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Short Communication

A study on Cu_2Co_2SOD and Co_2Co_2SOD by voltammetry and thin-layer spectroelectrochemistry

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Abstract

The electrochemistry of Co_2Co_2SOD and Cu_2Co_2SOD on a pyrolytic graphite electrode (PGE) without using mediators was investigated by cyclic voltammetry (CV) and differential pulse voltammetry (DPV). The reversible and quasi-reversible voltammetric responses were observed for Co_2Co_2SOD and Cu_2Co_2SOD, respectively. Their formal redox potentials and electron numbers involved in electrode reactions were obtained, and are in agreement with those by spectroelectrochemistry (SEC).

Keywords: Cu_2Co_2SOD; Co_2Co_2SOD; Voltammetry

1. Introduction

Cu_2Zn_2-superoxide dismutase (Cu_2Zn_2SOD) is a dimeric enzyme consisted of two identical subunits, each containing one copper(II) and one zinc(II) ions, which are bridged by imidazolate. The catalytical reaction occurs at the site of copper, and zinc plays a role in stabilizing protein structure [1]. When copper(II) and zinc(II) in the native enzyme were substituted partially or completely by cobalt(II), two derivatives, Cu_2Co_2SOD and Co_2Co_2SOD, were obtained. The catalytic activities of Cu_2Zn_2SOD and its derivatives are related to their redox properties. Therefore, many scholars have paid attention to the studies on their electrochemical behaviors [2–4]. Claire et al. [5] have studied the temperature dependence of the reduction potential of Cu_2Zn_2SOD by spectroelectrochemistry. Afterwards, the redox potentials of Cu_2Zn_2SOD and its mutants were also reported [6]. In their systems, the promoters or mediators were used in order to enhance the rate of electron transfer and to decrease the absorption of proteins. Recently, we investigated successfully the redox behaviors of Cu_2Zn_2SOD and its reconstituted products on dropping mercury electrodes in the absence of the mediators by cyclic voltammetry and direct current polarography [7]. Also, Daniele et al. [8] determined the activity of Cu_2Zn_2SOD by steady-state voltammetry using mercury microelectrodes. These two examples show that the direct method without using mediators also would obtain the satisfactory results. In this paper, the electrochemical behaviors of Co_2Co_2SOD and Cu_2Co_2SOD were investigated by cyclic voltammetry (CV) and differential pulse voltammetry (DPV) on a pretreated pyrolytic graphite electrode (PGE) as well as by spectroelectrochemistry (SEC). The redox potentials and electron numbers of Co_2Co_2SOD and Cu_2Co_2SOD involved in electrode reactions on PGE were obtained and were in accordance with those by spectroelectrochemistry. The experimental results showed that the direct voltammetry on PGE for the investigations of Co_2Co_2SOD and Cu_2Co_2SOD was feasible and gave the explanation of inactivity of Co_2Co_2SOD. Although Co_2Co_2SOD has been researched by ^{1}HNMR and other methods, but to our knowledge, it is studied by electrochemical method for the first time.

2. Experimental

2.1. Materials

All chemical reagents were of A. R. grade and were recrystallized in twice-distilled water before use. SOD was extracted from bovine erythrocyte by the methods of the

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previous authors [3,9]. The purified SOD had only one homogeneous and clear band in polyacrylamide gel electrophoresis. The specific activity was assayed by xanthine oxidase method to be 6000 units mg^{-1}. The parameters of EPR and peak values of UV spectra were in agreement with those in the previous reports [10]. The ratio of A_{280}/A_{680} was 31. Its Cu and Zn contents were measured by inductively coupled plasma spectrometry to be 0.398% and 0.402%, respectively, being consistent with the calculated values 0.387% and 0.399%. The Co_2Co_2SOD and Cu_2Co_2 SOD were prepared by the methods reported previously and were characterized by elemental analysis and spectroscopy [11,12]. The specific activity of Cu_2Co_2SOD were 5500 units mg^{-1}, being 95% of the native enzyme, and Co_2Co_2 SOD scarcely displayed activity.

