Thermotropic Fluid → Ordered "Discontinuous" Phase Separation in Microsomal Lipids of *Tetrahymena*. An X-Ray Diffraction Study†

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ABSTRACT: The thermotropism of total lipids extracted from microsomal membranes which were isolated from *Tetrahymena pyriformis* cells grown at either 18 or 28 °C was investigated by small- and wide-angle x-ray diffraction. Furthermore, the 18 °C lipids were chromatographically separated into different lipid fractions, i.e., total neutral lipids, total phospholipids, ethanolamine phosphoglycerides (PE), and PE-deficient phospholipids; these were studied as a function of temperature by small-angle x-ray diffraction. Our data show that total 18 and 28 °C lipids undergo a broad thermotropic fluid to ordered phase separation. At 27 °C, all the 18 °C lipids are in the fluid state, about 60% of which are transformed into the ordered state upon lowering the temperature to 0 °C; fluid and ordered lipids coexist in two separate lamellar phases. Remarkably, this phase separation reveals a discontinuity at ~10 and ~16 °C in the 18 and 28 °C lipids, respectively. Such a discontinuous separation also occurs in the 18 °C phospholipids. However, only one lamellar phase can be detected in the 18 °C PE-deficient phospholipids and the 18 °C PE; the Bragg spacings of the latter show a dramatic change at ~10 °C. Thus, the PE constituting ~55% of the total lipids is assumed to play a dominant role in the fluid to ordered "discontinuous" phase separation of total lipids. We suggest that the lipids which are transformed from the fluid into the ordered state above and below the discontinuity point differ in their percent distribution of PE. Finally, our present data support and extend our concept previously suggested for the thermotropic lipid clustering within *T. pyriformis* membranes [Wunderlich, F., Ronai, A., Speth, V., Seelig, J., and Blume, A. (1975), *Biochemistry 14*, 3730].

Temperature dramatically affects the structure and function of biomembranes. It is widely assumed that at physiological temperatures the bulk lipids within membranes are fluid (liquid-crystalline), whereas they become ordered (crystalline) upon cooling below a critical transition temperature. Such thermotropic liquid-crystalline to crystalline lipid phase transitions appear to induce changes in membrane-associated enzyme activities, including membrane transport processes (e.g., Esfahani et al., 1971; Linden et al., 1973; Thilo et al., 1977), as well as lipid–protein segregations in a wide variety of membranes which have been directly visualized by freeze-etch electron microscopy (e.g., Verkleij et al., 1972; Kleemann and McConnell, 1974; Shechter et al., 1974).

We thoroughly investigated thermotropic lipid–protein segregations in different endomembranes of the unicellular eukaryote *Tetrahymena pyriformis* GL (Speth and Wunderlich, 1973; Wunderlich et al., 1973a,b; 1974a; Nägel and Wunderlich, 1977; cf. also Kitajima and Thompson, 1977; Nozawa and Thompson, 1977). For instance, *T. pyriformis* cells grown at 28 °C show lipid–protein segregations, i.e., the emergence of smooth areas on the fracture faces of endoplasmic reticulum membranes upon lowering the temperature below ~17 °C (Wunderlich et al., 1975), while *T. pyriformis* cells grown at 18 °C have shifted this lipid–protein segregation temperature down to ~12 °C (Wunderlich and Ronai, 1975). These lipid–protein segregations coincide with discontinuities observed in glucose 6-phosphatase activity associated with microsomal membrane vesicles isolated from *T. pyriformis*. Our studies using electron-spin resonance, fluorescence spectroscopy, differential thermal calorimetry, and proton nuclear magnetic resonance suggested that these thermotropic changes in structure and function of *T. pyriformis* membranes are induced by a clustering of the bilayer-arranged bulk membrane lipids. This means that at the growth temperature the membranes contain two separate lipid phases of different fluidity: Rigidlike lipid domains are randomly dispersed within predominantly fluid lipids. Upon temperature lowering, the rigidlike domains enlarge at the expense of the fluid lipids; i.e., molecules from the fluid lipid domains transform into a rigidlike conformation and join the rigidlike domains and/or create new small rigidlike domains. The rigidlike domains remain randomly dispersed until they reach a critical concentration; then they cluster. Below this clustering temperature, these clusters extend two dimensionally within the membranes at the expense of the fluid lipids. We suggested that the rigidlike domains in the *T. pyriformis* membranes do not consist of crystalline but rather "rigid" liquid-crystalline lipids (cf. also Baldassare et al., 1976), since we could not detect any fluid to ordered phase transition in the lipids extracted from the *T. pyriformis* membranes by either scanning calorimetry or proton nuclear magnetic resonance (Wunderlich et al., 1975).

