  Bull. Chem. Soc. Jpn., 60, 3849—3853 (1987)

Interactions between a Synthetic Phospholipid Vesicle and Cationic Surfactants

Mitsuhiro TAKASAKI, Noboru TAKISAWA, and Keishiro SHIRAHAMA*
Department of Chemistry, Faculty of Science and Engineering, Saga University, Saga 840
(Received April 9, 1987)

The interaction between a synthetic phospholipid derivative, the 1,3-ditetradecyl-*rac*-glycerophosphatidylcholine ($2C_{14}$-Gly-PC) vesicle, and two cationic surfactants, *N*-tetradecylpyridinium bromide (TDPBr) and *N*-tetradecyltrimethylammonium bromide (TDTMABr), were investigated in the hope of obtaining a better understanding of the mechanism of the growth of the vesicle size by the "surfactant-removal" method. The binding isotherms of these surfactants to the lipid vesicle obtained by the surfactant selective electrode were characterized by two-step binding processes; the Langmuir adsorption mechanism in the initial binding, followed by a markedly positive cooperative process. The variation in the hydrodynamic radius, as measured by quasi-elastic light scattering, and the quenching kinetics of pyrene fluorescence with the TDPBr surfactant solution as measured by a stopped-flow method, share a tendency with the binding behavior. The van't Hoff plots of the initial binding data give a slightly negative enthalpy change, $\Delta H°$, for TDPBr and a relatively large positive $\Delta H°$ for TDTMABr. The microenvironmental polarity around pyrene in the molecular assemblies was assessed by means of fluorescence spectroscopy.

When amphiphiles such as lecithin are dispersed in water, they form molecular assemblies with a bilayer structure called a vesicle, which is excellent in mimicking biological membranes.[1-3] Vesicles comparable in size with a biological cell will facilitate study of the mechanism of cellular reaction. A vesicle formed in water is usually much smaller than biocells and a multilamellar structure, even if the vesicle is prepared by the sonication method. It is known that a vesicle is solubilized by a surfactant, which is subsequently removed by dialysis or some other method above the transition temperature of the gel-liquid crystal, T_C of the lipid; there, then produced a large homogeneous, unilamellar vesicle. However, the "surfactant-removal" method has not been well understood yet[4-6] in spite of its biochemical significance.[7-9] We have investigated the interaction of the 1,3-ditetradecyl-*rac*-glycerophosphatidylcholine ($2C_{14}$-Gly-PC) vesicle with two cationic surfactants, *N*-tetradecylpyridinium bromide (TDPBr) and *N*-tetradecyltrimethylammonium bromide (TDTMABr). It has been known that $2C_{14}$-Gly-PC behaves much like lecithin, having the corresponding alkyl chains,[10] and it is chemically stable and suitable for investigating the vesicle-surfactant interaction, since the ether bond between two alkyl chains and the phosphatidylcholine group is resistant against hydrolysis.

The binding of TDPBr and TDTMABr to the vesicle was measured by using surfactant-electrodes which have been successfully utilized in our laboratory,[11-13] and the thermodynamic aspects of binding were discussed. The hydrodynamic radii of the vesicle were obtained by means of quasi-elastic light scattering (QELS). Pyrene fluorometry was useful in the static and kinetic study of the interaction mechanism.

Experimental

Materials. The $2C_{14}$-Gly-PC (Fig. 1) was purchased from Sogo Pharmaceutical, Tokyo, and was used as received. The TDPBr was synthesized by a method similar to that used for dodecylpyridinium bromide[12] and was recrystallized three times from acetone. A triple recrystallization of TDTMABr (Tokyo Kasei) from acetone was also carried out. The critical micelle concentrations (cmc) in an aqueous solution, as determined by the electric conductivity method, are 2.82 and 3.60 mmol dm^{-3} at 30 °C for TDPBr and TDTMABr respectively; these values are in good agreement with the literature.[14] For convenience, we use the abbreviation 1 M=1 mol dm^{-3} throughout this paper.

Sample Preparation. The vesicle-forming agent, $2C_{14}$-Gly-PC, was dispersed in doubly distilled water and treated in a bath-type sonicater (VELVO CLEAR VS-20, Iuchi) for 1 hour at about 50 °C. Surfactant-containing samples were incubated at about 50 °C and used after they had been allowed to stand for a day or so for a complete equilibration of the system.

