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A highly selective colorimetric probe based on 2,2′,2″-trisindolylmethene for cysteine/homocysteine

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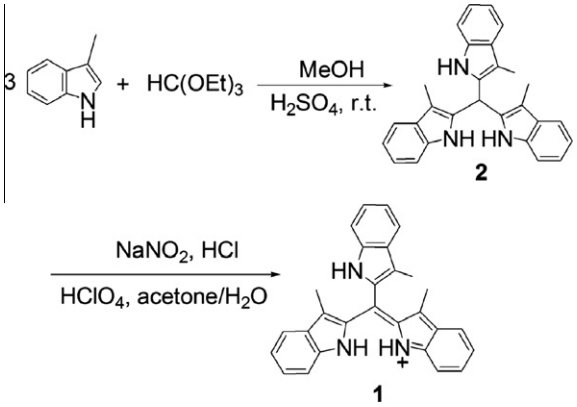
ABSTRACT

The interaction and colorimetric sensing properties of receptor **1**, tris(3-methylindole-2-yl)methene as the perchlorate salt, with amino acids in aqueous MeCN at neutral pH were investigated using UV–vis spectroscopic techniques. Specifically, receptor **1** behaves as a colorimetric probe for selective and sensitive detection of cysteine (Cys)/homocysteine (Hcy) based on the nucleophilic addition reaction between the sulphydryl group of Cys/Hcy and the *meso* carbon–carbon double bond of receptor **1**, leading to clear color change from violet to colorless. A more quantitative determination for Cys/Hcy was preliminary performed by flow injection analysis (FIA) coupled with spectrophotometry. The selective binding ability of receptor **1** toward Cys/Hcy has also been evaluated by electrochemical techniques.

Low molecular weight thiol-containing amino acids, such as cysteine (Cys) and homocysteine (Hcy), play crucial roles in the biological system.[1] Cys and Hcy are linked to various human disorders. Deficiency of Cys would cause hematopoiesis decrease, edema, skin lesions, slowed growth, etc.[2] An abnormal level of Hcy in human blood plasma is a risk factor for Alzheimer's and Parkinson's diseases,[3] cardiovascular disease,[4] coronary disease, and neural tube defect.[5] The safe, highly selective, and sensitive detections of small molecular thiols including Cys and Hcy in biological samples have been strongly desired. At present, many reported methods had been gone into, such as high performance liquid chromatography,[6] electrochemical detection,[7] capillary electrophoresis,[6e,8] and spectrometry identification,[9] etc. Among the methods, spectrometry techniques are very simple and rapid.[10] The probes containing an unsaturated double bond, such as maleimide group[11] or unsaturated ketone,[12] detect thiol-containing amino acids based on Michael addition reaction. Some probes can be used to label proteins and peptides by the cyclization reaction of aldehyde with Cys or Hcy.[13] In addition, the spectrometry detections for thiol-containing amino acids are based on the cleavage of sulfonamide or sulfonate ester,[14] metal complexes,[15] and other methods,[16] etc. In the various probes, compounds containing the carbon–carbon double bond are widely used in the determination of thiols by the nucleophilic addition of the sulfydryl group.[17] In our previous works, we have been interested in the research of colorimetric detection of amino acids using the charge-transfer complexes of pyrrole derivatives with tetracyanoquinodimethane (TCNQ) or *p*-chloranil (TCBQ) as an amino acid receptor.[18] Herein, we develop a colorimetric method for Cys and Hcy detection employing a new receptor based on tris(3-methylindole-2-yl)methane (**1**) in aqueous MeCN at neutral pH.

The synthesis of receptor **1** is shown in Scheme 1. Tris(3-methylindole- 2-yl)methane **2** was prepared in 47.4% yield by sulfuric-acid-catalyzed condensation reactions of 3-methylindole with triethyl orthoformate.[19] Trisindolylmethene **1** was formed by treating the precursor **2** with nitrous acid in the presence of a small



Scheme 1. Synthetic route of receptor **1**.

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amount of hydrochloric acid and perchloric acid in acetone in 90% yield. In fact, as described in the literature reported by French,[20] we isolated trisindolylmethene **1** as the perchlorate salt.