Xanthine oxidaze/xanthine system was employed for producing O_2^{-} [9], SOD competed with cytochrome *c* for O_2^{-}, resulting in the variation of the absorbance of cytochrome *c* at 550 nm. The variation of the absorbance at 550 nm was recorded for calculating the activity of SOD.

2.2. Electrochemical measurements

Cyclic voltammetry and differential pulse voltammetry measurements were carried out on an EG&G PAR 270 electrochemical analysis system with Model 273 potentiostat. A pyrolytic graphite electrode (area 0.054 cm^2) was employed as working electrode, a saturated calomel electrode as reference electrode, and a platinum coil wire as auxiliary electrode. A cell with content of 3 ml was used. All experiments were carried out under argon atmosphere and at 25 ± 0.10 °C. The blank solution containing 0.05 mol dm^{-3} HAc-NaAc buffer, (ca. pH = 5.6) and 0.1 mol dm^{-3} $NaClO_4$ was used for electrochemical and spectroelectrochemical experiments. The solution containing Co_2Co_2SOD or Cu_2Co_2SOD ca. 5.0×10^{-3} mol dm^{-3} was prepared by adding weighed sample into the blank solution and was injected into the cell and then deaerated for c.a.15 min before applying the voltage. The PGE was polished carefully by metallographic coated abrasive (1700 mesh, Electroanalytical Instruments, made in China), and then cleaned by ultrasonic for 10 min in twice-distilled water. The electrode was immersed immediately in a blank solution for ca.10–15 min, and then scanned cyclically from + 0.6 to − 1.5 V until a reproducible base current was obtained. Afterwards, the electrode with absorbed solution membrane was removed from the blank solution without washing and transferred to the solution to be assayed to record the CV and DPV curves. For CV experiments, the midpoint potential $E_{1/2}$ (or formal redox potential E^0) values were calculated from $(E_{pa}+E_{pc})/2$ (where E_{pa} and E_{pc} are the anodic and cathodic peak potentials, respectively). In DPV experiment, $E_{1/2}$ values were obtained by the Parry–Osteryoung relationship, $E_{1/2}=E_p+\Delta E/2$. Each measurement was repeated several times and the redox potentials were found to be reproducible within ± 5 mV. Unless otherwise stated, all the potentials reported are referred to the standard calomel electrode (SCE).

2.3. Spectroelectrochemical measurements

The spectroelectrochemical measurements of Co_2Co_2SOD and Cu_2Co_2SOD were performed by using a optically transparent thin-layer electrochemical cell as described by Heineman et al. [13]. The thin-layer electrochemical cell is consisted of a reticulated pyrolytic graphite electrode (OTTLE) which was sandwiched between two quartz slides separated by 0.8 mm. The OTTLE was made by cutting the pyrolytic graphite (Prifzer, USA) into minigrid. Its area within the thin-layer cell was approximately 0.8 × 2.8 cm^2, and the cell volume was ca. 0.6 ml. The open end of the cell was dipped into a small cup, several milliliters of the solution (2.07×10^{-3} mol dm^{-3} Co_2Co_2SOD in the blank solution) was poured into the cup. The saturated colomel and Pt coil wire electrodes were also immersed in the cup. The set was placed in the sample compartment of a shimadz UV-3100 spectrophotometer. The experiments were performed under anaerobic condition by varying the potentials applied across the cell. At each applied potential, a spectrum was recorded after equilibrium was established, namely, absorbance reached a constant value, it needed about 5 min. The redox potential E^0 and the electron number n involved in the reduction reaction were obtained by the following equation:

$$E_{app} = E^0 - (0.059/n)\log[(A - A_\infty)/(A_o - A)] \qquad (1)$$

where E_{app} denotes applied potential. A, absorbance at 596 nm and at given applied potential. A_o and A_∞, absorbance of oxidation form and reduction form for Co_2Co_2SOD, respectively.

