Study on the ET Process of SOD at Cysteine Modified Gold Electrode

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(Received August 3, 1999; CL-990685)

Cysteine-modifed gold wire electrode was used for the first time to examine the promoted electron transfer process of superoxide dismutase. Electrochemical Quartz Crystal Microbalance technique was combined with cyclic voltammetric study for the understanding of coupled adsorption/desorption process of different states of SOD molecules.

The electron transfer (ET) process of bovine erythrocyte copper-zinc superoxide dismutase (SOD) was very slow at conventional solid electrode due to its low activity responded to electrochemical stimulation. Some promising results had been reported when histidine or arginine was modified on gold electrode in advance. It indicates that suitable amino acids might function as effective promoter for the ET process of SOD.[1]

In this paper, the electrochemical behavior of SOD at cysteine-modified gold electrode in neutral aqueous solution is reported with cyclic voltammetry and electrochemical quartz crystal microbalance (EQCM) techniques.

In view of different categories of amino acids, and also considering their hydrophilic properties,[2] cysteine was used as a hopeful candidate used in promoting the ET process between SOD and gold electrode.

Cu-Zn SOD (8000 U/mg) and L-cysteine were purchased without further purification. All other reagents were of analytical grade and doubly distilled water was used for the preparation of aqueous solutions. The cysteine-modified gold electrode was prepared by dipping the electrode into a 2 mmol·L^{-1} cysteine solution for a given time, rinsed with doubly distilled water and then dried by airing.

Electrochemical measurements were run on M173 Potentiostat with M175 Programmer (EG&G PARC, USA) in phosphate buffer solution (pH=7.0). All measurements were undertaken at room temperature. The instrumentation and operating procedures for the EQCM measurement were as reported elsewhere.[3] The oscillator chip of the quartz crystal (9 MHz AT-cut) was coated with gold layers on both sides. One of them was used as working electrode for EQCM measurement.

Iyer[4] reported that an oxidation peak at 0.27 V(vs. SCE) could be observed for SOD system when the gold electrode was presetted with a potential of -0.1 V for a given duration (generally 10 min) followed by the initiation of an anodic scan and the subsequent reversal of scan at the anodic limit of potential. In this manner, however, no reduction peak response could be obtained.

As the ET process of SOD was studied in the presence of cysteine, inspiringly, apparent peak currents of both the anodic and cathodic directions were observed in author's lab. Figure 1 is the cyclic voltammograms of SOD at cysteine-modified gold wire electrode recorded under different scan rates. The peak currents of both directions increased with the continuous potential scans but repeated CV curves could be obtained after several cycles, with the peak current ratio (i_{Pa}/i_{Pc}) changing from 0.7 of the firstcycle to 1 of the reproducible cycle and the peak potential separation reduced to 120 mV under the scan rate of 10 mV/s. It depicts that this electrode process could be recognized as a quasi-reversible one. It was observed that the anodic peak potential E_{pa} and the cathodic peak potential E_{pc} were 0.12 V(vs. SCE) and 0 V(vs.SCE) respectively. Hence, the formal redox potential $E^{0'}$ of SOD could be estimated as 0.06 V (vs. SCE). The rate constant $k^{0'}$ for this heterogeneous electron transfer process and the diffusion coefficient D are calculated to be 5.56×10^{-4} cm.s^{-1} and 4.93×10^{-7} cm^{2}.s^{-1} respectively which are similar to the data obtained from microscale gold electrode.[1,5,6]

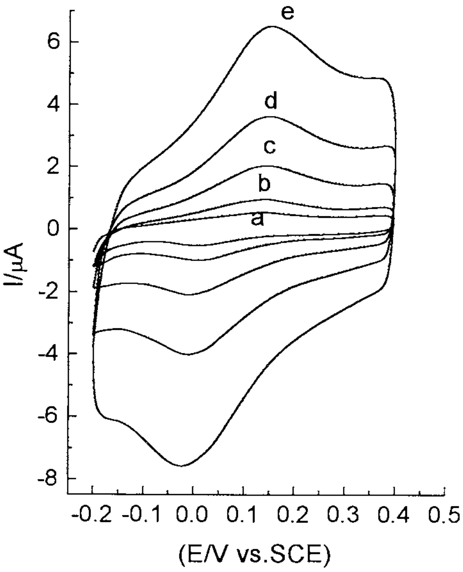

Figure 1. Cyclic voltammograms of SOD(0.26 mmol/L) at Cysteine-modified gold wire electrode at different scan rates. Scan rate(mV/s): a 10; b 20; c 50; d 100; e 200.

