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Fatty acid composition of lipids of *Escherichia coli* W 1655 F⁺ and its stable protoplast type L-form

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The comparative fatty acid analysis of extractable and non-extractable lipids of *Escherichia coli* W 1655 F^+ and its stable protoplast type L-form shows quantitative as well as qualitative differences. From 10 different fatty acids obtained 16:0, 17:0, and 18:0 are present at about the same quantities in the lipid fractions of the bacterial and L-form. The absence of larger amounts of 12:0, 14:0, and 14:βOH fatty acids in non-extractable L-form lipids reflects the loss of the cell wall in L-form cells. 16:1 fatty acid was found in L-form lipids only. This qualitative difference and the 2–3 times higher content of 18:1 in L-form lipids and the 7 times lower content of cyc 19:0 in extractable lipids of the L-form may be interpreted as alterations characteristic for the changed composition of the cytoplasmic membrane in L-form cells.

The transformation of bacteria into L-forms is accompanied by various morphologic, ultrastructural, metabolic and antigenic changes. In the protoplast type L-forms the cytoplasmic membrane is the only barrier of the cell towards its environment. It has to carry out many functions which are associated with the complete cell envelope in normal bacterial cells, for example maintaining cell integrity and osmotic stability, protection against environmental influences, regulation of transport processes. Nearly the entire lipid content of *E. coli* is found in the outer and inner membrane of the cell envelope (CRONAN and VAGELOS 1972). Changes in these structures should become visible in the fatty acid spectra of their lipid fractions.

Comparative studies of the lipid composition in bacterial and L-forms of different gram-negative species were carried out by several authors (CAVARD and SCHMITT-SLOMSKA 1976, GMEINER and MARTIN 1976, KREMBEL 1964, NESBITT and LENNARZ 1965, REBEL and MANDEL 1969, WEIBULL *et al.* 1967). Quantitative differences in both the lipid content as well as in its fatty acid composition have been found.

First investigations of the bacterial and L-form of *E. coli* W 1655 F^+ (BAYKOUSHEVA *et al.* 1980) showed that the L-form contains twice as much extractable lipid and several times less non-extractable lipid than the bacterial form. The main phospholipids phosphatidylethanolamine, phosphatidylglycerol, and cardiolipin are present in different amounts in both forms.

In this paper data concerning fatty acid composition of extractable and non-extractable lipids of bacterial and L-form cells will be summarized and discussed.

Materials and methods

Organism and growth conditions: The stable L-form of *E. coli* W 1655 F^+ was obtained from the bacterial form by induction with penicillin (SCHUHMANN and TAUBENECK 1969, GUMPERT

et al. 1971). Both strains were cultivated in nutrient broth composed of meat infusion (made in ZIMET Jena from bovine meat), 1% Proteose peptone II (DIFCO), and 0.5% NaCl. No serum was added to the cultures. The bacterial form was harvested after 18 h incubation at 37 °C on a rotatory shaker. L-form cells were incubated 42 h under the same conditions. In both cases harvested cells were in the stationary growth phase. Cells were lyophilized and washed in 0.15 M NaCl before extraction of the lipids.

Extraction of lipids and analysis of fatty acids: Lipids were extracted by the method of BLIGH and DYER (1959) with a mixture of chloroform and methanol (1:2). The lipid fractions of two extractions were washed with aqua dest. and evaporated to dryness. For isolation of non-extractable lipids (NEL) cell debris were hydrolyzed with 2 N alcoholic KOH in a boiling water bath (2 h) and after that treated with ethylether. Methylation of the fatty acids was carried out by treatment with diazomethane.

Fatty acid composition was determined by combined gas liquid chromatography — mass spectrometry of methyl esters using a JEOL JMS-D100 mass spectrometer equipped with a JGC-20 KP gas chromatograph. The fatty acid methyl esters were separated on a 2 m × 2 mm i. d. glass column packed with 3% of OV-101 on Gas Chrom Q (80—100 mesh) under the following operating conditions: injector temperature 250 °C, column temperature 150 °C programmed to 220 °C at 5°/min temperature of separator 230 °C, ion source temperature 200 °C. Helium was used as carrier gas. The mass spectra were recorded at 75 eV and an acceleration voltage of 3000 V. The electron emission energy was 300 μA. Identification of fatty acid methyl esters was confirmed by comparison of their mass spectra and their glc retention times with those of methyl ester standards. Unsaturated fatty acid methyl esters and cyclopropane fatty acid methyl esters were identified after selective hydrogenation according to BRIAN and GARDNER (1968).

Quantitative estimations of the fatty acid methyl esters were performed on a gas-liquid chromatograph GCHF 18.3 (VEB Chromatron, Berlin) under the same operating conditions described for the GLC-MS. The percentage of each acid was calculated from the ratio of the area of its peak to the total area of all peaks.

