Electrochemical Properties of Adriamycin Adsorbed on Pyrolytic Graphite Electrodes Modified by Phospholipid Monomolecular Membranes

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Synopsis. Electrochemical equilibrium and kinetic parameters have been determined by means of numerical simulation of d.c. voltammograms based on the theory of a two-step surface-redox reaction for the case of the redox system of adriamycin adsorbed on a basal-plane pyrolytic graphite electrode (BPGE) which is modified by a phospholipid monomolecular membrane. Comparison of these parameters with those obtained at a bare BPGE has shown that the semiquinone formation reaction is thermodynamically favorable and that the electron transfer rate constant decreases in these lipid membranes.

The toxic mechanism of adriamycin (anticancer drug), especially its cardiotoxicity, is not yet fully understood. The most acceptable mechanism is due to the bioreductive formation of the adriamycin semiquinone free radical as an intermediate catalyzed by NADPH-cytochrome P-450 reductase,[1] which is a membrane-bound enzyme. In addition, it is noted that the affinity of adriamycin for the phospholipid membrane is high.[2] These circumstances suggest that the elucidation of the redox property of adriamycin in the lipid membrane is fundamentally very important. In previous papers,[3,4] we have proposed a technique based on a numerical simulation of the quasi-reversible d.c. cyclic voltammograms in order to determine the electrochemical equilibrium and kinetic parameters pertinent to a two-step surface-redox reaction; $O_{ad}+e \rightleftarrows S_{ad}$ and $S_{ad}+e \rightleftarrows R_{ad}$. Here O_{ad}, S_{ad}, and R_{ad} stand for the oxidized, semiquinone, and reduced species in the adsorbed form, respectively.

In this study using the above analytical technique, we report the redox behavior of the quinone moiety in an adriamycin molecule adsorbed on a BPGE modified by two kinds of phospholipid monomolecular membranes, i.e., lecithin (phosphatidylethanolamine) and cardiolipin. The electrochemical equilibrium and kinetic parameters thus obtained are then compared with those determined at a bare BPGE.

Experimental

Chemicals. Adriamycin hydrochloride was kindly supplied by Kyowa Hakko Co. and used as received. Soybean lecithin (Wako Pure Chemical) and bovine heart cardiolipin (Sigma Chemical), both commercially available, were employed as received. Other chemicals were of reagent grade quality.

Preparation of BPGE Modified by a Phospholipid Monomolecular Membrane. BPGE's were prepared as described in a previous paper.[4] The phospholipid monomolecular layer was obtained according to the simplified Blodgett method.[5] The cleaned surface of triply-distilled water in a vessel was divided into two parts by a nylon thread. Upon the one part of the surface, a benzene solution of phospholipid was added dropwise and extended. On the other part, distilled rapeseed oil was extended as a piston oil.[6] The new plane surface of the BPGE was horizontally contacted to the phospholipid monomolecular layer. After the adhesion the modified electrode surface was sonicated for ca. 30 s in distilled water, and then air-dried at room temperature.

Electrochemical Measurement. The phospholipid membrane modified electrode was immersed in 3 ml of an acetate buffer (0.2 mol dm^{-3}, pH 4.5, ionic strength I=0.5) containing 3×10^{-5} mol dm^{-3} of adriamycin for 30 s under stirring. After the electrode had been washed by triply-distilled water, it was ultrasonically washed in distilled water and air-dried at room temperature. This electrode was immersed into a de-aerated electrolysis solution (0.2 mol dm^{-3} acetate buffer, pH 4.5, I=0.5) without adriamycin, and the voltammograms were then recorded. All other electrochemical measurements were described in previous papers.[3,4,7]