Indeed, some evidence exists that lipids, though in the fluid state, are not indefinitely miscible. For instance, Wu and McConnell (1975) and Galla and Sackmann (1975) have shown that fluid lipids in binary liposomes consisting of either dipalmitoylphosphatidylethanolamine and dielaidoylphosphatidylcholine or dipalmitoylphosphatidylcholine and dipalmitoylphosphatidic acid, respectively, exhibit a miscibility gap in a certain temperature and composition range (cf. also Kreutz, 1970). Moreover, Davis et al. (1976) found by wide-angle x-ray diffraction and proton nuclear magnetic resonance that the lipids in sarcoplasmic reticulum membranes are in the fluid state between 37 and 0 °C (cf. also Blazyk and Steim, 1972). Incidentally, in this temperature range, lipid–protein segregation and a discontinuity of the ATPase activity are observed in this membrane type (Davis et al., 1976; Duppel and Dahl, 1976). On the other hand, we have to keep in mind that

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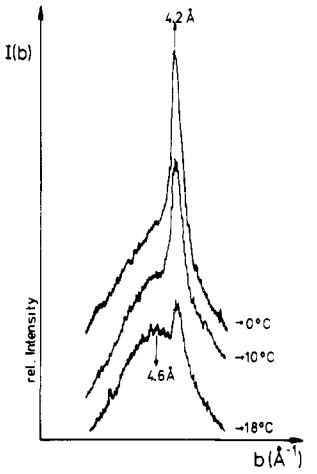

FIGURE 1: Densitometer tracings of wide-angle x-ray diffraction patterns of total 18 °C lipids at different temperatures: $I(\boldsymbol{b})$, scattering intensity; $\boldsymbol{b}$, scattering vector; $|\boldsymbol{b}| = (2 \sin \theta/\lambda)$; 2θ, scattering angle; λ, 1.54 Å.

our previous data obtained by differential scanning calorimetry and proton nuclear magnetic resonance could not exclude the possibility that the rigidlike domains in *Tetrahymena* membranes nevertheless contain ordered lipids. This would be the case if the lipid phase transition were very broad (i.e., proceeding over a very broad temperature range). In this case, wide-angle x-ray diffraction would be a more powerful method revealing ordered and fluid lipids as a sharp 4.2-Å reflection and a diffuse 4.6-Å reflection, respectively. We therefore decided to investigate the thermotropism of lipids extracted from microsomes isolated from *T. pyriformis* cells grown either at 18 or 28 °C by wide- and low-angle x-ray diffraction. Our present data indicate that, at least under certain conditions, these membrane-extracted lipids can indeed undergo a very broad fluid to ordered phase transition.

Materials and Methods

Cultures. Static cultures of the ciliate protozoan *T. pyriformis GL* were axenically grown at 28 or 18 °C up to midlate logarithmic growth phase (20 000–80 000 cells/mL) under the same conditions as described recently (Ronai and Wunderlich, 1975; Wunderlich and Ronai, 1975).

Isolation of Microsomes. The cells were disrupted by an osmotic shock treatment (Ronai and Wunderlich, 1975) and then subsequently homogenized in 0.4 M sucrose, 5 mM $MgCl_2$, 5 mM Tris-HCl[1] (pH 7.2) with a Potter-Elvejhem grinder at 0–4 °C. From this homogenate we isolated microsomal membrane vesicles according to our previous method (Ronai and Wunderlich, 1975), except for one modification. In order to raise the yields of microsomal membranes, we relinquished the microsomal subfraction; i.e., the 1.33 M sucrose layer was omitted when the postmitochondrial supernatants were centrifuged at 110 000*g* for 3 h. These microsomes retained their thermotropic character during isolation, for in control experiments we were able to show that the activity of glucose 6-phosphatase associated with microsomes isolated from 28 and 18 °C cells revealed a thermotropic discontinuity at ~18 and ~12 °C, respectively (cf. also Ronai and Wunderlich, 1975; Wunderlich and Ronai, 1975).

