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Voltammetric investigation of cytochrome *c* on gold coated with a self-assembled glutathione monolayer

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Abstract

The direct, reversible electrochemistry of horse-heart cytochrome *c* (cyt. *c*) was realized on a self-assembled glutathione (GSH) monolayer modified Au electrode. The voltammetric responses of cyt. *c* on GSH/Au electrode were found to be affected by pH during the electrode modification, metal ions and surfactants. Using potassium ferricyanide [$K_4Fe(CN)_6$] as a probe, these effects on the voltammetric responses of cyt. *c* were characterized by electrochemical methods. It was found that the pH during the electrode modification, metallic ions and surfactants changed GSH monolayer's charge state and the conformation on the electrode surface, and resulted in the influence on the voltammetric responses of cyt. *c*. The experimental results provided us information to understand the mechanism of the interfacial electron transfer of electrode-protein, as well as the electron transfer of cyt. *c* in life system.

Keywords: Cytochrome *c*; Glutathione; Self-assembled monolayers; Direct electrochemistry

1. Introduction

Self-assembled monolayers (SAMs) were thought to be a good model for the investigation to some kinds of interfaces, such as biomembrane [1,2]. Its characteristics of molecular size, structure model and natural formation are very similar to those of natural biomembrane. Redox proteins can be adsorbed via electrostatic interaction or covalently immobilized on SAM, which can avoid the influence of the mass transfer, and these systems display diffusionless electron-transfer processes. On the other hand, the distance between the electroactive group of the protein and the electrode can be regulated by changing the chain length of self-assembled molecules. Thus, the mechanism of the interfacial electron transfer of redox proteins is appropriate to be studied on the self-assembled monolayer modified electrode. Among various redox proteins, cyt. *c* has been widely studied on the SAM modified electrodes [3–7]. Song et al. have reported the value data of cyt. *c*, such as the surface formal potential, electron transfer rate, electron transfer distance and the reorganization energy for electron transfer on the 16-mercaptohexadecanoic acid, 11-mercaptoundecanoic acid and 6-mercaptohexanoic acid composite monolayer [5].

Glutathione is a well-known biological active tripeptide, which participates in the various functions of biological processes [8], for example, maintenance of normal redox potentials in the cells [9], radical scavenger [10], detoxification of various cytotoxic compounds [11], antioxidant [12], and so on. GSH molecule possesses three polar heads (two carboxyls and one amino group) besides its cysteinyl residue, which can act as the anchor on the Au electrode surface. The polar terminate heads of GSH are the key to interact with hydrogen or metallic ions [13,14]. Sometimes, it can also interact with some surfactants as reported by Wang et al. [15]. Fang and Zhou have used glutathione modified Au electrode to investigate its electrocatalytic oxidation of NADH [16] and its complexation with Cu^{2+} [17].

In this paper, the direct electrochemistry of cyt. *c* on the GSH SAM modified Au electrode was realized. GSH

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monolayer provided a good model of interfacial film to study the direct electrochemistry of cyt. *c*. GSH molecule not only has three polar tails facing to the solution, but also it has some degree of hydrophobicity. These characteristics make the charge state and conformation of GSH monolayer on Au electrode easily affected by pH during the electrode modification, metal ions and surfactants. In our experiment, these effects were investigated by electrochemical methods with potassium ferricyanide [$K_4Fe(CN)_6$] as a probe. It was found that the change of GSH monolayer on Au electrode surface led to the corresponding change of voltammetric responses of cyt. *c* on GSH/Au electrodes. The results provided us some useful information about the electrode-protein interfacial characteristics and helped us to understand the electron transfer process of cyt. *c* in life system.

2. Experimental

2.1. Reagents

The horse-heart cytochrome *c* and glutathione (γ-L-glutamyl-L-cysteinyl-glycine) were purchased from Sigma and used without further purification. Cytochrome *c* solutions (1.6×10^{-5} M) were made up in phosphate buffer or tris–HCl buffer (pH 7.0,) and stored in refrigerator at 4 °C. Glutathione solutions (1×10^{-2} M) were dissolved in phosphate or tris–HCl buffer at given pH. Cetyltrimethylammonium bromide (CTAB), sodium dodecyl benzene sulfonate (SDBS) and triton X-100 (purchased from Shanghai Reagent Factory, China) were dissolved in water aided by ultrasonication to prepare 1×10^{-2} M stock solutions. Other chemicals were of analytical grade and doubly distilled water was used throughout. The pH value of buffer was adjusted by adding appropriate NaOH or HCl.

2.2. Apparatus

All cyclic voltammetry experiments were performed with a computer controlled Model CHI 830A electrochemical analyzer (ChengHua Instrument Co., ShangHai, China). A conventional three-electrode cell was employed with a glutathione SAM-modified Au disk electrode (CHI, 2 mm in diameter) as working electrode, a platinum wire as counter electrode and a saturated calomel electrode (SCE) as reference electrode. All experiments were done under room temperature in conventional electrochemical cell. All experimental solutions were deaerated by bubbling nitrogen for 15 min, and a nitrogen atmosphere was kept over the solution during measurements.

For EIS experiments, a Model RDE 5 Bi-potentiostat (Princeton Applied Research, USA) and a Model 5210 Lock-in Amplifier interfaced to an EG and G 273 A Potentiostat/Galvanostat (Princeton Applied Research, USA) were used. The impedance measurements were performed in the presence of equimolar of 5 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ as a redox probe. The supporting electrolyte was 0.1 M KCl. The EIS experiments were done at the formal potential (0.20 V) at an amplitude of 5.0 mV (rms) with a wide frequency range of 100 KHz to 0.1 Hz.

2.3. Preparation of glutathione SAM modified gold electrode

The gold electrode was firstly polished on a slush of 0.05 μm alumina (Al_2O_3). Then this electrode was sonicated in water for 5 min and scanned in 1 M H_2SO_4 in the potential range of −0.2 to 1.5 V until stable signals were obtained. The area of the reduction peak at each bare gold electrode was compared to ensure that all the SAM-modified gold electrodes have the same effective area. Then the gold electrode was sonicated for 5 min in ethanol and water, successively. The above electrode was modified by dipping it into 10 mM glutathione solution for 30 min, followed by rinsing with doubly distilled water thoroughly before experiment. The voltammetric response of cyt. *c* was examined to be unaffected by the immersion time for periods of longer than 30 min.

