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Promoter effect of poly(4-vinylpyridine) on the direct electron transfer between cytochrome *c* and gold electrode

Xiaogang Qu, Tianhong Lu *, Shaojun Dong

Laboratory of Electroanalytical Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun, 130022, China

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Abstract

The electrochemistry of cytochrome *c* was studied at the PVP-modified gold electrode. It was found that the promoter effect is related to the amount of PVP at the gold electrode. From our results, it can be seen that the nitrogen element in the polymer is important for accelerating the electron transfer of cytochrome *c*.

Keywords: Cytochrome *c*; Electron transfer; Gold; Modified electrode; Pyridine derivatives

1. Introduction

Cytochrome *c* is integral in the respiratory chain of living organisms. It plays the role of electron transfer agent between cytochrome *c* oxidase and reductase [1]. At present, cytochrome *c* injection, which can stimulate the cell, has been used as first-aid in the clinic for organs which are lacking oxygen. However, the mechanism of the electron transfer between cytochrome *c* and its oxidase or reductase is not clear. Therefore, it is very important to study the electron transfer reaction of cytochrome *c*. Electrochemical techniques have been considered to be the most useful means to investigate the biomolecular electron transfer systems [2–4]. Previous studies have shown that cytochrome *c* was easily denatured on bare metal electrode surfaces and it was difficult to achieve the rapid electron transfer reaction of cytochrome *c* [5,6]. In 1977, Hill and his coworkers observed a quasi-reversible electrochemical reaction of cytochrome *c* at 4,4′-bipyridine modified gold electrodes [7]. 4,4′-Bipyridine was termed 'promoter'. Up till now, several promoters have been found [7–11]. But most of them were organic compounds. Since polymer modified electrodes are stable and the amount on the electrode surface can be verified in a controlled manner, they have been successfully applied in biosensors [12] and a few polymers which could promote the electrochemical reaction of cytochrome *c* have also been found [13–16]. Dong and her coworkers [13] studied the heterogeneous electron transfer of cytochrome *c* facilitated by polypyrrole and methylene blue polypyrrole film-modified electrodes. These modified electrodes showed high electrocatalytic activity and good stability for the electron transfer of cytochrome *c*. Caselli et al. also investigated the redox behaviour of horse heart

* Corresponding author. Tel. (+86-431)5682801, fax. (+86-431)5685653.

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cytochrome *c* immobilized into polypyrrole films and deposited on a Pt electrode [14]. But the mechanism of the electrochemical reaction of cytochrome *c* is still unclear. Bartlett and Farington reported [16] that poly-5-carboxyindole could promote the electron transfer of cytochrome *c*. It was believed that the presence of carboxylate groups on the conducting polymer facilitated the electrochemistry of cytochrome *c*. But the reason why the monomer, 5-carboxyindole, could not accelerate the electron transfer between cytochrome *c* and the electrode is unknown. Therefore, a series of studies about the mechanism of the electron transfer between cytochrome *c* and the polymer-modified electrodes have been carried out in our group.

In this paper, we report that a very well studied polymer, poly-4-vinylpyridine can also promote the electron transfer of cytochrome *c* and further studies illustrate that polymer promotion is much better than by the monomer, 4-vinylpyridine. Our results show that the promotion effect is related to the amount of promoter molecules on the electrode surface. Here, the mechanism of the electrochemical reaction of cytochrome *c* at poly-4-vinylpyridine modified gold electrodes is discussed in terms of an electrostatic model.

2. Experimental

Horse heart cytochrome *c* (type VI, 99%, Sigma Chemical Co.) was used without further purification. Poly(4-vinylpyridine) and poly(*N*-vinylpyridine) were from Aldrich. All other chemicals were reagent grade.

Cyclic voltammetry was performed using Model 276 potentiostat, Model 179 universal programmer (Princeton Applied Research), Houston Instruments Series 2000 Omnigraphic *X–Y* recorder and a conventional three-electrode electrochemical cell. The working electrode was constructed from a gold rod which was approximately 0.8 mm^2. A Pt wire was used as the auxiliary electrode. A saturated calomel electrode (SCE) served as the reference electrode and all potentials were reported with respect to the SCE.

