Direct Electron Transfer Reaction of d-Gluconate 2-Dehydrogenase Adsorbed on Bare and Thiol-modified Gold Electrodes

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Direct electron transfer-type bioelectrocatalytic oxidation of d-gluconate was observed with d-gluconate 2-dehydrogenase (GADH, EC 1.1.99.3, from Glucobacter frateurii) at bare and thiols-modified gold electrodes. Thiols used have different charges and various lengths of the alkyl chains. The catalytic current at every electrode arose at -0.05 V, which would correspond to the formal potential of the heme c site in GADH. The GADH-loading examined through quartz crystal microbalance measurements was practically independent of the surface properties. However, the current-potential curves were strongly affected by the electrode surface properties, and were interpreted in views of kinetics of enzymatic reaction and electrode reaction, and states of GADH on the electrode surface. The interfacial electron transfer rate constants of GADH depended on the alkane-chain lengths.

Key Words: d-Gluconate 2-Dehydrogenase, Au Electrode, Direct Electron Transfer, Quartz Crystal Microbalance

1 Introduction

Direct electron transfer (DET) reactions have received considerable attention not only for fundamental interest but also for construction of more practical, simplified and downscaled electrochemical applications, such as biosensors and biofuel cells. 1-5) The limited number of redox enzymes can realize a DET-reaction with electrodes, and it has been proposed that these enzymes have more than one redox center and one of the redox centers located near the protein surface acts as a built-in mediator for electron transfer (ET) between enzymes and electrodes. There are several approaches to achieve DET; one is the use of new materials such as carbon or metal nanoparticles. 6) It is also important to prepare electrodes with surface suitable for orientation and stabilization of adsorbed enzymes for efficient DET reaction. Functionalized thiols adsorbed spontaneously on Au surface to generate a monolayer exhibiting the functional group and to provide well-organized surface environment. 7) These electrodes have been utilized for the studies on the electrochemistry of redox proteins except redox enzymes, such as cytochrome c and azurin. For example, the effects of ET distance and electric field on the ET rate has been studied by varying the alkyl chain length. 8) In this study, thiol-modified Au electrodes were examined to elucidate the interfacial ET kinetics and the surface properties suitable for enzyme adsorption and orientation for DET reaction. We focused on d-gluconate 2-dehydrogenase (GADH; EC 1.1.99.3) from Glucobacter frateurii which is a membrane-bound enzyme catalyzing the oxidation of d-gluconate to 2-dehydro-d-gluconate. 9) In our preliminary study, we have demonstrated an anodic catalytic current arising from DET-reaction of GADH adsorbed on an indium-tin oxide electrode at pH 5. 10) Here, cyclic voltammetry of GADH was carried out in the presence of d-gluconate with bare and thiol-modified Au electrodes; positively or negatively charged thiols and hydrophobic alkanethiols were selected. The resultant voltammograms were analyzed in views of enzymatic reaction rate and interfacial ET reaction, and status of GADH on the electrode surface. Time-dependent adsorption behavior of GADH on the electrodes was also examined by quartz crystal microbalance (QCM) measurements.

2 Experimental

2.1 Materials and electrodes

d-Gluconate 2-dehydrogenase (GADH, 150 U mg⁻¹) was purified from Glucobacter frateurii NBRC 3271 as described previously. 10) The concentration of GADH in stock solution was determined by Lowry method. Thiols used for the surface modification of Au electrodes were as follows: 2-mercaptoethane sulfonic acid (MES) and mercaptoethylguanidine sulfate (MEG) from Wako, and 1-pentanethiol (PT), 1-octanethiol (OT) and 1-undecanethiol (UT) from Aldrich. 9 MHz AT-cut quartz crystal plates coated with Au were purchased from Seiko EG&G. The geometrical surface area of Au is 0.196 cm² with a roughness factor of 1.5 ~ 1.7. 11) The Au coated on quartz crystal plates were modified with thiol by immersing them overnight in ethanol solutions of 1 mM thiol, resulting in the formation of a self-assembled monolayer on the electrode surface.

2.2 Apparatus and electrochemical measurements

Cyclic voltammetry was performed on a BAS CV-50W electrochemical analyzer. A Pt wire was used as a counter electrode and Ag/AgCl/sat. KCl reference electrode was used. All potentials are referred to the Ag/AgCl/sat.
KCl electrode in this paper. All experiments were carried out in pH 4.0, 30 mM acetate buffer solution. QCM measurements were performed on a QCA-917 QCM analyzer (Seiko EG&G).

