Electrochemical Behavior and Sensitive Determination of L-Tyrosine with a Gold Nanoparticles Modified Glassy Carbon Electrode

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The electrocatalytic oxidation of L-tyrosine was investigated on a gold nanoparticles self-assembled glassy carbon electrode (gold nanoparticles/cysteamine/glassy carbon) using cyclic voltammetry and differential pulse voltammetry. Cyclic voltammetry was carried out to study the electrochemical oxidation mechanism of L-tyrosine, which showed an irreversible oxidation process at a potential of 0.681 V at a modified electrode and 0.807 V at a bare glassy carbon electrode. The anodic peak current linearly increased with the square root of the scan rate, suggesting that the oxidation of L-tyrosine at this kind of modified electrode is a diffusion-controlled process. A good linear relationship between the oxidation peak current and the L-tyrosine concentration in the range of $1.0 \times 10^{-3}$ to $3.0 \times 10^{-4}$ mol L$^{-1}$ was obtained in a phosphate buffer solution at pH 7.0. Good sensitivity, selectivity and stability of the modified electrode make it very suitable for L-tyrosine determination in a commercial amino acid oral solution.

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Introduction

L-Tyrosine (TS, see Scheme 1) is well known as a kind of essential amino acids in human and herbivores bodies. It is a vital constituent of proteins, which are indispensable in human nutrition for establishing and maintaining a positive nitrogen balance.¹ It is sometimes added to dietary and food products and to pharmaceutical formulations because it is scarcely present in vegetables. TS has been determined to be a precursor of dopa, dopamine, thyroxin, and epinephrine-hormone or neurotransmitters.²³ These studies have documented that a trace-level TS can modulate and control acetylecholine (ACh) receptor metabolic stability in muscle cells.⁴ Additionally, the absence of TS will cause albinism and alkaptonuria, while a high TS concentration in a culture medium results in an increased sister chromatid exchange. Thus, rapid and sensitive TS determination methods are necessary and of vital interest in pharmacology.

Currently, numerous methods have been reported for TS determination in pharmaceutical preparations or biological samples. These mainly include spectrophotometry,⁵ fluorometry,⁶ liquid chromatography-tandem mass spectrometry,⁷ gas chromatography-mass spectrometry and high-performance liquid chromatography.⁸⁹ These methods are quite accurate, but need multi-step sample clean-up procedures. Therefore, they are relatively expensive and time-consuming. On the other hand, TS can be determined by electrochemical methods, because it is an electroactive compound. The electrochemical methods have additional advantages over other methods because they are more convenient and cheaper. However, the main problem with electrochemical detection methods is that tryptophan, another kind of amino acid, is electroactive, and coexists with TS in biological systems, and can be oxidized at a near oxidation potential of TS on most conventional solid electrodes. It is thus very important for the TS determination to remove any interfering of tryptophan. Various modified electrodes have been reported for the determination of TS or tryptophan. Li et al. reported an l-serine polymer modified electrode for the voltammetric determination of TS in pharmaceutical tablets.¹⁰ Huang and his coworkers prepared a multi-walled carbon nanotubes/4-aminobenzenesulfonic acid film-coated glassy carbon electrode for the selective determination of tyrosine in amino acid injection.¹¹ Fang et al. reported a poly(9-aminoureidine) modified glassy carbon electrode for the determination of tyrosine in the presence of tryptophan.¹² Zinola et al. reported a polycrystalline platinum electrode for the electrocatalytic oxidation of tyrosine in alkaline solution.¹³

Nano-scaled particles can be applied to analytical chemistry for their special physicochemical characteristics. Some voltammetric sensors based on gold nanoparticles for the

Scheme 1

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determination of biological molecule, such as cysteine,14 carcinoembryonic antigen,15 norepinephrine16 and protein,17 have received much attention due to their good stability and biocompatibility. Huang et al. developed a glassy carbon electrode modified with poly(3-methylthiophene)/gold nanoparticle for the sensitive determination of dopamine and uric acid.18 Raj et al. reported a gold nanoparticle arrays for voltammetric sensing of dopamine.19 Yang et al. prepared a nano-Au self-assembled glassy carbon electrode for the determination of epinephrine in the presence of ascorbic acid.20 However, to the best of our knowledge, the application of a gold nanoparticles self-assembled glassy carbon electrode as an amino acid electrochemical sensor has not been reported.

