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Original article

Anaphthalimide-based fluorescent probe for mercapto-containing compounds

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ABSTRACT

A polarity-sensitive fluorescent probe MNP was rationally designed and synthesized with naphthalimides the fluorophore and maleimide as the receptor for thiols. MNP is weakly fluorescent due to the photoinduced electron-transfer (PET) from the fluorophore to the receptor, and it displays evidently solvatochromic UV–vis and fluorescence spectra: the emission shifted from 495 nm in *n*-hexane to 545 nm in phosphate buffer solution. Michael addition reaction between thiols and the maleimide in MNP inhibited the PET process, which led to about eight-fold fluorescence enhancement. In addition, MNP showed highly sensitivity to mercapto-containing proteins and it could detect as low as 20.4 μg/mL of BSA in PBS. MNP has potential in fluorescent imaging of thiols in living cells.

1. Introduction

Biothiols, including cysteine (Cys), homocysteine (Hcy), and glutathione (GSH), play pivotal roles in physiological and pathological events including redox homeostasis, biocatalysis, detoxification of xenobiotics, metal biding, signal transduction, *etc.* [1–3]. Abnormal levels of biothiols are thought to be implicated in various diseases, such as liver damage, cancer, hematopoiesis decrease, cardiovascular, Alzheimer's disease, and HIV [4–6]. Therefore, it is of intense interest to develop sensitive and selective methods for the detection of biothiols. Fluorescence technique is a frequently used method due to its advantages of the convenience, real time monitoring, in vivo and in vitro bioimaging. Many fluorescent probes have been developed for biothiols in view of their strong nucleophilicity, high binding affinity toward metal ions [7–9].

In the past few decades, maleimide is widely used as a receptor for thiols because of its merits of highly selective reaction with thiols, very fast response to mercapto-containing compounds and ease of being appended to different fluorophores. We present here a rationally designed fluorescent probe MNP for thiols. The probe consists of the following three units: 1) maleimide, a selective thiol receptor; 2) naphthalimide fluorophore with absorption and emission in visible region; 3) a morpholine molecule was incorporated into the 4-position of naphthalimide to improve the water solubility. The photoinduced electron transfer (PET) takes place from the fluorophore to the electron deficient maleimide receptor; therefore, MNP is expected to be weakly fluorescent. 1,4-Michael addition of a mercapto group to the C=C in maleimide blocks the PET process, which leads to a significant fluorescence enhancement. We envisioned that small weighted thiols and mercapto-containing proteins could be detected by MNP due to the high activity of maleimide.

Scheme 1

2. Experimental

MNP was synthesized according to the following procedures (Scheme 2).

MNP-M1: 4-Bromine-1,8-naphthalic anhydride (1.1 g, 4 mmol) and morpholine (1.4 g, 16 mmol) were added to 20 mL of ethylene glycol monomethyl ether. The mixture was refluxed for 4 h under a nitrogen atmosphere. The solvent was evaporated under vacuum. The remaining solid was purified through column chromatography (PE:DCM = 1:4, v/v) to give MNP-M1 as yellow powder, yield 71.2%. ^{1}H NMR ($CDCl_3$): δ 8.60 (d, 1H, J = 7.2 Hz), 8.54 (d, 1H, J = 8.1 Hz), 8.48

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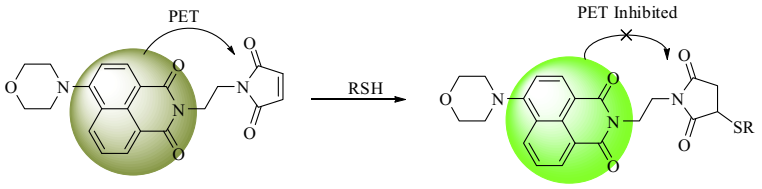
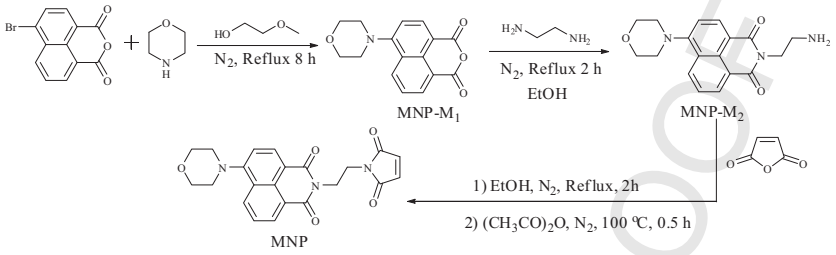


Scheme 1. The sensing mechanism of MNP toward thiols.



Scheme 2. The synthesis procedures of MNP.

(d, 1H, J = 8.5 Hz), 7.76 (t, 1H, J = 7.5 Hz), 7.26 (d, 1H, J = 8.0 Hz), 4.44 (t, 4H, J = 4.5 Hz), 4.04 (t, 1H, J = 4.1 Hz).