Results

The results of the fatty acid analysis of extractable (EL) and non-extractable lipids (NEL) are summarized in Fig. 1 and Table 1. There are similarities but also

Table 1

Fatty acid composition in extractable and non-extractable lipids of the bacterial (B) and stable L-form (L) of *Escherichia coli* W 1655 F+. Values in % calculated on the basis of peak areas.

Fatty acid		extractable lipids		non-extractable lipids	
		B	L	B	L
C 12:0	*n*-dodecanoic (lauric)	0	1.9	14.0	0
C 14:0	*n*-tetradecanoic (myristic)	3.1	4.9	15.6	6.7
C 14:βOH	3-hydroxytetradecanoic (β-hydroxymyristic)	0	0	27.8	6.6
C 16:1	hexadecenoic (palmitoleic)	0	9.5	0	8.6
C 16:0	*n*-hexadecanoic (palmitic)	53.2	54.2	17.5	46.8
cyc C 17:0	methylenehexadecanoic	25.5	20.1	3.5	9.4
C 17:0	*n*-heptadecanoic (margaric)	0	0	1.8	0
C 18:1	octadecenoic	2.9	6.1	2.3	7.0
C 18:0	*n*-octadecanoic (stearic)	2.9	1.4	17.0	14.8
cyc C 19:0	methyleneoctadecanoic	12.5	1.7	0	0
ratio of unsaturated to saturated fatty acids[1]		0.69	0.60	0.06	0.33

[1]) cyclopropane fatty acids are regarded as unsaturated

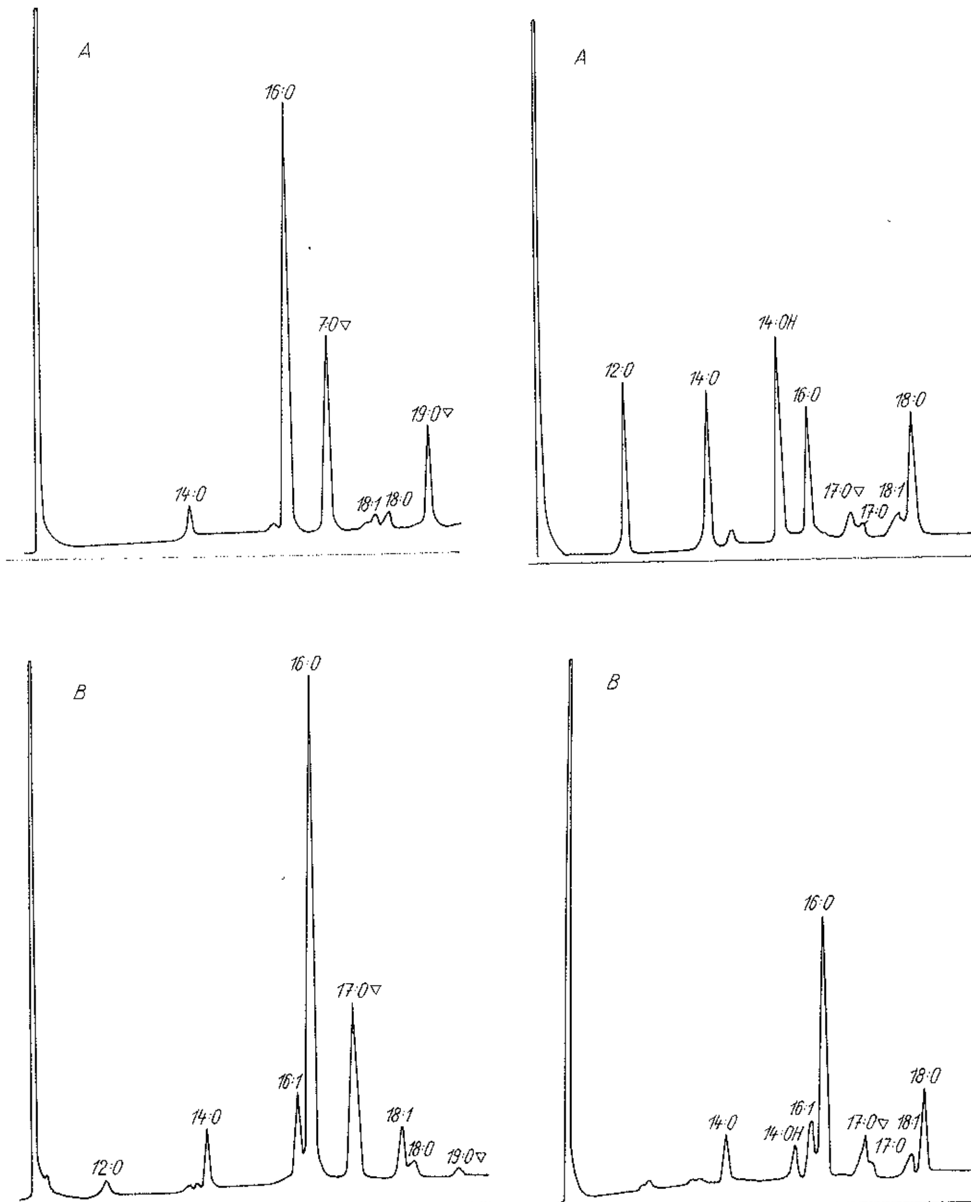

Fig 1. Gas-liquid chromatograms of fatty acid methyl esters in extractable lipids (left) and non-extractable lipids (right) from parent (A) and L-form (B) cells of *E. coli* W 1655 F+

