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A highly selective fluorescent probe for colorimetric recognition of cyanide anion based on heptamethine cyanine-triphenylamine conjugate

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ABSTRACT

In this work, a novel near-infrared (NIR) dyad T-Cy with a robust C—C bond connection between heptamethine cyanine and triphenylamine was synthesized via a modified Suzuki coupling reaction and applied for CN^- detection. The probe displayed significant changes in its UV–vis absorption and fluorescence spectra together with a naked-eye visible colour change when CN^- was added. The change of spectral signals was linearly proportional to CN^- concentration, and the detection limit was calculated to be 14 nM in CH_3CN and 0.23 μM in CH_3CN/H_2O (9/1, v/v), respectively. The probe exhibited high selectivity and anti-interference towards CN^- in the presence of other common anions (F^-, AcO^-, Br^-, NO_2^-, Cl^-, SO_4^{2-}, I^-, HCO_3^-, CO_3^{2-}, SCN^-) and biothiols (Cys/ Hcy/ GSH). Furthermore, the test strips of the probe were verified to be more convenient to detect CN^-, and the fluorescence imaging demonstrated that the probe has the potential application in the field of biological system.

1. Introduction

Cyanide is highly toxic to human beings and other living organisms. However, cyanide is widely used in industrial production such as mineral extraction, electroplating, polymer production and other industrial activities [1–3]. Extensive use of cyanide can cause serious contamination in environment, especially in water pollution. Exposure to cyanide may lead to a variety of serious health problems including damage to the central nervous system and visual nervous system, disturbance of metabolism, inhibition of respiration and even death [4,5]. According to the World Health Organization (WHO) criteria, the highest level of cyanide anion (CN^-) in drinking water is 1.9 μM and the lethal level is 20 μM [6]. Therefore, it is crucial to detect trace CN^- in environmental and biological systems for the protection of human health and the environment.

Recently, fluorescent CN^- probes have attracted considerable attentions due to their simplicity, rapidity, and real-time analysis compared to conventional methods [7,8]. In general, there are three main types of fluorescent CN^- probes with detection mechanism relating to coordination interaction, hydrogen-bonding interaction and nucleophilic addition reaction [9–15]. Among them, the reactive fluorescent CN^- probe is of especial interest because of its unique selectivity over other anions. In these probes, the double bonds such as C=C, C=N and C=O are usual recognition sites for the nucleophilic addition of CN^-. For example, Li et al. have reported a fluorescent probe using coumarin motif as the chromophore unit and dicyanovinyl group (C=C) as the reactive site, which exhibited good sensitivity and high selectivity to CN^- with visible colour change [16]. Guo et al. have synthesized a coumarin–hemicyanine hybrid dyad and applied to detect CN^- based on the nucleophilic addition of CN^- to C=N in the indolium unit [17]. Besides coumarin and its derivatives, fluorescent probes with phenazine [18], triphenylamine [19], quinolone [20], triarylborane [21], carbazole [22] as fluorogens and carbon–carbon/heteroatom double bonds as acceptors have also been developed for CN^- detection. However, most of these probes have absorption and fluorescence spectra in the short wavelength range in which the background interference is unavoidable.

NIR fluorescent dyes can significantly reduce background interference because their absorption and emission are in the long wavelength regions (> 700 nm). As a typical NIR fluorescent dye, heptamethine cyanine has been widely used in biomarker, cell imaging and fluorescence sensing [23–25]. There are several advantages in heptamethine cyanine-based fluorescent probes: (1) reduced auto-fluorescence and light scattering; (2) high extinction coefficients and strong fluorescence; (3) tunable molecular structure. Kim's group has reported a NIR fluorescent probe based on heptamethine-azo dye for the detection of mitochondrial GSH with a heptamethine unit as the biomarker [26]. Peng et al. have developed a ratiometric NIR fluorescent

