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Rhodamine based colorimetric and fluorescent probe for recognition of nucleoside polyphosphates through multi-hydrogen bond

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ABSTRACT

A novelty rhodamine B based chemosensor containing 2-amino-7-methyl-1, 8-naphthyridine moiety was designed and synthesized for colorimetric and fluorescent response on corresponding nucleoside polyphosphates through multi-hydrogen bond interaction in aqueous solution. The supramolecular recognition between the **RBS** and Cytidine-5′-diphosphate disodium hydrate (CDP) was investigated carefully: ^{1}H nuclear magnetic resonance confirmed that the formation of multi-hydrogen bonds between naphthyridine moiety and the nucleoside base group could untie the spiro structure of **RBS**, and the ESI-MS spectra proved the formation of the 1:1 complexation species between **RBS** and CDP. The strong emission response of the **RBS** toward CDP and Adenosine-5′-triphosphate disodium trihydrate (ATP) ensures its application in living cells imaging.

1. Introduction

The design of artificial molecular receptor for the recognition of nucleotide polyphosphates is very important since nucleotide polyphosphates play pivotal roles in various physiological events, such as transport across membranes, **DNA** synthesis, cell signaling and energy processes [1,2]. As the well known paragon, hydrogen bonds between purine and pyrimidine bases which contribute to delicate double helical structure of **DNA**, played important role in the supramolecular recognition of nucleotide polyphosphates. To realize the recognition for the nucleotides, constructing effectively hydrogen bonding platform to act as artificial receptor molecules could be judicious strategy. The 2-amino-1,8-naphthyridine derivatives shown to bind effectively with guanine or cytosine *via* triple hydrogen bonding mode [3–6] in accordance with the different microenvironment, respectively, have been well used in construction of efficient molecular sensors for nucleotide polymorphism.

On the other hand, the detection of nucleotide polyphosphates in real time and *in vivo* has gained particular importance to understand their physiological roles and the application in medicine. [7] Fluorescence measurement of specific biological molecules by artificial chemosensors, as a versatile technique with high sensitivity, rapid response, and easy performance, offering utility not only for in vitro assays but also for *in vivo* imaging studies, is a promising technique for elucidation of biological functions of nucleotide polyphosphates [8]. However, 1,8-naphthyridine itself with low quantum yields (0.01 *vs* **Rh6G**) is difficult to monitor biological events sensitively.

Because of the large molar extinction coefficient and the high fluorescence quantum yield, rhodamine-based dyes have been used as effective dual responsive optical probes *via* chromogenical and fluorogenical signals [9–13]. Herein, by incorporating 2-amino-7-methyl-1,8-naphthyridine (**AMND**) into the rhodamine B group, the colorless and nonfluorescent spirolactam platform could be obtained. Additionally, **AMND** possessing hydrogen bonding sites could form multiple hydrogen bonds with nucleic acid base. Herein, we use rhodamine B spirolactam complex with **AMND** as a new and practical luminescence chemosensor (**RBS**) for the detection of nucleoside polyphosphates in aqueous solution and in living systems with high contrast to background. Since rhodamine B is a promising signaling subunit emitting in the red region with high quantum yields, the chemosensor is also successfully applied to cells imaging for related nucleotide polyphosphates, respectively.

