Available online at www.sciencedirect.com
SCIENCE DIRECT®
Tetrahedron Letters
Tetrahedron Letters 45 (2004) 6477–6480

A specific colorimetric cysteine sensing probe based on dipyrromethane–TCNQ assembly

Yong Guo, Shijun Shao, Jian Xu, Yanping Shi and Shengxiang Jiang*

Key Laboratory for Natural Medicine of Gansu Province, Lanzhou Institute of Chemical Physics, Chinese Academy of Sciences, Lanzhou 730000, PR China

Received 30 April 2004; revised 21 June 2004; accepted 25 June 2004
Available online 20 July 2004

Abstract—A new charge-transfer complex, consisting of dihydroxymethyl di-(2-pyrrolyl)methane and tetracyanoquinodimethane (TCNQ), has been designed to high selectively distinguish cysteine from other amino acids in water/organic solvent mixtures through the visual color change from blue to nearly colorless. The excellent system properties make the supramolecular assembly a highly selective colorimetric probe for monitoring cysteine.

The design and synthesis of receptors for various important biological anionic and zwitterionic species is of current interest. One of the most attractive approaches in this field involves the construction of colorimetric chemosensors.[1] Most of the known colorimetric chemosensors are based on the synthetic receptors generally containing some combination of guest molecules recognition unit and chromophore, either covalently attached or intermolecularly linked.[2] Additionally, the noncovalent charge-transfer assembly can also be served as colorimetric chemosensors.[3] This kind of system is composed of two parts. One is the neutral molecule unit with electron-rich groups, the other is an electron-deficient neutral molecule species, which can accept an electron and induce a color change. Previously, we reported a new dipyrromethane–TCNQ based anion sensor, which allows for the colorimetric detection of PO_4^{3-} and CO_3^{2-}.[3b] In extensive experiments, we found that the noncovalent charge-transfer assembly formed by dihydroxymethyl di-(2-pyrrolyl)methane (**1**) with TCNQ (**2**) can high selectively sense cysteine from other amino acids in water/organic solvent mixtures. As we all know, cysteine and its derivatives play numerous important roles in the transmission of information and in the change of protein conformation by their oxidation–reduction reactions in biological systems. Since cysteine and many of its analogs containing thiol groups are found to be related to metabolism disorder diseases, the determination of cysteine and its derivatives in pharmaceuticals, urine, serum, and plasma has attracted increasing interest[4] and the development of new methods for detecting cysteine is obviously desirable. In this paper, we reported some interesting results of unique colorimetric detection of cysteine using the molecular complex **1**·**2**.

The synthesis of the dihydroxymethyl di-(2-pyrrolyl)methane was carried out by condensation of pyrrole with dihydroxyacetone in excessive pyrrole.[3b] The TCNQ-based complex **1**·**2** system could be obtained easily by dissolving colorless dipyrromethane **1** and TCNQ in acetonitrile at room temperature. The color of the mixed system changes gradually from pale yellow-green to dark blue (near-complete color conversion). In this case, the free TCNQ absorption peak at 397 nm vanished while two new absorption bands appeared at 317 nm and in long-wave region of the spectrum (λ_{max} at around 627 nm), respectively. The results in UV–vis spectra and the corresponding visual color changes indicate that dipyrromethane **1** forms π–π charge-transfer complex with TCNQ in MeCN. Further evidence that dipyrromethane **1** bind TCNQ in acetonitrile solution (the molar ratio 1:1) came from fast atom bombardment (FAB) mass spectrometric analyses, which revealed that in addition to the peak corresponding to free dipyrromethane **1**, the peak corresponding to the **1**·**2** adduct could be observed at *m*/*z* 411.3.

Keywords: Amino acids detection; Charge-transfer complex; Dipyrromethane; Sensor; TCNQ.
* Corresponding author. Tel.: +86-931-8275740; fax: +86-931-8277088; e-mail: shaoguo@ns.lzb.ac.cn

0040-4039/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2004.06.109

Figure 1. The color changes of the complex **1·2** (5.0×10^{-4} mol dm^{-3} **1**; 5.0×10^{-5} mol dm^{-3} **2**) in the presence of amino acids (1.0×10^{-3} mol dm^{-3}) in MeCN/H_2O (1:1, v/v) mixture.

