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The direct electrochemistry of cytochrome *c* at the nanometer-sized rare earth element oxide particle-modified gold electrodes

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Abstract

The direct electrochemistry of cytochrome *c* was studied at nanometer-sized rare earth element dioxide particle-modified gold electrodes. It was demonstrated that rare earth element oxides can accelerate the electrochemical reaction of cytochrome *c* and the reversibility of the electrochemical reaction of cytochrome *c* was related to the size of rare earth element oxide particles.

Keywords: Cytochrome *c*; Rare earth elements; Nanometer-sized particle

1. Introduction

The electron transfer reactions of biological molecules are fundamental to life processes. There has been substantial interest in understanding the reaction mechanisms by which biological molecules can efficiently and selectively transport electrons intermolecularly and intramolecularly. Electrochemical methods are particularly suited to the study of the electron transfer reactions of biomolecules. As early as in 1933, the electrochemical properties of cytochrome *c* were studied [1]. However, research relating to direct electron transfer between biomolecules and electrodes did not experience a rapid growth because the electrochemical reactions of most of biomolecules at bare metal electrodes are often irreversible and in some case undetectable [2]. The major breakthrough occurred when the direct quasi-reversible electrochemical reaction of cytochrome *c* was obtained at the tin-doped indium oxide electrode [3] and at the gold electrode in cytochrome *c* solution with 4,4′-bipyridine [4]. Since then, an extensive research effort has been devoted to the study of the direct electrochemical reaction between cytochrome *c* and promoter-modified electrodes.

To date, most of the promoters studied are organic compounds [5]. Only a few inorganic promoters, such as tin-doped indium oxide [3], S [6] and As adatoms [7], heteropolytunstates [8] and pyrolytic graphite electrode [9] have been reported. Among the inorganic promoters studied, the semiconductors, such as tin-doped indium oxide, are most interesting because the surface morphology and chemistry of many semiconductors mimics natural redox partners and photoelectrochemical studies on the semiconductor-protein

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interface can allow straightforward kinetic analysis [10].

The nanometer-sized crystalline material is a kind of very promising solid substance with the potential applications in catalysis, designing molecular devices and photoelectrochemistry [11]. In this report, the direct electrochemical behavior of cytochrome *c* was studied at the gold electrodes modified with nanometer-sized semiconductor particles of several rare earth element oxides. The results showed that all the rare earth element oxide studied are efficient promoters for the electrochemical reaction of cytochrome *c*. The promoter effect is found to be related to the size of the rare earth element oxide particles.

2. Experimental

Horse heart cytochrome *c* (type VI, 99%, Sigma Chemical Co.) was used without further purification. All other chemicals were of reagent grade. The 0.38 mM cytochrome *c* solution with 0.025 M phosphate buffer at pH 7.0 and 0.1 M sodium perchlorate was used for the electrochemical measurements. The solution was prepared with triple distilled water and deaerated before use by nitrogen bubbling.

Cyclic voltammetry was performed using a Model 276 potentiostat, Model 179 universal programmer (Princeton Applied Research, USA), Houston Instruments Series 2000 Omnigraphic XY recorder and a conventional three-electrode electrochemical cell. The working electrode was constructed from a gold rod which was sealed into glass tubing with Torr seal (Varian). The exposed area 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 surface was obtained. The electrode was then sonicated in deionized water. Surface modification of the working electrode was carried out by dipping the freshly polished working electrode into a rare earth element oxide suspension solution for a certain time, followed by rinsing two times with triple distilled water.

The nanometer-sized particles of rare earth element oxides were prepared by the sol–gel method [12]. The precursor of a rare earth element oxide was calcinated at 250–1000°C for 2 h in air. A Kigaku D/MAX-IIB X-ray diffractometer was used to analyse the phases. H-600 Transmission Electron Microscope was used to observe the morphology of the particles and to measure the size of the particles. The specific surface area of the particles of rare earth element oxide was measured with Beauner–Emmett–Teller (BET) method.

3. Results and discussion

Fig. 1 is the X-ray diffraction spectrum of CeO_2 particles synthesized by this group. The d values of CeO_2 particles are consistent with JCPDS standard card (4-0593). They are cubic in structure. CeO_2 particles are spherical in shape based on the morphology study with a transmission electron microscope. Fig. 2 shows the relationship between the particle size and the calcination temperature. It can be seen from Fig. 2 that the particle size increases with increase of calcination temperature. Using the BET method, the specific surface areas of CeO_2 particles were measured. Fig. 3 shows the relationship between the particle size and the specific surface area. The results indicated that the small-sized particles are of large specific surface areas and thus a low calcination temperature should be used if a large specific surface area is required.