Lipid Extraction. Total lipids of the microsomes were extracted three times with chloroform–methanol (2:1) at 0–4 °C according to Folch et al. (1957). Total lipids of microsomes isolated from 18 °C cells were further subfractionated chromatographically into total neutral lipids and total phospholipids; from the latter we separated the ethanolamine phosphoglycerides containing phosphatidylethanolamine and its phosphono analogue glycerideaminoethylphosphonate by two-dimensional thin-layer chromatography as described in detail by Ronai and Wunderlich (1975).

Lipid Composition. The lipids of *T. pyriformis* microsomes have been characterized in detail elsewhere (Ronai and Wunderlich, 1975; Wunderlich and Ronai, 1975). For instance, microsomal membranes isolated from 28 and 18 °C *T. pyriformis* cells under identical conditions exhibit the same amount of neutral lipids (~17%), which contain no cholesterol. The phospholipids of 28 °C microsomes (18 °C microsomes) are composed of 30.0% (31.8%) phosphatidylethanolamine, 23.0% (26.2%) glycerideaminoethylphosphonate, 14.2% (12.9%) ceramideaminoethylphosphonate, 3.9% (2.8%) phosphatidylinositol, 4.8% (2.2%) lysophosphatidylcholine, 18.1% (18.2%) phosphatidylcholine, 4.7% (4.4%) ceramidemonomethylaminoethylphosphonate, and 1.3% (1.2%) cardiolipin. The total fatty acids (mainly even-numbered ranging between C_{12} and C_{18}) of the 28 °C (18 °C) phospholipids contain 65.2% (71.3%) unsaturated acids, 70.9% (72.3%) of which are contributed by about equal portions of the octadecadienoic and octadecatrienoic acid.

X-Ray Diffraction Procedures. The chloroform/methanol fractions containing lipid were evaporated under vacuum (aspirator pump) for 1 h. Under nitrogen, the lipids were then brought into Mark capillaries of 0.1-cm diameter for the recording of wide- and small-angle x-ray diffraction. The x-ray experiments were carried out with a rotating anode generator (Rigaku Denki Ru-200) at 60 kV and 200 mA and partly also with a conventional x-ray generator (Müller Mikro 111) at 35 kV and 30 mA. For the small-angle x-ray diagrams, Kratky cameras with slit collimation (30-μm slit width) were used with a sample to film distance of 21 cm; wide-angle patterns were obtained by Kiesig cameras (pinhole slits of 0.3-mm diameter) with a sample to film distance of 10 cm. The exposure times of the small-angle x-ray patterns were 20 min only with the rotating anode generator and 7 h with the normal x-ray generator. Ilford industrial G film was used. The temperature regulation was performed with Peltier elements in the case of the Kratky cameras and by a streaming water/alcohol mixture in the case of the Kiesig cameras. Densitometer tracings were recorded with a microdensitometer of Joyce-Loebl, Mark III. Integral intensities were evaluated planimetrically.

Results

Lipids Extracted from 18 °C Microsomes. (1) Wide-Angle X-Ray Diffraction. Figure 1 shows the x-ray diffraction patterns of *total lipids* at different temperatures. At 18 °C, two reflections can be seen: a sharp 4.2-Å reflection which is typical for the ordered state and a diffuse 4.6-Å reflection which is characteristic for the fluid state of lipids. At lower temperatures, the intensity of the 4.6-Å reflection decreases, while the 4.2-Å reflection concomitantly increases. The latter predominates at 0 °C.

