Electrocatalytic Oxidation of NADH Using a Novel Modified Electrode with a Ruthenium Complex Containing Phenanthroline Quinone

Keiko Yokoyama, Yoshikazu Ueda, Nobuhumi Nakamura,* and Hiroyuki Ohno

Department of Biotechnology and Life Science, Tokyo University of Agriculture and Technology, Koganei, Tokyo 184-8588

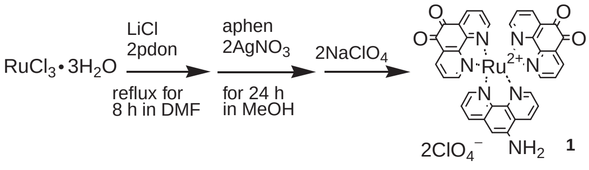
(Received June 3, 2005; CL-050719)

A ruthenium complex containing phenanthroline quinone, $[Ru(pdon)_2(aphen)](ClO_4)_2$ (pdon = 1,10-phenanthroline-5,6-dione, aphen = 5-amino-1,10-phenanthroline) (**1**), was prepared as an electrocatalytic adsorbate on a gold electrode. Immobilization of **1** on the gold electrode was confirmed by surface enhanced resonance Raman spectroscopy. The rate constant k_s was calculated as $10\,s^{-1}$ at pH 7.0. The electrocatalytic oxidation of NADH using this modified electrode was investigated.

Nicotinamide adenine dinucleotide (NADH, reduced form) is a coenzyme playing an important role in various biochemical redox reactions. The electrocatalytic oxidation of NADH has been a subject of great attention, because NADH-dependent enzymes are useful for constructing biodevices. However, the direct electrochemical oxidation of NADH on conventional electrodes is only possible with high overpotentials. Various types of mediators have been employed for the oxidation of NADH so far. Quinone compounds (e.g. naphthoquinone[1] and pyrroloquinoline quinone (PQQ)[2]) are one example of utilizable mediators. 1,10-Phenanthroline-5,6-dione (pdon) has two important parts: an *ortho*-quinone moiety that acts as a mediator for NADH, and a diiminic moiety at positions 1 and 10 having the ability to form stable metal complexes. It was reported that metal-free pdon shows electrocatalytic activity for the oxidation of NADH.[3] The reactivity of pdon is affected by coordination with a metal ion.[4] Indeed, transition-metal complexes with pdon show greater electrocatalytic activity for the oxidation of NADH.[5,6]

Since self-assembled techniques have been widely studied and can help to construct a highly-ordered monolayer designed on a nanoscale, it is beneficial to create a functional electrode surface.[7] Self-assembled monolayers (SAMs) containing transition-metal complexes have attracted attention because of their redox properties and catalytic ability.[8–11] Here, we report the construction of a novel electrode modified with a SAM of a ruthenium complex containing pdon, the confirmation of the complex immobilized on the electrode surface by surface-enhanced resonance Raman spectroscopy (SERRS), and the application of this modified electrode to the electrochemical oxidation of NADH.

A ruthenium complex containing pdon, $[Ru(pdon)_2(aphen)](ClO_4)_2$ (aphen = 5-amino-1,10-phenanthroline) (**1**), was synthesized (Scheme 1).[12] A gold electrode was immersed in a 10 mM mercaptopropionic acid (MPA) solution. Subsequently, the freshly prepared MPA-modified electrode was immersed in an aqueous solution of 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC) and *N*-hydroxysuccinimide (NHS) to activate the carboxyl group, followed by a 1 mM complex **1** acetonitrile solution overnight. For SERRS, a silver electrode was used instead of a gold electrode.



Scheme 1.

The complex **1** was obtained as dark brown crystals in a 37% yield. To confirm the immobilization of **1** on the electrode surface, we chose SERRS. SERRS is beneficial for detecting an electrode surface, since it can provide information on an adsorbate forming a monolayer. Figure 1a shows the resonance Raman (RR) spectrum of **1** in 0.1 M phosphate buffer, pH 7.0. Because an oxidation and reduction cycle (ORC)[13] was applied to the electrode, the system becomes SERRS active and a SERR spectrum of the adsorbed monolayer can be observed. The SERR spectrum of **1** immobilized on the electrode is shown in Figure 1b. The SERR spectrum of the novel modified electrode is very similar to the RR spectrum of **1** in buffer (Figure 1). In contrast, the characteristic vibration of the amide band at $1610\,cm^{-1}$ is clearly visible in the SERR spectrum. It is suggested that the reaction of an amino group of **1** and a carboxyl terminal of MPA immobilized on the electrode leads to the amide bond. Thus, we can conclude that **1** is immobilized on the MPA-modified electrode. Furthermore, this result demonstrates that SERRS can probe a specific functional group in a monolayer of a metal complex on the surface.

