Available online at www.sciencedirect.com
ScienceDirect
Journal of Electroanalytical Chemistry 614 (2008) 67–72
Journal of Electroanalytical Chemistry
www.elsevier.com/locate/jelechem

Mediated bioelectrocatalytic reaction at an ultrathin redox polymer film on a glassy carbon electrode surface and effect of the ionic strength on the catalytic current

Seiya Tsujimura, Akio Ishii, Tomohiko Abo, Kenji Kano *

Division of Applied Life Sciences, Graduate School of Agriculture, Kyoto University, Sakyo, Kyoto 606-8502, Japan

Received 20 August 2007; received in revised form 2 November 2007; accepted 9 November 2007
Available online 21 November 2007

Abstract

Intermolecular interactions between water-soluble pyrroloquinoline quinone-dependent glucose dehydrogenase (PQQ-GDH) and an Os complex-containing redox polymer coated on a glassy carbon electrode surface were investigated, especially by focusing on their electrostatic interaction, in which the electrostatic interaction was changed by varying the ionic strength in the solution. The redox polymer showed a strong repulsive interaction between the positively charged Os complexes, especially at low ionic strengths. The polymer worked as a good electron-shuttling mediator between PQQ-GDH and the electrode to produce a steady-state catalytic current ascribed to the D-glucose oxidation. The catalytic current drastically increased with an increase in the ionic strength, presumably due to the reduction of the repulsive electrostatic interaction between the active site of the enzyme and the Os complex in the polymer and between the Os complexes. The electrostatic interaction governs the electrostatic interaction between the redox site of PQQ-GDH and the Os complexes, and the dynamics of the Os complexes on the electrode surface.

Keywords: Modified electrode; Bioelectrocatalysis; Redox polymer; Mediator; Electrostatic interaction

1. Introduction

The bioelectrocatalytic reaction is an electrode reaction catalyzed by oxidoreductases, and is the basic principle of electrochemical biosensors, bioelectroorganic synthesis, and biofuel cells [1–7]. The reaction systems are classified into two types based on the electron transfer (ET) mechanism between the enzymes and electrodes: one is the direct electron transfer (DET) type and the other is the mediated electron transfer (MET) type. However, the catalytic redox center of most of the enzymes is buried in protein shells, and DET-based catalytic phenomena have been reported for a limited number of enzyme species [7–10]. The mediators, which are usually small redox active molecules, assist the ET between the enzyme active site of the enzymes and electrodes. The use of mediators has opened up a route for the use of a wide variety of redox enzymes.

The immobilization of mediators and enzymes on an electrode surface would facilitate practical bioelectrochemical applications. Many methods have been developed to immobilize polymerized mediators, such as electro-polymerization, electrostatic or covalent cross-linking to a polymer network, which may be conductive or insulating [11–15]. Heller's group first employed redox polymers for the immobilization and mediation of redox enzymes in the context of amperometric biosensors, in which enzymes and a redox polymer containing Os complexes were covalently connected with cross-linking agents, such as poly(ethylene glycol) diglycidyl ether [16–18], or coordinatively crosslinked [19].

When enzymes and mediators are co-immobilized in the film via covalent and/or non-covalent bonds, they would

* Corresponding author.
E-mail address: kkano@kais.kyoto-u.ac.jp (K. Kano).

0022-0728/$ - see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.jelechem.2007.11.003

be extremely concentrated and the distance between them would be shortened. This situation would strongly influence the bioelectrocatalytic activity due to limited mobility of the enzymes and the mediators and enhanced interactions between the enzymes and the mediators and between the enzyme and its substrate. It would be necessary to consider several interactions to accomplish the fast electron relay in the enzyme-mediator layer to design the enzyme-mediator-immobilized electrodes and optimize their performance. There are several inter- and intra-molecular non-covalent interactions in the redox polymer–enzyme film; electrostatic interaction would be very important because soluble macromolecules in the immobilized layer including enzymes, redox mediators, and polymer backbones are charged in most cases. A number of approaches have been investigated to optimize the ionic environments in the film for the desired operating conditions; they might be controlled by (1) adjusting the polymer backbone by mixing monomer units bearing various functional groups, (2) changing the length and chemical properties of the side chain, and (3) mixing several polymers, in which the polymer may be conductive or insulating, and charged or non-charged. For example, Heller's group has developed a new redox polymer linking an Os complex with long and flexible tether and has successfully enhanced the mediated electrocatalytic current [20].