3. Results and discussion

3.1. Cyclic voltammetric behaviors

The cyclic voltammograms of Co_2Co_2SOD and Cu_2Co_2 SOD were shown in Fig. 1. In the scan range from − 1.10 to − 1.45 V, only the Co_2Co_2SOD displayed a redox process, but the Cu_2Co_2SOD is inactive electrochemically. By subtracting CV curves of blank solution from curve 1, the difference spectrum was obtained (Fig. 1(a), inset). It showed one pair of well-defined redox peaks with peak potentials E_{pc} = − 1.289 V, E_{pa} = − 1.237 V and the ratio of cathode current to anode one i_{pc}/i_{pa} was close to unity. The separation of anodic from cathodic peaks ΔE_p was 52 mV, which was independent on the scan rates of in the range of 0.06–0.50 V s^{-1}. Both i_{pc} and i_{pa} were varied proportionally to the square root of potential scan rate $v^{1/2}$ (Fig. 2). The results indicated that the electrochemical process was reversible and the electrode reaction was controlled by diffusion

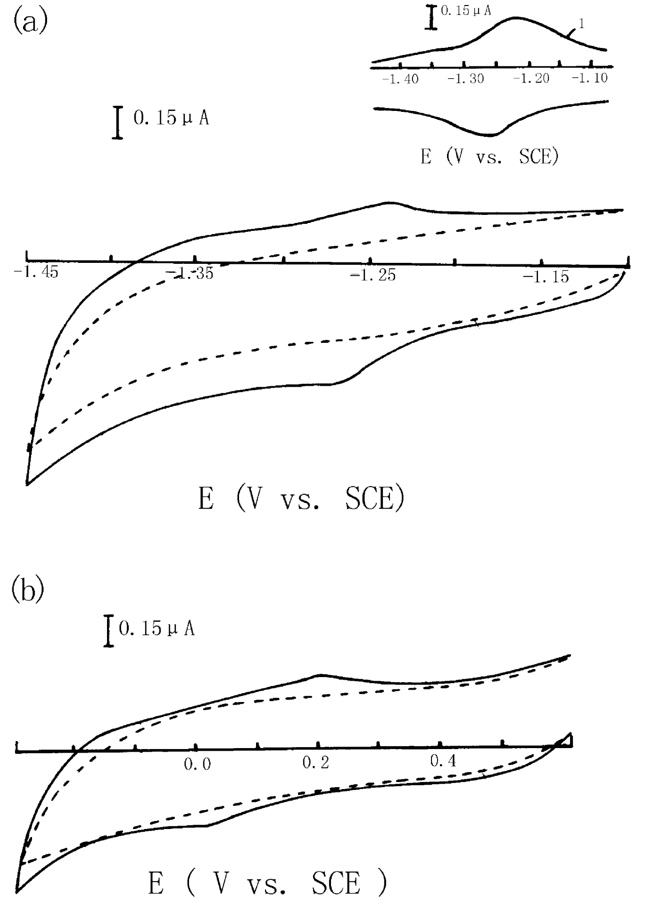

Fig. 1. Cyclic voltammograms of Co_2Co_2SOD and Cu_2Co_2SOD in the presence of 0.05 mol dm^{-3} HAc-NaAc and 0.1 mol dm^{-3} $NaClO_4$, scan rate 100 mV s^{-1}. Full line: Co_2Co_2SOD and Cu_2Co_2SOD, broken line: blank solution. (a) 4.88×10^{-5} mol dm^{-3} Co_2Co_2SOD. Inset: difference curve between the curve of test solution and the blank one. (b) 4.68×10^{-5} mol dm^{-3} Cu_2Co_2SOD.

of protein molecules from bulk solution to electrode surface. One pair of peaks in the voltammogram could be designated to be single-electron redox peaks of cobalt(II) in native copper site of Cu_2Zn_2SOD (see below). The electrode reaction was given as follows:

$$1/2Co^{II}_2Co^{II}_2SOD + e \rightarrow 1/2Co^{I}_2Co^{II}_2SOD$$
$$E^0 = -1.263 \text{ V (SCE)}$$

During scanning from −0.3 to+0.6 V, only Cu_2Co_2SOD gave one pair of redox peaks with E_{pc}=+0.209 V, E_{pa}=+0.028 V, i_{pa}=0.131 μA, i_{pc}=0.137 μA, i_{pa}/i_{pc}=1.0 and ΔE_p=+0.181 V (Fig. 1(b)). The results showed that the electrochemical process was quasi-reversible with $E_{1/2}$=+0.119 V (SCE), or +0.361 V (NHE). This pair of redox peaks was attributed to the reduction of copper(II) in Cu_2Co_2SOD. Azab et al. [6] have reported that the $E_{1/2}$ values of Cu_2Co_2SOD on modified gold electrode were in the range of 0.36–0.42 V (NHE). The $E_{1/2}$ values of Cu_2Co_2 SOD obtained on PGE is in agreement with those of Azab.

