Polarographic Reduction of Ubiquinone-10 Solubilized in Micelles

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Ubiquinone-10 solubilized in an aqueous solution containing sodium dodecyl sulfate as an anionic surfactant was polarographically investigated. Ubiquinone-10 solubilized in micelles shows a well-defined, diffusion-controlled, pH-dependent wave. Diffusion coefficient and apparent particles radii were calculated. The surfactant concentration dependence of the diffusion coefficient and of the apparent particle radii was also determined.

The electron transport system in respiratory chains and in the photosynthetic system, in which electron transfer takes place very smoothly, consists of various combinations of redox reaction. Electrochemical techniques are being developed for the analysis of the mechanism of electron transfer.

Underwood and his co-workers[1–3] investigated the electrode kinetics of NAD and its analogues by cyclic voltammetry. Takahashi *et al.*[4,5] reported that NADH produced from NAD by an enzymatic process can be anodically oxidized to NAD. Most of these studies were usually carried out in aqueous or nonaqueous media, and the difference from actual physiological conditions must be accounted for. Erabi *et al.*[6] investigated the polalographic behavior of redox components bound with chromatophores from photosynthetic bacterium, *Rhodospirillum rubrum*, under the physiological conditions, although reactions involved were not completely elucidated because of the difficulty of mass transfer.

In the photosynthetic organ, chromatophores, redox components have been arranged in good order. In such membrane systems, the usual electrochemical theory developed for homogeneous solution systems cannot be applied directly. No such development of electrochemical theory for the membrane systems have been carried out.

Hayano and Shinozuka[7–9] explained the polarographic behavior of the disperse-dyes solubilized in aqueous surfactant solutions. Westmoreland *et al.*[10] studied the polarography of *trans*-azobenzene in aqueous surfactant solutions, and discussed the boundary region between the properties of an aqueous system and those of a nonaqueous system. Micelle represents a good model for individual macromolecules and for a hydrophobic region within subcellular membrane systems. The electrochemical treatment of disperse systems is of interest not only in electrochemistry but also in biology. By accumulating the data for such disperse systems, we might obtain information on the membrane structure.

Experiments were carried out for ubiquinone-10 (UQ_{10}) solubilized in micelles, which plays an important role in the photosynthetic electron transport system.

Experimental

Materials. Pure UQ_{10} was extracted from chromatophores from photosynthetic bacterium, *R. rubrum*, by the usual method.[11] Sodium dodecyl sulfate (SDS, Marubishi Oil Co., Osaka) was washed with ether, and then recrystallized twice from ethanol. Tris(hydroxymethyl)aminomethane (Tris, Sigma Chemical Co., St. Louis) was used without further purification. Isooctane (Nakarai Chemicals Ltd., Kyoto) and other reagents (Wako Pure Chemical Industries Ltd., Osaka) were used.

Buffer solutions of various pH values were prepared with 0.12 M GTA (containing 0.04 M β-β-dimethylglutamic acid, 0.04 M Tris, and 0.04 M 2-amino-2-methyl-1,3-propandiol)–HCl and 0.12 M GTA–Tris, respectively. Water was used after five times distillation.

Apparatus. A solution containing a surfactant, 0.12 M GTA buffer solution, and UQ_{10} was irradiated with a 29kHz supersonic wave for 10 min. The solution was then stored for a day at room temperature. The equilibrium of the solution was thus attained. Excess UQ_{10} was removed by filtration through a G4 glass filter. The solid UQ_{10} remaining on the filter was dissolved with isooctane, and its quantity was determined by means of absorption spectrum at 271 nm[12] using a Hitachi Model 101 spectrophotometer. Polarographic measurements were carried out at room temperature for the filtrate, and UQ_{10} concentration in the filtrate was rechecked by the solid content on the filter. Polarograms were recorded with a high sensitive polarograph PA-202 (Yanagimoto Mfg. Co., Ltd., Kyoto).

Dissolved oxygen was removed from solutions by bubbling oxygen-free nitrogen for 2 hr.

A dropping mercury electrode (DME) was used as the working electrode, and a saturated calomel electrode (SCE) as the reference and counter electrode. The DME had the following characteristics; m=0.93 mg/s (in pure water at zero applied potential) and t=3.0 s. The height of mercury head was usually 75 cm.

The viscosity of the basic solution was measured with an Ostwald viscometer.

