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Electrochemical Properties of Coenzyme Pyrroloquinolinequinone Using a Di-(4-pyridyl) Disulfide Modified Gold Electrode under Acidic Conditions

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Synopsis. The reversible electron transfer behavior of pyrroloquinolinequinone (PQQ) using a di-(4-pyridyl) disulfide (4-pyds) modified gold electrode was observed by cyclic voltammetry and the optically transparent thin-layer electrode (OTTLE) method under acidic conditions.

A great variety of bacterial dehydrogenases (e.g., alcohol dehydrogenase from *Gluconobacter suboxydans* and glucose dehydrogenase from *Acinetobacter calcoaceticus*) have been known to contain PQQ (1*H*-pyrrolo[2, 3-*f*]quinoline-4,5-quinone-2,7,9-tricarboxylic acid) as an organic cofactor with respect to the redox reactions of biological systems.[1—9] To know the redox properties of PQQ is important for an understanding of the electron transfer processes in these oxidoreductases. Electrochemical properties of PQQ and its analogues have been reported in recent years.[10—15] However, the electrochemical behavior of PQQ at conventional electrodes (platinum, gold, and glassy carbon) shows quasi-reversible or irreversible process. Here we report the electrochemical behavior of PQQ in acidic solutions at a 4-pyds modified gold electrode. Various sulfur-containing promoters such as 4-pyds are generally employed for the acceleration of electron transfer between the electrodes and metalloproteins.[16—20] Spectroelectrochemistry of PQQ provides the redox potentials ($E^{\circ\prime}$), the number of electrons (n) involved in overall redox process, and the spectra of the electrogenerated species at the same time.

Experimental

Materials. PQQ was purchased from Ube Industries (Tokyo, Japan). Other chemicals were guaranteed reagent grade and were purchased from Wako Chemical Co.

Apparatus. All electrochemical measurements were carried out with a three-electrode system consisting of an Ag/AgCl reference electrode, a platinum-wire counter electrode, and a 4-pyds modified gold working electrode. Bioanalytical Systems (BAS) CV-27 Voltammograph, BAS PA-1 low-current module, and Graphtec WX-2400 X–Y recorder were used to record the voltammograms. The OTTLE cell was constructed according to the reports by Heineman and co-workers.[21,22] The size of the gold minigrid electrode was 8 mm (width)×25 mm (height)×0.3 mm (thickness). Electronic absorption spectra were measured with a Shimadzu MPS-2000 spectrophotometer.

Procedure. The 0.05—0.1 M ($1\ \mathrm{M}=1\ \mathrm{mol\,dm^{-3}}$) CH_3COOH/HCl buffer was used for electrochemical measurements. The pH value of the buffer solution was measured before and after each measurement. The gold disk electrode (electrode surface area=$0.0211\ \mathrm{cm^2}$) and the gold minigrid electrode were freshly modified with 4-pyds by dipping the electrode for 10 min into the saturated solution of 4-pyds before each measurement. The OTTLE cell was positioned in the Shimadzu MPS-2000 sample compartment (previously purged with nitrogen gas) and a blanket of nitrogen gas was maintained over the sample solution throughout the experiment. After deaeration with wet argon, all electrochemical measurements were carried out at 25°C. All of the electrode potentials in this paper are given with respect to an Ag/AgCl electrode.

Results and Discussion

Cyclic voltammograms of PQQ were obtained under different pH conditions in the 0.1 M buffer solution. In the case of a bare gold electrode, the voltammograms exhibit no obvious peak at pH>4.5. A couple of reduction and reoxidation peaks was observed below pH 4.5, although the electron transfer between PQQ and the electrode does not show the reversible process. Figure 1a depicts the voltammograms using a bare gold electrode at pH 2.70. Only one quasi-reversible response was observed with a midpoint potential, $E_{1/2}=+140$ mV and the potential separation between the reduction