(2) Small-Angle X-Ray Diffraction. Total lipids reveal two interference series of two separate lamellar phases in the temperature range between 0 and 24 °C. At 27 °C, however, only one lamellar phase is present (Figure 2). This result was reproduced in four different experiments. (The Bragg spacings of the first-order reflections, however, slightly vary from experiment to experiment, probably depending on the slightly differing amount of the lipid-bound water.) In the temperature

[1] Abbreviation used: Tris-HCl, 2-amino-2-hydroxymethyl-1,3-propanediol hydrochloride.

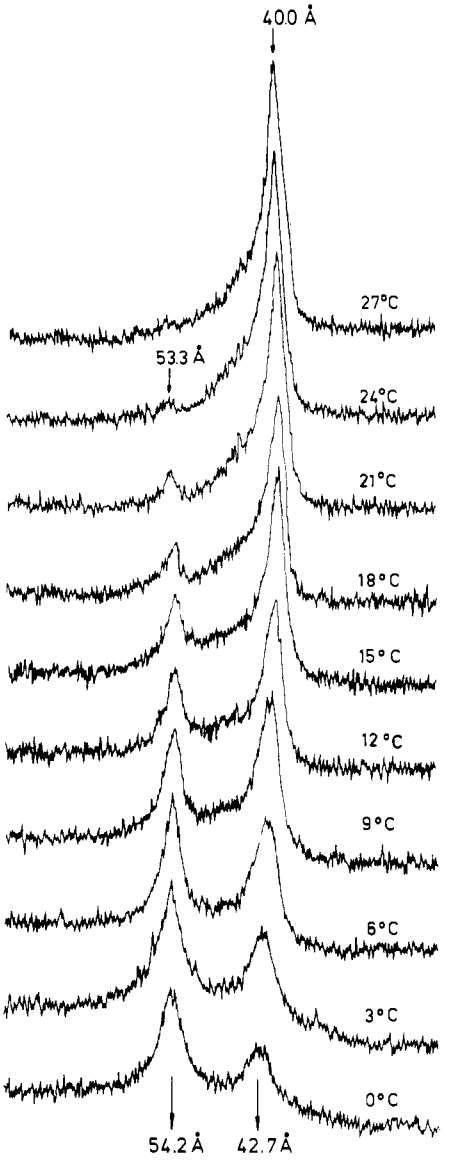

FIGURE 2: Densitometer tracings of a temperature series of small-angle first-order x-ray diffractions of total 18 °C lipids.

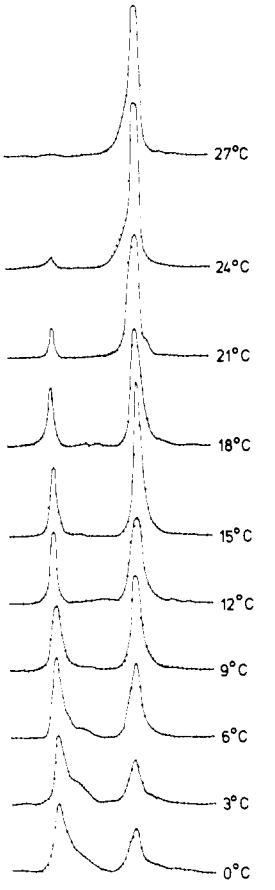

FIGURE 3: Temperature series of slit-corrected small first-order x-ray diffractions of total 18 °C lipids.

series of the experiment shown in Figure 2 the first-order reflections correspond to Bragg spacings of 54.2 and 42.7 Å, respectively, at 0 °C. Upon raising the temperature, these repeat distances decrease continuously to 53.3 Å at 24 °C and 40.0 Å at 27 °C, respectively, which is obviously due to the temperature-dependent reduction in the extension of the fatty acid chains. When the sample temperature is lowered from 27 again to 0 °C, the two initial Bragg spacings are refound. In comparison with the wide-angle x-ray patterns, we conclude that the fluid lipids form the 40-Å lamellar phase, whereas the 54-Å lamellar phase contains the ordered lipids. In other words, ordered and fluid lipids coexist in two separate phase spaces (= stacks of bilayers) in the temperature range between 0 and 24 °C, while only fluid lipids exist at and above 27 °C.

