  Bull. Chem. Soc. Jpn., 59, 1533—1537 (1986)

Adsorption of Cationic Surfactants on Phospholipid Membranes and Its Contributions to Membrane-Surface Potential

Hideo MATSUMURA,* Miyako IWAMOTO,† and Kunio FURUSAWA†
Electrotechnical Laboratory, Sakura-Mura, Ibaraki 305
†Department of Chemistry, The University of Tsukuba, Sakura-Mura, Ibaraki 305
(Received October 31, 1985)

The adsorption behavior of cationic surfactants (alkyltrimethylammonium ions) on the phospholipid (phosphatidylethanolamine) membranes has been studied by means of interfacial electrostatic measurements, i.e., the surface potential of the monolayer and the zeta potential of the vesicles, in order to elucidate the balance between hydrophobic and electrostatic interactions. The data have been analyzed in terms of the interfacial electrochemical concept; these analyses have revealed that the hydrophobic interaction of a surfactant with the phospholipid membrane is the main motive force for adsorption and there is a critical alkyl-chain length to promote the surfactant adsorption. Furthermore, it was found that the electrostatic interactions between the surfactant and the phospholipid membrane, and among the surfactants themselves, also play a role in their adsorption.

The interaction of surfactants with the lipid membrane, especially with the lipid vesicles, has been studied extensively out of interest in the solubilization or fusion of biological membranes and their antibacterial action. The nonionic surfactant has been utilized as a solubilizer of the biological membrane because of its mild property of not destroying the biological function of the membrane components. On the other hand, studies of ionic surfactants have focused on their antibacterial properties. Quaternary ammonium compounds are typical cationic surfactants, and the antibacterial activity of a series of aliphatic quaternary ammonium salts have been studied.[1,2] The length of the hydrocarbon chain is one of the most significant factors controlling the antibacterial activity.[1] Shelton et al.[2] reported that an alkyltrimethylammonium bromide which contains less than eight carbon atoms in its alkyl group shows little antibacterial activity.

As well as the above practical interest, the adsorption of ionic surfactants on various interfaces has been studied out of interest in interfacial electrochemistry. For example, the effect of the surfactant adsorption on the zeta potential of a solid–water interface[3] and that on the electrocapillary curve at the oil–water interface[4] have been studied by many researchers.

In this work, the adsorption behavior of alkyltrimethylammonium bromides on the lipid membranes has been studied from the viewpoint of interfacial electrochemistry. As lipid membranes, phospholipid vesicles and monolayers spread on the aqueous solution were employed, and the change in the interfacial electrostatic potentials, i.e., the zeta potential of the vesicles and the surface potential of monolayers, caused by the adsorption of cationic surfactants, was studied. The main object in this work is to elucidate the contribution of electrostatic and hydrophobic interaction to the adsorption of cationic surfactants on phospholipid membranes.

Experimental

Materials. The monolayers and vesicles were prepared using L-α-dipalmitoylphosphatidylethanolamine (DPPE), which had been purchased from the Sigma Chemical Co., Ltd. (USA). As cationic surfactants, several alkyltrimethylammonium bromide (carbon numbers: 6,10,12,and 16) and tetrapentylammonium chloride, which had been purchased from the Tokyo Kasei Kogyo Co. Ltd., were used. These were used without any further purification. All the solutions of these compounds and the subphase solution in the monolayer experiment were prepared with deionized (Barnstead, NANO pure system) and doubly distilled water.

Zeta Potential. The DPPE vesicles were prepared by sonication without using any buffer to eliminate any unexpected effect of the included buffer ions. Vesicle suspensions were mixed with various concentrations of cationic surfactants; the final salt condition was 10^{-3} mol dm^{-3} NaCl. The electrophoretic mobilities of large-sized vesicles were measured with a Rank Brother microelectrophoretic apparatus (Model MK-2) using a rectangular glass cell at 20 °C. The zeta potentials were calculated from the mobility data according to the method of Wiersema.[5] In this calculation, the radius (a) of the vesicle was assumed to be 1000 Å.