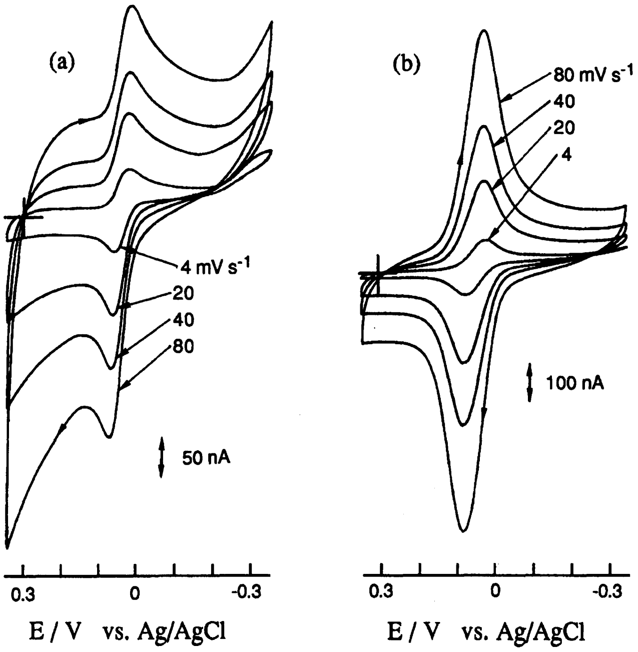

Fig. 1. Cyclic voltammograms of PQQ using a bare gold electrode (a) and a 4-pyds modified gold electrode (b) at pH 2.70. Scan rate: 4, 20, 40, and 80 mV s^{-1}, PQQ concentration: 30 μM.

peak and the oxidation one, ΔE_p=51 mV at a scan rate (v) of 20 mV s^{-1}. These data suggest that the electrochemically reversible response of PQQ at a bare gold electrode is difficult. The cyclic voltammetric response using a 4-pyds modified gold electrode was reversible in the region from pH 2 to 5, but not reversible above pH 5. The cyclic voltammograms at pH 2.70 are shown in Fig. 1b. $E_{1/2}$ and ΔE_p were evaluated to be +144 and 37 mV, respectively; the ΔE_p value proved that the redox reaction proceeds in a one-step two-electron transfer process. ΔE_p was practically independent of scan rate. The peak current of the oxidation is almost the same as that of the reduction.

Figure 2 exhibits the relationships between square root of $v^{1/2}$ and cathodic peak current (i_{pc}). A scan rate dependence of the reversible couple yields linear plots of i_{pc}/C vs. $v^{1/2}$ up to v=80 mV s^{-1} at pH 2.03—4.74. However, a plot of i_{pc}/C vs. $v^{1/2}$ at pH 1.52 displays to be nonlinear, which indicates that PQQ weakly adsorbs on the electrode.[23] These findings suggest that PQQ interacts with the 4-pyds modified gold electrode to receive two electrons and two protons at the *o*-quinone moiety under acidic conditions. It is considered that the pyridine moiety of 4-pyds modified gold electrode interacts with the *o*-quinone part of PQQ through hydrogen bonds, which accelerate the proton transfer as well as the electron transfer (Fig. 3).

Electronic absorption spectra during the course of PQQ reduction were recorded by the spectroelectrochemical method using OTTLE, as shown in Fig. 4. The PQQ and $PQQH_2$ (two electron reduced form of PQQ) exhibit absorption maxima at 249 and 318 nm, respectively. The absorption band of $PQQH_2$ coincides with that of the reduction product of PQQ with $Na_2S_2O_4$.[24] The absorbance changes at a given wavelength can be

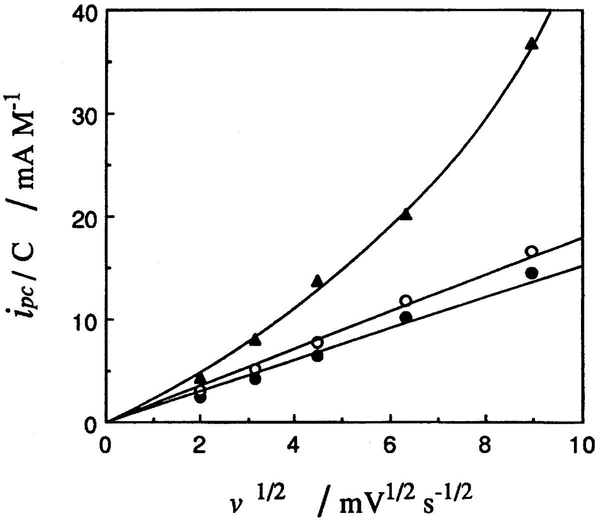

Fig. 2. Plots of i_{pc}/C vs. $v^{1/2}$ using a 4-pyds modified gold electrode at pH 1.52 (▲), pH 2.03 (○), and pH 4.74 (●). PQQ concentration (C): 30 μM.