In the present work, we demonstrated that there was a good electrochemical response towards TS at a glassy carbon electrode electrochemically pretreated with cysteamine (CA) and functionalized with gold nanoparticles (nano-Au/CA/GCE). Based on this phenomenon we established a sensitive and selective TS determination method. The peak current linearly increases with an increase of the TS concentration in the range of $1.0 \times 10^{-7}$ to $3.0 \times 10^{-4}$ mol L$^{-1}$, with a detection limit of $4.0 \times 10^{-8}$ mol L$^{-1}$. The modified electrode shows good stability and can be applied for the selective determination of TS in the presence of tryptophan. The proposed method can be applied to detect the concentration of TS in a commercial amino acid oral solution.

**Experimental**

**Apparatus and reagents**

Electrochemical measurements were carried out on a CHI 660C electrochemical workstation (Chenhua, China). A three-electrode system was used in an experiment with a bare and a modified glassy carbon electrode (3 mm in diameter) as the working electrode. A saturated calomel electrode (SCE) and a platinum electrode were used as a reference electrode and an auxiliary electrode, respectively. All of the potentials given in this paper were referred to SCE. A magnetic stirrer was used if necessary.

TS hydrochloride and CA hydrochloride were purchased from Sigma and used as received. Gold nanoparticles were prepared with hydrogen tetrachloroaurate (HAuCl$_4$).21 Other reagents were of analytical grade, and used without further purification. A commercial amino acid oral solution containing 18 kinds of amino acids, such as l-arginine, l-alanine, l-glycine, l-valine, l-leucine, l-isoleucine, l-glutamic acid, l-glutamine, l-proline, l-phenylalanine, l-tyrosine, l-tryptophan, l-serine, l-threonine, l-cystine, l-aspartic acid, l-glutamic acid, l-lysine and l-histidine, were purchased from Guangzhou Green Cross Pharmaceutical Co., China. All aqueous solutions were prepared in redistilled water and all electrochemical experiments were carried out at room temperature.

**Preparation of the nano-Au/CA/GCE**

Nano-Au/CA/GCE was prepared according to a former report,20 a bare glassy carbon electrode was polished to a mirror finish with 0.05 μm Al$_2$O$_3$ slurry on emery paper before a modification. It was then rinsed with redistilled water and sonicated in nitric acid (1:1), acetone and redistilled water for 5 min, respectively, then activated by cyclic voltammetry (CV) to stability from −1.0 to 1.0 V at 100 mV s$^{-1}$ in 0.1 mol L$^{-1}$ pH 7.0 PBS, and finally scanned with CV method from −1.2 to 2.5 V at 100 mV s$^{-1}$ for 20 cycles in a 0.1 mol L$^{-1}$ phosphate buffer solution (PBS, pH 7.0) containing 1.0 $\times$ 10$^{-3}$ mol L$^{-1}$ CA. The pretreated GCE was thoroughly rinsed with double-distilled water and immersed in a colloidal gold solution that had been obtained for 10 h at 4°C to acquire the gold nanoparticles self-assembled GCE.