3. Results and discussion

3.1. Direct electrochemistry of cytochrome c on the glutathione SAM modified Au electrode

The cyclic voltammograms of cyt. *c* at a bare Au electrode, and one modified by 10 mM glutathione in phosphate buffer are shown in Fig. 1. No electrochemistry attributable to cyt. *c* is observed at bare Au electrode (curve a), this shows that electron transfer between the bare Au electrode and cyt. *c* in this solution occurs very slowly or not at all. The electrochemical behavior of cyt. *c* may be different if cyt. *c* is further purified. When the Au electrode

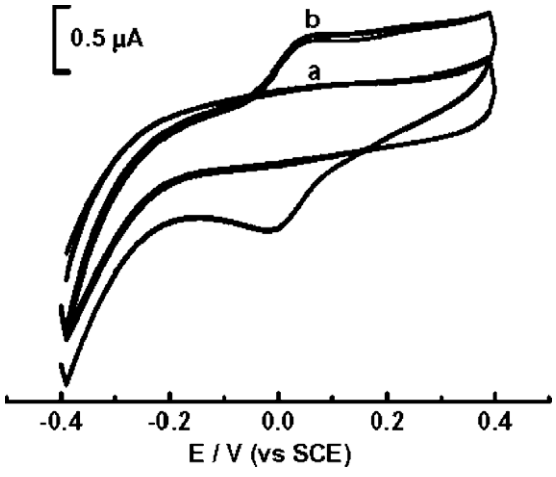


Fig. 1. Cyclic voltammograms of 8.0×10^{-5} M cytochrome *c* in pH 7.0 phosphate buffer on bare Au electrode (a); GSH/Au electrode (b). Scan rate 50 mV s^{-1}.

modified with glutathione, the electrochemistry of cyt. *c* is well behaved (curve b). This indicated that glutathione SAM on the electrode surface could act as "promotor" of electron transfer in the electrochemistry of cyt. *c* at the Au electrode. The cyclic voltammogram had a peak separation of 50 mV, at a scan rate of 50 mV s^{-1}, which was consistent with a reversible one-electron transfer. The formal potential ($E^{o'}$) of the reaction in 0.06 M phosphate buffer (pH 7.0) was 25 mV, a value similar to others reported [5]. The peak current gradually increased with the scan number in the first 3 cycles, then remained stable.

With the increase of the scan rate the peak-to-peak separation remains constant (Fig. 2), and both the anodic and cathodic peak currents are linear with the scan rates (the inset of Fig. 2), indicating a typical adsorption-controlled electrode process. Increasing the ion strength of the phosphate buffer, the redox peaks attenuated gradually, and the peaks completely vanished when the phosphate concentration was raised to 0.2 M, indicating the desorption of cyt. *c* from electrode.These experimental results can be taken as an indication that cyt. *c* adsorbed on the electrode surface through electrostatic immobilization. After immersing GSH/Au electrode in cyt. *c* solution for 10 min and transferring it to pure buffer solution, no cyt. *c* response was observed, indicating that the binging between GSH and cyt. *c* was reversible and weak.

3.2. Various influences on the direct electrochemistry of cytochrome c at GSH/Au electrodes

It is well known that a surface modifier, which promotes the electrochemistry of cyt. *c* at Au electrode, at least two functional groups are required, one of which, X, binds to the surfaces of the Au electrode and other, Y, interacts favorably with the electron-transfer domain of cyt. *c* [18]. The molecule structure of GSH and its diagram confined on an Au electrode surface are illustrated in Scheme 1. The sulfur

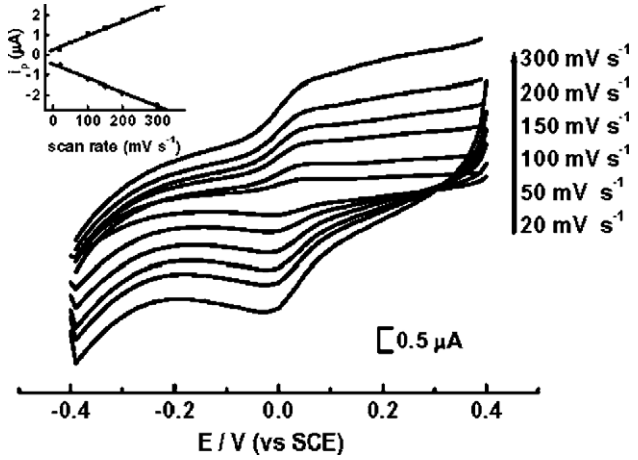
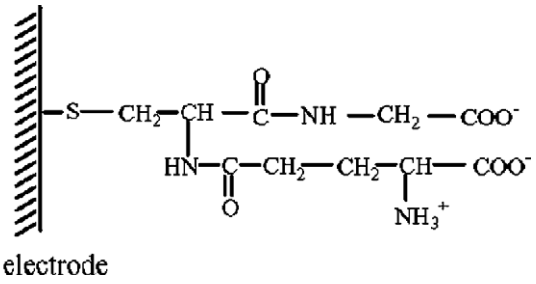


Fig. 2. Cyclic voltammograms of 8.0×10^{-5} M cytochrome *c* in pH 7.0 phosphate buffer on GSH/Au electrode at the scan rate of 20, 50, 100, 150, 200, 300 mV s^{-1}. Inset is the plot of cathodic and anodic peak currents vs. the scan rate.



Scheme 1. Schematic illustration of GSH molecule confined on an Au electrode surface.

atom of the cysteinyl residue is anchored on the Au electrode surface, and the three polar heads of two carboxyls and one amino groups face to the solution. The three polar heads play an important role to interact with the binding sites associated with electron transfer on cyt. *c* [3]. When the pH during the electrode modification is changed or metal ions or surfactants are added to the cyt. *c* solution, the charge state and conformation of GSH SAM on the electrode surface are altered, which influence the interaction of the GSH with cyt. *c*, resulting in the effect on the voltammetric responses of cyt. *c*. In order to study well on these influences, potassium ferricyanide was used as a probe to characterize the GSH SAM simultaneously.

