Biochimica et Biophysica Acta, 512 (1978) 472—479

BBA 78148

AN ESTIMATE OF THE MINIMUM AMOUNT OF FLUID LIPID REQUIRED FOR THE GROWTH OF *ESCHERICHIA COLI*

MEYER B. JACKSON and JOHN E. CRONAN, Jr.

Departments of Molecular Biophysics and Biochemistry and of Chemistry, Yale University, New Haven, Conn. (U.S.A.)

(Received March 17th, 1978)

Summary

The lipid phase transition of *Escherichia coli* was studied by high sensitivity differential scanning calorimetry. A temperature sensitive unsaturated fatty acid auxotroph was used to obtain lipids with subnormal unsaturated fatty acid contents. From these studies it was concluded that *E. coli* can grow normally with as much as 20% of its membrane lipids in the ordered state but that if more than 55% of the lipids are ordered, growth ceases. Studies with wild-type cells show that the phase transition ends more than 10°C below the growth temperature when the growth temperature is either 25°C or 37°C.

It has become well established that synthetic phospholipids and the lipids of cell membranes can exist in two states at physiological temperatures. The change from one state to the other occurs via a thermally induced phase transition with the ordered (gel) state occurring at lower temperatures and the disordered (liquid-crystal) state occurring at higher temperatures.

The most basic question concerning this property of phospholipids is whether cellular function depends on the ordered or disordered state of the membrane lipids. This question was first asked by Steim et al. [1] and Melchior et al. [2] in *Acholeplasma laidlawii*. These early studies indicated that the upper end of the lipid phase transition coincided with the temperature at which the organism was grown. Thus it appeared that most of the lipids were disordered at the growth temperature. A more detailed study by McElhaney [3] showed that this organism requires at least half of its lipids to be in the disordered state for normal growth, but that growth can continue at a reduced rate with only a small amount of fluid lipid.

Abbreviation: DSC, differential scanning calorimetry.

In *Escherichia coli* it was shown by Overath et al. [4] that an unsaturated fatty acid auxotroph cannot grow below the lipid transition temperature defined by incorporation of elaidate, an unnatural unsaturated fatty acid supplement. Cronan and Gelmann [6] have previously shown that *E. coli* can grow with only about one third its normal unsaturated fatty acid content [6]. This indicated that all essential cellular functions are operative under these conditions. Based on the data of Esfahani et al. [5], Cronan and Gelmann [6] suggested that *E. coli* could grow at a temperature below the beginning of its phase transition, that is, with all the membrane lipid in the ordered state.

However, subsequent work (reviewed by Cronan and Gelmann, see ref. 7) indicated that due to instrumental limitations, Esfahani et al. [5] were unable to detect the lower part of the phase transition when the transition was broad. These considerations argued against the previous suggestion and indicated that direct measurements of the lipid transition of the minimum unsaturate cultures were required. We have therefore measured the lipid phase transition of these cultures using the Privalov [8] calorimeter, an instrument of unusually high sensitivity. We find that the lipids of cells growing with a minimum content of unsaturated fatty acids have transitions which begin well below the growth temperature. We therefore conclude that the suggestion of Cronan and Gelman that *E. coli* can grow with all of the lipid in the ordered state is incorrect.

Materials and Methods

Bacterial strains

Strain UC1 is a wild-type *E. coli* K12 strain. Strain UC1098 was derived from UCl and contains mutations in two genes of fatty acid synthesis, *fabA* and *fabF*. The *fabA* gene is the structural gene for β-hydroxy-decanoyl thioester dehydrase, the enzyme responsible for the introduction of the double bond into the unsaturated fatty acids of *E. coli* [6]. The *fabF* (formerly called cvc) mutation [9] causes the loss of β-ketoacyl-acyl carrier protein synthetase II (Garwin, J. and Cronan, J., manuscript in preparation) and results in an inability to elongate palmitoleic acid to *cis*-vaccenic acid. Strain WNl is a $fabA^+$ transductant of UC1098 which retains the *fabF* lesion [10]. Further information on these strains can be found in these references [6,9,10].

Lipid preparation

These three strains were grown in the glycerol-casein hydrolysate medium in gyrorotary water bath shakers at various temperatures as previously described [6]. The cultures were harvested in log phase by centrifugation. The cells were then washed 4 times with 10 mM Tris · HCl (pH 8.0) and extracted with $CHCl_3/CH_3OH$ as described by Ames [11]. Dilute lipid suspensions were prepared by homogenization in 0.1 M potassium phosphate pH 6.6 as previously described [12]. DSC was done with a Privalov calorimeter operated as a scan rate of 1°C/min. The areas under the scan curves were quantitated by integration using a polar planimeter.

