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ORIGINAL ARTICLE

Side-chain oxidative damage to cysteine on a glassy carbon electrode

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Abstract In this paper, oxidative damage to the cysteine (CySH) side-chain on a glassy carbon electrode (GCE) was investigated. Voltammetric studies show that there are three anodic peaks for the oxidation of CySH, which arise from (1) the oxidation of the –SH side-chain, forming cystine (0.71 V, vs. SCE) and (2) $CySO_xH$, $x = 2$, 3 (0.98 V vs. SCE), and (3) the oxidation of the amino acid carboxyl group (around 1.51 V vs. SCE). The influence of dissolved oxygen, pH, scan rate, scan time, temperature and CySH concentration were investigated and the oxidative mechanism proposed. The peaks near 0.71 and 0.98 V are the promising candidates for measuring the oxidation of CySH on the GCE. This paper provides a new strategy for researching oxidative damage of amino acids, sulfur-containing peptides and proteins.

Keywords Cycle volt-ampere · Glassy carbon electrode · Cysteine · Oxidative damage

Introduction

There is increased interest in the redox reactions of various organic sulfur-containing compounds in cellular homeostasis and metabolism (Gazit et al. 2004; Davies 2005). CySH is the simplest sulfur-containing amino acid and plays important roles in protein structure and function (Hammermeister et al. 2000; Xu and Chance 2005a, b). It is widely used as a model for evaluating the toxicity of chemical contaminants and the function of thiol groups in peptides and proteins (Xu and Chance 2005a, b; Dean et al. 1997).

Numerous analytical methods have been developed for the analysis of amino acids, including capillary electrophoresis (Fan et al. 2007), ion-exchange chromatography (Zhou et al. 2007a, b; Sun et al. 2007), gas chromatography (Sun et al. 2007; Meng et al. 2000), liquid chromatography (Davey and Ersser 1990) and mass spectrometry (Hammermeister et al. 2000; Yu and Mou 2005). Ultraviolet, fluorescent and chemical luminescent detectors are mainly used in the above methods (Hammermeister et al. 2000; Fan et al. 2007; Yu and Mou 2005). Because CySH has the disadvantages of weak ultraviolet and fluorescent absorption, low volatility, and short retention time, derivation is needed to improve sensitivity and selectivity (Sun et al. 2007; Liu and Li 2003; Ding and Mou 2004). But derivation complicates CySH determination and brings in new contaminants.

Electrochemistry is simple to perform, highly sensitive, and requires no radioactive or toxic chemical additives (Liu and Pang 2002; Liu and Wu 2006). Electrochemical studies can simulate oxidative damage to CySH in vitro and in vivo. Prior research on cysteine has mostly been performed on mercury electrodes (Yu and Mou 2005), the Au electrode (Liu and Wu 2006; Tudös and Johnson 1995; Nazmutdinov et al. 2006) and modified electrodes (Spătaru et al. 2001; Zhou et al. 2007a, b) and little work has been done related to cysteine/cystine electrochemistry on the bare GCE. In this paper, the oxidation of CySH in 0.1 mol/L phosphate buffer was thoroughly studied with a GCE. The influences of dissolved oxygen, pH, scan rate,

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temperature and CySH concentration were also investigated. The GCE exhibits excellent behavior for the studies of CySH oxidation and provides a new strategy for researching oxidative damage to amino acids, sulfur-containing peptides and proteins.

Experimental section

Reagents and apparatus

Cysteine, cystine, glycine (Sinopharm Chemical Reagent Co. Ltd, Shanghai, China); H_3PO_4 (Tianjin Tianda Chemical Reagent Co. Ltd); ethanol, and Na_2HPO_4 (Tianjin Guangcheng Chemical Reagent Co. Ltd) were used as supplied.

A CHI 600A Electrochemistry Workstation (Shanghai Chenhua Instrument Co. Ltd) was used for all the experiments and was equipped with a standard tri-electrode system which consisted of a glassy carbon working electrode (CHI104 GCE), a saturated calomel reference electrode (SCE), and a platinum wire counter electrode. The pH buffers were prepared using a pHs-3C pH meter (Shanghai Pengshun Scientific Instrument Co. Ltd); Samples were degassed in a 2XZ-0.5 vacuum pump and stored in a vacuum desiccator.

Pretreatment of glassy carbon electrode

The GC electrode was polished using 1, 0.3 and 0.05 μm α-Al_2O_3 powders, successively (Li et al. 2005). After ultrasonic cleaning in distilled water, the GC electrode was stored in ethanol (Fu et al. 2004).

