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4,6–DIMETHYL–2–MERCAPTOPYRIMIDINE AS NEW PROMOTER FOR THE VOLTAMMETRIC RESPONSE OF HORSE HEART CYTOCHROME C AT A MICROBAND GOLD ELECTRODE

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Abstract A new promoter, 4,6–dimethyl–2–mercaptopyrimidine(DMMP), was used to modify a microband gold electrode for promoting the direct elctrochemistry of cytochrome c was reported. The experimental results indicated that DMMP is a more effective promoter for redox reaction of cytochrome c than others.

The electrochemistry of cytochrome c has been studied extensively at various electrode surface. Generally, cytochrome c is electrochemically inert at bare metal electrode surface. Hill et al [1] found that the well–defined reversible electrochemistry of cytochrome c at gold electrode can be observed in the presence of 4,4′–bipyridyl in solution. Since then, numerous further studies on the electrochemistry of cytochrome c at modified gold and other electrodes have been reported, many promoters were found to promote the direct electrochemistry of cytochrome c. However, the interaction between a promoter and cytochrome c is still a subject of question. Thus, it would be significant to find new promoter and enrich the knowledge about the structure of a promoter to understand better the suitable surface at which cytochrome c can be rapidly reduced and oxidized. Here we have proved that 4,6–dimethyl–2–mercaptopyrimidine (DMMP) is effective as a new promoter for rapid redox reaction of cytochrome c.

Experimental

Horse heart cytochrome c (Type VI, Sigma Chemical Co.) and DMMP (Aldrich Chemical Co.) were used as received without further purification. All other chemicals used were of analytical grade. All solutions were prepared with doubly distilled deionized water. Electrochemical experiments were carried out by using a PAR M270 electrochemical analysis system (EG& G, USA). A three electrode system, with a Pt wire as counter electrode, a

saturated calomel electrode (SCE) as a reference electrode and a microband gold electrode (ca. 0.1μm × 1.0cm) as the working electrode, was employed. The electrode was modified by the film transfer technique of immersing the electrode into 5×10^{-3} mol / L DMMP solution (pH 7.0) for some time, washed with water, and then transferred to cytochrome c solution to perform the cyclic voltammetric experiments in the potential range of −0.15～+0.3V (vs.SCE) at 25 ± 0.1℃. All solutions were deaerated by passing pure N_2 for 30 minutes before the experiment, and a continuous flow of highly pure N_2 was maintained over the solution during experiments. All the electrochemical experiments were carried out inside a Faraday cage.

Results and Discussion

Figure 1a shows a cyclic voltammogram for a DMMP adsorptively modified microband gold electrode (defined as DMMP / Au) in phosphate buffer. No redox peaks were observed. When cytochrome c is added to the buffer solution, two well-defined redox peaks appear with anodic and cathodic peak potential of 70mv and 2mv , respectively (Fig. 1b). The results indicate that DMMP is a promoter for the redox reaction of cytochrome c. The formal potential, defined as the mid-point of peak potentials, is 36mv (vs.SCE) which is in good agreement with literature[1]. The experimental results show that the ratio of anodic to cathodic current, i_{pa} / i_{pc} , obtained at various scan rates is almost unity. Both cathodic and anodic peak currents increase linearly as a function of the square root of scan rate (20～ 100mv / s), which shows that the electrode process is controlled by the diffusion of cytochrome c in solution. The diffusion coefficient (D) calculated from the slope of i_p vs. $v^{1/2}$ is 8.3×10^{-7} cm^2 / s . It is similar to those obtained by using other promoters[2]. The separation of peaks at a low scan rate is 68mv, which is consistent with a quasi-reversible one electron transfer reaction. A

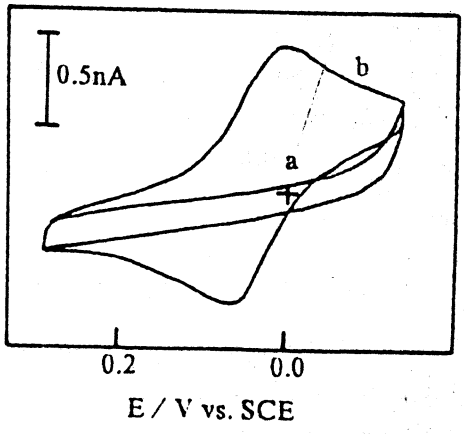

Fig. 1 Cyclic voltammograms of DMMP / Au electrode in phosphate buffer (pH 7.0). (a) in the absence of cytochrome c , and (b) in the presence of 3.4×10^{-4} mol / L cytochrome c. v = 20mv / s.