Fatty acid analysis

The fatty acid methyl esters were obtained from the phospholipids by trans-

esterification with sodium methoxide in methanol as described previously [13]. The methyl esters were separated on a diethylene glycol succinate column at 190° C and quantitated as previously described [9].

Results

Biological system

Cronan and Gelmann [6] determined the minimum amount of unsaturated fatty acid required to support the growth of *E. coli* by using strain UC1098 which has a temperature-sensitive mutation in unsaturated fatty acid synthesis. At 30° C, this strain grows normally. However, at 37° C this strain is unable to grow unless an appropriate unsaturated fatty acid is provided in the growth medium. At temperatures between 30° and 37° C, the rate of cell growth is a direct function of the rate of unsaturated fatty acid synthesis. At temperatures between 35° and 36° C, the minimum unsaturated fatty acid content, 15--20% of the total fatty acids, is reached. Since the unsaturated fatty acid content determined the state of the lipids [14], the membranes of these cells should contain the maximal amount of ordered lipid compatible with growth. The advantages of this method were given by Cronan and Gelmann [6,7]. Briefly, these are that the cells examined are (1) fully viable and (2) contain only the fatty acids normally found in *E. coli.* This method therefore, avoids the use of unnatural fatty acids such as elaidate and the cell death engendered by starvation of an auxotroph for an unsaturated fatty acid [15]. These attributes make the physiological interpretation of the results much more straightforward. The growth experiments in this paper were done exactly as described by Cronan and Gelmann [6] and the fatty acid compositions (Table I) found in this work are essentially identical to those of the previous report.

Maximal content of ordered lipid

Cultures of strain UC1098 were grown at various temperatures. The lipids were extracted and their phase transitions (Fig. 1) and fatty acid contents

TABLE I

FATTY ACID COMPOSITION OF MEMBRANE PHOSPHOLIPID FRACTION

Growth temperature (°C)	Fatty ester (wt %) *						Percent unsaturated **
	C_{14}	C_{16}	$C_{16:1}$	C_{17c}	$C_{18:1}$	C_{19c}	
Strain UC1098 lipids							
25.0	4	47	42	6	1	—	49
30.3	16	50	27	7	—	—	33
35.7	19	65	9	7	—	—	16
Strain WN1 lipids							
37.0	3	45	46	5	1	—	52
Strain UC1 lipids							
25.0	3	33	10	29	19	6	64
37.0	5	48	26	7	19	—	52

* $C_{16:1}$ and $C_{18:1}$ are palmitoleic and *cis*-vaccenic acids respectively. C_{17c} and C_{19c} are the cyclopropane derivation of the unsaturates.

** Percent unsaturated is the unsaturated plus cyclopropane fatty esters divided by the total fatty acids.

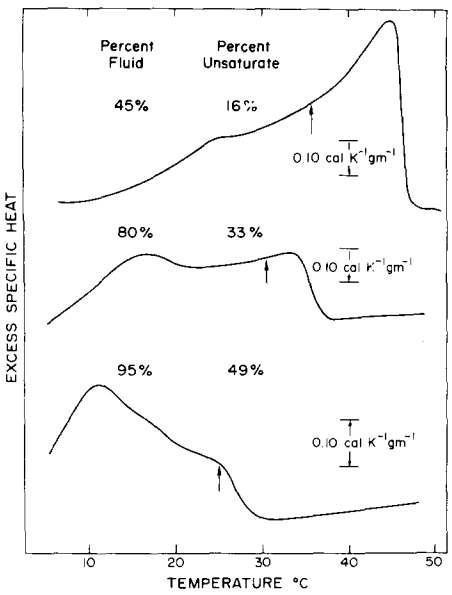

Fig. 1. Differential scanning calorimetry of lipids extracted from UC1098 grown at various temperatures. The growth temperatures are indicated by the arrows.

(Table I) were determined. Cultures grown at 35.7°C (the maximal temperature permitting growth) divide at about half of the normal rate due to a suboptimal rate of unsaturated fatty acid synthesis [6] and have only about one-third of the normal unsaturated fatty acid content. The transition of lipids extracted from these cells starts at about 8°C and ends at about 47°C (Fig. 1). The shape of the transition is asymmetric and somewhat triangular. The scans of lipids from cells grown at lower temperatures show the effects of a less severe reduction in the unsaturated fatty acid content. The transition and fatty acid content of lipids of UC1098 grown at 25°C is very similar to that of a wild-type strain grown at 37°C (see below).

