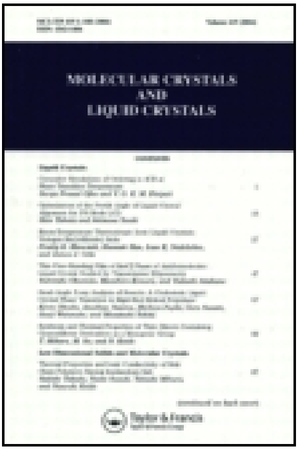

Molecular Crystals and Liquid Crystals

Successive Phase Transitions in Lipid Membranes

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Successive Phase Transitions in Lipid Membranes†

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On the basis of a statistical model previously proposed by the present authors, a new sort of phase transition recently observed in the fluid-like phase of DPL bilayer and mono-layer above the main transition temperature is investigated as successive orientational transitions accompanied with conformational changes. The effects of lateral pressure on the transition temperatures and latent entropies observed in DPL monolayer are also explained consistently. This type of phase transition is expected to occur in DML, DSL and DBL as well, although it has not been observed yet in the case of these molecules.

1 INTRODUCTION

Lipid bilayers have a low temperature solid-like phase and a high temperature fluid-like or liquid crystalline phase; and the transition between these is usually known as the "main" transition in the membrane. At the main transition, the hydrocarbon chains of lipid molecules change cooperatively their conformation from an extended long form to a bended short one and simultaneously the orientational transition occurs, where a fairly large latent entropy is observed.

Recently, a new weak phase transition has been found above the main transition temperature in bilayers of DPL, DOL‡ and DPL + DOL mixtures by Furuya and Mitsui,[1] and also in monolayer of DPL under a lateral pressure by Albrecht *et al.*[2] It has not been clarified yet experimentally what changes in the molecular ordering occurs at that transition.

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‡ DPL: dipalmitoyl lecithin, DOL: dioleyl lecithin.

In the present paper, we try to investigate theoretically such phase transitions. We have previously proposed a statistical model which describes the orientational phase transition in the system of flexible molecules such as lipids.[3] We can expect the orientational phase transition occurs twice successively in that model under an appropriate condition for the molecular parameters.

In section 2, the model and its consequences are briefly introduced. The observed results on the successive phase transition in bilayer and monolayer of DPL are explained theoretically on the basis of the model mentioned above, in section 3. The possibility of the similar phase transition phenomena in other lipids is also discussed in section 4.

2 MODEL AND GENERAL RESULTS

In previous papers,[3] by regarding a lipid membrane as an assembly of hydrocarbon chain molecules, we have assumed an attractive potential, between the i-th and j-th chain molecules in the form

$$\phi_{ij} = -C(r_{ij})P_2(\cos\,\theta_{ij})\sigma(q_i)\sigma(q_j), \tag{1}$$

where $C(r_{ij})$ denotes a coupling strength depending on the distance r_{ij} between the molecules, P_2 (cos θ_{ij}) the second order Legendre polynomial of the angle θ_{ij} between the largest principal axes of molecules and $\sigma(q_i)$ depends on the molecular conformation q_i, in particular on the chain length. Two order parameters are defined for a system of N molecules in a total volume V as

$$S = <\frac{1}{N}\sum_{i=1}^{N} P_2(\cos\,\theta_i)\sigma(q_i)>, \tag{2}$$

and

$$\sigma = <\frac{1}{N}\sum_{i=1}^{N} \sigma(q_i)>, \tag{3}$$

where θ_i denotes the angle between the largest principal axis of i-th molecule and the direction of the mean alignment and $<. . .>$ indicates the thermal average. The parameter S represents the orientational order of the system, whereas σ characterizes the mean conformation of the chains.