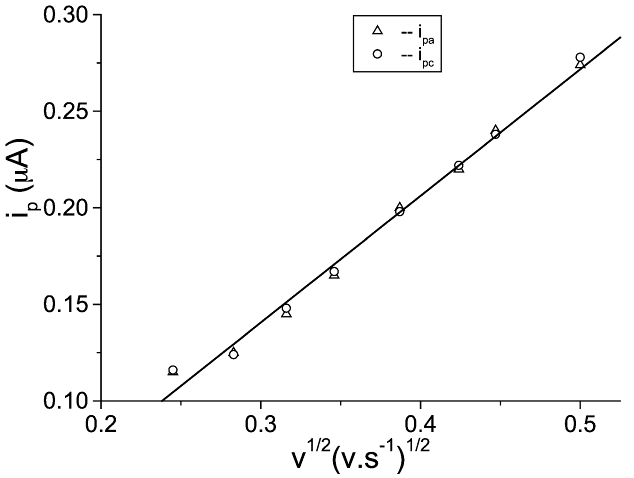

Fig. 2. Dependences of cathodic and anode peak current i_{pc} and i_{pa} on $v^{1/2}$ for Co_2Co_2SOD. The conditions were the same as Fig. 1.

We studied the electrode process by differential pulse voltammetry on PGE for deep insight. A DPV curve was illustrated in Fig. 3. The reduction peak potential E_p of Co_2Co_2SOD appeared at −1.225 V, the half-width value $W_{1/2}$ 0.085 V was close to reversible one-electron reduction process (0.090 V). The $E_{1/2}$ value −1.250 V was approximate to −1.263 V, which was obtained by CV. In Fig. 3 when the applied voltages were lower than −1.4 V, the current of blank solution increased rapidly with the decrement of the voltage. This resulted from the electrolysis of hydrogen ions in the solution with pH 5.6. Therefore, we obtained slightly asymmetrical curve in pulse voltammogram of Co_2Co_2SOD. As the same way, the $E_{1/2}$ value of redox of copper(II) in Cu_2Co_2SOD was obtained to be 0.110 V (SCE), or 0.352 V (NHE).

The diffusion coefficient of Co_2Co_2SOD in the solution was 7.45×10^{-7} cm^2 s^{-1}, calculated by Randles–Sevcik equation, using the slope of line (0.613×10^{-6} A (V $s^{-1})^{-1/2)}$ in Fig. 2. It is in agreement well with that of SOD (7.4×10^{-7} cm^2 s^{-1}) obtained by direct current

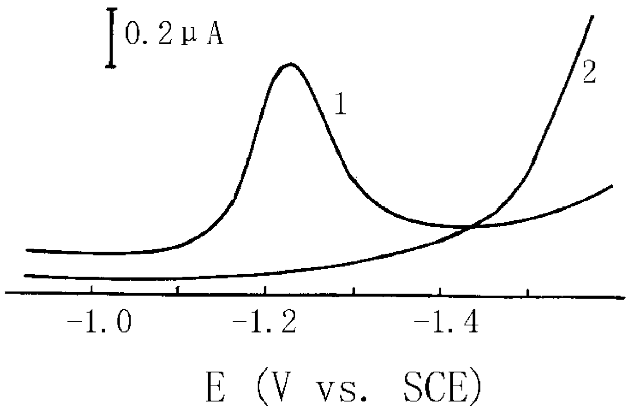

Fig. 3. Differential pulse voltammogram of 1—4.88×10^{-5} mol dm^{-3} Co_2Co_2SOD, 2—a blank solution. Scan rate: 0.5 mV s^{-1}, pulse height: 10 mV, pulse width: 50 mV.

polarograph [7]. The result demonstrated that diffusion coefficient was kept constant when all copper(II) and zinc(II) in Cu_2Zn_2SOD were replaced by cobalt(II).

