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Static and dynamic calorimetric studies on the three kinds of phase transition in the systems of L- and DL-dipalmitoylphosphatidylcholine/water

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Thermodynamic studies on the phase transitions of two systems composed of L- or DL-dipalmitoylphosphatidylcholine (DPPC) and water were performed by using the static adiabatic calorimeter, in addition to the dynamic differential scanning calorimeter (DSC) usually used in previous investigations, focusing our attention in particular on the subtransition phenomenon. The static method revealed that, compared with the L-DPPC system, the DL-DPPC system shows smaller transition entropies (ΔS) and enthalpies (ΔH) associated with the sub- and pretransitions, indicative of the heavy dependence on the main transition of the appearance of the liquid crystalline, L_α, phase. In particular, for the subtransition of the L-DPPC system, about 15-fold larger thermodynamic values were found as compared to those obtained for the DL-DPPC system, and a total of 145 h was required to complete this transition. This behavior of the L-DPPC system observed by using thermal equilibrium measurements demonstrated that the extremely low rate of the subtransition may be ascribed to the interaction between the L-DPPC and water molecules.

Introduction

It has been generally accepted that in the presence of water dipalmitoyl phosphatidylcholine (DPPC), one of the important constituents of cell membranes, undergoes two successive phase changes, which are called pretransition and main transition, respectively. Quite recently, Sturtevant et al. [1] revealed from dynamic thermal measurement of the L-type DPPC/water system by using the differential scanning calorimeter (DSC) that there exists a third phase transition at a temperature below that of the pretransition, which occurs after annealing treatment of a sample at around 0°C for 4 days. They also pointed out that this so-called 'sub-transition' is characterized by the kinetic nature, that is, the transition temperature as well as the peak width depend on the heating rate. Hereafter, conformational studies on the structural change of molecules associated with the subtransition phenomenon were carried out by several workers [2–4].

In order to clarify the kinetic nature of the subtransition in more detail, we carried out, in the present study, static thermal measurements using the adiabatic calorimeter for two samples of the DL-DPPC/water system, in addition to the L-DPPC/water system, which had been the object of all previous studies on the subtransition. Based on the heat capacity data obtained in this study, we investigated the nature of the subtransition concerning the difference in the method of measurement, static or dynamic, and the difference in the molecular configuration, DL- or L-DPPC.

Experimental

Samples

DL- and L-DPPC were obtained from Sigma Chemical Co., and were purified according to the procedure reported previously [5]. The purified DPPC was completely dehydrated under high vacuum at a temperature above its main transition temperature, in the same manner as reported previously [5]. The anhydrous DPPC thus obtained and the distilled water, both having controlled weights, were transferred into the calorimeter cell in a dry box filled with dry N_2 gas, and the cell was then sealed. Two samples of the DL-DPPC/water and L-DPPC/water systems were prepared with a water contents of 97.24 g% (DL-DPPC, 0.4733 g; H_2O, 16.6991 g) and 97.58 g% (L-DPPC, 0.4556 g; H_2O, 18.3757 g), respectively. Before the calorimetric measurements, the samples were preheated up to about 60°C in order to assure homogeneous mixing, after which they were cooled down to about 0°C, and they were annealed at this temperature for about 14 days.

Heat capacity measurements

The calorimeter used for the heat capacity measurements was of a standard adiabatic type with a can for the refrigerant [6]. Adiabatic temperature control was carried out by a five-channel automatic temperature controller. A platinum resistance thermometer (Tinsley, 5187L) and A.C. resistance bridge (Tinsley, 5840) were used for temperature measurements. The temperature scale was based on IPTS-68. Electric energy supplied to the sample cell was measured by using the digital multimeter (Takeda Riken, TR-6656). The sample cell, with a volume of 19 cm^3, was made of gold-plated copper and the platinum resistance thermometer was inserted into the cell through the sheath, around which KARMA wire (Driver Harris) was wound in order to supply the electric energy for the sample. After switching off the electric energy, it took 5–10 min to attain the thermal equilibration of the sample in a temperature region remote from that of the phase transition.