clear differences between both EL and NEL in bacterial and L-form cells. The major component in bacterial and L-form lipids is C 16:0, amounting to about 54% in EL and 46.8 or 17.5%, respectively in NEL. Further similarities are the concentrations of cyc C 17:0 and C 18:0 in EL and NEL of bacterial and L-forms.

An important qualitative difference is the presence of about 9% C 16:1 in L-form lipids only.

Clear quantitative differences concern the 2—3 times higher content of C 18:1 in both L-form lipids and the about 7 times higher content of cyc C 19:0 in EL of the bacterial form in comparison to the EL of the L-form.

Very striking are also the high amounts of C 12:0, C 14:0, and C 14:βOH in the NEL of the bacterial form. These fatty acids represent more than 50% of the total fatty acid content in this fraction, whereas they occur only as traces in EL and in an amount of 13% in NEL of the L-form.

Traces of C 17:0 could be found in both NEL fractions.

Discussion

The transformation of bacterial cells into stable protoplast type L-form cells is accompanied by a more or less complete loss of the cell wall and the periplasmic space (GUMPERT *et al.* 1971) as well as by changes in the cytoplasmic membrane. The adaptation of L-form cells to grow in liquid media is always a relatively long process. In the course of the adaptation those L-form cell types become selected which are more resistant against unsuitable environmental influences and which are able to carry out all essential metabolic processes under these conditions. Obviously biochemical and structural changes in the cytoplasmic membrane play an important role during isolation and adaptation of stable L-forms (GMEINER and MARTIN 1976, PANOS 1968).

The fatty acid spectra of *E. coli* W 1655 F^+ and its L-form lipids are qualitatively similar to those of other *E. coli* strains (CRONAN and VAGELOS 1972, HOEKSTRA *et al.* 1976, ISHINAGA *et al.* 1979, RAETZ 1978, SIJHER *et al.* 1980). Especially C 16:0 seems to be a major component in many *E. coli* strains and other gram-negative bacteria, *e. g. Proteus mirabilis* (GMEINER and MARTIN 1976, NESBITT and LENNARZ 1965).

Because its content is about 54% in EL of bacterial and L-form it obviously plays an essential role as constituent of the cytoplasmic membrane in *E. coli.* It does not appear to change during L-form transformation. The same conclusion can be drawn for cyc C 17:0 and C 18:0 which are present in nearly the same amount in the corresponding lipid fractions of the bacterial and L-form.

A remarkable qualitative difference is the presence of C 16:1 in L-form lipids exclusively. Two explanations are possible. It is known that there can be a conversion of C 16:1 into cyc C 17:0 during the transition from the exponential growth phase into the stationary growth phase (SIJHER *et al.* 1980). Because the L-form grows more slowly and inhomogeneously some lysis and cell propagation can occur also in later growth phases. In this case a certain portion of growing L-form cells in the culture after 42 h incubation may explain this C 16:1 content.

Another explanation would be that the C 16:1 content reflects one important alteration in the composition of the L-form membrane. It might be that this fatty acid plays an important role in strengthening the membrane structure. This is supported by the findings of NESBITT and LENNARZ (1965) in *Proteus mirabilis.* They obtained a 4—5 times higher content of C 16:1 in EL and NEL from L-forms in comparison to the bacterial form.

Very clear quantitative differences exist in the amount of C 12:0, C 14:0, and C 14:βOH between EL and NEL of bacterial forms as well as between NEL from bacterial and L-form. These 3 fatty acids represent more than 50% in the NEL of the bacterial form in comparison to 13% in that of the L-form. The conclusion that these fatty acids are mainly localized in the cell wall, is supported by the fact, that C 12:0, C 14:0 and C 14:βOH are known to be components of lipid A, the characteristic cell wall lipopolysaccharide in *Enterobacteriaceae* (Burton and Carter 1964, Goldfine 1972, Raetz 1978).

While there are no differences in the ratio of unsaturated to saturated fatty acids in the EL of both cell types, the NEL of the bacterial form has very low content of unsaturated fatty acids (Table 1). This result confirms findings of other authors that the outer membrane in *E. coli* strains contains more saturated fatty acids than the cytoplasmic membrane (Ishinaga *et al.* 1979, Lugtenberg and Peters 1976).

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