Cytochrome *c* does not undergo rapid electrochemical reaction at the bare gold electrode [2]. However, at CeO_2 particle-modified gold electrodes, a pair of redox peaks can be observed in the cyclic voltammogram (CV) of cytochrome *c* (Fig. 4). It was found that the CV response is related to the size of CeO_2 particles. Fig. 4 shows the CVs of cytochrome *c* at the gold electrodes

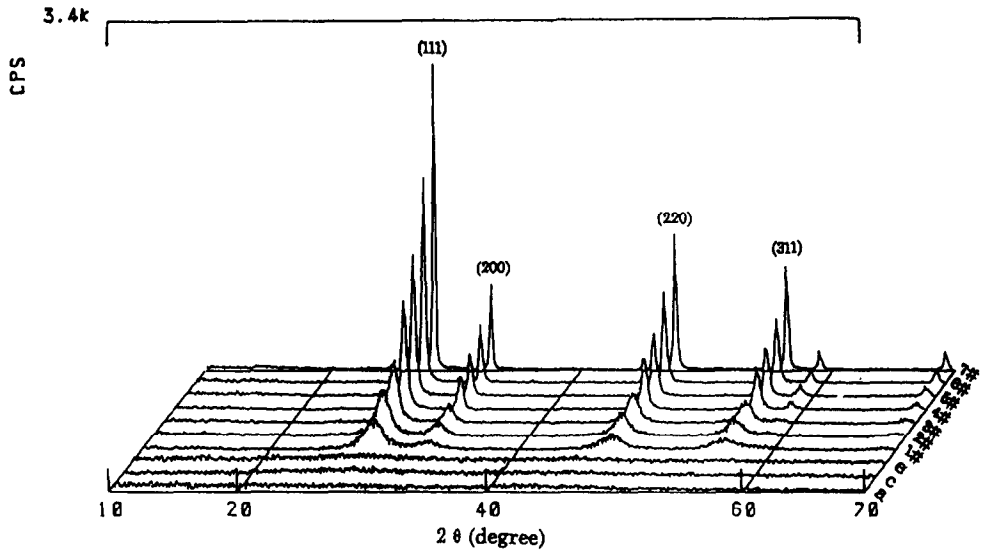

Fig. 1. The X-ray diffraction patterns of the dried gel calcinated at various temperatures for 2 h. (a) not calcinated, (c) 210°C, (e) 230°C; (1) 250°C, (2) 350°C, (3) 450°C, (4) 600°C, (5) 700°C, (6) 800°C, (7) 1000°C.

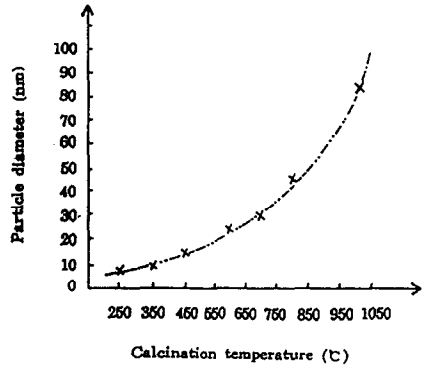

Fig. 2. Dependence of the average size of the CeO_2 particle on the calcination temperature.

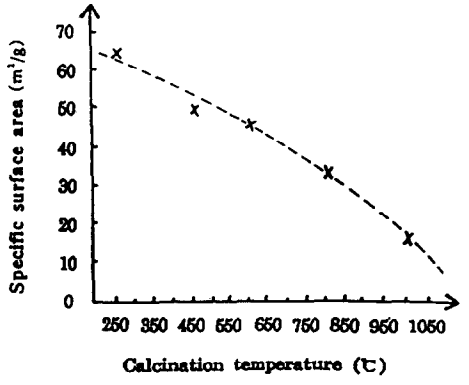

Fig. 3. Relationship between the specific surface area of CeO_2 particles and calcination temperature.

modified with CeO_2 particles with different sizes. It can be seen that the peak current increases and the separation between the cathodic and anodic peak potentials in the CV of cytochrome *c* decreases with decrease in the average particle size of CeO_2 particles. For example, the 110 mV peak separation for the 100 nm average particle

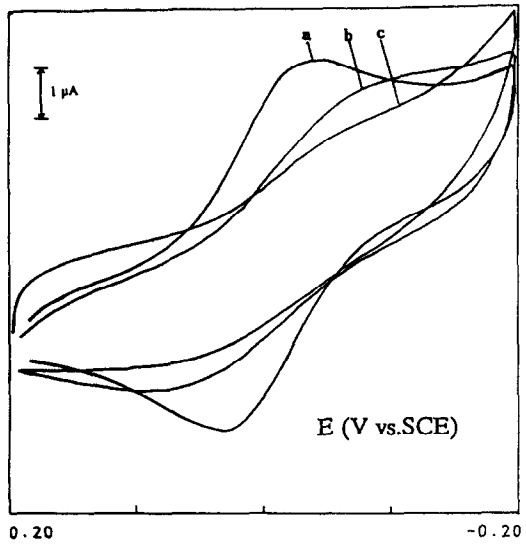

Fig. 4. Cyclic voltammograms of 0.38 mM cytochrome *c* in phosphate buffer solution (6.97) with 0.1 M $NaClO_4$ at (a) 8 nm sized, (b) 35 nm sized, (c) 100 nm sized CeO_2-modified gold electrodes. Scan rate: 50 mV/s.