Surfactant-Selective Electrode. Poly(vinyl chloride) (PVC; 0.9 g) and bis(2-ethylhexyl) phthalate (2.1 g) were mixed well, after which 6 ml of tetrahydrofuran was added to the slurry mixture. The whole mixture was then heated at 60 °C. After a while, a clear and viscous solution was obtained; it was cast on a flat glass plate. The solvent was gradually removed in a silica-gel desiccator for two days, and then the gel membrane (about 0.2 mm thick) was obtained. The membrane was cut out and glued on one end

1,3-ditetradecyl-rac-glycero-phosphatidyl choline
($2C_{14}$-Gly-PC)

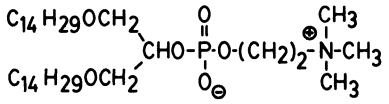

Fig. 1. Structure of $2C_{14}$-Gly-PC amphiphile.

of a PVC tube (9 mm in inner diameter, 11 cm long) and "annealed" at 40—50 °C under reduced pressure.

The binding of the surfactant to the vesicle was measured by using the surfactant-selective electrode in a thermostated cocylindrical cell. The concentration cell formula was as follows.

Ag/AgCl	KCl agar bridge	C_0 mM surfactant	**M**	C_1 mM surfactant (+vesicle)	KCl agar bridge	AgCl/Ag

in which **M** is the surfactant-selective electrode membrane; C_0, the inner (reference) surfactant concentration, and C_1, the outer surfactant concentration. The electromotive force, emf, was measured with a digital multimeter (Advantest TR-6845).

Pyrene Fluorometry. The fluorescence of pyrene was measured by means of a Shimadzu digital spectrophotomerer RF-510 (temperature controlled with LAUDA RM3 MODEL S-1 thermostat). The fluorometry was carried out from both static and kinetic points of view. The static experiment was done only with the system using TDTMABr. The variation in the I/III ratio found in the vibronic fine structure in the pyrene fluorescence spectrum was measured by changing the surfactant concentration in the 350—450 nm emission-wavelength region.[15] As for the kinetic experiment, the vesicle dispersions containing pyrene (p-dispersion) and TDPBr as a fluorescent quencher (q-dispersion) were quickly mixed by the used of a stopped-flow mixer (Union Giken MX-7) and the fluorescence intensity was followed with time, where the excitation wavelength, λ_{ex}=337 nm, and the emission one, λ_{em}=391 nm, were both used. The mixing was also carried out by a conventional "manual fast mixing"; i.e., the q-dispersion was poured into the p-dispersion in a cuvette cell, and the mixture was stirred with a tiny plastic rod as quickly as possible.

Quasi-Elastic Light Scattering (QELS). The hydrodynamic radii of the molecular assemblies were measured for the TDPBr system by the QELS method.

Results and Discussion

Figure 2 shows the relation between the emf and the logarithm of the TDPBr concentration. The electrode exhibits the Nernstian response for the TDPBr surfactant without a vesicle. In the coexistence of the $2C_{14}$-Gly-PC vesicle, however, a deviation from linearity is observed. The deviation may result from a partial binding of TDPBr to the vesicle. From the deviation, we can obtain the equilibrium surfactant concentration, C_f, and the bound surfactant concentration, $C_b=C_t-C_f$, where C_t is the concentration actually weighed-in. The surfactant-selective electrode responds equally well to TDTMABr. The binding isotherms were obtained by plotting the amount of binding per unit of lipid concentration, C_b/C_v, against C_f; they are shown in Figs. 3 and 4 for TDPBr and TDTMABr respectively. In the lower C_f region, the C_b/C_v amount is not so large, and it increases gradually with C_f before increasing sharply.

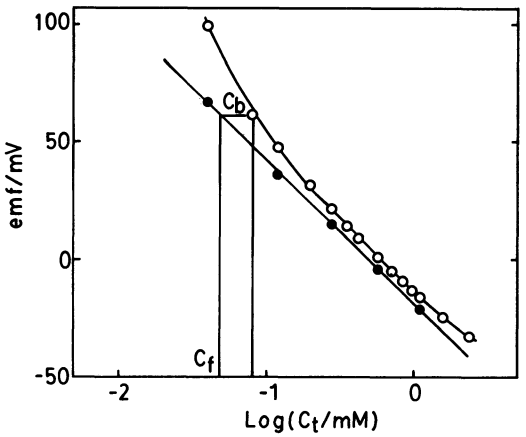

Fig. 2. Potentiogram of TDPBr at 303 K.
●: Without vesicle O: with 0.616 mM vesicle.