The color changes of receptor **1** (2.5×10^{-5} M) in MeCN/H_2O (1:1, v/v) solution at neutral pH buffered with HEPES-NaOH were first observed by the addition of 200 equiv of various amino acids and peptides, such as glycine (Gly), alanine (Ala), valine (Val), leucine (Leu), isoleucine (Ile), proline (Pro), phenylalanine (Phe), tyrosine (Tyr), tryptophan (Trp), serine (Ser), threonine (Thr), methionine (Met), aspartic acid (Asp), glutamic acid (Glu), lysine (Lys), arginine (Arg), Cys, Hcy, histidine (His) and reduced glutathione (GSH). As shown in Figure 1, receptor **1** in MeCN/H_2O (1:1, v/v) binary solution at neutral pH changes color from violet to colorless in the presence of Cys or Hcy. The dramatic combination of amino acids-specific response/nonresponse makes receptor **1** an especial and effective colorimetric probe for Cys/Hcy under the solution-phase conditions.

The amino acids binding and sensing properties of receptor **1** have been studied by using UV–vis spectroscopic techniques. Receptor **1** itself displays a strong and wide absorption band at 400–700 nm in MeCN/H_2O (1:1, v/v) mixture at neutral pH, which can be assigned to the ICT absorption band of the conjugated trisindolylmethene quaternary ammonium ion skeleton. Figure 2a shows the amino acid-induced spectral changes of the solution of receptor **1** (2.5×10^{-5} M). In the presence of 200 equiv of Cys or Hcy, the strong and wide absorption band almost disappeared with the effect that the mixed solution instantaneously changed color from violet to colorless. On the other hand, less remarkable spectral changes were also observed in the cases of other amino acids and peptides, but no obvious color changes occurred. The interaction of receptor **1** with Cys/Hcy was investigated in detail through the UV–vis spectroscopic titration. Upon addition of Hcy, the absorption band of receptor **1** at 400–700 nm gradually decreased and a new band centered at 286 nm appeared with distinct isosbestic points at 423 nm (Fig. 2b). Similar spectral features were also observed for Cys under the same conditions (Fig. S1). However, almost no obvious spectral and color changes were observed in the cases of receptor **2** in the presence of various amino acids and GSH (Fig. S2). Considering of the possible influence of common anions existing in the organism, the sensing properties of receptor **1** with various common anions in aqueous MeCN were also evaluated preliminarily,[21] with the effect that the presences of common anions have no effect on the selective colorimetric detection of receptor **1** for Cys/Hcy in MeCN/H_2O under neutral pH.

In the light of the mechanism of nucleophilic addition of carbon–carbon double bond with sulfhydryl,[17] we speculated that the nucleophilic addition reaction occurred between the sulphydryl group of Cys/Hcy and the carbon–carbon double bond of receptor **1**. As a control experiment, titration with the simple thiol compound, mercaptoethanol (MPA), also induced the same color and spectral changes of receptor **1** as those observed with Cys/Hcy (Fig. S3). Importantly, as a structurally related thiol biomolecule, reduced glutathione (GSH) exhibited low reaction activity to receptor **1** under the same conditions, which may be due to its steric hindrance.

The disappearance of the absorption band at 400–700 nm and the occurrence of a new band at 286 nm pertained to the formation of the *meso*-position carbon–carbon single bond of addition products (Scheme 2). Receptor **1** associates with Cys/Hcy in a 1:1 stoichiometry addition product, which is confirmed by the Benesi–Hildebrand analysis (Fig. S4), the binding constants of receptor **1** with Cys and Hcy (Ks(Cys) = 1.35×10^4 M^{-1}, Ks(Hcy) = 6.48×10^4 M^{-1}) have been calculated by Benesi–Hildebrand plot.[22]

