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Highly selective colorimetric sensor for cysteine and homocysteine based on azo derivatives

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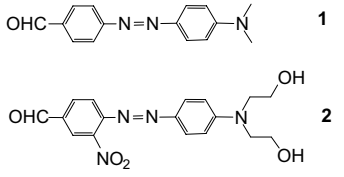
Abstract—A simple colorimetric method for the determination of cysteine and homocysteine has been developed. The reaction of the azo dyes containing an aldehyde group with cysteine or homocysteine afforded very stable derivatives thiazolidines or thiazinanes under neutral pH conditions. The method is selective and sensitive for cysteine and homocysteine detection without the interference of other amino acids. Importantly, the recognition of Cys and Hcy could be observed by naked eyes.

As one kind of important amino acids, cysteine (Cys) and homocysteine (Hcy) play a crucial role in living systems.[1] The deficiency of Cys is associated with slowed growth, hair depigmentation, edema, lethargy, liver damage, muscle and fat loss, skin lesions, and weakness.[2] Additionally, Cys-induced hypoglycemic brain damage has been studied as an alternative mechanism to excitotoxicity.[3] At elevated levels in plasma, Hcy is a risk factor for Alzheimer's,[4] cardiovascular diseases, neutral tube defects, and osteoporosis.[5] Therefore, the determination of Cys and Hcy in body fluids is very important from the biological and pharmacological stand points. The cysteine and homocysteine analyses have been often carried out with high performance liquid chromatography (HPLC),[6] capillary electrophoresis (CE),[7] electrochemical detection,[8] UV–vis detection,[9] fluorescent detection,[10] fourier transform infrared (FTIR) detection,[11] and mass spectrometry identification.[12]

Recently, colorimetric sensors are popular due to their capability to detect analyte by the naked eye without resorting to any expensive instruments.[13] One key problem for Cys and Hcy detections is the discrimination of Cys and Hcy from other amino acids, amines, and thiols. Although fluorescent and visual detection methods for free amino acids,[14] amine,[15] and thiols[16] have been reported, selective detection of Cys and Hcy by color change is quite limited.[17,18] Strongin's group have reported the xanthene dyes containing aldehydes that afforded thiazolidines or thiazinanes by the reaction with Cys or Hcy, resulting in a color change,[17] however, this detection of Cys or Hcy has to be carried out under alkaline condition. Cys and Hcy usually are obtained in physiological condition, therefore, the development of selective colorimetric sensors for Cys and Hcy is required under neutral pH. In this letter, we report a naked-eye sensor for Cys and Hcy employing azo dyes 4-[[4′-(*N*,*N*-dimethylamino)phenyl-1′-]azo]benzaldehyde (**1**) and 4-[[4′-(bis(2-hydroxyethyl)amino)phenyl-1′-]azo]-3-nitrobenzaldehyde (**2**) (Scheme 1) under neutral pH with high selectivity and sensitivity.

Sensor **1** was synthesized according to the previous literature.[19] Sensor **2** was prepared by diazo-coupling of 4-formyl-2-nitroaniline[20] to *N*-phenyldiethanolamine, and was characterized by NMR and elemental



Scheme 1. Chemical structures of compounds **1** and **2**.

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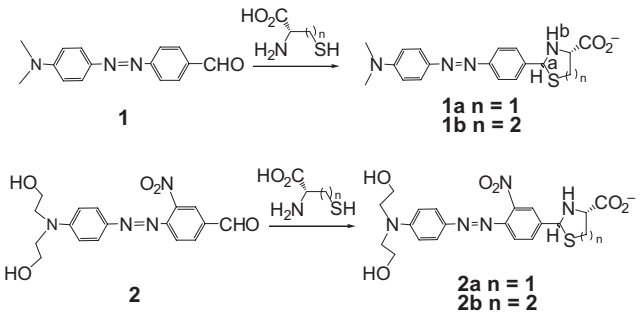
analysis.[21] The complexation abilities of **1** and **2** with Cys and Hcy were investigated by the UV–vis absorption technique.

As shown in Figure 1, the DMF solution of **1** showed maximum absorbance at 465 nm with the extinction coefficient of 3.4×10^4 mol^{-1} L cm^{-1}, corresponding to the intramolecular charge-transfer character (ICT) of the chromophore due to the push–pull effect.[22] Upon addition of Cys, the absorption maximum of **1** shifted from 465 to 442 nm (Fig. 1a), corresponding to an isosbestic point at 416 nm, indicating the formation of thiazolidine **1a** (Scheme 2). Furthermore, this transformation was confirmed by ^{1}H NMR and FT-IR spectra. Upon addition of Cys, the aldehyde signal at 10.05 ppm disappeared and the thiazolidine resonances (protons a and b, Scheme 2) centered at δ 5.74 and 5.57 ppm appeared (see ESI). Moreover, FT-IR spectra showed that the strong vibration band at 1701 cm^{-1}, the signal ascribed to C=O stretching of the aldehyde group of **1**, disappeared upon addition of Cys, indicating the interaction of aldehyde group with Cys. Similar spectral changes were also observed upon addition of Hcy (see Fig. 1b). These data indicated that **1** could be used as a chemosensor for Cys or Hcy.