Experimental method

A series of 0.1 mol L^{-1} phosphate buffers (pH 5–8) were prepared. Selected amounts of CySH were dissolved in the phosphate buffers and then scanned in the tri-electrode cell. All solutions were degassed under vacuum before scanning. Scans were performed from −2 to +2 V at scan rates from 25 to 250 mV/s etc.

Results and discussion

Cyclic voltammograms

The cyclic voltammograms of phosphate buffer, cystine and CySH are shown in Fig. 1.

For phosphate buffer alone (Fig. 1, curve a), the oxidative peak near 0.46 V and the reductive peak near −0.61 V are the redox peaks of GCE (phosphate buffer

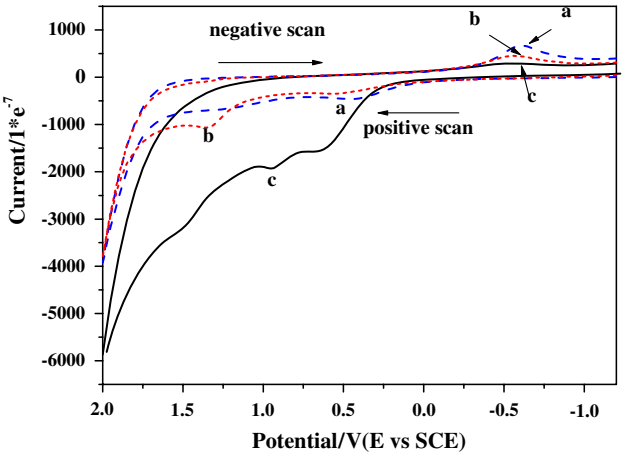

Fig. 1 Cyclic voltammograms of phosphate buffer, cystine and CySH. Conditions: *curve a* 0.1 mol/L phosphate buffer, *curve b* cystine saturated solution, *curve c* 0.01 mol/L CySH, scan rate 100 mV/s, scan range −2.0 to +2.0 V, scan time 10th, pH 6.0, temperature 25°C

cannot be oxidized at such a low potential). The oxidative peak arises from the formation of oxygen-containing groups (for example, hydroxybenzene and carboxyl) and the reductive peak corresponds to the reductive process (Yang and Lin 1994; Zhang et al. 1996).

From the voltammogram of cystine (Fig. 1 curve b), it can be seen that the current of the reductive peak of GCE is lower, meaning a lower oxidative peak than that for phosphate buffer alone. The redox process of GCE is restrained by cystine. Saturated cystine has only one characteristic oxidative peak near 1.36 V, indicating it is an irreversible process. The oxidative peak comes from the oxidation of the S–S bond (Xu and Chance 2005; Tudös and Johnson 1995). This reaction occurs by the following mechanism:

$$\mathrm{CySSCy} + \mathrm{H_2O} \rightarrow \mathrm{CySO}_x\mathrm{H} + \mathrm{e^-} + \mathrm{H^+} \quad (1)$$

CySH (Fig. 1, curve c) has three oxidative peaks and their potentials are near 0.71, 0.98 and 1.51 V. Compared with curves a and b, the lowered GCE reduction current means a lower background. The first oxidative peak, close to 0.71 V, is due to the oxidation of –SH, forming cystine (Liu and Wu 2006; Spătaru et al. 2001). At pH 6, the main form of cysteine is CySH (Liu and Wu 2006). A possible mechanism can be proposed as follows:

$$\mathrm{CySH} \rightarrow \mathrm{CyS^-} + \mathrm{H^+} \quad (2)$$

$$\mathrm{CyS^-} \rightarrow \mathrm{CyS}\cdot + \mathrm{e^-} \quad (3)$$

$$\mathrm{CyS}\cdot + \mathrm{CyS}\cdot \rightarrow \mathrm{CySSCy} \quad (4)$$

Because the oxidative peak of saturated cystine is near 1.36 V, the oxidative peak close to 0.98 V arises from the further oxidation of CySH, forming sulfonic acid and

sulfinic acid (Zen et al. 2001). It is unrelated to the oxidation of the cystine formed from cysteine.

