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INFLUENCE OF LIPIDS WITH BRANCHED-CHAIN FATTY ACIDS ON THE PHYSICAL, MORPHOLOGICAL AND FUNCTIONAL PROPERTIES OF *ESCHERICHIA COLI* CYTOPLASMIC MEMBRANE

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Summary

Escherichia coli cells (unsaturated fatty acid auxotroph) have been adapted to grow on branched-chain fatty acids. Membrane vesicles were isolated from cells grown on a mixture of branched-chain fatty acids isolated from the lipids of *Bacillus subtilis* (*E. coli* (*B. subtilis*) membranes) and on a pure synthetic *anti*-isononadecanoic acid (*E. coli* (aC19) membranes).

We have shown, using wide-angle X-ray diffraction and differential scanning calorimetry, that the ordered state of the lipids is perturbed in the case of *E. coli* (*B. subtilis*) membranes but is unperturbed in the case of *E. coli* (aC19) membranes. The perturbation leads to the presence of a large wide-angle X-ray diffraction at 4.25—4.3 Å, as opposed to the presence of a sharp 4.2 Å reflection in unperturbed systems.

We have shown, using freeze-fracture electron microscopy, that a protein segregation exists in the case of *E. coli* (aC19) membranes (at low temperature the integral membrane proteins aggregate in the membrane domains containing the disordered lipids); we do not observe such segregation in the case of *E. coli* (*B. subtilis*) membranes. We conclude that in cases where the branching of the fatty acids introduces a perturbation of the lipid order, the integral membrane proteins can still be accommodated in membrane domains containing the 'perturbed' ordered lipids.

Finally, we have determined the rate of β-galactoside transport in *E. coli* (aC19) and *E. coli* (*B. subtilis*) membranes as a function of temperature. We have shown that, in both cases, the Arrhenius representations display an

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increased slope in the region of the disorder-to-order transition. We conclude that such an increased slope may have different origins. In the case of *E. coli* (aC19) membranes, it is the result of the aggregation of the β-galactoside carriers together with other integral membrane proteins which may lead to the inactivation of the carriers; in the case of *E. coli* (*B. subtilis*) membranes, it is the result of the partial immobilisation of the carriers embedded in a lipid environment, of which the fluidity, despite the perturbation of its lipid order, is still much less than that associated with lipids in a totally disordered state.

Introduction

Most biological membrane functions are controlled to some extent by the membrane structure (for a review, see Ref. 1). Currently, the best understood structural parameter is that describing the conformation of the hydrocarbon chains of the lipids [2]. So-called disorder-order conformational transitions may occur, during which the lipids change from a state in which the hydrocarbon chains are 'liquid-like' (disordered α-conformation) to one in which the chains are stiff and 'rigid-like' (ordered β-conformation) [2].

In natural membranes, due to the heterogeneity of the lipids and the presence of proteins and, in some cases cholesterol, only a fraction of the lipids takes part in the conformational transition. As a consequence, these transitions are accompanied by a lateral phase separation of the lipids leading to the coexistence of membrane domains containing ordered and disordered lipids, respectively [3]. In many cases, the lipid segregation is accompanied by a segregation of proteins in which the intrinsic membrane proteins are excluded from the ordered lipid domains and aggregated in the disordered lipid domains [3].

The transport of solute across membranes has frequently been shown to be sensitive to the disorder-to-order transition of the lipids, and discontinuities in the rate of transport as a function of temperature have been reported [4,5]. These discontinuities have been interpreted in various ways, in terms of the ordering of the lipids per se, or in terms of the protein segregation which accompanies the lipid segragation, or in terms of both [5—7].

Haest et al. [8] initially showed that in *Bacillus subtilis* and *Staphylococcus aureus* cells, the disorder-to-order transition of the membrane lipids takes place without concomitant segregation of the proteins. Since then, this phenomenon has been reported in other systems [9,10]. Haest et al. [8] proposed that the absence of the protein segregation was a result of the perturbation of the ordering of the lipids due to the large amount of branched-chain fatty acids present in these membranes. Membranes containing large amounts of phospholipids with branched-chain fatty acids in addition to linear fatty acids may also be obtained from mutants of *Escherichia coli* (unsaturated fatty acid auxotroph) adapted to grow on branched-chain fatty acids [11]. Clearly, such membranes are well suited to the study of the perturbation by branched-chain fatty acids of the disorder-to-order transition of membrane lipids and its effect upon solute transport under conditions in which the conformational transition is or is not accompanied by segregation of the proteins.

In this report, we demonstrate the relative importance of lipid ordering and protein segregation on β-galactoside transport in *E. coli*. The disorder-to-order transitions of the *E. coli* membrane lipids possessing various branched-chain fatty acids were characterized by wide-angle X-ray diffraction and differential scanning calorimetry. Freeze-fracture electron microscopy was used to determine the extent of protein segregation occurring during these transitions. Active transport of β-galactoside was found to be affected by both the ordering of the lipids and the segregation of the membrane proteins.

