A New Fluorescent Turn-on Dual Interaction Position Probe for Determination of Hydrazine

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Hydrazine is an important catalyst and chemical raw material. But it is highly toxic and potentially carcinogenic. We designed a new hydrazine probe based on a synergistic effect by introducing acetate and phthalimide into 2-phenyl-benzimidazole (PBI). Comparative experiments proved that "the dual position interaction" had a "synergistic effect" on fluorescence enhancement. The fluorescence enhancement caused by the probe (15.0 fold) is much larger than the sum of the fluorescence enhancement of the two monomer compounds (2.6 and 1.4 folds, respectively). A theoretical calculation showed an inhibition of the PET process and a recovery of the ICT process led to a fluorescence enhancement. The probe was specific to hydrazine and showed a linear response to it in the concentrations range of 0.2 – 200 μM with a LOD of 0.062 μM (1.99 ppb). Moreover, the probe could detect hydrazine in tap water; the recovery of hydrazine from the tap water was between 98.86 – 103.28%.

Keywords Hydrazine, fluorescent probe, 2-phenyl-benzimidazole, synergism effect

(Received June 21, 2019; Accepted August 6, 2019; Published December 10, 2019)
sulfamic acid (20 mg) in HOAc. Reflux for 12 h. Cool to room-temperature. Adjust the pH to 7. Extract the mixture with EtOAc. Concentrate to afford compound 2 (500 mg, yield, 70%).

1H NMR (DMSO-d6) δ 12.89 (s, 1H), 10.02 (s, 1H), 8.27 – 7.76 (m, 6H), 7.62 (s, 2H), 7.21 (d, J = 8.3 Hz, 1H), 6.94 (d, J = 8.3 Hz, 2H). 13C NMR (DMSO-d6): δ 168.60, 163.21, 136.15, 132.92, 131.21, 130.69, 128.55, 125.32. HRMS (ESI, m/z): [M+H]+ calcd for C21H14N3O3: 356.1030; found: 356.1031.

After concentration, compound was purified by chromatography. A/uni00A0mixture of Ac2O (370 μL) in DCM. A/uni00A0solution of hydrazine (2 mM) was prepared in deionized water. The tap water was obtained from “Baoding water supply Corporation”. The tap water was needed to be filtered through microporous membrane (0.22 μm) before it was used. A certain concentration of hydrazine was added to the tap water. After interaction with the probe, the fluorescence intensity at 450 nm was recorded.

Result and Discussion

The identification performance of the probe was studied by the UV spectra. The probe had absorption at 308 nm (Fig. S1, Supporting Information). After reacting with hydrazine, the absorption at 330 nm is gradually enhanced. Meanwhile, the absorption at 308 nm decreased. Next, we performed a fluorescence titration study on the probe (Fig. 1a). The probe had extremely weak fluorescence. After interaction with hydrazine, the fluorescence intensity at 450 nm increased significantly (up to 15.0 fold). Accordingly, the fluorescent color of the probe solution changed from dark to bright blue. There was a linear relationship (R² = 0.996) between the concentration of hydrazine (0.2 – 200 μM) and the fluorescence intensity (at 450 nm) (Fig. 1b). According to the 3Δκ method, we calculated the LOD of the probe was 0.062 μM (1.99 ppb). We made a comparison of the probe with the reported ones for hydrazine (Table S1, Supporting Information). The statistical data showed that the probe was more sensitive to hydrazine.

The pH value of the system was an important factor affecting the identification performance of the probe. We measured the fluorescence intensity of in different acidity and alkalinity conditions. The fluorescence of the probe did not change at pH 3.0 – 12.0 (Fig. 2). After interaction with hydrazine, the fluorescence increased significantly at pH 6.0 – 9.0. The results show that the probe can be used in a wide pH range.

Specificity is the basic requirement of probe identification. We tested various substances including nucleophilic molecules (hydrazide, isoniazid, urea, methylamine, ethylenediamine, isoniazid, urea, methylamine, ethylenediamine, serine, cysteine, glutathione, citric acid, glutamic acid, glycine, histidine, alanine, proline, arginine, aspartic acid, threonine, serine, cysteine, glutathione, citric acid, glutamic acid, glycine, histidine, alanine, proline, arginine, aspartic acid, threonine) with the probe. The synthesized probe was more sensitive to hydrazine than these above-referred molecules.