Reactions are as follows:

$$\mathrm{CyS}\cdot + 2\mathrm{H_2O} \rightarrow \mathrm{CySO}_x\mathrm{H} + \mathrm{H}^+ + \mathrm{e}(x = 2, 3) \quad (5)$$

$$\mathrm{CyS}^- + 2\mathrm{H_2O} \rightarrow \mathrm{CySO}_x\mathrm{H} + \mathrm{H}^+ + \mathrm{e}(x = 2, 3) \quad (6)$$

Under the same conditions, glycine has only one oxidative peak (near 1.5 V), which is due to the oxidation of carboxyl. So the oxidative peak close to 1.51 V can be assigned to the oxidation of the carboxyl in CySH. There is only one free carboxyl per peptide or protein molecule, except the carboxyls from the Glu or Asp side-chains. In addition, the activity of carboxyl side-chain is not very high (Xu and Chance 2004). Therefore, the oxidation of carboxyl groups in amino acids, peptides and proteins is rarely reported.

The influence of dissolved oxygen, number of scans, scan rate, pH and temperature on the redox process

Influence of dissolved oxygen

With vacuum deaeration, the potential of the oxidative peaks shifts negatively (Fig. 2) and the currents increase slightly, but the potential and current of the reductive peaks level off. O_2 blocks the oxidation of CySH because it can adhere to the GCE and change the external redox state (Liu et al. 2003). To get reliable and steady results, all samples should be degassed before scanning.

The influence of scan times

With the increase of scan cycles, the currents of all peaks gradually increase. The number of scans has a strong influence on the potential of the oxidative peak at 0.71 V, but has less influence on other redox peaks (Fig. 3). Hence, the oxidative peak around 0.71 V was chosen for further study.

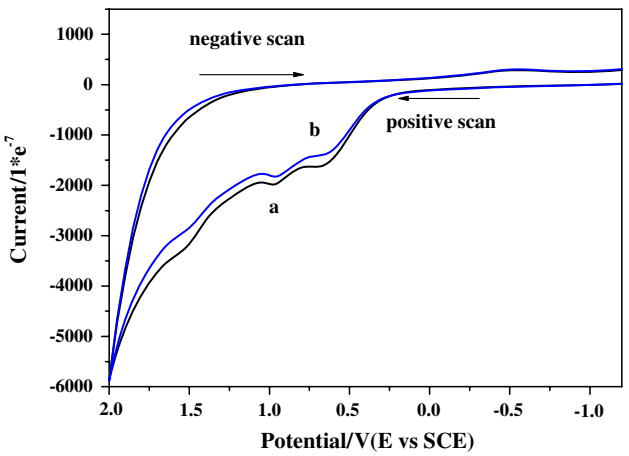

Fig. 2 Influence of dissolved oxygen on the redox process. Conditions: *curve a* 0.01 mol/L CySH (with vacuum disposal), *curve b* 0.01 mol/L CySH (without vacuum disposal), scan rate 100 mV/s, scan range −2.0 to +2.0 V, scan time 10th, temperature 25°C, pH 6.0

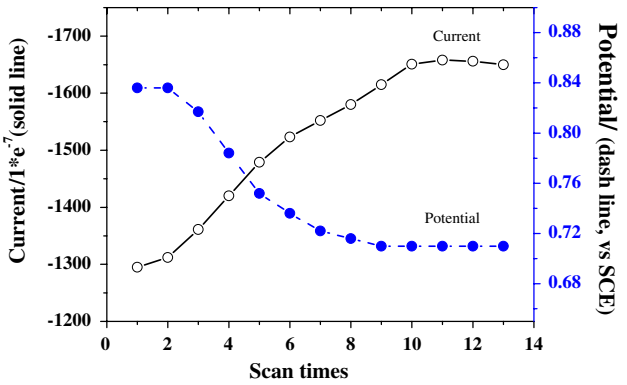

Fig. 3 Influence of number of scans on the oxidative peak near 0.71 V. Conditions: 0.01 mol/L CySH, scan rate 100 mV/s, scan range −2.0 to +2.0 V, pH 6.0, temperature 25°C

In Fig. 3, as the number of scans increases, the potential reduces, the current enhances, and both level off after the tenth scan (0.71 V, 1.65×10^{-4} A). The redox state of GCE is restricted by the electrochemical process and certainly influences the oxidation of CySH. After ten scan cycles, the results are steady, so we selected the tenth scan of each further experiment for analysis and discussion.

The influence of scan rate

The influence of scan rate on the redox peaks is shown in Fig. 4.