Material and Methods

Cell-growth conditions and membrane preparation. E. coli K 1059 ($i^+z^+y^+a^+$), an unsaturated fatty acid auxotroph unable to degrade or elongate unsaturated fatty acids [12], was grown on a Cohen-Rickenberg mineral-salt medium [13] supplemented with 0.4% glycerol, 0.3% casamino acids and 0.005% of exogenous fatty acids solubilized in 0.04% Brij 35. The strain was first adapted for growth on branched-chain fatty acids by successive precultures at 37°C, first with oleic acid as exogenous fatty acid (*cis* Δ^9, C18:1) and then with the total fatty acids extracted from the *B. subtilis* phospholipids. The adapted cells were then grown in the presence of either a well defined branched-chain fatty acid (here, *anti*-isononadecanoic acid, 16-Me-C18:0), or again in the presence of the total fatty acids extracted from *B. subtilis* phospholipids.

Membrane vesicles were prepared from whole cells according to the method of Kaback [14]. The vesicles are referred to as *E. coli* (aC19) membranes and *E. coli* (*B. subtilis*) membranes. For comparative purposes, membrane vesicles were prepared from *E. coli* cells grown on oleic acid and elaidic acid (*trans* Δ^9, C18:1) and from *B. subtilis* cells. These membrane vesicles are referred to as *E. coli* (oleate), *E. coli* (elaidate) and *B. subtilis* membranes, respectively.

Chemical composition. Lipid and protein compositions were determined as described previously [3].

Wide-angle X-ray diffraction. A Phillips generator was used as the X-ray source and was coupled with a Guinier-type camera operating under vacuum. The CuKα line (λ = 1.54 Å) was isolated and focussed by a bent-quartz monochromator.

Lipid/water samples were prepared by mixing equal amounts of water and dried lipids isolated from the membrane preparations. Although the transition characteristics of the lipids may depend on the nature of the solvent, we have shown previously that they are similar in *E. coli* membranes and in water-dissolved lipids [3]. Therefore, no attempt was made to adjust the ionic strength.

Differential scanning calorimetry (DSC). DSC experiments were performed with a highly sensitive DSC 111 Setaram calorimeter (minimum sensitivity 10 μW/mm) operating on the Tian-Calvet principle [15]. The heating rate was 5°C/min. 10—20 mg of the lipid/water mixtures (1 : 1, w/w) were sampled in 0.3 ml stainless-steel sealed cells.

Freeze-fracture electron microscopy. Membrane samples were prepared and the replicas were examined as described previously [3].

Transport. Thiomethylgalactoside ([^{14}C]methylthio-D-galactose, specific

activity 17.5 mCi/mmol, $8 \cdot 10^{-4}$ M final concentration) transport across the membrane vesicles as a function of temperature was determined according to the method of Kaback [14]. Initial rates were measured over the linear part of the uptake curves and the data reported here are the mean values of five independent experiments.

Results and Discussion

Incorporation of branched-chain fatty acids in phospholipids of E. coli membranes

Table I shows the fatty acid composition of the membrane phospholipids of adapted *E. coli* cells grown at 37°C on a mixture of branched-chain fatty acids (isolated from *B. subtilis* membranes) and on a pure branched-chain fatty acid (*anti*-isononadecanoic acid). In the former case, the branched-chain fatty acid composition is complex and reflects the heterogeneity of the exogenous fatty acids supplemented during the growth; in the latter case, the only significant branched-chain fatty acid found in the membrane phospholipids is that supplemented in the medium. In both cases, the amount of branched-chain fatty acid is approx. 60% of the total fatty acids, the remaining 40% being saturated fatty acids synthesized by the cells. Attempts to increase the incorporation of branched-chain fatty acid by shifting the growth temperature failed: whatever the growth temperature between 30 and 40°C, we observed no significant variation in the branched-chain fatty acid composition of the membranes (data not shown). The existence of a maximum in the amount of branched-chain fatty acids incorporated in the cells has been reported by Silbert et al. [11]. These authors have shown that in *E. coli*, branched-chain fatty acids, like *cis*-unsaturated fatty acids, are mainly incorporated in position 2 of the phospho-

TABLE I

FATTY ACID COMPOSITION OF *E. COLI* MEMBRANE ISOLATED FROM CELLS GROWN ON VARIOUS EXOGENOUS FATTY ACIDS

Values are expressed as percentages.

	E. coli supplemented with		
	B. subtilis fatty acids	*anti*-Isononadecanoic acid	Oleic acid
Fatty acid composition			
Exogenous	57	63	45
Saturated	41	34	53
Unknown	2	3	2
Different fatty acids species present			
iC15 + aC15	29	4	
iC17 + aC17	17	1	
aC19	11	58	
C14 : 0	2	5	17
C16 : 0	35	25	36
C18 : 0	4	4	
C18 : 1			45

lipids and that growth stops as the amount of phospholipids with two branched-chain fatty acids becomes significant.

Table I also shows, for comparative purposes, the fatty acid composition of the phospholipids of *E. coli* (oleate) membranes. At a growth temperature of 37°C, the exogenous fatty acid makes up 45% of the total fatty acids [3].

