

# Determination of cysteine concentration by fluorescence increase: reaction of cysteine with a fluorogenic aldehyde

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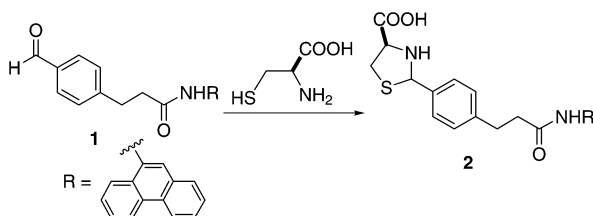
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## A fluorogenic method for the determination of cysteine concentration has been developed.

Spectroscopic detection of small organic compounds using sensors designed either to monitor covalent-bond formation or to detect in non-covalent fashion has attracted increased interest recently.<sup>1–4</sup> Although fluorescent and visual detection methods for free amino acids,<sup>1</sup> amines,<sup>2</sup> and thiols<sup>3</sup> have been reported, selective detection of cysteine by fluorescence or by color change has only been reported by the Strongin group.<sup>5</sup> Development of simpler methods for the determination of cysteine concentration is of current interest.<sup>5,6</sup> One key for useful cysteine detection is discrimination of cysteine from other amino acids, amines, and thiols. The Strongin group used a xanthene dye possessing aldehydes that afforded thiazolidine by the reaction with cysteine under alkaline conditions, resulting in a decrease in fluorescence and a color change.<sup>5</sup> Their results stimulated us to examine the utility of our recently developed fluorogenic aldehyde<sup>7</sup> for the selective detection of cysteine. Our fluorogenic aldehydes can be used to monitor the progress of aldehyde transformations through increased fluorescence.<sup>7</sup> Monitoring of an increase in fluorescence from a low level of fluorescence is typically more reliable than monitoring reduction in fluorescence intensity of a highly fluorescent sample when changes in fluorescence are small.<sup>7</sup> Here we report a fluorescence growth method for cysteine detection employing our fluorogenic aldehyde **1** at neutral pH (Scheme 1).<sup>8,9</sup>

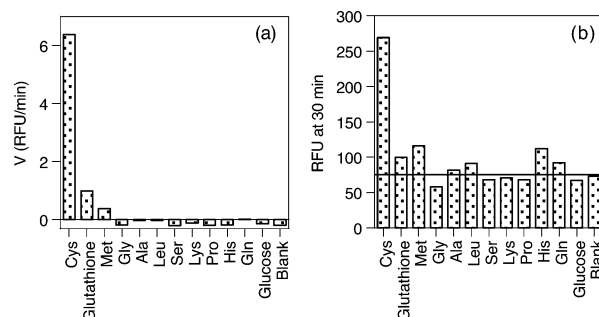
Aldehyde **1** was mixed with a series of amino acids, glutathione (reduced,  $\gamma$ -L-glutamyl-L-cysteinylglycine), and glucose in aqueous buffer (pH 7) and the fluorescence ( $\lambda_{\text{ex}}$  250 nm,  $\lambda_{\text{em}}$  380 nm) was monitored (Figs. 1 and 2). The reaction with cysteine resulted in an increase in fluorescence, while mixture with other amino acids or with glucose did not change the fluorescence; the reaction with glutathione showed only a small increase in fluorescence (Fig. 1a). When the fluorescence intensity was measured at 30 min, only the reaction with cysteine provided values meaningfully higher than the blank (without amino acid) (Fig. 1b). These results indicate that this fluorescence assay selectively detects cysteine and discriminates the aminothiol of cysteine from other simple thiols. Other amino acids including methionine, serine, lysine, proline, and histidine, as well as glutathione were all inactive in this assay, indicating that formation of thiazolidine **2** is key for the selective detection of cysteine. Although iminium or hemiaminal formation from aldehydes and amines (including amino acids) and monothiohemiacetal formation from aldehydes and thiols have been used for the detection of amines and thiols, these reactions were reversible and higher concentrations of analytes were typically required for the detection.<sup>9</sup> Our results indicated that the level of