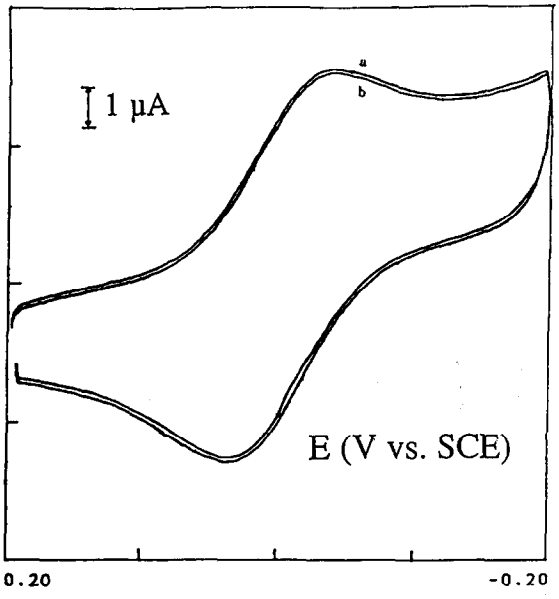

Fig. 5. Cyclic voltammograms of 0.38 mM cytochrome *c* at nanometer-sized CeO_2 particle-modified gold electrode in phosphate buffer solution (6.97) with 0.1 M $NaClO_4$. (a) First, (b) 25th cycle. Particle size: 8 nm, scan rate: 50 mV/s.

size of the CeO_2 particles (Fig. 4, curve c), 85 mV for the 35 nm average particle size (Fig. 4, curve b) and 70 mV for the 8 nm size (Fig. 4, curve a).

For curve a in Fig. 4, the peak separation in the CV of cytochrome *c* is 70 mV which is slightly larger than that for a reversible one-electron transfer reaction. The midpoint of the potentials of the cathodic and anodic peaks is about 0.01 V, which is close to the formal potential of the native cytochrome *c* [13]. The ratio of the cathodic and anodic peak currents is in proximity to one. A plot of the peak current for both the cathodic and anodic peaks shows a linear increase in the peak current as a function of the square root of the scan rate demonstrating that the electrochemical reaction rate of cytochrome *c* is diffusion controlled. In addition, CeO_2 particles have no electrochemical reaction in the potential range of −0.2 V to +0.2 V. Therefore, all the above characteristics suggest a quasi-reversible direct electron transfer reaction between cytochrome *c* and the gold electrode modified with nanometer-sized CeO_2 particles. The performance of the nanometer-sized CeO_2 particle-modified electrode is very stable. For example, after 25 continuous cycles, the CV response was nearly the same as that observed initially (Fig. 5). Thus, the nanometer-sized CeO_2 particles are the effective promoter for the electrochemical reaction of cytochrome *c*.

Table 1
The peak separations in the CV of cytochrome c at the different nanometer-sized rare earth element particle-modified gold electrodes

	Particle					
	CeO_2	Eu_2O_3	Nd_2O_3	Er_2O_3	Yb_2O_3	Dy_2O_3
Peak separation(mV)	70	80	75	82	74	80

The promoter effects of the nanometer-sized particles of the other rare earth element oxides, such as Eu_2O_3, Nd_2O_3, Er_2O_3, Yb_2O_3 and Dy_2O_3, were also studied. The peak separation in the CV of cytochrome *c* at the gold electrodes modified with these oxide particles of the 8 nm average particle size is somewhat larger than that for CeO_2 particles with the same average particle size (Table 1) indicating that these rare earth element particles are also the good promoters for the electrochemical reaction of cytochrome *c*.

After an extensive investigation, Hill et al. [4] proposed a model of the promoter function: on the one hand, the promoter can be adsorbed on the electrode surface and on the other hand, it can interact with cytochrome *c* through salt bridge or hydrogen bond. The function is favorable for forming an electron transfer pathway between the electrode and cytochrome *c* so that the rate of electron transfer between the electrode and cytochrome *c* can be accelerated. Cytochrome *c* is a highly ionic protein with a net charge of +9 in the oxidized state at pH 7. The 9 net positive charges are distributed on the lysine residues near the redox center of heme group. When the gold electrodes are modified with nanometer-sized rare earth element oxide particles, the interfacial oxygen atom in the lattice of the crystalline structure of an oxide is the functional group. It could form hydrogen bonds with positively charged lysine residues in the cytochrome *c* molecule. When small-sized particles are used, a large specific sur-

face area is obtained and more oxygen atoms would be distributed on the interface in the crystalline structure [14]. Then, the promoter effect of the small-sized oxide particles is better than that of the large-sized particles.

Acknowledgements

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