Surface Potential of Monolayers. Phospholipid monolayers were prepared by spreading DPPE from a chloroform–ethanol (3:1) solution on the surface of a subphase solution containing 0.1 mol dm^{-3} NaCl in 80 ml a Teflon trough. The packing density of the monolayers prepared in these experiments was about 1/60 (molecule/Å^2). After the addition of a concentrated surfactant solution to the subphase solution, the solution was stirred for more than 30 min; then the surface potentials of the monolayers were measured by the vibrating-electrode method at 23 °C.

Results and Discussion

The electrostatic potential profile through the membrane-solution interface can be described ap-

proximately as a combination of an inside membrane potential which originates from dipoles of polar-head groups of phospholipid molecules and an ionic diffuse-double layer potential.[6] These potentials and their combination will change upon the adsorption of ionic compounds (a cationic surfactant in this case). Therefore, it is expected that their adsorption behavior can be analyzed by measuring both the surface potential of the monolayers and the zeta potential of the vesicles.[7]

Contribution of Alkyl-Chain Length. In Fig. 1, the change in the surface potential of the monolayer ($\Delta\Delta V$) is plotted against the concentrations of several kinds of alkyltrimethylammonium ions (abbreviated as alkyl-TMA) on a log scale. It is found that decyl-(C_{10}) and hexyl-(C_6) TMA have little effect on $\Delta\Delta V$, whereas dodecyl-(C_{12}) and hexadecyl-(C_{16}) TMA make a considerable contribution on $\Delta\Delta V$ in the present experimental concentrations. It is interesting to notice that there is a great difference in their contributions to $\Delta\Delta V$ between C_{10} and C_{12} TMA. This fact suggests that there is a critical hydrocarbon-chain length for the interaction between a surfactant and phopholipid membranes. A simillar tendency has been observed in the electrophoresis experiments. (Fig. 2). The electrophoretic mobility of DPPE vesicles changes the sign of its value with the concentration of C_{12} and C_{16} TMA, but not with that of C_{10} TMA. These data indicate that hydrocarbon numbers larger than ten are necessary for the alkyl chain of the surfactants to interact effectively with the DPPE membrane. Figure 3 shows the relationship between the $\Delta\Delta V$ of the DPPE monolayer membrane and the concentration of tetrapentylammonium bromide in the solution. As is shown in Fig. 3, this molecule has only a slight effect on the surface potential of the DPPE monolayer membrane. Those results suggest that these four alkyl chains are too short to enter the membrane interior of DPPE deep enough to interact with the hydrophobic part of the membrane and that a certain bulky effect of this molecule may play a role in this phenomenon.

Effect of Ionization State of DPPE. A DPPE molecule is an amphoteric molecule, and it has two dissociable groups, i.e., $-\overset{+}{N}H_3$ and $-PO_4H$. Depending on the pH in the bulk solution, these groups change their ionization state, as is shown in Fig. 4. (The ionization behavior of the PE membrane is

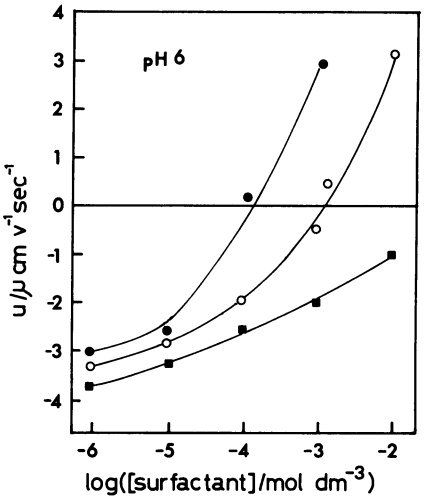

Fig. 2. Electrophoretic mobility of DPPE vesicles bathed in various concentration of several alkyltrimethylammonium bromide aqueous solution. (●): C_{16}, (○): C_{12}, (■): C_{10}.