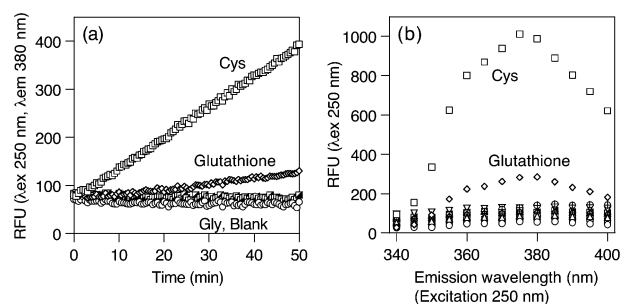
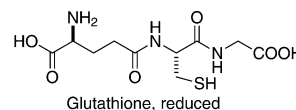
**Scheme 1** Reaction of fluorogenic aldehyde and cysteine to afford thiazolidine **2**.

formation of hemiaminal or monothiohemiacetal was far below the formation of thiazolidine **2**.

The time course of the fluorescence assay is shown in Fig. 2a. At 30 min, less than 0.5  $\mu\text{M}$  of **2** was formed in the reaction with cysteine, determined based on the fluorescence intensity of **2** in the same buffer. The fluorescence of the reaction with cysteine was still increasing at 200 min. The fluorescence spectra of the reaction mixtures at 200 min are shown in Fig. 2b. Under these conditions complete conversion of **1** to **2** was not necessary to detect cysteine. In many fluorescence-based detection methods where decreases in fluorescence were monitored, it was often difficult to detect the small decrease in fluorescence at the initial stages of the reactions because of the intense fluorescence of the fluorescent reactant. Since our detection method monitored increase in fluorescence,



**Fig. 1** Fluorescence assay for cysteine using aldehyde **1**. (a) Velocities of the fluorescence increase. (b) Fluorescence intensity at 30 min. The fluorescence ( $\lambda_{\text{ex}}$  250 nm,  $\lambda_{\text{em}}$  380 nm) was recorded on a microplate spectrophotometer. Reaction conditions: [aldehyde **1**] 5  $\mu\text{M}$ , [amino acid, glutathione, or glucose] 5 mM in 1%  $\text{CH}_3\text{CN}$ –1% 2- $\text{PrOH}$ –46.5 mM Na phosphate (pH 7.0), total volume 100  $\mu\text{L}$  in a 96-well polypropylene plate at 26  $^\circ\text{C}$ . RFU = relative fluorescence unit. Glutathione = reduced,  $\gamma$ -L-glutamyl-L-cysteinylglycine. The purity of glutathione used in the experiments was 98%.

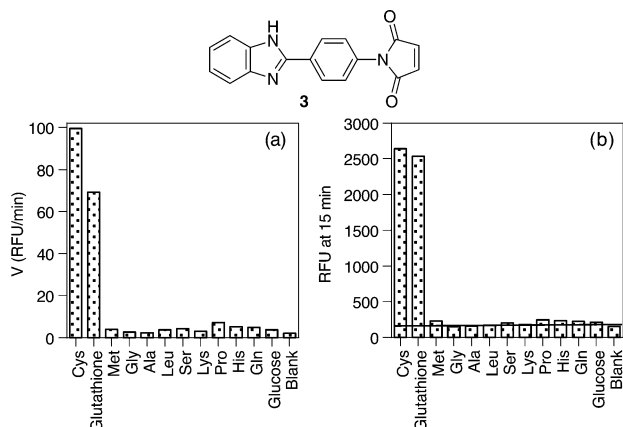


**Fig. 2** Fluorescence assay for cysteine using aldehyde **1**. (a) Time course, (b) fluorescence emission spectra of the reaction mixtures at 200 min. Conditions see Fig. 1 legend. In (a), reactions with cysteine, glutathione, glycine, and blank are shown. In (b), all reactions indicated in Fig. 1 are included with the exception of methionine, but only reactions with cysteine and glutathione are marked.