The proportion of fluid lipid in each of the lipid preparations was determined by integration of the areas enclosed by the baseline and the calorimetric scan curve above and below the growth temperature. The proportions of fluid lipid given in Fig. 1 are slight underestimates since the lipids with higher transition temperatures usually have higher transition enthalpies. With this reservation, it seems clear that *E. coli* is still able to grow with about 55% of its lipid in the ordered state. With this quantity of ordered lipids, growth is reduced to half the rate of the wild-type strain. When UC1098 is grown at 30.3°C, its growth is the same as that of the wild-type strain [6] even though 20% of its lipids are ordered.

These experiments were done with isolated lipids for several reasons. First, it is difficult to observe broad phase transitions in isolated membranes from *E. coli* by DSC (for review see Steim, ref. 16). An example of the signal to noise problems given by broad transition can be seen in the work of Haest et al. [17]. Using DSC, these workers observed a sharp phase transition for elaidate enriched membranes similar to that seen by other workers (for review see ref.

7). but were unable to detect any transition in oleate enriched membranes. It seems likely that the difficulties in the detection of broad transition arise from background heat changes in non-lipid membrane components. We particularly wished to avoid heat uptake by protein since this would accentuate the upper end of the transition. It should be noted that in *E. coli* the transition seen in isolated lipids have invariably been found to agree with those detected in membranes (for review see ref. 7). This has also been found using the present methods. Jackson and Sturtevant [12] have shown similar sharp transitions in the isolated lipid membranes and whole cells of elaidate-enriched *E. coli*. We therefore, believe that DSC of the isolated lipids in an accurate reflection of the in vivo situation.

Wild-type E. coli lipid transitions

In wild-type *E. coli*, the transition has been reported to be complete at temperatures below the growth temperature. However, other workers have reported that the transition is centered about the growth temperature. This disagreement prompted us to measure the transition of the lipids of a wild-type strain. The wild-type strain, UCl, was grown at either 25° or 37° C (Fig. 2). The cells grown at 37° C have a transition which begins below 0° C and ends at about 25° C whereas with the cells grown at 25° C only the end of the transition is visible at about 8° C. It therefore seems that *E. coli* normally grows with all of the membrane lipid in the fluid state.

Fig. 2 also shows that lipids from strain WNl, grown at 37° C, have a transition which is only slightly lower than the transition of lipids from wild-type cells grown at the same temperature. WNl is defective in the elongation of palmitoleic acid to *cis*-vaccenic acid (Table I) [8]. The slightly lower transition temperature of WNl lipids relative to UCl lipids is consistent with the slightly greater average fatty acid chain length of the latter lipids.

A possible complication in these studies is the conversion of unsaturated fatty acids to their cyclopropane derivatives. This alteration which is dependent on grow phase, could potentially alter the phase properties of the lipids.

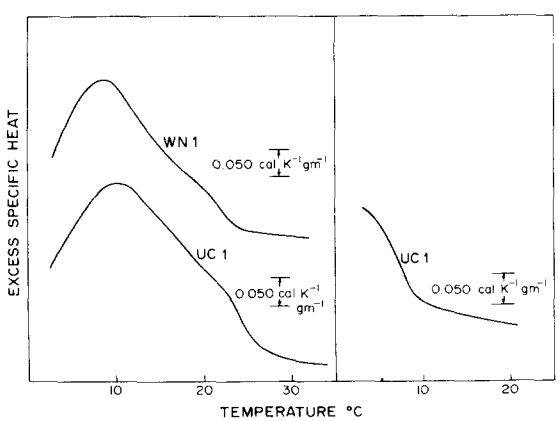

Fig. 2. Differential scanning calorimetry of UCl and WNl lipids. The scans on the left are of lipids from cells grown at 37° C and on the right are scans of lipids from cells grown at 25° C.

Although previous studies had shown that the physical properties of a cyclopropane fatty acid are extremely similar to the homologous unsaturate [18,19] we have obtained direct evidence to show that cyclopropanation does not alter the lipid phase transition. These experiments which are to be published elsewhere, used the cyclopropane deficient mutant of *E. coli* isolated by Taylor and Cronan [20]. The lipids isolated from this mutant contain <0.1% of the normal cyclopropane fatty acid content and gave a DSC scan indistinguishable from that of the parental strain in which virtually all the unsaturates had been converted to cyclopropane acids. These scans were done on lipids from cultures grown at 37°C and are very similar to the scan of UCl lipids given in Fig. 2. (left panel).

