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CHANGES IN THERMAL PHASE TRANSITION OF VARIOUS MEMBRANES DURING TEMPERATURE ACCLIMATION IN *TETRAHYMENA*

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Changes in the thermal phase transition temperature of membrane lipids were studied by X-ray wide-angle diffraction during adaptation of *Tetrahymena pyriformis* to a lower growth temperature. After a shift in growth temperature from 39 to 15°C, the phase transition temperature was lowered gradually in microsomal and pellicular phospholipids, whereas that in mitochondrial phospholipids was unchanged for 10 h after the temperature shift. Only a small decrease in the transition temperature of mitochondrial phospholipids was observed, even after 24 h following the shift. Transition temperatures of microsomal, pellicular and mitochondrial phospholipids reached the growth temperature (15°C) about 6, 10 and 24 h after the temperature shift. The temperature dependence of the solid phase in membrane phospholipids was estimated from the 4.2 Å peak of the X-ray diffraction pattern. In the case of the phospholipids extracted from cells grown at 39°C, the solid phase was increased upon lowering temperature in a similar manner in all three membrane fractions: mitochondria, pellicles and microsomes. However, in the case of the phospholipids from cells exposed to a lower growth temperature (15°C) for 10 h, the increase in the solid phase was significantly smaller in mitochondrial phospholipids than in two other membrane fractions. The difference in the thermal behaviour of mitochondrial lipid from pellicular and microsomal lipids is discussed in terms of phase transition and phase separation.

Introduction

It is well known that the functions of membranous proteins are closely related to the physical states of membrane lipids [1,2]. In response to external stimuli such as temperature change, various microorganisms strive to maintain the optimal physical state of their membranes through modification of lipid composition [3,4]. For example, a unicellular eucaryote, *Tetrahymena pyriformis*, has been proved to be a useful system in which to survey the molecular mechanism of temperature adaptation. When *Tetraahymena* cells were exposed to a lower growth temperature, they performed modification of membrane lipid composition, and the appropriate state of membranes was recovered by the adaptation mechanism [5]. In the case of *T. pyriformis* strain NT-1, not only the molecular species of the fatty acyl chains but also the composition of phospholipid headgroup were altered by acclimation to a lower growth temperature [6]. In the process of acclimation, a rapid alteration of lipid composition occurred in the fatty acyl chains of phospholipids following the temperature shift. The relative proportion of phosphatidylethanolamine and 2-aminoethylphosphonolipid began to change in a counterbalanced fashion [6]. Hence it has been suggested that fatty acid composition would play a major role in controlling the physical state of membranes in this

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organism. In order to clarify the relative roles of the fatty acyl chain and the phospholipid headgroup in membranes, the changes in physical states of different membrane fractions during cold acclimation were examined in detail.

Yamauchi et al. [7] have previously shown that the ratio of total unsaturated to saturated fatty acid content increased progressively after temperature shift in mitochondrial, microsomal and pellicular membranes, while the proportional composition of phospholipid headgroup was found to remain unchanged. Fluidities of microsomal and pellicular lipids decreased in response to the change in unsaturation, but the fluidity of the mitochondrial lipid was quite constant within 10 h after the temperature shift. This characteristic behaviour of mitochondrial lipids was ascribed to cardiolipin, which is present exclusively in mitochondrial membranes.

In the present report, phospholipids were extracted from mitochondria, microsomes and pellicles of a thermotolerant strain, *T. pyriformis* NT-1, at different stages during temperature acclimation, and the solid-to-liquid phase transition temperatures of their phospholipids were examined by the wide-angle X-ray diffraction method. Comparison of lipid composition and fluidity was made in order to elucidate the role of and the difference in the physical state of various membranes during temperature acclimation.

Materials and Methods

Cell growth

Cells of a thermotolerant strain of *T. pyriformis*, NT-1, were grown in an enriched medium at 39°C. The cells in logarithmic growth phase were used in all experiments. All procedures for the temperature shift-down were carried out as discribed in a previous paper [6]. For temperature shift experiments, cells grown at 39°C in 200 ml medium to a cell density of approx. $(2.0–3.0) \cdot 10^5$ cells/ml were cooled to 15°C over a period of 30 min by swirling the flask in an ice-water slurry. The rate of cooling was essentially linear (0.8 K/min) and the culture temperature was monitored by placing a sterile thermometer directly into the medium. After cells had been cooled to 15°C, cell division did not occur for approx. 10 h.

