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Short Communication

Chromogenic sensing of biological thiols using squarylium dye

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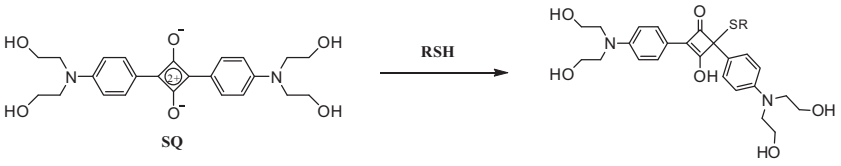
HIGHLIGHTS

- A new biothiol probe SQ was synthesized.
- The probe displayed a color change from Cys, Hcy and GSH.
- SQ was highly selective for Cys detection.
- SQ could serve as a "naked-eye" probe for Cys with a minimum concentration of approximately 4 μM.

GRAPHICAL ABSTRACT



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ABSTRACT

A new highly selective probe for biothiols, squarylium dye (SQ), was designed and synthesized. The probe displayed a color change from blue to colorless upon reaction with biothilos such as cysteine (Cys), homocysteine (Hcy) and glutathione (GSH). The competition experiments revealed that no obvious interference was observed by performing the titration with the mixtures of Cys and other amino acids. The results indicated that SQ was highly selective for Cys detection. Moreover, SQ could also serve as a "naked-eye" probe for Cys with a minimum detectable concentration of approximately 4 μM.

Introduction

Naturally occurring thiols exhibit a variety of structures and important physiological properties. In which, Cys, Hcy, GSH have the similar structures (Fig. 1).

Biological thiols such as Cys, Hcy and GSH play crucial roles in the cellular antioxidant defense system [1]. Their abnormal levels have been directly linked to some diseases and cancers. Deficiency of Cys would lead to many diseases, such as hematopoiesis decrease, leucocyte loss [2], and psoriasis, while Hcy is a risk factor for cardiovascular [3] and Alzheimer's disease [4]. GSH is a major component of the cellular antioxidant system, and it plays an important role in the detoxification of xenobiotic compounds and in the antioxidation of reactive oxygen species and free radicals [5]. The determination of biothiols in body fluids is very important from the biological and pharmacological stand point. Some Cys and Hcy analyses have been developed in conjunction with HPLC [6], capillary electrophoresis [6], immunoassay [6], colorimetric and fluorescence detection [7–11], etc.

Recently, colorimetric sensors are popular due to their capability to detect analyte by the naked eye without resorting to any expensive instruments. Therefore, the design of ratiometric and colorimetric biothiol probes has been the focus of numerous research efforts because of their remarkable importance in the qualitative and quantitative detection. Squarylium dyes and derivatives are 1,3-disubstituted compounds synthesized from squaric acid and two equivalents of various types of electron donating carbocycles or heterocycles such as azulene [12], pyrroles [13], or heterocyclic methylene bases [14]. Squarylium dyes are expected to have

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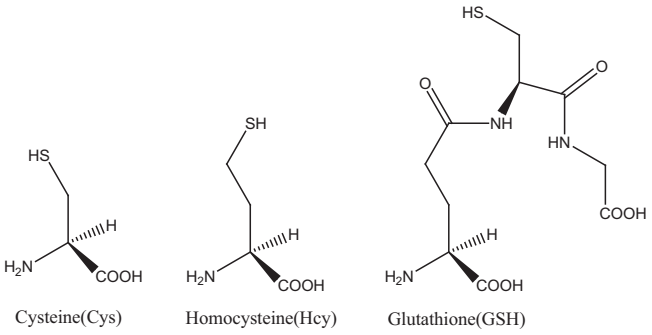


Fig. 1. Structure of biothiol.

significant technological applications in many areas such as electrophotography [15–18], solar energy conversion [19], optical recording [20], nonlinear optics [21], and electroluminescence [22]. We now present a squarylium dye (SQ), having chromogenic responses for fast, selective and sensitive detection of biothiols in aqueous media.

Experimental

Synthesis of SQ

N-phenyldiethanolamine **1** (3.62 g, 20 mmol) and squaric acid **2** (1.14 g, 10 mmol) was heated under reflux for 24 h in a mixture of 60 ml of n-butanol/benzene (5:5/v:v). Water was removed azeotropically using a Dean–Stark trap. The reaction mixture was cooled to room temperature. The product is collected by filtration and washed with n-hexane to give 1.05 g (24%) of pure product. ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.64–3.67 (m, 20H), 7.00 (*d*, *J* = 9.32, 4H), 8.10 (*d*, *J* = 9.2, 4H). EA: Anal.Calcd. for $C_{24}H_{32}N_2O_6$: C, 64.85; H, 7.26; N, 6.30. Found: C, 64.99; H, 6.32; N, 6.02%. IR (Kbr): ν = 3358 cm^{-1} (OH), 1756 cm^{-1} (C═O), 1374, 1353 cm^{-1} (Ar—N). FAB Mass: 440.1.

