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THE UNSATURATED FATTY ACID REQUIREMENT IN *ESCHERICHIA COLI*

TEMPERATURE DEPENDENCE AND TOTAL REPLACEMENT BY BRANCHED-CHAIN FATTY ACIDS

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SUMMARY

The present study evaluates the unsaturated fatty acid requirement in *Escherichia coli*. A derivative of a double mutant defective both in unsaturated fatty acid biosynthesis and in fatty acid degradation has been selected which grows equally well on anteisopentadecanoate (12-*Me*-14:0) or *cis*-Δ^9-octadecenoate (*cis*-Δ^9-18:1). When this strain is grown for many generations on 12-*Me*-14:0, there is extensive incorporation of this analogue into the membrane phospholipid and essentially no detectable unsaturated fatty acids residues in any lipid-containing structures of the cell envelope. Secondly, as the maximal growth temperature of *E. coli* is approached, the minimum content of unsaturated fatty acid required by this strain for growth decreases to a few percent and is associated with the appearance of substantial amounts of 12:0 (8%) and 14:0 (50%) in the phospholipid. These experiments demonstrate that the *cis* unsaturated fatty acids of *E. coli* phospholipids can be replaced by residues which possess no special electronic configuration. Hence, the unsaturated fatty acids do not participate in specific interactions with other membrane components but serve a general role of controlling the packing of paraffin chains in the membrane bilayer.

INTRODUCTION

Many prokaryotic and eukaryotic cells appear to have an essential requirement for *cis* unsaturated fatty acids. Unsaturated fatty acid auxotrophs of *E. coli* K-12 have permitted the systematic investigation of what structural features of *cis* unsaturated fatty acids are essential. Fatty acid analogues which have been shown to effectively substitute for these acyl residues share some of their steric and/or electronic properties; for example, fatty acids with a cyclopropane ring, a *trans* ethylenic bond, or a bromosubstituent in the middle of the hydrocarbon chain[1–6]. A number of years ago van Deenen postulated that the fatty acyl residues on the β position of naturally occurring phospholipids had a special role in regulating the distance between paraffinic chains[7]. The specific fatty acyl group on this position varied with the biological

source of the lipid but they were considered equivalent in their physical properties. Thus, in monolayer studies, *cis* unsaturated, anteiso and isomethyl branched chain, and 12 and 14 carbon unsaturated fatty acyl residues prevented very close packing of the acyl chains, increasing the minimum cross-sectional area per phospholipid molecule[7]. This approach to phospholipid structure, then, focused on the common physical attributes of diverse acyl groups found in the β position. If one could replace the one type of residue with another in the membrane phospholipid of the same organism and demonstrate that membrane functions are preserved, then the *in vivo* findings would provide direct support for the van Deenen hypothesis. McElhaney and Tourtellotte[8] have found that isohexadecanoate (14-*Me*-15:0) or *cis*-octadecenoate (*cis*-Δ^9-18:1) can be utilized extensively by *Mycoplasma laidlawii B*. However, *cis* unsaturated fatty acids which are present in the complex medium required for growth could not be entirely excluded from the membrane phospholipids when the branched-chain fatty acid supplement was provided. Furthermore, since this organism does not synthesize *cis* unsaturated fatty acids, its requirement for membrane function is less clear. In the present study we approached this question using *E. coli* unsaturated fatty acid auxotrophs.

MATERIALS AND METHODS

Chemicals and other reagents

All fatty acids were obtained at greater than 99% purity from Nuchek Prep. (Elysian, Minn.) except for the branched-chain fatty acids which were purchased from Supelco (Penn.). *Crotalus adamanteus* venom, a product of Sigma Chemical Co. (St. Louis, Mo.), was used as a source of phospholipase A.

Bacterial strains and growth conditions

The strains of *E. coli* K-12 used in the present study are listed in Table I. AB1623-1, LA1-6, and LA2-22 were grown on medium 63[11] containing 0.4% glycerol, 5 mM potassium glutamate, 0.00005% thiamine, 0.00005% yeast extract, 0.04% Brij 58 and 0.01% fatty acid. The same medium with 0.3% casamino acids in place of potassium glutamate and yeast extract was used for growing K1060 and K1060-B5 unless indicated otherwise. Cultures were grown in a New Brunswick gyratory water bath shaker in which the desired temperature was maintained within 0.3 °C. Growth was monitored with a Klett–Summerson colorimeter at 660 nm.

