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DNA facilitating electron transfer reaction of xanthine oxidase

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Abstract

Xanthine oxidase has potential multi electrochemical active centers (one molybdenum center, two Fe_2–S_2 centers and one flavin adenine dinucleotide (FAD)). The FAD and molybdenum group can exchange electron with electrode, because the active centers have cave connecting to the protein surface. Fe_2–S_2 centers bury in the protein framework and act as wire conduction the electron transfer between FAD and molybdenum groups. But there is still no report that electrochemical signals of these two active sites have been observed simultaneously. In this paper, we use DNA as a matrix to embed xanthine oxidase, and obtained the electrochemical responds of FAD and molybdenum center of xanthine oxidase. Moreover, the enzyme keeps its native catalytic activity to hypoxanthine in the DNA film. So, DNA can be considered as a "conductive molecular wire" to facilitate electron transfer between the enzyme and electrode.

Keywords: Cyclic voltammetry; DNA; Electron transfer; Xanthine oxidase

1. Introduction

Some physical mechanisms that might assure the function of DNA as a molecular wire have been considered on the basis of recent progress in understanding charge transfer of biological molecules [1–8]. So, the advent of molecular electronics has stimulated the interest in the possibility to exploit this molecule in functional mesoscopic electronic devices [9–12] and considerable interest has focused on the application of DNA based on its π-stacked base pairs [6,8,13–16]. This key structure feature is similar to the conductive one-dimensional aromatic crystals, which prompts the proposal that in the interior of the double helix, the stack of base pairs can provide a one-dimensional pathway for charge migration and acts as a "π way" for the efficient transfer of electrons [17–19].

Recently, direct electrochemistry of redox enzymes has aroused increasing interest since it can establish a model for the mechanism study of electron exchange among enzymes in biological systems. Protein film voltammetry has proven to be provided a powerful way and means to obtain the direct electrochemistry of proteins as well as to investigate how electron-transfer coupling occurs at active sites and how catalytic electron transfer through an enzyme is controlled. In this approach, protein is immobilized on an electrode surface as an absorbed electroactive film and by applying a potential, electrons are driven in and out of the active sites. Electrochemical signals can be obtained from these redox processes.

Xanthine oxidase (XOD) has been implicated as a key oxidative enzyme in many physiological processes. The enzyme can catalytically oxidize many substrates, including purines, pteridines, heterocyclic molecules and aldehydes [20]. The oxidation of xanthine takes place at the molybdenum center and the electrons distributed to other redox centers. The reoxidation of the

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reduced enzyme by the oxidant substrate, either NAD^+ or molecular oxygen, occurs through FAD [21]. Furthermore, the electron transfers of O_2, NAD^+, methylene blue, quinines and nitrate are also associated with XOD. So, electrochemical researchers have studied the electron transfer property of XOD [22–24], and used this enzyme to detect xanthine, hypoxanthine and other biological molecules [25–27]. Nonetheless, the electron transfer reaction of XOD is still very difficult to be achieved. Although various methods have been employed, there is no finding that electrochemical signals both of FAD and molybdenum centers can be observed simultaneously.

In this paper, we use DNA to facilitate electron transfer of FAD and molybdenum centers, which are both electrochemically active. Meanwhile, XOD keeps its enzymatic activity to hypoxanthine. In DNA, because π-conjugated nucleic acid bases are stacked, it is naturally considered to act as an effective molecular wire for electron transfer. Our experimental results indicate that DNA can simultaneously activate both the FAD and the molybdenum centers of XOD.

2. Experimental

Lyophilized powder xanthine oxidase from buttermilk (E.C. 1.1.3.22, MW = 275,000) was obtained from Sigma and used as received. Salmon sperm DNA was from the Chemical Plant of Huadong Normal University in Shanghai (China). Other reagents were of analytical grade. Water was purified with a Milli-Q purification system and was used to prepare all solutions.

Electrochemical experiments were carried out with a PAR 283 Potentiostat/Galvanostat (EG&G, NJ, USA). In a one-compartment glass cell with 5 ml reagent, a three-electrode system is used for measurement consisting of a modified pyrolytic graphite (PG) working electrode, a saturated calomel electrode (SCE) used as reference and a platinum wire as a counter electrode. All the following potentials reported in this work were versus SCE.

The PG electrode was prepared by putting a PG rod into a glass tube with fixing it by epoxy resin. Electrical contact was made by adhering a copper wire to the rode with the help of Wood alloy. The XOD/DNA modified PG electrodes were made by following procedures. The substrate PG electrode was first polished using rough and fine aluminum oxide papers. Then, it was polished to mirror smoothness with aluminum oxide (particle size of about 0.05 μm)/water slurry on silk. Finally, the electrode was thoroughly washed with doubly distilled water and treated in an ultrasonic bath for 5 min. Mixture of 10 μL 0.04 U/ml XOD and 10 μL 1.0 mg/ml DNA solution was spread on the PG electrode surface. The film was then dried overnight at room temperature. The modified electrode was thoroughly rinsed with nanopure water and was ready for use. When not in use, the modified electrode was stored at 4 °C in the refrigerator.

When electrochemical measurements were in an anaerobic measurement, the test solution was first bubbled thoroughly with high purity nitrogen, and then a stream of nitrogen was blown gently across the surface of the solution in order to maintain the solution anaerobic throughout the experiment. Cyclic voltammetry was employed to record and monitor the signals.

3. Results and discussion

It has been known that XOD has two independent subunits each containing four redox centers: a molybdenum (VI) center, two iron–sulfur (Fe_2–S_2) clusters called Fe/S I and Fe/S II and a flavin adenine dinucleotide (FAD) unit. The molybdenum and the FAD centers can accept two electrons each while the iron–sulfur clusters can accept one electron each [28–34]. When XOD catalyze the oxidation of xanthine or hypoxanthine, xanthine or hypoxanthine reacts at the molybdenum site while oxygen reacts with the flavo group [32]. In previous studies, the solution must be aerobic so that reduced XOD can be oxidized by dissolved O_2.

Same as the previous reports, under an aerobic condition, only a pair of peaks attributed to FAD group of XOD can be observed (Fig. 1). However, when XOD/DNA modified electrode is immerged in pH 5.0 phosphate buffer solution under an anaerobic condition, two pairs of peaks can be observed, as shown in Fig. 2(a). One pair of peaks, E_{pc} and E_{pa}, values at −0.380 and −0.310 V, and the other pair of peaks locates at −0.480 and −0.420 V, respectively. In contrast, there is no visible peak when the electrode is modified DNA

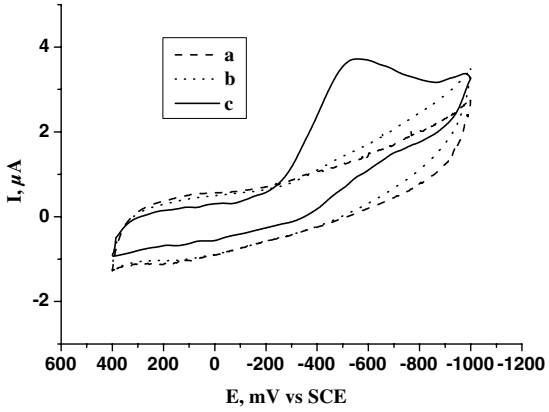

Fig. 1. The CVs of (a) bare PG electrode; (b) DNA alone modified PG electrode; (c) XOD/DNA modified PG electrode in pH 5.0 buffer solution (under the aerobic condition).