We focused in this study on the effect of the ionic strength on a bioelectrochemical reaction occurring on an electrode coated with an ultrathin film of a redox polymer complexed with an Os complex. Pyrroloquinoline quinone-dependent glucose dehydrogenase (PQQ-GDH, EC 1.1.99.17), which catalyzes the oxidation of D-glucose, was used as the model enzyme. The enzyme was not immobilized within the redox polymer, because it would be difficult to separately analyze the effects of the bulk ionic strength on the electrochemical response of redox polymers and the enzymatic activity in the film.

2. Experimental

2.1. Materials

PQQ-GDH (from *Acinetobacter* sp., "Amano"5; 4200 unit mg^{-1}) was purchased from Amano Enzyme, Inc. (Nagoya, Japan). The D-glucose solution was prepared from a stock solution (2.0 M) that was stored overnight to achieve the mutarotative equilibrium. PVI–[OsCl(DMe-BPy)$_2$] (PVI–Os) (PVI = poly(*N*-vinylimidazole); DMe-BPy = 4,4′-dimethyl-2,2′-bipyridine) was synthesized and characterized according to the literature [21]. There is one [OsCl(DMe-BPy)$_2$] unit for every 10 imidazole monomer units. $[Os(4\text{-}CaPy)_6]^{4-}$, $[Os(3,4\text{-}DCaPy)_6]^{10-}$, $[Os(4\text{-}AmPy)_6]^{8+}$, and $[Os(4\text{-}MeIm)_2(DMe\text{-}BPy)_2]^{2+}$ (4-CaPy = 4-carboxypyridine; 3,4-DcaPy = 3,4-dicarboxypyridine; 4-AmPy = 4-aminopyridine; 4-MeIm = 4-methylimidazole) were synthesized according to the literature [22–24]. All other reagents were used without further purification.

2.2. Apparatus

A glassy carbon electrode (GCE, 3 mm diameter, BAS) was used as the working electrode. A BAS-100B electrochemical analyzer (BAS, USA) and a conventional three-electrode system, with an Ag|AgCl|sat. KCl electrode and a platinum wire as the reference and counter electrodes, were used for the electrochemical experiments. All potentials in this paper are referred to the Ag|AgCl|sat. KCl electrode. The measurements were carried out in a water-jacketed electrolysis cell at 25 ± 1 °C without deaeration.

2.3. Preparation of an ultrathin film of PVI–Os on GCE

PVI–Os was immobilized on an electrode surface according to the literature [19]. In brief, GCE was polished to a "mirror-like" finish with 1 and 0.3 μm alumina powder in this order, and then sonicated and rinsed with distilled water. The modified electrodes were prepared by a potential scanning method in the range between −0.05 and 0.25 V at 0.01 V s^{-1} for 100 cycles in a phosphate buffer (50 mM, pH 7) containing PVI–Os (0.05 mg ml^{-1}). In the bioelectrocatalytic D-glucose oxidation experiments using the PVI–Os-modified electrode, PQQ-GDH (0.2 mg ml^{-1}) and D-glucose (0.2 M) were added to a buffer solution of MOPS (30 mM, pH 7.0) containing 3 mM $CaCl_2$.

3. Results and discussion

3.1. Electrochemical adsorption of PVI–Os on GCE

Fig. 1A shows the cyclic voltammograms (CVs) of PVI–Os adsorbed on GCE in pH 7 MOPS buffer (30 mM) containing 0.1 M KCl at several scan rates. It was found that the CVs behaved almost ideally as the surface-confined redox wave; i.e., the anodic and cathodic peak currents were proportional to the potential scan rate (v) (Fig. 1B), while the peak potential and peak separation were almost independent of v. It is believed that the Os polymer is immobilized on the GCE by forming a hydrophobic polymer network by exchanging the coordinated Cl to the neighboring imidazole [19].

The peak separation was 0.01 V and the midpoint potential was 0.115 V at $v = 0.05$ V s^{-1}. The peak-half-width of the CVs was about 0.11 V, which was somewhat greater than the theoretical one for the ideally reversible case with $n = 1$ (0.0906 V at 25 °C), n being the number of electrons [25]. This deviation in principle might be ascribed to slow heterogeneous ET rate and the interactions between the grafted Os complexes. However, the heterogeneous ET rate seemed to be fast because the peak separation did not depend on v. Therefore, the result suggests that the intramolecular interaction seems to be a predominant factor affecting the electrode reaction of the Os complexes on the electrode surface.

The peak current ascribed to the adsorbed Os complex increased with the increasing number of potential cycles