By means of the method of symmetry breaking potential,[4] the equilibrium value of the order parameter S can be determined from the self-consistent equations

$$\eta_0 = CS/k_BT, \tag{4}$$

$$S = (\partial/\partial\eta_0)\ \ln\ \varphi(\eta_0). \tag{5}$$

with

$$C \equiv \rho \int C(r) d\mathbf{r}, \tag{6}$$

$$\varphi(\eta_0) \equiv \sum_q I_0\{\eta_0 \sigma(q)\} \exp\{-E(q)/k_B T\}, \tag{7}$$

where we have made use of $\rho = N/V$,

$$I_0(x) \equiv \int_0^1 \exp\{x P_2(\cos\theta)\} d(\cos\theta), \tag{8}$$

and the conformational energy $E(q)$ of a chain in the state q. The summation with q in (7) is taken over all possible conformations. Similarly, σ can be calculated from

$$\sigma = \sum_q \sigma(q) I_0\{\eta_0 \sigma(q)\} \exp\{-E(q)/k_B T\}/\varphi(\eta_0). \tag{9}$$

The free energy $F(S)$ is expressed as

$$F(S) = F(0) + N k_B T\{\tfrac{1}{2} S\ \eta_0 - \ln \varphi(\eta_0)/z\}, \tag{10}$$

where use has been made of the conformational partition function for an isolated chain given by

$$z = \sum_q{}' \exp\{-E(q)/k_B T\}, \tag{11}$$

and the free energy $F(0)$ in the disordered state, $S = 0$. In order to proceed further, we should specify the conformation parameter $\sigma(q)$. In the previous paper,[3] the molecular conformations have only been classified into two categories: viz. longer extended and shorter bended forms, which are represented by $\sigma(q) = \sigma_1$ and $\sigma(q) = \sigma_2$, respectively ($0 < \sigma_2 < \sigma_1 \leq 1$). Then Eq. (7) is rewritten down as

$$\varphi(\eta_0) = I_0(\sigma_1 \eta_0) z_1 + I_0(\sigma_2 \eta_0) z_2, \tag{12}$$

where z_1 and z_2 are partial partition functions for the longer and shorter conformations given by (11) with the respective conditional summations satisfying $\sigma(q) = \sigma_i (i = 1,2)$.

As has been shown in the previous paper,[3] the phase transition in such a system can be classified into three cases, according to the values of

$$\alpha \equiv \sigma_2/\sigma_1 \text{ and } z^* \equiv z_2/z_1. \tag{13}$$

In the regions (I) or (II) on the plane of those molecular parameters shown in Figure 1, only a single transition occurs, whereas in the upper wedge-like region (III), two transitions occur successively. In Figure 2, we show temperature dependences of the order parameters S and σ in the case of region (III)

