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Highly selective and sensitive fluorescent sensing of N-acetylcysteine: Effective discrimination of N-acetylcysteine from cysteine

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ABSTRACT

A highly selective fluorescent probe for the effective discrimination of N-acetylcysteine (NAC) from cysteine (Cys) is proposed. Probe **1** contains an N,N-diethylrhodol (**DER**) dye and a dinitrophenyl ether moiety. Upon mixing with NAC in aqueous cetyltrimethylammonium bromide (CTAB) micellar solution, **1** was thiolyzed by NAC to release **DER**, thus affording a significant increase in fluorescence emission. Whereas for Cys, it gives only a dim response at the same reaction conditions. The significant difference in reaction rates can be explained via the fact that NAC shows more hydrophobicity than Cys, therefore the Meisenheimer complex intermediate (**2a**) of its nucleophilic aromatic substitution with **1** can embed in CTAB micelles effectively, which will facilitate the formation of **2a** and hence affords an acceleration of reaction rates. The proposed method shows an excellent selectivity for NAC over Cys, homocysteine (Hcy) and other amino acids.

1. Introduction

N-acetylcysteine (NAC) is a thiol-containing antioxidant and has been used as a mucolytic agent in chronic respiratory disorders as well as treating acute paracetamol poisoning [1]. It also acts as an antioxidant by raising intracellular levels of glutathione or by the scavenging of oxidant species itself [2]. In addition, in vitro and in vivo studies indicate that NAC can protect the cells against damages promoted by free radicals [3]. Therefore, the sensitive and selective detection of NAC is very important from the biological and pharmacological stand point.

Several analytical techniques have been developed for the determination of NAC, such as high performance liquid chromatography (HPLC) [4–9], capillary electrophoresis [10], colorimetric [11–13] and electroanalytical method [14,15]. Although HPLC is widely used for detecting NAC in different samples, the method usually needs chemical derivatization, which is time-consuming and inconvenient to operate.

In recent years, several fluorescent probes and chemosensors for thiol-containing compounds have been reported. Most of them are developed based on the strong nucleophilicity of thiol group, and various mechanisms have been employed [16]. Though these probes show high sensitivity toward thiol-containing compounds, they cannot discriminate them from each other due to non-specific thiol nucleophilicity. Therefore, the direct detection of target biothiols is highly challenging due to interference from other biothiols.

In 2004, Strongin et al. introduced an aldehyde-appended fluorophore to serve as a fluorescent probe for both cysteine (Cys) and homocysteine (Hcy) [17,18]. Because both the sulfhydryl and the amino groups contribute to the sensing mechanism, it enables selectivity for Cys and Hcy over other common thiols such as glutathione (GSH). Later, research in this area has been extended and some aldehyde-containing fluorescent probes selective for Cys or Hcy have been developed [19–22]. In 2007, Wang et al. reported the first fluorescent probe which can distinguish aliphatic thiols and thiophenols by using 2,4-dinitrobenzenesulfonamide as the recognition moiety [23]. This can be attributed to the distinct pK_a values of benzenethiols ($pK_a = 6.5$) and aliphatic thiols ($pK_a = 8.5$), and to the thiolysis of dinitrophenyl ethers proceeding via nucleophilic substitution by the nucleophilic thiolate. Very recently, we developed fluorescent probes using α,β-unsaturated carbonyl as the recognition unit, which can discriminate Cys and Hcy based on their relatively different intramolecular cyclization rates [24,25]. However, so far as we know, fluorescent method that can distinguish NAC from Cys has not been realized. And this is still very challenging due to similar chemical structures of NAC and Cys (Fig. 1).

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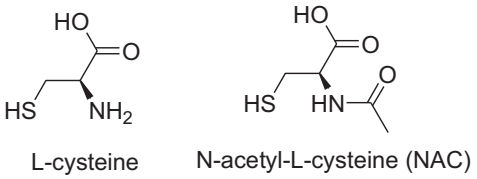


Fig. 1. Chemical structure of NAC and Cys.

On the other hand, it was reported that cetyltrimethylammonium bromide (CTAB) can catalyze the nucleophilic aromatic substitution reaction between thiols and 1-chloro-2,4-dinitrobenzene [26–28], and the reaction rates increased with increasing the hydrophobicity of thiols [29]. This phenomenon provides a unique opportunity to develop a chemical sensing system to discriminate thiols based on differences in their hydrophobicity. Thus, we can distinguish thiol-containing compounds based on their two independent characteristics (nucleophilicity and hydrophobicity). This design strategy may lead to the double molecular recognition-based fluorescence sensing systems, which in principle, may enhance the selectivity of the probe toward the target compound over other potentially competing species as two independent molecular recognition events are involved. As a proof of concept, we utilize this finding to develop a new fluorescent sensing system for discriminating NAC from Cys.