**Results and Discussion**

**Characterization of the modified electrode**

SEM images of CA/GCE and nano-Au/CA/GCE are shown in Fig. 1. It is found that gold nanoparticles have been equably modified onto the CA/GCE, which indicates that they could be chemically fixed to the CA membrane to form a nano-Au self-assembly glass carbon electrode. This is consistent with the literature.20

**Electrochemical behavior of TS at a nano-Au/CA/GCE**

The electrochemical behaviors of TS at three different working electrodes (bare GCE, CA/GCE, nano-Au/CA/GCE) in PBS at pH 7.0 were compared by cyclic voltammetry; the results are shown in Fig. 2. It is found that only one oxidation peak can be seen at three electrodes in the potential range from 0 to 0.9 V. No reduction peak was observed in the reverse scan, which suggests that the electrochemical reaction is a totally irreversible process. As shown in Fig. 2, the oxidation peak of TS at the bare GCE is broad due to its slow electron transfer, and a poorly defined oxidation peak with very low current is observed at +0.801 V (curve a). Also, an oxidation peak at +0.688 V with a larger current is observed at the CA/GCE (curve b). However, under identical conditions, the oxidation peak current of TS at the nano-Au/CA/GCE increases significantly, the anodic peak
current (I_\text{pa}) is the largest among the three electrodes and the oxidation peak potentials is 0.681 V. The probable reason is that the formation of CA film makes the CA/GCE surface be rich in electroactive groups, with a porous structure of the electrode surface. Thus CA/GCE shows better properties than a bare GCE. When fixed with gold nanoparticles, the nano-gold/CA/GCE has excellent conductivity and a larger surface area than CA/GCE, and more TS molecules can be oxidized on the nano-gold/CA/GCE surface. The nano-gold/CA/GCE shows better response towards TS than CA/GCE.

We also explored the relation between the anodic peak current and the scan rate. It is found that the anodic peak current is proportional to the square root of the scan rate in the range of 10 – 210 mV s^{-1}, with a correlation coefficient of 0.998. This result indicates that the electrocatalytic oxidation process of TS at the nano-Au/CA/GCE is controlled by diffusion.\(^{22}\)

**Effect of solution acid and the stability of the modified electrode**

In most cases, the solution pH is an important factor concerning the electrochemical reaction. The effect of the solution pH on the TS response signal was also examined in the range of 3 to 10 (shown in Fig. 3). As shown in Fig. 3A, it is obvious that the anodic peak current increases with an increase of the solution pH until it reaches 6. When the pH value of the solution exceeds 7.0, the anodic peak current rapidly decreases along with an increase of the solution pH. Hence, we chose pH 7.0 as the optimum pH value for electrochemical TS detection. The probable reason may be explained as follows: in a low-pH solution, the H\(^+\) concentration is high enough that the nitrogen atom of the polymer and the phenol hydroxyl of TS are protonated in the forms of \(-\text{NH}_2^+\) and \(-\text{OH}^+\).\(^{23}\) With an increase of the pH, more TS molecules can interact with the nitrogen atom of the polymer, so the anodic peak current becomes larger. At a pH of over 7.0, the TS molecule starts to be oxidized, leading to a decrease of the TS peak current.

Figure 3B shows the relationship between the anodic peak potential of TS and the solution pH. It is found that the potentials (E_{\text{pa}}) shifted negatively with increasing pH, indicating that protons take part in the oxidation process of TS at the nano-Au/CA/GCE. The anodic peak potential (E_{\text{pa}}) is proportional to the solution pH in the range of 3 to 10. The linear-regression equation of TS is described as:

\[
E_{\text{pa}} (V) = 1.096 - 0.0567\text{pH} \tag{1}
\]

with a correlation coefficient of 0.998, indicating that the electrode process is equal proton-electron transfer. Thus, the probable mechanism is as follows:

\[
\text{H}_2\text{O} + \text{C}_4\text{H}_6\text{N}_2\text{O}_2 \rightarrow \text{C}_4\text{H}_5\text{N}_2\text{O}_2^+ + \text{H}^+ 
\]

The stability of nano-Au/CA/GCE is a very import factor for its real application. The nano-Au/CA/GCE was stored in the 0.10 mol L\(^{-1}\) PBS (pH 7.0) after each electrochemical determination of TS. The CV method was carried out once a day under the same operation conditions. The oxidation current of TS at the CA/GCE decreased to 35% two weeks later. However, at the nano-Au/CA/GCE, the oxidation currents of TS did not change for almost two month, indicating a nicer stability of the latter.

