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Cathodic Reduction of Ubiquinone-10 Dissolved in Carbon-paste Electrode

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Carbon-paste electrodes were prepared with use of ubiquinone-10 dissolved in organic liquids, 1-chloronaphthalene, 1-bromonaphthalene, 1-methylnaphthalene and undecane. Ubiquinone-10 shows a well-defined, pH-dependent cathodic reduction wave in each liquid system and its reduction seems to proceed with two electrons and a proton transfer around neutral pH. The experimental results indicate the wave to be diffusion-controlled, and the apparent diffusion coefficient was additionally calculated in each liquid system.

It is generally accepted[1)] that ubiquinones and the reduction products take part in the formation of pH gradient across chromatophore membrane, that is indispensable for ATP production, in addition to the role as an electron pool in the photosynthetic electron transport system. Moreover, it has become apparent[2—7)] that ubiquinones also function as primary or secondary electron acceptors in the photosynthetic reaction centers. The majority of ubiquinones bound to chromatophores are reduced with two electrons and two protons transfer[8)] around neutral pH, but in the case of primary or secondary acceptor, it was suggested that they were reduced with an electron transfer to form corresponding semiquinones,[7,9)] similar to a cathodic reduction in an aprotic nonaqueous solvent.[10)] Although most of these studies have been carried out by means of analyzing their absorbance[8,9)] and/or EPR spectrum,[7)] disadvantages of these methods may be that its absorbance and difference spectra are not so distinctive, and the measurements should be carried out at temperature as low as liquid nitrogen in order to sharpen the spectra. On the other hand, electrochemistry provides essentially the knowledge on oxidation-reduction properties of a material of interest. On this viewpoint, we studied electrochemically the reduction of ubiquinone-10 in an aqueous ethanol[11,12)] and in a SDS micelle[12)] as a model of chromatophore membrane, and all of these reductions seemed to proceed with two electrons transfer. A cyclic voltammetric study of ubiquinone-10 bound to chromatophores was also carried out,[11)] but the reaction mechanism is not clear yet because of the difficulty of mass transfer in the suspension. Usually, use of a carbon-paste electrode has been limited to aqueous solutions because of flowing the paste. But, this limitation, as applied to water-insoluble, electroactive organic compounds, can be circumvented by dissolving these organics in electrode itself.[13)] In addition, a carbon-paste electrode dissolving ubiquinone-10 is supposed to mimic chromatophore membrane, most of ubiquinones being present at membrane-water interface.[11)] So, we report a cathodic behavior of ubiquinone-10 dissolved in a carbon-paste electrode, prior to elucidating the reactivities of ubiquinone-10 bound to chromatophores.

Experimental

Ubiquinone-10 was extracted and purified by the usual method.[14,15)] The exactly weighed ubiquinone-10 was dissolved in 1 cm^3 of an organic solvent (1-chloronaphthalene, 1-bromonaphthalene, 1-methylnaphthalene or undecane), 1.7 g of graphite was then added and mixed until the carbon appeared uniformly wetted. The paste was stored in water for a few days at 4 °C. A working electrode was prepared by packing the Teflon cavity (ϕ 2.56 mm$\times$0.24 mm depth) with the paste, smoothing the surface with a filter paper and removing excess paste from the edge surface of the Teflon with tissue paper. After each measurement, it was necessary to remove all of the paste in which the concentration of ubiquinone-10 had been changed by electrolysis, and the cavity was washed with petroleum ether. Commercially obtained ferrocene was further purified chromatographically on an alumina column with spectroscopic-quality hexane as eluent, and then recrystallized from redistilled methanol. Ferrocene thus purified was used for preparation of a carbon-paste electrode in a similar manner as above, and a peak current for ferrocene oxidation was measured for comparison of specific limiting currents.[16)]

A conventional H-type cell was used with the working electrode and a saturated calomel reference electrode located in the main compartment. A platinum disk electrode was served as auxiliary electrode in the other compartment. Buffer solutions of various pH values were prepared with 0.6 mol/dm^3 GTA–HCl or GTA–NaOH.[12)] Dissolved oxygen in this solution was removed by bubbling nitrogen for 30 min before electrolysis, and the electrolysis was carried out at 25 °C. A current-potential curve was recorded using a potentiostat PS-1000, a linear potential scanning unit LS-1D (Hokuto Denko Ltd.) and a Hitachi Model 056 recorder. Potential sweep rate was 250 mV/min unless otherwise noted. A coulometric measurement was carried out at a peak potential for ubiquinone-10 reduction controlled by a Hokuto Model HA-104 potentiostat using a working electrode packed with a known weight of carbon-paste containing ubiquinone-10 until the electrolytic current decreased to 1—2% of the initial current. Usually, 100 min of the electrolytic time was required, and the quantity of electricity was measured using a coulometer HF-201 (Hokuto Denko Ltd.).