Absorbance and fluorescence titration procedure

The solution of probe (2 mM) was prepared in DMF. The solution of hydrazine (2 mM) was prepared in deionized water. A solution of DMF-PBS (1:1 = v/v, 10 mM, pH = 8.0, 2 mL) to the cuvette (2 mL). 2 μL of the solution of hydrazine (2 mM) was added to the cuvette. Next, various concentrations of hydrazine were added to the system.
hydroxylamine), metal ions (Mg$_2^+$, Ca$_2^+$, Hg$_2^+$, Ba$_2^+$, Na$^+$, K$^+$), anions (Br$^-$, I$^-$, Cl$^-$, OAc$^-$, NO$_3^-$, NO$_2^-$, S$_2^-$), and biological species (Cys, Gsh, Hcy, Inh, Pro, Gly, Ile), which might interfere with identification (Fig. 3). The results showed that hydrazine alone could create an obvious fluorescence response at 450 nm. Other distracters did not hamper the identification of the probe to hydrazine. Therefore, the probe had the potential to analyze actual samples.

To clarify the recognition mechanism, we studied various spectral changes of the probe and the product in detail. As shown in Fig. 4, the acetyl signal at 2.33 ppm disappeared. Besides, the phthalimide signal at 7.93 and 8.00 ppm vanished. By comparing the carbon spectra of the probe (Fig. S2, Supporting Information) with the product (Fig. S3, Supporting Information), we found that the peaks at 167.48 and 167.44 ppm assigned to phthalimide carbonyl carbon disappeared. Meanwhile, the acetyl signals at 169.05 and 20.85 ppm vanished.

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**Fig. 1** (a) Fluorescence spectra of the probe (10 μM) upon the addition of hydrazine (0 – 50 equiv.) in DMF-PBS (1:1, v/v, 10 mM, pH = 8.0). (b) Linear relationship of the fluorescence intensities at 450 nm of the probe (10 μM) depending on the hydrazine concentrations (20 – 40 equiv.). $\lambda_{em} = 310$ nm.

**Fig. 2** Effect of the pH on the fluorescence intensity at 450 nm of the probe (10 μM) in the absence and presence of N$_2$H$_4$ (500 μM) in DMF-PBS (1/1, v/v, 10 mM).

**Fig. 3** Fluorescence spectra and fluorescence photos of the probe (10 μM) in the presence of 500 μM hydrazine and 5 mM different species respectively (hydrazine, isoniazid, urea, methylamine, ethylenediamine, hydroxylamine, Mg$^{2+}$, Ca$^{2+}$, Hg$^{2+}$, Ba$^{2+}$, Na$^+$, K$^+$, Br$^-$, I$^-$, Cl$^-$, OAc$^-$, NO$_3^-$, NO$_2^-$, S$_2^-$, Cys, Gsh, Hcy, Inh, Pro, Gly, Ile) in DMF-PBS solution (1:1, v/v, 10 mM, pH = 8.0) at room temperature. ($\lambda_{em} = 310$ nm).

**Fig. 4** Partial of $^1$H NMR spectra of the probe (up) and product (down).

hydroxylamine), metal ions (Mg$_{2}^{2+}$, Ca$_{2}^{2+}$, Hg$_{2}^{2+}$, Ba$_{2}^{2+}$, Na$^{+}$, K$^{+}$), anions (Br$^{-}$, I$^{-}$, Cl$^{-}$, OAc$^{-}$, NO$_{3}^{-}$, NO$_{2}^{-}$, S$_{2}^{-}$), and biological species (Cys, Gsh, Hcy, Inh, Pro, Gly, Ile), which might interfere with identification (Fig. 3). The results showed that hydrazine alone could create an obvious fluorescence response at 450 nm. Other distracters did not hamper the identification of the probe to hydrazine. Therefore, the probe had the potential to analyze actual samples.