further increase in the scan rate resulted in a larger separation of the peaks. For example, a separation of 94mv occurs at 100mv / s. The electron transfer rate constant (k_s) was ca. 6.6×10^{-3} cm / s . It is about six times larger than that reported in literature[3]. The voltammetric behaviour of cytochrome c depends strongly on the immersing time of electrode in DMMP solution. The separation of peaks decreases with the increasing of immersing time. It corresponds to an increase in k_s . From Fig. 2a, it can be seen that the value of k_s increases rapidly with increasing of the immersing time, and then reaches a relatively stable value after escaping time of about two hours. The experimental results suggest that the coverage of DMMP on the surface of gold electrode depends upon the immersing time, and also the adsorption–modified layer probably requires some time for the self–assembly of its molecules to facilitate the enhancement of promotional function.

The lifetime of DMMP / Au electrode for promoting the redox reaction of cytochrome c was examined by continuous scanning. The experimental results show that almost no changes of the CV response of cytochrome c and the separation of peaks occur within 120 cycles of repetitive scanning, whereas for the other promoter such as 2,2′–bipydine, the CV response of cytochrome c changes after only 25 cycles of repetitive scanning[4]. After 120th scanning, the CV response starts to change slowly and the separation of peaks also increases slowly.Figure 3 shows the comparison of the cyclic voltammograms between the 10th (solid line) and

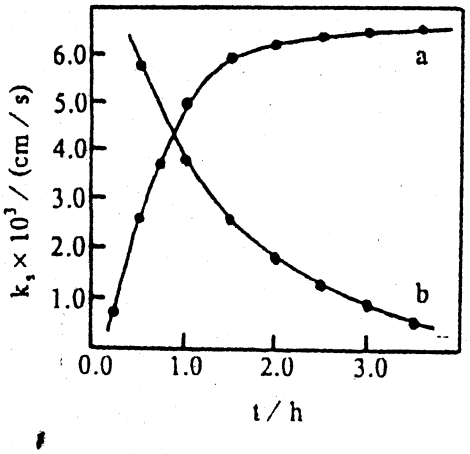

Fig. 2 Plots of k_s against the immersing time of gold electrode the DMMP solution (a) and the exposure time of DMMP / Au electrode to air (b).

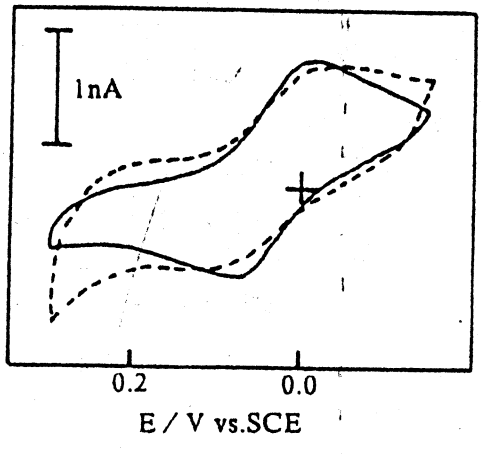

Fig. 3 Cyclic voltammograms of cytochrome c at DMMP / Au electrode at 50mv / s in phosphate buffer. Solid line, 10th cycle; dashed line, 300th cycle.

the 300th (dashed line) scanning (50mv / s). The stability of DMMP / Au electrode for promoting the redox reaction of cytochrome c could be ascribed to the high stability of thio–Au bond formed between DMMP and Au atoms. If the freshly prepared DMMP / Au electrode is exposed to air, the separation of peaks increases with the increase of exposure time because of the effect of contamination from air, hence the k_s decreases (Fig.2b). However, the DMMP / Au electrode can still retain the promotional activity for redox reaction of cytochrome c, even though it has been exposed in air for over 3 hours.

All the above characteristics suggest that DMMP can act as an effective promoter for the redox reaction of cytochrome c as long as it it adsorbed on the electrode surface.

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