Results

Static heat capacity measurements

Figs. 1 and 2 show the heat capacity at saturated vapor pressure, C_s, (per gram of water + DPPC) versus temperature curves for the L-DPPC/water and DL-DPPC/water systems, respectively. Both curves exhibit successively three kinds of anomaly, which correspond to the so-called sub-, pre- and main transitions, with increasing temperature. However, the comparison of both curves demonstrates remarkable differences in the mode of these phase transitions. The most striking difference appears in the subtransition phenomenon, where the peak area of the L-DPPC system is considerably larger than that of the DL-DPPC one. Furthermore, the peaks of the L-DPPC system are clearly isolated from each other, showing a sharply distinct behavior. While the peaks of the DL-DPPC system are different from those of the L-DPPC system, the pretransition peak of the DL-DPPC

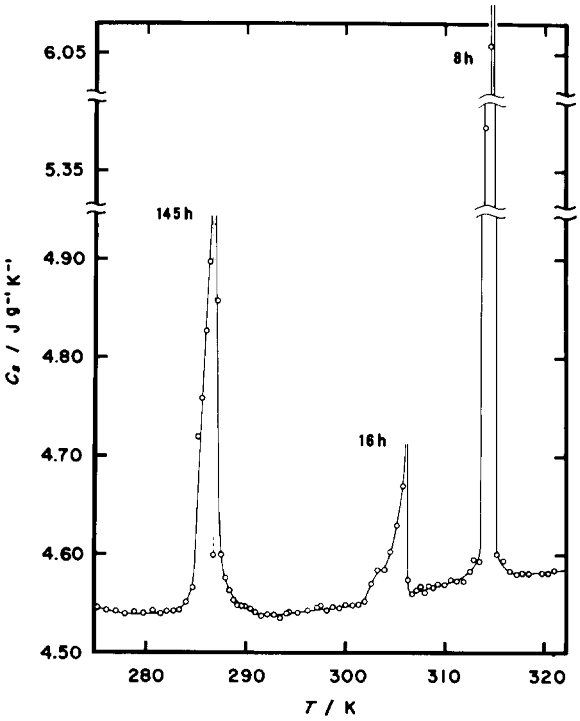

Fig. 1. Heat capacity curve of the L-DPPC/water system at a water content of 97.58 g%. Each figure attached at the transition points expresses the time required for the completion of the phase change.

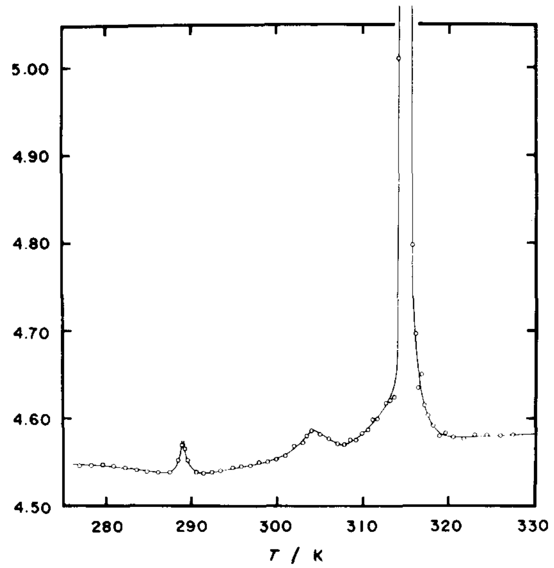

Fig. 2. Heat capacity curve of the DL-DPPC/water system at a water content of 97.24 g%.

system shows a rather gradual rise and descent and is overlapped by the skirt of the following main transition peak, which is much broader than that of the L-DPPC system. In order to clarify these differences between the two systems, each of the excess heat capacities associated with the three kidns of phase transition was separated by subtracting from the total heat capacity the normal one, which is given by joining smoothly the heat capacity curve below and above the transition temperature regions. By a numerical integration of the excess heat capacity thus obtained, the excess enthalpies, ΔH_t, accompanying these phase transitions were calculated. A plot of the excess enthalpy (per gram of water + DPPC) against the temperature is shown in Figs. 3 and 4 for the L-DPPC and DL-DPPC systems, respectively. In addition, the results of the thermodynamic quantities associated with these phase transitions are summarized in Table I, where the values of apparent enthalpy and entropy per mole of L- and DL-DPPC are shown. There, each transition temperature was determined as the one at which the heat capacity reached its maximum value.