The currents of redox peaks and $v^{1/2}$ have a linear relationship. For example, the linear regression curve of peak a is $i_p = -417.17 - 102.2\ v^{1/2}$ (i_p: 10^{-7} A, v: mV/s), $R = 0.9985$. We also find that the currents of the redox peaks and scan rate are linearly related. Taking peak a as an example, the linear regression curve is $i_p = -904.3 - 4.76\ v$ (i_p: 10^{-7} A, v: mV/s), $R = 0.9857$. These results make it clear that the oxidation of CySH and the redox processes of GCE are controlled by diffusion and adsorption (Huang et al. 2004). The phenomena relates to the high concentration of CySH. The peak value of line b in Fig. 4 also proves this explanation. Taking efficiency and stability into account, 100 mV/s was chosen as the most suitable scan rate.

The influence of pH

pH has clear influence on the oxidation peaks at 0.71 and 0.98 V, but has little influence on the peaks at 1.51 and −0.54 V. For the oxidation peaks at 0.71 and 0.98 V, the current reaches a maximum value at pH 7.4 (Fig. 5). These

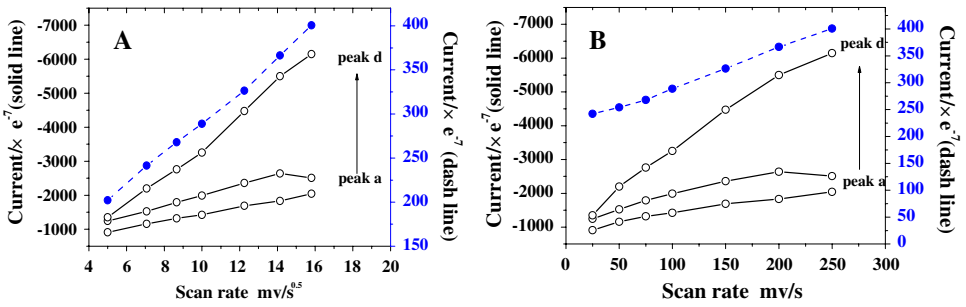

Fig. 4 Influence of scan rate on the redox process. Conditions: 0.01 mol/L CySH, *peak a* (around 0.71 V), *peak b* (around 0.98 V), *peak c* (around 1.51 V), *peak d* (around −0.54 V), scan rate 100 mV/s, scan range −2.0 to +2.0 V, scan time 10th, pH 6.0, temperature 25°C

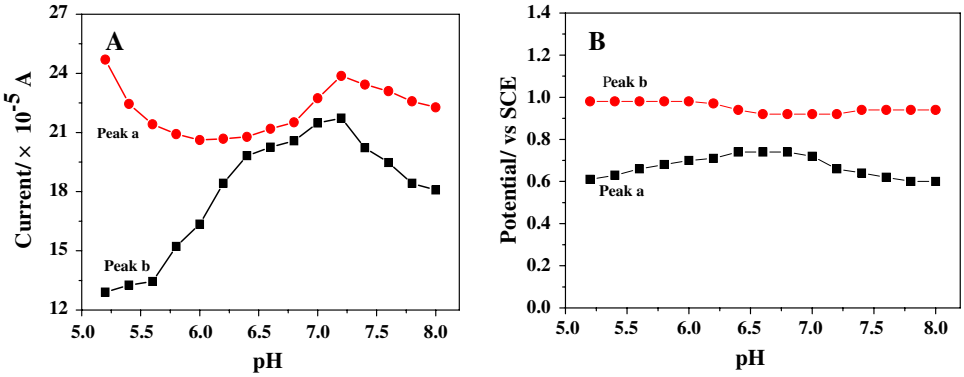

Fig. 5 Influence of pH on the redox process. Conditions: 0.01 mol/L CySH, *peak a* (around 0.71 V), *peak b* (around 0.98 V), scan rate 100 mV/s, scan range −2.0 to +2.0 V, scan time 10th, temperature 25°C

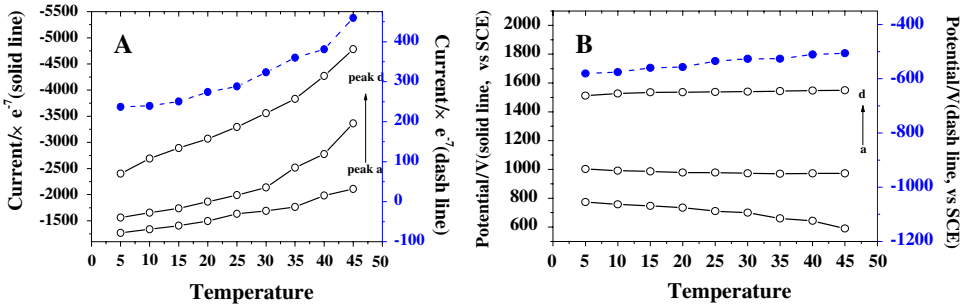

Fig. 6 Influence of temperature on the redox process. Conditions: CySH 0.01 mol/L, *peak a* (around 0.71 V), *peak b* (around 0.98 V), *peak c* (around 1.51 V), *peak d* (around −0.54 V), scan rate 100 mV/s, scan range −2.0 to +2.0 V, scan time 10th, pH 6.0