Herein, we develop compound **1** by incorporating the 2,4-dinitrophenyl group to N,N-diethylrhodol (**DER**) fluorophore. Probe **1** shows both colorimetric and fluorescent "turn-on" response for NAC in CTAB micelles, but there is no gain for Cys under identical reaction conditions due to its low hydrophobicity compared with that of NAC. Based on this strategy, a highly selective fluorescent method for NAC detection was developed, which showed an excellent selectivity for NAC over Cys, Hcy and other amino acids.

2. Material and methods

2.1. Materials

N-Acetyl-L-cysteine (NAC) was purchased from Sinopharm Chemical reagent Co., Ltd (Shanghai, China); N,N-diethyl-*m*-aminophenol was obtained from Aladdin reagent Co. (Shanghai, China); phthalic anhydride and resorcinol were obtained from Xi'an chemical reagent factory; 1-chloro-2,4-dinitrobenzene was obtained from Shanghai Darui fine chemical Co., Ltd. Flash chromatography was performed using Qingdao Haiyang silica gel (200–300 mesh). Unless otherwise stated, all reagents were purchased from commercial suppliers and used without further purification. Solvents were purified and dried by standard methods prior to use. Double-distilled water was used throughout the experiments.

2.2. Instrumentation

The fluorescence spectra and relative fluorescence intensity were measured with a Shimadzu RF-5301 spectrofluorimeter with a 10 mm quartz cuvette. Unless specific noted, the excitation and emission band passes were set at 3.0/3.0 nm. The absorption spectra were measured using a Shimadzu UV-2550 spectrophotometer. High-resolution mass spectra were collected using a Bruker micrOTOF-Q II mass spectrometer (Bruker Daltonics Corp., USA) in electrospray ionization (ESI) mode. ^{1}H and ^{13}C NMR spectra were recorded on an INOVA-400 spectrometer (Varian Unity), using tetramethylsilane (TMS) as the internal standard. All pH measurements were made with a Sartorius PB-10 pH meter.

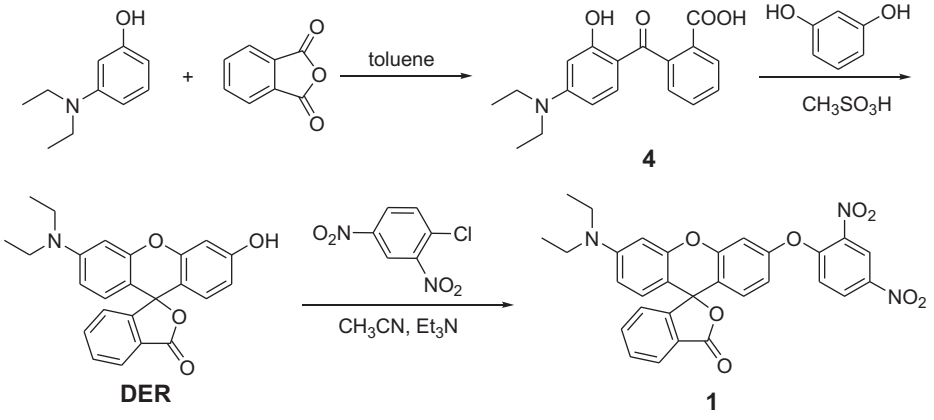
2.3. Synthesis of compound *1*

2.3.1. Preparation of *DER*

2-(4-Diethylamino-2-hydroxybenzoyl) benzoic acid (**4**) was prepared according to the literature procedures [30]. Then, a suspension of **4** (120 mg, 0.38 mmol) and m-resorcinol (42 mg, 0.38 mmol) in methanesulfonic acid (3 mL) was stirred at 90 °C for 24 h. The reaction mixture was cooled to room temperature and then poured in ice-cold water (15 mL). The precipitate was filtered and washed with brine (3 × 10 mL), then dried under vacuum. The target compound is isolated by flash column chromatography on silica gel using CH_2Cl_2:EtOAc:MeOH (20:20:3, v/v/v) for elution. Yield 51 mg, 34%. ^{1}H NMR (400 MHz, d_6-DMSO): δ 10.11 (s, 1H), 7.97 (d, 1H, J = 7.2 Hz), 7.77 (t, 1H, J = 7.0 Hz), 7.70 (t, 1H, J = 7.2 Hz), 7.26 (d, 1H, J = 7.2 Hz), 6.66 (s, 1H), 6.52–6.45 (m, 5H), 3.35 (q, 4H, J = 7.2 Hz), 1.08 (t, 6H, J = 7.2 Hz). HRMS (ESI) calc. for $C_{24}H_{20}NO_4$ $[M - H]^-$ 386.1398, found 386.1412.

2.3.2. Preparation of compound *1*

To a 25 mL flask, **DER** (0.1 g, 0.26 mmol), 1-chloro-2,4-dinitrobenzene (0.06 g, 0.31 mmol) and Et_3N (0.1 mL) in acetonitrile (10 mL) were mixed and refluxed for 6 h [31] (Scheme 1). After evaporation of the solvent, the residue was dissolved by $CHCl_3$ (50 mL). The solution was washed with brine (3 × 10 mL), dried over anhydrous Na_2SO_4. After that, the solvents were dried in vacuo



Scheme 1. Synthesis of probe **1**.