**Differential pulse voltammetric determination of TS**

The differential pulse voltammetry (DPV) method is usually used for the determination of test samples because of its high sensitivity.\(^{24-26}\) A TS concentration determination was performed with the DPV method. Figure 4 shows the dependence of the anodic peak current on TS concentration in 0.1 mol L\(^{-1}\) PBS at pH 7.0. The results prove that the anodic peak current is linear with the TS concentration in the range of 1.0 \times 10^{-7} to 3.0 \times 10^{-4} mol L\(^{-1}\).
mol L^{-1} under the optimum conditions. The linear regression equation is described as

$$I_p (\mu A) = 0.0473 + 0.989C_{TS} \times 10^{-5} \text{ mol/L}$$

with a correlation coefficient of 0.996. The detection limit is 4.0 \times 10^{-8} \text{ mol L}^{-1} (S = 3).

Amperometric response of TS was also investigated. The response current (see Fig. 5) was obtained at a fixed potential of +0.7 V via successive additions of 0.5 \mu mol TS into a stirring PBS (pH 7.0). The nearly equal current step for each addition of TS demonstrates a stable and efficient electrocatalytic oxidation of TS at the nano-Au/CA/GCE. The oxidation peak current linearly increases with an increase of the TS concentration, showing that this kind of electrode possesses a rapid response to TS, with a response time of less than 5 s. Meanwhile, a low noise level accompanies the favorable signals. Thus, the nano-Au/CA/GCE can be used as a highly sensitive amperometric sensor for TS detection.

**Influence of interruption**

Among the amino acids, the anodic potential of tryptophan is near to that of TS. Under the optimum conditions, the interference of tryptophan at the nano-Au/CA/GCE is shown in Fig. 6. Since the peak potential of tryptophan is at 0.98 V on the modified electrode, the oxidation peak of tryptophan is not observed at the selected potential window. It was found that a 80-fold excess of tryptophan had no substantial effect on the determination of TS. Furthermore, 100 fold of 18 amino acids showed no effect in our experiments. The influence of other substances on the oxidation currents of TS was also investigated. The experimental results showed that no interference occurred in the presence of the following: 1000 fold sodium nitrate, 1000 fold potassium chloride, 800 fold Ca^{2+}, 750 fold Br^{-}, 500 fold SO_{4}^{2-}, 100 fold ascorbic acid, with the deviations below 5%.

**Analysis application**

The TS content in a commercial amino acid oral solution was determined with the nano-Au/CA/GCE. The DPV method was used to detect the TS concentration with a standard-addition method. The results are listed in Table 1, suggesting that the nano-Au/CA/GCE is quite reliable, selective and sensitive enough for TS determination in real samples.

**Fig. 5** Amperometric response of TS on the nano-Au/CA/GCE in 0.1 mol L^{-1} PBS; each addition, 0.5 \mu mol L^{-1}.

**Fig. 6** CV voltammograms of 1.0 \times 10^{-6} \text{ mol L}^{-1} TS at a modified electrode without (a) and with (b) 2.0 \times 10^{-4} \text{ mol L}^{-1} tryptophan in a mixture of 1.0 \times 10^{-5} \text{ mol L}^{-1} 18 amino acids; scan rate, 100 mV s^{-1}.

<table>
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<th>Sample</th>
<th>Labeled/ mg L^{-1}</th>
<th>Found/ mg L^{-1}</th>
<th>RSD, %</th>
<th>Recovery, %</th>
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<tr>
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<td>0.495</td>
<td>1.8</td>
<td>99.9</td>
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<tr>
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</table>

**Conclusion**

The electrochemical response of TS at a gold nanoparticles self-assembled glassy carbon electrode was investigated. The nano-Au self-assembly modified glassy carbon electrode showed excellent response to TS. Tryptophan does not disturb the accurate determination of TS, illuminating that the nano-Au self-assembled glassy carbon electrode can be used for TS determination among 18 amino acids. The proposed method has been successfully applied for the selective and sensitive determination of TS in a commercial amino acid oral solution.

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**References**