Structural characteristics of phospholipids with branched-chain fatty acids

Previous studies by Haest et al. [8] and Letellier [16] have shown that the wide-angle X-ray diffraction spectra of *B. subtilis* and *S. aureus* membranes are different from those of *E. coli* membranes (wild-type or unsaturated fatty acid auxotroph). At sufficient low temperatures, *E. coli* membranes display a sharp 4.2 Å reflection characteristic of the well ordered hydrocarbon chain of the lipids [2] while *B. subtilis* and *S. aureus* membranes display a wide 4.3 Å reflection. At sufficiently high temperatures, all membranes display a broad band centered at 4.5 Å characteristic of lipids with totally disordered hydrocarbon chains [2]. It has been postulated that the position and width of the reflection

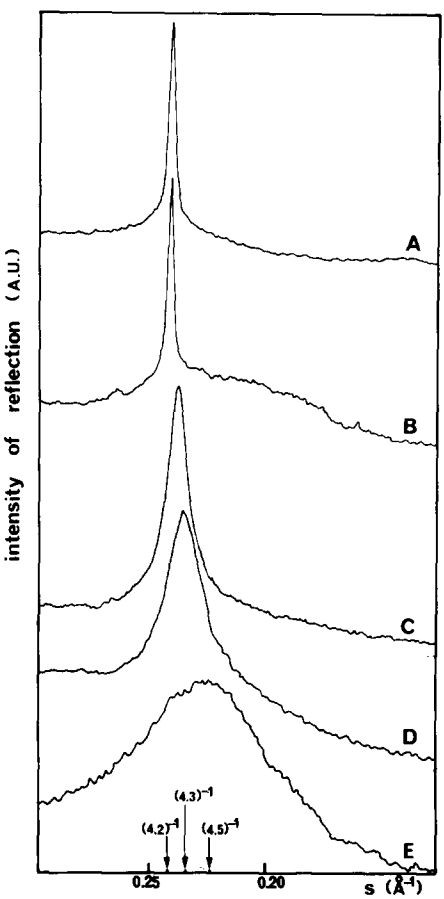


Fig. 1. Wide-angle X-ray diffraction of various total lipid extracts. (A) *E. coli* (oleate) membrane, (B) *E. coli* (aC19) membrane, (C) *E. coli* (*B. subtilis*) membrane, (D) *B. subtilis* membrane at a temperature below the transition. (E) *B. subtilis* membrane at a temperature above the transition. A.U., Arbitrary units.

at low temperatures of *B. subtilis* and *S. aureus* membranes, which is intermediate between those of *E. coli* membranes at low temperatures and those of all membranes at high temperatures, are a consequence of the perturbation of the lipid order due to the large amounts of branched-chain fatty acids present in the former two membranes [8].

The same type of structural perturbation can be observed in *E. coli* mutant membranes depending on the nature of the branched-chain fatty acid provided during growth of the cell. This is shown by the wide-angle X-ray diffraction spectra of the total lipid extracts of *E. coli* (*B. subtilis*) membranes and *E. coli* (aC19) membranes as compared with the total lipid extracts of *B. subtilis* membranes and *E. coli* (oleate) membranes (Fig. 1). At sufficiently high temperatures, all lipid extracts present a broad band centered at 4.5 Å (Fig. 1E). At sufficiently low temperatures, the lipid extract of *E. coli* (*B. subtilis*) membranes (Fig. 1C) presents a reflection, of which the position and breadth are intermediate between that of the lipid extract of *E. coli* (oleate) membrane (Fig. 1A) and that of the lipid extract of *B. subtilis* membranes (Fig. 1D). On the other hand, at sufficiently low temperatures, the lipid extract of *E. coli* (aC19) membranes (Fig. 1B) presents, like the lipid extract of *E. coli* (oleate) membranes, a sharp 4.2 Å reflection. Qualitatively similar wide-angle X-ray diffraction characteristics are observed for a given lipid extract and the corresponding membrane from which it has been extracted (data not shown). Thus, the breadth and position of the reflection are determined by the nature of the lipid hydrocarbon chains independently of the presence of the proteins.

The structural transition of the lipids from the conformational state characterized either by the sharp 4.2 Å reflection or the broader reflection at narrower angles (4.25—4.3 Å) to the conformational state characterized by the broad 4.5 Å band can be followed by DSC (Fig. 2). The DSC measurements of the lipid extracts as a function of increasing temperature show the presence of an endothermic transition characterized by its temperature range and its enthalpy of fusion (Table II). This transition is usually attributed to the 'melt-

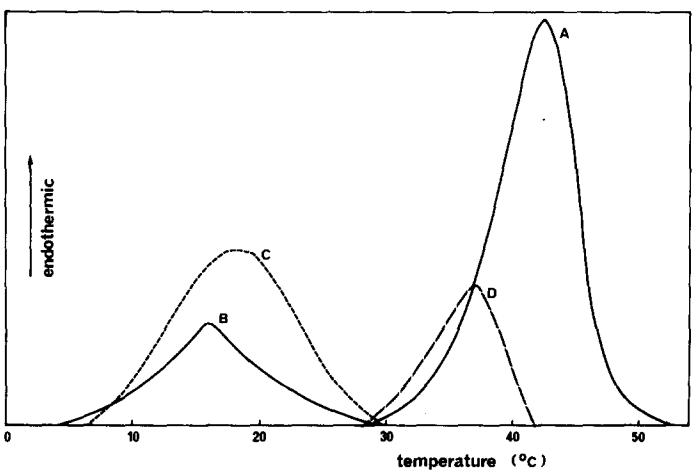


Fig. 2. Differential scanning calorimetry of various total lipid extracts. (A) *E. coli* (elaidate) membrane, (B) *B. subtilis* membrane, (C) *E. coli* (*B. subtilis*) membrane, (D) *E. coli* (aC19) membrane.