In the light of the insolubility of **1** in water, **2** with two hydroxyl groups was synthesized to increase the solubility in water, furthermore, the presence of a nitro group in **2** would enhance the intramolecular charge-transfer character, and give rise to red-shift in the absorption spectrum compared with **1**. The absorption spectrum of **2** showed a band centered at 515 nm with an extinction coefficient of 3.6×10^4 mol^{-1} L cm^{-1}, indicating that **2** had a strong ability of absorbing light. The absorption spectrum of **2** and its titration with Cys and Hcy were recorded in DMF–H_2O solution (9:1, v/v) at pH 7 maintained with HEPES buffer (50 mM). As shown in Figure 2a, upon addition of Cys, the absorption intensity at 515 nm decreased with the concomitant formation of a hypsochromically shifted band peaked at 475 nm, corresponding to a color change from pink to yellow, which were clearly visible to the naked eyes (see Fig. 3). Moreover, a clear isosbestic point at 488 nm was observed, indicating thiazolidine **2a** was



Scheme 2. Reactions of **1** and **2** with Cys or Hcy to afford thiazolidines (**1a**, **2a**) or thiazinanes (**1b**, **2b**), respectively.

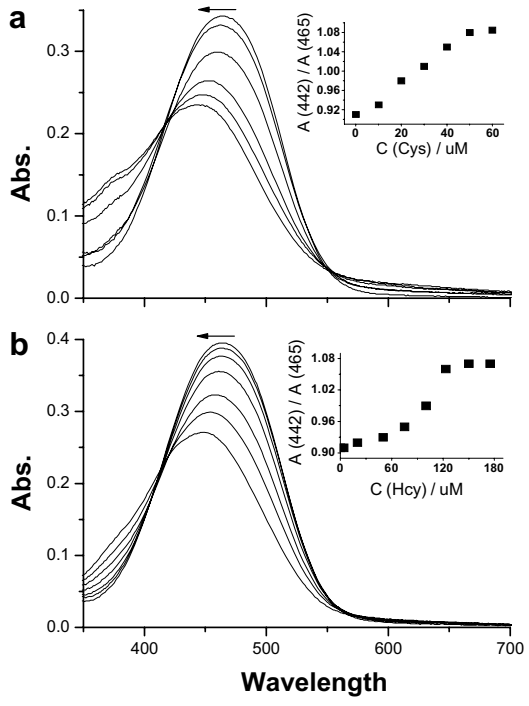


Figure 1. UV–vis spectra of **1** (10 μM) in the presence of different concentrations of Cys or Hcy in DMF solution at room temperature. Each spectrum is recorded 5 min after Cys or Hcy addition. As the concentration of Cys or Hcy increases, a blue shift from 465 to 442 nm was observed. Inset: ratiometric calibration curve A442/A465 as a function of Cys or Hcy concentration.

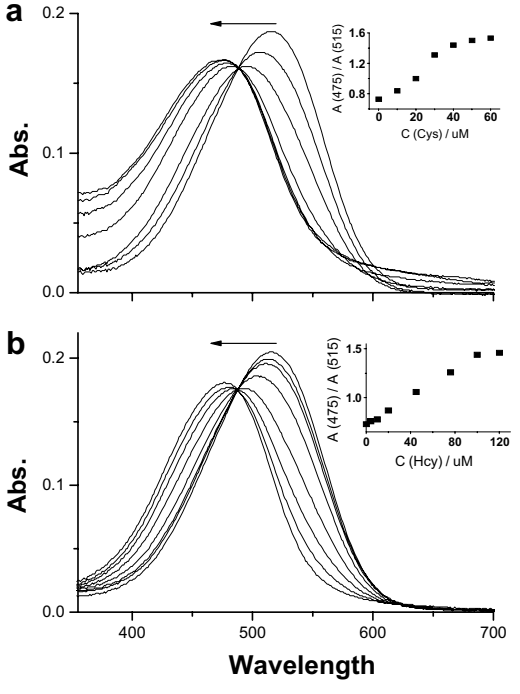


Figure 2. UV–vis spectra of **2** (5.6 μM) in the presence of different concentrations of Cys or Hcy in DMF–H_2O solution (9:1, v/v, 50 mM HEPES, pH 7.0) at room temperature. Each spectrum is recorded 5 min after Cys or Hcy addition. As the concentration of Cys or Hcy increases, a blue shift from 515 to 475 nm was observed. Inset: ratiometric calibration curve A475/A515 as a function of Cys or Hcy concentration.