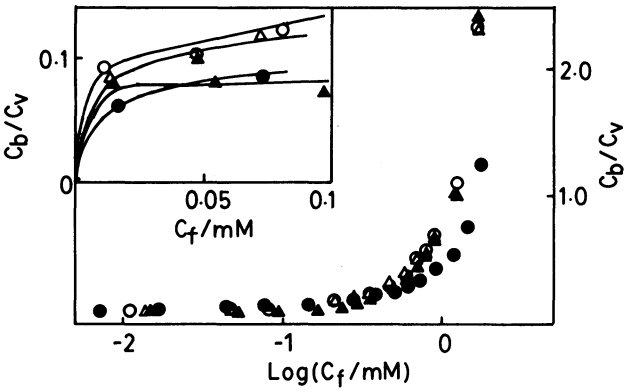

Fig. 3 Binding isotherms for TDPBr.
Temperature at ▲: 283 K, △: 293 K, O: 303 K, ●: 313 K.

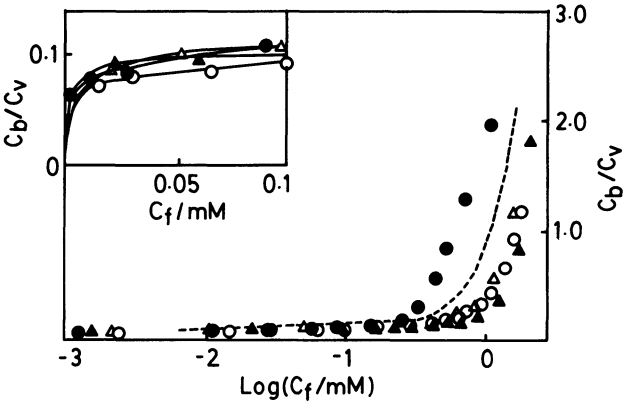

Fig. 4. Binding isotherms for TDTMABr.
Temperature at ▲: 283 K, △: 293 K, O: 303 K, ●: 313 K. ----: For TDPBr at 303 K.

In Fig. 4, a typical isotherm for TDPBr at 303 K is also shown by a dashed line for the sake of comparison. It appears that the C_b/C_v amount for TDPBr are slightly larger than those of TDTMABr, indicating a stronger

affinity of the pyridinium analog to the lipid vesicle. These isotherms depend a little on the temperature.

When the surfactants bind multiply and independently to the binding sites, which are mainly located on the surface of the vesicle, binding is expressed by the Langmuir isotherm:[16]

$$C_b/C_v = \frac{mKC_f}{1+KC_f} \quad (1)$$

where m is the maximum amount of binding and K, a sort of equilibrium constant. The Scatchard equation[16] derived from Eq. 1,

$$\frac{C_b/C_v}{C_f} = -K(C_b/C_v) + Km \quad (2)$$

is plotted for TDPBr and TDTMABr in Fig. 5. The data points support a straight line, with a negative slope at the lower concentrations; a different behavior

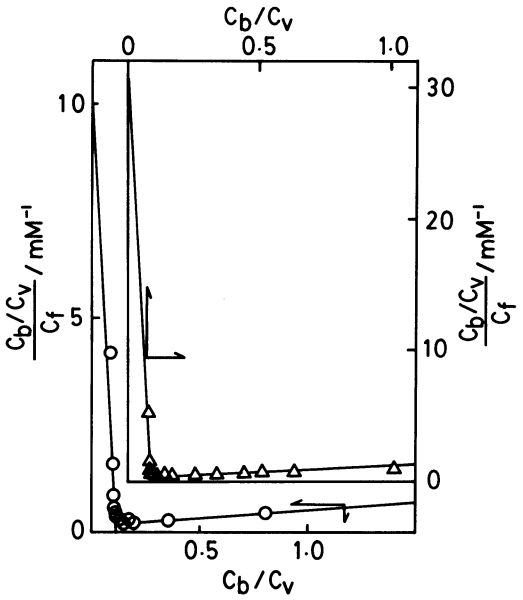

Fig. 5. Scatchard plots at 283 K.
△: For TDPBr, ○: for TDTMABr.