TABLE II

ORDER-DISORDER STRUCTURAL TRANSITION OF THE MEMBRANE PHOSPHOLIPIDS: DSC AND WIDE-ANGLE X-RAY DIFFRACTION DATA

Lipid extracts of *E. coli* membranes supplemented with the following fatty acid		Calorimetric data			Wide-angle X-ray diffraction data		
		Mid-transition temperature, T_t (°C)	Temperature range ΔT (°C)	Enthalpy of fushion ΔH (kJ/mol)	Below T_t		Above T_t, position (Å)
					Relative width *	Position (Å)	
B. subtilis fatty acids		18	6—30	20	3	4.25	4.5
aC19		35	28—42	4	1	4.20	4.5
oleic acid		21	11—32	13	1	4.19	4.5
Artificial mixtures of lipid extracts of *B. subtilis* and *E. coli* (elaidate) membranes							
B. subtilis (%)	*E. coli* (elaidate) (%)						
100	0	17	5—29	8	5.5	4.32	4.5
80	20	19	8—30	14		4.27	4.5
60	40	24	11—37	19		4.25	4.5
50	50	30	17—42	22		4.20	4.5
0	100	38	26—51	28	1	4.20	4.5

* The width is calculated at half-maximum intensity and is compared to that of the lipids of *E. coli* (elaidate) membranes.

ing' of the hydrocarbon chains from an ordered state at low temperature to a disordered state at high temperature [15].

We have tried to determine the nature and extent of the structural pertubations of the lipids due to the presence of branched-chain fatty acids by analyzing the order-disorder transition of artificial lipid systems formed by mixing branched-chain fatty acid-containing lipids with linear chain-containing lipids. We have taken as representative of branched-chain fatty acid-containing lipids those isolated from *B. subtilis* membranes, since over 90% of the fatty acids of their phospholipids are branched. The composition is approx. 50% *anti*-isopentadecanoate, 20% *anti*-isoheptadecanoate, 10% isopentadecanoate, 7% isoheptadecanoate, the remaining 10% being saturated (18:0 and 16:0) fatty acids [8]. Representative of linear saturated fatty acid-containing lipids are those isolated from *E. coli* (elaidate) membranes, elaidic acid being a *trans*-unsaturated fatty acid, of which the structural properties are similar to those of the saturated fatty acids. The composition is approx. 70% elaidic acid and 30% saturated (14:0 and 16:0) fatty acids [3]. We have analyzed by DSC and wide-angle X-ray diffraction the lipid extract of *B. subtilis* membranes, the lipid extract of *E. coli* (elaidate) membranes and three mixtures of these two lipid extracts containing 40, 60 and 80% of the former lipid extract, respectively.

Before analyzing the data in Table II, it should be stressed that independently of differences in fatty acid composition, *B. subtilis* and *E. coli* lipids differ in the nature of the phospholipids. While both are a mixture of phosphatidylglycerol, cardiolipin and phosphatidylethanolamine, *B. subtilis* is mainly composed of the two former lipids while *E. coli* is mainly composed of the latter. Although it cannot be excluded that differences in wide-angle X-ray diffraction and differential scanning calorimetry characteristics may be related to the difference in the nature of the phospholipids, it seems reasonable to assume that for these lipid mixtures the characteristics will be governed mainly by the difference in fatty acid composition.

The DSC parameters are quite different for the pure lipid extracts of *B. subtilis* and *E. coli* (elaidate) membranes (Table II and Fig. 2). The enthalpy of fusion of the lipid extract of *E. coli* (elaidate) membranes (Fig. 2A) is greater than that of the lipid extract of *B. subtilis* membranes (Fig. 2B). The experimentally observed enthalpy of fusion is a combination of the number of lipid molecules taking part in the order-disorder transition and the effective intrinsic enthalpy of fusion of these lipid molecules. We have shown elsewhere [3] that all the hydrocarbon chains of the total lipid extract of *E. coli* (elaidate) membranes participate in the order-disorder transition. Thus, the experimentally observed enthalpy of fusion of 28 kJ/mol reflects the intrinsic enthalpy of fusion of these lipid molecules. The enthalpy of fusion of the lipid extract of *B. subtilis* membranes is 8 kJ/mol. This may indicate that only a fraction of the lipids participate in the transition or that the intrinsic enthalpy of fusion of these lipids is smaller than that of the lipids of *E. coli* (elaidate) membranes, or both. Although no experimental data are available regarding the intrinsic enthalpy of fusion of lipids with branched-chain fatty acids, it seems reasonable to assume, in view of the perturbation of the lipid order introduced by the branching, that their enthalpy of fusion is smaller than that of lipids with linear fatty acids. We may thus conclude that a large fraction of the lipid extract of *B. subtilis* mem-

branes participates in the order-disorder transition. The DSC parameters of the three mixed systems are intermediate between those of the two extremes (see data in Table II). Within the limits of our experimental precision, we did not observe the simultaneous presence of the transitions associated with the two extreme systems (data not shown). This suggests, that in the mixtures, the different lipid species mix within a single phase.