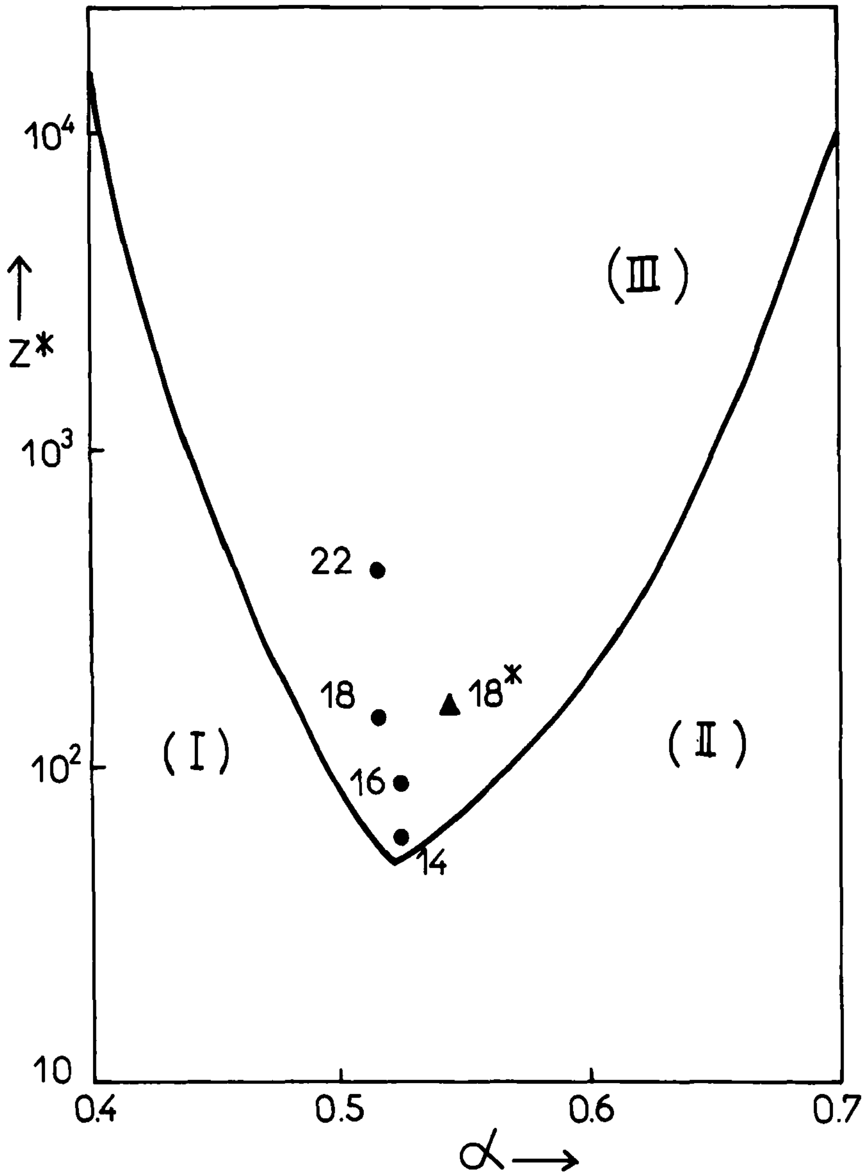

FIGURE 1 Classification of the phase transition on the $z^* - \alpha$ plane with the estimates of (α, z^*) for several lipid bilayers: closed cirles numbered 14, 16, 18 and 22 represent DML, DPL, DSL and DBL, respectively, and the closed triangle with 18* DOL. The successive orientational phase transition is expected to occur in the region (III).