The working electrode was sequentially polished with 5, 0.3, 0.05 μm alumina/water slurries until a shiny mirror-like finish was obtained. The electrode then sonicated in deionized water and washed thoroughly with deionized water. The film transfer method [8,10] was used to evaluate the promotion of 4-vinylpyridine. The concentration of 4-vinylpyridine was O.1 mM. Poly-4-vinylpyridine modified electrodes were made by dropping the poly-4-vinylpyridine (PVP) iso-butyl alcohol solution on the gold electrode surfaces and dried.

The electrochemical studies of cytochrome *c* were carried out at the modified electrode in 0.38 mM cytochrome *c* solution with 0.025 M phosphate buffer at pH 7.0 and 0.1 M sodium perchlorate. Oxygen was purged from solution by bubbling with nitrogen for 10 min prior to the electrochemical measurement. The scan rate was usually 50 mV/s and the potential range of scanning was from −0.2 V to +0.2 V.

3. Results

Cytochrome *c* can realize the direct electrochemical reaction on the 4-vinylpyridine-modified gold electrode (Fig. 1, b). From this curve, the difference between the cathodic and anodic

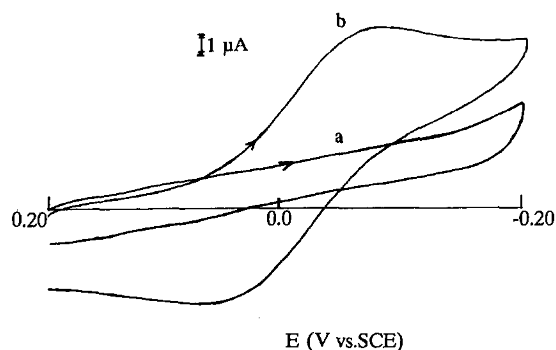

Fig. 1. Cyclic voltammograms of 0.38 mM cytochrome *c* at (a) a freshly polished gold electrode and (b) a 4-vinylpyridine-modified gold electrode in phosphate buffer solution (pH 7.0) with 0.1 M $NaClO_4$. The scan rate was 50 mV/s and the initial potential was +0.2 V.

peak potentials is about 130 mV. During the continuous scanning, the difference of the peak potentials is becoming larger and larger, at the same time, the peak current is becoming smaller and smaller. On the 5th scan, the peak current disappears. In order to obtain further information about the electrochemical reaction of cytochrome *c*, we prepared its polymer (PVP)-modified gold electrode. Fig. 2 shows the cyclic voltammogram of cytochrome *c* on PVP-modified gold electrode. It can be seen that the difference between the cathodic and anodic peak potentials is about 85 mV, which is much smaller than 130 mV on the monomer-modified gold electrode. The midpoint between the cathodic and anodic peak potentials is near 0.01 V which is in good agreement with the formal potential of cytochrome *c* [17]. The ratio of the anodic to the cathodic peak current is approximately one. The cathodic and anodic peak currents are proportional to the square root of the scan rate in the range of 10–200 mV/s, indicating that the reaction is diffusion-controlled. All the characteristics mentioned above demonstrate that a quasi-reversible, direct electrochemical reaction occurs at the PVP-modified gold electrode. From Fig. 3, the diffusion coefficient D_o, 8×10^{-7} cm^2/s, and the heterogeneous electron transfer constant K_s, 2×10^{-3} cm/s, can be calculated by using Nicholson's method [18], and it is in agreement with that reported in a previous paper [19].

Furthermore, the promoter effect of PVP is related to the amount of the polymer on the gold electrode surface. Fig. 4 shows that a quasi-reversible electrochemical reaction of cytochrome *c* can be obtained when there are enough polymer molecules on the gold electrode surface.