The wide-angle X-ray diffraction data of the artificial lipid systems are shown in Table II. As already mentioned, the diffraction spectra at low temperatures are different for the lipid extracts of *B. subtilis* and *E. coli* (elaidate) membranes; the former system displays a broad 4.3 Å reflection, the latter system displays a sharp 4.2 Å reflection. The characteristics of the diffraction pattern of the mixed systems are intermediate between those of the two extremes; as the amount of branched-chain fatty acid increases, the reflection broadens and is displaced from 4.2 to 4.3 Å. Within our experimental limits we did not observe the simultaneous presence of the 4.2 and 4.3 Å reflections in any case.

The wide-angle X-ray diffraction and DSC data for the natural lipid systems isolated from the various membranes, *E. coli* (*B. subtilis*), *E. coli* (aC19) and *E. coli* (oleate), may be analyzed in terms of the results obtained for the artificial lipid systems.

The lipids of *E. coli* (*B. subtilis*) possess about 40% linear saturated fatty acids and about 60% branched-chain fatty acids, of which the composition is reminescent of that of the lipids of *B. subtilis* membranes. Thus, this natural lipid system has a fatty acid composition similar to that of the artificial lipid system formed by the mixture of the lipids of *B. subtilis* membranes (60%) and *E. coli* (elaidate) membranes (40%). Indeed, we observed that both systems have similar calorimetric and wide-angle X-ray diffraction parameters, particularly a broad wide-angle X-ray diffraction reflection at low temperature situated between 4.2 and 4.3 Å (see Table II).

The lipids of *E. coli* (aC19) membranes possess, like those of *E. coli* (*B. subtilis*) membranes, about 60% branched-chain and 40% linear fatty acids. In contrast, however, this system presents, at temperatures below the transition, a sharp 4.2 Å reflection reminiscent of that of the lipids containing only linear fatty acid (see Table II). This may be explained by the location of the branching on the fatty acid occurring near the methyl end of the chain which is, in addition, the longest fatty acid chain. In most natural systems, in order to accommodate fatty acids with various chain lengths, there exists a highly disordered layer in the middle of the lipid leaflet; this layer remains disordered even at temperatures below the transition [2]. In the lipids of *E. coli* (aC19) membranes, the branching occurs in this region and thus will not introduce a perturbation in the ordering of the lipids. The enthalpy of fusion of this lipid system is very low (4 kJ/mol). Since there is no structural perturbation, this small value must be accounted for by the existence of a large fraction of the lipid which does not participate in the transition. This is substantiated by the wide-angle X-ray diffraction spectra at low temperatures which display together with the 4.2 Å sharp reflection a broad 4.5 Å band characteristic of disordered hydrocarbon chains (see Fig. 1B).

The lipids of *E. coli* (oleate) membranes present at low temperature a sharp

4.2 Å reflection which is expected, since this system contains only linear fatty acids (see Fig. 1A). The enthalpy of fusion is 13 kJ/mol. We have shown elsewhere that in this system, 50% of the lipids participate in the order-disorder transition [3]. Thus, the intrinsic enthalpy of fusion of these lipids is 26 kJ/mol, similar to that of the lipids of *E. coli* (elaidate) membranes.

Morphological characteristics of E. coli ((B. subtilis), aC19 and oleate) membranes

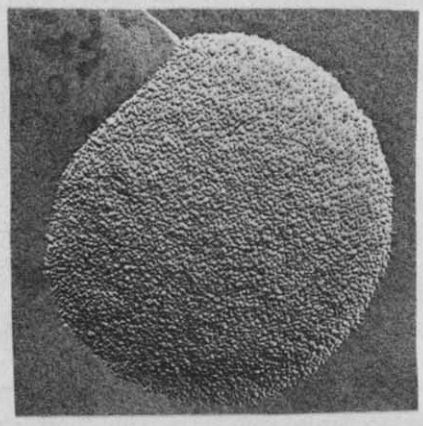
We have reported previously the morphological characteristics as shown by freeze-fracture electron microscopy of *E. coli* membranes isolated from cells grown on various linear unsaturated fatty acids [3].

The freeze-fracture electron micrographs of *E. coli* (oleate) membranes are shown in Fig. 3. At temperatures above the transition of the lipids (30°C), the freeze-fracture surfaces display a homogeneous and random distribution of particles (Fig. 3A) representing the intrinsic membrane proteins which become exposed during the fracturing [3]. At temperatures below the transition of the lipids (0°C), the freeze-fracture surfaces display a mixture of smooth surfaces devoid of particles separated by surfaces containing aggregated particles (Fig. 3B). The interpretation widely accepted is that the intrinsic proteins, which are excluded from the ordered lipid containing membrane domains, aggregate preferentially in the membrane domains containing the disordered lipids [3].

