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ADSORPTION AND DESORPTION STUDIES OF PHOSPHOLIPID AT THE AIR/WATER INTERFACE*

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SUMMARY

The desorption and adsorption properties of phosphatidylserine (extracted from beef brain) at the air/water interface were studied through surface pressure measurements. The rate of dissolution of phosphatidylserine monolayer into the underlying water of natural pH is extremely slow at room temperature but increases rather suddenly around 40°C. This sudden increase of dissolution rate might be explained as the Kraft point phenomena analogous to the ionic surfactants.

The energy of activation for the desorption processes of phosphatidylserine monolayer to the underlying water is calculated to be 28.6 kcal/mole from the temperature dependent characteristics of the dissolution rate. This is larger than the kT by a factor of about 50, but is good compared with the expected desorption energy 27.4 kcal/mole of amphiphiles of 39 carbon atoms which is calculated by applying Traube's rule.

INTRODUCTION

The solubilities of phospholipids in water have usually been thought to be negligibly small, and in many physicochemical experiments of reconstituted membranes such as in monolayer or bilayer studies, little attention has hitherto been paid concerning adsorption and desorption equilibria of phospholipids between membrane and surrounding aqueous media in spite of its expected importance in the growth or juvenescent[1] action of the living membrane[2], and also in the physicochemical properties of the natural and reconstituted[3] membranes.

In preliminary experiments we have qualitatively measured the limits of dissolution of phosphatidylserine monolayer in water at several temperatures by applying the benzene solution of phosphatidylserine from the microsyringe to the water surface in a petri dish, until the spreading of the benzene solution ceases. Fig. 1 shows the temperature dependence of the amount of phosphatidylserine necessary to saturate the surface of distilled water of 5-mm depth at pH 6. The titration

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curve rises steeply around 37 °C. Above this temperature it is difficult to find an end point of spreading as far as the one or two hours time of titration is concerned. This suggests the dissolution of phosphatidylserine in water to be considerable. In this report, the desorption and adsorption characteristics of phosphatidylserine at the air/water interface were further studied.

MATERIALS AND METHODS

The phosphatidylserine* used in this experiment was extracted from beef brain and refined to a purity[4] of more than 99 % for phosphatidylserine and more than 86 % for alkyl chain of 18 carbon atoms, as shown in Table I, which was sealed in an ampoule with nitrogen gas as a benzene solution, and stored in a refrigerator until needed. The monolayer of phosphatidylserine is prepared by spreading an aliquot of the benzene solution on aqueous surface from a microsyringe, and the surface pressure was measured after the evaporation of benzene. The benzene, for a spreading solvent, was purified over silica gel (Fischer, Scientific grade) and left no measurable degree of surface pressure increase after evaporation when spread on aqueous surface, even for the surface compressed to 1/10 of the original area. The water used for substrate was distilled three times, including the process of alkaline permanganate treatment (pH 5.5–6.0). The surface tension of the distilled water after prolonged aging of the surface compared well with that of the fresh one, indicating no organic substance contaminates the surface during the whole experimental procedures. The surface tension was measured with an accuracy of ± 0.3 dyne/cm by Cenco du Noüy surface balance using 17 mm × 17 mm × 0.2 mm microscopic cover glass as a Wilhelmy plate[5]. The greatest care was taken for the wettability of glass plate to ensure the contact angle of zero[6] and the receding condition to be always satisfied.

TABLE I

FATTY ACID COMPOSITION OF BOVINE BRAIN PHOSPHATIDYLSERINE

*Fatty acid**	*Percent*
16:0	—
18:0	48.9
18:1	37.2
18:2	—
20:1	4.8
20:4	4.0
20:6	3.8

* Number of carbon atoms: number of double bonds.

RESULTS AND DISCUSSION

The amounts of phospholipid spread for unit area of surface at the temperature higher than 36 °C in Fig. 1 are larger than the corresponding value of monolayer, but rather correspond to bilayer value. We are not sure at present whether this high value actually shows the bilayer formation at the aqueous surface, or simply means

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* Kindly given by Dr Papahadjopoulos of Roswell Park Memorial Institute at Buffalo, N.Y.

the loss of monolayer into the bulk solution by the multiple addition of spreading solution to the surface, because of the not well qualified process of spreading.

There may be an explanation for the sudden increase of dissolution of monolayer shown in Fig. 1 which attributes this increase to the abrupt change of collapse pressure of the monolayer with temperature. In order to see whether the abrupt increase in Fig. 1 can be explained by the lowering of the collapse pressure[7,8] of monolayer, the surface pressure–molecular area (F–A) relation was measured for phosphatidylserine at both 25 and 40 °C as shown in Fig. 2. Each collapse pressure from 45–50 dynes/cm is not enough to explain the abrupt increase of monolayer dissolution shown in Fig. 1. If we keep these phosphatidylserine monolayers standing for a long time, the pressure goes down to almost zero at various rates depending on the temperature. We have finished the measurement for each F–A curve within 10–15 min. (The rate is 1.2 Å^2/molecule per s for high pressure regions.) In such a short course of time the errors involved by the dissolution of phosphatidylserine may not exceed more than 1 dyne/cm.