Isolation of phospholipid and characterization of fatty acid composition of phospholipid or of other lipid containing envelope structures

Lipids were extracted from the cells and fractionated by thin-layer chromatography as described earlier[9] with the following modifications. After spotting the chloroform extracts on activated silica gel G, the plates were developed initially with *n*-pentane–ethyl ether–acetic acid (40:60:1, by vol.) to move any free fatty acids (R_F=0.8) well ahead of the phospholipids which remain at the origin. The solvents were allowed to evaporate from the plate which was then developed a second time in the usual fashion with chloroform–methanol–acetic acid (65:25:8, by vol.) except that the run was stopped before the solvent front reached the position to which the fatty acid had migrated in the first development. The chromatographic separation

TABLE I

CHARACTERISTICS OF BACTERIAL STRAINS

Strain	*Sex*	*Genotype*	*Source and reference*
AB1623-1	F−	gltA−oldE−thi−gal−xyl−mtl−T4^{r}T6^{r}strA^r	Silbert *et al.*[9]
LA1-6	F−	gltA−fab^{ts}thi−ara−lac−gal−xyl−mtl−T4^{r}T6^{r}strA^r	Harder *et al.*[10]
LA2-22	F−	gltA−fab^{ts}thi−ara−lac−gal−xyl−mtl−	Harder *et al.*[10]
K1060	F−	fabB−oldE−thi−str^r*	Overath *et al.*[4]
K1060-B5	F−	fabB−oldE−thi−str^r**	Spontaneous mutation, this paper

* Contains an additional mutation allowing growth on *trans* unsaturated fatty acids.
** A derivative of K1060 which contains a mutation allowing growth on branched-chain fatty acids.

in both systems was monitored with fatty acid, cardiolipin, phosphatidylglycerol and phosphatidylethanolamine standards spotted at the outer margins and in the center of the 20 cm × 20 cm plates. The plate was lightly exposed to I_2 vapors (blown on with a pipette) to reveal just the standard fatty acid location after the first run and then just that of phospholipid and fatty acid standards after the second run. To determine the fatty acid composition of the intact phospholipids, the appropriate silica gel fractions were scraped from the plate and the fatty acids released as methyl esters by transesterification in redistilled methanol containing 2% H_2SO_4 at 70 °C for 2 h followed by extraction into *n*-pentane. When the positional distribution of the fatty acids was to be measured, the intact phospholipids were recovered from the silica gel and treated as described elsewhere[9].

The cell pellets remaining after removal of the extractable lipids were dried under vacuum in a N_2 atmosphere and then hydrolyzed under vacuum at 105 °C in 6 M HCl for 3 h. The hydrolyzate was extracted with ethyl ether, traces of acid removed by washing the ether phase with water, and the fatty acids present in the ether converted to methyl esters by treatment with diazomethane[12].

The fatty acid composition from the phospholipids and from other lipid containing structures in the cell envelope was analyzed by gas–liquid chromatography as described previously[13].

RESULTS

Growth response to and utilization of branched-chain fatty acids by E. coli mutants affecting saturated and unsaturated fatty acid biosynthesis (fab)

Temperature-sensitive *fab* mutants described previously[10] grow normally at 37 °C when supplemented with a combination of saturated and *cis* unsaturated fatty acids or with a *trans* monoenoic acid (Fig. 1). Anteisopentadecanoate (12-*Me*-14:0) substitutes for *cis* monounsaturated (16:1) but not for *n*-saturated (16:0) fatty acid in supporting growth (Fig. 1). As previously observed with *cis*-Δ^9-16:1[10], 12-*Me*-14:0 by itself does not maintain growth of these mutants (not shown). Anteisoheptadecanoate (14-*Me*-16:0) serves as a growth factor when present with 12-*Me*-14:0 but transiently or not at all when supplied alone (not shown), with *cis* 16:1 or with 16:0.

The positional distribution of various acyl groups in phosphatidylethanolamine has been previously characterized for AB1623 (ref. 9). The results show that long-chain saturated (16:0 and 17:0) and *trans*-unsaturated (Δ^{11}-18:1) fatty acids are principally in Position 1 (from 74–78% when the fatty acid arises from synthesis and/or supplementation); certain long-chain *cis* monoenoic (Δ^{10}-17:1 and Δ^{11}-18:1) fatty acids appear equally in both positions; and other long-chain *cis* monoenoic (Δ^{9}-16:1) are found in Position 2 (66% when the fatty acid is derived from synthesis and/or supplementation). Due to the nature of the defect, it is not possible to examine under exactly comparable conditions the fatty acid distributions in the phospholipid of LA1-6, one of the *fab*^{ts} mutants obtained from AB1623. However, when 12-*Me*-14:0 and 14-*Me*-16:0 are provided individually as supplements at 37 °C for this strain, 72 and 46% of that incorporated into phosphatidylethanolamine is in Position 2, respectively for the two fatty acids. These results suggest that 12-*Me*-14:0 is recognized by the cell as an analogue of *cis*-Δ^{9}-16:1 and 14-*Me*-16:0, as an analogue of *cis*-Δ^{11}-18:1.