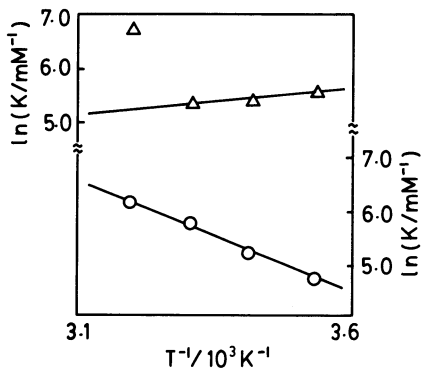

Fig. 6. The temperature dependence of K.
△: TDPBr, ○: TDTMABr.

with a positive slope is seen in the higher-concentration region, indicative of cooperative binding.[16,17] From the straight line in the lower-concentration region, the equilibrium constant, K, and the maximum amount of binding, m, were obtained. The maximum amount of binding is about 10 mol% irrespective of the kind of surfactant and the temperature; the further addition of a surfactant exceeding m may induce the formation of a mixed micelle. The van't Hoff plot for TDPBr in Fig. 6 has a slightly positive slope from 283 to 303 K, and the point at 313 K deviates from linearity, while all the data points for the TDTMABr system support linearity, with a relatively large negative slope. The different behavior at 313 K between the two systems may be related to the difference in the binding enthalpy, which is very much affected near the gel-liquid crystal transition temperature[10] of the vesicle, but the details remain still to be studied. The Langmuir parameters and ΔH°'s are listed in Table 1. In the Langmuir region, TDPBr binds to the vesicle exothermically, and TDTMABr, endothermically. This difference probably originates from the difference in the head group, since the two surfactants have the same hydrophobic chain-length. The difference in the ΔH° of binding between the surfactants, $\Delta\Delta H^\circ = \Delta H^\circ_{\text{TDPBr}} - \Delta H^\circ_{\text{TDTMABr}}$, may be governed by the electrostatic interaction between the phosphate anion and the cationic head group of each surfactant, and may result from the electrostatic energy difference. The phosphate ion group may approach the pyridinium ring plane closer so that electrostatic energy becomes less. The three bulky methyl groups of TDTMABr may interfere with such a close approach to the vesicular phosphate group.

QELS Measurement. The hydrodynamic radii, R_H, are obtained from the observed autocorrelation function, which decays single-exponentially, as

Table 1. Thermodynamic Parameters in the Langmuir Binding Process for (a) TDPBr and (b) TDTMABr Systems

(a)

T/K	m	K/mM^{-1}	ΔH°/kJ mol^{-1}
283	0.093	258	-7.7×10^0
293	0.112	220	
303	0.125	209	
313	0.105	801	

(b)

T/K	m	K/mM^{-1}	ΔH°/kJ mol^{-1}
283	0.107	115	3.43×10^1
293	0.118	183	
303	0.092	313	
313	0.096	450	

shown by an insert in Fig. 7. The variation in the R_H with C_f is shown in Fig. 7. In the lower-surfactant-concentration region, the R_H's are nearly invariant with C_f; the growth in the molecular assembly size appears above the same concentration range, where the binding deviates from the Langmuir isotherm. A similar constancy was previously observed with the system of a synthetic surfactant vesicle and nonionic surfactants.[4]

Pyrene Fluorescence. Pyrene shows a significant fine structure: the five well-defined peaks are numbered I—V from th lowest wavelength.[15] Since the intensity ratio, I/III, changes with the microenvironmental polarity around a pyrene molecule,[15] it will be used to assess the environment of pyrene molecules. Figure 8 shows how the polarity changes with the surfactant concentration. Pyrene dissolved in water shows a I/III ratio of about 1.5, as is indicated by the arrow in Fig. 8. The pyrene containing TDTMABr solution shows a sharp decrease in its I/III ratio near the cmc of TDTMABr and then a leveling-off. In contrast, pyrene shows a very low polarity in the vesicular medium at a low TDTMABr concentration, but its polarity increases to as much as that of pure micelle with an increase in the TDTMABr concentration, suggesting the formation of mixed micelles with the lipid molecules.