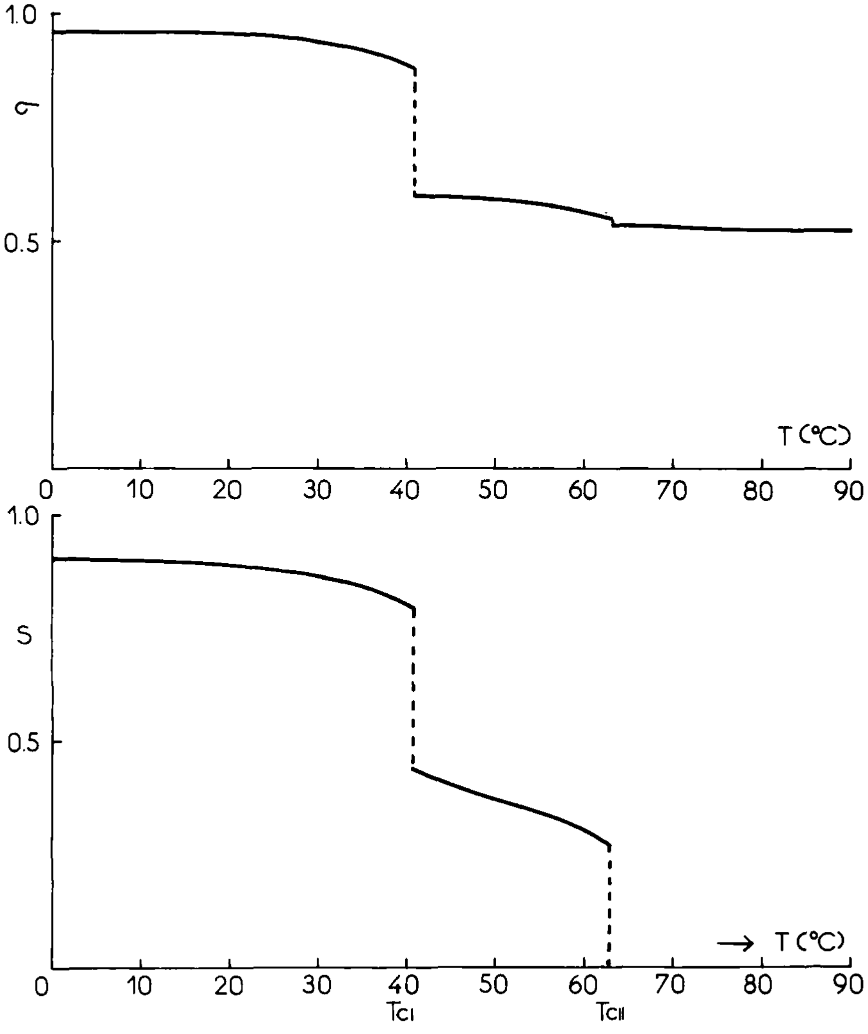

FIGURE 2 Temperature dependences of the order parameters σ and S calculated from the parameters $\alpha = 0.53$, $z^* = 90$ and $C = 10{,}400$ cal/mole for DPL, where T_{cI} and T_{cII} are the main transition and secondary transition temperatures, respectively.

with the parameters, $\alpha = 0.53$, $z^* = 90$ and $C = 10{,}400$ cal/mole. In the low temperature region $T < T_{cI}$, both σ and S are near to 1 and the system is regarded to be in an ordered solid-like phase. At $T = T_{cI}$, the order parameter S decreases with temperature and jumps to a smaller value, being accompanied with a change in the molecular conformation or in the parameter σ. With further increase of temperature, S vanishes discontinuously at a higher transition temperature T_{cII}, where the jump of the conformation parameter σ is much smaller. In this way, the character of the transition occuring at T_{cI} quite resembles the main transition in lipid membranes and the secondary transition at T_{cII} is similar to the above mentioned transition observed recently in certain membranes.

3 DISCUSSION ON THE SUCCESSIVE TRANSITION

(1) DPL bilayer

The molecule of DPL has two hydrocarbon chains each with 16 C—C bonds, where the main transition temperature T_{cI} and the latent entropy $\Delta\Sigma_{cI}$ at that temperature have been found as $T_{cI} = 314°\text{K}$, $\Delta\Sigma_{cI} = 27.6$ cal/mole °K[5]. The secondary transition temperature has been observed by Furuya and Mitsui as $T_{cII} = 335°\text{K}$. On the basis of these data, we can determine the relevant molecular parameters α, z^* and the coupling constant C in the present model. By making use of Eqs. (4), (5), (9), (10) and (12) with these parameters, we calculate the order parameters S and σ as functions of temperature and determine the inverse transition temperatures C/k_BT_{cI} and C/k_BT_{cII}, where α and z^* are assumed to be independent of temperature. Further, the latent entropy at T_{cI} is calculated from

$$\Delta\sum_{cI} = \left\{\frac{1}{2}\, C(S_{cI}^2 - S_{cI}'^2) + \langle E\rangle_0 \left(\frac{\sigma_{cI} - \sigma_{cI}'}{1 - \sigma_0}\right)\right\} N/k_BT_{cI} \qquad (14)$$

where $S_{cI}(S'_{cI})$ and $\sigma_{cI}(\sigma'_{cI})$ are the order parameters adjacently below (above) the temperature T_{cI} and $\langle E\rangle_0$ and σ_0 denote respectively the mean conformational energy and parameter of a free chain at that temperature. The values of $\langle E\rangle_0$ for hydrocarbon chains with n C—C bonds ($n = 14 \sim 22$) have already been calculated in the previous paper[3] by making use of the rotational isomeric state approximation. Then we can determine α, z^* and C by comparing the results of calculation with the experimental values of T_{cI}, T_{cII} and $\Delta\Sigma_{cI}$. Thus, we obtain the following values for DPL bilayer:

$$\alpha = 0.53,\ z^* = 90 \quad \text{and} \quad C = 10{,}400 \text{ cal/mole}. \qquad (15)$$