As shown in Fig. 3, the L-DPPC system shows stepwise, abrupt increases of the excess enthalpy,

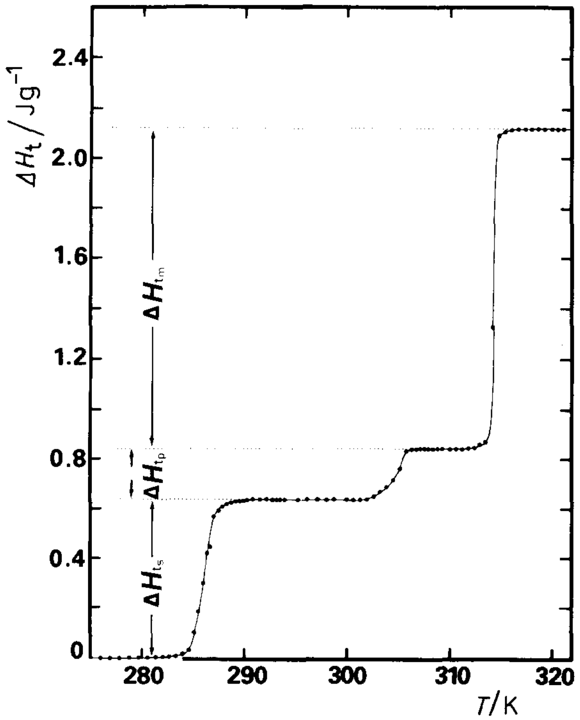

Fig. 3. A plot of excess enthalpies associated with three kinds of phase transition against the temperature of the L-DPPC/water system at a water content of 97.58 g%.

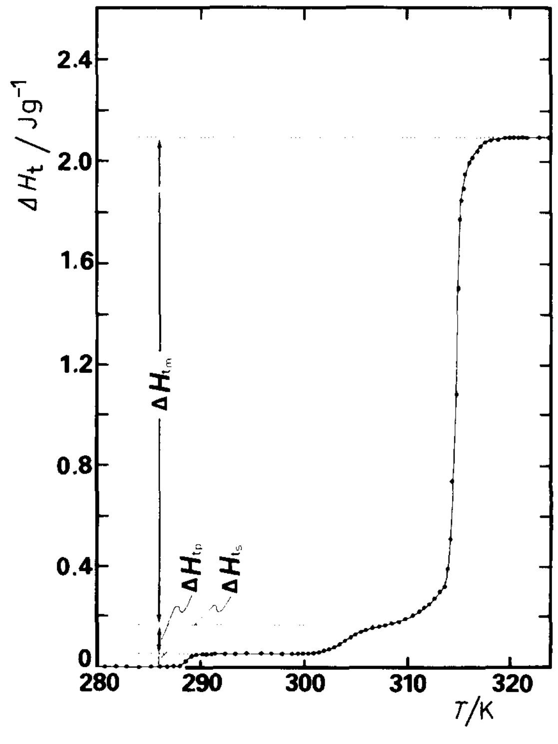

Fig. 4. Excess enthalpy curve of the DL-DPPC/water system at a water content of 97.24 g%.

TABLE I

COMPARISON OF THE CALORIMETRIC DATA OF THREE KINDS OF PHASE TRANSITION BETWEEN THE L-DPPC/WATER AND THE DL-DPPC/WATER SYSTEMS

Sample	Subtransition			Pretransition			Main transition		
	T_s (K)	ΔH_s ($kJ \cdot mol^{-1}$)	ΔS_s ($J \cdot K^{-1} \cdot mol^{-1}$)	T_p (K)	ΔH_p ($kJ \cdot mol^{-1}$)	ΔS_p ($J \cdot K^{-1} \cdot mol^{-1}$)	T_m (K)	ΔH_m ($kJ \cdot mol^{-1}$)	ΔS_m ($J \cdot K^{-1} \cdot mol^{-1}$)
DL-DPPC/water (97.244 g%)	288.8	1.27	4.41	304.2	2.92	9.61	314.9	51.54	163.8
L-DPPC/water (97.580 g%)	286.5	19.47	67.95	306.0	6.18	20.27	314.9	38.81	123.5

