Direct electron transfer reactions of glucose oxidase and D-amino acid oxidase at a glassy carbon electrode in organic media

By: Wei Jianjun, Qin Yiqin, Liu Haiying, Deng Jiaqi

J. Wei, Y. Q. Qin, H. Y. Liu, and J. Q. Deng. “Direct Electron Transfer Reactions of Glucose Oxidase and D-Amino Acid Oxidase at a Glass Carbon Electrode in Organic Media” *Journal of Shanghai University (Science Edition, English), 1998*, 2 (1) 77-80.

Abstract:

Cyclic voltammetry is employed to demonstrate feasibility of direct electron transfer of glucose oxidase and D-amino acid oxidase at a glassy carbon electrode in organic media. The reversible slight conformational change of glucose oxidase is observed by changing 0.1 mol/L phosphate buffer to acetonitrile containing 10% v/v of water and 0.05 mol/L tetrabutyalammonium perchlorate, and vice versa.

Keywords: glucose oxidase | D-amino acid oxidase | direct electron transfer

Article:

1. Introduction

The electrochemistry of proteins is attracting increasing attention as a tool for study of the modulation of their catalytic activity by the redox potential and also for the development of biosensors [1-6]. Much work has been focused on reversible interracial reactions involving small proteins such as, particularly cytochrome C, at electroinactive modifier electrode. Such modifiers appear to facilitate rapid and reversible adsorption of proteins at the interface via hydrogen bonding and the formation of salt bridges [5-7]. However, direct electrochemistry of glucose oxidase with Ca. 160,000 Da molecular weight from Aspergillus niger has been less reported because the redox centers of glucose oxidase (GOD), two identical and noninteracting flavin groups [8], is electrically insulated by thick glycoprotein shells, preventing direct electrical communication between the centers and electrodes. Considerable work has involved electrical communication between glucose oxidase and electrodes via a soluble or imobilized electron transfer mediator [9,10]. In recent years, quasi-reversible electrochemistry of GOD has been reported at a variety of suitably modified carbon [2,11] and gold surfaces [1]. In this paper, we will report, for the first time, the reversible direct electrochemistry of GOD or D-amino acid oxidase immobilized on the surface of a glassy carbon electrode in organic media. The reversible slight conformational change of GOD was observed by changing 0.1 mol/L phosphate buffer (7. 0) to acetonitrile containing 10% v/v of water and 0. 1 mol/L tetrabutyalammonium perchlorate, and vice versa. Moreover, immobilized GOD held an active conformation in acetonitrile with 10% v/v of water.

2. Experimental Section

2.1 Materials

Glucose oxidase (EC 1.1.3.4, type Vll-S) and Damino acid oxidase were obtained from Sigma. Glutaraldehyde (25%) was obtained from Merck. Glucose and bovine serum albuming (BSA) were purchased from Shanghai Chemical Reagent Company (Shanghai). Glucose solutions were stored overnight to allow the attainment of mutarotational equilibrium before use. All other chemicals employed were of analytical grade and used without further purification. Doubly distilled water was used to prepare the solutions.

2.2 Apparatus

Cyclic voltammetry was performed by using an EG&G potentiostat/galvanostar 273A (Princenton, NJ) controlled with an IBM-286 personal computer running the EG & G PARC Model 270/250 electrochemical software package for parameter setup and data acquisition and processing. The graphics output was sent to a Hewlett-Packard Model HP 7440A ColorPro plotter or a type 3086 x-y recorder (Tokyo, Japan). EG & G PARC Model 270/250 electrochemical software employed different line styles to differentiate the results for various conditions. A three-electrode configuration was employed in all the measurements. A saturated calomel electrode (SCE) serves as a reference electrode, against which all potentials were quoted in this paper. A thermostatic, stirred electrochemical cell consisted of a vial of 5 ml of volume with a PTFE cap. A platinum wire served as counter electrode.