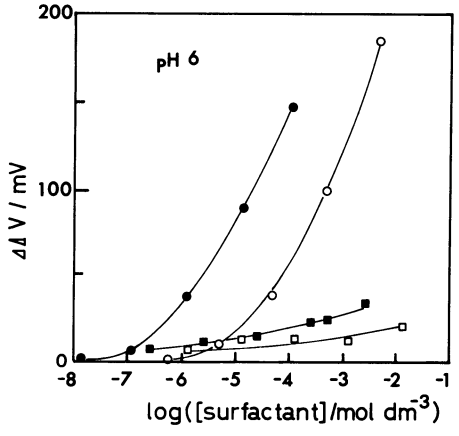

Fig. 1. Surface potential change of DPPE monolayer membrane vs. concentration of alkyltrimethyl ammonium bromide. (●): C_{16}, (○): C_{12}, (■): C_{10}, (□): C_6.

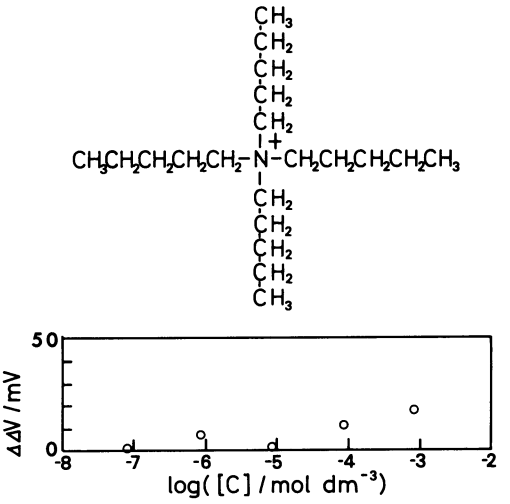

Fig. 3. Surface potential change of DPPE monolayer membrane vs. concentration of tetrapentylammonium bromide.

represented in Fig. 1 of Ref. 9.) Moreover, the ionization state of the phosphatidylethanolamine membrane has been reported by many researchers[8] to be different from that of the single molecule itself. In this work, three pH values, i.e., 3, 6, and 10, were selected in order to examine the effect of the ionization state on the adsorption of C_{12}–TMA onto the membrane. Figure 5 shows the change in the surface potential of the monolayer caused by the adsorption of C_{12}–TMA at the three pH values. $\Delta\Delta V$ begins to increase at a lower concentration of C_{12} TMA with an increase in the pH, i.e., in the order of: pH 10>pH 6>pH 3. This order is due to the change in the ionization state of the DPPE membrane; that is, at pH 10, the DPPE membrane has the most negative charges on the surface, whereas at pH 3, the DPPE membrane has the least negative charges or positive charges on it. As a result of the electrostatic interaction between C_{12} TMA and the DPPE membrane, the adsorption is promoted by the electrostatic attraction at pH 10, but is depressed by the repulsion at pH 3. Electrophoretic studies support this picture. Figure 6 shows the electrophoretic mobility of DPPE vesicles bathed in various concentrations of the C_{12} TMA solution at those three pH values. At pH 3, the DPPE vesicles have a positive value of mobility in the surfactant-free system. At pH 10, the electrophoretic mobility of the DPPE vesicles is comparable to that at pH 6. The adsorp-

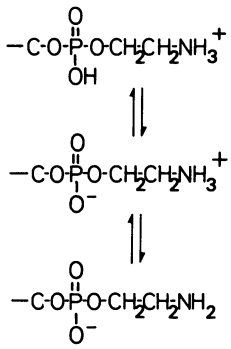

Fig. 4. Ionization state of PE.