Total replacement of cis monounsaturated fatty acids by branched-chain analogues of intermediate chain length in a derivative of an unsaturated fatty acid auxotroph

The fab^{ts} mutants continue to synthesize small amounts of *cis* unsaturated fatty

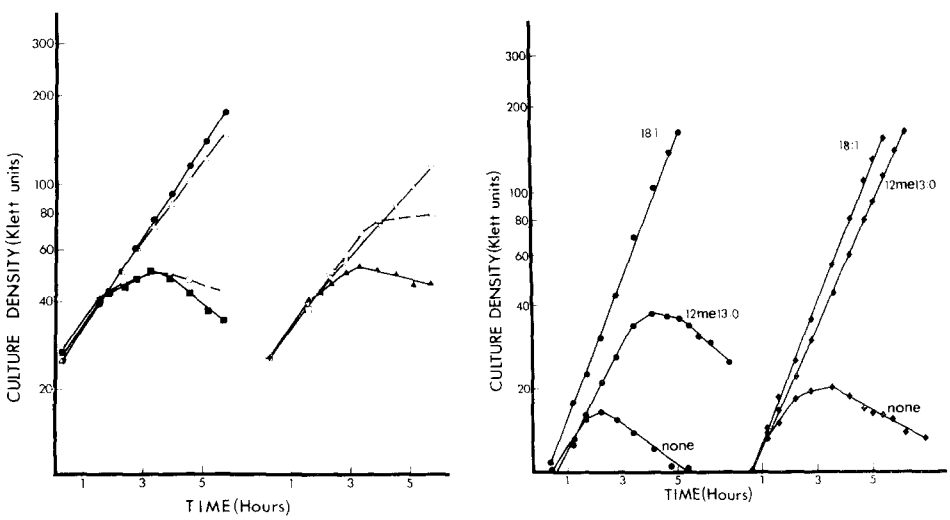

Fig. 1. Branched-chain fatty acids as a growth factor for *fab*^{ts} mutants at 37 °C. LA2-22 (or LA1-6) was grown overnight at 30 °C in minimal medium supplemented with 0.4 mM potassium oleate as described in the text and elsewhere[10]. The cells were harvested, washed and resuspended at about $2.5 \cdot 10^{8}$/ml in fresh medium without potassium oleate. The cell suspension was divided into portions to which various fatty acid supplements were added (0.4 mM when present alone and 0.3 mM if added in combination) and subcultured at 37.0 ± 0.3 °C. Growth curves on the left of the figure: without supplement, ○; *trans*-Δ^{9}-16:1, ●; 16:0 and 12-*Me*-14:0, △; 16:1 and 12-*Me*-14:0, ■. Growth curves on the right of the figure: 14-*Me*-16:0 and 12-*Me*-14:0, ▽; 16:0 and 14-*Me*-16:0, □; 16:1 and 14-*Me*-16:0, ▲.

Fig. 2. Growth of K1060 and K1060-B5 with *cis* monounsaturated, branched-chain, or no fatty acid supplement. Experimental details are described in legend to Table II. K1060, ●; K1060-B5, ◆.

TABLE II

GROWTH PROPERTIES OF K1060 AND K1060-B5

The starting inoculum were cells grown overnight at 37 °C with *cis*-Δ^9-18:1 as supplement as described in the text. The cells were harvested, washed and subcultured at a concentration of about 10^8/ml in fresh medium to follow the growth response with the indicated supplements over a period of up to four generations. To measure the ability of these strains to sustain growth over more than ten generations, the cells were subcultured at lower concentrations as indicated in the lower half of the Table.

