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Correlation of *In Vivo* and *In Vitro* Phase Transitions of Membrane Lipids in *Escherichia coli*

P. Overath, H. U. Schairer, and W. Stoffel

INSTITUT FÜR GENETIK UND PHYSIOLOGISCH CHEMISCHES INSTITUT DER UNIVERSITÄT KÖLN, KÖLN-LINDENTHAL, GERMANY

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Abstract. A double mutant of *Escherichia coli* unable to synthesize or degrade unsaturated fatty acids can incorporate fatty acids with various hydrocarbon chain structures into the membrane phospholipids. The temperature characteristic of three physiological properties of cells grown with different fatty acids (growth, respiration, and efflux of thiomethylgalactoside) is compared with the physical properties of the isolated phosphatidylethanolamines in monolayers at an air–water interface. Breaks in the temperature characteristic of the properties measured *in vivo* correspond to phase transitions in the lipid films from a liquid-expanded to a condensed form. It is concluded that a liquid-like state of the lipid phase is required for proper membrane function.

The isolation of mutants of *Escherichia coli* that cannot synthesize unsaturated fatty acids[1] made possible a new approach to the functional role played by the hydrocarbon chains in membrane phospholipids. The incorporation of a variety of fatty acids into the lipid[2-4] shows that the proper function of the membrane is not prevented by rather severe alterations in the hydrocarbon chain structure. Extensive investigations[5,6] on the physical properties of phospholipids and artificial model membranes have emphasized the notion that the fatty acid chains in membranes are probably in a disordered, liquid-like state. The fluidity of the phospholipids depends on the interaction energy of the hydrocarbon chains, which in turn is a consequence of their structure and the temperature. Insight into the physical state of the fatty acid chains in the membrane might be obtained by comparing the temperature dependence of various membrane-associated functions with the physical properties of the isolated lipids in suitable model systems.

In a previous communication[3] the rate of uptake of thiomethyl-β-galactoside (TMG) into *E. coli* cells was measured in the temperature range between 15 and 45°C. A break in the temperature characteristic in cells containing *trans*-unsaturated fatty acids suggested that the hydrocarbon chains undergo a phase transition from an expanded to a more condensed form at approximately 30°C. By measuring the transport of *o*-nitrophenyl-β-galactoside and *p*-nitrophenyl-β-glucoside Wilson *et al.*[7] obtained similar changes at 13 and 7°C for cells grown with oleic acid and linoleic acid, respectively. Moreover, phase transitions have been shown to occur in membranes of *Mycoplasma laidlawii* by the use of differen-

tial scanning colorimetry[8] and by x-ray diffraction.[9] In this paper an attempt is made to relate the changes in the temperature characteristic of *in vivo* parameters to the physical properties of the isolated phospholipids in monolayers at an air–water interface.

Materials and Methods. Bacterial strains and media: Strain K1059 is a derivative of strain K1001 described previously.[3] Since the lesion in strain K1001 maps near the *dsd* locus[3] (presumably[10] between the loci *aroC* and *purF*), it is of the *fabB* type[11] and its enzymatic defect in the synthesis of unsaturated fatty acids is unknown. (For gene designations see note on Abbreviations.) A requirement for proline was induced in strain K1001 by nitrosoguanidine mutagenesis and penicillin selection. Into this strain the mutation *old-62* from strain K19 was introduced by cotransduction with bacteriophage P1 and analysis of the *pro*$^+$ recombinants for the unselected *old* trait. *old-62* is a derivative of the prototrophic strain K12Ymel,[12] which is unable to convert the C_1 atom of oleate to CO_2 and is cotransducible with the *proB* locus.[13] The double mutant K1059 obtained in this way can therefore neither synthesize nor degrade unsaturated fatty acids. K1059, like the parent K1001, is constitutive for the synthesis of the enzymes of lactose degradation.