2.3 Construction of chemically modified electrodes

The glassy carbon electrode (3.5ram in diameter) was prepared and its clean reflective finish was obtained [10,12]. 12 mg of glucose oxidase, 10 mg of bovine serum albumin, 0.21 ml of 0.1 mol/L phosphate buffer (pH 7.0) were completely mixed and then 15 μl of a 5% glutaraldehyde solution was added. The solution was completely mixed again. Aliquots (10 μl) of the solution were pipetted onto glassy carbon electrode, letting it dried in air.

D-amino acid oxidase was immobilized on glassy carbon electrodes in the similar way. 25 ml of 0.1 mol/L phosphate buffer (pH 7.5) was placed in a 10-ml plastic centrifuge tube. followed by 15 mg D-amino acid oxidase and 10 mg of bovine serum albumin. After vortexing for 15 minutes and then 16 μl of a 5% glutaraldehyde solution was added. An aliquot (15 μl) of the mixture was pipetted onto the surface of a glassy carbon electrode after vortexing for 15 minutes.

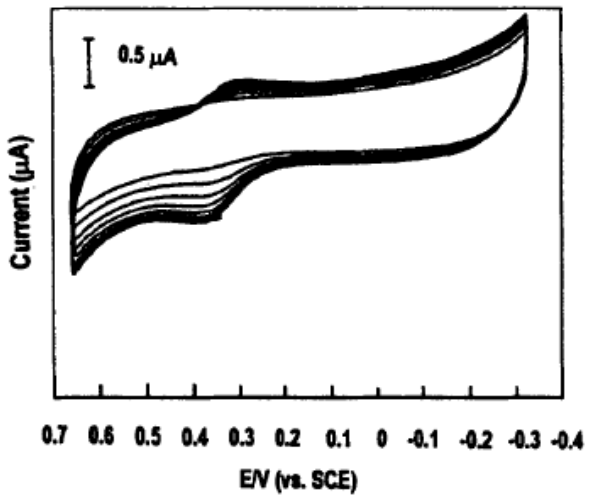

Fig. 1 Cyclic voltammograms of the GOD-electrode in acetonitrile solution containing 10% v/v water and 0. 05 mol/L tetraethylammonium chloride at a scan rate of 65 mV/s.

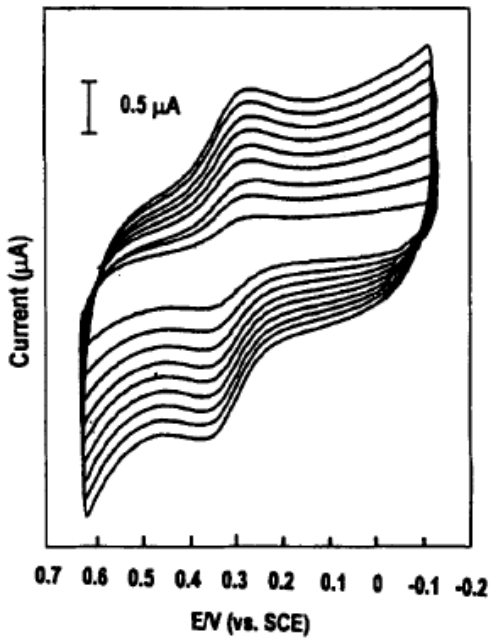

Fig. 2 Cyclic voltammograms of the GOD-electrode in acetonitrile solution containing 10% v/v water and 0. 05 mol/L tetraethylammonium chloride at various scan rate of 25, 45, 65, 85, 105, 125, 145, 165 and 185 mV/s (from inner curve to outer one).

3. Results and Discussion

3.1 Direct redox reaction of glucose oxidase in acetonitrile containing 10% water

No direct electrochemistry of immobilized GOD was observed at a glassy carbon electrode in 0.1 mol/L phosphate buffer (pH 4.5, 6.0, 7.0 and 8.0) or in pure acetonitrile containing 0.05 mol/L tetraetyalammonium perchlorate. However, immobilized GOD underwent direct electron transfer reactions in acetonitrile with 10% v/v of water and 0. 05 mol/L tetraethylammonium chloride

