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LIPID PHASE TRANSITIONS IN CYTOPLASMIC AND OUTER MEMBRANES OF *ESCHERICHIA COLI*

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SUMMARY

The cytoplasmic and outer membranes containing either *trans*-Δ^9-octadecenoate, *trans*-Δ^9-hexadecenoate or *cis*-Δ^9-octadecenoate as predominant unsaturated fatty acid residues in the phospholipids were prepared from a fatty acid auxotroph, *Escherichia coli* strain K1062. Order-disorder transitions of the phospholipids were revealed in both fractions of the cell envelope by fluorescent probing or wide angle X-ray diffraction. The mid-transition temperatures, T_t, and the range of the transition, ΔT, are similar in the outer and cytoplasmic membrane. Relative to the corresponding extracted lipids, 60–80 % of the hydrocarbon chains take part in the transition in the cytoplasmic membrane whereas in the outer membrane only 25–40 % of the chains become ordered. The results suggest that in the outer membrane part of the lipids form fluid domains in the form of mono- and/or bilayers.

INTRODUCTION

Lipid phase transitions have been detected by a variety of indirect methods in isolated phospholipids, membranes and whole cells of *Escherichia coli* fatty acid auxotrophs grown in the presence of different fatty acid supplements [1–9]. For membranes, several of these studies employed fluorescent or spin label probes to reveal the phase transition. The agreement between these various methods regarding the transition temperature, T_t, of the phase transition and characteristic changes in the activation energy of membrane associated functions was quite satisfactory [1–4]. Nevertheless, a procedure not relying on probe molecules, i.e. the direct observation of the order-disorder transition of the fatty acid chains by X-ray diffrac-

Abbreviations: 12 : 0, dodecanoic acid; 14 : 0, tetradecanoic acid; 16 : 0, hexadecanoic acid; *trans*-16 : 1, *trans*-Δ^9-hexadecenoic acid; *trans*-18 : 1, *trans*-Δ^9-octadecenoic acid; *cis*-16 : 1, *cis*-Δ^7-hexadecenoic acid; *cis*-18 : 1, *cis*-Δ^9-octadecenoic acid; 19Δ, 9,10-methylene-octadecanoic acid; β-OH-14 : 0, β-hydroxy-myristic acid.

tion, is able to provide more direct and independent evidence. Earlier X-ray studies by Esfahani et al. [10] and Shechter et al. [11] were recently followed by the extensive analysis of Shechter et al. [12] using a new X-ray detector of greatly improved sensitivity [13]. Thus, not only the transition temperature, T_t, and the range of the transition, ΔT, but in addition the amount of hydrocarbon chains taking part in the transition could be determined.

So far, the cytoplasmic membrane preparations used for the physical studies were prepared by the method of Kaback [14] or by a related method developed in C. F. Fox's laboratory [15, 16]. Recently, an improved procedure yielding relatively pure inner (cytoplasmic) and outer membranes of *Salmonella thyphimurium* has been published [17]. We have adopted this method for separating the two membranes of the envelope of an *E. coli* fatty acid auxotroph grown in the presence of different fatty acid supplements. This paper serves two purposes: firstly, a comparison of X-ray diffraction and fluorescent probing as methods for detecting phase transitions and, secondly, the demonstration of a lipid phase transition in the outer membrane of the cell envelope.

MATERIALS AND METHODS

Organism and growth conditions

E. coli strain K1062 [18] was grown in Cohen-Rickenberg mineral salts medium [19] supplemented with 0.5 % glycerol, 0.3 % casamino acids (Difco, vitamin free), 0.2 % Brij 35 (polyethylene glycolmonolauryl ether) and 0.02 % of either *trans*-18 : 1, *cis*-18 : 1 (both from C. Roth, Karlsruhe, Germany) or 0.01 % *trans*-16 : 1 (Hormel Institute, Austin, Minn.). Cells were grown as described before [2] to an absorbance at 600 nm of about 0.6, harvested at room temperature, and washed three times with Cohen-Rickenberg buffer.

Preparation of phospholipids

The total lipids were extracted from the cells according to Ames [20]. The non-polar lipids were removed by silicic acid chromatography [2].