In contrast, as for *B. subtilis* and *S. aureus* membranes, *E. coli* membranes may display a lack of protein segregation (evidenced by fracture surfaces with a homogeneous distribution of particles) at temperatures below the transition depending on the nature of the branched-chain fatty acid provided during the growth of the cell. At temperatures above the phase transition of the lipids, the fracture surfaces of *E. coli* (*B. subtilis*) and *E. coli* (aC19) membranes present a statistical and homogeneous distribution of particles (data not shown). At temperatures below the transition, the fracture surfaces of *E. coli* (aC19) membranes present, like those of *E. coli* (oleate) membranes, a mixture of smooth surfaces devoid of particles and surfaces where the particles are aggregated (Fig. 3C). However, the fracture surfaces of *E. coli* (*B. subtilis*) membranes present a homogeneous and statistical distribution of particles similar to that observed at temperatures above the transition (Fig. 3D).

These data are easily explained in terms of the perturbation of the lipid order introduced by the branching of the fatty acids. Since *anti*-isononadecanoic acid does not perturb the lipid order at low temperatures, as evidenced by wide-angle X-ray diffraction, one expects for *E. coli* (aC19) membranes (like for *E. coli* (oleate) membranes), at temperatures below the phase transition, the exclusion of intrinsic proteins from the ordered lipid domains of the membrane (appearance of smooth fracture surfaces) and their aggregation in the disordered lipid membrane regions (appearance of fracture surfaces highly particulated). It should be mentioned, as shown previously, that the size of the

Fig. 3. Freeze-fracture electron microscopy of membrane vesicles. (A) *E. coli* (oleate) membrane above the transition temperature, (B) *E. coli* (oleate) membrane below the transition temperatre, (C) *E. coli* (aC19) membrane below the transition temperature, (D) *E. coli* (*B. subtilis*) membrane below the transition temperature. Magnification: A, ×57 750; B, ×69 300; C, ×103 950; D, 57 750.



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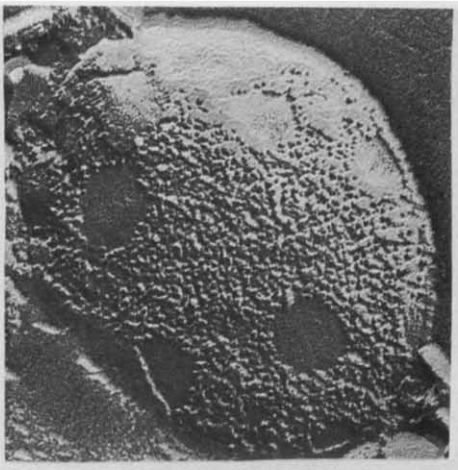

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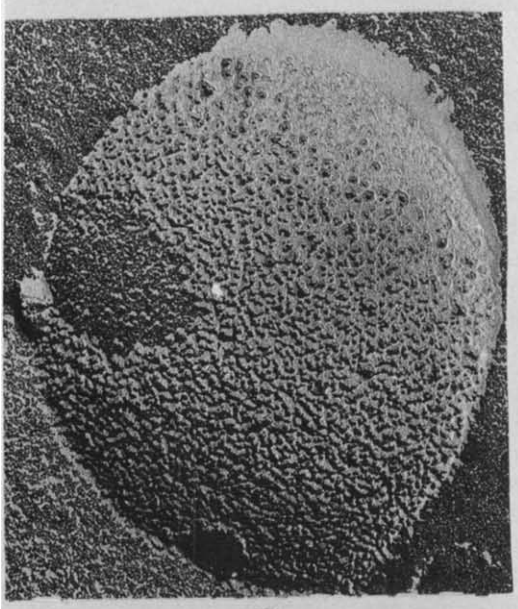

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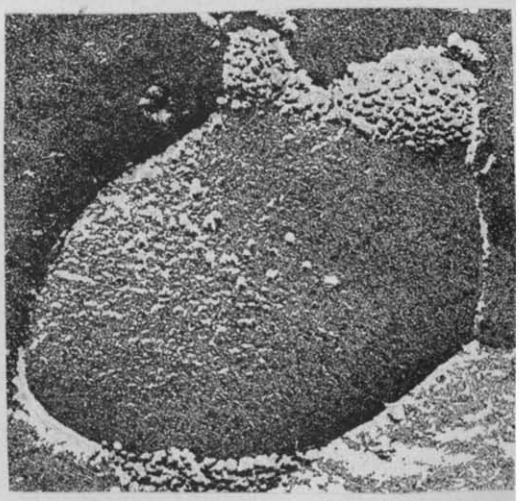

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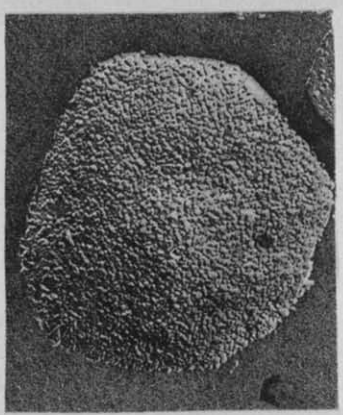

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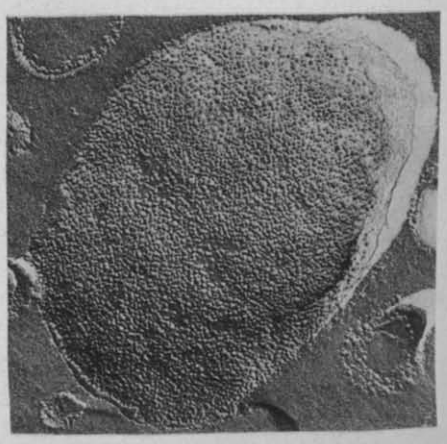