that is, ΔH_{t_s}, ΔH_{t_p} and ΔH_{t_m} at each transition point. In contrast to this behavior, the excess enthalpy of the DL-DPPC system increases with a gentle slope, in particular, in the course of the pre- to main transitions, as shown in Fig. 4. These findings indicate that the phase transitions of the DL-DPPC system are less cooperative than those of the L-DPPC one. Furthermore, in the DL-DPPC system, the enthalpy change (ΔH_{t_m}) associated with the main transition accounts for more than 90% of the total excess enthalpy, which corresponds to the summation of ΔH_{t_s}, ΔH_{t_p} and ΔH_{t_m}. This large portion reveals that the DL-DPPC system relies mostly on the main transition for the appearance of the L_α (liquid crystalline) phase. Corresponding with this result, the enthalpy change (ΔH_{t_s}) of the subtransition in this system is extremely small and accounts for less than 3% of the total excess enthalpy. On the other hands, the subtransition enthalpy of the L-DPPC system exhibits a large portion of approx. 30%. This remarkable difference in the subtransition phenomenon between both systems becomes more apparent in Table I. The apparent molar enthalpy and entropy changes due to the subtransition of the L-DPPC system are about 15-times larger than those of the DL-DPPC system. In connection with this result, the adiabatic calorimetry provided, furthermore, the important information that at each stage of the subtransition process for the L-DPPC system the temperature drift is quite large and, in the longest case, requires 24 h at the maximum point of the subtransition peak. That is, a total of 145 h is required to complete this transition for the L-DPPC system, while for the DL-DPPC system it takes only 4 h. These facts seem to prove that the subtransition of the L-DPPC system is an extremely low-rate process, which reflects the necessity of a quite long incubation to attain the stable low-temperature phase.

Dynamic DSC measurements

Parallel to the static thermal measurements by the adiabatic calorimeter mentioned above, dynamic DSC measurements were performed in order to study the effects of different kinetic natures and of differences in molecular configuration on the subtransition in the L-DPPC and DL-DPPC systems. Fig. 5 shows the DSC curves around the subtransition temperature for both annealed DL- and L-samples at a heating rate of 0.5 K/min. In this figure, the L-DPPC system gives a large endothermic peak due to the subtransition, and the

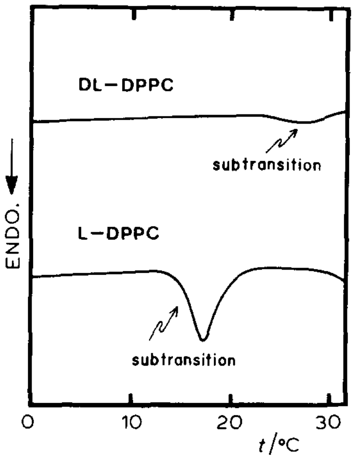

Fig. 5. DSC curves around the subtransition temperature of the L-DPPC/water and DL-DPPC/water samples (both with a water content of approx. 98 g%) at a heating rate of 0.5 K/min.

transition enthalpy ($\Delta H = 14.0$ kJ/mol) as well as the transition temperature are in good agreement with the results ($\Delta H = 13.5$ kJ/mol) obtained by Sturtevant et al. [1]. The DL-DPPC system exhibits a faint peak at a temperature higher than that of the L-DPPC system, which is assumed to correspond to the subtransition. The dynamic method also indicates a remarkable difference in the subtransition between the L-DPPC and DL-DPPC systems due to their molecular configurations.

Discussion

Difference in the subtransition phenomenon of the L-DPPC system between the static and dynamic methods

In this study, both the static and dynamic thermal methods were able to detect the difference in the subtransition phenomenon between the L-DPPC and DL-DPPC systems. However, focusing our attention on the thermal data concerning the subtransition of the L-DPPC system, we note that the calorimetric data shown in Table I are 1.4-times larger than the DSC data, indicating an apparent discrepancy due to the difference in the measuring method, static or dynamic. As is well known, the static calorimetric method provides the absolute values of the heat capacity at the thermal equilibrium state of a sample. In our present study, it has proved the subtransition of the L-DPPC system to be really an extremely low rate process, requiring a total of 145 h for the completion of the transition. On the other hand, the DSC method requires only about 30 min to pass through the subtransition temperature region. Accordingly, the lower values obtained by the dynamic method may be looked upon as the result of partial non-equilibrium nature caused by such a low-rate process.