The blue charge-transfer complexes **1·2** could stand in corresponding MeCN/H_2O (1:1, v/v) mixture. The approach of using water-containing medium is quite important to show applicability of the molecular aggregation for the selective determination of amino acids normally existing in aqueous phase. The inducted color changes between the complex **1·2** and commercially available 19 amino acids (such as Gly, Ala, Val, Leu, Ile, Phe, Thr, Cys, Gln, Asn, Met, Ser, Pro, Trp, Glu, Asp, Lys, Arg, and His) were first studied in MeCN/H_2O (1:1, v/v) mixture. The results were shown in Figure 1. No obvious changes in color were observed when the solution of the complex **1·2** was exposed to various above-mentioned amino acids, except for cysteine, which could instantly make the initially blue solutions turn almost colorless. Our previous experiments showed that the complex **1·2** system is unstable in the condition of strong basicity, and then it could be used for the colorimetric detection of strong basic inorganic anions PO_4^{3-} and CO_3^{2-}. However, the dipyrromethane–TCNQ assembly could not change color by adding aqueous solution (buffered with HEPES for pH 7) of PO_4^{3-} and CO_3^{2-}. The addition of the H_2O solutions of basic amino acids (Lys, Arg, and His) to the CH_3CN solution of the complex **1·2** induced no distinct changes in color, maybe due to the weak basicity of amino acids and low affinity for the subunits of the molecular complex **1·2**. Figure 2 showed the changes in the absorption spectrum of the complex **1·2**, obtained by adding aqueous solutions (buffered with HEPES for pH 7) of different amino acids (2.0×10^{-3} mol dm^{-3}) to the CH_3CN solution of the complex **1·2** (1.0×10^{-3} mol dm^{-3} **1**, 1.0×10^{-4} mol dm^{-3} **2**) at the volume ratio of 1:1. In the presence of cysteine, the charge-transfer absorption band of the complex **1·2** distinctly decreased, which means the disassembly of the complex **1·2**. However, cystine, serine, and methionine induced neglectable changes in color and the absorption spectra in contrast with cysteine. The control experiments suggest that the mercapto group in the side chain of cysteine plays a crucial role in colorimetric recognition.

As expected, the intensity of the charge-transfer absorption band of the complex **1·2** decreased with increasing the amounts of cysteine. The addition of 150 equiv (relative to TCNQ concentration) of cysteine make the charge-transfer absorption band completely disappear, and a new absorption band, albeit very weak, could be observed in the region of 400–550 nm ($\lambda_{max}=484$ nm) as a result of the intermolecular interactions between cysteine and the TCNQ moiety. This was supported by a control experiment carried out using individual TCNQ under the same conditions, as shown in Figure 3. In the presence of cysteine, the TCNQ absorbance ($\lambda_{max}=$

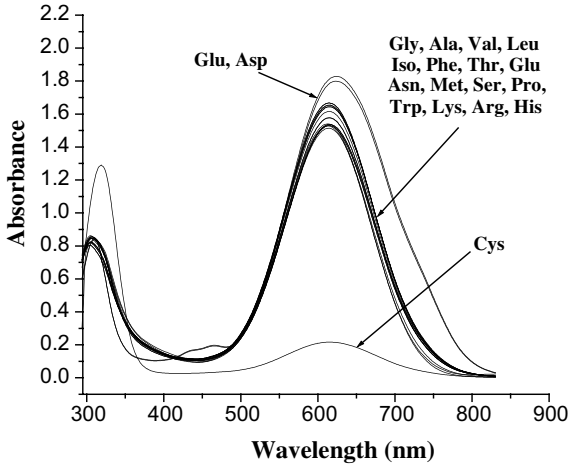


Figure 2. UV–vis spectra of the complex **1·2** (5.0×10^{-4} mol dm^{-3} **1**; 5.0×10^{-5} mol dm^{-3} **2**) in the presence of various amino acids (1.0×10^{-3} mol dm^{-3}) in MeCN/H_2O (1:1, v/v) mixture at pH 7 buffered with HEPES.