3.2.1. Effect of pH during the electrode modification

Effect of GSH SAM prepared in different pH phosphate buffer on inhibitive efficiency to ferricyanide ions was examined. Fig. 3 shows the cyclic voltammograms of 0.5 mM $K_4Fe(CN)_6$ in 0.1 M KCl solution on a bare Au electrode (solid line) and the GSH/Au electrodes (dashed

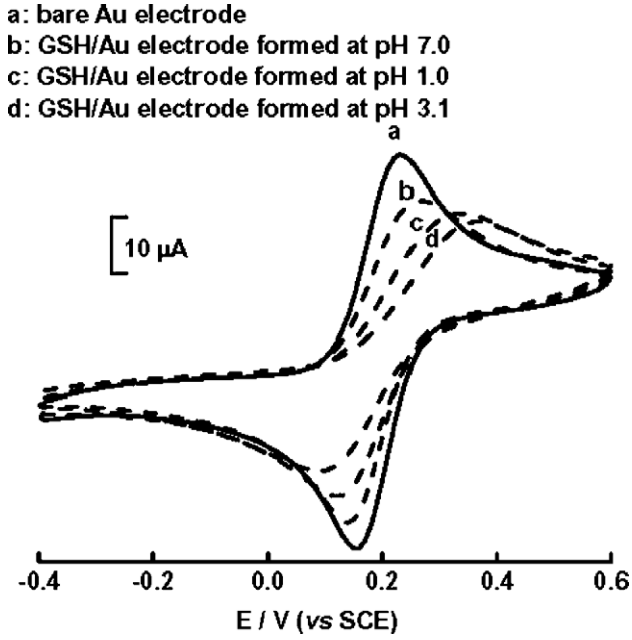


Fig. 3. Cyclic voltammograms of 5×10^{-4} M $K_4[Fe(CN)_6]$+0.1 M KCl on bare Au electrode (a) and on GSH/Au electrodes which modified at pH 7.0 (b); pH 1.0 (c); pH 3.1 (d). Scan rate 50 mV s^{-1}.

line). Comparing with the bare Au electrode (curve a), GSH/Au electrodes make ΔE_p larger and decrease the CV response, which indicates that GSH has assembled on the Au electrode surface and acts as a blocking layer to $Fe(CN)_6^{4-}$. But it is interesting that GSH prepared at different pH has a different blocking behavior to $Fe(CN)_6^{4-}$. At pH 3.1 GSH SAM produces the most drastic decrease in the peak current and the largest ΔE_p of $Fe(CN)_6^{4-}$ in the CV response (curve d). Comparatively, GSH SAM formed at pH 7.0 has the least effect on $Fe(CN)_6^{4-}$ in the CV response (curve b).

To investigate the structure of the GSH monolayer on the electrode surface prepared at various pH, EIS was performed. The corresponding impedance spectra (Nyguist plot) are shown in Fig. 4 and the R_{ct} (charge transfer resistance) can be estimated. The average results based on seven replicate determinations are listed in Table 1.

From the values of R_{ct}, the apparent electrode coverage (θ) of GSH SAM on the Au electrode surface can be approximately calculated [19]:

$$\theta = 1 - (R_{ct}/R'_{ct})$$

where R_{ct} is the redox probe on a bare Au electrode and R'_{ct} is the corresponding probe on the GSH/Au electrodes. The value of R_{ct} on a bare Au electrode is about 40.92 Ωcm^{-2} (assuming the transfer coefficient α as 0.5) [19].

The cyclic voltammetry of cyt. *c* in pH 7.0 phosphate buffer on the different pH prepared GSH/Au electrodes was investigated and the results are shown in Fig. 5. The redox peak potential of cyt. *c* on all modified electrodes is the same, but the peak current makes a difference. On the contrary to $Fe(CN)_6^{4-}$, the largest voltammetric response comes from the GSH/Au electrode modified at pH 3.1 (curve c), and the GSH/Au electrode modified at pH 7.0 inhibits the response of cyt. *c* mostly (curve a).

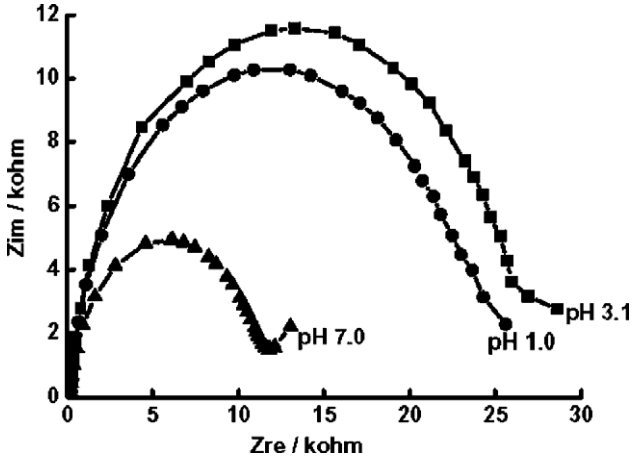


Fig. 4. Electrochemical impedance plots of 0.1 mM $Fe(CN)_6^{3-}/Fe(CN)_6^{4-}$ on the GSH/Au electrodes which modified at pH 3.1; pH 1.0; and pH 7.0 with 0.1 M KCl as the supporting electrolyte. The electrode potential was 0.20 V vs. SCE; the frequency was from 100 KHz to 0.1 Hz and the amplitude was 5.0 mV (rms).

Table 1
Different parameters obtained for the GSH/Au electrodes modified at various pH

pH when modified Au electrode	pH 1.0	pH 3.1	pH 7.0
Charge transfer resistance, R_{ct} ($\Omega \cdot cm^{-2}$)[a]	23070	25680	10890
Apparent electrode coverage, θ (%)	99.82	99.85	99.63

[a] Average of seven replicate determinations.