K1059 was grown in Cohen–Rickenberg (CR)–mineral salts medium[14] supplemented with 0.5% glycerol, 0.3% casamino acids (Difco, vitamin-free), 2% Brij 35 (polyethyleneglycol monolauryl ether) and 0.02% fatty acids. Cells were grown at 37°C with *cis*-18:1 and 19:0[15] and at 30 and 40°C with 18:3 and *trans*-18:1, respectively (fatty acids are designated by no. of carbon atoms:no. of double bonds). For the growth studies (Fig. 1) exponentially growing cells were shifted to the desired temperature and the turbidity was measured for several generations.

Respiration experiments: The cells were grown overnight to exponential phase (*A* at 420 nm about 2) and harvested and washed with CR-buffer. They were starved in CR for 3 hr by shaking (at 40°C for cells grown with *trans*-18:1, at 37°C for *cis*-18:1 and 19:0 and at 30°C for 18:3), washed by centrifugation, and resuspended in CR at an absorbance (at 420 nm) of 20–40. Respiration was measured with a Clark oxygen electrode (Rank Bros., Bottisham, England) in the presence of 0.27 M glycerol as substrate.

TMG efflux: The cells were incubated in growth medium containing 1.2 mM [^{14}C] TMG (0.5 Ci/mole; 40 min at 32°C for cells grown with *trans*-18:1 and 30 min at 25°C for cells grown with the other fatty acids). 0.5-ml samples were centrifuged and the pellet was resuspended in 55 ml of CR buffer, prewarmed to the desired temperature. Nine 5-ml samples were pipetted at appropriate time intervals on to membrane filters covered with 5 ml of CR. A tenth sample was incubated for about 45 min at 37°C and was used to correct the other samples for labeled sugar not available for the efflux.

Results. The double mutant K1059 is unable to synthesize and to degrade unsaturated fatty acids. Table 1 summarizes the fatty acid composition of the major membrane phospholipid of *E. coli*, phosphatidylethanolamine. Since K1059 cannot degrade the C_{18} fatty acids added to the medium, no C_{16} derivatives appear in the lipid. The amount of *cis*-18:1, *trans*-18:1, and 19:0 cyclopropane acid incorporated is quite similar in K1059 and K1001.[3] 18:3 is incorporated more extensively into K1059 (46%) than into K1001 (25%). As noted by others[2,4] the ratio of saturated to unsaturated fatty acids varies considerably. Moreover, the chain length of the saturated fatty acids incorporated into the phospholipid is influenced by the fatty acid added to the medium. Both effects indicate that so far unspecified control mechanisms operate in the cell which tend to maintain the physical properties of phospholipids within certain limits.

TABLE 1. *Fatty acid composition (%) of phosphatidylethanolamines from E. coli mutant grown with various fatty acids.*

	Fatty acid supplemented			
	cis-18:1	*trans*-18:1	19:0	18:3
16:0	22	6	27	50
14:0	6	11	17	3
12:0	...	2	1	...
cis-18:1	58	...	...	...
19:0	14	...	54	...
trans-18:1	...	81	...	...
18:3	...	...	...	46
Unidentified	...	<1	2	1
$\frac{16:0}{14:0}$	3.7	0.55	1.6	17
Ratio* of fatty acids	0.39	0.23	0.83	1.2

* Sum of the saturated fatty acids divided by the sum of the unsaturated and the cyclopropane derivatives. Phosphatidylethanolamine was isolated[16,17] from cells grown to late exponential phase.