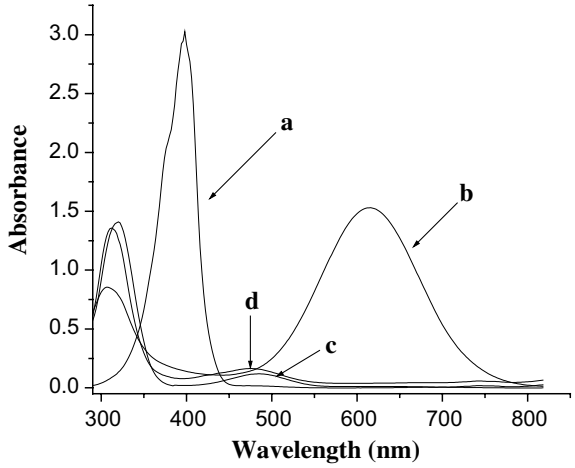


Figure 3. UV–vis spectra: (a) TCNQ (5.0×10^{-5} mol dm^{-3}); (b) the complex **1·2** (5.0×10^{-4} mol dm^{-3} **1**, 5.0×10^{-5} mol dm^{-3} **2**); (c) the complex **1·2** (5.0×10^{-4} mol dm^{-3} **1**, 5.0×10^{-5} mol dm^{-3} **2**) containing cysteine (TCNQ concentration of 150 equiv); (d) TCNQ (5.0×10^{-5} mol dm^{-3}) containing 150 equiv of cysteine, in MeCN/H_2O (1:1, v/v) mixture, at pH 7 buffered with HEPES.

397 nm) vanished and a new absorption peak at 317 nm grew while a very weak absorption bands in the region of 400–550 nm (λ_{max} = 481 nm) could be observed, which is rationally attributed to the n–π charge-transfer interaction between the mercapto group with lone pair electrons of cysteine and TCNQ. In this case, the solution induced no noticeable color changes. In pH 7 HEPES buffer solution, the protonated amino groups of amino acids have difficulty forming n–π charge-transfer complexes with TCNQ. In fact, addition of other amino acids to the solution of TCNQ at pH 7 caused no any changes in absorption spectra. The association constant of $7.9\times10^2\,dm^3\,mol^{-1}$ was determined for cysteine and TCNQ in MeCN/H_2O (1:1, v/v) mixture at pH 7 by UV–vis spectroscopy, using the Benesi–Hildebrandt analysis[5] of the absorbance changes at the new band maximum. The complex **1·2** has an association constant of $4.6\times10^2\,dm^3\,mol^{-1}$.[3b] The above results indicated that the perturbation of the complex **1·2** in the presence of cysteine is mainly ascribed to the competitive binding of cysteine to TCNQ subunit (Scheme 1).

On the other hand, it was found that no significant color-based responses were observed when urea, aliphatic amines (ethylamine, diethylamine, and triethylamine), and aromatic amines (*p*-amino phenol, *m*-amino phenol, *p*-aminophenol sulfonic acid) were added to the complex **1·2** system under the same conditions. The hyposensitive absorption spectra changes were shown in Figure 4. However, the results are also quite important to show applicability of the complex **1·2** system for the determination of cysteine existing in some biological samples.