Quenching Kinetics of Pyrene Fluorescence. As soon as the pyrene-containing vesicle (p-vesicle) and the quencher-containing vesicle (q-vesicle) are mixed, quenching begins. It is considered to be controlled by the collision between the two kinds of vesicles. The frequency of collision may be very slow because of the large vesicular size, and so the quenching may also be very slow. The fluorescent intensity, $I(t)$ at 391 nm, decayed immediately until it reached about half the initial intensity. This may be due to the quenching of the free pyrene by the free quencher. Following this very rapid process, a relatively slow single-exponential decay was observed in the lower-TDPBr-concentration region, while in the higher-surfactant-concentration region the quenching kinetic trace seems a double-exponential process. By plotting the quantity, $\ln(I(t)-I(\infty))/(I(0)-I(\infty))$ against the time, the first order reaction constant, k, is obtained; it is shown in Fig. 9, where it may be seen that k is independent of C_f when C_f is low, but the k value increases rather abruptly at higher C_f values. It may be considered that the slow process results from a collision between the p-vesicle and the q-vesicle. At a higher cationic surfactant concentration, a part of the lipid is solubilized to form mixed micelles with the lipid and pyrene; the small molecular assemblies are efficient for quenching since they are moving quickly. The labile structure of a mixed micelle may also be preferable for efficient quenching.

All these experimental results for TDPBr are summarized in Fig. 10, where the various observed

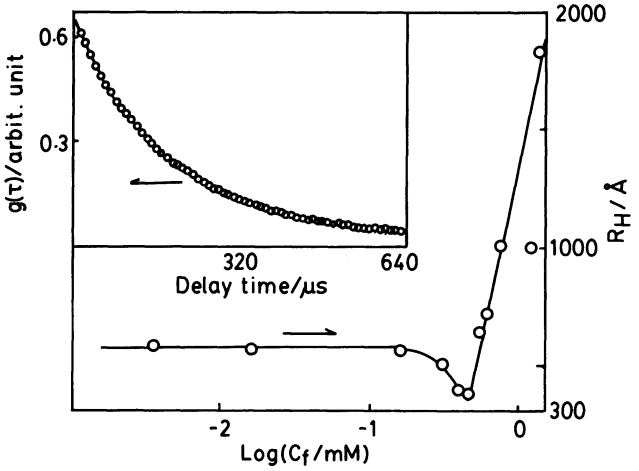

Fig. 7. An autocorrelation function and the variation of R_H with log C_f measured at 303 K.

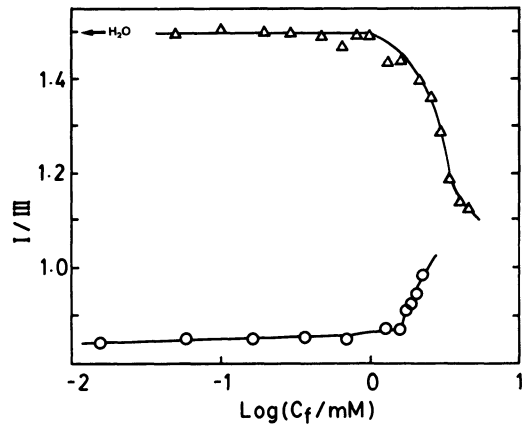

Fig. 8. The change of I/III ratio of pyrene molecule with C_f measured at 303 K.
○: With 0.616 mM lipid, △: without lipid.

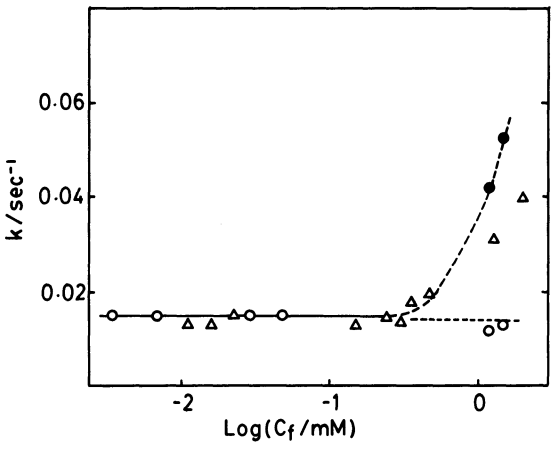

Fig. 9. Variation of quenching rate constant of pyrene fluorescence with logarithm of C_f.
○●: Stopped-flow method, △: manual mixing.