The acid dissociation constants of the two carboxyl terminals and the amino terminal of GSH were reported to be 2.4±0.2, 3.6±0.2 and 8.8±0.4, respectively [20], which indicated that at pH 3.1 only one of the carboxyl terminals of GSH deprotonated and the whole GSH molecule was assumed to have no net charge, so that there was no electrostatic repulsion interaction between GSH molecules during the monolayer formation process and accordingly a more packed monolayer was obtained. On the contrary, at pH of 1.0 or 7.0, the GSH had a positive net charge or a negative net charge. When the net charged GSH molecules were attached on the electrode surface, it was obvious that there existed electrostatic repulsion between GSH molecules, resulting in less concentration of GSH and a looser monolayer on the electrode surface. It was noticed that the apparent electrode coverage (θ) of GSH monolayer formed at pH 7.0 is less than that at pH 1.0. The each GSH molecule has two negative charges due to the deprotonated of the two carboxyl groups and a positive charge due to the protonation of the amino group at pH 7.0. So there was more complicated electrostatic interaction within GSH molecules at pH 7.0 than at pH 1.0 because the GSH molecule had only one positive charge on amino group at pH 1.0.

Speculating on the different influence of GSH SAM prepared at various pH on the voltammetric response between cyt. *c* and $Fe(CN)_6^{4-}$, conformation of GSH SAM rather than its charge states are the more critical factor. $Fe(CN)_6^{4-}$ takes electrode reaction on the GSH/Au electrode by diffusing directly into the pinholes or defects of the

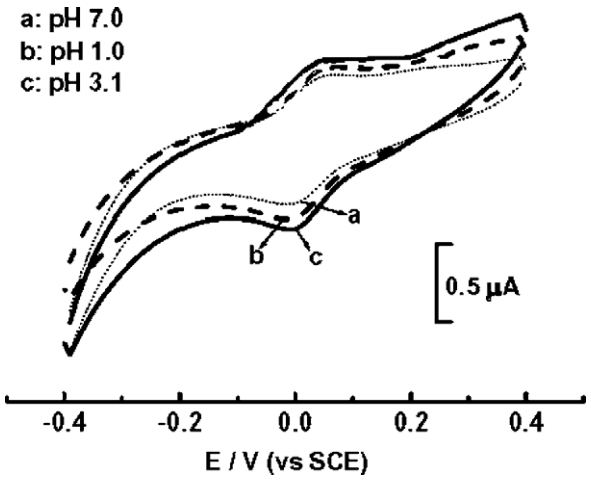


Fig. 5. Cyclic voltammograms of 8.0×10^{-5} M cytochrome *c* in pH 7.0 phosphate buffer on GSH/Au electrode which modified at pH 7.0 (a); pH 1.0 (b); pH 3.1 (c). Scan rate 50 mV s^{-1}.

monolayer or by tunneling transfer via GSH molecule. Based on the result that larger apparent electrode coverage and tighter adsorption of GSH SAM resulted in its high inhibiting efficiency to $Fe(CN)_6^{4-}$, the conclusion can be drawn that $Fe(CN)_6^{4-}$ takes electrode reaction mainly via pinholes or defects of GSH SAM. To cyt. *c*, its direct electron transfer must be dependent on GSH monolayer, which provides the electro-active sites for cyt. *c* to bind with and prevents the irreversible of degradative adsorption of cyt. *c* or impurities, thus larger apparent electrode coverage and tighter adsorption of GSH SAM on Au electrode result in better voltammetric response to cyt. *c*.

3.2.2. Effect of Ca^{2+} ion

The effects of biologically relevant redox-inactive cations such as Mg^{2+} or Ca^{2+} have been investigated in the studies on the redox chemistry of metalloproteins including cyt. *c*. [21–23]. Bond et al. have concluded that metal ion binding with cyt. *c* took place as a result of proton replacement and the overall charge on the native protein and metalated protein remained the same. Neither the iron-containing porphyrin nor the iron center were replaced or removed by the added cations [23]. Mcleod et al. have showed the addition of Mg^{2+} had a significant effect on the reversible potentials for both negatively and positively charged plastcyanins, but to cyt. *c* the added Mg^{2+} affected its wave shape dramatically and not altered its reversible potential [22].

In our work, Ca^{2+} ion was chosen to investigate its effect on voltammetric response of cyt. *c* at GSH/Au electrode. To examine the effect, GSH and cyt. *c* were dissolved in tris–HCl buffer instead of phosphate buffer to avoid Ca^{2+} ion precipitated by phosphate. The experiments were performed

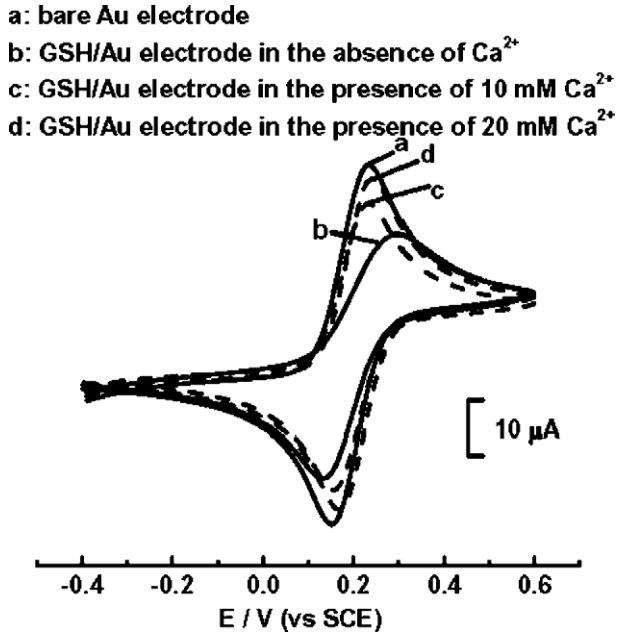


Fig. 6. Cyclic voltammograms of 0.5 mM $K_4[Fe(CN)_6]$ + 0.1 M KCl on bare Au electrode (a); GSH/Au electrode (b); GSH/Au electrode with addition of 10 mM Ca^{2+} (c); GSH/Au electrode with addition of 20 mM Ca^{2+} (d).