Supplement	*Generation time (h)*	
	K1060	*K1060-B5*
18:1	1.2 (1.5)*	1.2 (2.0)
12-*Me*-14	1.3 slowing (2.0)	1.5 (1.9)
12-*Me*-13	1.4 lysis (1.5) lysis	1.4 (2.2)

Supplement	*Inoculum*	*Time to full growth (h)*	
		K1060	*K1060-B5*
18:1	$4 \cdot 10^4$/ml	≤15	≤15
	$4 \cdot 10^5$/ml	≤15	≤15
12-*Me*-14	$4 \cdot 10^4$/ml	≥72	26
	$4 \cdot 10^5$/ml	44	20
12-*Me*-13	$4 \cdot 10^4$/ml	≥72	22
	$4 \cdot 10^5$/ml	≥72	17

* Growth rate in the same minimal medium without casamino acids is shown by the numbers in parentheses.

acids at 37 °C (ref. 10). In order to demonstrate that total replacement of this class of acyl residues was possible, we utilized an unsaturated fatty acid auxotroph to derive a strain which could grow equally well on 12-*Me*-14:0 or *cis*-Δ^9-18:1. Table II shows that the auxotroph K1060 and its derivative K1060-B5 are very similar with respect to initial growth rates on *cis* monoenoic and branched-chain fatty acids in a casamino acid enriched medium, but that growth of K1060 on branched-chain fatty acids, unlike that of K1060-B5, stops after a few generations. This difference is shown dramatically in Fig. 2 where growth in the presence of 12-*Me*-13:0 and *cis*-Δ^9-18:1 is compared. When cultures are started with a very small inoculum of K1060, visible growth on 12-*Me*-14:0 is detected only after a very long lag consistent with the emergence of a derivative strain (Table II). If the cells which appear are cloned on *cis*-Δ^9-18:1 and a colony subsequently subcultured starting again with a low inoculum, then

growth on 12-*Me*-13:0 and 12-*Me*-14:0 is just slightly slower than on *cis*-Δ^9-18:1. Thus, the ability to grow on a branched-chain fatty acid supplement is an inherited trait.

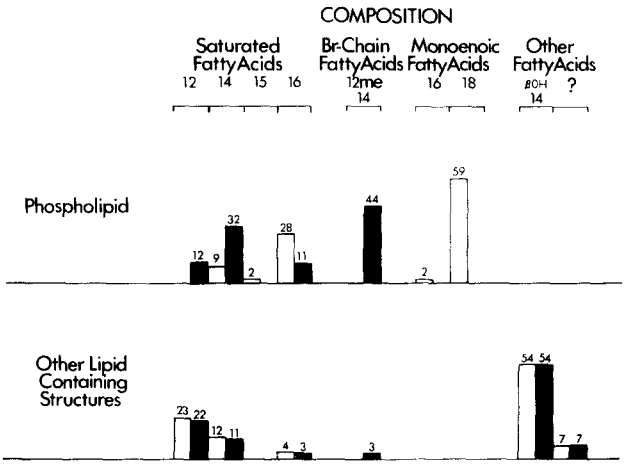

Fig. 3. Fatty acid composition of phospholipid and other lipid containing structures of the cell envelope of K1060-B5. The cells were grown at 37 °C for more than ten generations on *cis*-Δ^9-18:1 (open bars) or on 12-*Me*-14:0 (filled bars). The procedures used for characterization of the fatty acid composition are described in the text. The phospholipid fraction consists of cardiolipin, phosphatidylglycerol, and phosphatidylethanolamine, the three glycerophosphatides found in significant amounts of *E. coli*. The numbers above the bar graphs are the weight percentage of each component obtained by gas–liquid chromatographic analysis. Components which are totally undetectable or are $<0.5\%$ are not shown.

When K1060-B5 is grown on *cis*-Δ^9-18:1, the phospholipid of the cell contains a moderate content (28%) of 16:0 and a large fraction (59%) of *cis*-18:1 (Fig. 3). If these cells are now grown for a number of generations on 12-*Me*-14:0, one finds moderate amounts (44%) of 12-*Me*-14:0, increased levels of 12:0 (12%) and 14:0 (32%), decreased amounts of 16:0 (11%), and an essentially undetectable ($<0.5\%$) amount of *cis* monounsaturated fatty acids in the phospholipid. Examination of the fatty acid composition of other lipid containing structures in the cell envelope, which normally do not contain *cis* monoenoic residues, reveals essentially no compositional change in response to the shift in growth supplements (except for the appearance of a very small amount of 12-*Me*-14:0 in this fraction of the cell envelope). Thus, there are two principal findings in this experiment: (1) the essential attributes of *cis* monounsaturated fatty acids of *E. coli* can be replaced at 37 °C by fatty acid chains which are shorter or have bulky substituents but possess no special electronic configuration; (2) the change in the fatty acyl composition of membrane phospholipid also results in a reduction in the average acyl group chain length from 17 to 14 carbons.

While K1060-B5 grows equally well on 12-*Me*-14:0 and *cis*-Δ^9-18:1 at temperatures above approximately 30 °C, K1060-B5 grows less well on the branched-chain *versus* the unsaturated fatty acid supplement at lower temperatures. In this connection it is notable that many *Bacillus* species which contain virtually only branched-chain residues in their phospholipids at 35 °C, have been shown to produce fatty acyl desaturases and monounsaturated fatty acids below 30 °C (ref. 14).