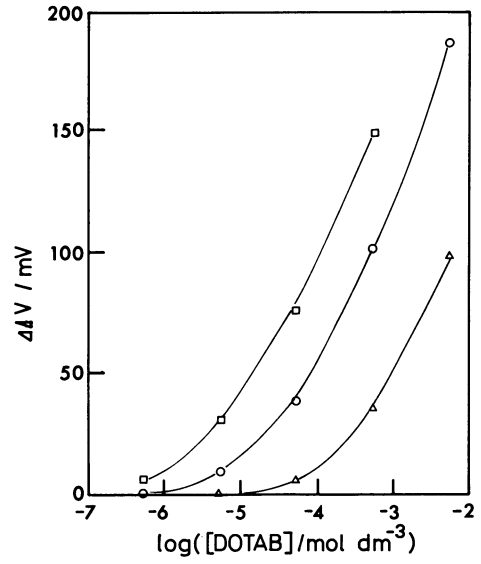

Fig. 5. Surface potential change of DPPE monolayer membrane vs. concentration of dodecyltrimethylammonium bromide at (△) pH 3, (○) pH 6, (□) pH 10.

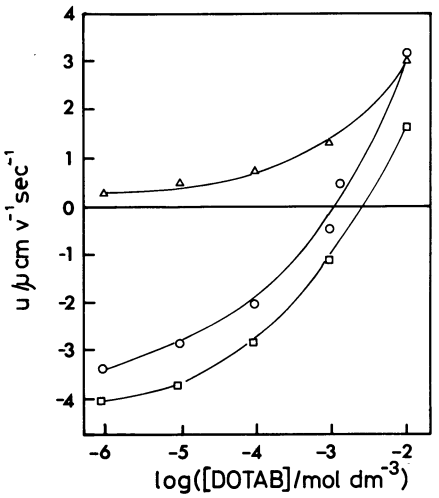

Fig. 6. Electrophoretic mobility of DPPE vesicles bathed in various concentration of dodecyltrimethylammonium bromide at (△) pH 3, (○) pH 6, (□) pH 10.

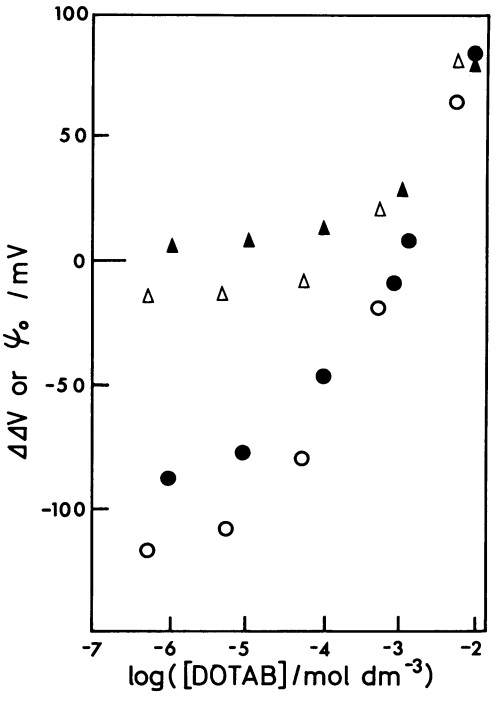

Fig. 7. Membrane surface potential (ψ_0; ▲,●) calculated from the data of electrophoretic mobility or surface potential change ($\Delta\Delta V$; △,○) of monolayer at pH 3 and pH 6, respectively.