The similar CV plots could also be obtained in SOD solution after the sonication treatment of this cysteine-modified gold electrode. This interesting result means that the Au-S bond formed between gold electrode surface and the thiol group of cysteine molecules is stable enough for such modified electrode to be used repeatedly, and also inspired us that this promoting effect of cysteine might resulted from the strong adsorption of cysteine molecules onto the surface of gold electrode, which could be estimated as a special bridge between the SOD. At this point, the gold substrate electrode could be used effectively to create an optimal interface for the ET process.

In Figure 1, the peak currents (i_{Pc}) under different scan rates were found to be varied with scan rate v linearly, rather than $v^{1/2}$ in the range of v=10~200 mV/s . Moreover, it could be observed that the peak potential separation was somewhat larger than the data recognized as the criteria of reversible ET process. These phenomena seemed contradictive to the criteria of the 1:1 peak current ratio obtained above. As all the above evidences are weighed, this complicated process of SOD might be analyzed as a quasi-reversible ET process coupled by an adsorption/ desorption process.

In order to acquire deep understanding related to the ET process of SOD, EQCM was used to study the adsorption/ desorption process during the potential scan described above. The oscilating frequency shift at the cysteine-modified Au-QCM during the potential scan in SOD solution was detected for process estimation.

As the Au-QCM was applied with potential scan between -0.2 V and 0.4 V, either in the pure SOD solution or in the pure cysteine solution, the frequency almost didn't shift with potential scan. But as the measurement was performed in the mixed solution of SOD and cysteine or in the SOD solution with the cysteine-modified Au-QCM, distinct difference was produced.

In Figure 2, the upper part is the CV plot of SOD at the cysteine-modified Au-QCM, it is similar to that obtained at the cysteine-modified gold wire electrode but only a few shift lies in the peak potentials. The lower part is the frequency shift of the cysteine-modified Au-QCM in the SOD solution corresponding to the electrochemical process listed above. It was observed that as the potential was scanned positively to the anodic peak potential of 0.22 V, the frequency dropped by 30 Hz, then the frequency kept constant as the potential changed from 0.22 V to 0.40 V and back to 0.08 V afterwards. As the potential was scanned negatively from 0.08 V (near the formal potential $E^{0'}$) to -0.20 V, the frequency increased to its initial level again. The frequency shift of the oscillator could not be observed either in the pure SOD solution or in the pure cysteine solution, but an obvious frequency decrease was observed in the mixed solution of above two species during the positive potential scan. It depicts the existed adsorption of SOD in its oxidized form. On the contrary, there existed a comparable frequency increase during the reverse scan, which might reflect a corresponding desorption process. This result indicates that an optimal interface for the facilitated ET process between the SOD and the gold substrate electrode can be established in the presence of cysteine.

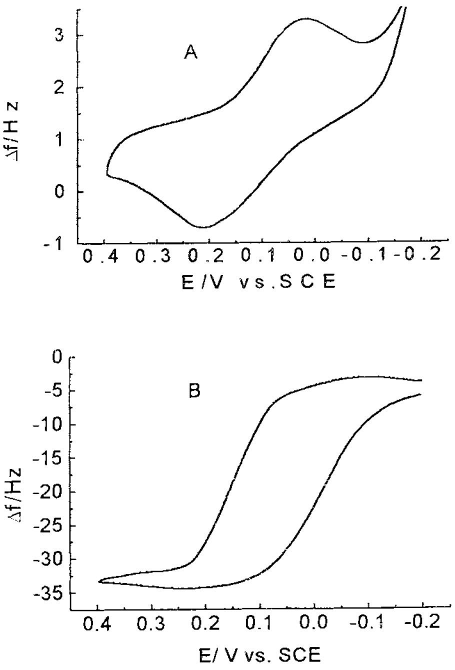

Figure 2. Cyclic voltammogram (A) and the frequency shift (B) of SOD at cysteine-modified Au-QCM. Scan rate: 10 mV/s.

In conclusion, the well-defined redox response of Cu-Zn SOD could be observed at the cysteine-modified gold electrode, and the adsorbed cysteine molecules could be recognized as an effective promoter for the slow ET process of SOD. Additionally, the frequency shift observed in EQCM measurement might be related to the adsorption/desorption process of different states of SOD molecules coupled to the ET process of SOD simultaneously. Further study for deeper understanding of discussed process is under investigation continously.

Financial support from Natural Science Fundation of Shanghai and Shanghai Education Commission is grateful.

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