(shown in Fig. 1). The redox peak of cyclic voltammogram increased with each cycle scan as two identical and noninteracting flavin groups of GOD got closer to the electrode, which may result from the influence of acetonitrile and water. They reached a steady state after 20 minutes' scan. Glucose oxidase changed its conformation under the influence of acetonitrile and water so that the redox active center (flavin groups) of glucose oxidase gradually approached to a glassy carbon electrode, resulting in direct electron transfer and an increase of redox currents at the electrode. The welldefined redox peak currents for immobilized GOD were found to be directly dependent on the scan rate when they reached a steady state (shown in Fig. 2). These facts indicate that GOD undergoes reversible charge transfer in acetonitrile with 10% v/v water, $E_{1/2}$ of 0. 32 V vs. SCE, Peak-to-peak separation of 60 mV at 35 mV s^1. In addition, the redox potential of flavin adenine dinucleotide (FAD) bound within GOD in acetonitrile with 10% v/v water shifts anodically by 0. 65 V with respect to that of the unbound species in aqueous solution (--0. 2 V). Following 3 minutes' incubation of the GOD-electrode in 0.1 mol/L phosphate buffer (pH 6.0), the characteristic electrochemistry disappeared in 0.1 mol/L phosphate buffer, indicating that GOD changed its conformation under influence of water, causing its redox centers to depart from the surface of the electrode. This long distance between the redox centers of GOD and the electrode in the phosphate buffer prevented the direct electron transfer between GOD and the electrode and thus no redox reaction was observed at the electrode in the phosphate buffer. However, the direct electrochemical behavior of immobilized GOD similar to the phenomena mentioned above was observed again when the GOD-electrode was conducted in acetonitrile with 10% v/v water, which indicates that GOD undergoes a slight reversible conformational change during the change of two kinds of solution. Addition of glucose resulted in an increase of the oxidation current and a decrease of reduction current of GOD under aerobic conditions (shown in Fig.3), indicating that GOD holds an active conformation in acetonitrile with 10% v/v water.

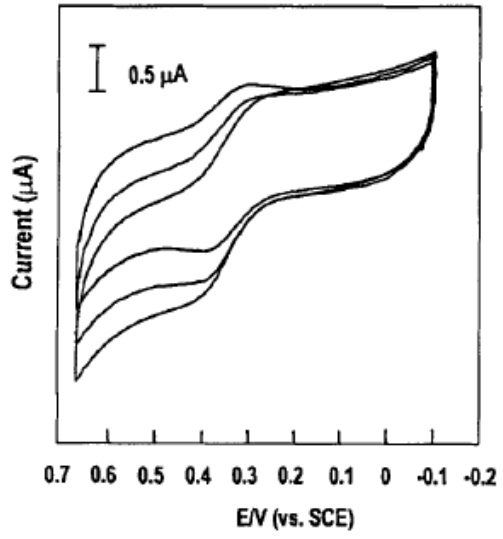

Fig. 3 Cyclic voltammograms of the GOD-electrode in acetonitrile solution containing 10% v/v water and 0. 05 mol/L tetraethylammonium chloride at a scan rate of 65 mV/s in the absence (a) and presence of glucose (b) 10 mmol/L and (c) 20 mmol/L.

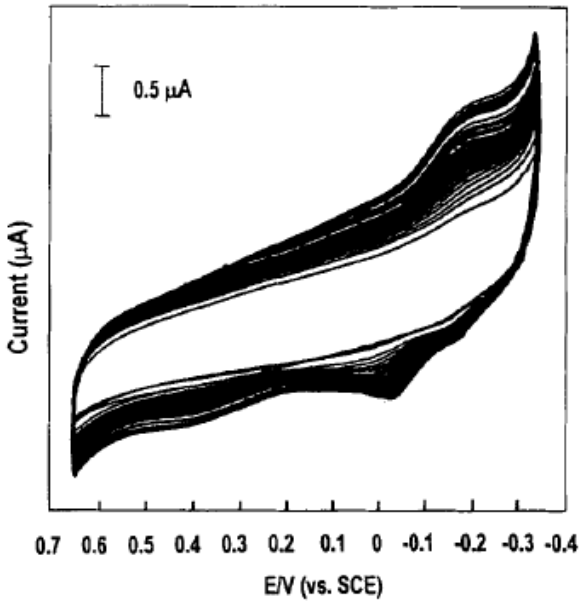

Fig. 4 Cyclic voltammograms of the D-amino acid oxidase-electrode in acetonitrile solution containing 10% v/v water and 0. 05 mol/L tetraethylammonium chloride at a scan rate of 65 mV/s.