Results and Discussion

Current-potential curves of ubiquinone-10 dissolved in carbon-paste electrodes always showed typical well-defined, pH-dependent reduction waves. Table 1 shows a comparison of specific limiting currents[16)] for ferrocene oxidation and ubiquinone-10 reduction dissolved in 1-chloronaphthalene at pH 7.0. Since the oxidation of ferrocene is known to proceed with one electron transfer,[13)] the total number of electrons transferred in ubiquinone-10 reduction can be determined by comparing the both specific limiting currents. The results suggest that the reduction proceeds with two electrons transfer. This presumption is supported by a coulometric measurement summa-

TABLE 1. COMPARISON OF SPECIFIC LIMITING CURRENTS FOR FERROCENE OXIDATION AND UBIQUINONE-10 REDUCTION AT pH 7.0

Exptl No.	$I_{SL}/\mu A\ mmol^{-1}\ dm^3\ cm^{-2}$ a) Ferrocence oxidation[b)]	Ubiquinone-10 reduction[b)]	Ratio
1	2.67	−5.34	
2	2.31	−5.58	
3	2.31	−5.44	
	av. 2.43	av. −5.45	2.24

a) I_{SL} is specific limiting current. b) The both concentrations in 1-chloronaphthalene were kept constant at 4.0×10^{-2} mol/dm^3, and potential sweep rate was 250 mV/min. Similar results were obtained in the other liquids.

TABLE 2. COULOMETRIC DATA FOR UBIQUINONE-10 REDUCTION IN 1-CHLORONAPHTHALENE

Exptl No.	Weight of carbon-paste/mg[a)]	Q/mC Calculated as $n=1$	Measured	Ratio
1	3.8	4.94	8.85	1.79
2	3.6	4.68	7.35	1.57
3	3.3	4.29	6.94	1.62

a) The ubiquinone-10 concentration was kept constant at 4.0×10^{-2} mol/dm^3. Controlled potential electrolyses were carried out for 100 min at −260 mV *vs.* NHE. Similar results were obtained in the other liquids.

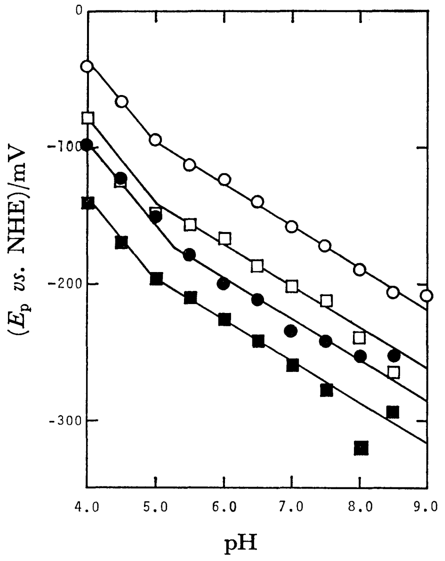

Fig. 1. Plots showing the pH dependence of the peak potentials for ubiquinone-10 reduction.
Symbols in this figure represent the data in each organic liquid as follows: ■; 1-chloronaphthalene, ●; 1-bromonaphthalene, □: 1-methylnaphthalene, or ○; undecane. Potential sweep rate was 250 mV/min. Ubiquinone-10 concentration was 4×10^{-2} mol/dm^3 in each liquid.

rized in Table 2 in which the ratio of measured quantity of electricity to calculated one assuming the number of electrons transferred was 1, could be shown to be about 2. When the plots showing the pH dependence of the peak potentials for ubiquinone-10 reduction were made with each pasting liquid; 1-chloronaphthalene, 1-bromonaphthalene, 1-methylnaphthalene or undecane (Fig. 1), the slope of the straight lines was −60 mV/pH in the more acidic than about pH 5.0 and −30 mV/pH in the more basic region. Therefore, the number of protons transferred is estimated to be 2 in the more acidic than about pH 5.0 and to be 1 in the more basic region because the number of electrons transferred from the electrode is presumed to be 2 from the comparison of the specific limiting currents and the coulometric data. By titrating with mixture of succinate and fumarate, and determining the redox state of ubiquinone-10 spectrophotometrically, Kakuno *et al.*[8)] measured a midpoint potential of +23 mV for the endogenous ubiquinone-10 of chromatophore membrane. The reduction was two electrons process, coupled to the uptake of one proton per electron around neutral pH. Our present data indicate the reduction occurs in two electrons transfer, and as expected, ubiquinone-10 dissolved in the carbon-paste seems to be in the similar environment to most of those bound to chromatophore membrane rather than that in an aprotic nonaqueous solvent.[10)] However, its reduction potential is quite different from that bound to chromatophores. The peak potentials of ubiquinone-10 reduction at pH 7.0 were −260 mV with 1-chloronaphthalene, −240 mV with 1-bromonaphthalene, −205 mV with 1-methylnaphthalene and −160 mV with undecane, respectively. These potentials in the organic liquids are fairly lower than that in chromatophores even considering that the reaction is totally irreversible electron transfer process, because the transfer coefficient was calculated to be 0.2—0.3 from the slope of Tafel plots. Since Loach[17)] reported that the redox potential of ubiquinones became more negative in the more hydrophobic medium, it seems possible that the microenvironment around ubiquinone-10 molecule in chromatophore membrane is more hydrophilic than that in the organic liquids. In any event, the reduction of ubiquinone-10, though that is the reaction in a model system, was first characterized electrochemically in the present study to the best of our knowledge except those in acetonitrile[10)] and ethanolic solution.[11,12,18)] Studies of the redox properties of the components in photosynthetic and other biological electron transport systems, such as those in nitrogen fixation and oxidative phosphorylation, are very important to the understanding of the reaction mechanism.[19)] Especially, the knowledge of the redox potential levels and the number of electrons and protons transferred of the electron carriers in the photosynthetic electron transport system has greatly aided in the assessment of their physiological functions. That is, for instance, determining the phosphorylation sites along the photosynthetic electron transport system because the energy necessary for ATP synthesis must come from the free energy derived from the potential difference and the proton concentration gradient across the biological membrane.[20)] So, the development of