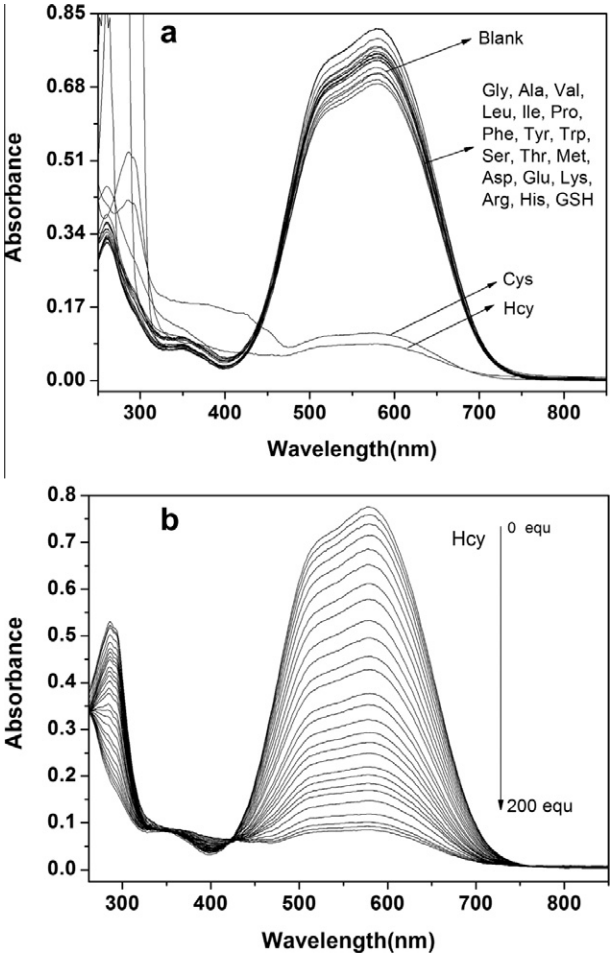


Figure 2. UV–vis absorption spectral changes of receptor **1** (2.5×10^{-5} M) in MeCN/H_2O (1:1, v/v) mixture at neutral pH buffered with HEPES-NaOH in the presence of, (a) 200 equiv of various amino acids and GSH, and (b) 0–200 equiv of Hcy.

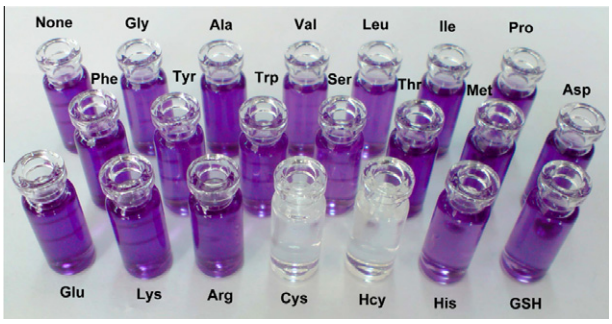
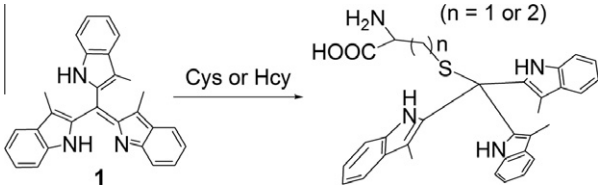


Figure 1. The color changes of receptor **1** (2.5×10^{-5} M) upon addition of 200 equiv of various amino acids and peptide in MeCN/H_2O (1:1, v/v) mixture at neutral pH buffered with HEPES-NaOH, including Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Met, Asp, Glu, Lys, Arg, Cys, Hcy, His and GSH, respectively.



Scheme 2. Possible reaction mechanisms of receptor **1** with Cys/Hcy.

Table 1
Quantitative analysis results of detection of Cys and Hcy using FIA

	Equation of calibration curves	Linear range ($\times 10^{-5}$ M)	LOD[a] ($\times 10^{-5}$ M)	RSD[b] (%)	Recovery (%)	Sampling rate (samples/h)
Cys	$Y = 845703.5X - 62.5$ ($r^2 = 0.997$, $n = 6$)	2.0–200	2	1.4	97.5	126
Hcy	$Y = 16358.8X + 740.5$ ($r^2 = 0.989$, $n = 7$)	1.4–300	1.4	0.9	106.8	128

[a] Limit of detection (S/N = 3).
[b] Relative standard deviation at 0.01 M using 10 replicated injections.