It was essential for both practical and theoretical reasons to study the temperature conditions that allow a fatty acid to serve as a growth factor. The Arrhenius plots of reciprocal temperature against the logarithm of the relative growth rate given in Fig. 1 reveal the following properties. (1) The minimum temperature of growth is about 10°C when the medium is supplemented with *cis*-18:1, 18:2 (not shown), and 18:3. It is increased to 22 and 37°C when the cells grow with 19:0 and *trans*-18:1, respectively. (2) The optimal rate of growth is similar for cells grown with *cis*-18:1 (doubling time 57 min at 38°C), 19:0 (65 min at 38°C), and *trans*-18:1 (70 min at 40°C). The optimum was shifted to 37°C (generation time 82 min) and 36°C (95 min) when the growth medium was supplemented with 18:2 and 18:3, respectively. (3) Growth is observed up to a maximum temperature of 44–45°C for *cis*-18:1, *trans*-18:1, and 19:1 and about 40°C for 18:2 and 18.3. (4) In the temperature range 20–30°C a temperature characteristic μ of 15.2 kcal/mol can be calculated for *cis*-18:1, 18:2, and 18.3.

The growth properties of K1059 with *cis*-18:1 are identical to those of wild type *E. coli*.[18] The argument[19] that the lipid composition of *E. coli* does not define the minimal temperature of growth is supported by the identical behavior of cells grown with *cis*-18:1 and 18:3. In the case of *trans*-18:1 and 19:0 the hydrocarbon chain composition clearly determines the minimal temperature of growth. The maximal temperature for growth of wild type *E. coli* is likewise not determined by the properties of the hydrocarbon chains of the membrane lipids. A shift to higher temperatures might otherwise have been expected with cells containing *trans*-18:1. The lowering of the maximum to 40°C in cells containing 18:3 indicates that there is an upper limit of fluidity of phospholipid compatible with growth.

Since phospholipids are known to be required for the activity of the electron-transport systems of mitochondria[20] and of *E. coli*[21] the influence of the hydrocarbon chain composition of phospholipids on the rate of respiration with glycerol as substrate was investigated (Fig. 2). The curves for 18:3 and 19:0 do not have any characteristic breaks in the temperature range between 3 and about

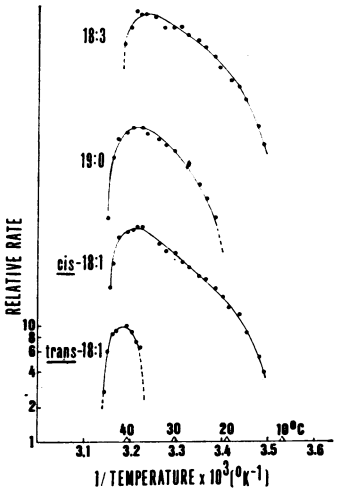

FIG. 1. Arrhenius plot of relative growth rates. The growth rates (number of generations per hour) were normalized to a temperature of 40°C. The doubling times in minutes at this temperature are: *cis*-18:1, 63; 18:3, 175; *trans*-18:1, 70; 19:0, 70.

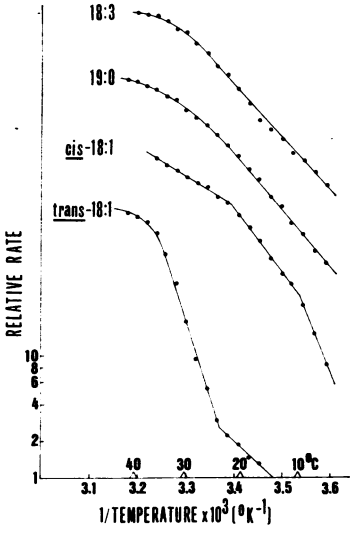

FIG. 2. Arrhenius plot of relative rates of respiration. The rates of oxygen consumption were normalized to a temperature of 36°C. Absolute rates of respiration at 36°C in μg O_2/mg protein/min: *cis*-18:1, 24.8; 18:3, 25.6; *trans*-18:1, 9.5; 19:0, 29.2.