In connection with this result, in particular concerning the true equilibrium measurements, we should like to refer to the result of dilatometric studies of the subtransition in the L-DPPC system by Nagle et al. [7], which is shown in Fig. 6. In this figure, their dilatometric curve indicated by black circles is compared with our calorimetric one shown by open circles. They have confirmed that at each point in the subtransition region, it took 10–14 h to attain the equilibrium state, while requiring only 15–20 min at each point far removed from this transition. Thus, their dilatometric equilibrium measurement also indicates that the volumetric equilibration of the sample proceeds very slowly in the subtransition region, which is similar to the behavior of temperature equilibration observed by our calorimetric equilibrium measurement. Furthermore, comparison of the calorimetric and dilatometric curves shown in Fig. 6 gives excellent agreement in the starting point as well as the end point of the subtransition temperature region and in addition, the midpoint temperature (286.6 K) found by the dilatometry is the same as that determined by our calorimetry as shown in Table I. These agreements between the calorimetric and dilatometric results have proved that the equilibrium measurements are essential to clarify the nature of the subtransition phenomenon.

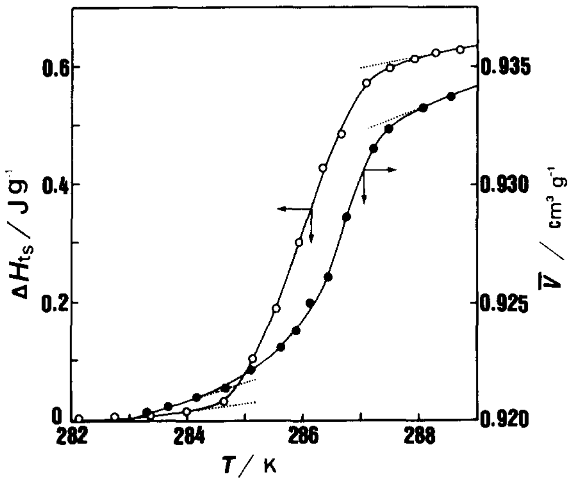

Fig. 6. Comparison of our equilibrium data on the process of subtransition of the L-DPPC/water system with the dilatometric data obtained by Nagle et al. [7]. $\bar{V}$, specific volume.

Kinetic nature of the subtransition in the L-DPPC system

Next, we should like to proceed to a discussion of the kinetic nature of the subtransition in the L-DPPC system. The presence of the subtransition implies that in the temperature region below the subtransition there exists a stable-form alternative to the gel $L_{\beta'}$ phase, which exists as the supercooled, metastable state in this temperature region. Structural investigations of the stable, low-temperature phase below the subtransition temperature in the L-DPPC system have been carried out by several workers [2–4]. In their papers, the concordant result is that the dehydration phenomena of the polar head groups as well as of the carbonyl hydrogen bonding groups take place in the conversion process of the metastable $L_{\beta'}$ phase into the stable phase during a long period of annealing treatment. Summarizing these results, it may be predicted that the stable, low-temperature form as the result of dehydration corresponds to a highly ordered structure, close to a bilayer crystal, due to the strengthened intermolecular interactions of the polar head groups and of the stacking hydrocarbon chains, which is induced by the tightening of the polar head groups. The phase transition from a more dehydrated state of the low temperature phase to the $L_{\beta'}$ gel phase in the heating direction would be expected to be accompanied by an increase of hydration of the polar head and carbonyl groups. Here, it is worth noting that water has a predominant role in the subtransition phenomenon, similar to that in the pretransition previously reported to us *. Thus, an extremely low rate of the subtransition in the L-DPPC system may be attributed to the interaction between the water and L-DPPC molecules. Presumably, such an interaction consists of the swelling action of water, which induces a principal configurational change of the polar parts (their disordered arrangements), followed by an additional configurational change of the hydrocarbon chains (their lateral, loosed packing). That is, these cooperational processes, caused by the combined action of the water, zwitter ion, hydrogen bonding polar groups and hydrocarbon chains at the subtransition, proceed in harmony with each other and very sluggishly, as revealed by the equilibrium measurements by us and Nagle et al. [7]. Because of the very highly ordered packing in the lowest temperature phase, the subtransition requires such a long time to be performed, compared to the pretransition [1,8].