Based on colorimetric sensing of receptor **1** toward Cys/Hcy by instantaneous response, a more quantitative determination for Cys and Hcy was preliminarily performed by flow injection analysis (FIA) coupled with spectrophotometry.[18b] The MeCN/H_2O (1:1, v/v) solution of receptor **1** (2.5×10^{-5} M) at neutral pH buffered with HEPES-NaOH was used as the reagent stream. As shown in Table 1, the determination results obtained for Cys and Hcy indicated that the method is simple, rapid, and high sensitive, and can provide a high sampling rate, a wide linear range, (2.0×10^{-5}–2.0×10^{-3} M for Cys, and 1.4×10^{-5}–3.0×10^{-3} M for Hcy) and good precisions (RSD = 1.4% for Cys, and 0.9% for Hcy). The limits of detection are 2.0×10^{-5} M and 1.4×10^{-5} M for Cys and Hcy, respectively. However, the new methodology of determination of Cys/Hcy was only preliminarily evaluated, and further studies relating to the interference of other co-existing amino acids are currently in progress.

In addition, the selective binding ability of receptor **1** toward Cys/Hcy has also been studied by electrochemical techniques. Receptor **1** was utilized for constructing receptor **1**-modified pyrolytic graphite electrode (**1**/PGE), and the electrochemical behaviors of potassium ferricyanide ($K_3Fe(CN)_6$) probe were evaluated at the **1**/PGE treated with various amino acids by cyclic voltammetry (CV) and electrochemistry impedance spectroscopy (EIS). The detailed experimental methods were described in the Supplementary data.

As shown in Figure 3a, a bare PGE shows a strong electrochemical response toward potassium ferricyanide, with the anodic and cathodic peak potential at 0.24 and 0.15 V, respectively. No significant variations in voltammetric responses were observed after bare PGE was treated with various amino acids, including Cys/Hcy, which indicated a weak interaction between the bare PGE surface and amino acids. However, **1**/PGE showed the increase of oxidation potential and a significant decrease of oxidation current, as a result of the weakened electron transfer between potassium ferricyanide and **1**/PGE surface.

Based on **1**/PGE, the competitive binding experiments of receptor **1** toward amino acids were further tested. Compared with the **1**/PGE, no obvious changes of peak current and peak potential were detected at **1**/PGE, which was treated with a buffer solution containing Ala, Asp, and Arg mixture. Dissimilarly, after the **1**/PGE treated with a buffer solution of Cys or Hcy, as well as a mixture of Cys (or Hcy) and other amino acids, the oxidation potential of potassium ferricyanide further increased and the oxidation current significantly decreased. The results indicated that receptor **1** on PGE surface selectively adsorbed Cys/Hcy in the mixture of amino acids, with the effect that the electron transfer between potassium ferricyanide and the electrode surface was badly retarded. Furthermore, the electrochemistry impedance spectroscopies (EIS) of different modified electrodes have also been carried out (Fig. 3b) to get the same information about the high selectivity of receptor **1** for Cys/Hcy.

In summary, we have developed a new colorimetric probe based on 2,2′,2″-trisindolylmethene for the detection of amino acids. The receptor featured excellent sensitivity and selectivity for Cys/Hcy over other amino acids and GSH by visible color change from violet to colorless, which is presumably attributed to nucleophilic addition of the sulphydryl group to the *meso*-position carbon–carbon double bond of receptor **1**. Flow injection analysis (FIA) method using the new receptor for detecting Cys and Hcy is simple, rapid, and highly selective and sensitive, and it can provide wide linear ranges and good precisions. Moreover, the electrochemical results further indicated that receptor **1** can selectively bind Cys or Hcy in the interference of other co-existing amino acids. We trust that this receptor will be beneficial to biomedical researchers studying the levels of Cys or Hcy in biological systems.