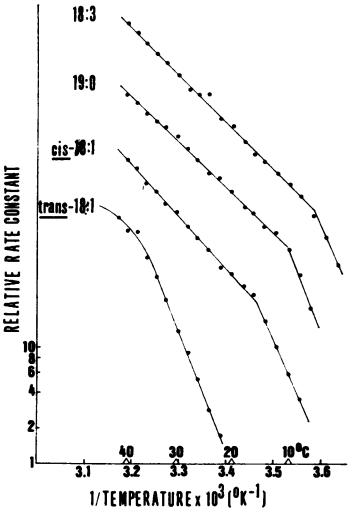

FIG. 3. Arrhenius plot of relative rate constants of [^{14}C]TMG efflux. The figure gives rate constants normalized to a temperature of 40°C. The rate constants in min^{-1} at this temperature are: *cis*-18:1, 1.35; 18:3, 1.47; *trans*-18:1, 1.65; 19:0, 1.0.

30°C (μ = 21 kcal/mol). Cells containing *cis*-18:1 show changes in the temperature characteristic at 22 and 10°C. A very sharp transition occurs with cells grown with *trans*-18:1 at 36°C. After a steep fall (μ = 58 kcal/mol) down to about 24°C, a μ of 16 kcal/mol is observed at very low rates of respiration. Since glycerol-grown cells take up glycerol extremely rapidly,[22] permeation is probably not rate-limiting in the overall process. However, permeation can be

the rate-limiting step in respiration when glucose is used as a substrate: the ratio of glycerol- to glucose-stimulated respiration is 50–100 in *cis*-18:1-grown cells between 3 and 7°C. This ratio changes to a value of 2 between 7 and 20°C and remains constant at higher temperatures.

The efflux of [^{14}C]TMG from *E. coli* cells is independent of the supply of metabolic energy and is known to be highly temperature-dependent.[23–25] Fig. 3 demonstrates that this process gives well-defined breaks in the temperature characteristic at 38, 15, 10, and 6°C for *trans*-18:1-, *cis*-18:1, 19:0-, and 18:3-containing cells respectively. Above the transition points, a μ of 17–20 kcal/mol can be calculated; below the breaks, values of 42–46 kcal/mol are found. The increase in the transition point for *trans*-18:1-grown cells, from 25 to 30°C for [^{14}C]TMG uptake in K1001[3] to 38°C for the efflux in K1059, is probably caused by the absence of degradation products of *trans*-18:1 in the lipid. The exit experiments clearly indicate that the membrane becomes impermeable at low temperatures.

The physical properties of phosphatidylethanolamines shown in Table 1 were determined with a Langmuir balance.[26] Force/area isotherms of phospholipid films provide information on the arrangement of the hydrocarbon chains at an air–water interface.[27] Fig. 4 shows that all four lipids give phase transitions from the liquid-expanded to a more condensed state that are highly dependent on

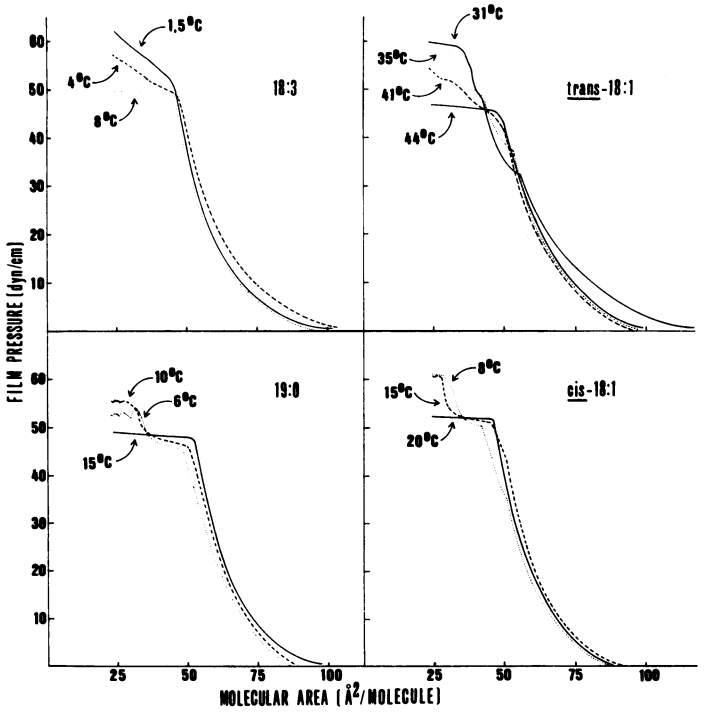

FIG. 4. Force–area isotherms of phosphatidylethanolamine, measured as described in Ref. 28. The temperature of the air above the lipid film was kept constant by a thermostated cover.

