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Adsorption of Hydrophobic Anions onto Phospholipid Membranes. The Effect of Membrane Surface Charge

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Hydrophobic adsorption of tetraphenylborate ions (TPB) onto lipid membranes were studied at different pH's of aqueous phase by using lipid vesicles and monolayers. The amount of adsorbed TPB is much higher at pH 2 than at pH 7.4. This result indicates that the adsorption of hydrophobic ions is affected by the ionization state of surface polar groups of lipids, *i.e.*, that the membrane surface potential plays a role in the adsorption of hydrophobic ions.

Interactions of ions with phospholipid membranes have been intensively studied, especially, the binding of metal cations to negatively charged phospholipid membranes, which is now fairly well understood.[1-4] Interactions of organic ions with phospholipid membranes, however, have not yet been explored. Hydrophobic ions may be permeable through lipid bilayers *via* their hydrophobic interaction with lipid hydrocarbon chains. Therefore, hydrophobic anions and cations have been used as tracers to estimate the barrier height of membrane's electrical potential. The transport mechanism for hydrophobic ions passing through a lipid bilayer membrane has been studied by Ketterer *et al.*[5] They measured the conductance of tetraphenylborate ions through a black bilayer membrane of dioleoyllecithin, and proposed the "three-step model" that consists of adsorption, translocation, and desorption steps. In this model, the adsorption sites are considered to be located near the membrane–aqueous solution interface on the membrane side. Therefore, it is possible to determine the membrane potential by measuring the amount of these ions adsorbed from the aqueous phase. Further, it is significant to elucidate exactly the feature of adsorption of these ions from the aqueous phase.[6]

Andersen *et al.*[7] measured surface potential change of bacterial phosphatidylethanolamine monolayer on aqueous solution as a function of aqueous TPB concentration and proposed the discrete charge effect for the boundary potential model with a limiting number of adsorbed ions. In this sense, electrical charges near the membrane surface have a remarkable influence on the adsorption behavior of charged molecules toward the membrane. It is believed that charges on the membrane surface play a role in the binding of charged molecules such as metal ions and ionic detergents to the membrane.[8] In this work, the effect of the ionization state of surface polar groups on the adsorption of TPB onto a phospholipid membrane has been studied systematically by using phospholipid vesicles and monolayers as membrane samples.

Experimental

Bovin brain phosphatidylethanolamine (BPE), dipalmitoylphosphatidylethanolamine (DPPE), and dimyristoylphosphatidic acid (DMPA) were purchased from Sigma Chemical Co. (USA) to be used as phospholipids. Sodium tetraphenylborate was purchased from DOJINDO Laboratories (Japan). These were employed without further purification. The other reagents used were of analytical grade. All solutions of these substances were made with deionized and doubly distilled water.

BPE vesicles were prepared by sonication without using any buffer to eliminate the spurious effect of included buffer ions.[9] The amount of TPB adsorbed on these vesicles was measured as follows: 2 ml of a vesicle suspension was added to 2 ml of a TPB solution of known concentration in a glass-stoppered vial (5 ml). After the mixture had been left standing overnight at room temperature, the vesicle particles were separated from the medium liquid by ultrafiltration (MILLIPORE, PTGC). The concentration of TPB in the filtrate as well as in the original solution was measured by the inductively coupled Argon plasma method (Jarrell-Ash 975). From these measurements, the amount of adsorbed TPB per gram of vesicles was calculated by using a calibration curve.

Electrophoretic mobilities of large vesicles were determined at various pH values in an aqueous solution of 1×10^{-3} mol dm^{-3} KCl at 25 °C. The measurments were performed in a Rank Brother microelectrophoresis apparatus (MK-2) using a rectangular glass cell.

Monolayers of phospholipids were prepared by spreading a chloroform/ethanol (3:1) solution of DPPE or hexane/ethanol (9:1) solution of DMPA on the surface of a subphase solution containing 0.1 mol dm^{-3} NaCl and 0.01 mol dm^{-3} *Tris*-HCl buffer at pH 7.4 or HCl at pH 2 in an 80 ml Teflon trough. The subphase solution was continuously stirred with a glass-sealed 5 mm needle by a magnetic stirrer, except while the surface potential change being measured. A concentrated TPB solution was added to the solution, and this mixed solution was stirred for more than 30 min. Surface potentials of the monolayer were measured with a vibrating electrode against an Ag/AgCl reference electrode as a function of TPB concentration.