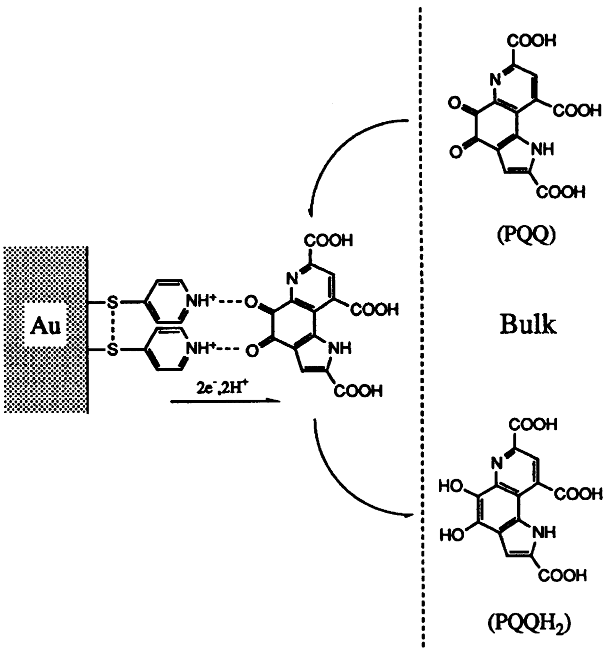

Fig. 3. Proposed reduction mechanism of PQQ at a 4-pyds modified gold electrode.

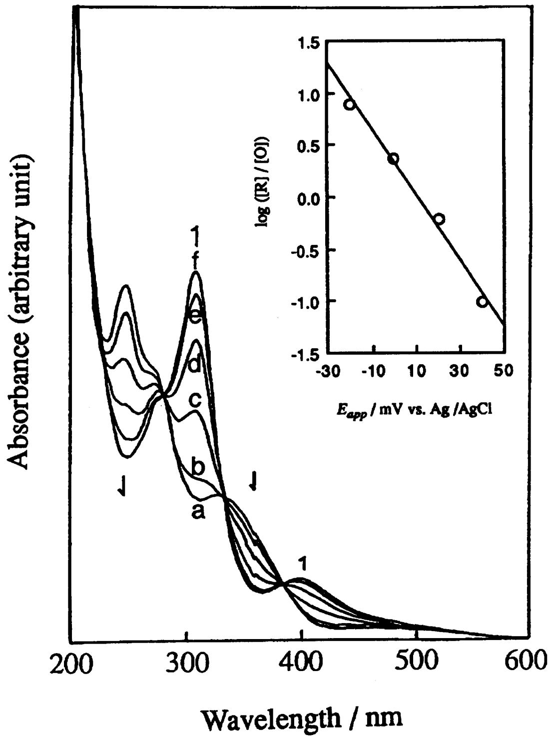

Fig. 4. Electronic absorption spectra recorded during OTTLE spectropotentiostatic experiment in 0.05 M CH_3COOH/HCl buffer containing 0.21 mM PQQ at pH 4.31. Applied potentials in mV vs. Ag/AgCl are follows: (a) 100, (b) 40, (c) 20, (d) 0, (e) −20, (f) −100. Inset: Nernst plot at 318 nm.

related to the concentration ratio of reduced to oxidized species by Eq. 1,

$$[\mathrm{R}]/[\mathrm{O}] = (A - A_{\mathrm{ox}}) / (A_{\mathrm{red}} - A)\,, \tag{1}$$

where A is the absorbance of OTTLE at a given applied potential (E_{app}), A_{ox} is the absorbance of the fully oxidized form, and A_{red} is the absorbance of the fully reduced form. The Nernst Eq. 2 indicates that a plot of E_{app} vs. log ([R]/[O]) should be linear

$$E_{\mathrm{app}} = E^{\circ\prime} - (0.059/n)\log([\mathrm{R}]/[\mathrm{O}]), \tag{2}$$

with a slope of $59/n$ mV and an intercept of $E^{\circ\prime}$ (the standard redox potential), assuming Nernstian reversibility. The Nernst plot at 318 nm in Fig. 4 gives $E^{\circ\prime}=+10$ mV and $n=1.88$ at pH 4.31.[25] The well-defined isosbestic points (at 272, 332, and 380 nm) indicate that one-electron reduced form of PQQ (pyrroloquinoline semiquinone) does not occur in this process. Therefore, this OTTLE measurement also suggests that the one-step two-electron transfer between PQQ and a 4-pyds modified gold electrode proceeds reversibly. The unclear voltammetric response was observed at pH>5, which is presumably due to the deprotonation of pyridine moiety in 4-pyds or the hydration[26,27] of the *o*-quinone moiety in PQQ or both.

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