Requirement for cis unsaturated fatty acids as a function of temperature in starved K1060-B5

In the course of investigating the behavior of different branched-chain fatty acids as analogues for the unsaturated fatty acids in *E. coli* membrane phospholipids, we found that the extent of growth of K1060-B5 during starvation for any fatty acid supplement is a function of temperature (Figs 4 and 5). Growth ceases after 1.0, 1.5, 2.5-3.0, and more than 4.0 generations at 28, 37, 43 and 45 °C, respectively. At increasing temperatures it becomes apparent that cessation of growth is preceded by a period of slower growth. When the fatty acid composition of the membrane phos-

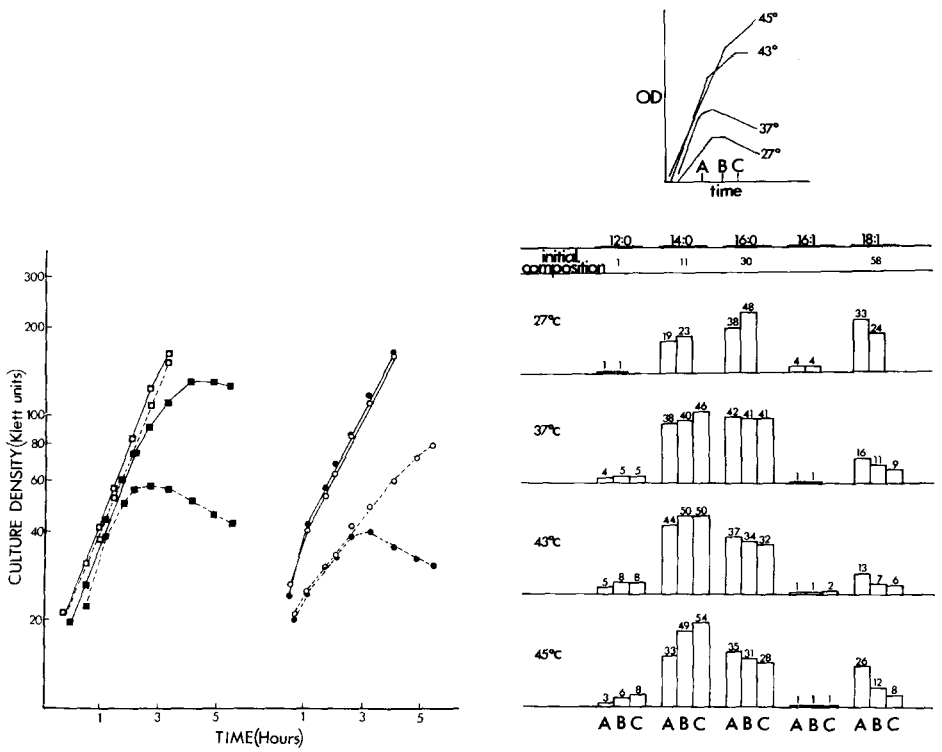

Fig. 4. Growth of K1060-B5 without fatty acid supplement at various temperatures. K1060-B5 was grown overnight at 37 °C with *cis*-Δ^9-18:1 supplement as described in the text. The cells were harvested, washed twice, resuspended at a concentration of 10^8 per ml in fresh medium, and subdivided for growth at the indicated temperatures with and without *cis*-Δ^9-18:1 supplement. Growth curves on the left of the figure: no supplement at 37 °C (---■---) and 43 °C (—■—); *cis*-Δ^9-18:1 at 37 °C (dotted line with open squares, containing circle) and 43 °C (solid line with open squares, containing circle). Growth curves on the right of the figure: no supplement at 28 °C (---●---) and 45 °C (—●—); *cis*-Δ^9-18:1 at 28 °C (---○---) and 45 °C (—○—).

Fig. 5. Fatty acid composition of phospholipid from K1060-B5 during growth without supplement at various temperatures. Conditions of growth of K1060-B5 are the same as described in Fig. 4. At Intervals A, B, and C, aliquots of each culture were taken and the cells collected by centrifugation and washed twice with medium 63. The lipid extraction and fractionation and the analysis of fatty acid composition were performed as described in the text. The fatty acid composition is expressed as weight % which is shown by the relative heights of and the number above the bars.