Preparation of membranes

The preparation of membranes followed the procedure of Osborn et al. [17] with slight modifications: 10 g of cells (wet weight) were rapidly suspended in 100 ml 0.75 M sucrose/10 mM Tris · HCl (pH 7.8) at 4 °C for *cis*-18 : 1-grown cells (at 23 °C for *trans*-16 : 1-grown cells, at 32 °C for *trans*-18 : 1-grown cells). 5 ml lysozyme solution (2 mg/ml) were added from a pipette. After 2 min stirring, 210 ml 1.5 mM sodium EDTA, pH 7.5, were added to the *cis*-18 : 1-grown cell suspension at 4 °C continuously through a tubing under the surface of the solution within 10 min. The suspension was then heated to 20 °C in a water bath. After stirring for another 20 min, spheroplast formation was > 90 %. In the case of the *trans*-16 : 1-grown cells the suspension was quickly heated to 32 °C (to 40 °C for *trans*-18 : 1-grown cells) before adding the pre-warmed EDTA solution at 32 °C (or 40 °C, respectively) as described above. Some lysis of cells could not be prevented, but spheroplast formation was essentially complete after another 20 min stirring at 32 °C (or 40 °C). All

preparations were then cooled with an ice/salt bath to 0 °C which caused extensive lysis of the spheroplasts. After sonication with stirring in 150-ml portions for 5–7 min with a Branson sonifier at 0–5 °C, the lysates were centrifuged for 20 min at $1500 \times g$. The membranes, obtained from the supernatant by centrifugation (60 min at 300 000 $\times g$ in a Beckman 60 Ti-rotor), were resuspended in 100 ml 0.25 mM sucrose/3.3 mM Tris/1 mM EDTA (pH 7.8) and collected after another low speed centrifugation as described above. Separation of the membranes was performed on six tubes in a Beckman SW41-rotor exactly as described by Osborn et al. [17]. A combined $L_1 + L_2$-fraction, called L-fraction (cytoplasmic membrane), an intermediate density M-band and the H-fraction (outer membrane) were obtained in this way. The membranes were collected by centrifugation, resuspended in water and stored in liquid N_2. Yield in mg of protein/10 g cells: *trans*-18 : 1-membranes: L: 14.9, M: 12, H: 22.1; *trans*-16 : 1-membranes: L: 7.3, M: 9.5, H: 22.3; *cis*-18 : 1-membranes: L: 19.7, M: 6.0, H: 31.8.

Analysis of membrane fractions

For protein determinations the procedure of Lowry et al. [21] was employed using bovine albumin as a standard. The fatty acid composition was determined in the following way: A defined amount of pentadecanoic acid was added to samples of the total lipid extract or to the L- and M-membrane fractions. After saponification with methanolic KOH, the fatty acids were extracted with chloroform, esterified with diazomethane and analysed by gas chromatography on an Apiezon column at 240 °C. The absolute amount of each fatty acid was obtained from the gas chromatogram relative to the pentadecanoic methylester standard. In the case of the H-fractions, a sample was lyophilized and the phospholipids extracted three times with chloroform/methanol (2 : 1, v/v) leaving the lipopolysaccharide in the pellet after centrifugation. Pentadecanoic acid was added to both the residue and the extract and the solvent removed by a stream of N_2. After acid hydrolysis (4 M HCl, 100 °C, 5 h) according to Rietschel et al. [22], the fatty acids in the lipopolysaccharide were determined as described above.

2-Keto-3-deoxyoctonic acid was determined as described by Weissbach and Hurwitz [23]. 1 mg of lipopolysaccharide contains 0.45 μmol of 2-keto-3-deoxyoctonic acid [24]. The relative cytochrome content of the membrane fractions was estimated from the Soret band absorption at 427 nm (dithionite-reduced versus H_2O_2-oxidized difference spectra at the temperature of liquid N_2). D-Lactate dehydrogenase and succinate dehydrogenase were measured at 40 °C as described by Futai [25].

Measurement of phase transitions

For the fluorescence measurements aliquots of the membrane fractions were diluted with 3 ml Cohen-Rickenberg buffer [2] to a final phospholipid hydrocarbon chain concentration of $2 \cdot 10^{-4}$ M. After addition of 3 μl 10^{-2} M methanolic *N*-phenyl-1-naphthylamine the temperature dependence of fluorescence was determined as described previously [2]. The fluorescence change at the phase transition was normalized as described in the legend to Fig. 3 of ref. 2.