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Next, we studied the IR spectra of the probe (Fig. S4, Supporting Information) and the product (Fig. S5, Supporting Information). The peaks at 1719 and 1731 cm\(^{-1}\) were the characteristic of phthalimide and ester respectively of the probe. In the product, the absorptions at 1719 and 1731 cm\(^{-1}\) disappeared. The infrared absorptions at 3400 and 3500 cm\(^{-1}\) showed the presence of the amino group. Moreover, the infrared absorptions at 3300 and 1171 cm\(^{-1}\) were the characteristic of the hydroxyl group. Based on the above experimental results, we speculate that the recognition mechanism was as follows. Hydrolysis of ester group produced the hydroxyl groups. Meanwhile, hydrazinolysis of phthalimide liberated the amino group (Scheme 2).

To explain the fluorescence enhancement of the probe, theoretical calculations had been carried out by DFT with the hf/3-21g method (Gaussian 09 program). In the probe, the electron cloud was mainly distributed in the PTB part in the ground state. Under excitation, the electron cloud was transferred to the phthalimide moiety (Fig S6, Supporting Information). Comparatively, in the case of the product, the electron cloud was distributed throughout the molecule in the HOMO and LUMO states, which inhibited the PET process. In addition, acetyl reduced the electron density of the PTB moiety of the probe. The hydroxyl increased the electron density of the PTB moiety in the product, which restored the ICT procedure. The inhibited PET and restored ICT co-induced fluorescence enhancement.

To explore the contribution of the synergism effect to the performance of the probe, the fluorescence response of monomer compounds (compound 2 and compound 5) to hydrazine was tested (Fig. S7, Supporting Information). The reaction products of compound 2 and compound 5 with hydrazine were tested. The \(^1\)H NMR spectra showed that the reaction products were the same as that of the probe and hydrazine (Figs. S8 and S9, Supporting Information). In addition, the fluorescence intensity-reaction time curve was measured. Kinetic experiments showed that the interaction between the probe and hydrazine was 60 min (Fig. S10, Supporting Information). The reaction time between compound 2 and compound 5 with hydrazine were 35 and 60 min respectively (Fig. S11, Supporting Information). So the reaction of the probe, compound 2 and compound 5 with hydrazine could be ended in 60 min. Compound 2 displayed weak fluorescence. The addition of hydrazine could enhance the fluorescence intensity (up to 2.6 folds). Compound 5 showed a moderate fluorescence. Addition of hydrazine could increase the fluorescent intensity to 1.4 times. As for the probe, the fluorescence enhancement at 450 nm was up to 15.0 fold. Compared with compound 2 and compound 5, the probe had obvious and higher extent of fluorescence enhancement. The result showed that synergism effect could increase the sensitivity of the probe to hydrazine.

Based on the good recognition performance of the probe, the practical application of the probe was further explored. Using this probe, we detected the hydrazine in actual water samples (tap water). In the contrast experiment, we chose distilled water as the reference and tap water as the object. A certain amount of hydrazine was added to the two systems respectively, and the pH value was adjusted to 8.0. Then, the fluorescence performance was tested. The experimental results showed that the fluorescence intensity of the two solvent systems agrees well within a certain concentration range (0 - 100 \(\mu\)M) (Fig. S12, Supporting Information). The recovery of hydrazine from the tap water was between 98.86 - 103.28% (Table S2, Supporting Information). The intra- and inter-day precision was between 2.59 - 4.03 and 3.70 - 4.29%, respectively. The results show that the probe can be used to detect hydrazine in real water samples.

Conclusions

A new hydrazine fluorescent probe based on the synergism effect was developed. By introducing acetate and phthalimide into PBI, the fluorescence of the probe was significantly enhanced after interaction with hydrazine. The comparative experiment shows that the sensitivity of the probe to hydrazine is greatly improved by the additive effect and the LOD was 0.062 \(\mu\)M (1.99 ppb). A theoretical calculation proved that the inhibited PET and restored ICT co-induced fluorescence enhancement. Moreover, this probe could detect \(\text{N}_2\text{H}_4\) quantitatively in tap water.

Acknowledgements

The work was supported by the Hebei Province Science Foundation for Youths (B2017201093), National Natural Science Foundation of China (Nos. 21702043 and 21807021).

Supporting Information

This material is available free of charge on the Web at http://www.jsac.or.jp/analsci/.

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