TABLE III

CORRELATION OF GROWTH TEMPERATURE AND MINIMUM UNSATURATED FATTY ACID REQUIRED FOR GROWTH UNDER CONDITIONS OF FATTY ACID STARVATION AND 14-*Me* BRANCHED-CHAIN SUPPLEMENTATION

The unsaturated fatty acid contents shown in the right hand column are derived from weight percentages given in Figs 5–7. The minimum unsaturated fatty acid content is taken as the value obtained when growth rate changes since addition of unsaturated fatty acid up to this point preserves the initial growth rate. Since samples were not obtained always precisely at this point, the values shown are estimated from data derived from samples taken on either side of this critical point.

Growth temperature and supplement	*Estimated minimum unsaturated fatty acid content for growth*
27 °C, none	≥32
37 °C	
None	>17
14-*Me*-15	14–7
14-*Me*-16	16–9
43 °C	
None	12 (8)*
14-*Me*-15	< 3
14-*Me*-16	(5)
45 °C, none	11 (3)

* The numbers in parentheses are the weight percentages found in the 43 °C cultures when growth stopped and in a 45 °C culture from a separate experiment after four generations of growth without supplement.

pholipids is analyzed at various times during the growth (Fig. 5), one finds an inverse correlation between the relative amount of unsaturated fatty acid which is present at the time growth slows or stops and the temperature of growth (see also summary, Table III). In addition, as the growth temperature is increased, there is a buildup of 14:0 (and 12:0) and a decrease in 16:0. These changes are consistent then with the conclusion that the minimum amount of unsaturated fatty acid essential for growth is a function of temperature and that the intermediate chain length fatty acids (12:0 and 14:0) may substitute for unsaturated fatty acyl residues at the higher temperature.

Utilization of long branched-chain fatty acids as growth factors for K1060-B5

Isohexadecanoate (14-*Me*-15:0) and anteisoheptadecanoate (14-*Me*-16:0) have only a limited capacity to substitute for the *cis* monounsaturated fatty acid requirement of K1060-B5. 14-*Me*-15:0 and 14-*Me*-16:0 are incorporated extensively (up to approximately 80% of the total composition) at 37 and 43 °C (Figs 6 and 7, respectively). However, only 14-*Me*-15:0, and only at the higher temperature, stimulates growth and lowers the unsaturated fatty acid levels much beyond that experienced by the unsupplemented culture (Fig. 7 and Table III). In this culture, there is also a moderate buildup in 12:0 and 14:0 which combined with 14-*Me*-15:0 may contribute to the continued growth of the cells. Growth ceases in these branched-chain supplemented cultures when the relative amount of branched-chain fatty acids exceeds 50%

indicating that the membrane has begun to accumulate at least some phospholipid molecules that carry two identical iso- or anteisobranched chains.

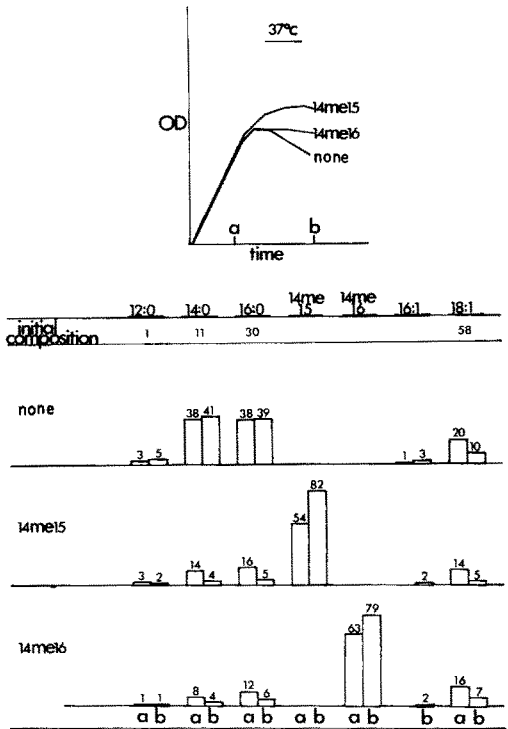

Fig. 6. Growth response to and incorporation of 14-*Me*-branched-chain fatty acid by K1060-B5 at 37 °C. Conditions of growth are the same as described in Fig. 4. At time Points a and b, aliquots of each culture were obtained and subjected to the same procedures described in Fig. 5 to determine the fatty acid composition of the phospholipids. The same definitions given in Fig. 5 apply here to the numbers shown above the bars.