Wide angle X-ray diffraction was performed as described in ref. 12. Membrane fractions of known composition in Cohen-Rickenberg buffer were collected by high

speed centrifugation yielding pellets with a water content of about 70 %. From dry weight determinations (drying to constant weight in high vacuum), the concentration of hydrocarbon chains in the pellet could be calculated. The pellets were transferred to a sample holder and X-ray diffraction spectra were recorded for 6–15 min at various temperatures. The amount of ordered paraffin chains in the various samples were determined by comparison of the integrated intensity of their 4.2 Å reflection to that of standards consisting of total *trans*-18 : 1 or *trans*-16 : 1 phospholipids mixed with 10 % water. Simultaneous low and high angle X-ray diffraction of these standards, at 5 °C, indicates that only one phase is present in which 100 % of the paraffin chains are in the β-type-ordered conformation.

Freeze-etch electron microscopy was performed as described in ref. 12.

RESULTS

Characterization of membrane fractions

The procedure of Osborn et al. [17] for separating the cytoplasmic and outer membrane of the *S. thyphimurium* cell envelope is critically dependent on good spheroplast formation after the lysozyme-EDTA treatment. The whole preparation is normally performed at a temperature of 0–5 °C. Under these conditions we observed extensive lysis of *E. coli* strain K1062 grown in the presence of *trans*-16 : 1 or *trans*-18 : 1. This behavior is caused by the brittleness of the membrane at temperatures well below the lipid phase transition. Extensive damage to the barrier properties of the cytoplasmic membrane about 10 °C below the transition temperature of transport [1] was previously observed by H. U. Schairer (unpublished observations, see also Haest et al. [26] and Steim [9]) when studying the temperature dependence of thiomethyl-β-galactoside efflux from *E. coli* cells. Under these conditions an immediate efflux of the sugar ensued. The altered temperature regime in spheroplast formation used here assures that the lipids in the membrane are in a liquid-like state. However, the subsequent sonication was performed at 0 °C in order to prevent hybridization of the inner and outer membrane [16].

Tables I and II give the composition of cytoplasmic (L)- and outer (H)-membrane fractions from *E. coli* cells grown in the presence of either *trans*-18 : 1, *trans*-16 : 1 or *cis*-18 : 1. The lipid to protein ratio in the L-fractions was consistently higher than observed for *S. thyphimurium* [17]. A lipid to protein ratio of 0.24 to 0.36 for the H-fraction is similar to previously observed values [17]. The contamination of the cytoplasmic membrane fractions (combined L_1 and L_2, see ref. 17) by lipopolysaccharide is also in the same range as observed by others [17]. For the following, the contamination of the outer-membrane preparation by components of the cytoplasmic membrane is critically important. The cytochrome content and the specific activity of two membrane-bound enzymes suggest that the H-bands contain about 3 % L-band components in the *trans*-16 : 1- and *cis*-18 : 1-containing membranes and $\leqq 9$ % contamination in the *trans*-18 : 1-containing fraction. The fatty acid composition in the phospholipids of the L- and H-fractions is very similar and in accordance with previous results [1, 3]. The lipopolysaccharide contains in addition to the typical lipid A components (12 : 0, 14 : 0, 16 : 0 and β-OH-14 : 0) minor amounts of the unsaturated fatty acids added to the growth medium. For the present report, the fatty acid composition in the lipopolysaccharide was only

TABLE I

COMPOSITION OF MEMBRANE FRACTIONS FROM *E. COLI* STRAIN K1062

Fatty acid supplement	*trans*-18 : 1		*trans*-16 : 1		*cis*-18 : 1	
	L	H	L	H	L	H
Fatty acid chains in phospholipids (μmol/mg protein)	2.2	0.67	3.7	0.93	2.3	0.69
Phospholipids (mg/mg protein)	0.80	0.24	1.2	0.36	0.83	0.27
Lipopolysaccharide (mg/mg protein)	0.03	0.26	0.10	0.54	0.05	0.38
Molar ratio of 2-keto-3-deoxy-octonic acid to fatty acids	—	0.61	—	0.54	—	0.44
Cytochrome content (%)	58	5	123	4	100*	3
D-Lactate dehydrogenase (μmol/min per mg protein)	0.21	0.04	0.95	0.058	1.8	0.067
Succinate dehydrogenase (μmol/min per mg protein)	0.81	0.028	1.85	0.055	1.58	0.023

* The cytochrome content of *cis*-18 : 1-containing L-fraction is arbitrarily given as 100 %.

needed for an estimation of the phospholipid content. The small amount of unsaturated fatty acids in the lipopolysaccharide fraction may be due to contamination by phospholipid. Silbert et al. [27] have also observed that the fatty acid composition of the lipopolysaccharide remains constant in fatty acid auxotrophs in spite of the extensive alteration in the composition of the phospholipid hydrocarbon chains.