Moreover, the experiments confirmed that the color change of the dipyrromethane–TCNQ complex solution induced by cysteine is a reversible procedure. When the selected cysteine was eliminated from the ensemble solution, the solution could slowly become blue again, which can be ascribed to the renewal of the noncovalent

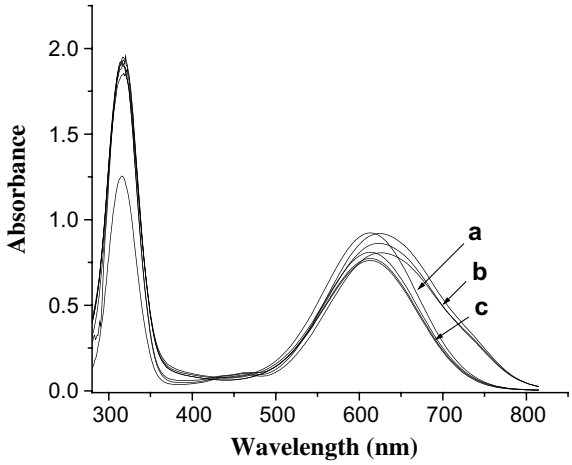
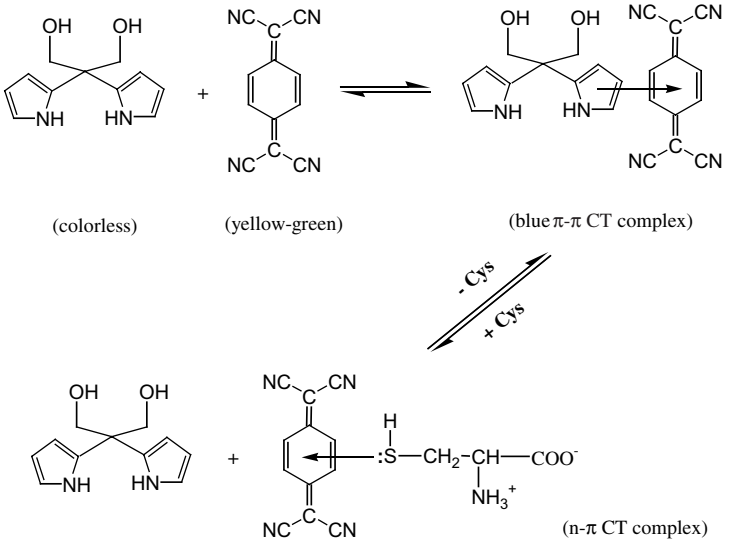


Figure 4. UV–vis spectra of the complex **1·2** (2.5×10^{-4} mol dm^{-3} **1**; 5.0×10^{-5} mol dm^{-3} **2**) in the absence (a) and in the presence of 1.0×10^{-3} mol dm^{-3}; (b) aromatic amines (*p*-amino phenol, *m*-amino phenol, *p*-aminophenol sulfonic acid); (c) aliphatic amines (ethylamine, diethylamine, triethylamine, and urea), in MeCN/H_2O (1:1, v/v) mixture at pH 7 buffered with HEPES.

charge-transfer interaction between dipyrromethane **1** and TCNQ. The reversible changes in color could be more easily observed under a condign complex solution concentration.[6]

In summary, we have developed a novel colorimetric amino acids sensor based on the dipyrromethane–TCNQ molecular complex, which can uniquely distinguish cysteine from other amino acids and/or amine compounds in water/organic solvent mixtures. The present results may provide a useful approach for the development of a simple, inexpensive, high selective method without tedious separation for the rapid identification and detection of cysteine. Further works on spectrophotometric determination of cysteine are in progress in our group.



Scheme 1.

Acknowledgements

We thank the National Natural Science Foundation of China for financial support (No. 20372067).