The change of surface pressure, that should be caused by the dissolution of the monolayer, was traced with time at various temperatures. The rate of surface pressure depression $-\mathrm{d}F/\mathrm{d}t$ at the early stage of desorption[9] increased with temperature as shown in Fig. 3 (initial pressures for these monolayers were chosen to have the value between 40 and 44 dynes/cm). The steep rise was also observed around 40 °C similar to the curve shown in Fig. 1. The sudden increase of the rate can be understood as an increased dissolution of phosphatidylserine monolayer at that temperature which might be explained as a similar phenomenon to the so-called "Kraft point"[10], well-known for the common ionic surface active agent. The Kraft point is generally understood as a melting point at which the hydrated crystal[11] of paraffin chain salt liquefies[12], and it is above this temperature the micelle formation or swelling of crystalline[13] with water are probable for ionic surface active substances. The formation of phosphatidylserine micelles in bulk solution should necessarily bring the increase of net concentration of phosphatidylserine which is in equilibrium with the monolayer. The rate of dissolution of the monolayer into substrate water, should, therefore, be larger when equilibrium bulk concentration is increased. Similar critical change in physicochemical properties of phospholipid with temperature is also observed for the swelling of various phospholipid gels by Van Deenen[14], and for the phase diagram from the X-ray diffraction studies by Luzzati[15]. Recently Krasne *et al.*[16] reported the melting and freezing of bilayer membrane of glyceryl distearate and dipalmitate

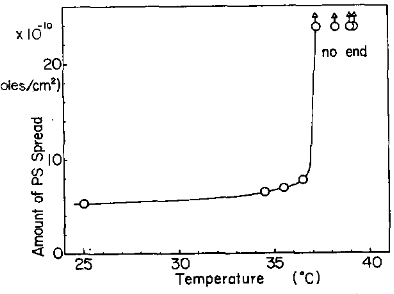


Fig. 1. Dissolution of phosphatidylserine (PS) in water.

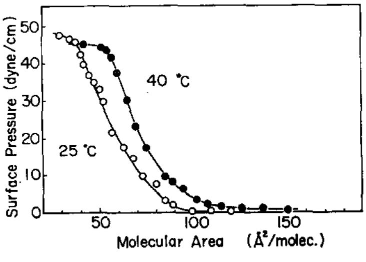


Fig. 2. Surface pressure–molecular area relations of phosphatidylserine.

mixture at 40 °C as a sudden change of the fluidity of bilayer and of the conductance across the membrane. The melting point is very high for non-hydrated phosphatidylserine, 154–155 °C[13], but might be lowered to around 40 °C when phosphatidylserine is hydrated. This is implicative enough if we notice the fact that the temperature is very close to that of cow which is around 38.5 °C[17], though the condition of the present experiment is somewhat different from the physiological one containing fairly high concentration of electrolytes.

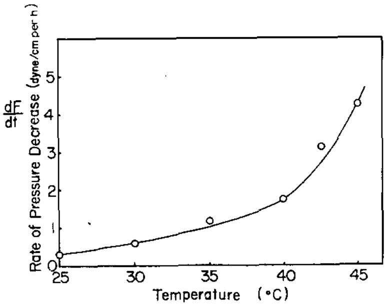


Fig. 3. Initial rate of surface pressure decrease for phosphatidylserine monolayer against temperature.

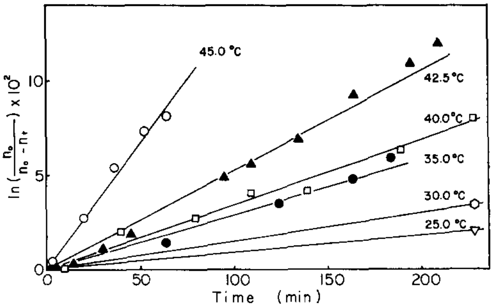


Fig. 4. Rate of dissolution of phosphatidylserine monolayer.

Applying the first order rate equation[18] to each desorption process, the linear relations shown in Fig. 4 were obtained. Here, n_0 and n_t indicate the number of molecules in unit area of the monolayer at time 0 and t, respectively, which are calculated from the corresponding F–A curve at each temperature. The rate constant, which is the slope of this linear relation, increases with temperature. The Arrhenius plot of these data shows a straight line (Fig. 5) from which we can calculate the activation energy for the desorption process of phosphatidylserine monolayer into the aqueous substrate. That is 28.6 kcal/mole, or $2.0 \cdot 10^{-12}$ erg/molecule which is far larger than $kT = 4.2 \cdot 10^{-14}$ erg/molecule (40 °C) by a factor of 48, but is good compared with the expected value of desorption energy $1.9 \cdot 10^{-12}$ for phosphatidylserine calculated after Langmuir[19] applying Traube's rule[20]. This calculation is based on an assumption that one phosphatidylserine molecule has a single long alkyl chain of 39 carbon atoms, and each methylene group contributes to the desorption energy 625 cal/mole and for those functional polar groups, ester 470, amine 600, phosphate 600 and carboxyl group 437 cal/mole are assumed[21,22]. Some ambiguities are still left in regarding the phosphatidylserine molecule to have one long alkyl chain instead of two, and also concerning the coordinate role between the functional groups of phosphatidylserine and the role of the double bond in alkyl chains on the solubility phenomena. The activation energy obtained here is also in a fair agreement with the value by Krasne[16] 29 kcal/mole for the transition of the bilayer of glyceryl distearate and dipalmitate mixture in their conductance experiments.