Results and Discussion

Lecithin-Monomolecular-Layer Modified BPGE (LT-BPGE). Adriamycin adsorbed or bound strongly on an LT-BPGE shows a reversible surface redox wave at a sweep rate v=10 mV s^{-1}. The peak potential, E_p^{rev}, and the half-peak width, $\Delta E_{p/2}^{rev}$, of these reversible waves are −0.500 V vs. SCE and 77—82 mV, respectively. These waves can be ascribed to the redox reaction of the quinone moiety in the adriamycin[7] and are analyzed on the basis of the theory regarding a two-step one-electron surface-redox reaction.[3,8] Assuming that the surface activity coefficients of O_{ad}, S_{ad}, and R_{ad} of adriamycin are equal, the semiquinone formation constant, K, is defined as:

$$K = [S_{ad}]^2/[O_{ad}][R_{ad}] = \exp[F(E'_{o1}-E'_{o2})/RT], \quad (1)$$

where $[j_{ad}]$'s (j=O, S, R) are the surface concentrations of j_{ad}, and E'_{o1} and E'_{o2} are the standard surface redox potentials of the couples O_{ad}/S_{ad} and S_{ad}/R_{ad}, respectively. The K value was estimated to be 2.2—2.8 from $\Delta E_{p/2}^{rev}$.[7,8]

At large v such as 1 V s^{-1} a typical quasi-reversible voltammogram is recorded as shown in Fig. 1A (solid line). Here we observed that the cathodic and anodic peak currents and also the cathodic and anodic half-peak widths are respectively almost the same within an experimental error. The average of the anodic and cathodic peak potentials is equal to E_p^{rev}. In other words, this quasi-reversible wave is symmetrical about the point of $E=E_p^{rev}$ and i=0. These results confirm the following two assumptions[3,4]: a) the apparent rate constants, k_{sap}, of the first and the second single electron transfer steps at E'_{o1} and E'_{o2} are equal and b) the transfer coefficient for the electron transfer of each step is 0.5. Under these conditions, the current (i)-potential (E)-surface concentration (Γ) characteristics can be expressed by the Butler-Volmer equation:[3,8]

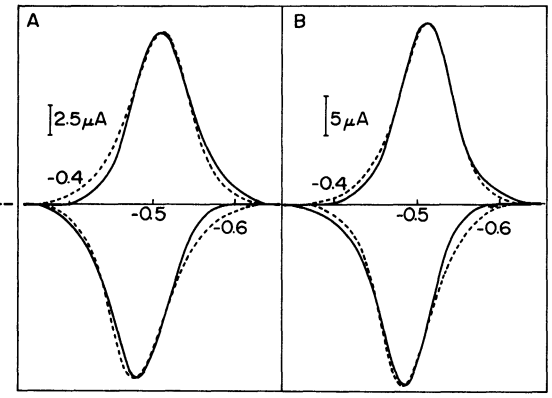

Fig. 1. Background-corrected cyclic d.c. voltammograms of adriamycin adsorbed on A) LT-BPGE and B) CL-BPGE. Voltage scanning was done with v=1 V s^{-1}. The solid lines and broken lines represent the experimental and the regression curves, respectively. See text for the parameters used for calculation.

$$i = FAk_{sap}\{\Gamma_S[(\rho/\sqrt{K})^{0.5}-(\rho\sqrt{K})^{-0.5}] - \Gamma_O(\rho/\sqrt{K})^{-0.5}+\Gamma_R(\rho\sqrt{K})^{0.5}\} \quad (2)$$

where Γ_j's (j=O, S, R) are the surface concentrations of j species, and A is the surface area. The standard surface redox potential of the O_{ad}/R_{ad} couple is given by E'_o [$=(E'_{o1}+E'_{o2})/2=E_p^{rev}$], and $\rho=\exp[F(E-E'_o)/RT]$. Other symbols have their usual meanings. Using Eq. 2 and the other fundamental equations with regard to the cyclic voltammetry on the surface-redox reaction, we can draw a quasi-reversible current-potential curve by the technique of numerical calculations.[3]