MNP-M2: Ethanediamine (120 mg, 2 mmol) was dissolved in 5 mL EtOH, MNP-M1 (566 mg, 2 mmol) was dissolved in 10 mL EtOH. MNP-M1 solution was added dropwise into the ethanediamine solution. The mixture was refluxed under nitrogen atmosphere for 2 h. The solvent was evaporated under vacuum. The remaining solid was purified through column chromatography (DCM:MeOH:Et_3N = 500:10:1, v/v/v) to give MNP-M2 as yellow powder, yield 60.9%. ^{1}H NMR ($CDCl_3$): δ 8.60 (d, 1H, J = 7.2 Hz), 8.54 (d, 1H, J = 8.1 Hz), 8.43 (d, 1H, J = 8.4 Hz), 7.73 (t, 1H, J = 7.5 Hz), 7.24 (d, 1H, J = 8.0 Hz), 4.33 (t, 2H, J = 6.3 Hz), 4.03 (t, 4H, J = 3.8 Hz), 3.28 (t, 4H, J = 4.0 Hz), 3.14 (t, 2H, J = 6.3 Hz).

MNP: MNP-M2 (325.1 mg, 1 mmol) was dissolved in 8 mL anhydrous EtOH, maleic anhydride (98 mg, 1 mmol) was dissolved in 2 mL anhydrous EtOH. MNP-M2 solution was added dropwise into the maleic anhydride solution. The mixture was refluxed under nitrogen atmosphere for 2 h. The solvent was evaporated under vacuum. The remaining solid was dissolved in 5 mL acetic anhydride, and then sodium acetate (820 mg, 10 mmol) was added into the solution. The mixture was stirred at 100 °C for 0.5 h. After evaporating the solvent, the remaining solid was purified through column chromatography (DCM:MeOH:HOAc = 500:10:2, v/v/v) to give MNP as yellowy powder, yield 53.2%. ^{1}H NMR ($CDCl_3$): δ 8.55 (d, 1H, J = 7.3 Hz), 8.49 (d, 1H, J = 8.0 Hz), 8.42 (d, 1H, J = 8.4 Hz), 7.70 (t, 1H, J = 7.8 Hz), 7.21 (d, 1H, J = 8.1 Hz), 6.62 (s, 1H), 4.42 (t, 2H, J = 4.6 Hz), 4.03 (t, 4H, J = 4.1 Hz), 4.00 (t, 2H, J = 5.1 Hz), 3.27 (t, 2H, J = 4.1 Hz). ^{13}C NMR (DMSO-d_6): δ 163.42, 163.24, 149.08, 148.94, 146.85, 145.40, 143.80, 139.31, 133.44, 132.70, 132.58, 131.75, 131.56, 130.45, 130.39, 129.88, 129.33, 129.18, 125.25, 123.40, 123.18, 44.88. ESI: calcd for $[M + H]^+$: 406.1403, found: 406.1400.

3. Results and discussion

3.1. UV–vis absorption and fluorescence responses of MNP toward thiols

With probe MNP in hand, we first measured its spectral responses toward small weighted thiols. Q2

Fig. S3a–S3b in supporting information shows the absorption and emission spectra of MNP and MNP mixed with Cys. MNP is weakly fluorescent and absorbs at 405 nm. The addition of Cys led to about eight-fold fluorescence enhancement at 545 nm, however, the absorption spectrum almost kept the same. The fluorescence intensity of Cys-MNP system had no obvious change after 1 min (Fig. S3c), demonstrating that the reaction between MNP and Cys can be finished within 1 min. Similar spectral changes of MNP were observed when Hcy and GSH were added instead of Cys.

Biological samples are composed of many kinds of components; other coexisted species may interfere with the detection of thiols. Consequently, the high selectivity is very important for the probe applied in biosamples. To evaluate the selectivity and competition of MNP toward thiols over other biologically relevant species, a series of amino acids and anions were examined. As shown in Fig. 1

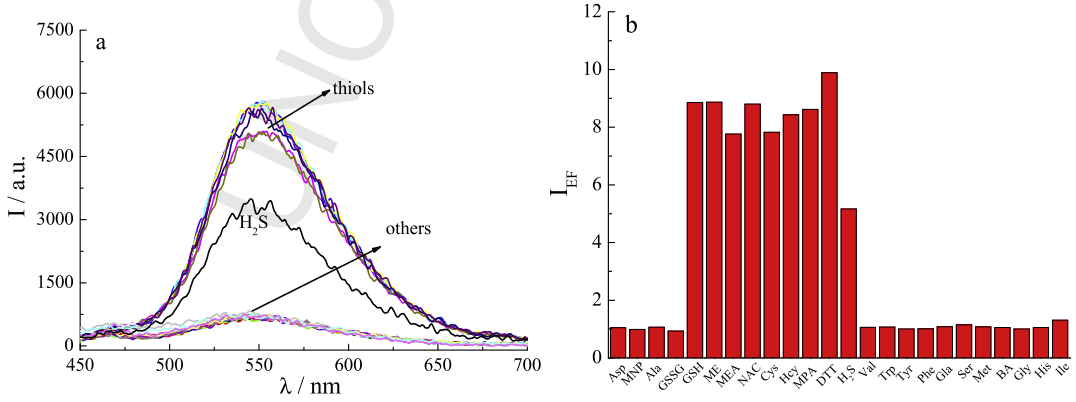


Fig. 1. The emission spectra (a) and the fluorescence intensity at 550 nm (b) of MNP in the presence of different additives. [MNP] = 20 μmol/L, [analyte] = 400 μmol/L, recorded 1 min after the addition of the reagent, PBS (20 mmol/L, pH 7.4) containing 2% MeCN, 25 °C, λ_{ex}= 405 nm.

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