TABLE II

COMPOSITION OF FATTY ACID CHAINS (MOL %) IN PHOSPHOLIPIDS AND LIPOPOLYSACCHARIDE

At the top of the table the fatty acids used as supplements in the medium are listed.

	trans-18 : 1			*trans*-16 : 1			*cis*-18 : 1		
	L	H		L	H		L	H	
	Phospholipids	Phospholipids	Lipopolysaccharide	Phospholipids	Phospholipids	Lipopolysaccharide	Phospholipids	Phospholipids	Lipopolysaccharide
12 : 0	1.8	5.4	34.3	1.2	1.0	28.8	0.6	0.8	26
14 : 0	4.3	6.1	15.1	4.9	6.1	21.4	5.9	9.4	22.9
16 : 0	4.0	5.3	5.5	17.6	12.9	3.3	25.8	35.5	12.8
trans-16 : 1	—	—	—	76.3	79.9	14.6	—	—	—
trans-18 : 1	89.9	83.6	12.1	—	—	—	—	—	—
cis-16 : 1	—	—	—	—	—	—	4.3	3.0	—
cis-18 : 1	—	—	—	—	—	—	61.5	51.3	7.5
19Δ	—	—	—	—	—	—	1.9	—	—
β-OH-14 : 0	—	—	32.9	—	—	31.8	—	—	30.8

Lipid phase transitions in membrane fractions

The upper part of Fig. 1 shows X-ray diffraction spectra of a cytoplasmic membrane fraction containing predominantly *trans*-16 : 1 in the phospholipids. The disappearance of the sharp 4.2 Å reflection over the temperature range indicated is evident. The lower part of the figure gives spectra for *trans*-16 : 1-containing lipids in the ordered (4 °C) and the disordered (45 °C) state.

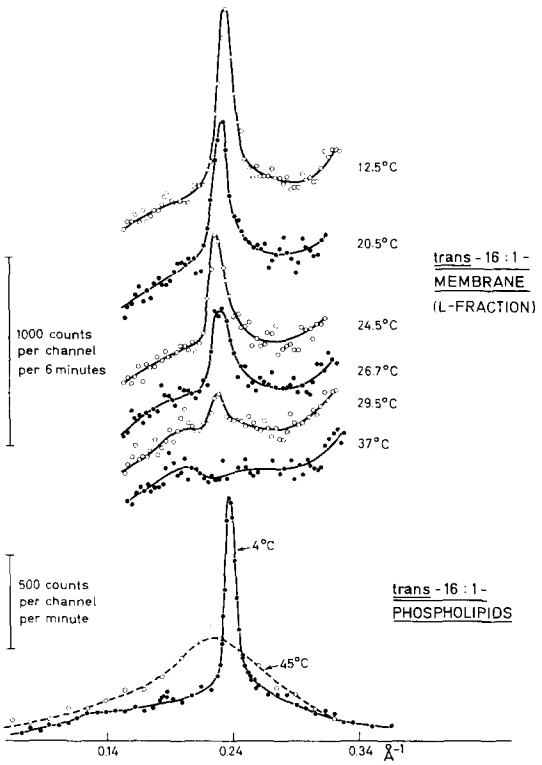

Fig. 1. Temperature dependence of wide angle X-ray diffraction of cytoplasmic membrane and isolated phospholipids containing *trans*-Δ^9-16 : 1 as predominant fatty acid.

Figs 2 and 3 compare the order-disorder transition as observed by X-ray diffraction with fluorescence measurements using *N*-phenyl-1-naphthylamine as a probe. The transition temperatures, the range of the transition and the amount of hydrocarbon chains taking part in the transition in membranes relative to lipid standards are summarized in Table III.