Figure 2 clearly shows that, upon temperature lowering, the fluid lipids become transformed into ordered lipids. In order to quantify this fluid to ordered transformation process, the ratios of the integral intensities of the fluid lipids to those of total lipids are plotted vs. temperature in Figure 4. At 0 °C, only 40% of total lipids are still in the fluid state; i.e., about 60% of the total lipids undergo a transformation from the fluid into the ordered state upon lowering the temperature from 27 to 0 °C. Conspicuously, this fluid to ordered transformation does not proceed continuously but rather reveals a discontinuity at ~10 °C.

We are aware that this type of evaluation may bear some errors because the two lipid lamellar reflections arise from two different products of lattice factors and structure factors of bilayer electron-density profiles (unit cells). A correct evaluation would be therefore, for example, to plot the percentage of fluid lipids in relation to the 100% fluid lipids at 27 °C as a function of temperature. In order to determine exactly the integral intensities of the fluid lipid reflections, we have thus first performed a slit correction according to the method of Lake (1967) as modified by Pape (manuscript in preparation). (The small-angle x-ray reflections are slightly smeared because the x-ray patterns have been recorded with a slit-collimated x-ray beam in order to minimize the exposure time of the lipid sample.) The slit-corrected x-ray patterns are shown in Figure 3. The portion of the integral intensities of the fluid lipids from the 100% fluid lipids at 27 °C is plotted vs. decreasing temperatures (Figure 4). In comparison with the first evaluation modus, the latter evaluation yields slightly different amounts of transformed fluid lipids at the different temperatures, but this curve follows principally the same slope pattern of the first curve also with a discontinuity at ~10 °C. The first evaluation modus can therefore be considered correct at a first approximation. We thus only use this less troublesome evaluation type in the following.

Total phospholipids separated from total lipids also coexist in two separate lamellar phases in the temperature range between 18 and 0 °C. In comparison with total lipids, however, the half-width of the two first-order reflections is distinctly smaller (Figure 5). This indicates larger bilayer stacks of the

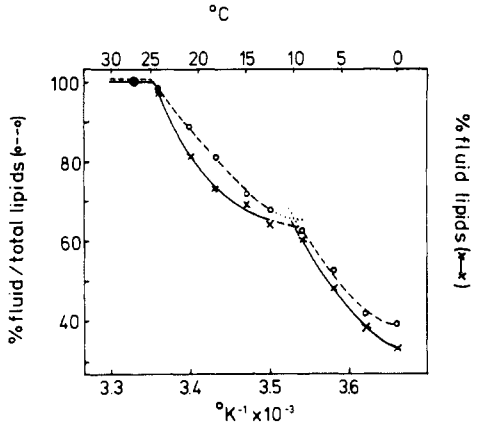

FIGURE 4: Percentage of fluid from total lipids (evaluated from the integral intensities of the small-angle x-ray reflections shown in Figure 2) and the portion of fluid lipids from the 100% fluid lipids at 27 °C (evaluated from the integral intensities of the slit-corrected small-angle x-ray reflections shown in Figure 3) as a function of temperature.

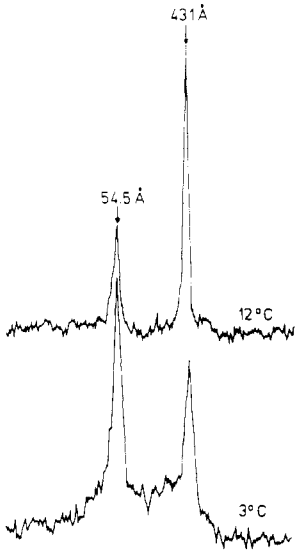

FIGURE 5: First-order reflections of two small-angle x-ray diffraction patterns of 18 °C phospholipids at 3 and 12 °C.

phospholipids. Probably, the neutral lipids which reveal only some diffuse diffraction (not shown) exert a slight disorganizing effect on the bilayer stacking of total lipids. The repeat distances of the fluid and ordered phospholipids are 41.9 and 54.5 Å at 18 °C. At this temperature, 72% of the phospholipids are fluid (Figure 6). Upon temperature lowering, the fluid lipids transform into ordered lipids with a discontinuity at ~10 °C. At 0 °C, 67% of the lipids are ordered; i.e., 39% of the total phospholipids transform from the fluid into the ordered state in the temperature range between 18 and 0 °C (Figure 6).