Supplementation of K1060-B5 simultaneously with two different branched-chain fatty acids characteristic of Bacillus subtilis

The fatty acid composition of *Bacillus subtilis* grown at 37 °C is characterized by a predominance (94%) of branched-chain fatty acids, a small amount (6%) of *n*-saturated fatty acid, and the absence of unsaturated acyl residues[15]. The growth response of K1060-B5 to the several conditions of supplementation shown in Table IV is essentially identical. Multiple supplementation with 12-*Me*-14:0 and 14-*Me*-15:0 or 14-*Me*-16:0 over many generations leads to effective incorporation of both supplements into the phospholipid. However, there is not a complete transformation to the fatty acid composition characteristic of *Bacillus subtilis* due to the continued presence of moderate amounts of intermediate-chain length *n*-saturated fatty acids. The relative absence of this latter class of acyl groups from the phospholipid of *Bacillus subtilis* reflects the difference in specificity of *E. coli* and *Bacillus subtilis* fatty acid synthetase for acetyl thioester and branched-chain acyl thioester as initiators of long-chain fatty acid synthesis[16]. Thus, in order to reduce the level of *n*-saturated

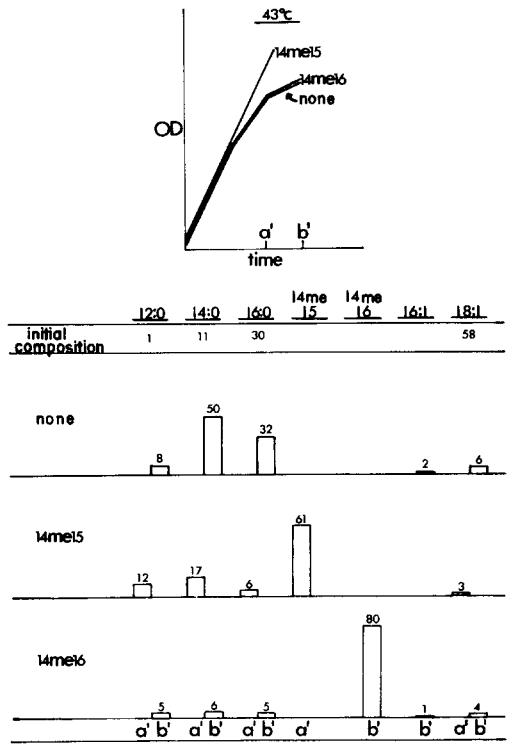

Fig. 7. Growth response to and incorporation of 14-*Me*-branched-chain fatty acids by K1060-B5 at 43 °C. Conditions of growth are the same as described in Fig. 4. At time Points a′ or b′, an aliquot of each culture was obtained and treated as described in Fig. 5 to determine the fatty acid composition of the phospholipids. The same definitions given in Fig. 5 apply here to the numbers shown above the bars.

TABLE IV

E. COLI PHOSPHOLIPID CONTAINING BRANCHED-CHAIN FATTY ACIDS CHARACTERISTIC OF *B. SUBTILIS*

The conditions for growth of K1060-B5 and the methods of analysis of the fatty acid composition are the same as those described in Fig. 3. 0% means the components are <0.5%. The data from Fig. 3 is reproduced here for purposes of comparison with the results obtained with cultures supplemented with two different branched-chain fatty acids.

Supplement	*Saturated fatty acids*			*Branched-chain fatty acids*			*Unsaturated fatty acids*	
	12	*14*	*16*	*12me 14*	*14me 15*	*14me 16*	*16:1*	*18:1*
cis-Δ^9-18:1	0	9	28	0	0	0	2	59
12-*Me*-14	12	32	11	44	0	0	0	0
12-*Me*-14+14-*Me*-15	10	31	13	22	23	0	0	0
12-*Me*-14+14-*Me*-16	7	34	19	27	0	13	0	0

fatty acids and completely transform the composition of *E. coli* phospholipids, it is probably necessary to alter the availability of acetyl CoA or the specificity of the synthetase for it as a primer.