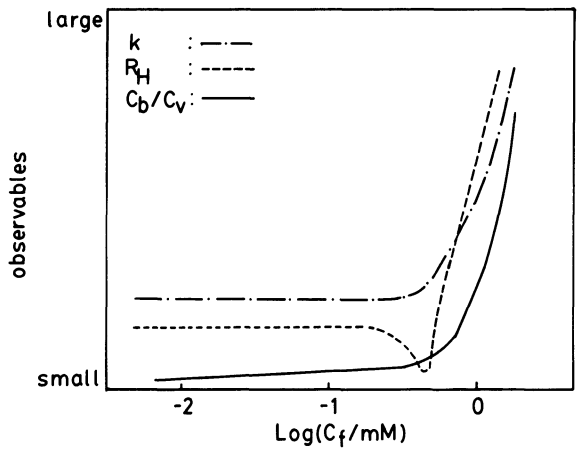

Fig. 10. Various observable vs. log C_f plot for TDPBr. —·—; k, ----; C_b/C_v, ——; R_H.

results are plotted as a function of the logarithm of C_f. It is notable that there is a close resemblance among the tendencies of all these observed data; the addition of the surfactant to the Langmuir binding limit causes drastic change in the behavior of the system. It may be concluded that the cationic surfactants are bound to the vesicle that remains the original vesicular structure in the lower-surfactant-concentration regions, and that, with an increase in the surfactant concentration, the sudden change in the observables in the present work may result from the formation of the mixed micelle. The growth of the vesicular size may also occur by the collision between the mixed micelle and the vesicular aggregation.[18]

In the QELS measurements we are grateful to the kind support of Professor Mitsuru Tanaka's laboratory of Fukuoka University. We are also thankful to Professor Masao Okubo of Saga University for lending the stopped-flow mixer. The author (K.S.) thanks "the Asahi Glass Foundation for Industrial Technology" for partially supporting this work.

References

1) J. H. Fendler, *Acc. Chem. Res.*, **1**, 7 (1980).
2) C. Tanford, "The Hydrophobic Effect: Formation of Micelles and Biological Membranes," John Wiley & Sons, New York (1973).
3) L. Stryer, "Biochemistry," Freeman, San Francisco (1981), p. 205.
4) K. Shirahama, K. Takashima, N. Takisawa, K. Kameyama, and T. Takagi, "Phenomena in Mixed Surfactant Systems," ed by John F. Scamehorn, American Chemical Society, Washington D.C. (1985), p. 299.
5) G. C. Kresheck, K. Kale, and J. Erman, "Solution Behavior of Surfactants," ed by K. L. Mittal and E. J. Fendler, Plenum, New York (1982), Vol. 1, p. 677.
6) P. Schurtenberger, N. Mazer, and W. Kanzig, *J. Phys. Chem.*, **89**, 1042 (1985).
7) H. Hauser, N. Gains, H.-J. Eibl, M. Muller, and E. Wehrli, *Biochemistry*, **25**, 2126 (1986).
8) S. Almog, T. Kushner, S. Nir, and D. Lichtenberg, *Biochemistry*, **25**, 2597 (1986).
9) R. Schubert, K. Beyer, H. Wolburg, and K.-H. Schmidt, *Biochemistry*, **25**, 5263 (1986).
10) T. Kunitake, Y. Okahata, and S. Tawaki, *J. Colloid Interface Sci.*, **103**, 190 (1983).
11) K. Shirahama, and M. Tashiro, *Bull. Chem. Soc. Jpn.*, **57**, 377 (1984).
12) K. Shirahama, T. Masaki, and T. Takashima, "Microdomains in Polymer Solutions," ed by P. Dubin, Plenum, New York (1985), p. 299.
13) K. Shirahama, K. Takashima, and N. Takisawa, *Bull. Chem. Soc. Jpn.*, **60**, 43 (1987).
14) P. Mukerjee and K. J. Mysels, "Critical Micelle Concentrations of Aqueous Surfactant Solutions," U.S. Government Printing Office, Washington D.C. (1971).
15) J. K. Thomas, *Chem. Rev.*, **80**, 283 (1980).
16) G. M. Barrow, "Physical Chemistry," ed by D. C. Jackson and S. Wagley, McGraw-Hill, Auckland (1979), p. 731.
17) K. E. Van Holde, "Physical Biochemistry," ed by L. Hager and F. Wold, Prentice-Hall, Englewood Cliffts, New Jersey (1971), Chap. 3.
18) D. Lasic, *Biochim. Biophys. Acta*, **692**, 501 (1982).