The x-ray diffraction patterns of the *PE-deficient phospholipids,* i.e., phospholipids lacking the ethanolamine phosphoglycerides, are shown in Figure 7 at different temperatures. These PE-deficient phospholipids reveal only some diffuse diffraction between ~85 and ~52 Å below 3 °C. With increasing temperatures, however, a distinct interference ring appears. Its intensity and repeat distance increase, while its half-width decreases upon raising the temperature. The PE-deficient phospholipids obviously form an increasing number of increasingly better stacked bilayers, exhibiting a thickness of about 53 Å at 18 °C. This value is close to that of the ordered lipids observed in total lipids and total phospholipids.

The ethanolamine phosphoglycerides also show only one

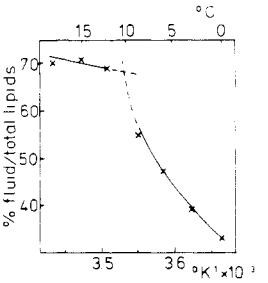

FIGURE 6: Temperature dependence of the integral intensities of the small-angle x-ray patterns of 18 °C phospholipids (cf. Figure 5) with a discontinuity at ~10 °C.

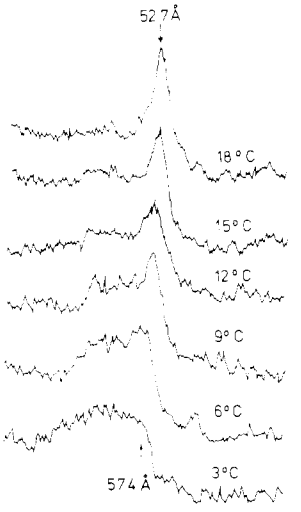

FIGURE 7: A series of small-angle first-order x-ray reflections of the 18 °C PE-deficient phospholipids as a function of temperature.

interference ring (Figure 8). Its half-width decreases at lower temperatures. This indicates that the bilayer stacking increases with decreasing temperatures. Most remarkable, however, the repeat distance decreases at higher temperatures (Figures 8 and 9). Between 0 and 10 °C, it remains nearly constant at ~48.5 Å. At ~10 °C, however, the repeat distance begins to decrease; it reaches 39.5 Å at 18 °C. This thermotropic change of the repeat distance of the ethanolamine phosphoglycerides reflects conformational changes of the single lipid molecules within the stacked bilayers. The fatty acid residues are surely involved in this conformational change. These, however, are very heterogeneous (cf. Materials and Methods). Thus, it is reasonable to assume that also a rearrangement of the polar head group is involved in, if not even majorly responsible for, this conformational change (cf. Kreutz, 1972; Phillips et al., 1972; Seelig and Gally, 1976).

Lipids Extracted from 28 °C Microsomes. (1) Wide-Angle X-Ray Diffraction. Total lipids contain lipids in the fluid and ordered state at 28 and 0 °C (Figure 10). At 0 °C, the ordered reflection predominates, while the reverse situation is observed at 28 °C.

(2) Small-Angle X-Ray Diffraction. The fluid and ordered lipids in *total lipids* are segregated into two lamellar phases in the temperature range between 28 and 0 °C, as is also the case with the above 18 °C lipids. At 28 °C, the repeat distance of the fluid and ordered lipids amounts to 40.4 and 54.0 Å. As can be seen in Figure 11, about 89% of total lipids are in the fluid state at 28 °C, whereas only 24% are fluid at 0 °C. This means that 65% of the total lipids transform from a fluid into an ordered state in the temperature range between 28 and 0

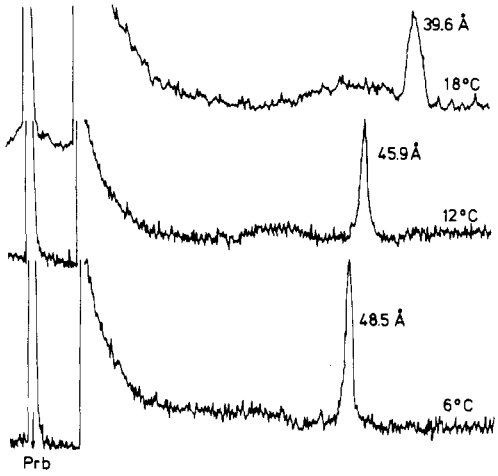

FIGURE 8: Small-angle x-ray diffraction patterns of the 18 °C ethanolamine phosphoglycerides at 6, 12, and 18 °C: Prb, primary x-ray beam.