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hydrazine probe by modulating the conjugated polymethine π-electron of the probe [27]. Meso-crown ether-bearing heptamethine cyanine dyes have also been synthesized and applied to monitor lithium ion and mercury ion via complexation [28,29]. These heptamethine cyanine derivatives are usually synthesized through the nucleophilic substitution reaction between the central chlorine of cyanine derivatives and functional molecules containing −OH, $-NH_2$, and −SH, and thus allow the two parts connecting with each other by the C–X (X═O, N, S) bond [30,31]. Previous studies have verified that the direct C—C bond connection can effectively enhance the chemical stability and photostability of these dyes [32,33]. But so far, only a few reports have described the heptamethine cyanine dyes having direct C—C linkage owing to the very low activity of central chlorine. For example, with unsubstituted heterocyclic nitrogen in heptamethine cyanine dye, the direct conjugation of phenylcarboxylic acid at the meso- position should be performed by a two-step method including a Suzuki-coupling between Vilsmeier reagent and 4-carboxyphenyboronic acid and followed by a condensation reaction [34]. In contrast, when the heterocyclic nitrogen was substituted, heptamethine cyanine dyes were simply synthesized by a one-step method of modified Suzuki-Miyaura, from which NIR fluorescent probes were derived and applied for imaging in tumour cells [35,36] and modulation of the fluorescence lifetime of quantum dots [37]. According to our knowledge, there is no report on the synthesis and application of the robust C—C bond connected heptamethine cyanine derivative in the field of CN^- detection.

Considering that iminium cation moiety in the heptamethine cyanine dye can be served as an effective recognition site for CN^- [38,39], we herein reported the design and synthesis of novel heptamethine cyanine-triphenylamine conjugate (T-Cy) for the detection of CN^-. The T-Cy probe was synthesized by the improved Suzuki couple reaction. Triphenylamine, as a common fluorophore, is well known for its good electron-donating and hole-transporting capabilities in the field of optical- and electronic- materials [40]. In the present study, triphenylamine was directly linked to the heptamethine cyanine dye through the C—C bond, yielding T-Cy probe. In this probe, indolium and triphenylamine groups can concurrently act as fluorophore and electron-donating units, while indolium salt serves as recognition and electron-withdrawing group. Upon the addition of CN^-, the probe showed a rapidly and significantly spectroscopic response with naked-eye discernable colour change, which was confirmed to be high sensitivity and selectivity. Furthermore, the practical application of the probe on test strips and in living cells was also systematically investigated.

2. Experimental

2.1. Materials and apparatus

All the raw materials and solvents were bought from commercial source and directly utilized without further purification unless otherwise stated. NMR spectra were measured using a Bruker AV-400 NMR spectrometer. MALDI-TOF mass spectra were performed by a Bruker BIFLEXeIII mass spectrometer using a nitrogen laser (337 nm) with a 20 kV of accelerating potential. UV–vis spectra were tested through an Agilent Cary 100 UV–vis spectrometer at ambient temperature. Photoluminescence emission spectra were measured using an Agilent QM 100 luminescence spectrometer. The bioimaging of the probe was investigated by an FV 1000-IX81confocal laser scanning microscope (CLSM, Olympus) with an excitation wavelength of 650 nm.