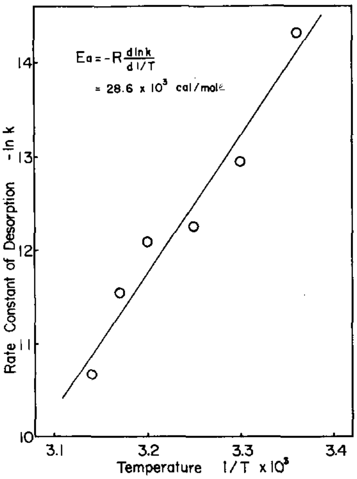
The theoretical calculation[23] of the cohesive energy based on the second order perturbation theory gave 27.3 kcal/mole at 62 Å²/molecule for the hexagonally packed monolayer of lipid which has two alkyl chains of 18 carbon atoms. The value is very close to the observed activation energy and suggests the activation process of the desorption depends on the breaking of the cohesive bond between the lipid molecules in the monolayer.

The atmospheric oxygen should have an appreciable effect on the solubility properties of phosphatidylserine. The oxidation of phosphatidylserine generally makes the monolayer less soluble in water, and also gives the *F–A* curve an expanded feature. Great care was taken to avoid exposing the sample to the open air by doing the experiment in nitrogen atmosphere. The effect of oxidation on the present experiment is proved to be negligibly small by the comparative monolayer measurements done in atmospheres of both air and nitrogen as far as 1 or 2 h time course of experiment is concerned.

In order to see the adsorption properties of phospholipid at air/water interface, the surface tension was measured at different concentrations of phosphatidylseiine in 145 mM aqueous NaCl solution of pH 6.

The full line in Fig. 6 shows the results obtained at 40 °C. All the solutions at different concentrations were kept in nitrogen atmosphere and the effect of evaporation[24] was also prevented for about 35 h until the equilibrium is reached.

The surface tension measurement for such extremely dilute solution often leads to an erroneous expression of the isotherm[25,26] due to the change of equilibrium concentration of solute by adsorption. In Fig. 6, the broken line shows the isotherm corrected for equilibrium concentration which was calculated by assuming the identity of spread monolayers to the adsorbed one, and by utilizing the *F–A* curve to convert the surface pressure to the amount of adsorption. The surface tension decreases gradually with the concentration increase and shows almost constant value as low as 30 dynes/cm above $5.0 \cdot 10^{-5}$ M. From the analogy to the general feature of the adsorption isotherm[27] of ionic surface active agents the critical change of surface tension at c_m in Fig. 6 is explained as follows. The constant surface tension γ of the solution of micellar surface-active substance at the concentration above c_m implies in Gibbs adsorption isotherm $(\partial\gamma/\partial \ln \alpha)_T = -nRT\Gamma$ the constant activity α of surface-



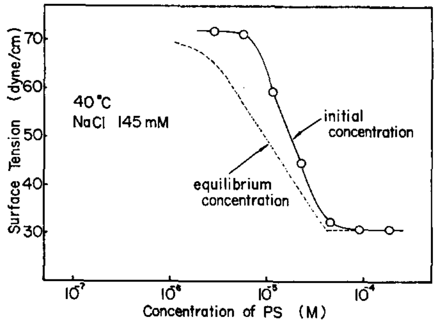


Fig. 5. Arrhenius plots for the desorption process of phosphatidylserine monolayer.

Fig. 6. Adsorption of phosphatidylserine (PS) at air/water interface.

active species[28]. Here, Γ is the amount of adsorption of surface-active solute, R the gas constant, T the absolute temperature and n the constant. As the micelles are reasonably thought to be surface-inactive, the lowering of surface tension suggests the presence of some surface active species other than the micelles. The surface-active species might be the single ions of phospholipid dispersed molecularly, or the aggregates in lower degree of association, lower enough to give the aggregate particle an asymmetric conformation in regard to the hydrophobic and hydrophilic nature. Above the critical concentration, the surface active solutes tend to form micelles, and the activity of surface active species stays unchanged that keeps the surface tension constant. It is still difficult to conclude, however, from this adsorption experiment that the surface film of phospholipid was formed only through adsorption and not by the spreading of surface-inactive micelles or minute crystalline particles[29] that has happened to come to the air/water interface. But, the adsorption seems to be more likely from the gradual change of surface tension in Fig. 6 with the concentration increase.

Application of Gibbs' isotherm to Fig. 6, by assuming the analytical concentration of lipid to be equal to the activity, and $n = 2$, gives an amount of adsorption far smaller than the corresponding monolayer value at the same surface pressure and shows that the simple thermodynamic relation is not applicable to such systems[30].

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