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individual smooth fracture surfaces is independent of the amount of ordered hydrocarbon chains [3]. A small fraction of the lipids of *E. coli* (aC19) membranes is ordered at temperature below the phase transition, yet the smooth fracture surfaces are very large and extend over a large portion of the fracture. In contrast, wide-angle X-ray diffraction demonstrates a perturbation of the *E. coli* (*B. subtilis*) membrane lipid order at temperatures below the transition. The lack of intrinsic protein segregation (absence of appearance of smooth freeze-fracture surfaces) must be related to these perturbations. Possibly, the perturbations lead to a weakening of the interactions between adjacent lipid molecules as compared to the interactions prevailing at temperatures below the transition in systems containing only linear fatty acids; as a consequence, intrinsic membrane proteins may still be accommodated in the membrane regions possessing this perturbed lipid order.

Temperature dependence of β-galactoside transport by E. coli ((B. subtilis), aC19 and oleate) membranes

E. coli membrane vesicles (like whole *E. coli* cells) are able to convert oxidative energy (oxidation of electron donors) into electrochemical energy in the form of a proton gradient across the membrane [18]. The dissipation of this gradient is in turn coupled to the active transport of a variety of solutes via specific membrane carrier proteins [18]. The study of this transport as a function of temperature in the case of β-galactoside (via the lactose carrier) has shown that the transport depends on the conformational state of the lipids [5,6]. The Arrhenius plots for β-galactoside transport are biphasic, the change in slope occurring at a temperature that correlates well with the mid-order-to-disorder transition of the lipids [5,6]. The more pronounced slope at low temperatures was originally presumed to reflect the increase in the energy of activation of transport as a fraction of the membrane lipids becomes ordered [3]. However, more recently, it has been shown that the Arrhenius plots of transport are in fact triphasic and that at temperatures below the transition the slope again assumes a smaller value similar to that observed in regions above the transition temperature [5]. Thus, the original interpretation is no longer applicable in these cases. It has been proposed that the steep intermediate part of the Arrhenius plot, extending over the order-disorder transition temperature range, results from a redistribution of the membrane carriers (lactose carrier) between the membrane domains containing the ordered and disordered lipids. The redistribution of the carriers (exclusion from the membrane domains containing the ordered lipids) and their concomitant aggregation in the membrane domains containing the disordered lipids alter the rate of transport either by affecting (decrease) the frequency factor of the rate constant of the rate-limiting step [5] or by affecting the number of effective functional carriers as their aggregation with other intrinsic membrane proteins may inactivate a fraction of the lactose carriers [6].

A comparison of β-galactoside transport as a function of temperature in *E. coli* (aC19) and *E. coli* (*B. subtilis*) membranes is useful to understand the Arrhenius plots, since in the former case the lipid phase transition is accompanied by a protein segregation similar to that observed for *E. coli* (oleate) membranes while in the latter case there is no protein segregation associated with