In order to study the electron transfer mechanism of cytochrome *c* on the PVP-modified gold electrode, three more experiments are carried out. The first was carried out to detect the change of the open-circuit potential before and after surface modification of the gold electrode with poly-4-vinylpyridine. It was found that the open-circuit potential becomes more negative when the electrode is modified with PVP. This result is similar to that reported in a previous paper [10]. It means

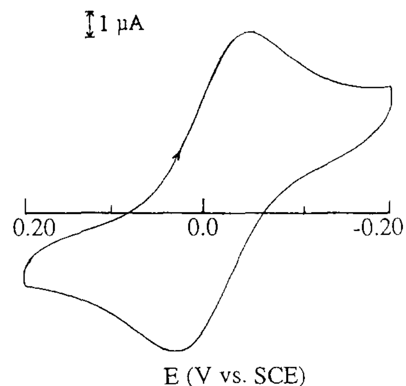

Fig. 2. Cyclic voltammogram of 0.38 mM cytochrome *c* at PVP-modified gold electrode. The scan rate was 50 mV/s. Other conditions as same as in Fig. 1.

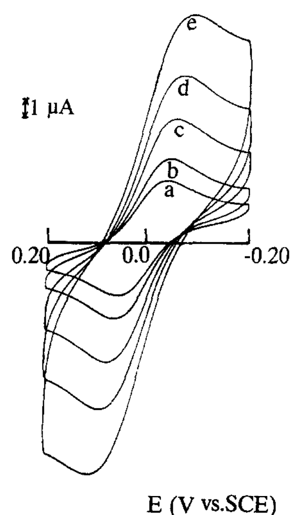

Fig. 3. Cyclic voltammograms of 0.38 mM cytochrome *c* at PVP-modified gold electrode with different scan rates (a) 10, (b) 20, (c) 50, (d) 100, (e) 200 mV/s.

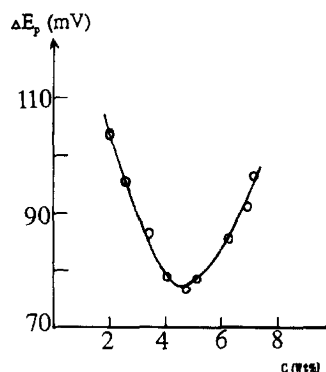

Fig. 4. Relationship between concentration of PVP and the separation of cathodic and anodic peak potentials in the cyclic voltammograms of cytochrome *c* at PVP-modified gold electrodes.

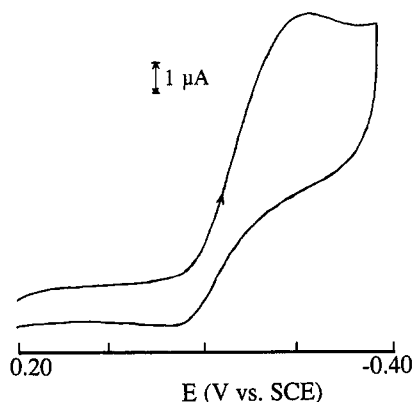

Fig. 5. Cyclic voltammogram of iron at PVP-modified gold electrode with 0.1 M sulphuric acid. The scan rate was 50 mV / s. PVP-modified gold electrode, dipping time for 10 min in 0.1 M $FeCl_3$.

that the charges on the gold electrode surface would change after modification with promoters. In the second experiment the PVP-modified gold electrode was placed in the $FeCl_3$ solution for 10 min. Then, the electrode was removed from the solution and washed thoroughly with deionized water. After that, the electrochemical measurement was carried out in cytochrome *c* solution using the immersed electrode. There was no electrochemical response at all. But if the immersed electrode is placed in a 0.1 M sulphuric acid solution, the electrochemical reaction of Fe ion can be observed (Fig. 5). This shows that Fe^{3+} cations can diffuse into the PVP layer. For the third experiment, another polymer-modified gold electrode, a poly(*N*-vinylcarbazole)-modified electrode was used. Cytochrome *c* does not undergo electrochemical reaction at a poly(*N*-vinylcarbazole)-modified electrode in the range of potentials studied. Therefore, nitrogen elements in the polymer molecule are important for accelerating the electron transfer of cytochrome *c*. If the position of the N element is open in the polymer, such as in poly(4-vinylpyridine), this modified electrode can promote the electrochemistry of cytochrome *c*. In contrast, when the position of N is occupied by vinyl group, as in the case of poly(*N*-vinylcarbazole), this polymer will lose its promoter ability for the electrochemistry of cytochrome *c*.