Results and Discussion

UQ_{10} in an Aqueous Ethanol Solution. The polarogram of UQ_{10} in an 80% (v/v) ethanolic solution showed pH-dependent wave. In the pH region pH 4.7—8.7,

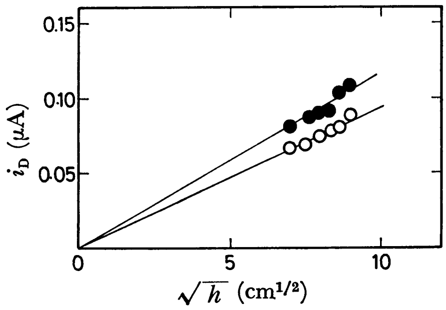

Fig. 1. Dependence of current on mercury height at pH 7.5.

●, and ○ represent the data in 80% ethanolic solution and in 4×10^{-2} M SDS solution, respectively.

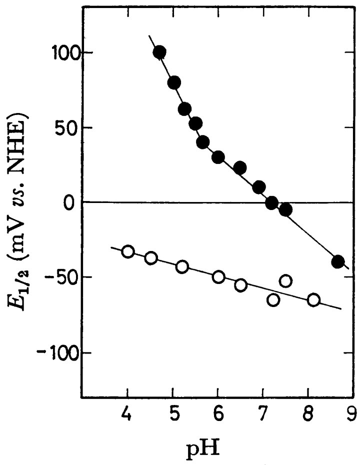

Fig. 2. Plots showing the pH dependence of the half-wave potentials for UQ_{10} reduction.
●, and ○ represent the data in 80% ethanolic solution and in 4×10^{-2} M SDS solution, respectively.

a typical well-defined cathodic wave was observed, and the limiting current was proportional to the square root of the mercury-column height as shown in Fig. 1.

The half-wave potential ($E_{1/2}$) was plotted against the solution pH (Fig. 2). The slope of the straight line for UQ_{10} was 60 mV/pH in the more acidic region than pH 5.7 and 27 mV/pH in the more basic region. When the Tafel plots and Heyrovskii-Ilkovic plots were made for the polarogram obtained, a value of αn=1.0 (α being the transfer coefficient) was obtained, the number of electrons exchanged being presumed to be 2. The α values was thus calculated to be 0.5. The number of proton transfer was therefore estimated to be 2 in the more acidic region than pH 5.7 and 1 in the more basic region. The proportionality of the limiting current to the square root of the mercury height and to UQ_{10} concentration indicates the wave to be diffusion-controlled. The Ilkovic equation was applied to calculate the diffusion coefficient, and the value was 1.04×10^{-6} cm^2/s at pH 7.5.

UQ_{10} Solubilized in SDS Solution. The polarogram of UQ_{10} solubilized in SDS solution also showed a pH-dependent wave. A typical well-defined cathodic wave was observed and the current was also proportional to the square root of the mercury-column height (Fig. 1).

The slope of straight line for UQ_{10} solubilized in a SDS solution was 8 mV/pH in the pH region 4.0—8.2

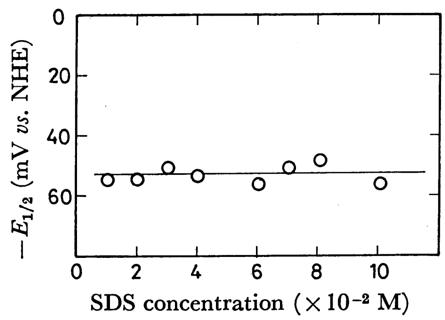

Fig. 3. Effect of surfactant concentration upon half-wave potential at pH 7.5.

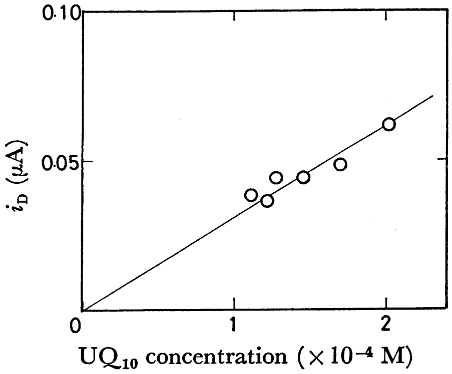

Fig. 4. Relationship of limiting current to UQ_{10} concentration at pH 7.5.

(Fig. 2). In Fig. 3 the half-wave potential for UQ_{10} reduction is shown as a function of SDS concentration. The half-wave potential was not affected by SDS concentration ($1—10\times10^{-2}$ M), that is, the solvent effect discussed by Mairanovskii[13] was not observed. Thus, UQ_{10} seems to be merely solubilized in micelles.