Results and discussion

Symmetrical squarylium dyes are normally synthesized by condensation of squaric acid **2** and electron rich aromatic or heteroaromatic compounds. SQ dye used in this work was synthesized from N-phenyldiethanolamine **1** and squaric acid **2**, according to previous described procedures [15–18] (Scheme 1).

In order to demonstrate the potential application of SQ as a highly colorimetric probe for biothiols, absorption spectral studies of SQ in the presence of biothiols were performed.

At first, the time course of the reaction between probe SQ and biothiols was studied by monitoring the absorption intensities of the reaction mixture at 654 nm (Fig. 2).

SQ presents a sharp and intense absorption band at 654 nm. Upon treatment with 1 equiv. of Cys in pH 7.4 DMSO/HEPES(4-(2-Hydroxyethyl) piperazine-1-ethanesulfonic acid) (9:1, v/v) at room temperature, the absorption band at 654 nm decreased within

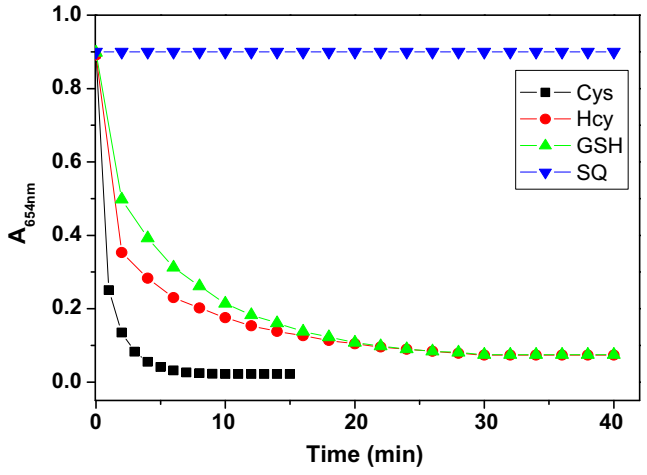


Fig. 2. Time dependant absorption intensity change of SQ (4 μM) in the absence and presence of 1 equiv. Cys, Hcy, GSH at 654 nm in a mixture of DMSO and HEPES. (9:1,v/v, pH7.4).

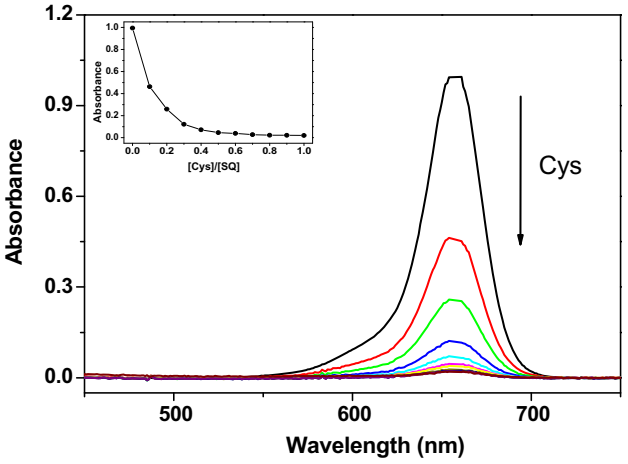
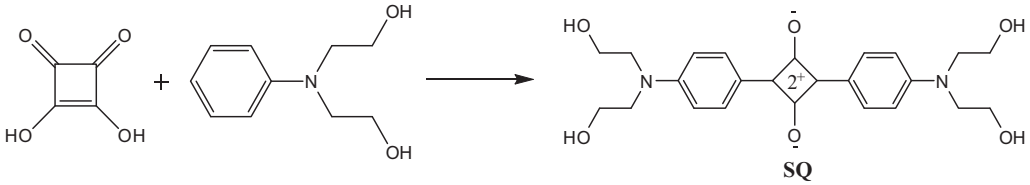


Fig. 3. Absorption spectra change of SQ(4 μM) upon addition of Cys (0–1 equiv.) in DMSO/HEPES (9:1v/v, pH7.4). Each spectrum is recorded 10 min after Cys addition. Inset:change in absorption intensity profile of SQ at 654 nm.

10 min. After 30 min the decrease of the absorption intensity of SQ induced by Hcy/GSH leveled off. Therefore, all samples were equilibrated for 30 min before measurements. Changes in the UV–visible absorption spectra upon additions of Cys to the solution of SQ are shown in the Fig. 3.

We examined the kinetic profiles of the reaction under first-order reaction with 1 equiv. of Cys, Hcy and GSH over probe SQ. The first-order rate constant k was calculated according to the following equation:

$$\ln(A_t - A_\infty)/(A_i - A_\infty) = kt \quad (1)$$



Scheme 1. Synthesis of SQ.