(a) *Cytoplasmic membrane. trans*-18 : 1-Containing membranes give a transition temperature, T_t (see legend to Table III for definition), of 34–35 °C using fluorescent probing or X-ray diffraction for detection. The range of the transition is sharper for the fluorescence ($\Delta T = 7$ °C) than for the X-ray measurements ($\Delta T =$ 15 °C). In previous studies involving both fluorescent and spin label probes or calorimetry T_t values of 36–41 °C ($\Delta T =$ 3–15 °C) were obtained [2, 4–6, 8]. In the *trans*-16 : 1-containing L-fraction the values for both T_t and ΔT are the same using both

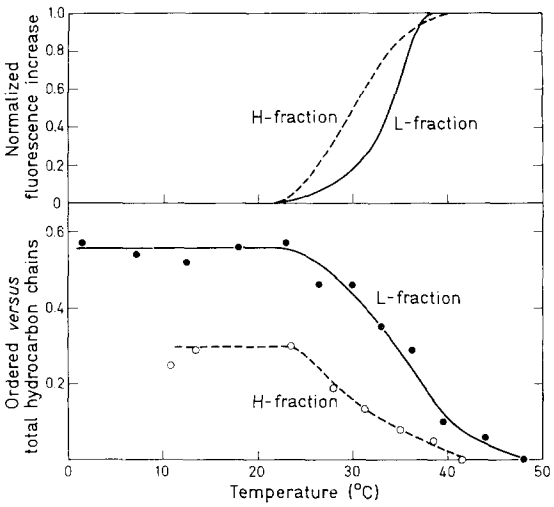

Fig. 2. Temperature dependence of fluorescence increase (upper part) and X-ray diffraction (lower part) in L- and H-membrane fractions of *trans*-18 : 1 grown cells. The temperature dependence of the fluorescence change at the phase transition was continuously recorded as described previously (see Materials and Methods and ref. 2) using *N*-phenyl-1-naphthylamine as a probe. Only the rising temperature scans are shown; decreasing scans have the same shape with T_t shifted by 3 °C to lower temperatures.

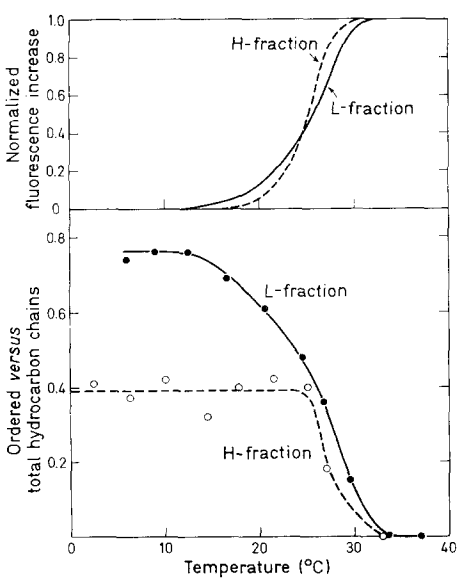

Fig. 3. Temperature dependence of fluorescence increase (upper part) and X-ray diffraction (lower part) in L- and H-membrane fractions of *trans*-16 : 1-grown cells. Compare legend to Fig. 2 for explanation.

TABLE III

CORRELATION OF X-RAY DIFFRACTION AND FLUORESCENCE MEASUREMENTS

T_t values correspond to the temperature at a normalized fluorescence increase of 0.5. Similarly, T_t of the X-ray diffraction data is defined as the temperature where half of the chains are ordered, relative to the percentage of ordered chains at $T \ll T_t$. As a measure of the sharpness of the transition, ΔT is defined by intercepts of the tangent in T_t with the extrapolated, horizontal lines above and below the transition [2]. The data in the table are obtained from the curves in Figs 2 and 3. *trans*-18 : 1, *trans*-16 : 1 and *cis*-18 : 1 refer to the fatty acid supplements used in the growth medium. In calculating the amount of ordered hydrocarbon chains present at $T \ll T_t$ in the H-fractions, it is assumed that only phospholipids take part in the transition.