3 Results and Discussion

3.1 GADH adsorption on the electrodes

In order to investigate the amount and behavior of GADH adsorption on the electrode surfaces, QCM measurements were performed. Figure 1 shows typical time-dependence of the resonance frequency shift (Δf) using bare and thiol-modified Au electrodes; thiols selected in this study were hydrophobic (PT, OT, UT), negatively charged (MES), and positively charged (MEG). After the addition of GADH at the point indicated with the arrow and stirring solution for a few seconds, the resonance frequency decreased rapidly within few minutes and then gradually. It reached a plateau value of approximately −100 Hz at 2,500 s. The Δf was converted to the adsorbed mass of GADH (Δm) using Sauerbrey equation expressed by:

\[ \Delta f = -\frac{2f_0^2 \Delta m}{A(\mu \rho)^{1/2}} \]  

(1)

where A is the surface area of the electrode on the quartz crystal plate (0.196 cm²), \( f_0 \) is the fundamental resonance frequency (9 MHz), \( \mu \) is the density of quartz (2.648 × 10³ kg m⁻³), and \( \rho \) is the share module (2.947 × 10¹⁰ kg m⁻¹ s⁻²). The surface concentration of the adsorbed GADH (\( \Gamma_{GADH} \)) was calculated by regarding the molecular mass of GADH as 130,000 g⁻¹ \( \Gamma_{GADH} \) at bare and thiols-modified Au plates were summarized in Table 1. There were no significant differences in \( \Gamma_{GADH} \) values ((4.1 ± 0.65) × 10⁻¹² mol cm⁻²) and the time-dependent adsorption behavior at pH 4 seems to be practically independent of the functional group of thiols on the Au surface. When GADH is assumed to be a sphere with an average diameter of ca. 8 nm (assumed from the molecular weight of 130,000) and packed together as a monolayer on the electrodes as closely as their geometry allows, the maximum \( \Gamma_{GADH} \) value can be estimated as ca. 3 × 10⁻¹² mol cm⁻². GADH seems to be adsorbed on the electrodes as a monolayer by considering the roughness of the Au disk surface.

3.2 Electrochemistry of GADH on the electrodes

Figure 2 shows cyclic voltammograms (CVs) of GADH-adsorbed on bare and thiol-modified Au electrodes after 2,500 s of QCM measurements. No faradaic current attributed to the redox reaction of GADH itself was observed at every electrode. When D-glucuronate was added into the buffer solution as a final concentration of 50 mM (>> its Michaelis constant value for D-glucuronate¹⁰), catalytic currents were observed. The limiting value of the catalytic current density and the shape of potential-current curve strongly depended on the functional group of the thiols.

The shape of CVs should be affected by not \( \Gamma_{GADH} \) but the state of GADH on the electrode surface. In order to better understand and evaluate the DET-reaction, the current-potential curves were interpreted in views of kinetics and thermodynamics.

Assuming a simple DET-type GADH catalytic reaction, the current density (i) can be expressed by the following equations¹¹,¹⁴

\[
i = \frac{nFk_i \Gamma_{GADH}}{1 + \frac{k_m}{k_i} + \frac{k_m}{k_i}}
\]  

(2)

Table 1 Surface concentration and ET kinetic parameters of GADH at various electrodes.

<table>
<thead>
<tr>
<th>Electrode</th>
<th>( \Gamma_{GADH} ) (10⁻¹² mol cm⁻²)</th>
<th>( k_m/k_i ) (cm⁻¹)</th>
<th>( \lambda k_e ) (s⁻¹)</th>
<th>( \alpha )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bare Au</td>
<td>4.8</td>
<td>2.2</td>
<td>10</td>
<td>0.85</td>
</tr>
<tr>
<td>MES-Au</td>
<td>3.8</td>
<td>2.2</td>
<td>19</td>
<td>0.85</td>
</tr>
<tr>
<td>MEG-Au</td>
<td>3.9</td>
<td>2.2</td>
<td>65</td>
<td>0.85</td>
</tr>
<tr>
<td>PT-Au</td>
<td>3.3</td>
<td>8.3</td>
<td>15</td>
<td>0.80</td>
</tr>
<tr>
<td>OT-Au</td>
<td>3.9</td>
<td>100</td>
<td>10</td>
<td>0.85</td>
</tr>
<tr>
<td>UT-Au</td>
<td>5.0</td>
<td>600</td>
<td>10</td>
<td>0.85</td>
</tr>
</tbody>
</table>