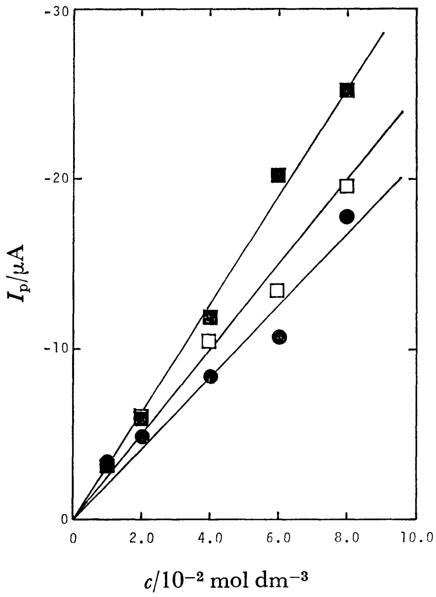

Fig. 2. Relationship of peak current to ubiquinone-10 concentration.
Symbols and experimental conditions are the same as those in Fig. 1, except that ubiquinone-10 concentration is varied and the solution pH is kept constant at 7.0.

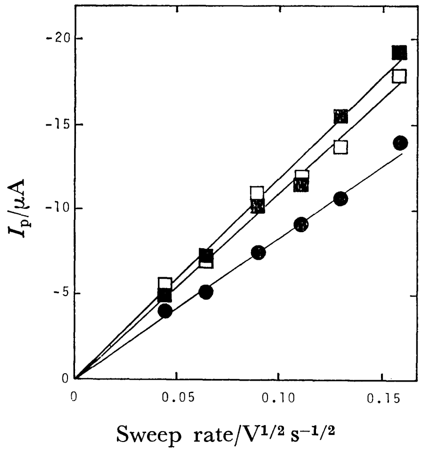

Fig. 3. Relationship of peak current to the square root of potential sweep rate.
Symbols and experimental conditions are the same as those in Fig. 1, except that the potential sweep rate is varied and the solution pH is at 7.0.

other new better model system of chromatophore membrane is still necessary to elucidate clearly the role of ubiquinone-10 in the photosynthetic electron transport system.

In addition, the reduction mechanism of ubiquinone-10 in the organic liquids was briefly examined. The linear relationship of peak current to varying concentration of ubiquinone-10 was observed with each pasting liquid (Fig. 2), and the peak current was proportional to the square root of the potential sweep rate (Fig. 3). It is evident from these results that the observed wave is due to the reduction of ubiquinone-10 itself, and the mass transfer rate is governed by diffusion from the paste. If mass transfer rate is governed by diffusion, the viscosity of the pasting liquid should affect this rate and consequently peak current. The Stokes-Einstein relation predicts a linear relationship between the diffusion coefficient and the reciprocal of viscosity. The diffusion coefficient was calculated by an ordinary method,[21,22] and the plots of D *vs.* $1/\eta$, as shown in Fig. 4, are linear but cannot extrapolate to the origin. Since the actual, effective concentration in the pasting liquid is not known, the calculated values of the diffusion coefficient must be considered as apparent values.

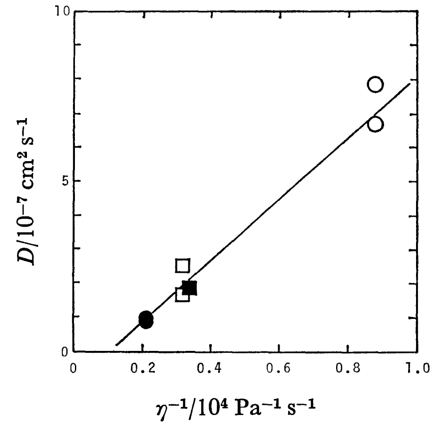

Fig. 4. Relationship of diffusion coefficient to the viscosity of pasting liquids.
Symbols are the same as those in Fig. 1, and values of viscosity are cited from the literture.[13]

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