DISCUSSION

The conclusion that phospholipid molecules in biological membranes are arranged in a bilayer with their apolar chains directed inward and more or less perpendicular to the plane of the membrane is supported by considerable experimental evidence and is firmly established by X-ray diffraction studies[17,18]. In this arrangement, two physical attributes of the fatty acid residues, namely the geometry and the length of the chains, have particular importance to membrane structure. Several different physical measurements indicate that the stereochemistry and/or length of the apolar chains affect their packing within natural and model membranes[4,6,7,19,20]. In addition, the length of the hydrocarbon residues has been shown by X-ray diffraction studies to influence the width of the bilayer in natural membranes[18]. In the present study we wished to determine in organisms which synthesize *cis* monounsaturated fatty acids whether or not there is an essential requirement, however small, for fatty acyl chains possessing centrally located electron rich regions. It is possible that modifications in the electronic structure located in the middle of the hydrocarbon chain are essential for specific interactions between membrane components, or alternately, that they serve only to disrupt potential van der Waals interactions and thus restrict the packing of paraffin chains. The latter explanation is supported by the results reported here. *Cis* unsaturated fatty acids can be totally replaced within the limit of detection by branched-chain fatty acids bearing bulky methyl substituents which disrupt packing but do not possess the π bonds, "banana" orbitals, or electronegative atoms, which characterize, respectively, the *trans* monounsaturated, the cyclopropane derivatives of *cis* monoenoic, and the bromo substituted fatty acids previously shown to serve as growth factors for *E. coli* unsaturated fatty acid auxotrophs[1–6]. It would seem that carbon atoms associated with *cis* or *trans* ethylenic bonds have reduced van der Waals interactions. Hence, *trans* monoenoic acids but not the homologous *n*-saturated fatty acids, which are very similar to the *trans* unsaturated residues in certain of their physical properties, can replace *cis* unsaturated fatty acids in the phospholipids. In keeping with the view that reduced van der Waals interactions are important, we have also found that the requirement for unsaturated fatty acyl groups is markedly reduced and probably replaced in large measure by the increased content of intermediate-chain length of saturated residues in the phospholipids at growth temperatures near maximum for *E. coli*. The wild type *E. coli* strains used in this study do not grow for long periods much above 43 °C. Thus, we encountered an upper limit in growth temperature as we attempted to abolish the requirement for *cis* monounsaturated fatty acids at higher temperatures.

Wide variation in fatty acid composition are compatible with normal growth at 37 °C (ref. 9). The minimum amount of unsaturated fatty acid required for growth at this temperature as estimated here is in good agreement with earlier results from this laboratory[13] and with recent measurements by Cronan and Gelmann[21] using a temperature-sensitive unsaturated fatty acid auxotroph grown at slightly lower temperatures. These estimates of minimum unsaturated fatty acid required for growth

at 37 °C or other temperatures have been based on the fatty acid composition of phospholipids from whole cells. The synthesis of outer membrane in *Enterobacteriae* involves the translocation of lipid from inner membrane[22]. If the critical cell function(s) requiring an expanded bilayer structure resides in the inner membrane, the physiologically significant minimum amount of unsaturated fatty acid may be either higher or lower than that obtained with whole cells if there is asymmetry in the distribution of the residual *cis* monoenoic residues between inner and outer membranes.

The mutant strain described in this paper provides a system for exploring not only the physical and functional properties of membranes containing fatty acid residues of diverse stereochemistry but also for examining the consequences of the reduction in average chain length of acyl groups in the membrane phospholipids from cells grown on 12-*Me*-14:0. From the earlier studies with *Mycoplasma* membranes by Engelman[18], inner membranes prepared from K1060-B5 would be expected to show a narrowing of the width of the bilayer which might produce changes in the organization or exposure of membrane proteins inserted into or transversing the bilayer.

The nature of the mutation in K1060-B5 which results in the ability to utilize branched-chain fatty acid supplements in place of *cis* unsaturated fatty acids is not known. K1060, the parent strain, incorporates the branched-chain analogues as extensively as K1060-B5. When one compares the fatty acid composition of the phospholipid of the two strains at the time when K1060 stops growing, they are identical. These results indicate that the two strains are probably not different with respect to the ability to transport and activate the branched-chain fatty acids or in the specificity of their acyl transferases involved in phospholipid synthesis. The correlation between the extent of growth without fatty acid supplement and growth temperature, which is dramatically seen with K1060-B5, is less prominant in K1060 and almost absent in L010 and UC1098, all unsaturated fatty acid auxotrophs of *E. coli*. As observed earlier with K1060 and L010[23] and recently confirmed with other mutants[24], *E. coli* fatty acid auxotrophs release lipid into the medium (especially in the presence of detergent) which comes from the outer membrane. The release of phospholipid–lipopolysaccharide–protein complexes by *E. coli* was first described in connection with conditions affecting protein synthesis by Knox *et al.*[25] and more recently by Rothfield and Pearlman-Kothencz[26]. Thus, one possible explanation for the strain difference in the extent of growth without fatty acid supplement may be differences in their rates of loss of membrane lipid to the medium[23]. In K1060-B5, such losses must be low since the reduction in unsaturated fatty acid content follows closely the increase in cell mass. Furthermore, the ability of unsaturated fatty acid auxotrophs starved of fatty acid supplement to accumulate very high levels of 12:0 and 14:0 and to grow at higher temperatures with very low levels of unsaturated fatty acids may be lost in strains which have a sustained loss of membrane phospholipid to the medium.

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