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2. Experiment

2.1. Materials and methods

All the reagents and solvents unless otherwise stated, were purchased from commercial sources and used without further purification. Adenosine-5′-triphosphate disodium trihydrate (**ATP**), Adenosine-5′-diphosphate disodium (**ADP**), Adenosine-5′-monophosphate acid monodydrate (**AMP**), Cytidine-5′-triphosphate disodium dihydrate (**CTP**), Cytidine-5′-diphosphate disodium hydrate (**CDP**), Cytidine-5′-diphosphate acid (**CMP**), Guanosine-5′-triphosphate disodium hydrate (**GTP**), Guanosine-5′-diphosphate disodium (**GDP**), Guanosine-5′-monophosphate disodium (**GMP**), Uridine-5′-triphosphate trisodium (**UTP**), Uridine-5′-diphosphate disodium (**UDP**) and Uridine-5′-monophosphate disodium (**UMP**) were purchased from Bio Basic Inc. (**BBI**) company. ^{1}H NMR spectra were measured on a VARIAN INOVA-400 spectrometer with chemical shifts reported as ppm (in d_6-DMSO, $CDCl_3$ or D_2O, TMS as internal standard). ESI mass spectra were carried out on an HPLC-Q-Tof MS spectrometer. The solution fluorescent spectra were measured on EDINBURGH FS920. Optical absorption spectra were measured on a TU-1900 Uv−Vis spectrophotometer at room temperature. The fluorescence imaging for intracellular nucleoside polyphosphates in HeLa cells was observed under Nikon eclipse TE2000-5 inverted fluorescence microscopy with a 20× objective lens (excited with green light) and Olympus FV1000 laser scanning microscopy with a 40× objective lens (excited with 510 nm).

Single crystals data of compound **RBS** was collected on a BRUKER SMART APEXCCD diffractometer with graphite-monochromated Mo-Kα (λ = 0.71073 Å) using the SMART and SAINT programs. The skeleton non-hydrogen atoms were refined anisotropically and hydrogen atoms within the ligand backbones were fixed geometrically at calculated distances and allowed to ride on the parent non-hydrogen atoms.

Cell imaging was measured on Nikon eclipse TE2000-5 inverted fluorescence microscopy and Olympus FV1000 laser scanning microscopy. HeLa cells were cultured in 1640 supplemented with 10% FCS (Invitrogen). Cells were seeded on 18 mm glass cover slips for fluorescence imaging and in 24-well flat-bottomed plates. After 12 h, HeLa cells were incubated with 10 μM compound **RBS** buffer solution (pH = 6.04) for 30 min at room temperature and then washed with physiological brine three times before incubating with 40 eq nucleoside polyphosphates (pH = 6.04) for another 30 min, and cells were rinsed with physiological brine three times again. The fluorescence imaging of intracellular nucleoside polyphosphates in HeLa cells was observed under Nikon eclipse TE2000-5 inverted fluorescence microscopy with a 20× objective lens (excited with green light) and Olympus FV1000 laser scanning microscopy with a 40× objective lens (excited with 510 nm).

The binding constant was calculated from the fluorescent titration curve according to the equation.

$$\mathrm{Log}((F - F_{\min})/(F_{\max} - F)) = \log k + n \log[c]$$

where F is fluorescent intensity of **RBS** at 583 nm upon the addition of different amount of nucleoside polyphosphates. [c] stands for the concentration of nucleoside polyphosphates.

2.2. Synthesis of 2-amino-7-methyl-1,8-naphthyridine (*AMND*) [14,15]

2,6-Diaminopyridine (3.00 g, 27.5 mmol) was dissolved in 35 mL of H_3PO_4 at 90 °C under Ar atmosphere, 3-ketobutanal dimethyl acetal (3.70 g, 28.2 mmol) was slowly added from a pressure-equalizing addition funnel, and the mixture was heated at 115 °C. Reactions were monitored by TLC. After cooling, neutralized with NH_4OH (15%) and sodium hydroxide until pH = 8, extracted with $CHCl_3$ for several times, while the extraction solution was monitored, washed with brine, dried over anhydrous magnesium sulfate, and concentrated to yield a dark-red solid which was recrystallized from toluene to afford 2.30 g of the products (52%); ^{1}H NMR ($CDCl_3$, ppm): 7.82 (d, 1H, J = 4.0 Hz), 7.80 (d, 1H, J = 4.0 Hz), 7.07 (d, 1H, J = 8.0 Hz), 6.71 (d, 1H, J = 8.4 Hz), 5.08 (s, 2H), 2.69 (s, 3H).

Table 1
Crystal data and structure refinement for **RBS**.