3.2. Spectroelectrochemical studies

Cu_2Co_2SOD prepared by us has three absorption bands in electronic spectra which were contributed by cobalt(II) in Cu_2Co_2SOD. A maximum absorption band at 595 nm ($\varepsilon = 432$ mol^{-1} dm^3 cm^{-1}) [11], a distinguished band around 565 nm and a shoulder band at 532 nm were observed, implying that the cobalt(II) was bound in a tetrahedral coordination environment. In addition, a broad absorption centered at 680 nm ($\varepsilon = 298$ mol^{-1} dm^3 cm^{-1})^7 was assigned to d → d transition of copper(II) bound to protein. When the solution of Cu_2Co_2SOD in the spectroelectrochemical cell was reduced at about − 1.4 V, the broad absorption band at 680 nm rapidly decreased with time and disappeared finally, but the absorption band characteristic of cobalt(II) in Cu_2Co_2SOD scarcely varied (Fig. 4(a)). This showed that only copper(II) in Cu_2Co_2SOD was reduced completely at about − 1.4 V, but cobalt(II) in zinc site of Cu_2Co_2SOD did not display electroactivity. Therefore, it is undoubted that the pair of redox peaks with $E_{1/2} = +0.361$ V (NHE) in Fig. 1(b) ought to be designated to the reduction of copper(II) and re-oxidation.

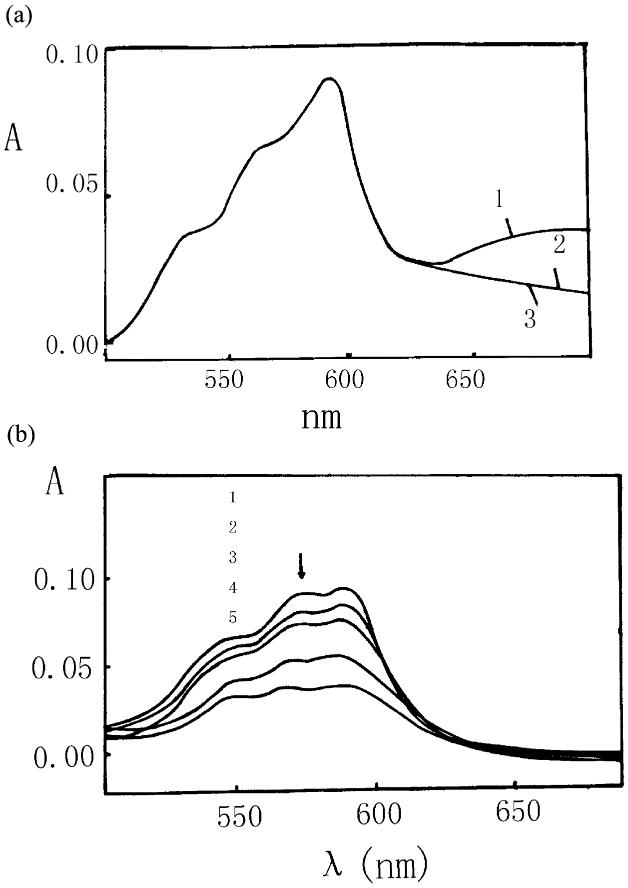

Fig. 4. Spectra of Co_2Cu_2SOD (a) and Co_2Co_2SOD (b) solution containing SOD 2.07×10^{-3} mol dm^{-3}, $NaClO_4$ 0.1 mol dm^{-3} and HAc-NaAc 0.05 mol dm^{-3}. Cell thickness: 0.8 mm. Applied potentials: 0 0.000 V, 1—1.100 V, 2—1.200 V, 3—1.250 V, 4—1.300 V, 5—1.400 V.