by two methods involving Ca^{2+} ions added to GSH solution when monolayer was formed (GSH-Ca^{2+}/Au electrode) or added to cyt. *c* solution directly. Fig. 6 shows the effect of Ca^{2+} ions on the CV response of $Fe(CN)_6^{4-}$ at the GSH/Au electrode. The CV response increases with the addition of Ca^{2+}. It was found that Ca^{2+} could be removed difficultly from GSH monolayers by washing with water because there was little change in CVs, suggesting the strong interaction between GSH monolayer and Ca^{2+} was a complex interaction. The carboxyl groups of GSH coordinate with Ca^{2+} strongly; the interaction not only reduces the electrostatic interaction between carboxyl groups of GSH and $Fe(CN)_6^{4-}$, but also lessens the intermolecular electrostatic interaction of GSH monolayer, which eventually leads to an open channel in GSH monolayer. So more reversibility and higher peak currents of $Fe(CN)_6^{4-}$ were observed with increasing addition of Ca^{2+}. Fig. 7 compares the CVs of $Fe(CN)_6^{4-}$ on the GSH/Au electrode (curve b) and the GSH-Ca^{2+}/Au electrode (curve c). Comparing with a bare Au electrode (curve a), both modified electrodes show inhibitive efficiency to $Fe(CN)_6^{4-}$, which suggests that the complex GSH-Ca^{2+} can be assembled on the electrode surface as easily as GSH molecules. It was also noticed the larger inhibitive behavior of GSH-Ca^{2+} monolayer to $Fe(CN)_6^{4-}$. An ion of Ca^{2+} has the ability to bind two carboxyl terminals from one GSH molecule or two neighbor GSH molecules, which faces a great stereo inhibition of GSH molecule. This kind of structure configuration undoubtedly results in the compact GSH/Ca^{2+} monolayer formed on the electrode surface, resulting larger inhibitive efficiency to $Fe(CN)_6^{4-}$.

Cyclic voltammograms of cyt. *c* on GSH/Au electrode (curve a) and GSH-Ca^{2+}/Au electrode (curve b) are shown in Fig. 8. At both modified electrodes CVs of cyt. *c* are peak-shaped, however, the $E^{0\prime}$ is positively shifted and the peak currents are decreased on GSH-Ca^{2+}/Au electrode. At GSH-Ca^{2+}/Au electrodes, because of the carboxyl groups complexed with Ca^{2+}, at pH 7.0 each GSH molecule is changed from one net negative charged to one positive charged, thus altering the overall charge of the electrode surface from negative to positive. The positive charged GSH-Ca^{2+}monolayer became unfavorable for the interaction of cyt. *c* (positively charged at pH 7.0) with the GSH monolayer, which results in the decreased peak currents and the positively shifted formal potential.

Curve c in Fig. 8 shows the cyclic voltammogram of cyt. *c* on GSH/Au electrode with the addition of Ca^{2+} into tris–HCl buffer containing cyt. *c*. Comparing with the CV of cyt. *c* on GSH/Au electrode in the absence of Ca^{2+} (curve a), the voltammetric responses changed from peak shaped to sigmoidal shaped, and it was found that after addition of 30 mM Ca^{2+}, no response above the background current was observed. The change of the cyt. *c* voltammetric shape in the presence of Ca^{2+} is due to a change in the electrode surface activity. The Au electrode modified with GSH is to provide electroactive sites at the electrode interface by allowing

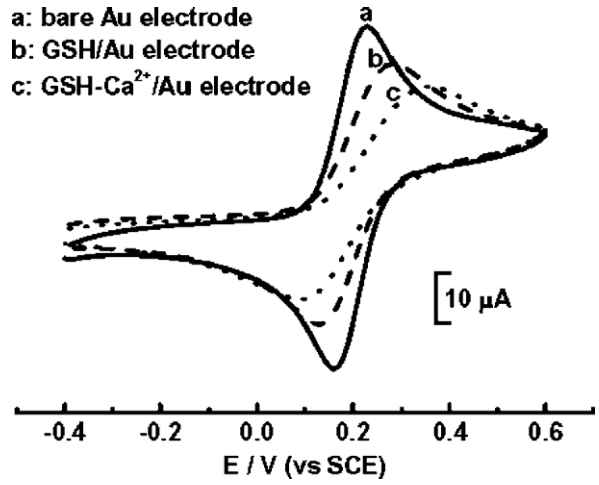


Fig. 7. Cyclic voltammograms of 0.5 mM $K_4[Fe(CN)_6]$+0.1 M KCl on bare Au electrode (a); GSH/Au electrode (b); GSH-Ca^{2+}/Au electrode (c). Scan rate 50 mV s^{-1}.

necessary binding interaction of cyt. *c* with the electrode interface and preventing irreversible and degradative adsorption of cyt. *c*. In the presence of low concentration of Ca^{2+}, they bind with GSH molecules, change the electrode surface charge state and cause GSH SAM to appear larger pinholes, which result in the unfavorable interaction of cyt. *c* with the electrode interface and partially blocking the electrode surface, leading to the voltammetric response of cyt. *c* changed from peak shaped to sigmoidal shaped. In the presence of high concentration of Ca^{2+}, excess free Ca^{2+} ions blocking the electroactive sites of the modified electrode surface and no voltammetric response of cyt. *c* was observed.