The values of α and z^* are indicated in Figure 1 by a closed circle marked by 16. The results from the parameters (15) are shown in Figure 2, which shows that the secondary transition is a weak first order one accompanied by the latent entropy $\Delta\Sigma_{cII}$ estimated only one tenth of $\Delta\Sigma_{cI}$ in the main transition (Figure 5b). This is consistent with the experiment on DPL bilayer.[1]

(2) Effect of lateral pressure on the transition in monolayer

From experiment on the DPL monolayer, Albrecht *et al.*[2] have proposed such a temperature vs pressure phase diagram as partially shown in Figure 3(b).

The curve T_M indicates the transition temperature which corresponds to the main transition in bilayers. They observed a secondary phase transition occurs at higher temperature T_F at sufficiently high lateral pressures. Under a lateral pressure π, a term πA is added to the free energy of the system with the surface area A of the membrane. In the present model, we assume that

$$A = \sum_i A(q_i), \tag{16}$$

where $A(q_i)$ is the area occupied by the i-th chain in the conformation q_i. The partition function z and the parameter z^* should be replaced respectively by

$$z(\pi) = \sum_q \exp[-\{E(q) + \pi A(q)\}/k_B T], \tag{17}$$

$$z^*(\pi) = z^*(0)\exp(-\pi \Delta A/k_B T), \tag{18}$$

where we have assumed that

$$\begin{aligned} A(q) &= A_1 \quad \text{for} \quad \sigma(q) = \sigma_1, \\ &= A_1 + \Delta A \quad \text{for} \quad \sigma(q) = \sigma_2, \end{aligned} \tag{19}$$

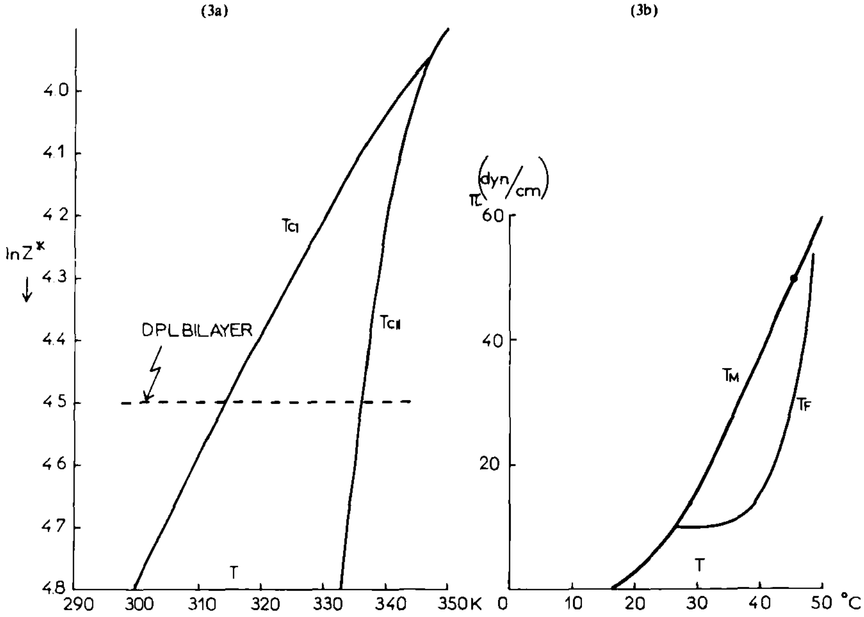

FIGURE 3 (a) Phase diagram for DPL membrane calculated with the values $\alpha = 0.53$ and $C = 10{,}400$ cal/mole. The ordinate represents the relative lateral pressure, which is at the level of the broken line in DPL bilayer. (b) Phase diagram for DPL monolayer from the experiment by Albrecht *et al.*; T_M: the main transition temperature, T_F: the secondary transition temperature, π: the lateral pressure.