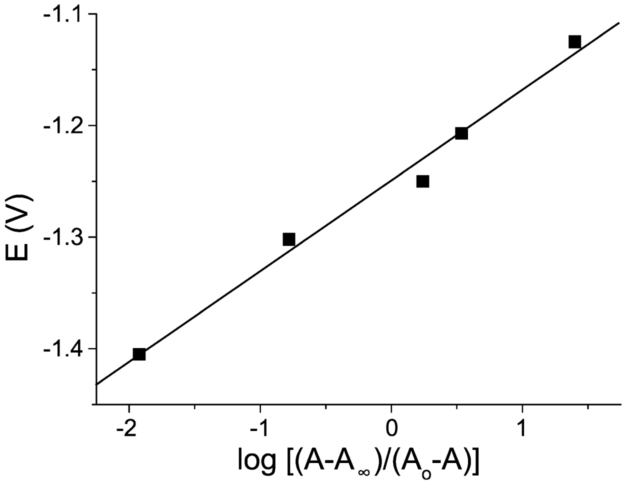

Fig. 5. The plot of E_{app} vs. $\log[(A - A_\infty)/(A_o - A)]$.

Three visible absorption bands of Co_2Co_2SOD were observed at 539 nm ($\varepsilon = 608$ mol^{-1} dm^3 cm^{-1}), 568 nm ($\varepsilon = 790$ mol^{-1} dm^3 cm^{-1}) and 596 nm ($\varepsilon = 804$ mol^{-1} dm^3 cm^{-1}). Their peak values and molar absorption coefficients were close to those reported previously [12]. Fig. 4(b) showed visible absorption spectra of Co_2Co_2SOD obtained using different applied potentials. According to Eq. (1), the values of formal redox potential $E^0(Co^{II}/Co^{I})$ and the electron number n were obtained by plotting E_{app} vs. $\log[(A - A_\infty)/A_o - A]$ (Fig. 5). The plot is linear as predicated by Eq. (1), n was 0.85 calculated by the slope and E^0 was − 1.245 V obtained by the intercept. The formal redox potentials of Co_2Co_2SOD and Cu_2Co_2SOD obtained by three methods were listed in Table 1.

In Table 1, the reaction of Co_2Co_2SOD was one-electron process with very negative potential relative to that of Cu(II)/Cu(I) couple in Cu_2Co_2SOD. Therefore, the Co_2Co_2 SOD was shown to be inactive in biological significance. It is remarkable that the values of E^0 obtained by cyclic voltammetry and differential pulse voltammetry were in agreement well with that by spectroelectrochemistry. This means that none of Cu_2Co_2SOD and Co_2Co_2SOD were denatured on the pretreated pyrolytic graphite electrode.

It is well known that the rates of heterogeneous electron transfer between proteins and electrode surfaces not only

Table 1
The formal redox potentials of Co_2Co_2SOD and Cu_2Co_2SOD in the presence of 0.1 mol dm^{-3} $NaClO_4$ and 0.05 mol dm^{-3} HAc-NaAc

Method	Co_2Co_2SOD			Cu_2Co_2SOD	
	CV	DPV	SEC	CV	DPV
E^0 (V, SCE)	− 1.262	− 1.250	− 1.245	+0.119	+0.110
N	1.04	1.07	0.85		

pH = 5.6, 25 °C.

depend on the purity of proteins [3,14], the pretreatment of electrodes [15], but also on the ionic strength of solutions [16], because the ionic strength plays an important role in adjusting the coulombic attraction between proteins and electrode surfaces, and also in enhancing the rates of electron transfer. In our experiments, the PGE was immersed for 10–15 min in blank solution with definite ionic strength before measurement, and the clear voltammograms were obtained. However, if the electrode was not treated, only a very weak and insignificant current signal was obtained. We consider that a homogeneous absorbed ionic membrane was formed during immersing the PGE in blank solution, the absorbed membrane played an important role in reducing the coulombic attraction between proteins and electrode surface, in decreasing the adsorption of proteins on the electrode surface and in enhancing the rate of electron transfer between the electrode and proteins. So we could obtain the well-defined peaks of Cu_2Co_2SOD and Co_2Co_2SOD.

Thin-layer spectroelectrochemical method has been used effectively in the study of biological molecules, which are difficult to be investigated by electrochemical methods [5]. We have compared the results of direct electrochemistry of Co_2Co_2SOD with those of spectroelectrochemistry. They confirm that this direct voltammetry on pretreated pyrolytic graphite electrode is feasible to investigate Co_2Co_2SOD and Cu_2Co_2SOD.

Acknowledgements

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