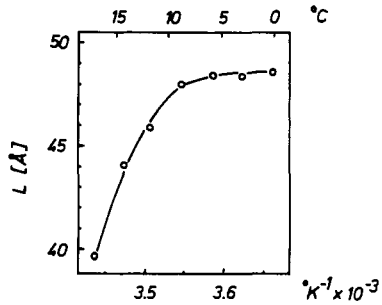

FIGURE 9: Repeat distance L (Bragg spacing) of the first-order reflections of the 18 °C ethanolamine phosphoglycerides as a function of temperature.

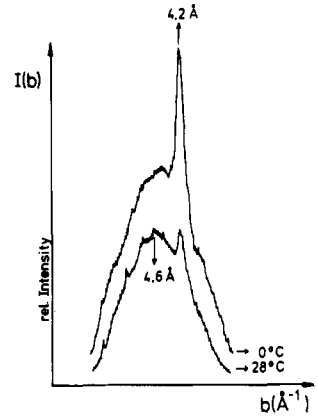

FIGURE 10: Wide-angle x-ray diffraction patterns of total 28 °C lipids at 0 and 28 °C.

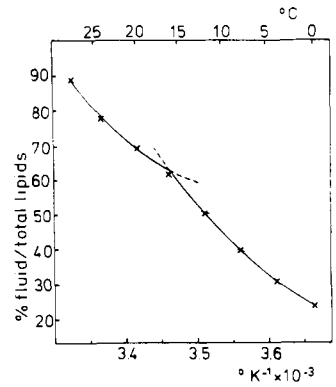

FIGURE 11: Temperature dependence of the integral intensities of small-angle x-ray patterns (first-order reflections) of total 28 °C lipids. Note the discontinuity at ~16 °C.

°C. This transformation process reveals a discontinuity at ~16 °C.

Discussion

Fluid to Ordered "Discontinuous" Lipid Phase Separation. Our low- and wide-angle x-ray diffraction studies indicate that total lipids extracted from microsomal membrane vesicles isolated from 28 and 18 °C grown *T. pyriformis* cells contain both fluid and ordered lipids coexisting in two separate lamellar phases. The 18 °C lipids are all in the fluid state at 27 °C. About 60% of these fluid lipids become transformed into the ordered state upon lowering the temperature to 0 °C, as can be approximated from our small-angle x-ray data. A similar situation is found with the 28 °C lipids, 65% of which are transformed from the fluid into the ordered state upon lowering the temperature from 28 to 0 °C. These findings indicate that at least a considerable portion of the 28 and 18 °C lipids undergoes a thermotropic fluid to ordered phase transition over a very broad temperature range; the ordered lipids are segregated from the fluid lipids. Thus, the term transition is better substituted by the term separation (Shimshick and McConnell, 1973).

Furthermore, our data indicate that this fluid to ordered phase separation is a discontinuous process; i.e., the transformation of fluid into ordered lipids does not proceed continuously but rather reveals a discontinuity at ~16 °C in the 28 °C lipids and at ~10 °C in the 18 °C lipids. One possible explanation for this behavior could be that both above and below the discontinuity point the lipids in the fluid state do reveal the same compositional quality but differ slightly in their conformation. This, however, seems unlikely, since one would then expect a discontinuous change in the repeat distance of the fluid lamellar phase, indicating a conformational change of the fluid lipids, which we observed neither in total lipids of 28 °C microsomes nor in total lipids and phospholipids of 18 °C microsomes. At the moment, however, we therefore prefer the more plausible explanation that the compositional quality of that amount of fluid lipids which transforms into the ordered lipids above the discontinuity point differs from that transformed below the discontinuity point. In consequence, both the fluid and the ordered lipids exhibit a different composition above and below the discontinuity point.