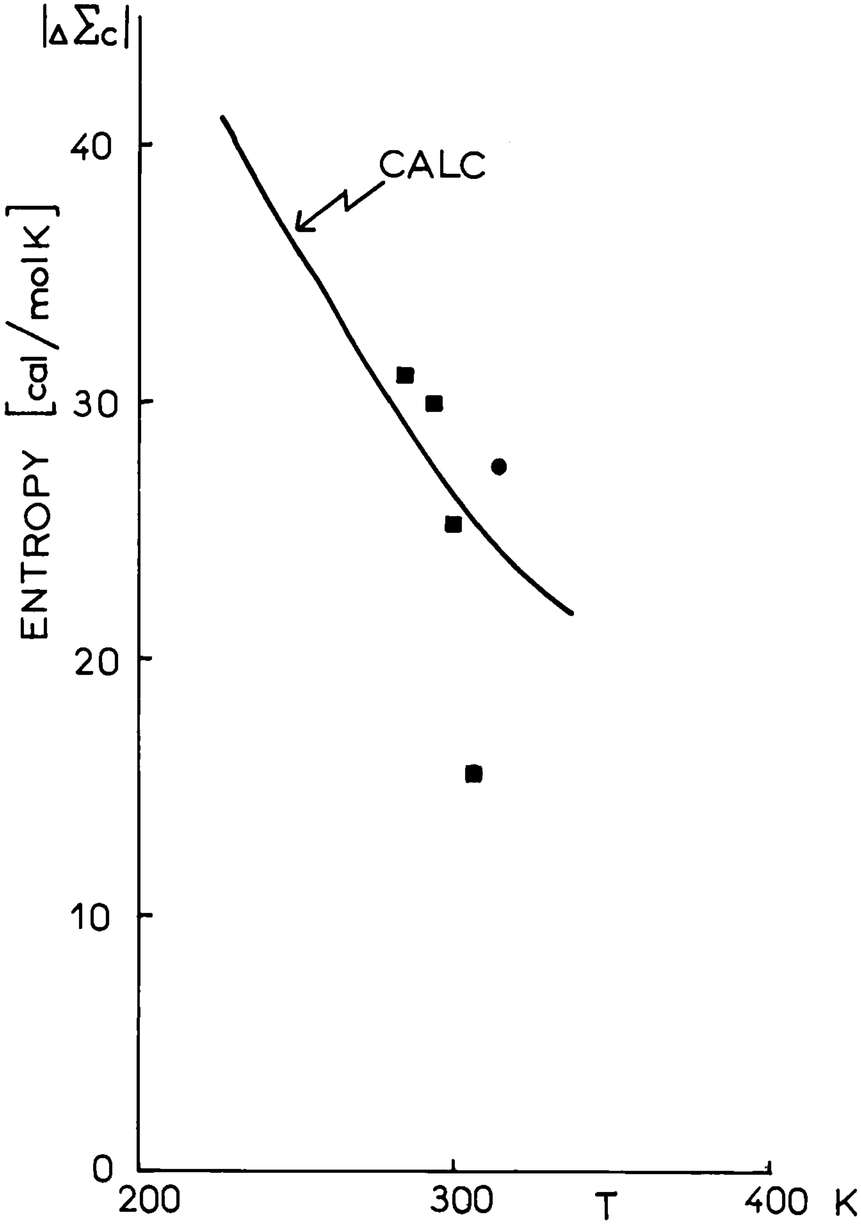

FIGURE 4 Latent entropies along the main transition temperature on the temperature-pressure plane in Figure 3, calculated from the values $\alpha = 0.53$ and $C = 10{,}400$ cal/mole. Experimental values are also shown by closed squares and circle for mono- and bi-layers, respectively.