tion of C_{12} TMA on the membrane causes a change in the zeta potential of the vesicles. The contribution of this surfactant to the mobility is greater at pH 6 and pH 10 than at pH 3. This behavior is almost the same as that shown in Fig. 5. In Fig. 7, the membrane surface potential (ψ_0), calculated from the data of electrophoretic mobilities by the method of Wiersema and the relation of $\psi_0=\zeta e^{\kappa d}$ (ζ: zeta potential, κ: Debye-Hückel parameter), is plotted together with the surface potential of the monolayer ($\Delta\Delta V$). In this calculation, the distance from the membrane surface to the slipping plane (d) is assumed to be 10 Å.[9] The two sets of data are compared with each other on the assumption that their values are equal at the surfactant concentration, 10^{-3} mol dm^{-3} (around this concentration the zeta potentials have a zero value at pH 6, and the surface potential can be taken to be zero.). This figure shows that $\Delta\Delta V$ resembles the change in the ψ_0 value of the vesicles (at pH 3 and pH 6). This means that the surfactants are adsorbed on the membrane surface in such a manner as their charges of the head group are located at the membrane-solution interface. The data of electrophoretic mobilities at pH 10 are not very different from those at pH 6. This may be due to the relaxation effect, because $\kappa a \simeq 5$ under these experimental condition; i.e., the electrophoretic mobility does not increase with the increase in the zeta potential above 100 mV.

Analytical Considerations

The adsorption behavior of charged molecules by the membrane can be described by the following simple relation:

$$[\text{surfactant}]_m = K_w K_e f [\text{surfactant}]_b, \quad (1)$$

where $[\text{surfactant}]_m$ is the concentration of C_{12} trimethylammonium ions adsorbed on the membrane; $[\text{surfactant}]_b$, its concentration in the bulk aqueous solution; K_w, the adsorption coefficient based on hydrophobic interaction, and f, the factor which originates from the mutual electrostatic repulsion of adsorbed ions and the next adsorbing ion and which determines the line shape of the adsorption curve. In other words, f denotes the discrete charge effects on the charged surface.[10] K_e is the constant of electrostatic interaction between the DPPE membrane and the surfactant molecules which arises from the ionization of DPPE molecules; it is denoted as follows:

$$K_e = e^{-\psi_0 e/kT}, \quad (2)$$

where ψ_0 is the membrane-surface potential.

The change in the surface potential of the monolayer ($\Delta\Delta V$) is approximated by the following equation:

$$\Delta\Delta V = \Delta q/C_c = e \cdot \Delta[\text{surfactant}]_m/C_c, \quad (3)$$

where Δq is the amount of charges adsorbed; e, the elementary electric charge, and C_c, the specific capacitance of the outer region of the monolayer.

The f factor, which determines the curvature of the figure, can be expressed as follows:

$$f = e^{-a \cdot \Delta\Delta V e/kT}, \quad (4)$$

where $0 \leqq a \leqq 1$. $a=0$ means that the mutual interaction of ions is absent, while $a=1$ means that the electrostatic potential produced by the adsorption of ions greatly influences the adsorption of the next adsorbing ion.

If one adopts here the diffuse double-layer theory instead of the simple capacitor model, Eq. 3 can be rewritten by the following equation:[11]

$$\sinh\frac{\Delta\Delta V}{51.4} = \frac{136.6 \cdot \Delta q}{\sqrt{C}}, \quad (5)$$

where C the concentration of indifferent ions.

These equations allow the analytical calculation of the a factor. The experimental data of $\Delta\Delta V$ for C_{12}-TMA are plotted, with their analytical curves, in Fig. 8. The solid lines are for Eq. 3, while the dashed ones are for Eq. 5. When we compare these data with the theoretical curves, $a=0.5$ is adequate. This means that these surfactant ions adsorb on the DPPE membrane partially hindered by electrostatic repulsion among the adsorbed ions themselves.

By comparing the data of $\Delta\Delta V$ and the electrophoretic mobility for C_{12}-TMA with those for C_{16}-TMA in Figs. 1 and 2, one can estimate the hydrophobic interaction per $-CH_2$ group. The adsorption coefficient of the hydrophobic part, K_w, is expressed as follows:

$$K_w = K \cdot e^{-(N-N_0)w/kT}, \quad (6)$$

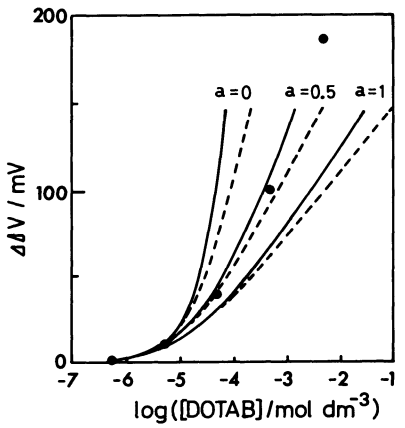

Fig. 8. Calculated curves from Eq. 3 —— and Eq. 5 ---- for surface potential change and experimental data of dodecyltrimethylammonium bromide.