2.2. Synthesis of heptamethine cyanine-triphenylamine dyad (T-Cy)

Heptamethine cyanine dye (Cy) was synthesized according to the reported literature [39]. The typical synthetic procedure for T-Cy: under an atmosphere of dry nitrogen, Cy (1.22 g, 2.00 mmol), 4-(diphenylamino)phenylboronic acid (1.04 g, 3.60 mmol), $Pd(PPh_3)_4$ (0.23 g, 0.20 mmol) and potassium carbonate (0.28 g, 2.0 mmol) in 50 mL $EtOH/H_2O$ (5:1, v/v) were refluxing for 20 h. After cooling to room temperature, the solvent was evaporated under vacuum. The crude product was purified by silica gel chromatography using ethyl acetate/methanol (5/1, v/v) as an eluent to isolate pure dark-green compound T-Cy (1.20 g, 73%). ^1H NMR (400 MHz, $CDCl_3$) δ (ppm): 8.10–8.06 (d, J = 14.4 Hz, 2H, CH), 7.40–7.33 (m, ArH, 10 H), 7.32–7.31 (d, J = 3.2 Hz, ArH, 2 H), 7.23–7.22 (d, J = 3.2 Hz, ArH, 2 H), 7.21–7.16 (m, ArH, 4 H), 7.14–7.13 (d, J = 4.4 Hz, ArH, 2 H), 7.12–7.11 (d, J = 4.4 Hz, ArH, 2 H), 6.07–6.04 (d, J = 14.4 Hz, 2H, CH), 3.68 (s, 6H, CH_3), 2.66–2.63 (t, 4H, CH_2), 1.93–1.90 (m, 2H, CH_2), 1.70 (s, 12H, CH_3).^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 172.11, 170.03, 147.68, 143.43, 141.10, 140.38, 135.55, 134.83, 133.81, 131.19, 130.79, 130.22, 128.93, 127.47, 126.85, 125.00, 123.85, 122.76, 111.38, 100.87, 48.91, 31.72, 28.02, 24.61, 21.31. MALDI-TOF MS ($C_{50}H_{50}IN_3$) *m/z*: calcd for 819.30, found: 692.33 $[M-I]^+$.

2.3. General spectroscopic procedures

The probe solution (T-Cy) (5 μM) was prepared in CH_3CN or CH_3CN/H_2O (9/1, v/v, HEPES buffer, pH 7.4) solution. Titration experiments were carried out in 10-mm quartz cell at room temperature. The cyanide solution (as the tetrabutylammonium salt) in CH_3CN or CH_3CN/H_2O (9/1, v/v) was added to the probe solution and used for the titration experiments. Other anions solutions were prepared (as the sodium salt or potassium salt) and used for the selectivity and anti-interference experiments.

2.4. Binding stoichiometry

The binding stoichiometry of the probe T-Cy with CN^- was investigated through the Job's titration experiments. For the Job's plot analyses, the total concentration of probe and CN^- was set to 5 μM, while mole fraction of the probe to CN^- was changed. The UV–vis absorbance at 750 nm was monitored for each spectrum.

2.5. Bioimaging of living cells

The potential biological application of the probe T-Cy was measured by CLSM. In the given condition of Roswell Park Memorial Institute (RPMI)-1640 medium and 10% fetal bovine serum (FBS) at 37 °C under an atmosphere of 5% CO_2, the mouse fibroblast cells L929 were culture and used to image for the probe solution. At first, L929 cells were treated with 20 μM probe in culture media for 30 min at 37 °C and washed three times with phosphate-buffered saline (PBS, pH = 7.4). Then, the cells were further incubated with 20 μM CN^- for another 30 min at 37 °C. After washing three times with PBS solution, the cells were observed under CLSM.

3. Results and discussion

3.1. Synthesis and solubility of T-Cy

Generally, central chlorine of heptamethine cyanine derivatives can be easily replaced by amine, alcohol and thiol via nucleophilic substitution reaction, but the connection bonds (C—N, C—O and C—S) are susceptible to strong nucleophiles and biothiols [41,42]. Direct C—C bond connection at the central position of heptamethine cyanine dye was confirmed to be more stable [43]. In this work, the probe T-Cy was designed by incorporating triphenylamine moiety at the central position through a robust C—C bond and synthesized via a modified Suzuki coupling reaction (Scheme 1). The central chlorine of heptamethine cyanine was found to be efficiently coupled with 4-(diphenylamine) phenylboronic acid in environmentally friendly solvents (ethanol/water) and afforded the target dyad T-Cy in a relatively high yield. The structure of T-Cy was completely characterized by ^1H NMR (Fig. S1), ^{13}C NMR (Fig. S2) and MALDI-TOF mass spectra (Fig. S3).