Isolation of membrane fractions and extraction of membrane lipids

Various membrane fractions, mitochondria, pellicles and microsomes, were isolated according to the method of Nozawa and Thompson [8] using a phosphate buffer (0.2 M K_2HPO_4/0.2 M KH_2PO_4/3 mM EDTA/0.1 M NaCl (pH 7.2)). Lipids were extracted from individual membrane fractions by the method of Bligh and Dyer [9], and then phospholipids were separated from neutral lipids (mainly tetrahymanol) by silicic acid column chromatography [8]. The resulting lipids were stored in $CHCl_3/CH_3OH$ (6 : 1, v/v) at −20°C.

X-ray diffraction

Dried phospholipids in vacuo were hydrated by 1–2 μl of buffer (50 mM NaCl 10 mM Tris-HCl (pH 7.5) deoxygenated by nitrogen replacement) per mg of lipid. X-rays were obtained from Rigaku rotating anode microfocus generator RU-100 at 40 kV and 25 mA, and nickel-filtered CuK_α radiation (= 1.542 Å) was used. Diffracted X-rays were collected through a position-sensitive proportional counter (Rigaku Denki Co.) set at an angle of 1.20 rad to the direct beam in order reflections of our interest, and transmitted to and processed by PDP 11/34 minicomputer. Detailed procedures were described previously [10].

Results

Changes in phase transition temperature of membrane phospholipids during acclimation to a lower growth temperature

It was shown in a previous paper [10] that phospholipid dispersions of various membrane fractions *T. pyriformis* have given a typical pattern of wide-angle X-ray diffraction and a temperature dependence as observed with lipids obtained from nautral sources: the sharp 4.2 Å peak ($1/d = 0.24$ Å^{-1}) was increased and the broad 4.6 Å peak ($1/d = 0.22$ Å^{-1}) was concomitantly decreased on lowering temperature. Fig. 1 shows the X-ray diffraction patterns of microsomal phospholipids from the cells exposed to 15°C for 2 h after the shift. The sharp 4.2 Å peak was used as a measure of the solid phase, and the transition temperature, T_t, was defined as a temperature at which the solid phase disappears. Changes in transition tempera-

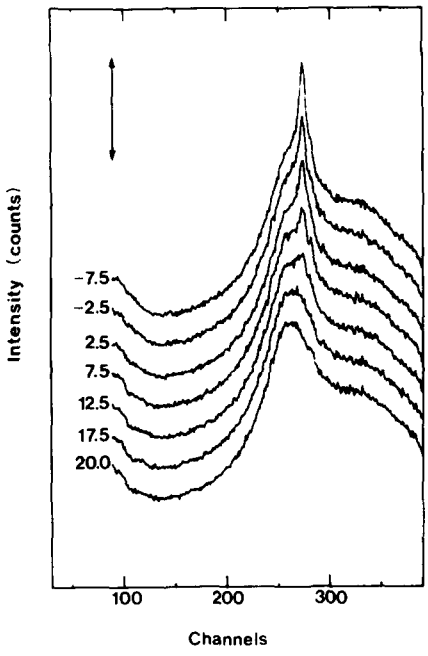

Fig. 1. Wide-angle X-ray diffraction patterns from microsomal phospholipids. The sample is from the cells of 2 h after the temperature shift. Abscissa: channel number. This corresponds to the position on the proportional counter. Ordinate: intensity. Patterns are shifted about 1600 counts from each other. The bar indicates 5000 counts. Figures at the left edge of each pattern are sample temperatures (°C).

tures during acclimation to a lower growth temperature are shown in Fig. 2. In pellicles and microsomes, T_f values gradually decreased in accordance with the changes in the ratio of unsaturated to saturated fatty acyl chain content as shown in Table II and Fig. 1 of Ref. 7. On the other hand, the T_f value of the mitochondrial fraction remained constant for 10 h after the temperature shift, despite changes in fatty acyl chain composition similar to those observed with two other membrane fractions [7]. Hence, in *Tetrahymena* cells grown at 39°C, the order of transition temperatures in these membrane fractions was as follows: pellicles > microsomes > mitochondria. However, 10 h after the temperature shift to 15°C, the T_f value of mitochondria surpassed that of microsomes because of the difference in the temperature dependence of the T_f values. The delayed response to the growth temperature change in mitochondria was also observed in membrane