	X-ray diffraction			Fluorescence		
	T_t (°C)	ΔT (°C)	Ordered hydrocarbon chains at $T \ll T_t$ (%)	T_t (°C)	ΔT (°C)	Amount of lipid taking part in phase transition (%)
trans-18 : 1						
L-fraction	35	15	57	34	7	80*
H-fraction	33	14	25	30	10	—
trans-16 : 1						
L-fraction	26	10	76	26	9	—
H-fraction	27	5	39	25	6	—
cis-18 : 1						
L-fraction	approx. 10	approx. 20	27	approx. 9	>15	—
H-fraction	n.d.	n.d.	16	approx. 16	11	—

* From Träuble and Overath [3].

techniques. These values are in agreement with previous studies [2, 4]. *cis*-18 : 1-Containing L-fraction shows a broad reversible transition starting at about 20 °C and extending close to or below 0 °C. Only approximate values are, therefore, given in Table III. The X-ray data reported here are in good agreement with previous results [12] on cytoplasmic membrane vesicles prepared by the Kaback method (14) from the same fatty acid auxotroph.

(b) *Outer membrane.* With both methods and in all three preparations a thermal transition is observed (cf. Figs 2 and 3 and Table III). These transitions appear to occur in roughly the same temperature range as in the cytoplasmic membrane. It should be noted that the 4.5 Å-4.2 Å change in the wide angle X-ray pattern observed in these preparations is the typical transition observed upon ordering of phospholipid hydrocarbon chains. A participation of part or all of the hydrocarbon chains of the lipopolysaccharide component in the lipid transition cannot be excluded. It remains, furthermore, unknown if the manipulations necessary for isolating the outer membrane (EDTA-lysozyme treatment, sonication) lead to changes in the arrangement of the various membrane constituents.

Amount of lipid taking part in the transition

X-ray diffraction allows an estimation of the percentage of ordered hydrocarbon chains below the thermal transition. The values in Table III are calculated

relative to a standard having essentially 100 % of the chains in an ordered state. In the extracted lipids containing *trans*-unsaturated fatty acids nearly all chains are ordered at $T \ll T_t$. Therefore, the values for *trans*-18 : 1-containing membranes (57 %, previous estimate 55 % [12]) and *trans*-16 : 1-containing cytoplasmic membranes (76 %) directly give the percentage of chains taking part in the membrane transition. These values are in fair agreement with a previous estimate (80 % for *trans*-18 : 1 membranes [3]) using a different method. In *cis*-18 : 1-containing lipid dispersions only 49 % of the chains are ordered at $T \approx 0$ °C [12]. The value of 27 % in Table III (previous value 33 % [12]) for the L-fraction therefore means, that relative to the extracted lipids 55 % (or 67 % [12]) of the chains take part in the membrane transition. In all three H-fractions investigated, the percentage of ordered chains observed at low temperature is about 50 % of that in the cytoplasmic membrane. In summary, relative to the extracted lipids 60–80 and 24–40 % of the hydrocarbon chains become ordered in the cytoplasmic and outer membrane, respectively.

Freeze-etching electron microscopy

Recent studies in several laboratories have shown [6, 8, 12, 28–31] that lipid phase transitions may result in gross alterations of membrane structure involving an aggregation of integral membrane proteins. The preparations used in this study indicate the following: When quenched from temperatures above the thermal transition partial aggregation is observed in *trans*-16 : 1- and *trans*-18 : 1-containing cytoplasmic membranes. This patchy appearance is artefactual since a random arrangement of particles is observed after previous fixation with glutaraldehyde [6, 31]. When quenched from low temperatures one observes large areas denuded of particles. The outer membrane vesicles give densely particulated fracture planes when quenched from high temperatures. Contrary to the cytoplasmic membrane, the particles are mainly associated with the concave fracture face. When cooled from 0 °C, the outer membrane also displays mainly smooth surfaces.

DISCUSSION

Cytoplasmic membrane

It appears rather gratifying that from experiments described in this and previous [1–6, 8, 12] publications a fair amount of agreement regarding the range, ΔT, and the transition temperatures, T_t, at least for the more simply substituted *E. coli* membranes containing *trans*-18 : 1, *trans*-16 : 1 or *cis*-18 : 1 has been obtained. Throughout the transition there is a gradual increase in the amount of ordered paraffin chains. Presumably, ordered domains of lipid molecules arranged as monolayers or bilayers are formed. Both cases have been observed in lipid-water model systems [32]. A simple mechanism involving essentially no separation of lipid molecules with different hydrocarbon substituents may be approached in the *trans*-fatty acid-containing membranes. If the lipid composition of the membrane is more heterogeneous, e.g. if it contains double-saturated, saturated-*cis*-unsaturated and double-*cis*-unsaturated molecules with widely different packing properties, the transition may involve separation of domains with different hydrocarbon chain composition. In interpreting their spin label partitioning experiments with both artificial and natural membrane systems [5, 6, 33] McConnell and co-workers have introduced the term "lateral phase separa-