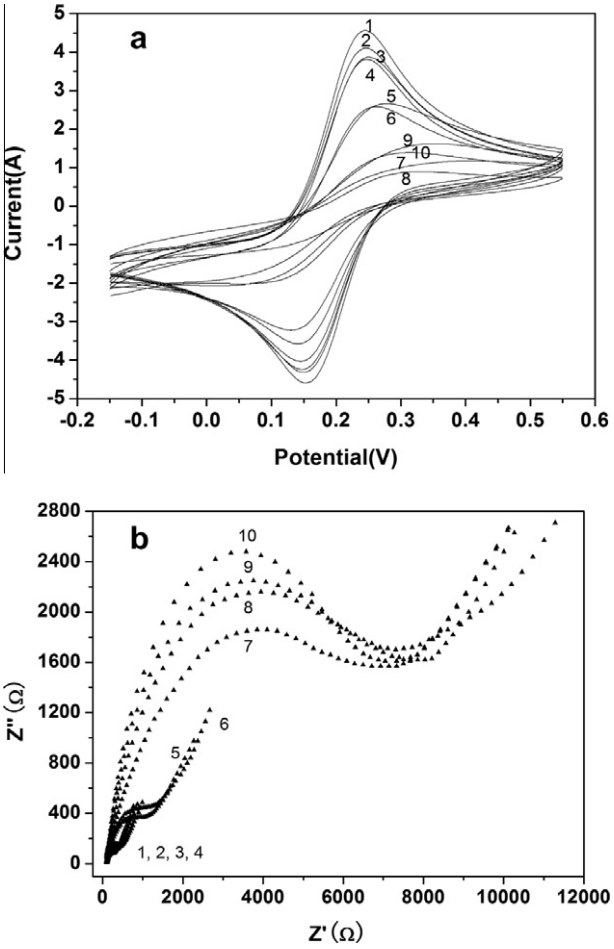


Figure 3. CV (a) and EIS (b) characteristics of $K_3Fe(CN)_6$ at differently modified pyrolytic graphite electrode (PGE), scan rate: 50 mV/s. (1) Bare PGE; (2) PGE treated with a mixed solution of Ala, Asp and Arg; (3) PGE treated with Cys; (4) PGE treated with Hcy; (5) PGE modified with receptor **1** (**1**/PGE); (6) **1**/PGE treated with a mixed solution of Ala, Asp and Arg; (7) **1**/PGE treated with Cys; (8) **1**/PGE treated with a mixed solution of Ala, Asp, Arg and Cys; (9) **1**/PGE treated with Hcy; (10) **1**/PGE treated with a mixed solution of Ala, Asp, Arg and Hcy.

Acknowledgment

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Supplementary data

Supplementary data (synthesis and characterization data of compounds **1** and **2**, the procedure of electrochemistry experiments, absorption titration of receptor **1** with Cys, absorption spectral changes of receptor **2** with various amino acids and GSH, absorption spectral changes of receptor **1** with MPA, Benesi–Hildebrand plots of receptor **1** with Cys and Hcy) associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2010.07.182.

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21. The anion binding and sensing properties of receptor **1** were also evaluated. The preliminary experiments indicated that, in MeCN solution, the remarkable spectral changes of receptor **1** were observed in the presence of 200 equiv of F^- AcO^-, $H_2PO_4^-$, HSO_4^- and Cl^- (see Figs. S5 and S6), along with the color changes from violet to yellow for F^-, from violet to colorless for AcO^-, $H_2PO_4^-$, HSO_4^- or Cl^-. However, less significant spectral and color changes of receptor **1** were observed after adding various common anions (i.e., F^-, Cl^-, Br^-, I^-, AcO^-, $H_2PO_4^-$, HSO_4^- and ClO_4^-) in MeCN/H_2O (1:1, v/v) mixture system. The results showed that the presences of common anions have no effect on the selective colorimetric detection of receptor **1** for Cys/Hcy in MeCN/H_2O (1:1, v/v) under neutral physiological conditions. Moreover, the tests indicated that receptor **1** is very sensitive to the basic environment in MeCN/H_2O (1:1, v/v) (see Fig. S7), therefore, the colorimetric detections of Cys/Hcy were carried out under neutral buffer solution system, similar to physiological environment.
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