4. Discussion

According to the above experimental results, it can be concluded that the promotion of poly-4-vinylpyridine is better than its monomer, 4-vinylpyridine, since the adsorption strength of the monomer is much weaker than its polymer, cytochrome *c* would replace the monomer molecules on the electrode surface [20]. Since there are not enough monomer molecules on the electrode surface, the promotion effect of the monomer becomes rather poor and unstable. This means that the promoter effect of a compound is related to the adsorption strength and the amount in which it is present at the electrode surface. Fig. 4 clearly demonstrates the relationship between the concentration of PVP and its promoter effect for the electrochemical reaction of cytochrome *c*. Although the same amount of PVP was used, the reversibility of the electrochemical reaction of cytochrome *c* was quite different at different concentrations of the PVP solution-modified gold electrode. If the concentration is too low, PVP molecules on the gold electrode cannot completely prevent the strong adsorption of cytochrome *c*. The difference of cathodic and anodic peak potentials in the cyclic voltammogram of cytochrome *c* is larger. Meanwhile, if the concentration is too high, there will be too much resistance to the electron transfer between cytochrome *c* and the electrode. The reversibility also becomes poor. Another experiment on different thicknesses of PVP layer at the gold electrode for the same concentration of PVP gives a similar result. Then, enough promoter molecules are required on the electrode surface to prevent the strongly denatured adsorption of cytochrome *c*. Cotton et al. [21] and Hildebrant et al. [22] identified, by using surface enhanced Raman spectroscopy scattering technique, that cytochrome *c* was denatured when it adsorbed on bare metal electrodes. They found that the spin state of cytochrome *c* was related to the applied potential during the adsorption on the electrode surface [22]. Niki and his coworkers also found that the formal potential of cytochrome *c* at a bare elec-

trode was quite different from the value in the bulk state [23].

Cytochrome *c* is a highly ionized metal protein with a net charge of +9 in the oxidized state at pH 7.5. The positively charged residues are fairly homogeneously distributed on the protein surface. On the other hand, the distribution of the negative surface charges is asymmetric, with nearly all the negatively charged residues located in the small area on the back surface of cytochrome *c* [24,25]. The heme group, the plane of which is nearly perpendicular to the protein surface, sits in a crevice surrounded by the polypeptide chain of 104 amino acids. The solvent-exposed surface of the heme corresponds to a small proportion (0.06%) of the total molecular surface and its edge is located approximately 0.3 nm below the molecular surface [26]. This region of the protein surface is surrounded by positively charged lysines and constitutes an electron-transfer domain for interaction with cytochrome *c* oxidase or reductase [27].

On the basis of our experimental results and the properties of cytochrome *c*, a mechanism for the electron transfer of cytochrome *c* at the PVP-modified gold electrode can be proposed. When the Au electrode is modified with PVP, the position of nitrogen in the polymer chain is exposed. The negative shift about open-circuit potential when the gold electrode is modified with PVP demonstrates that more negative charges are distributed on the electrode surface than before modification. The positively charged lysine residues in cytochrome *c* molecules will interact with the nitrogen element in the polymer by forming hydrogen bonds. This is probably important for the electron transfer of cytochrome *c* [28]. The redox centre, the heme group, is proximal to the electrode surface and the rapid electron transfer reaction of cytochrome *c* can take place on the PVP-modified gold electrode. When the electrode is modified with poly(*N*-vinylcarbazole), the position of nitrogen in this polymer is occupied by vinyl group. Therefore, there is no suitable group which can bind to the lysine residues. The rapid electron transfer between cytochrome *c* and the electrode cannot take place.

When the Fe^{3+} cation diffuses into the PVP layer, the promoter effect of PVP is lost. This illustrates that the property of the charges in the polymer on the electrode surface also affects the electrochemistry of cytochrome *c*. Since the distribution of charges in cytochrome *c* is asymmetric, electrostatic repulsion may result if cytochrome *c* adsorbs at the electrode with its positively charged lysine residues which are near the redox centre, heme group. Therefore, in this case, cytochrome *c* would tend to orient with the negatively charged back surface to the electrode surface [29] and the distance between the heme group and the electrode becomes longer. As a result, the electrochemistry of cytochrome *c* is blocked.

Acknowledgements

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