Figure 3. The color change of **2** (5.6 μM) in DMF–H_2O 9:1 (v/v) in the presence of 20 equiv of certain amino acids. From left to right and top to bottom: absence of amino acid, phenylalanine, threonine, arginine, histidine, asparagine, leucine, alanine, proline, valine, glycine, lysine, glutamine, methionine, isoleucin, serine, cysteine, tryptophan, homocysteine, and aspartic acid.

formed by the interaction of aldehyde with Cys, reducing the electron-withdrawing ability of **2**, resulting in a relative weak ICT of the chromophore (see Scheme 2). The similar experimental phenomena were also observed when Hcy was added into the solution of **2** (see Fig. 2b). As shown in Figure 2, the dependence of the absorption ratios at 475 nm to that of 515 nm (A475/A515) on the concentration of Cys or Hcy indicated the obvious change upon addition of Cys or Hcy. In the presence of electrolyte (phosphate buffer, pH = 7.2, NaCl, 0.145 mol L^{-1}, K_2CO_3, 5.5 mmol L^{-1}, $CaCl_2$, 12 mmol L^{-1}), a similar spectral shift of **2** was observed upon addition of Cys or Hcy (see ESI).

For an excellent chemosensor, high selectivity is a matter of necessity. Photophysical properties of **2** in DMF–H_2O (9:1, v/v) were also investigated upon addition of other amino acids (phenylalanine, threonine, arginine, histidine, asparagine, leucine, alanine, proline, valine, glycine, lysine, glutamine, methionine, serine, isoleucine, tyrosine, tryptophane, glutamic acid, and aspartic acid). As shown in Figures 3 and 4, no obvious color changes were observed upon addition of other amino acids compared with the hypsochromic shift of **2** with Cys and Hcy, indicating that the formation of thiazolidine **2a** and thiazinane **2b** was a key for the selective recognition of Cys and Hcy. Furthermore, compared with the assays using thiol reagents designed for the detection of thiols by the Michael addition,[16b,23] no response of **2** upon addition of glutathione was observed (see ESI). These phenomena suggested the high selectivity of **2** for Cys and Hcy over other amino acids.

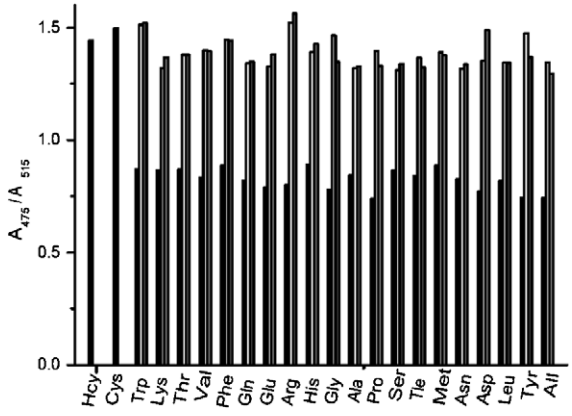


Figure 4. A475/A515 change profile of **2** (5.6 μM) in DMF–H_2O solution (9:1, v/v, 50 mM HEPES, pH 7.0) in the presence of various amino acids (20 equiv) (black bars), one of other amino acids or the mixture of them with Cys (white bars) and with Hcy (gray bars).

Achieving high selectivity for the analyte of interest over a complex background of potentially competing species is a challenge in sensor development. Thus, the competition experiments were conducted in the presence of 50 equiv of one of the above other amino acids or the mixture of them, at 5.6 μM of **2** mixed with Cys or Hcy (20 equiv), respectively. No significant variation in absorption intensity was found in comparison of that containing only Cys or Hcy (Fig. 4). Moreover, no obvious interference in its absorption was observed when performing the titrations with Cys or Hcy in the mixture of other amino acids. These facts indicated a high selectivity of **2** in sensing Cys and Hcy.

In conclusion, we have developed a highly selective colorimetric chemosensor for Cys and Hcy under neutral pH. The recognition of Cys and Hcy gave obvious color changes from pink to yellow, which was clearly visible to the naked eyes. Moreover, the competition experiments showed that the interference from other amino acids was minimal. Due to the simplicity and sensitivity of the analysis, this sensor would have many opportunities in a variety of settings requiring rapid and accurate Cys and Hcy analysis. This preliminary understanding on the Cys and Hcy sensing mechanism would actually help to find possible structural modification to achieve new probes that show Cys and Hcy sensing capacity in pure water.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2006.07.080.

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21. Data for **1**: Yield 85%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.06 (s, 1H), 7.90–7.99 (m, 6H), 6.76 (q, 2H), 3.12 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): 191.9, 157.1, 153.3, 143.9, 136.3, 130.9, 125.9, 122.9, 111.7, 40.5; Anal. Calcd for $C_{15}H_{15}N_3O$ (253.12): C, 71.13; H, 5.97; N, 16.59. Found: C, 71.00; H, 5.90; N, 16.62.
Data for **2**: Yield 40%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.06 (s, 1H), 8.32 (s, 1H), 8.10 (d, $J = 8.4$ Hz, 1H), 7.84–7.89 (m, 3H), 6.76 (d, $J = 9.2$ Hz, 2H), 3.97 (t, 4H), 3.76 (t, 4H), 3.03 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): 191.7, 153.8, 148.5, 147.5, 143.6, 135.9, 133.6, 127.5, 125.9, 119.8, 112.7, 58.9, 54.1; Anal. Calcd for $C_{17}H_{18}N_4O_5$ (358.13): C, 56.98; H, 5.06; N, 15.63. Found: C, 56.88; H, 5.00; N, 15.61.
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