3.2 Effect of conducting salts, organic solvents and content of water on redox reaction of GOD

Similar phenomena were found by using tetrabutyalammonium perchlorate as a conducting salt in place of tetraethylammonium chloride or tetramethylammonium chloride. No change of redox currents was found by varying the conducting salt concentration between 0.1 mol/L and 0.05 mol/L. In addition, no direct electrochemistry of GOD was found in acetone, butane and tetrahydrofuran with 10% v/v of water and 0. 1 mol/L tetraethylammonium chloride, which indicates that direct electrochemistry of GOD is greatly dependent on organic solvent.

Redox currents of GOD increased with an increase of water in acetonitrile and reached a maximum value when the water was 10% in the solution. The redox currents keep changed with further increase of water.

3.3 Direct redox reaction of D-amino acid oxidase in acetonitrile containing 10% water

Immobilized D-amino acid oxidase underwent the direct electron transfer reactions in acetonitrile with 10% v/v water and 0.1 mol/L tetraethylammonium chloride (shown in Fig.4). The results similar to those of GOD were obtained. Immobilized D-amino acid oxidase showed the formal potential ($E_{1/2}$) of --0. 12 V vs. SCE and peak-to-peak separation of 0.18 V vs. SEC, which was less reversible than glucose oxidase. The difference between formal potentials of glucose oxidase and D-amino acid oxidase is due to their different environments.

Cyclic voltammograms of horseradish peroxidase modified electrode were observed unchanged in acetonitrile solution containing 10% v/v water and 0.05 mol/L tetraethylammonium chloride, which indicates that immobilized horseradish peroxidase with heme as coenzyme does not undergo direct reaction at the electrode, neither does cytochrome C according to our experiment.

In conclusion, many immobilized enzymes with flavin adenine dinucleotide ($FAD/FADH_2$) coenzyme such as glucose oxidase and D-amino acid oxidase can undergo direct electron transfer reaction at a glassy carbon electrode in acetonitrile solution containg 10% v/v water and 0.05 mol/L tetraethylammonium chloride.

Acknowledgment

Project supported by the Natural Science Foundation of Shanghai for Young scientists (96QF 36)

References

1. Jiang L., McNeil C. J. and Cooper J. M., J. Chem. Soc., Chem. Commun., 1995:1293
2. Chi Q., Zhang J., Dong S. and Wang, E., J. Chem. Soc., Faraday Trans., 90:2057 (1994)
3. Armstrong F. A., Hill H. A.O. and Walton N. J.,Acc. Chem. Res., 21:407(1988)
4. Bourdillon C., Bourgeois J.P. and Thomas D., J. Am. Chem. Soc., 102: 4231(1980)
5. Taniguchi I., Hirakawa Y., Iwakiri K., Tominaga M. and Nishiyama K., J. Chem. Soc., Chem. Commun., 1994:953
6. Armstrong F.A., Hill H.A.O. and Oliver B.N., J. Chem. Soc., Chem. Commun., 1984:976

7. Lu T., Yu X., Dong S., Zhou C., Ye S. and Cotton T. M., J. Electronic Chem., 369:79 (1994)
8. Pazur J.H. and Kleppe K., Biochemistry, 3:578 (1964)
9. Degani Y. and Heller A., J. Am. Chem. Soc., 111:2357 (1989)
10. Liu H. and Deng J. , Anal. Chim. Acta. , 300:65(1995)
11. Chi Q., Zhang J., Dong S. and Wang E., Electrochemical Acta, 39: 2431(1994)
12. Liu H. and Deng J., Anal. Lett., 28:563 (1995)