where w is the hydrophobic interaction energy per CH_2 group; N, the number of CH_2 groups in the hydrocarbon chain; N_0, the critical number of CH_2 for hydrophobic interaction with the membrane, and K, the constant from other parts. The $\Delta\Delta V$ curves of C_{16}-TMA are shifted to the lower-concentration side by more than 1 order from that of C_{12}-TMA. Furthermore, the electrophoretic mobility curve of C_{16}-TMA is also shifted to a lower side by more than 1 order from that of C_{12}-TMA. These data make possible the calculation of the w-value through this relation: (shift value)$=e^{\Delta n \cdot w/kT}$; where $\Delta n=N(C_{16})-N(C_{12})=4$; i.e., Δn is the difference in CH_2 number. Then, w is obtained as $w=0.6-1.2\,kT$. By comparing this value with that of the hydrophobic interaction energy (1.1 kT) for the micelle formation of various surfactants, it can be said that the main motive force of adsorption is also hydrophobic interaction between the membrane and the ionic surfactants. In fact, Zaslavsky et al.[12] have studied the interaction between a series of N-acyl derivatives of amino acids and egg lecithin liposome and have reported that the binding free energy per CH_2 ranges from 0.24 Kcal mol^{-1}† (0.4 kT) to 1.05 Kcal mol^{-1} (1.8 kT).

† 1 cal=4.184 J.

References

1) J. A. Cella, D. N. Eggenberger, D. R. Noel, L. A. Harriman, and H. J. Harwood, *J. Am. Chem. Soc.*, **74**, 2061 (1952).

2) R. S. Shelton, M. G. Van Campen, C. H. Tilford, H. C. Lang, L. Nisomger, F. J. Bandelin, and H. L. Rubenkoenig, *J. Am. Chem. Soc.*, **68**, 753 (1946).

3) A. Watanabe, F. Tsuji, and S. Ueda, *Denki Kagaku*, **29**, 777 (1961).

4) A. Watanabe, M. Matsumoto, H. Tamai, and R. Gotoh, *Kolloid-Z*, **220**, 152 (1967).

5) P. H. Wiersema, A. L. Loeb, and J. TH. G. Overbeek, *J. Colloid Interface Sci.*, **22**, 78 (1966).

6) R. Aveyard and D. A. Haydon, "An Introduction to the Princples of Surface Chemistry," Cambridge Univ. Press, London/New York (1973), p. 34.

7) D. A. Haydon and V. B. Myers, *Biochim. Biophys. Acta*, **307**, 429 (1973).

8) H. Hauser and M. C. Phillips, "Progress in Surface and Membrane Science," 13, Academic Press, New York (1979), p. 297.

9) H. Matsumura, H. Kakoki, and K. Furusawa, *Bull. Chem. Soc. Jpn.*, **58**, 78 (1985).

10) O. S. Andersen, S. Feidberg, H. Nakadomari, S. Levy, and S. McLaughlin, *Biophys. J.*, **21**, 35 (1978).

11) A. G. Lee, *Biochim. Biophys. Acta*, **472**, 237 (1977).

12) B. Y. Zaslavsky, A. A. Borovskaya, A. K. Lavrinenko, A. Y. Lisichkin, Y. A. Davidovich, and S. V. Rogozhin, *Chem. Phys. Lipids*, **26**, 49 (1980).