Of course, our data cannot discriminate which compositional quality the lipids exhibit, which are transformed above and below the discontinuity point. They indicate, however, that the ethanolamine phosphoglycerides play a dominant role in—even if they are not predominantly responsible—this discontinuous phase separation. For these phospholipid species extracted from the 18 °C lipids dramatically changed their conformation at ~10 °C. At this temperature, we also observe the discontinuity in the total 18 °C lipids. Further circumstantial evidence for such a dominant role comes from our previous findings that the 18 °C *Tetrahymena* cells, in comparison to 28 °C cells, have shifted the lipid-protein segregation temperature within their membranes from ~17 to ~12 °C by replacing the more saturated phospholipid species with the more unsaturated ethanolamine phosphoglycerides (Wunderlich and Ronai, 1975; cf. also Fukushima et al., 1976). For this reason, we tend to speculate that the percent distribution of the ethanolamine phosphoglycerides in the two lamellar phases differs above and below the discontinuity point of the fluid to ordered phase separation.

Biological Significance. Membrane proteins very probably influence the conformational state and the distribution of the lipids in biomembranes. It is therefore always problematic to transform data obtained with free lipids on the lipid situation in membranes. Our present findings, however, could deliver a rational basis for extending our concept previously suggested for the thermotropism of lipids bound within *T. pyriformis* membranes in two major points concerning the lipid conformation and clustering of the rigidlike domains. (1) The fact that total 28 and 18 °C lipids undergo a broad fluid to ordered phase separation indicates that the rigidlike domains within membranes do not contain "rigid liquid-crystalline" lipids as previously suggested but rather lipids in the ordered state. (2) The fact that the fluid to ordered phase separation is a discontinuous process permits a plausible explanation for the cause of the lateral clustering of the ordered lipid domains within the *T. pyriformis* membranes. The latter could be attributed to a change of interactions between ordered and fluid lipid domains conditioned by the change in composition of these domains at the clustering temperature. A logical consequence of this conclusion is that the lipid composition in the smooth areas in freeze-fractured membranes exhibits another quality than that in the particle-rich regions. Indeed, this was recently shown in plasma membranes of *Escherichia coli*. Membrane vesicles derived from smooth areas contain different lipids than those from particulated areas (van Heerikhuizen et al., 1975; Letellier et al., 1977).

Our present view of the lipid thermotropism within membranes is as follows. The poikilothermic eukaryote *T. pyriformis* adjusts the fluidity of its endomembranes within a broad fluid to ordered discontinuous phase separation. This means that the membrane fluidity is heterogeneous (cf. also Oldfield and Chapman, 1973). At the cells' growth temperature, the bulk membrane lipids are predominantly fluid, in which domains of ordered lipids are dispersed. The number and/or the size of these ordered lipid domains increase at the expense of the fluid lipids upon temperature lowering. At a critical temperature the ordered lipid domains gain another compositional quality. This leads to a change in interactions between ordered and fluid lipid domains, thus causing a lateral clustering of the ordered lipid domains. This clustering manifests itself in lipid–protein segregations observed in freeze-fractured membranes, in discontinuities of the activities of membrane-associated enzymes such as glucose 6-phosphatase, in discontinuities of fluorescence spectroscopical signals, in discontinuities of the partition coefficient of electron-spin resonance probes and in discontinuities of membrane lipid ordering, as detected by direct membrane labeling in electron-spin resonance (cf. Wunderlich et al., 1975). Below the clustering temperature the clusters of ordered lipids extend two dimensionally at the expense of the fluid lipids. Though, for instance, thermotropic lipid–protein segregations can be also visualized in other cell types, including mammalian cells (e.g., Duppel and Dahl, 1976; Wunderlich et al., 1974b), it remains to be seen whether our present model for lipid thermotropism can be applied as a rational basis for the interpretation of the lipid thermotropism within other membranes also revealing a heterogeneous lipid composition.

Acknowledgment

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