and $z^*(0)$ is equal to z^* defined by (13). In Figure 3(a), the result of calculation of temperature vs $\ln z^*$ diagram for DPL membrane is shown in the case of the parameters $\alpha = 0.53$ and $C = 10{,}400$ cal/mole. This can be read as the temperature vs pressure phase diagram, by consulting the relation (18). A considerable similarity can be found between Figures 3(a) and 3(b) except at very low pressures. The transition temperature T_{cI} is surely an increasing function of pressure, whereas T_{cII} hardly depends on pressure. This result is quite analogous to the tendency of the temperatures T_M and T_F in the observation. The theoretical temperatures T_{cI} and T_{cII} join to each other at a sufficiently high pressure, similarly to the observed temperatures T_M and T_F. The entropy change at the main transition calculated along the T_{cI} line in Figure 3(a) is shown in Figure 4, with the experimental points due to Albrecht *et al.* According to Albrecht *et al.*, the main transition in the monolayer reduces to the second order one at pressures higher than a critical pressure, which is nearly 50 dyne/cm and indicated by an open circle in Figure 3(b).

(3) DOL bilayer

The values of α, z^* and C are similarly estimated from experimental values, $T_{cI} = 251$°k, $\Delta\Sigma_{cI} = 30.3$ cal/mole °K[5] and $T_{cII} = 301$°K by Furuya and Mitsui. The result for α and z^* are indicated in Figure 1 by a closed triangle numbered 18*; the length of the chains of DOL molecule are equal to 18, including one double C—C bond. The coupling constant C is estimated as 9,000 cal/mole, which is rather smaller than (15) for DPL which is shorter than DOL. This corresponds to an experimentally well-known fact that the main transition temperature for bilayer of lipids with unsaturated bonds are considerably lower than those without unsaturations.

4 EXISTENCE OF THE SECONDARY TRANSITION

If we simply assume the coupling constant C is proportional to the chain length n, we can obtain from (15)

$$C = 650\text{n cal/mole}, \tag{20}$$

which is near to the estimation by Marčelja[6] from the heat of freezing of polyethylene ($C = 680$n cal/mole). By making use of the coupling constants thus estimated and experimental data of T_{cI} and $\Delta\Sigma_{cI}$, we can determine the parameters α and z^* for some lipids: e.g. DML (dimyristoyl lecithin, $n = 14$), DSL (distearoyl lecithin, $n = 18$) and DBL (dibehenoyl lecithin, $n = 22$). The results are indicated in Figure 1 by closed circles with the respective numbers of C—C bonds. As all of these fall in the region (III), the secondary transition can be expected to occur in all these substances too, though such a transition has not been observed yet for these lipids. The results of calculation of the transition temperatures T_{cI} and T_{cII} and the latent entropies $\Delta\Sigma_{cI}$ and $\Delta\Sigma_{cII}$ are shown as functions of bond numbers of the chains, in comparison with the experimental data in Figures 5(a) and 5(b). According to the results shown in Figure 1 and Figure 5(a), we can expect the successive transition in lipid membranes with chain length $n \geqq 16$: e.g. DPL, DSL, DBL and so on, whereas we cannot in the case $n < 14$. This estimate is consistent with Marčelja's results,[6] which are all for the case $n < 14$. He calculated for the chain length $n = 9, 10, 11, 12$, and found no other transitions than a main transition. The case $n = 14$ (DML) near the border line in Figure 1 is quite uncertain, although the present calculation indicates the case. The study of the behavior of DSL and DBL are desired at temperatures higher than the main transition point to examine the validity of the present model.

Lastly, we discuss on our estimate of z^*, which is necessarily smaller than z given by (11) for the free molecule. Our estimates of z^* and z for various lipid bilayers are shown in Table 1. The values of z have been already calculated by means of the rotational isomeric state approximation in the previous paper.[3] From these values we can estimate z_1 and z_2 separately: for example, $z_1 = 22.9$