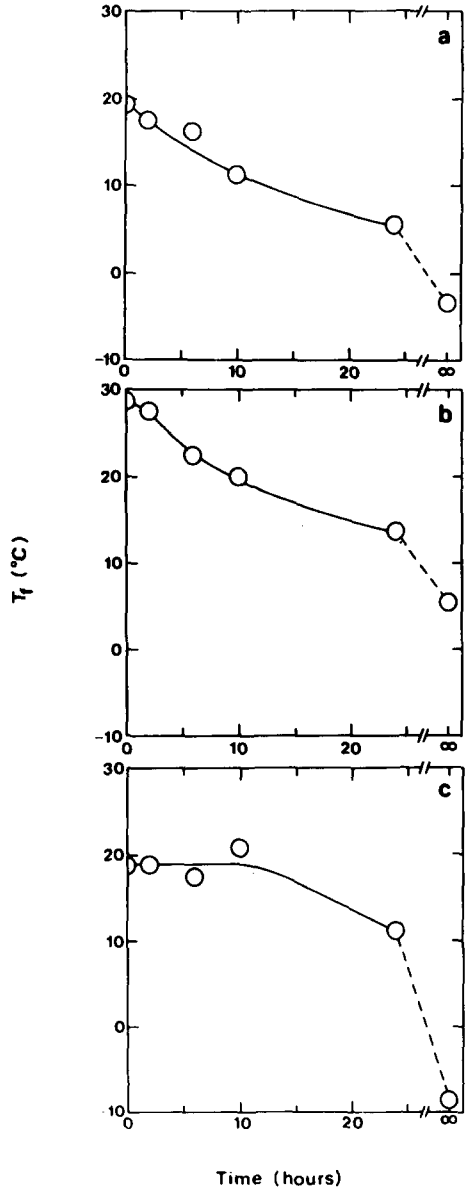

Fig. 2. Time-course of transition temperatures. Abscissa: time after the growth temperature change (h). ∞ is the value of fully acclimated cells. Ordinate: transition temperature (°C). (a) Microsomal phospholipids; (b) pellicular phospholipids; and (c) mitochondrial phospholipids.

fluidity measured by a spin probe. The membrane fluidity of mitochondrial phospholipids was kept at a constant value for 10 h after the temperature shift, irrespective of the changes in fatty acyl chain composition [7]. This correspondence between the fluidity change and the transition temperature