The dependence of the limiting current on UQ_{10} concentration is shown in Fig. 4, where a linear relationship is indicated. From the above results, it is evident that the observed waves are due to the reduction of UQ_{10} itself, and indicated to be diffusion-controlled.

The Tafel plots were drawn for the polarograms obtained at each SDS concentration, and αn values calculated from these results were plotted against the SDS concentration (Fig. 5). The αn value was not

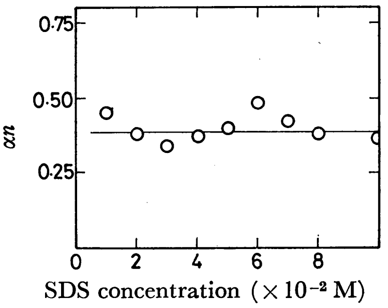

Fig. 5. Relationship of αn value to surfactant concentration at pH 7.5.

strongly affected by SDS concentration, being about 0.4 throughout the measurements in this study. Since the number of electrons exchanged for UQ_{10} in SDS solution was presumed to be 2 as well as in ethanolic medium, the α value was estimated to be 0.2. It is clear from experimental results that the diffusion particle is the micelle with UQ_{10}; this causes the diffusion current. On the one hand, adsorption and re-orientation of the surfactant molecules and micelles on the electrode were also considered to occur,[14] and micelles containing no UQ_{10} might exist in the neighborhood of the electrode. Futhermore, it was indicated by Kuta and Smoler[15] that the α value was lowered in the presence of a surface-active substance. As in the above case, the α value for UQ_{10} reduction is lowered to a greater extent in an aqueous surfactant solution than in an ethanolic one.

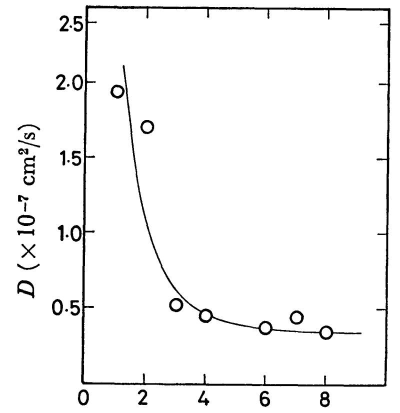

Fig. 6. Dependence of diffusion coefficient on surfactant concentration at pH 7.5.

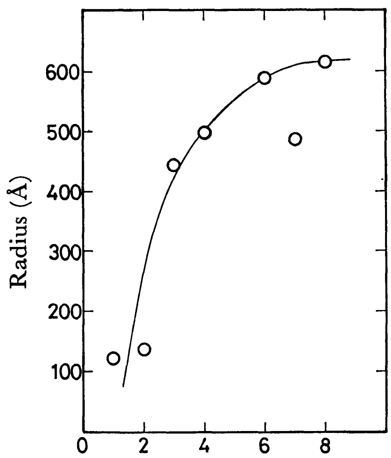

Fig. 7. Dependence of apparent mean particle radii on surfactant concentration at pH 7.5.

Since the limiting current of solubilized UQ_{10} was thought to be diffusion-controlled, the Ilkovic equation was applied to calculate the diffusion coefficient at each SDS concentration. Figure 6 shows the dependence of diffusion coefficient on SDS concentration. The apparent diffusion coefficient of solubilized UQ_{10} was about one-tenth compared with that in the ethanolic solution. Applying Stokes-Einstein's law for the diffusion coefficient (Fig. 6), the apparent mean radii of micelles were evaluated at various SDS concentrations, assuming that the micelles were perfectly spherical. The results are shown in Fig. 7. The obtained diffusion coefficients of solubilized UQ_{10} were more or less smaller than those of disperse-dyes reported by Hayano and Shinozuka.[9] However, in view of the size of a micelle which solubilized UQ_{10} containing a very long side-chain, these values seem to be appropriate. Tartar[16] and Tanford[17] reported that the theoretical micelle's radius for SDS is 16.68 Å and the aggregation number of molecules is 56. However, the radius and aggregation number of a micelle increase with increasing concentration of the electrolytes and, if a distortion in the micelle shape arises, the aggregation number of micelle increases still further. Therefore, the values for the particle radii (Fig. 7) do not seem too large if we take both existence of the electrolytes and situation of solubilized UQ_{10} into consideration.

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