It should be noted that the inner and outer membranes of *E. coli* have very similar fatty acid contents and lipid phase transitions [21]. The unsaturated fatty acid contents of inner and outer membrane from UC1098 grown under unsaturate limiting conditions are almost identical (ref. 22 and Johnson, C. and Cronan, J., unpublished data).

Discussion

E. coli normally grows with all of its lipid in the fluid state. When cultures of this organism are grown at low temperatures the amount of fluid lipid is increased via an increased synthesis of unsaturated fatty acids. These observations suggest that the amount of fluid lipid could be of vital importance to the cell. However, we have found that *E. coli* is viable and can grow with more than half of its lipid in the ordered state. A similar conclusion was recently reported by Thilo and Overath [23] who examined the phase transition of cells of an *E. coli* auxotroph which had been starved for unsaturated fatty acids. Since the procedure used by Thilo and Overath [23] is known to result in nonviable cells [21], we feel our estimate is physiologically more valid. Despite this caveat, it is satisfying that there is good quantitative agreement between our DSC results and those of Thilo and Overath obtained by fluorescence studies of starved cells. It therefore seems that *E. coli* can tolerate rather large variations in the amounts of ordered lipid without large effects on vital processes. It seems possible that this property of keeping the transition temperature far below the growth temperature may serve to protect the organism from sudden temperature decreases.

Unsaturated fatty acid auxotrophs can grow with brominated [24] or branched chain [25] fatty acids as supplements, replacing fatty acids with double bonds and demonstrating that the requirement of these auxotrophs is for any fluidizing perturbant of the lipids as opposed to a steric or electronic requirement for a carbon-carbon double bond (for review see ref. 7). The experiments with strain UC1098 demonstrate that there are essential cell functions which require at least some fluid lipid. Apparently no cell functions which limit the growth rate are inhibited when 20% of the lipids are ordered, but as the fraction of ordered lipids is increased beyond 20%, some cell functions are restricted and when the proportion of ordered lipids exceeds 55%, growth ceases. At present, one can only speculate as to what these cell functions are and what is the nature of their dependence on lipid fluidity.

The shape of the transition of lipids from UC1098 grown at 35.7°C is somewhat triangular. Such a triangular shape has been seen in the transition of lipids from an unsaturated fatty acid auxotroph of *E. coli* enriched with elaidate, although that transition is considerably more narrow than that reported here [12]. It was suggested that the triangular shape of the elaidate enriched lipids can be attributed to head group heterogeneity when the fatty acyl chains of the lipids are nearly homogeneous [12]. The fatty acyl moieties of UC1098 grown at 35.7°C are also rather homogeneous being greatly enriched in palmitate, thus offering additional support for that contention.

There has been considerable disagreement in the literature on the phase transition of the lipids of wild-type *E. coli.* Using X-ray diffraction, Shechter et al. [26] reported a transition between 20° and 30°C for membranes isolated from cells grown at 37°C whereas Steim [27] observed a transition from 0° to 30°C in lipids from cells grown at 20°C using calorimetry. Both of these results are inconsistent with our results with isolated lipids (in Fig. 2). Incomplete hydration of the lipids may have been a problem in the studies of Shechter et al. [26] and of Steim [27]. It should also be noted that the other calorimetric studies [27] were done with an instrument which is much less sensitive at the lower end of the transition than that used in these studies.

In a study using an electron spin resonance probe, Sinensky [28] measured the lipid phase transition of wild-type *E. coli* grown at different temperatures. Although the growth temperatures chosen in our study are not identical to those chosen by Sinensky and the interpretation of his data in terms of phase transition is not straightforward [7,28], our results are in qualtitative agreement with the transition temperature being well below the growth temperature. The calorimetric data presented here show more clearly that the lipid transition is definitely completed more than 10°C below the growth temperature. The result of our experiments with variations in the growth temperature confirms Sinensky's [28] observation that the increased unsaturated fatty acid synthesis which occurs at lower temperatures affects a lowering in the lipid transition temperature. However, the fact that UC1098 with 20% of its lipid ordered, can grow at the same rate as the wild-type strain means that maintenance of a completely fluid membrane with a specific 'microviscosity' (as determined by the rate of motion of the electron spin resonance probe) is not necessary for optimal growth.