Fig. 2 CVs of a GADH-adsorbed bare and thiol-modified electrodes at pH 4 in the presence of 50 mM D-glucuronate, at a scan rate 0.02 V s⁻¹. The open circles represent the regression curves obtained based on eqs. (2)-(4) with parameters listed in Table 1.
\[ k_i = k' \exp\left(1 - \alpha(nF/RT)(E - E^*)\right) \]  
\[ k_b = k' \exp\left(- \alpha(nF/RT)(E - E^*)\right) \]

(3) (4)

\( n \) and \( F \) are the number of electrons (= 1 for the heme \( e \) in GADH) and the Faraday constant, respectively. \( \Gamma_{\text{GADH}} \) evaluated from the QCM measurements was used in this work. The parameter \( \lambda \) denotes the ratio of the enzyme actually contacting the DET reaction to the total amount of the enzyme adsorbed on the electrode surface. The catalytic constant \( k_c \) is a function of the intermolecular ET rate constant for \( \beta \)-glucanate oxidation at the flavin active center and intramolecular ET rate constant from the flavin to the heme \( e \) site. The \( k_c \) and \( k_b \) indicate the surface ET rate constants (subscripts \( f \) and \( b \) mean forward and backward, respectively). \( E^* \) is the formal potential of the heme \( e \) site of GADH and was assumed to be 0.05 V according to a previous report.\(^{11} \) \( k' \) is the standard surface ET rate constant. The transfer coefficient (\( \alpha \) value) was not fixed in this study, although further consideration should be required. Eqs. (2)-(4) were satisfactorily fitted to the experimental current-potential curves with \( k_c, k_b/k_c \) and \( \lambda \), as adjustable parameters using a non-linear least-squares method. The refined parameters are summarized in Table 1.

The \( \lambda k_c \) value obtained at the electrode modified with a thiol having a positively charged functional group (MEG) was largest among those at other electrodes and was \( \alpha \) \( 6 \) times as large as that in the case of bare Au electrode. Assuming that \( k_c \) is constant, these data indicate that \( \lambda \) is strongly affected by the characteristics of thiols on Au surface. It is considered that the hydrophilic surface, especially positively charged surface, would improve the orientation of the adsorbed GADH and/or prevent the denaturation or aggregation of the adsorbed GADH.

In the case of alkanethiol-modified electrodes, although the \( \lambda k_c \) values were comparable to the one at bare Au electrode, the \( k_c/k_b \) values were larger than that at bare and charged thiol-modified Au electrodes. The \( k_c/k_b \) values depended on the alkane-chain lengths. The distance between the heme \( e \) site in GADH and the electrode surface would affect the \( k_c \) value from a viewpoint of the long-range tunneling ET. The \( k_c \) can be expressed as a function of the tunneling distance (\( d \)).\(^{15} \)

\[ k_{\text{tunneling}} = k_{\text{tunneling}}(d) \]

(5)

\( \beta \) value represents the decay constant. \( \beta \) value for electron tunneling through the alkane spacers was assumed as 1 \( \text{Å}^{-1} \).\(^{13,16} \) The difference between the chain lengths of PT and OT is 3–4 \( \text{Å} \), and therefore, the \( k' \) value at OT-Au electrode would be as small as \( \alpha \) \( 2 \)–5% of the one at PT-Au electrode. The small catalytic current densities at OT- and UT- modified electrodes might be due to the small value of \( k' \), and therefore it would require large overpotential to reach a limiting current. On the other hand, there is small difference in \( k' \) values at bare Au, MES-Au and MEG-Au electrodes assuming a constant \( k_c \) value. Similar behavior is reported in the previous studies on the electrochemistry of cytochrome \( e \) and azurin on alkanethiol-modified Au electrode surface; the ET rate is independent of the carbon chain length at such short carbon chains.\(^{33} \)

The \( \alpha \) values estimated here are much larger than that assumed for the typical electrochemical reaction (\( \alpha = 0.5 \)). However, setting \( \alpha \) as an adjustable parameter is essential to well reproduce the experimental curves. The physical meaning of the \( \alpha \) evaluated here would not be the same as that of simple electrode reaction, but involve several factors such as the structural characteristics of enzyme. The redox center is buried in a large protein shell and the rate of DET is governed by the distance between the redox center and electrode surface. Thus the DET reaction would be determined by (1) the location of the redox center in the enzymes, (2) the orientation of the enzymes on the electrode surface, and (3) the distribution of the orientation of enzyme on the electrode surface. All these factors might influence the \( \alpha \) value with respect to the DET-type electro-enzymatic reactions and \( \alpha \) is very important parameter characterizing the DET-type bioelectrocatalysis, although further study is needed.

References