Empirical formula	C_{37} $H_{39.50}$ N_5 $O_{3.25}$
Formula weight	606.24
Crystal size	0.24 × 0.26 × 0.28 mm^3
Temperature (K)	293(2)
Wavelength (Å)	0.71073 Å
Crystal system	Monoclinic
Space group	*C*2/*c*
a(Å)	24.7312(12)
b(Å)	25.7108(12)
c(Å)	20.9872(12)
β(deg)	91.845(4)
V(Å^3)	13,338.0(12)
Z	16
Dc(g cm^{-3})	1.208
μ(mm^{-1})	0.078
F(000)	5160
Theta range for data collection	1.86–25.00 deg.
Reflections collected	34,605
Independent reflections	11,668 (Rint = 0.0470)
Completeness to theta = 25.00	99.2%
Absorption correction	None
Refinement method	Full-matrix least-squares on F^2
Goodness-of-fit on F^2	1.031
Final Rindices [$I > 2\sigma(I)$]	R_1 = 0.0845[a], wR_2 = 0.2178[a]
Rindices (all data)	R_1 = 0.2180[a], wR_2 = 0.2546[a]
Largest peak and hole (e Å^{-3})	0.453 and −0.277
CCDC number	954044

[a] $R_1 = \sum(|F_0| - |.F_c|.)/\sum|.F_0|.$; $wR_2 = [\sum w(|F_0| - |.F_c|.)^2/\sum wF_0^2]^{1/2}$.

2.3. Synthesis of *RBS* [16–18]

To a solution of rhodamine B (1.0 g, 2.1 mmol) in dry 1,2-dichloroethane (15 mL) under stirring phosphorus oxychloride (1.9 mL, 21 mmol) was added dropwise over a period of 10 min, with a pressure-equalizing addition funnel under Ar. After being heated to reflux for 4 h, the solvent and excess amount of phosphorus oxychloride was removed by rotary evaporation to give the corresponding acid chloride, which was dried over high vacuum and used for the next step without further purification. To a solution of the acid chloride in dry acetonitrile (5.0 mL) was added dropwise a solution of **AMND** (0.5 g, 3.15 mmol) and triethylamine (5 mL) in dry acetonitrile (10.0 mL), the resulting mixture was heated to reflux for 5 h. The reaction mixture was then concentrated under vacuum, and the residue was purified by column chromatography (ethyl acetate/dichloromethane 1/1, v/v) to give the crude product, which was further purified by recrystallization from dichloromethane/hexane (25/1, v/v) to give a pure compound **RBS** as a white solid in 22% yield (0.275 g); ^{1}H NMR (d_6-DMSO, ppm): 1.03 (12H, m), 2.57 (3H, s), 3.24–3.34 (8H, m), 6.15 (1H, d, J = 2.6 Hz), 6.17 (1H, d, 2.6, J = 8.8 Hz), 6.40 (4H, m), 7.05 (1H, d, J = 8.8 Hz), 7.32 (1H, d, J = 7.7 Hz), 7.59 (1H, t, 8.8 Hz), 7.64 (1H, t, J = 7.7 Hz), 7.97 (1H, d, J = 7.2 Hz), 8.12 (1H, d, J = 8.0 Hz), 8.25 (1H, d, J = 8.8 Hz), 8.47 (1H, d, J = 8.8 Hz); ^{13}C NMR 168.67, 162.27, 154.63, 154.20, 153.52, 152.57, 148.50, 137.32, 135.75, 133.73, 129.90, 128.12, 128.05, 124.30, 123.41, 121.35, 118.25, 116.04, 107.98, 107.40, 98.01, 66.85, 44.26, 25.60, 12.61; TOF-ESI-MS: Calcd for $[M + 2H]^{2+}$: 292.6552 *m/z*. Found: *m/z* 292.7144; Calcd for $[M + H]^+$: *m/z* 584.3026. Found: *m/z* 584.4264.