the temperature. Above 8, 15, 20, and 44°C only liquid-expanded films are observed for phosphatidylethanolamine containing 18:3, 19:0, *cis*-18:1, and *trans*-18:1, respectively. Below these temperatures condensed phases appear. The 18:3-containing lipid gives a condensed phase around 4°C. In 19:0 containing lipid films a transition first appears at about 10°C, in *trans*-18:1-containing lipid at about 41°C. At 35 and 31°C a transition from the liquid-expanded to the liquid-condensed state is observed with *trans*-18:1-containing phosphatidylethanolamine at a film pressure of 37 and 32 dynes/cm, respectively; another transition to the condensed state occurs at 48–49 dynes/cm. A similar behavior is observed with the *cis*-18:1-containing phosphatidylethanolamine at 15 and 8°C. Unfortunately, the arrangement of the hydrocarbon chains between the liquid-expanded and the condensed, solid state is poorly understood at the present time. The collapse point of the expanded films always occurs at a molecular area of about 50 Å²/molecule and at a pressure of 50 dynes/cm.

Discussion. Table 2 summarizes the critical temperatures related to the

TABLE 2. *Correlation of in vivo and in vitro transition points.*

	Fatty acid incorporated into phospholipid			
	18:3	19:0	*trans*-18:1	*cis*-18:1
Growth	...	22°C	37°C	...
Respiration	...	...	36°C	10°C
Efflux of [^{14}C]-TMG	6°C	10°C	38°C	15°C
Appearance of phase transition in monolayers	4°C	10°C	41°C	15°C

hydrocarbon chain composition of the phospholipids. The transitions from the liquid-expanded to the condensed state(s) occur in the same temperature range as the breaks in the temperature characteristic of [^{14}C]TMG exit. This correlation extends with *trans*-18:1 to the breaks in the temperature dependence of growth and respiration. The transition in respiration for *cis*-18:1-containing lipids at 10°C might also be caused by the hydrocarbon chain composition. Similar transitions in the rate of respiration for 19:0- and 18:3-containing cells might occur below 4°C. The cessation of growth of 19:0-grown cells at 22°C cannot be explained from the monolayer studies. Thus, it appears that transport processes are most sensitive to changes in the packing properties of the hydrocarbon chains.

The results indicate that there is lipid–lipid interaction in the membrane which influences the function of membrane-bound proteins and that proper membrane function requires mobile hydrocarbon chains in the lipid. Moreover, the force–area isotherms suggest that a lipid molecule in the membrane occupies a molecular area of about 50 Å²/molecule. The incorporation of a *trans* fatty acid into the lipid might provide a more general tool for revealing lipid–protein interaction in membranes.

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Abbreviations: TMG, thiomethyl-β-D-galactoside; *dsd, old, fab, aro, pur*, genes involved in the breakdown of D-serine and oleate and the synthesis of unsaturated fatty acids, aromatic amino acids, and purines, respectively; 18:3, *cis,cis,cis*-$\Delta^{9,12,15}$-octadecatrienoic acid; 19:0-DL-*cis*-9,10-methylene-octadecanoic acid; *cis*-18:1, *cis*-Δ^{9}-octadecenoic acid; *trans*-18:1, *trans*, Δ^{9}-octadecenoic acid; 18:2, *cis,cis*-$\Delta^{9,12}$-octadecadienoic acid.

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