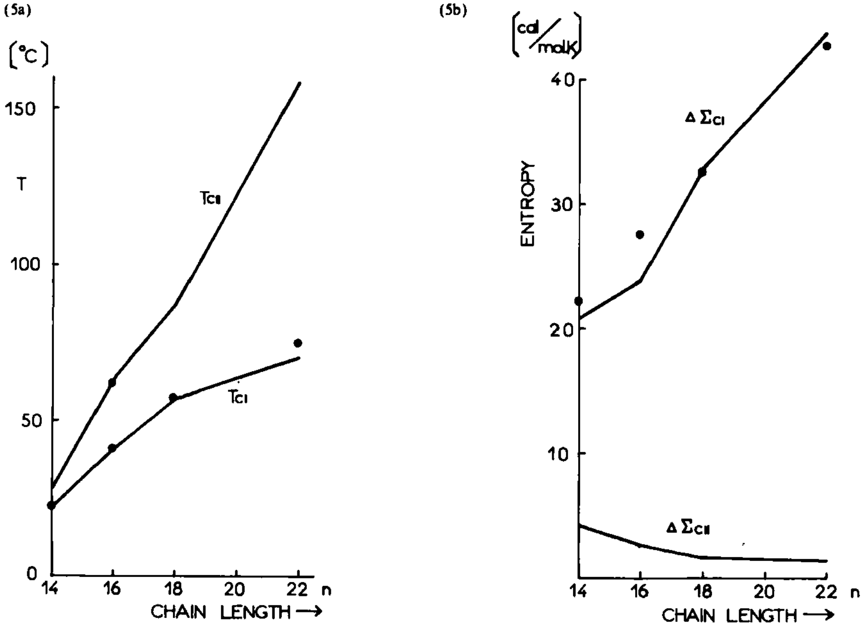

FIGURE 5 (a) Transition temperatures calculated for lipid bilayers DML ($n = 14$), DPL ($n = 16$), DSL ($n = 18$) and DBL ($n = 22$), compared with the experimental values indicated by closed circles.[1,5] (b) Latent entropies calculated for lipid bilayers DML ($n = 14$), DPL ($n = 16$), DSL ($n = 18$) and DBL ($n = 22$), in comparison with the experimental values.[5]

TABLE I

Estimations of α, z^* and z for several lipid bilayers at the main transition temperature T_{cl}.

Lipid	DML	DPL	DSL	DBL	DOL
Number of bonds, *n*	14	16	18	22	18.*
α	0.53	0.53	0.52	0.52	0.52
z^*	60	90	145	430	100
z	576	2,082	7,694	88,690	506

and $z_2 = 2{,}060$ for DPL. By making use of these values and assuming the energy required to produce a gauche bond in hydrocarbon chain to be 500 cal/mole, we can roughly estimate that the DPL molecule has 1.5 and 5.2 gauche bonds per chain in the average in the longer and shorter conformations, respectively. As these numbers seem to be reasonable, the present estimation of z^* can be considered to be plausible. It is noted that $\ln z^*$ is nearly proportional to the chain length n, whereas α scarcely depends on n.

References

1. K. Furuya and T. Mitsui, *J. Phys. Soc. Jpn.*, **46**, 611 (1979).
2. O. Albrecht, H. Gruler and E. Sackmann, *J. de Phys.*, **39**, 301 (1978).
3. H. Kimura and H. Nakano, *J. Phys. Soc. Jpn.*, **43**, 1477 (1977); *ibid.* **46**, 1695 (1979).
4. H. Nakano and M. Hattori, *Prog. Theor. Phys.*, **49**, 1752 (1973). The application to the problem quite similar to the present is given in H. Kimura, *J. Phys. Soc. Jpn.*, **36**, 1280 (1974); *ibid.* **37**, 1204 (1974). Many applications are in the followings: M. Ueda and H. Nakano, *J. Phys. Soc. Jpn.*, **46**, 1701 (1979), and articles cited therein as Ref. 4).
5. D. Chapman, Biological Membrane (Academic Press, London, 1973), ed. D. Chapman and D. F. H. Wallach, Chap. 2, p. 93.
6. S. Marčelja, *J. Chem. Phys.*, **60**, 3599 (1974); *Biochim. Biophys. Acta*, **367**, 165 (1974).