Acknowledgements

We should like to thank Professor Julian M. Sturtevant for his encouragement, support and the use of his laboratory facilities. This work was supported by grants from the National Institutes of Health (GM-04725 and AI 10186) and the National Science Foundation (PCM76-81012). J. Cronan was an NIH Research Career Development Awardee (GM70, 411).

References

1 Steim, J.M., Tourtelotte, M.E., Reinert, J.C., McElhaney, R.N. and Rader, R.L. (1969) Proc. Natl. Acad. Sci. U.S. 63, 104—109

2 Melchior, D.L., Morowitz, H.J., Sturtevant, J.M. and Tsong, T.Y. (1970) Biochim. Biophys. Acta 219, 114—122
3 McElhaney, R.N. (1974) J. Mol. Biol. 84, 145—157
4 Overath, P., Schairer, H.U. and Stoffel, W. (1970) Proc. Natl. Acad. Sci. U.S. 67, 606—612
5 Esfahani, M., Limbrick, A.R., Knutton, S., Oka, T. and Wakil, S.J. (1971) Proc. Natl. Acad. Sci. U.S. 68, 3180—3184
6 Cronan, Jr., J.E. and Gelmann, E.P. (1973) J. Biol. Chem. 248, 1188—1195
7 Cronan, Jr., J.E. and Gelmann, E.P. (1975) Bacteriol. Rev. 39, 232—256
8 Privalov, P.L., Plotnikov, V.V. and Filimonov, V.V. (1975) J. Chem. Thermodyn. 7, 41—47
9 Gelmann, E.P. and Cronan, Jr., J.E. (1972) J. Bacteriol. 112, 381—387
10 Nunn, W.D. and Cronan, Jr., J.E. (1974) J. Biol. Chem. 249, 724—731
11 Ames, G.F. (1968) J. Bacteriol. 95, 833—843
12 Jackson, M.B. and Sturtevant, J.M. (1977) J. Biol. Chem. 252, 4749—4751
13 Polacco, M.L. and Cronan, Jr., J.E. (1977) J. Biol. Chem. 252, 5488—5490
14 Chapman, D., Williams, R.M. and Ladbrooke, B.D. (1967) Chem. Phys. Lipids 1, 445—475
15 Henning, U., Dennert, G., Rehn, F. and Deppe, G. (1969) J. Bacteriol, 98, 784—796
16 Steim, J.M. (1974) Meth. Enzymol. 32B, 262—272
17 Haest, C.W.M., Verkleij, A.J., De Gier, J., Scheek, R., Ververgaert, P.H.J. and van Deenan, L.L.M. (1974) Biochim. Biophys. Acta 356, 17—26
18 van Deenan, L.L.M. (1965) in Progress in the Chemistry of Fats and Other Lipids (Halman, R.T., ed.), Vol. VIII, pp. 1—115, Pergamon Press, N.Y.
19 Christie, W.W. (1970) in Topics in Lipid Chemistry (Gunstone, F.D., ed.), Vol. I, pp. 1—89, Whiley-Interscience, N.Y.
20 Taylor, F. and Cronan, Jr., J.E. (1976) J. Bacteriol. 125, 518—523
21 Overath, P., Brenner, M., Galik-Krzywicki, U., Schechter, E. and Letellier, L. (1975) Biochim. Biophys. Acta 389, 258—269
22 Lugtenberg, E.J.J. and Peters, R. (1977) Biochim. Biophys. Acta 441, 38—47
23 Thilo, L. and Overath, P. (1976) Biochemistry 15, 328—334
24 Fox, C.F., Law, J.H., Tsukagoski, J. and Wilson, G. (1970) Proc. Natl. Acad. Sci. U.S. 67, 598—606
25 Silbert, D.F., Ladenson, R.C. and Honeggar, J.F. (1973) Biochim. Biophys. Acta 311, 349—361
26 Shechter, E.T., Gulik-Krzywicki, U. and Kaback, H.R. (1972) Biochim. Biophys. Acta 274, 466—477
27 Steim, J.N. (1972) in Mitochondria and Biomembranes (van den Bergh, S.C., Borst, P., van Deenan, L.L.M., Kiermersma, J.C., Slater, E.C. and Tager, J.M., eds), pp. 185—196, North-Holland, Amsterdam
28 Sinensky, M. (1974) Proc. Natl. Acad. Sci. U.S. 71, 522—525