Cyclic voltammetric measurements of the modified elec-

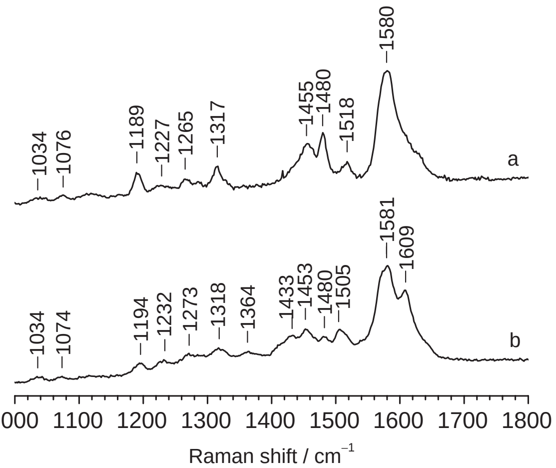


Figure 1. (a) The RR spectrum of 1 in 0.1 M phosphate buffer and (b) the SERR spectrum of **1** immobilized on the MPA-modified gold electrode. Spectra were obtained using 413.1-nm excitation (2 mW).

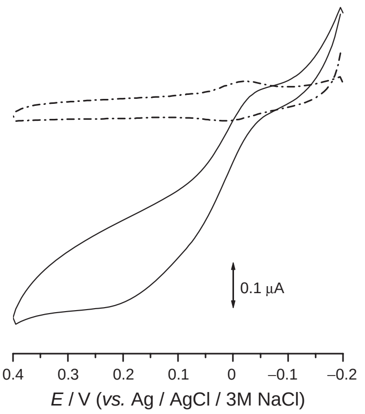


Figure 2. Cyclic voltammograms for the **1**-modified electrode without NADH (dashed and dotted line) and with 1.5 mM NADH (solid line) at $v = 4\,\mathrm{mV/s}$.

trode were carried out in 0.1 M phosphate buffer, and one of the results is presented in Figure 2 (dashed and dotted line). The formal potential of the complex **1** modified on a gold electrode is −15 mV (vs Ag/AgCl/3 M NaCl). This response was assigned to the quinone/hydroquinone couple. The rate constant k_s for the electron transfer between the electrode and the quinone moiety was calculated from peak separations,[14] and a value of 10 s^{-1} was obtained at pH 7.0. This value was slightly larger than that reported for immobilized PQQ at the same pH.[2] Stable redox responses were observed over a wide pH range (pH 3–9). The surface coverage of **1** was estimated by integrating the cathodic and anodic peaks after subtracting the background current and by assuming a four-electron transfer process. This assumption was based on the experimental results, which were reported by Wu et al.[6] An average surface coverage value of 8.9×10^{-11} mol/cm^2 was obtained. Since the theoretical value expected for a closely packed monolayer is 2×10^{-10} mol/cm^2 based on the calculation assuming 9.6 Å for the diameter of **1**, the present result is approximately 45% of the theoretical value.

In the presence of NADH, the cyclic voltammogram obtained using the **1**-modified electrode at a slow potential scan rate exhibited a drastic enhancement of the anodic current because of the catalytic oxidation of NADH (Figure 2 solid line). The potential value for the direct oxidation of NADH at a bare gold electrode surface was 720 mV (vs Ag/AgCl/3 M NaCl), which was measured in our laboratory. When we used this novel modified electrode, the oxidation took place at around $E_{p/2} =$ 80 mV (vs Ag/AgCl/3 M NaCl), which represents a significant diminution in the overpotential. Linearity of the plot of the anodic electrocatalytic current vs NADH concentration was obtained below 1.5 mM NADH, and saturation was achieved above 10 mM. The electrocatalytic reaction on the modified electrode surface proceeds according to the following reaction scheme:

$$[\mathrm{Ru(pdon)_2(aphen)}]^{2+} + \mathrm{NADH} + \mathrm{H}^+$$
$$\xrightarrow{k_{\mathrm{obs}}} [\mathrm{Ru(pdon)(pdol)(aphen)}]^{2+} + \mathrm{NAD}^+, \qquad (1)$$

where pdol is 1,10-phenanthroline-5,6-diol. A rough estimate of the second order rate constant for the electron transfer reaction between NADH and the immobilized quinone moiety, k_{obs}, was calculated from cyclic voltammetry according to the approach of Andrieux and Saveant.[15] A k_{obs} of $1.2 \times 10^3\,\mathrm{M^{-1}\,s^{-1}}$ was obtained for the electrocatalytic oxidation of NADH (1.5 mM). This value was greater than that ($1.6 \times 10^2\,\mathrm{M^{-1}\,s^{-1}}$; 5 mM NADH) for the PQQ-modified gold electrode,[16] which is another example of a modified electrode formed by a SAM of quinone compound for electrocatalytic oxidation of NADH, at the same pH (7.0).