Results and Discussion

The degree of ionization of phospholipid polar groups depends on the pH of solution. It has been reported that a phosphatidylethanolamine (PE) membrane has net negative charges at neutral pH due to the deprotonating effect of the lower amino group on the vesicle surface[3] or due to hydrolytic degradation of PE molecules.[4] To confirm the state of ionization of our PE vesicles, electrophoretic mobility was measured in 0.001 mol dm^{-3} KCl aqueous solution. Figure 1 indicates that the PE vesicles have net negative surface charges at neutral pH and that the value decreases with decrease in pH value. This trend agrees qualitatively with the result of Papahadjopoulos[4] shown in Fig. 1, which was obtained with 0.145 mol dm^{-3} NaCl aqueous solution. Both data exhibit that electrophoretic mobilities converge to zero at pH 2. The membrane surface potential (or more exactly, the potential at the Stern layer) at pH 7.4 can be calculated, on the assumption of a Boltzmann distribution for the counter ions from our data and that of Papahadjopoulos, according to

$$\zeta = \psi_0 e^{-\kappa d}, \quad (1)$$

where ζ is the zeta potential, κ Debye's reciprocal length, d the distance from membrane surface to slipping plane, and ψ_0 the membrane surface potential. By this analysis using the ζ value at pH 7.4, the result of d=11 Å and ψ_0=−60 mV is obtained. Considering the value of d=10 Å for silica particles in 0.01 mol dm^{-3} NaCl solution, the value of d=11 Å is not so unrealistic as the distance in 0.1—0.001 mol dm^{-3} salt solution. Furthermore, as shown later, the estimated ψ_0 value (ψ_0=−60 mV) is in close agreement with the value derived from adsorption experiments.

In Fig. 2, adsorption isotherms for TPB on PE vesicles are shown in the form of semi-log plot, which were obtained at pH's 7.4 and 2 in 0.001 mol dm^{-3} KCl solution. From these data, it is possible to examine the surface potential effect on the adsorption by using the following relation:

$$[\mathrm{TPB}]_m = K[\mathrm{TPB}]_b e^{\psi_0 F/RT}, \quad (2)$$

where $[\mathrm{TPB}]_m$ is the concentration of TPB adsorbed on the membrane, $[\mathrm{TPB}]_b$ the residual concentration of TPB in the bulk solution, ψ_0 the membrane surface potential, K a constant independent of the surface potential term (K can be expressed by $K_{ad}e^{\Phi F/RT}$, where K_{ad} is the adsorption coefficient and Φ the electrostatic potential difference between membrane surface and adsorbing site), and the other symbols have the usual meanings. The solid lines in Fig. 2 are the curves obtained by calculation from Eq. 2 for two $Ke^{\psi_0 F/RT}$ values, which are different from each other by a factor of

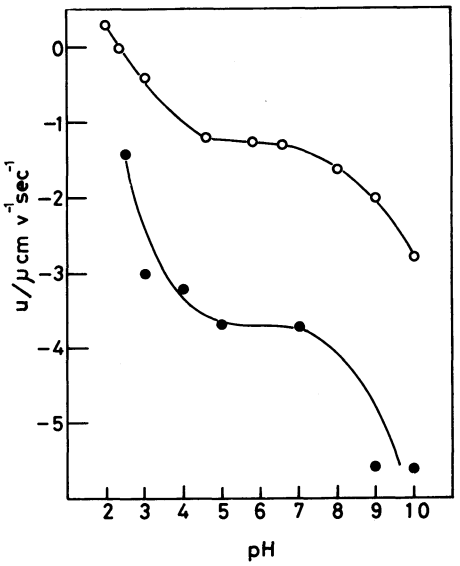

Fig. 1. Electrophoretic mobility-pH curves for PE vesicles.
(●): 0.001 mol dm^{-3} KCl, (○): Papahadjopoulos' data: 0.145 mol dm^{-3} NaCl.

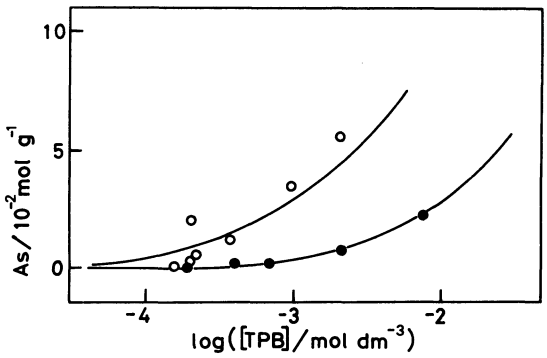

Fig. 2. Adsorption isotherms of TPB to PE vesicles.
(●): pH 7.4, (○): pH 2.

10, indicating that between the two cases there is a difference of about 60 mV in surface potential. As seen from Fig. 2, the two analytical curves fit the respective data very well, suggesting that the membrane surface potential at pH 7.4 is nearly −60 mV since the potential at pH 2 will become zero (see Fig. 1). An analogous discussion can be developed by using the data from the potential measurements on monolayer membranes. Potential change owing to TPB adsorption onto the monolayer can be given as follows on the simple capacitor model:

$$\Delta\Delta V = [\mathrm{TPB}]_m(F/C) = K_{ad}[\mathrm{TPB}]_b e^{\psi_0 F/RT} e^{\Phi F/RT}(F/C), \quad (3)$$

where Φ is the potential difference between membrane surface and adsorbing site, C the specific capacitance of the outer region of monolayer, K_{ad} the adsorption