3.2.3. Effect of surfactants

Wang et al. have studied the influence of surfactants on the electron-transfer reaction on the self-assembled thiol monolayer modifying a gold electrode [15]. They found that surfactants can interact with thiol monolayer in different ways, changing the structure and properties of the monolayer, and can further affect the electron transfer of analytes at the modified gold electrode. Surfactants adsorption on hydrophobic electrode surfaces have also characterized by various research groups [24–26]. It was

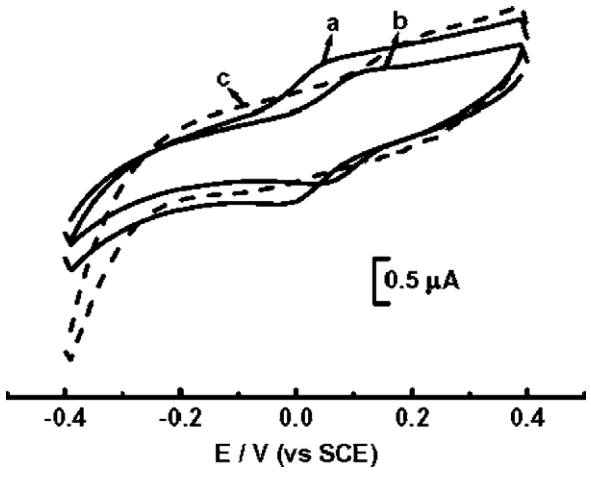


Fig. 8. Cyclic voltammograms of 8.0×10^{-5} M cytochrome *c* in pH 7.0 tris–HCl buffer on GSH/Au electrode without Ca^{2+}(a); GSH–Ca^{2+}/Au electrode (b); GSH/Au electrode with addition of 10 mM Ca^{2+}(c). Scan rate 50 mV s^{-1}.

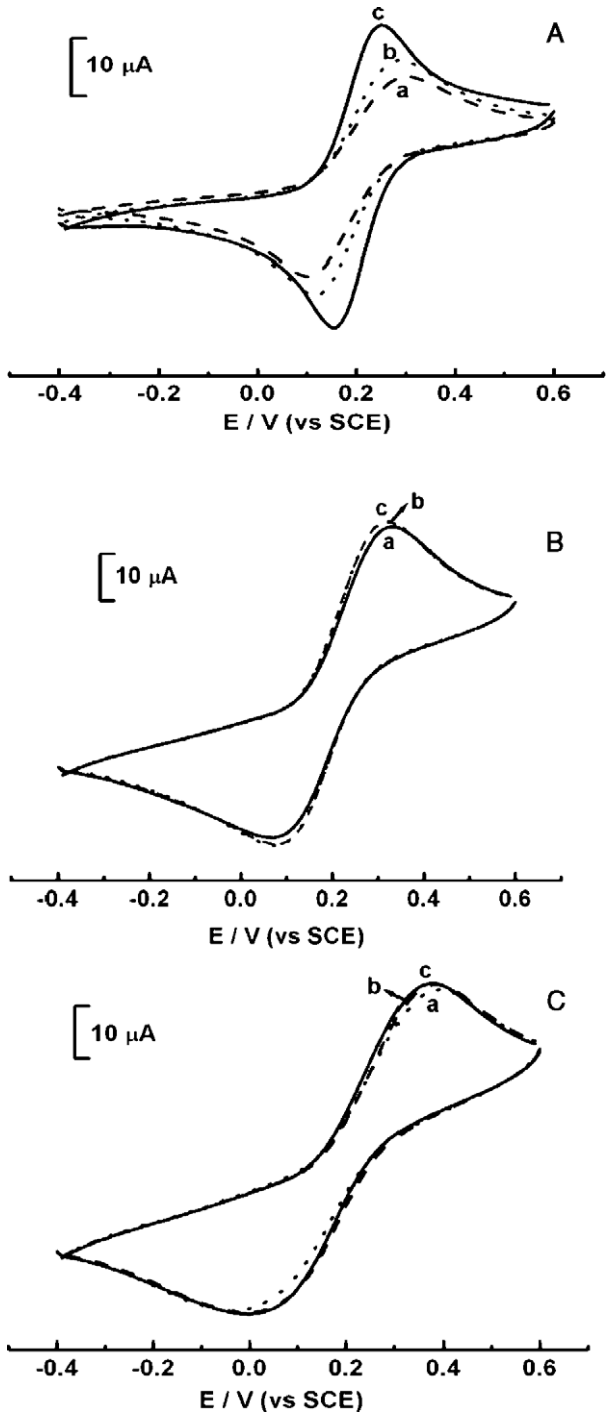


Fig. 9. Cyclic voltammograms of 0.5 mM $K_4[Fe(CN)_6]$ on the GSH/Au electrode in the presence of CTAB (A) ; SDBS (B); and triton X-100 (C). The concentration of surfactant in each CV: 0 M (a); 1×10^{-5} M (b); 2×10^{-4} M (c). Scan rate 50 mV s^{-1}.

found that surfactants showed different adsorptive behavior on the hydrophobic electrode surface, including monomer adsorption and monolayer adsorption. Surfactants have also been employed in the immobilization of proteins on the electrode surface to realize their direct electrochemistry [27–29]. The results showed that surfactant molecules interacted with the electrode surface in a specific manner and anchored the protein molecules to align in a suitable orientation, which promoted electron transfer between the protein molecules and the electrode. But the influence of surfactants on the electron transfer between redox proteins and self-assembled monolayer modified electrode has not been investigated.