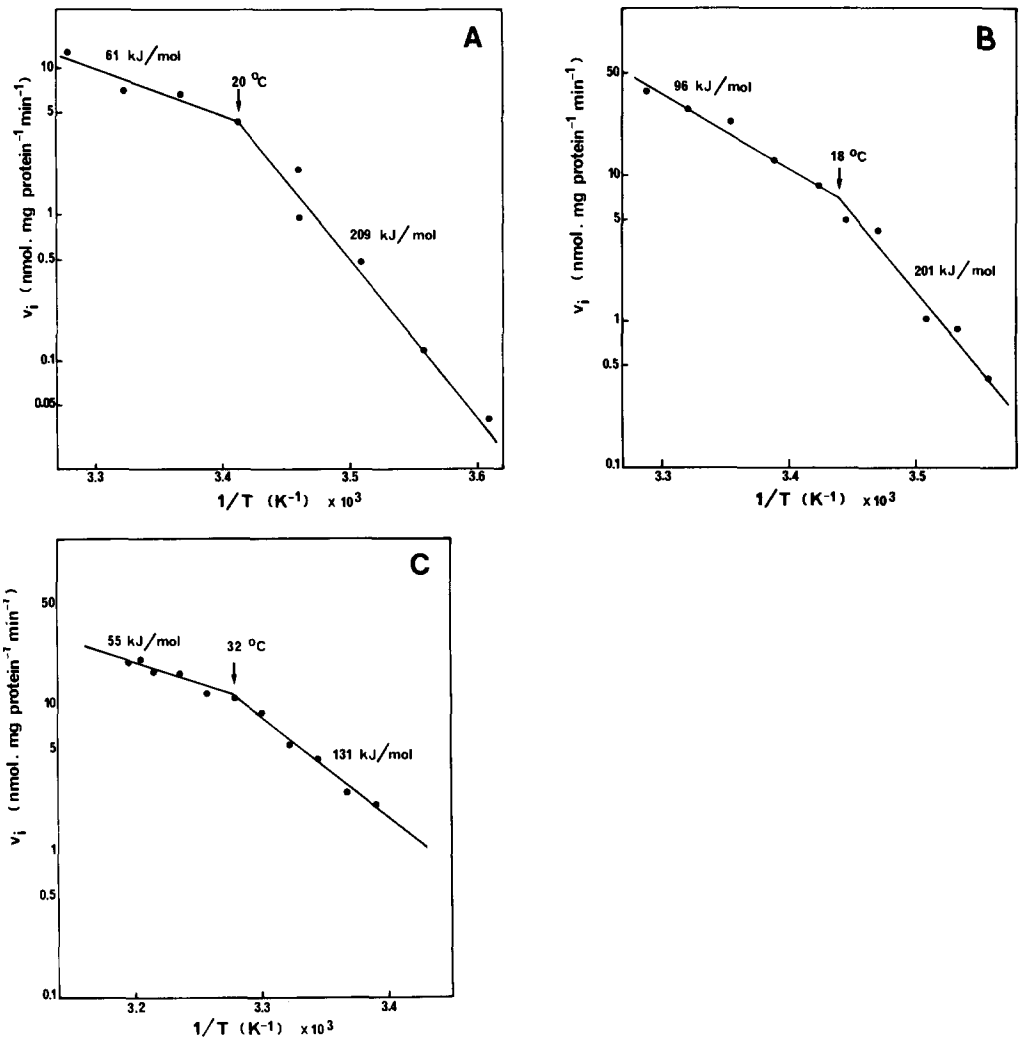

Fig. 4. Arrhenius representation of thiomethylgalactoside transport in *E. coli* (oleate) membrane (A), *E. coli* (*B. subtilis*) membrane (B) and *E. coli* (aC19) membrane (C).

the lipid phase transition. The Arrhenius representations of β-galactoside transport in *E. coli* (*B. subtilis*) membranes, *E. coli* (aC19) membranes, and, for comparison, *E. coli* (oleate) membranes, are shown in Fig. 4A, B and C, respectively. Similar behaviour is observed in all three cases; the Arrhenius plots display a steeper slope in the low temperature range extending through the order-disorder transition.

E. coli membrane vesicles are more permeable to solutes that whole cells [3]. This permeability depends on the nature of the fatty acid and is particylarly conspicuous at temperatures below the transition. Thus, in the case of *E. coli* (aC19) membranes at low temperature, the uptake is linear with time for only 5—10 s (data not shown). Since the initial rates of uptake are determined over the linear portion of the uptake curve, this will limit the measurements of

initial rates at low temperature. It is, therefore, not possible in the case of membrane vesicles to extend the uptake studies at temperatures well below the transition temperature and to detect an eventual triphasic Arrhenius plot.

In the case of *E. coli* (aC19) membranes, there exists a protein segregation accompanying the lipid phase transition and thus the increased slope in the temperature range of the transition can be explained (as for *E. coli* (oleate) membranes) in terms of a change in the rate of transport as a result of the redistribution and the aggregation of the lactose carrier in the membrane. On the other hand, in the case of *E. coli* (*B. subtilis*) membranes, no protein segregation takes place during the phase transition of the lipids and the lactose carriers probably remain associated with the lipids, of which the order has been perturbed by the existence of the branched-chain fatty acids. Therefore, the increased slope cannot be attributed to the redistribution of the carrier. In this case, at low temperature, the carrier is in a lipid environment, of which the fluidity, despite the structural perturbation, is much less than that of totally disordered lipid prevailing at temperatures above the transition. This decreased fluidity may hinder the movement and/or the conformational change of the lactose carrier which takes place during the translocations of β-galactosides. The increased slope in the temperature range of the transition may truly represent here an increase in the energy of activation of transport.

Conclusions

The present study shows that the incorporation of branched-chain fatty acids in the membrane phospholipids of *E. coli* cells may result in structural and morphological characteristics which are different from those prevailing in *E. coli* cells, of which the membrane phospholipids possess only linear fatty acids.

The structural differences are conspicuous primarily at low temperatures where the branching, as a result of steric hindrances, introduces a perturbation of the ordered state of the lipids as demonstrated by wide-angle X-ray diffraction. The existence of such perturbations depends on the nature of the branched-chain fatty acids, none being observed when the branching occurs in a portion of the fatty acid chain located in the middle of the lipid leaflet, a membrane region which is anyway highly disordered [2].

The perturbation of the lipid order results in a situation where integral membrane proteins are not excluded at low temperatures from the membrane domains containing these ordered lipids. This is in opposition to the large segregation of integral membrane proteins which accompanies the disorder-to-order transition of membrane lipids in the absence of perturbation [3]. Most probably, the segregation of integral proteins, when observed at low temperatures, is the result of an increase in lipid-lipid interactions at the expense of lipid-protein interactions; when the ordered state of the lipids is perturbed, there is a weakening of the lipid-lipid interactions and integral membrane protein may still be accommodated in membrane domains containing these ordered lipids.

Interestingly, active transport of β-galactosides responds to the disorder-to-order transition of the membrane lipids in the same way, whether or not the

transition is accompanied by a segregation of the integral membrane protein, i.e., by an increase in the slope of the Arrhenius representation. We are led to conclude that this increase in slope, which is observed for numerous membrane functions associated with integral membrane proteins, may have different origins, either an aggregation of membrane proteins, or a partial immobilization of the same proteins remaining embedded in a medium, of which the fluidity is intermediate between that characteristic of totaly disordered and totally ordered lipids.

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