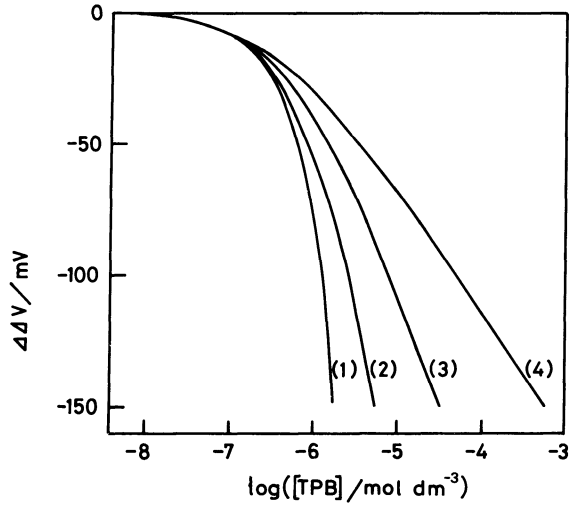

Fig. 3. Calculated curves from Eq. 3 for surface potential change of monolayer membrane. (1): $\Phi=0$, (2): $\Phi=0.2\Delta\Delta V$, (3): $\Phi=0.5\Delta\Delta V$, (4): $\Phi=\Delta\Delta V$.

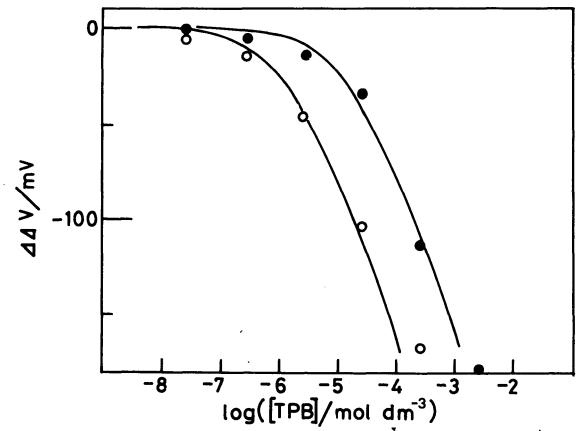

Fig. 5. Surface potential change of DMPA monolayer membrane *vs.* concentration of TPB (●): pH 7.4, (○) pH 2.

coefficient, ψ_0 the surface potential, and the other symbols have the usual meanings.

Figure 3 shows several calculated curves obtained from Eq. 3 by assuming ψ_0 is constant. In this case, Φ, is the electrostatic potential produced by adsorption of TPB on the membrane and expressed by the equation $\Phi=a\cdot\Delta\Delta V$. In the case if $a=1$, the full electrostatic potential produced by the adsorption of TPB molecules affects the adsorption of the next TPB molecule onto the membrane. In the case of $a=0$, the adsorption is affected by the chemical potential difference between solution and membrane and by the surface potential. In the experiments, intermediate cases with $0<a<1$ were observed. In Fig. 4, data of $\Delta\Delta V$ are plotted against the solution concentration of TPB (not against the residual concentration, though actually both are equal to each other) for the two values of pH. In these experiments, DPPE monolayers which have a packing density of

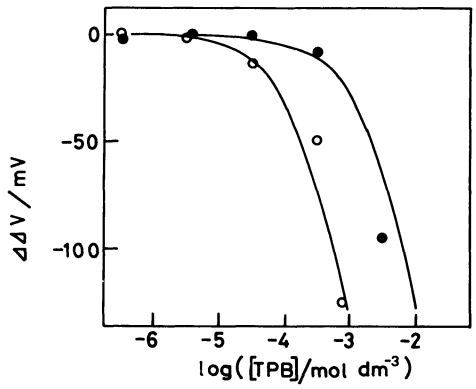

Fig. 4. Surface potential change of DPPE monolayer membrane *vs.* concentration of TPB. (●): pH 7.4, (○): pH 2.

1/80 molecule/Å^2 were used as the membrane sample. The solid lines in the figure are calculated curves from Eq. 3 in the case of $\Phi=0.2\Delta\Delta V$ for the two $K_{ad}(F/C)e^{\psi_0 F/RT}$ values differing from each other by a factor of 10. The agreement between the observed and calculated values is excellent if we take the experimental errors in consideration. If the capacitance value (C) in the boundary region is kept constant, there is expected to be about 60 mV difference in the surface potential of DPPE monolayer membrane between pH's 7.4 and 2. This value is in close agreement with the result estimated by the zeta potential analysis.

Similar behavior has been observed with DMPA monolayer membranes which have a packing density of 1/60 molecule/Å^2. In this case, calculated curves for $\Phi=0.5\Delta\Delta V$ were in close agreement with experimental data as shown in Fig. 5. These data suggest that there is a difference of about 60 mV in the surface potential of DMPA monolayer membrane between pH's 7.4 and 2.

The present analysis indicates that the adsorption equilibrium of TPB may be reasonably expressed by Eq. 3. This means that adsorption equilibrium exists between the concentration of TPB in the membrane, $[TPB]_m$, and the concentration of TPB at the membrane surface, $[TPB]_0$, which is represented by $[TPB]_b e^{\psi_0 F/RT}$. This is in good agreement with observations made on other charged molecules.

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