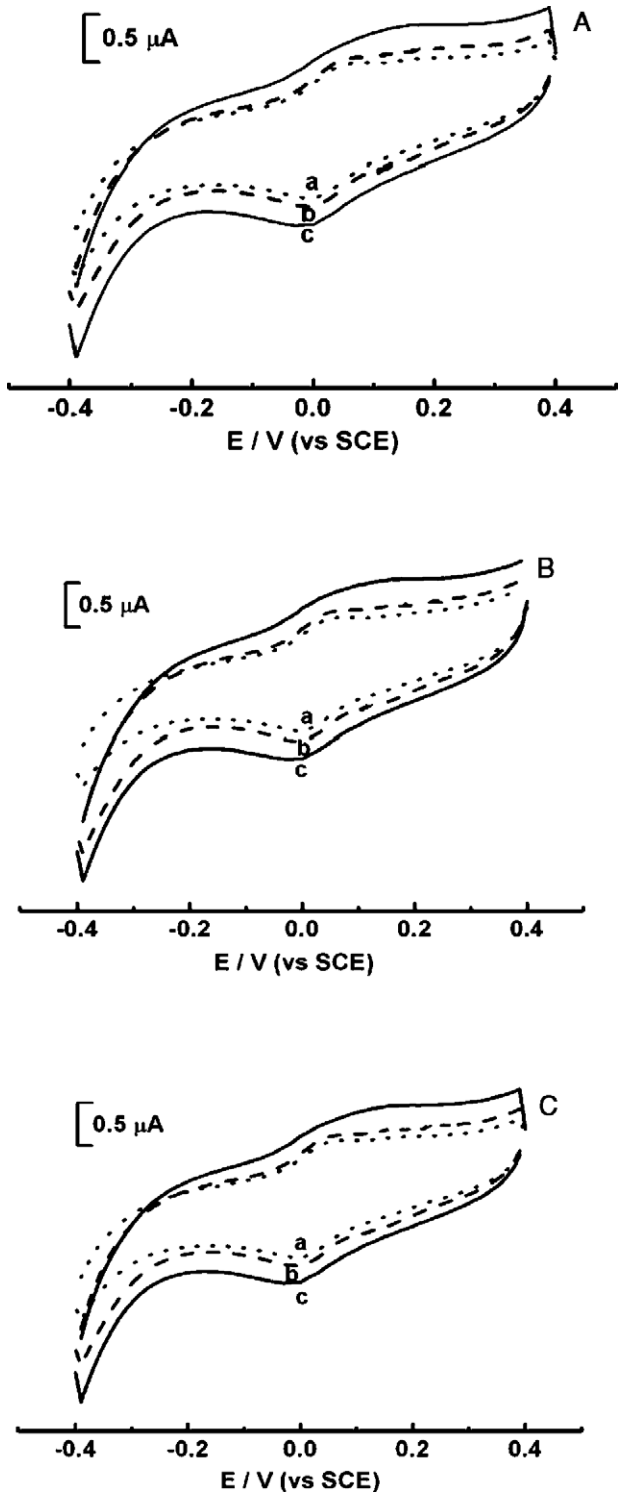


Fig. 10. Cyclic voltammograms of 8.0×10^{-5} M cytochrome *c* in pH 7.0 phosphate buffer on GSH/Au electrode in the presence of CTAB (A); SDBS (B); and triton X-100 (C). The concentration of surfactant in each CV: 0 M (a); 1×10^{-5} M (b); 5×10^{-4} M (c). Scan rate 50 mV s^{-1}.

In our work, three surfactants, cetyltrimethylammonium bromide (CTAB), sodium dodecyl benzene sulfonate (SDBS) and triton X-100, were chosen to investigate their influence on the electron transfer of cyt. *c* at GSH/Au electrodes. In order to study the change of the electrode interface affected by surfactants, $Fe(CN)_6^{4-}$ was also used as a probe. Fig. 9 shows the cyclic voltammograms of $Fe(CN)_6^{4-}$ on the GSH/Au electrode in the presence of CTAB (Fig. 9A), SDBS (Fig. 9B) and triton X-100 (Fig. 9C). Compared with the CVs without surfactant, the peak currents increase and ΔE_p decrease with the increase of CTAB concentration (curve b and c in Fig. 9A) . The optimal concentration of surfactant is around 10^{-4} M, this value is related to the cmc of the solution. While anionic surfactant (SDBS) and neutral surfactant (triton X-100) have little influence on voltammetric response of $Fe(CN)_6^{4-}$ at the GSH/Au electrode(curve b and c in Fig. 9B and Fig. 9C). $Fe(CN)_6^{4-}$ can form ion pairs with cationic surfactant and the adsorption of the surfactant on the GSH monolayer facilitates the electron transfer between $Fe(CN)_6^{4-}$ and the electrode surface. Anionic and neutral surfactants can't form ion pairs with $Fe(CN)_6^{4-}$, on the contrary, their adsorption on the GSH SAMs leads the denser membrane on the electrode surface, which can block the electron transfer of $Fe(CN)_6^{4-}$.

Cyclic voltammograms of cyt. *c* in the presence of surfactants on GSH/Au electrodes are shown in Fig. 10. Being different from their influence on $Fe(CN)_6^{4-}$, it was observed that cationic, anionic and neutral surfactants in the concentration range of 1×10^{-5} – 2×10^{-4} M could increase the peak currents of cyt. *c*, and the reversible potential of cyt. *c* was not changed (curve b in Fig. 10A, B and C). Cyt. *c* is a hydrophobic macromolecule; surfactants can bind it with their hydrophobic tails. The adsorption layer of surfactants on the GSH/Au electrode surface leads to more cyt. *c* attaché to the electrode surface. Furthermore, surfactants can change the orientation of cyt. *c* on the GSH/Au electrode surface to be prone to its electron transfer reaction. All these results led to higher peak currents of cyt. *c* in CVs at the GSH/Au electrodes in the presence of proper amount of surfactants. When the concentration of surfactants was above 5×10^{-4} M, the peak shape of cyt. *c* was not well-developed and the peak potential separation became larger (curve c Fig. 10A, B and C), indicating that excess amount of surfactants would form micelle in solution and had a bad influence on the electrode response of cyt. *c* on the GSH/Au electrode.

4. Conclusions

The direct, reversible electrochemistry of cyt. *c* was realized on GSH/Au electrodes. GSH monolayer provided a good model of interfacial film to study the direct electrochemistry of cyt. *c*. The pH value during the electrode modification, Ca^{2+} ion, and surfactants could change the state of GSH monolayer on the Au electrode surface, leading to the corresponding change of voltammetric response of cyt. *c* on GSH/Au electrode . The experimental results provided us information to understand the mechanism of the interfacial electron transfer of electrode-protein, as well as the electron transfer of cyt. *c* in life system.