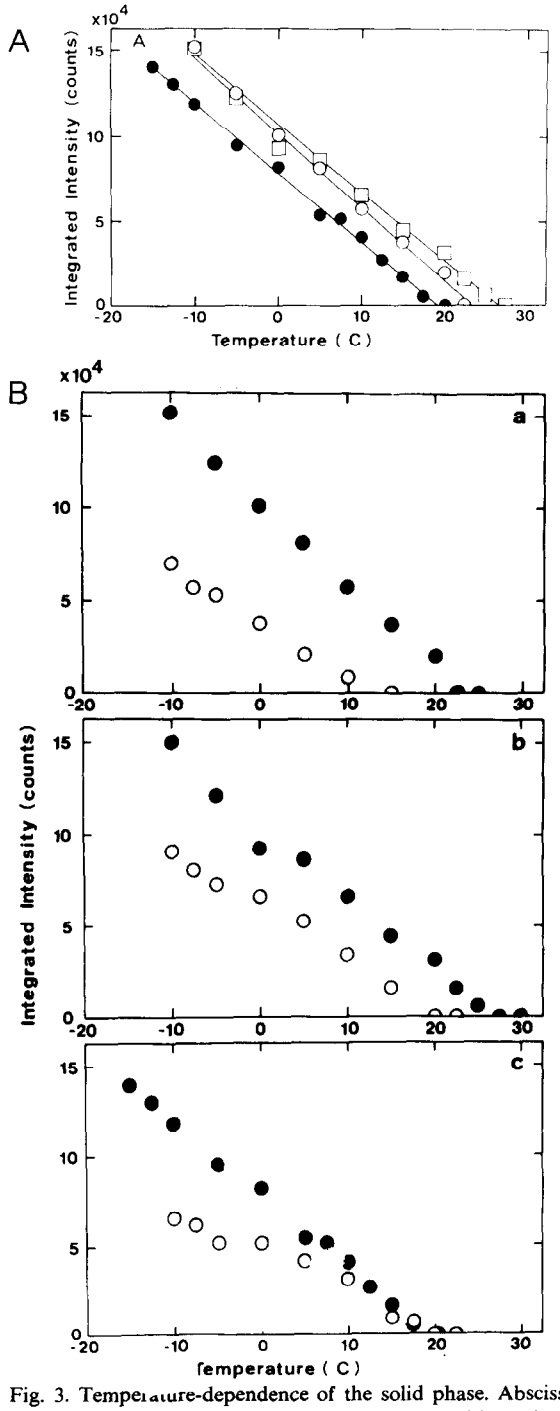

Fig. 3. Temperature-dependence of the solid phase. Abscissa: temperature of samples (°C). Ordinate; integrated intensity of the 4.2 Å peak (counts). Units for ordinate were normalized using a complete fluid phase pattern among different samples. (A) Before temperature shift. ○, microsomal phospholipid; □, pellicular phospholipids; and ●, mitochondrial phospholipids. (B) During temperature acclimation. ●, at zero time (before temperature shift); and ○, at 10 h after temperature shift. (a) Microsomes; (b) pellicles; and (c) mitochondria.

change is somewhat striking, since absolute values of order parameter in ESR experiments reflect rather the intrinsic mobility of the membrane lipids than the phase they are in, while X-ray diffraction can reveal the existence of respective phases but is insensitive to the absolute value of fluidity. One plausible explanation is that a finite amount of solid phase domain emerged in each membrane when exposed to a lower temperature, and that this solid domain was abolished by the modification of the fatty acyl chain composition thereafter. Increased unsaturation of phospholipids caused the solid phase to disappear and an increase in membrane fluidity. Abolition of the solid phase was detected by X-ray diffraction, and fluidity change was observed by the spin-labeling technique. In a previous paper [7], the characteristic response of mitochondrial phospholipid to cold acclimation was ascribed to the effect of cardiolipin. It was assumed that cardiolipin 'absorbed' remodelled lipids and formed a non-bilayer membrane structure (H_{II} phase). Therefore, the remaining lipid exhibited the same fluidity as did the initial lipid before the cold shift. This hypothetical concept is based on the phase separation between cardiolipin plus remodelled lipids and original lipids. In order to examine this possibility, changes in features of the phase transition were investigated.

Temperature dependence of the solid phase

The 4.2 Å peak profile was obtained from wide-angle diffraction patterns following the procedure described in a previous paper [10], by subtracting from the observed profiles parasitic scatterings, scatterings from the buffer solution and a broad 4.6 Å peak. Its integrated intensity was denoted as a measure of the solid phase. The temperature dependence of the formation of a solid phase in the phospholipids is shown in Fig. 3. Before subtraction of the 4.6 Å peak, patterns at the highest temperatures of every series (samples were normalized to each other and the normalization factor was applied to all patterns of the respective series. Hence, the relative intensities of different samples can be compared with each other, although the ordinate in Fig. 3 is in arbitrary units. In the case of the phospholipids extracted from cells grown at 39°C, the trend to an increase