Even after 4 days of scanning between −200 and +200 mV at pH 7.0, a clear electrochemical response was still obtained. On adding of NADH to this buffer solution, the electrocatalytic current was observed. The immobilization of the complex **1** through the covalent bond seems to cause this stability.

In summary, a novel electrode was constructed using a SAM of a ruthenium complex containing phenanthroline quinone. Quinone groups on the surface of this electrode retained their redox activity and mediated the oxidation of NADH. This novel quinone-modified electrode was stable and showed an electrochemical response even after few days.

This study was supported by grants from the Ministry of Education, Culture, Sports, Science and Technology through the Tokyo University of Agriculture & Technology as a part of the 21st century COE (Center of Excellence) program of the “Future Nano-Materials” research and education project, the Japan Society for the Promotion of Science (No. 14592001 to N. N.).

References and Notes

1 A. Sato, K. Kano, and T. Ikeda, *Chem. Lett.*, **32**, 880 (2003).
2 E. Katz, D. D. Schlereth, and H.-L. Schmidt, *J. Electroanal. Chem.*, **367**, 59 (1994).
3 S. Itoh, H. Fukushima, M. Komatsu, and Y. Ohshiro, *Chem. Lett.*, **1992**, 1583.
4 E. C. Constable, in “Metals and Ligand Reactivity,” VCH, Weinheim and New York (1995), Chap. 8, p 245.
5 G. Hilt, T. Jarbawi, W. R. Heineman, and E. Steckhan, *Chem.—Eur. J.*, **3**, 79 (1997).
6 Q. Wu, M. Maskus, F. Pariente, F. Tobalina, V. M. Fernández, E. Lorenzo, and H. D. Abruña, *Anal. Chem.*, **68**, 3688 (1996).
7 A. Ulman, *Chem. Rev.*, **96**, 1533 (1996).
8 K. Kanaizuka, M. Murata, Y. Nishimori, I. Mori, K. Nishio, H. Masuda, and H. Nishihara, *Chem. Lett.*, **34**, 534 (2005).
9 S. Ye, W. Zhou, M. Abe, T. Nishida, L. Cui, K. Uosaki, M. Osawa, and Y. Sasaki, *J. Am. Chem. Soc.*, **126**, 7434 (2004).
10 M. Haga, H.-G. Hong, Y. Shiozawa, Y. Kawata, H. Monjushiro, T. Fukuo, and R. Arakawa, *Inorg. Chem.*, **39**, 4566 (2000).
11 K. Yokoyama, A. Wakabayashi, K. Noguchi, N. Nakamura, and H. Ohno, *Inorg. Chim. Acta*, in press.
12 Selected physical data for **1**: ^{1}H NMR (500 MHz, DMSO-d_6): δ 6.984 (s, 2H), 7.103 (s, 1H), 7.559–7.613 (m, 2H), 7.630–7.679 (m, 2H), 7.757–7.801 (m, 4H), 7.900 (dd, 1H), 8.031 (dd, 2H), 8.079 (d, 1H), 8.371 (d, 1H), 8.500 (d, 2H), 8.563–8.586 (m, 2H), 8.959 (d, 1H); UV–vis ($\mathrm{CH_3CN}$): λ_{max}/nm ($\varepsilon/10^4\,\mathrm{M^{-1}\,cm^{-1}}$) 249.2 (5.76), 294.7 (2.51), 306.1 (2.35), 358.9 (1.31), 434.0 (1.23); ESI MS $[\mathrm{M}]^{2+}$, m/z 358.5. CAUTION: All the perchlorate salts are explosive and should be handled with care.
13 A. Otto, *Appl. Surf. Sci.*, **6**, 309 (1980).
14 E. Laviron, *J. Electroanal. Chem.*, **101**, 19 (1979).
15 C. P. Andrieux and J. M. Saveant, *J. Electroanal. Chem.*, **93**, 163 (1978).
16 E. Katz, T. Lötzbeyer, D. D. Schlereth, W. Schuhmann, and H.-L. Schmidt, *J. Electroanal. Chem.*, **373**, 189 (1994).