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References

[1] A. Ulman, Formation and structure of self-assembled monolayers, Chem. Rev. 96 (1996) 1533–1554.
[2] T. Wink, S.J. Zulien, A. Bult, W.P. Bennekom, Self-assembled monolayers for biosensors, Analyst 122 (1997) 43R–50R.
[3] K.D. Gleria, H.A.O. Hill, V.J. Lowe, D.J. Page, Direct electrochemistry of horse-heart cytochrome *c* at amino acid–modified gold electrodes, J. Electroanal. Chem. 213 (1986) 333–338.
[4] M. Collinson, E.F. Bowden, Voltammetry of covalently immobilized cytochrome *c* on self-assembled monolayer electrodes, Langmuir 8 (1992) 1247–1250.
[5] S.H. Song, R.A. Clark, E.F. Bowden, Characterization of cytochrome *c*/alkanethiolate structures prepared by self-assembly on gold, Langmuir 97 (1993) 6564–6572.
[6] T. Ruzgas, L. Wong, A.K. Gaigalas, V.L. Vilker, Electron transfer between surface-confined cytochrome *c* and an *n*-acetylcysteine–modified gold electrode, Langmuir 14 (1998) 7298–7305.
[7] K. Niki, W.R. Hardy, M.G. Hill, H. Li, J.R. Sprinkle, E. Margoliash, K. Fujita, R. Tanimura, N. Nakamura, H. Ohno, J.H. Richards, H.B. Gray, Coupling to lysine-13 promotes electron tunneling through carboxylate-terminated alkanethiol self-assembled monolayers to cytochrome *c*, J. Phys. Chem., B 107 (2003) 9947–9949.
[8] L. Stryer, Biochemistry, 3rd edn., Freeman, New York, 1988, pp. 436–592.
[9] S. Izawa, Y. Inoae, A. Kimura, Oxidative stress response in yeast: effect of glutathione on adaptation to hydrogen peroxide stress in *Saccharomyces cerevisiae*, FEBS Lett. 368 (1995) 73–76.
[10] P.J. Deschavanne, E.P. Malaise, L. Revesz, Radiation survival of glutathione deficient human fibroblasts in culture, Br. J. Radiol. 54 (1981) 361–362.
[11] Y.K. Chen, Glutathione detoxification and its toxic metabolites, Prog. Biochem. Biophys. 21 (1994) 395–399.
[12] D.W. Stephen, D.J. Jamieson, Glutathione is an important antioxidant molecule in the yeast *Saccharomyces cerevisiae*, FEMS Microbiol. Lett. 141 (1996) 207–212.
[13] K. Takehara, Y. Ide, M. Aihara, E. Obuchi, An ion-gate response of the glutathione monolayer assembly formed on a gold electrode: Part 1. The effect of pH, K^+ and Ca^{2+}, Bioelectrochem. Bioenerg. 29 (1992) 103–111.
[14] K. Takehara, Y. Ide, M. Aihara, E. Obuchi, An ion-gate response of the glutathione monolayer assembly formed on a gold electrode: Part 2. The effect of alkaline earth ions, Bioelectrochem. Bioenerg. 29 (1992) 113–120.
[15] J. Wang, B.Z. Zeng, C. Fang, X.Y. Zhou, The influence of surfactants on electron-transfer reaction at self assembled thiol monolayer modifying a gold electrode, J. Electroanal. Chem. 484 (2000) 88–92.
[16] C. Fang, X.Y Zhou, The electrochemical characteristics of C_{60}-Glutathione modified Au electrode and the electrocatalytic oxidation of NADH, Electroanalysis 13 (2001) 949–954.
[17] C. Fang, X.Y. Zhou, Voltammetry and EQCM investigation of glutathione monolayer and its complexation with Cu^{2+}, Electroanalysis 15 (2001) 1632–1638.
[18] W.H. Koppenol, E. Margoliash, The asymmetric distribution of charges on the surface of horse heart cytochrome *c*, J. Biol. Chem. 257 (1982) 4426–4437.
[19] E.B. Troughton, C.D. Bain, G.M. Whitesides, R.G. Nuzzo, D.L. Allara, M.D. Porter, Monolayer films prepared by the spontaneous self-assembled of symmetrical and unsymmetrical diakyl sulfides from solution onto gold substrates, Langmuir 4 (1982) 365–385.
[20] A.M. Corrie, D.R. Williams, Thermodynamic considerations in Coordination: Part XXIV. Gibbs free-energy charges, enthalpies, and entropies of formation of complexes of glycinate, glycylglycinate, glycylglycylglycinate, cysteinate, and glutathionate with hydrogen and lead (II) ions and suggested aqueous structure, J. Chem. Soc. Dalton Trans. (1976) 1068–1072.
[21] F.A. Armstrong, P.A. Cox, H.A.O. Hill, V.J. Lowe, B.N. Oliver, Metals and complexes as modulators of protein-interfacial electron transport at graphite electrodes, J. Electroanal. Chem. 217 (1987) 331–366.
[22] D.D.N. Mcleod, H.C. Freeman, I. Harvey, P.A. Lay, A.M. Bond, Voltammetry of plastocyanin at a graphite electrode: effects of structure, charge, and electrolyte, Inorg. Chem. 35 (1996) 7156–7165.
[23] A.M. Bond, R. Colton, A.D. Agostino, J.C. Traeger, A.J. Downard, A.J. Canty, An electrospray mass spectrometric and voltammetric study of horse heart cytochrome *c* in the presence of metal ions, Inorg. Chim. Acta 267 (1998) 281–291.
[24] K. Hu, A.J. Bard, Characterization of adsorption of sodium dodecyl sulfate on charge-regulated substrates by atomic force microscopy force measurements, Langmuir 13 (1997) 5418–5425.
[25] M.E. Montgomery, M.J. Wirth, Spectroscopic investigation of sodium dodecyl sulfate adsorption on a hydrocarbon monolayer, Langmuir 10 (1994) 861–869.
[26] G.B. Sigal, M. Mrksich, G.M. Whitesides, Using surface plasmon resonance spectroscopy to measure the association of detergents with self-assembled monolayers of hexadecanethiolate on gold, Langmuir 13 (1997) 2749–2755.
[27] K. Chattopadhyay, S. Mazumdar, Direct electrochemistry of heme proteins; effects of electrode surface modification by neutral surfactants, Bioelectrochemistry 53 (2000) 17–24.
[28] J.F. Rusling, Enzyme bioelectrochemistry in cast biomembrane-like films, Acc. Chem. Res. 31 (1998) 363–369.
[29] S. Boussaad, N.J. Tao, Electron transfer and adsorption of myoglobin on self-assembled surfactant films: an electrochemical tapping-mode AFM study, J. Am. Chem. Soc. 121 (1999) 4510–4515.