Difference in the mode of phase transitions between the L-DPPC and DL-DPPC systems

We should now like to explain the differences in the mode of phase transitions, in particular those between the subtransitions of the L- and that of the DL-DPPC system. For this purpose, Fig. 7 shows the entropy-histograms obtained from the calorimetric data shown in Table I, by assuming that the entropy values of the L_α liquid crystalline phase between both systems are the same. The total increments of the apparent entropy due to the three kidns of phase transition are 211.7 and 177.8 J/kmol for the L- and DL-DPPC systems, respectively. The gap between these entropy values indicates a more ordered packing structure of the low-temperature phase for the L-DPPC system, which may be ascribed to the stronger intermolecular forces operating between the same molecular species in L-DPPC, compared with the DL-DPPC system composed of the two species, L- and D-DPPC molecules. On the other hand, both the $L_{\beta'}$ and $P_{\beta'}$ phases of the L-DPPC system are, respectively, situated at higher entropy states than those of the DL-DPPC system, contrary to the case of the low-temperature crystal phase. In other words, compared with the L-DPPC system, the DL-DPPC system shows smaller entropy changes at the sub- and pretransitions, but a larger one at the main transition, indicating the heavy dependence on the

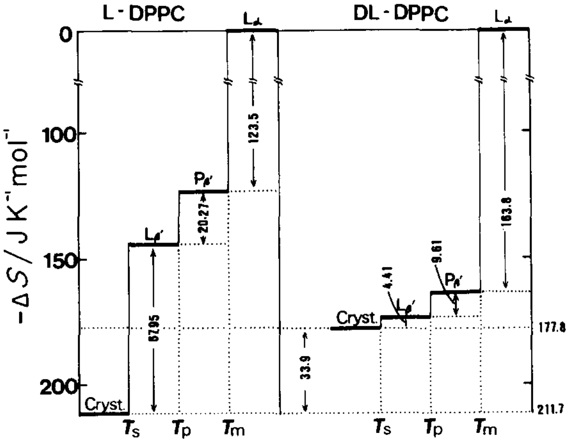

Fig. 7. Entropy histograms explaining the differences in entropy changes associated with the sub-, pre- and main transitions between the L-DPPC/water and DL-DPPC/water systems, assuming the same entropy values for both L_α states.

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* In our previous paper [5], it has been revealed from thermoanalytical investigations that the free water coexisting with the $L_{\beta'}$ phase is interposed between the bilayers of the DPPC molecules at the elevated pretransition temperature, which has also been proved by the sudden increase in the thickness of the water layer at that temperature, as found by Mitsui et al. [9]. This finding indicates a predominant role of water in the appearance of the pretransition.

main transition in the course of reaching the L_α phase, which has been shown in Figs. 3 and 4. These facts suggest that the sub- and pretransitions of the system composed of two molecular species of DL-DPPC are less cooperative phenomena with respect to the interaction between water and DPPC molecules than those of one and the same molecular species of L-DPPC. Consequently, the DL-DPPC system cannot obtain enough entropy increments at the sub- and pretransitions, while this entropy loss is compensated for by the large entropy increment at the main transition.

References

1 Chen, S.C., Sturtevant, J.M. and Gaffeny, B.J. (1980) Proc. Natl. Acad. Sci. USA 77, 5060–5063

2 Füldner, H.H. (1981) Biochemistry 20, 5707–5710

3 Ruocco, M.J. and Shipley, G.G. (1982) Biochim. Biophys. Acta 684, 59–66

4 Cameron, D.G. and Mantsch, H.H. (1982) Boiphys. J. 38, 175–184

5 Kodama, M., Ogawa, Y., Kuwabara, M. and Seki, S. (1982) in Ions and Molecules in Solution, Studies in Physical and Theoretical Chemistry, Vol. 27 (Tanaka, T., Oktaki, H. and Tamamushi, R., eds.), pp. 449–456, Elsevier, Amsterdam

6 Tsuji, K., Sorai, M., Suga, H. and Seki, S. (1979) Mol. Cryst. Liq. Cryst. 55, 71–88

7 Nagle, J.F., Wilkinson, D.A. (1982) Biochemistry 21, 3817–3821

8 Lentz, B.R., Freire, E. and Biltonen, R.L. (1978) Biochemistry 17, 4475–4480

9 Inoko, Y. and Mitsui, T. (1978) J. Phys. Soc. Jap. 44, 1918–1924