results are due to the structural changes of CySH at different pH (Spătaru et al. 2001; Zen et al. 2001). At low pH (5–5.6), CySH is the main form and it is resistant to self-oxidation, corresponding to a reduced current. With the increase of pH, CyS^- increases and the oxidation of CySH is promoted, meaning a higher current. Then CyS^{2-} increases, inhibiting the adsorption of CyS^-. So the current drops back down above neutral pH.

From Fig. 5, we can also see that pH has a regular effect on the potentials of both the peaks. All the oxidative peaks of CySH have favorable contrast when the pH is adjusted between 5.00 and 6.40, so pH 6.00 was chosen as a suitable experimental condition.

The influence of temperature

The influence of temperature on the oxidative current is shown in Fig. 6.

When the temperature increases from 5.0 to 45.0°C, the oxidative peaks shift in the negative direction and their currents gradually increase. A higher temperature can promote the diffusion of cysteine onto GCE, thus enhancing the redox processes on the GCE. This result is consistent with the diffusion-controlled redox processes occurring at the GCE. CySH is spontaneously oxidized at higher temperature, so the temperature is controlled around 25°C in our experiment.

The effect of CySH concentration on the cyclic voltammograms

To investigate the mechanism of oxidative damage of CySH on the glassy carbon electrode, the influence of CySH concentration was determined (Fig. 7).

At higher CySH concentrations, the currents of the oxidative peaks increase, but the reductive peak falls. The explanation for this fall is that the oxidation of CySH

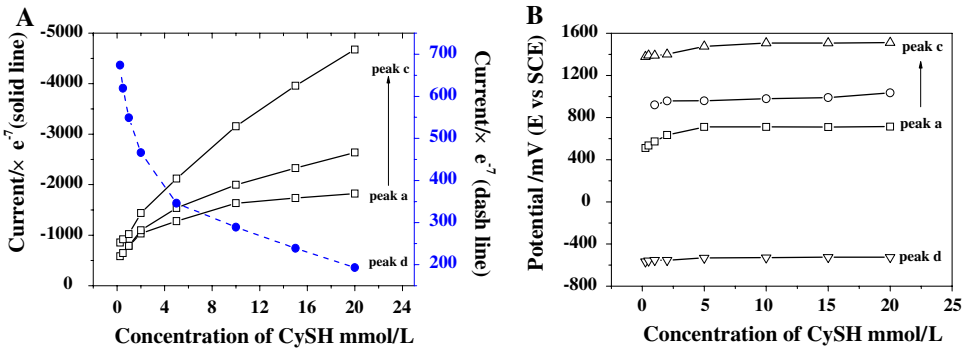

Fig. 7 Cyclic voltammograms of cysteine at different concentrations. Conditions: *peak a* (around 0.71 V), *peak b* (around 0.98 V), *peak c* (around 1.51 V), *peak d* (around −0.54 V), scan rate 100 mV/s, scan range −2.0 to +2.0 V, scan time 10th, pH 6.0, temperature 25°C

restrains the redox process of GCE (according to the result in "Cyclic voltammograms"). And when the concentration reaches 0.015 mol/L, the current of peak a levels off,indicating that reactions 2–4 are controlled by adsorption. The currents of peak b and peak c are limited by the concentration in the selected range. The potentials of all peaks first increase, then level off. The phosphate buffers have a distinct influence on the results when the CySH concentration is low.

Conclusions

We presented here an electrochemical method that focuses on the oxidative damage of CySH side-chain on glassy carbon electrode. Results demonstrate that CySH has three characteristic oxidative peaks and the oxidation mainly occurs by the –SH side-chain (around 0.71, 0.98 V) and the carboxyl terminus (around 1.51 V). The oxidative products of –SH include cystine (0.71 V) and $CySO_xH$, $x = 2$, 3 (0.98 V). Both the peaks are promising candidates for measuring oxidative damage to CySH in peptides and proteins. This paper provides a novel strategy for researches on the oxidative damage mechanisms of sulfur-containing peptides or proteins and simulating the oxidative stress process in biologic tissues or cells.

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