in the size of the solid phase with decreasing temperature was observed in all three fractions (Fig. 3A). 10 h after the shift, pellicular and microsomal phospholipids showed no substantial change in the increase of solid phase but a shift of the transition to the lower temperatures. This indicates that decreases in the T_f in microsomes and pellicles after temperature shift are realized through a shift of the transition (Fig. 3B). On the other hand, mitochondrial lipids demonstrated an appreciable increase in solid phase as the temperature decreased, whereas the T_f was not altered. This may imply that the relative proportion of lipids taking part in the phase transition of mitochondrial lipids decreased in the temperature region between T_f and about 30 K below T_f. Such a phenomenon can be induced by (i) broadening of single transition or (ii) segregation of a domain of higher transition temperature in the fluid phase. A detailed analysis of the temperature dependence of the 4.2 Å peak shows that initial increases of solid phase around T_f are same in both curves, and the curve of 10 h after the temperature shift decays to lower temperatures. This specific feature of transition would not be expected in the case of a simple broadening of transition temperature. However, this was not observed. Therefore it is strongly suggested that the decrease in the proportion involved in inducing transition results from the segregation of different classes of lipid in mitochondrial lipids. Hence, these findings suggest that a large segregation emerges between the domain of lipids taking part in the phase transition around T_f and the other domain remaining as fluid phase in mitochondrial membranes.

Discussion

In response to temperature change, *Tetrahymena* cells modify their membrane lipid compositions to maintain the physical state of membrane within an optimal state [4]. In fact, our previous studies [5,6] on *T. pyriformis* have shown that exposure of *Tetrahymena* cells to a lower temperature resulted in an increase in the relative proportion of unsaturated fatty acids in membrane phospholipids; consequently, membrane fluidity increased and the thermal transition temperature of the membrane lipid decreased. Furthermore, dependent on the different unsaturation of phospholipid fatty acyl chains in different cellular organelles, differences of fluidity and transition temperature among different membrane fractions were maintained, regardless of the growth temperature.

The present results demonstrating changes in phase transition temperature during the course of temperature acclimation apparently agree with previous observations on the change in fluidity [7]: both the fluidity and the transition temperature of pellicular and microsomal lipids were gradually altered, whereas those mitochondrial lipids were not changed within 10 h after the temperature shift. But in such a complex system as lipids extracted from biomembranes, there is no a priori reason for changes in fluidity and transition temperature to be parallel, because different lipid mixtures generally may not have the same transition temperature, and vice versa. Furthermore, in the case of microsomal and pellicular lipids, a decrease in transition temperature occurred upon an increase in solid phase. This fact implies that (i) the previous measurement of fluidity reflects the fluidity of overall membranes, and (ii) fatty acid composition changes in such a manner that membrane fluidity and phase transition shift in parallel. In mitochondrial phospholipids, the slope of the temperature-dependence plot of solid phase was progressively reduced with increasing time after the temperature shift, which implies that the proportion of lipids taking part in the transition appreciably decreased in the temperature region examined in the present study.

As for the broadening of the phase transition, it was likely that the variety of lipid components induces gradual phase transitions over a wide range of temperature [1,11]. In the mitochondrial phospholipid, we observed a decreased proportion of lipids participating in the transition, which can be expected from the broadened transition. Consequently, we have attempted to estimate the variation in lipid composition which can cause broadening of transition as discussed above. The previous work has demonstrated that cardiolipin, most abundantly localized in mitochondria, is responsible for this specific behaviour of mitochondrial lipids. Indeed, our recent work has shown that cardiolipin interacts preferentially with phos-

pholipids containing a high proportion of unsaturated fatty acids [12]. The domain which does not participate in the transition observed in the present study should correspond to the area containing cardiolipin. Upon the acclimation to a lower temperature, *Tetrahymena* cells are thought to discriminate the solid phase in the membrane lipid bilayer in various organelles including mitochondria. It is then tempting to speculate that the function of this two-domain separation would be to keep a part of the membrane in the fluid-like state induced by cardiolipin for maintaining the solid phase. Recent studies using ^{31}P-NMR and freeze-fracture electron microscopy [13,14] have shown that cardiolipin has a tendency to induce the hexagonal II phase, and De Kruijff and Cullis [15] have proposed functional rôles of hexagonal II phase such as flip-flop, fusion and channel. Since fatty acids in hexagonal II phase cannot be assumed to produce the two-dimensional hexagonal lattice, which defines the solid phase (L_β phase), the domain which does not take part in transition may be the hexagonal II phase. In order to investigate the existence of the hexagonal II phase, further studies have been carried out in the presence and the absence of Ca^{2+}. The present results suggest that one of the functions of a constant transition temperature can be the emergence of an annular structure, such as in the hexagonal II phase, for immediate adaptation, leaving the other part in the solid phase, i.e., with low fluidity.

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