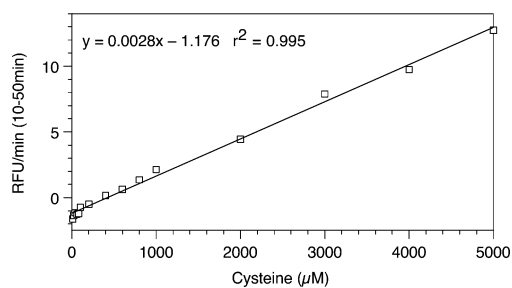
very early stages of the reaction progress between **1** and cysteine were detected.

This method has a significant advantage for the selective detection of cysteine compared with assays using thiol reagents designed for the detection of thiols by the Michael addition: When thiol-detecting reagent **3**<sup>3a,10</sup> was used, both cysteine and glutathione were similarly detected (Fig. 3).

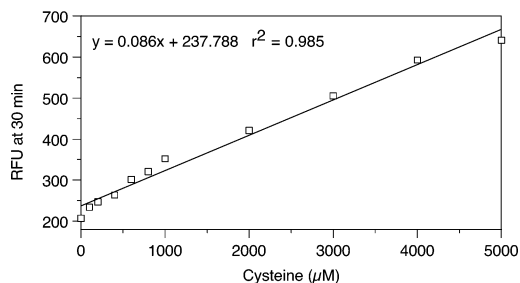
The detection system for cysteine using fluorogenic aldehyde **1** was useful for the determination of cysteine concentrations (Fig. 4). When aldehyde **1** (10  $\mu\text{M}$ ) and cysteine were mixed in aqueous buffer (pH 7) in a 200  $\mu\text{L}$  volume in a 96-well plate and the



**Fig. 3** Fluorescence assay using **3**. (a) Velocities of the fluorescence increase. (b) Fluorescence intensity at 15 min. The fluorescence ( $\lambda_{\text{ex}}$  315 nm,  $\lambda_{\text{em}}$  360 nm) was recorded on a microplate spectrophotometer. Reaction conditions: [**3**] 5  $\mu\text{M}$ , [amino acid, glutathione, or glucose] 5 mM in 2%  $\text{CH}_3\text{CN}$ –46.5 mM Na phosphate (pH 7.0), total volume 100  $\mu\text{L}$  in a 96-well polypropylene plate at 26  $^\circ\text{C}$ . RFU and glutathione; see Fig. 1 legend.



**Fig. 5** Plots of velocity of increase in fluorescence versus cysteine concentration in the assay using aldehyde **1**. The velocity (RFU per min.) was determined as the slope of the increase in fluorescence ( $\lambda_{\text{ex}}$  250 nm,  $\lambda_{\text{em}}$  380 nm) over the time range from 10 min to 50 min. The x-axis refers the final concentration of cysteine in the assay mixture. Reaction conditions, see Fig. 4 legend.



**Fig. 4** Plots of fluorescence versus cysteine concentration in the assay using aldehyde **1**. The relative fluorescence ( $\lambda_{\text{ex}}$  250 nm,  $\lambda_{\text{em}}$  380 nm) intensity (RFU) at 30 min. was recorded on a microplate spectrophotometer. The x-axis refers the final concentration of cysteine in the assay mixture. Reaction conditions: [aldehyde **1**] 10  $\mu\text{M}$ , in 1%  $\text{CH}_3\text{CN}$ –1% 2-PrOH–44 mM Na phosphate (pH 7.0), total volume 200  $\mu\text{L}$  in a 96-well polypropylene plate at 26  $^\circ\text{C}$ .

fluorescence was measured at 30 min, the concentration of cysteine in the range of 100  $\mu\text{M}$ –5 mM was well correlated with the fluorescence intensity. Using varied concentrations of fluorogenic aldehyde **1** (5  $\mu\text{M}$  and 40  $\mu\text{M}$ ) in the same procedure provided essentially the same detection range. More accurate results especially for lower concentration range (20–100  $\mu\text{M}$ ) in a 96-well plate format were obtained when the velocities of the increase in fluorescence were used (Fig. 5).

We have developed a rapid and simple reaction-based method for the determination of cysteine concentration. The method allows for the selective detection of cysteine by fluorescence growth at neutral pH on a small scale.

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