tion" for such phenomena. However, in attempting to interpret the width of the transition in the membrane it is impossible to differentiate between two alternatives: (a) The broadness of the transition reflects the degree of cooperativity of the system. (b) The range of the transition defines the beginning and end of a phase separation. The ultimate goal, namely a quantitative description of the spacial distribution of lipid molecules in the membrane throughout the phase transition, is beyond the scope of presently available experimental techniques.

The aggregation of proteins is a possible but not necessary phenomenon accompanying the phase transition. Whereas aggregation is observed for the membrane preparations used in this study, Haest et al [8] have recently shown that branched-chain substituents in the membrane lipids prevent aggregation although a thermal transition is clearly indicated by calorimetry. Kleemann and McConnell [6] were likewise unable to detect aggregation in wild type *E. coli*. Thus, "lipid-protein phase separations" are also dependent on the hydrocarbon chain composition of the lipids.

Protein aggregation caused by an order-disorder transition has also a bearing on estimates of the amount of lipids taking part in the phase transition [3, 11, 34]. It has been assumed in an earlier study [3] that the amount of lipids taking part in the transition is an adequate measure for the percentage of molecules in the "free", i.e. non-protein-associated state above the transition. Although the similar transition temperatures in membranes and isolated lipids are consistent with this assumption, aggregation of proteins may cause an increase of lipid molecules available for lipid-lipid interaction. For this reason, estimates of the amount of lipid molecules taking part in the transition (Table III and refs 3, 12 and 34) should be considered as upper limits.

Outer membrane

The permeability properties of the outer membrane of Gram-negative bacteria suggest a molecular sieve-like structure: It is permeable to small molecules like ions or sugars and impermeable to larger compounds like certain antibiotics. Furthermore, this outmost layer of the envelope protects the proteins of the periplasmic space. Most models of this membrane, therefore, assume a phospholipid bilayer with embedded proteins and an asymmetric arrangement of the lipopolysaccharide towards the surrounding medium (see refs 35 and 36 for reviews). Lipid-lipid interaction in the outer membrane as shown in this study is consistent with such models. Furthermore, only about half as many lipid molecules take part in the transition in the outer membrane as compared to the cytoplasmic membrane. This is in accordance with the lower lipid to protein ratio in the outer membrane, that is a higher proportion of lipid molecules interacts with proteins and is not available for lipid-lipid interaction. It is reasonable that the transition temperature and the range of the transition are similar in the outer and inner membranes because phosphatidylethanolamine is the predominant lipid class in both membranes [17]. It has been shown before [2] that the transition temperature of *E. coli* lipids is essentially determined by phosphatidylethanolamine. The demonstration of lateral mobility [37] of the lipopolysaccharide in the *S. thyphimurium* cell wall also indicates that there are fluid domains in the outer membrane which will likely be formed by phospolipid. As noted already, our measurements allow no conclusion regarding the participation of lipopolysaccharide in the phospholipid phase transition.

The demonstration of a thermal transition in the outer membrane raises the question how this transition will influence measurements involving functions associated with the cytoplasmic membrane. While such an influence can only be ruled out by a comparative study using whole cells, spheroplasts and cytoplasmic membrane vesicles, the following experiment (Overath, P., unpublished) suggests that the outer membrane does not change its permeability properties to low molecular weight compounds throughout the phase transition: A fatty acid auxotroph (K1062) was grown with *cis*-18 : 1 or *trans*-18 : 1 and de-repressed for the synthesis of alkaline phosphatase, a periplasmic enzyme. Although the specific activity of this enzyme using *p*-nitrophenyl phosphate as a substrate was six times higher in crude sonicates as compared to whole cells, Arrhenius plots for extracts and cells were linear throughout the temperature range of the phase transition in both cases. Thus, while the access of the substrate to the enzyme is hindered in an unknown way in whole cells, the ordering of the paraffin chains in the outer membrane does not change this behavior.

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