References and notes

1. (a) Schmidtchen, F. P.; Berger, M. *Chem. Rev.* **1997**, *97*, 1609; (b) Metzger, A.; Anslyn, E. V. *Angew. Chem., Int. Ed.* **1998**, *37*, 649–652; (c) Lavigne, J. J.; Anslyn, E. V. *Angew. Chem., Int. Ed.* **1999**, *38*, 3666–3669; (d) Gale, P. A. *Coord. Chem. Rev.* **2001**, *213*, 79–128; (e) Martínez-Máñez, R.; Sancenón, F. *Chem. Rev.* **2003**, *103*, 4419–4476.
2. See for example: (a) Black, C. B.; Andrioletti, B.; Try, A. C.; Ruiperez, C.; Sessler, J. L. *J. Am. Chem. Soc.* **1999**, *21*, 10438–10439; (b) Gale, P. A.; Twyman, L. J.; Handlin, C. I.; Sessler, J. L. *Chem. Commun.* **1999**, 1851–1852; (c) Miyaji, H.; Sato, W.; Sessler, J. L. *Angew. Chem., Int. Ed.* **2000**, *39*, 1777–1780; (d) Anzenbacher, P., Jr.; Try, A. C.; Miyaji, H.; Jursíková, K.; Lynch, V. M.; Marquez, M.; Sessler, J. L. *J. Am. Chem. Soc.* **2000**, *122*, 10268–10272; (e) Hennrich, G.; Sonnenschein, H.; Resch-Genger, U. *Tetrahedron Lett.* **2001**, *42*, 2805–2808; (f) Kato, R.; Nishizawa, S.; Hayashita, T.; Teramae, N. *Tetrahedron Lett.* **2001**, *42*, 5053–5056; (g) Lee, K. H.; Lee, H.-Y.; Lee, D. H.; Hong, J.-I. *Tetrahedron Lett.* **2001**, *42*, 5447–5449; (h) Lee, C.; Lee, D. H.; Hong, J.-I. *Tetrahedron Lett.* **2001**, *42*, 8665–8668; (i) Han, M. Su.; Kin, D. H. *Angew. Chem., Int. Ed.* **2002**, *41*, 3809–3811; (j) Piatek, P.; Jurczak, J. *Chem. Commun.* **2002**, 2450–2451; (k) Tsubaki, K.; Morimoto, T.; Otsubo, T.; Fuji, K. *Org. Lett.* **2002**, *4*, 2301–2304.
3. (a) Shao, S. J.; Guo, Y.; He, L. J.; Jiang, S. X.; Yu, X. D. *Tetrahedron Lett.* **2003**, *44*, 2175–2178; (b) Guo, Y.; Shao, S. J.; Xu, J.; Shi, Y. P.; Jiang, S. X. *Inorg. Chem. Commun.* **2004** (7), 333–336.
4. See for example: (a) Shimada, K.; Mitamura, K. *J. Chromatogr. B* **1994**, *659*, 227–241; (b) Kataoka, H.; Takagi, K.; Makita, M. *J. Chromatogr. B* **1995**, *664*, 421–425; (c) Kaniowska, E.; Chwatko, G.; Glowacki, R.; Kubalczyk, P.; Bald, E. *J. Chromatogr. A* **1998**, *798*, 27–35; (d) Vieira, C.; Filho, O. F. *Anal. Chim. Acta* **1999**, *399*, 287–293; (e) Zaia, D. A. M.; Ribas, K. C. L.; Zaia, C. T. B. V. *Talanta* **1999**, *50*, 1003–1010; (f) Chwatko, G.; Bald, E. *Talanta* **2000**, *52*, 509–515; (g) Zhao, C.; Zhang, J.; Song, J. *Anal. Biochem.* **2001**, *297*, 170–176; (h) Nie, L.; Ma, H.; Sun, M.; Li, X.; Su, M.; Liang, S. *Talanta* **2003**, *59*, 959–964; (i) Amarnath, K.; Amarnath, V.; Amarnath, K.; Valentine, H. L.; Valentine, W. M. *Talanta* **2003**, *60*, 1229–1238.
5. Benesi, H. A.; Hildebrandt, J. H. *J. Am. Chem. Soc.* **1949**, *71*, 2703–2707.
6. For example, the addition of excess cysteine to the blue complex **1**·**2** solution (containing 2.0×10^{-3} mol dm^{-3} **1** and 1.0×10^{-3} mol dm^{-3} **2**) in MeCN/H_2O (1:1, v/v) mixture instantly caused color changes of the ensemble solution from blue to almost colorless. Subsequently, adding dry ether to the mixture, shaking carefully, the aqueous phase containing a mass of cysteine was separated out from the mixture, and the organic phase gradually turned color from colorless to pale blue.