphate groups. The protons of the nucleic bases and the aromatic rings corresponding to the 1,8-naphthalimide all exhibited small but significant upfield shifts, demonstrating the possible stacking interactions between the nucleic base and the aromatic moiety of 1,8-naphthalimide groups, like that reported by Yoon et al. The TIA2 response with ATP in HeLa cells was examined (Fig. 4). HeLa cells incubated with TIA2 (10 μM) for 30 min at room temperature showed a weak green intracellular fluorescence, which suggested that TIA2 was cell permeable. The cells remained viable and no apparent toxicity and side effects were observed throughout the imaging experiments. Fluorescence enhancement was observed when cells stained were further incubated with ATP (0.4 mM) for 30 min, indicating the possible usage in fluorescence images involving nucleoside polyphosphates within living cells.

Fig. 4 Confocal fluorescence images of HeLa cells (λex = 488 nm) incubated with (a) TIA2 (10 μM) and (b) their images after further incubated with ATP (0.4 mM).

In summary, we have reported two new types of chemical sensors TIA1 and TIA2 for ADP and nucleotide triphosphates, respectively. TIA1 exhibits a selective “turn-on” fluorescent property for ADP over other ribonucleotide polyphosphates. TIA2 exhibits a same fluorescent property for ATP, GTP and UTP. These sensors are also successfully applied to cell imaging for the corresponding nucleotide polyphosphates.

Acknowledgements

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Notes and references