We have now analyzed the voltammogram shown in Fig. 1A by applying the technique of numerical simulation using two parameters,[3,4] k_{sap} and K, at E'_o=−0.500 V and the electricity Q=1.29 μC, here $Q=2FA\Gamma_t$, Γ_t being the sum of Γ_O, Γ_S, and Γ_R. The refined parameters are k_{sap}=65.7±5.0 s^{-1} and K=2.61±0.29. The resultant regression curve is depicted in Fig. 1A as a broken line. The K value obtained is in agreement with those estimated from the reversible voltammogram (K=2.2—2.8). The analysis of the voltammograms at various Q's shows that, within the experimental error, the values of K and k_{sap} are independent of Q in the range of 1.3 to 2.6 μC[9] that is in the possible limit of the present experiment. The average K value evaluated in this Q range is 2.32, and the k_{sap} is 62.4 s^{-1} in the same Q range. Alternatively, we calculated the theoretical current-potential curves[3] at v=100 and 200 mV s^{-1} by using the above average values of K and k_{sap}. These theoretical curves well reproduce the experimental one. These results indicate that the electrochemical behavior of adriamycin adsorbed on an LT-BPGE can be well interpreted on the base of the theory of a two-step one-electron surface-redox reaction, and that these refined parameters would be considered to be physically significant.

Cardiolipin-Monomolecular-Layer Modified BPGE (CL-BPGE). The electrochemical redox behavior of adriamycin adsorbed on a CL-BPGE was fundamentally the same as that observed on an LT-BPGE. In Fig. 1B the solid line shows an observed quasi-reversible wave at v=1 V s^{-1} and Q=2.00 μC (solid line), and the regression curve is given by the broken line. The refined parameters are k_{sap}=73.7±4.8 s^{-1} and K=1.75±0.17. The K value is equivalent to 73 mV of $\Delta E_{p/2}^{rev}$,[7,8] which agrees well with the experimental value (71—76 mV) of the reversible wave recorded at v=10 mV s^{-1}. It was also observed that the K and k_{sap} values at this electrode are independent of Q within an experimental error in the range of the experimentally obtainable Q (1.5—2.0 μC), and the average values are 1.69 and 77.9 s^{-1} for K and k_{sap}, respectively, in the above Q range. The reliability of the parameters was supported by the same method as in the case of LT-BPGE.

Table 1. Electrochemical Data of Adriamycin on a Bare BPGE and Two Kinds of Phospholipid Membrane Modified Electrodes

	E'_o	k_{sap}	K	$\Delta G°$
	V vs. SCE	s^{-1}		kJ mol^{-1}
Bare-BPGE	−0.497	115[a]	0.21	+3.85
LT-BPGE	−0.500	62	2.32	−2.10
CL-BPGE	−0.500	78	1.69	−1.30

a) The value at θ_A (the surface coverage of adriamycin)→1 is used.

Comparison with the Behavior at Bare BPGE. Table 1 summarizes the electrochemical data of the adriamycin adsorbed on the two types of phospholipid membrane modified BPGE as well as those on a bare BPGE. The E'_o values are almost the same in all three cases. However, it is to be noted that the K values at the phospholipid membrane modified electrodes are almost ten times larger than that at the bare one, and the free energy changes, $\Delta G°(=-RT\ln K)$, of the semiquinone radical formation equilibrium ($O_{ad}+R_{ad}\rightleftarrows 2S_{ad}$) are lower by about 5—6 kJ mol^{-1} than that at the bare one. In other words, the semiquinone radical is stabilized in the hydrophobic environment in the phospholipid membranes. On the other hand, the k_{sap} values at the phospholipid membrane modified electrodes are about one-half the value of that obtained at the bare electrode. The above decrease in k_{sap} may be attributed in part to the attractive interaction between adriamycin and the phospholipid molecule,[3,8,10] and in part to the slow rate of the proton transfer in phospholipid membranes immediately following the electron transfer.

The aforementioned properties of adriamycin in phospholipid membranes are important from the viewpoint of the cardiotoxicity of this drug. It is evident that the toxicity is associated with the inhibition of mitochondrial electron transport in the lipid bilayer and with the generation of oxyradicals, such as superoxide anion radical, by an auto-oxidation of the semiquinone radical intermediate.[11] It has been suggested that the adriamycin-mediated oxyradicals are responsible for the adriamycin-caused DNA and enzyme damage and also for the adriamycin-enhanced

peroxidation of unsaturated phospholipids.[12] The high stability of the semiquinone radical in lipid membranes and the high affinity of adriamycin for the membranes may bring about the increase of the semiquinone-related cardiotoxicity.

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