Osmium Complex Grafted on a Carbon Electrode Surface as a Mediator for a Bioelectrocatalytic Reaction

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A novel electrochemical modification method of carbon electrodes with redox active amines is proposed for mediated electron transfer between enzymes and electrodes. Histamine-coordinated Os complex was synthesized as a model redox amine. The electro-oxidation of the complex generated a stable covalent linkage between the nitrogen atom of the amino group and the carbon atom of the electrode surface. The covalent-linked Os complex on the electrode showed a good redox response and a high activity as an electron-transfer mediator between the electrode and a model enzyme: PQQ-dependant water-soluble glucose dehydrogenase.

Several oxidoreductase reactions can be coupled with an electrode reaction. The coupled reactions enable the oxidation/reduction of intrinsically electro-inactive biological compounds under very mild conditions. The system is called "bioelectrocatalysis" and would be utilized for several bioelectrochemical devices such as biosensors, biofuel cells, and bioreactors. The electron-transfer process between enzymes and electrodes can be classified into two categories from the viewpoint of the electron-transferring mode; one is the direct electron transfer (DET) and the other is the mediated electron transfer using mediators (MET).

The DET-type system is very simple in the structure and operates at potentials close to the formal potential of the enzyme because of the absence of mediators. These properties are very convenient for construction of miniaturized system. However, the rate of DET is usually very slow due to the long distance between an electrode surface and the redox site buried deeply in enzymes. Therefore, there are a limited number of examples for practical use, which include multi-copper oxidases and flavin (or quinone) and heme-containing dehydrogenases.[1]

The MET-type system using various kinds of redox mediators can realize fast electron transfer between the active site of enzymes and electrodes and has been utilized in bioelectrochemical devices for practical use. However, it is usually necessary to immobilize mediators as well as enzymes on the electrode surface. There have been a number of approaches for constructing catalytic enzyme films based on van der Waals interaction, electrostatic interaction, or cross-linkage with backbone polymers.[2,3] Such films, however, sometimes lacks long-term stability.

In this paper, a method has been investigated for chemical modification of carbon electrodes with amino group-containing redox compounds for the electron transfer between enzymes and electrodes. The method is based on the electrochemical oxidation of amines at carbon electrodes to form a covalent linkage between the N atom of the amine and the C atom of the electrode surface.[4,5] In this work, histamine (His) was used one of ligands of an Os complex. The complex was successfully linked to carbon electrodes and functioned well as a surface-confined mediator of the electron transfer from PQQ-dependant water-soluble glucose dehydrogenase (PQQ-GDH) to the carbon electrode.

Histamine-coordinated Os complex (OsHis(4,4′-dimethyl-2,2′-bipyridine)$_2$Cl) was synthesized by the following procedure.[6,7] $(NH_4)_2[OsCl_6]$ (1 mmol) and 2.0 equiv. of 4,4′-dimethyl-2,2′-bipyridine (4-dmbpy) (2 mmol) were refluxed in 1,2-ethanediol (18 mL ($L = dm^3$)) for 1 h under Ar atmosphere. After cooling, the reaction mixture was treated with 30 mL of 1 M ($M = mol\,L^{-1}$) $Na_2S_2O_4$. The reaction mixture was cooled for 30 min in an ice bath to precipitate the Os complex. The precipitate was thoroughly washed with cold water and diethyl ether, and then dried in vacuum. This was used as *cis*-[$OsCl_2$-(4-dmbpy)$_2$] without further purification. Histamine was complexed with *cis*-[$OsCl_2$(4-dmbpy)$_2$] in the following procedure. *cis*-[$OsCl_2$(4-dmbpy)$_2$] (0.2 mmol) and 50 equiv. of histamine$\cdot$2HCl (10 mmol) were refluxed in 1,2-ethanediol (6 mL) for 2 h under Ar. Saturated NH_4PF_6 solution (10 mL) was added to the cooled reaction mixture and the mixture was filtered with a membrane filter (pore size 1.0 μm, Millipore), yielding a dark brownish solid. The product was dried in vacuum and then purified by column chromatography on basic alumina using acetonitrile followed by water as eluents. A dark brown band was collected and the solvent was evaporated. The product was used as [OsCl(His)(4-dmbpy)$_2$]$(PF_6)_2$.

Electrochemical experiments were carried out using BAS 50W (BAS). Glassy carbon electrode (GCE, 3 mm diameter, BAS) was polished with emery papers (No. 600, 1000, and 2000) followed by alumina suspension (diameter: 3000 Å) on a polishing pad (Buehler) before use. In all electrochemical experiments, a single compartment cell was used with an Ag|AgCl|KCl (sat.) reference electrode and a platinum counter electrode. One mM ($M = mol\,L^{-1}$) of [Os(4-dmbpy)$_2$HisCl]-$(PF_6)_2$ solution was prepared by dissolving the complex in 100 μL of ethanol containing 0.1 M $LiClO_4$ and 900 μL potassium phosphate buffer (10 mM, pH 12.5). Electrochemical modification of a GCE with $[OsCl(His)(4\text{-}dmbpy)_2]^{+/2+}$ was performed in cyclic potential scanning mode between 0 and 1.1 V at a scan rate of $0.01\,V\,s^{-1}$.

The midpoint potential of the Os complex was 0.01 V, which is very close to that expected from the ligand effects.[8] $[OsCl(His)(4\text{-}dmbpy)_2]^{+/2+}$ was electrolyzed on a GCE in the range between 0 and 1.1 V to form the covalent linkage between the N atom of histamine and the C atom of the GCE as shown in the inset of Figure 1 by referring to the literature.[4,5] As shown by curve b in Figure 1, the complex showed an irreversible oxidation response above 0.8 V. Considering the literature on electrochemical oxidation of amines,[4,5] this wave is ascribed to the oxidation of the amino group in histamine coordinated to the Os complex, while the irreversible wave above 1.2 V corresponds to the oxidation of the solvent as shown by curve a in Figure 1.

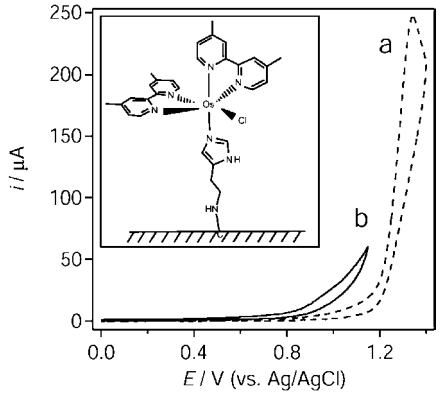

Figure 1. Voltammetric response of $OsCl(His)(4\text{-}dmbpy)_2$ to form a covalent bonding between the N atom in histamine and the C atom in the carbon electrode (inset). CVs obtained in the buffer solution of pH 12.5 in the absence (a) and presence (b) of the Os complex.

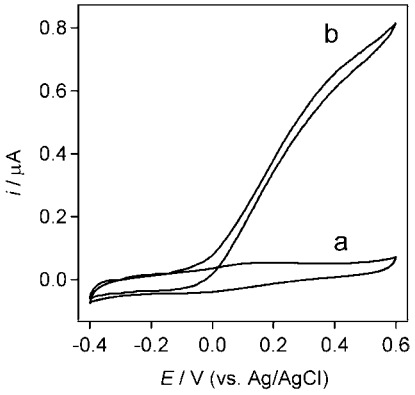

Figure 2. CVs of a Os complex-modified GCE in pH MOPS buffer (pH 7.0) containing 3 mM Ca^{2+} and PQQ-GDH ($0.02\,mg\,mL^{-1}$) in the absence (a) and the presence (b) of 0.1 M D-glucose at scan rate $0.005\,V\,s^{-1}$.

The electrode used for the oxidation of the amino group of the complex was thoroughly washed and sonicated in water and ethanol. The electrode showed a couple of the oxidation and reduction peaks in the absence of the Os complex in the MOPS buffer (Figure 2, curve a). The peak separation was 0.11 V and the midpoint potential was 0.05 V at a scan rate (v) of $0.05\,V\,s^{-1}$. The distance from the center metal to the electrode surface would reduce the electron-transfer kinetics. The peak separation depended on the ionic strength and would be also attributed to the electrostatic interaction. The peak current increased in proportion to v (data not shown). The covalent-linked Os complex was very stable during repeated measurements. The redox signal was unchanged for at least 1 week. The electricity of the oxidation of the Os complex attached to the GCE was evaluated by integrating the oxidation current in the cyclic voltammogram corrected the background current. The electricity corresponded to $0.12\,nmol\,cm^{-2}$ of the surface coverage of the Os complex. The value is close to that of the monolayer with full coverage assuming a surface roughness of unity.

The ability of the surface-confined Os complex as a mediator was examined using PQQ-GDH as an enzyme, since PQQ-GDH cannot pass the electrons from PQQ to electrodes directly. Using the Os-complex-modified GCE, cyclic voltammetry was carried out from −0.4 to 0.6 V in the MOPS buffer (30 mM, pH 7.0) containing $CaCl_2$ (3 mM), D-glucose (0.1 M) and PQQ-GDH (Amano Enzyme Inc.; GLUCDH "Amano"5; 4200 unit mg^{-1}; $0.02\,mg\,mL^{-1}$) at $v = 0.005\,V\,s^{-1}$. The large catalytic current was obtained as shown by curve b of Figure 2. In the absence of PQQ-GDH or glucose, no catalytic current was observed. The results indicate that the system allows rapid electron transfer from the reduced PQQ in PQQ-GDH to the immobilized Os complex. The acceptable function of the immobilized Os complex as a mediator might be in part due to the relatively large size of the active site pocket (oval shape from 1- to 3-nm length and ca. 0.8 nm in depth), in which the glucose oxidation reaction occurs and PQQ locates at the bottom of the pocket, as judged from the crystallographic structure.[9] The Os complex would be expected to be a sphere of ca. 1-nm diameter, which is somewhat smaller than the active site pocket.

4-Amino pyridine (NH_2-py) was used instead of histamine and the complex ($OsCl(NH_2\text{-}py)(4\text{-}dmbpy)_2$) was electrochemically immobilized on a GCE using similar procedure. However, the catalytic current was decreased to 10–20% of that observed with the $OsCl(His)(4\text{-}dmbpy)_2$-modified electrode. This result would suggest the significance of the distance from the center metal to the electrode surface. In addition, some extent of the flexibility is expected for $OsCl(His)(4\text{-}dmbpy)_2$ compared with $Os(4\text{-}NH_2\text{-}py)(4\text{-}dmbpy)_2$. These properties of $OsCl(His)(4\text{-}dmbpy)_2$ is convenient as a mediator between PQQ-GDH and electrode, compared with $OsCl(4\text{-}NH_2\text{-}py)(4\text{-}dmbpy)_2$. Detailed study on the factors governing the mediator function will be published elsewhere.

The procedure proposed in the present work would open routes to prepare carbon electrodes with redox functions. The method is very easy and applicable to variety of redox-active amines including metal complexes and organic compounds. The Os complex immobilized on carbon electrodes functions as a good mediator, as if the enzyme showed DET-type catalysis at modified electrode.

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