Biochimica et Biophysica Acta, 426 (1976) 581—586

BBA Report

BBA 71245

FREEZE ETCH MORPHOLOGY OF OUTER MEMBRANE MUTANTS OF *ESCHERICHIA COLI* K12

A.J. VERKLEIJ, E.J.J. LUGTENBERG and P.H.J.Th. VERVERGAERT

Institute of Molecular Biology, Department of Microbiology and Biological Ultrastructure Research Unit, State University, Transitorium 3, Padualaan 8, Utrecht (The Netherlands)

(Received November 28th, 1975)

Summary

Freeze etching showed that the loss of each of the major outer membrane proteins b, c or d in mutants of *Escherichia coli* K12 does not influence the morphology of fracture faces of the outer membrane.

Mutants that possess a heptose-deficient lipopolysaccharide and which in addition are deficient in one or more major outer membrane proteins exhibit a reduction in the number of intramembranous particles of the outer membrane.

Moreover it was shown that lipid phase transitions induce a lateral lipid protein separation in the outer membrane, similar to that found in the cytoplasmic membrane.

Mutants of *Escherichia coli* K12 have been isolated that lack one or more of their major outer membrane proteins and/or possess a heptose-deficient lipopolysaccharide [1,2]. The present freeze etch electron-microscopic study was undertaken in order to investigate whether these defects cause changes in the architecture of the outer membrane.

The isolation and characterization of the mutants will be described extensively elsewhere [1]. Cells were grown at 37°C in yeast broth [2], harvested at the end of the exponential growth phase and washed with 0.9% NaCl. They were fixed with 2% glutaraldehyde in 0.9% NaCl. Before quenching glycerol was added up to 25% to prevent freeze damage. The fracture faces were not influenced by the presence of glycerol. The samples were quenched in a mixture of liquid and solid nitrogen and fractured in a Denton freeze etch apparatus as described before [3]. Electron micrographs were made with a Siemens Elmiskop 1 A.

Composition of outer membrane proteins and lipopolysaccharide. Fig. 1 shows the polyacrylamide gel electrophoresis patterns of the cell envelope

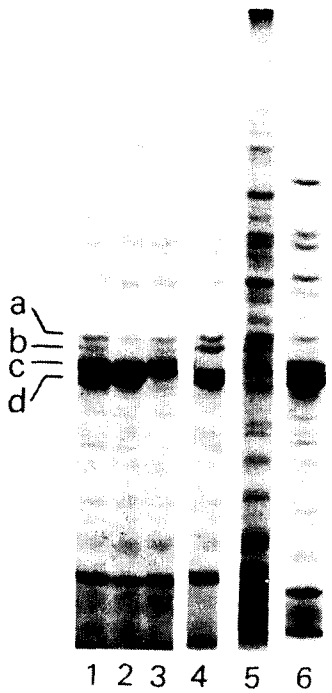

Fig. 1. Polyacrylamide gel electrophoresis of cell envelopes. Protein profiles of the following strains are shown. 1, AB1859; 2, CE1032; 3, CE1034; 4, CE1036; 5, CE1042; 6, AB1859 grown in yeast broth HS medium. Experiments were carried out as described previously [2].

proteins of the strains under investigation. The most significant differences between cell envelope proteins of parent and mutant strains are the deficiencies in or loss of one or more of the proteins b, c and d, which are located in the outer membrane [2]. The deficiencies of the strains with respect to major outer membrane proteins and lipopolysaccharide are listed in Table I.

TABLE I

OUTER MEMBRANE DEFICIENCIES IN *E. COLI* K12 STRAINS

Strain designation	Relevant characteristics	Composition of cell envelopes	
		Deficiency in outer membrane protein	Deficiencies in lipopolysaccharide
AB1859	Parent strain	none	none
AB1859 grown in YBHS medium		b	none
CE1032	Bacteriophage T_3-, T_4, and T_7- resistant mutant	b	Heptose-deficient
CE1034	Bacteriophage K_3- resistant mutant	d	none
CE1036	Bacteriophage T_6- resistant mutant	c	none
CE1042	Triple mutant	b,c,d	Heptose-deficient
PC0479	Parent strain	none	none
CE1007	*galE* mutant	none	Galactose-deficient
CE1018	*galU* mutant	b	Glucose-deficient
CE1023	Bacteriophage T_3-, T_4-, and T_7- resistant mutant	b	Heptose-deficient

A comparison of nomenclature of protein bands has been presented in a previous paper [2].

Freeze etch morphology. Fig. 2 shows the fracture faces of the outer membrane of the parent strain AB1859 quenched from both 37°C and 20°C as the temperature of quenching appeared to influence the freeze etch morphology. At 37°C the convex or inner fracture face of the outer membrane ($\widehat{OM}$) is rather rough and appears to have predominantly pits. In addition it contains a few particles with a diameter of about 80 Å (Fig. 2A). The concave or outer fracture face of the outer membrane ($\breve{OM}$) is completely occupied by particles with a diameter of about 80 Å (probably complementary to the pits on the $\widehat{OM}$) (Fig. 2B). At 20°C the morphology differs from that at 37°C in that on the $\breve{OM}$ small smooth areas are visible, probably as a result of aggregation of the particles (Fig. 2D). At lower temperature no further changes were observed. The freeze etch morphology of the cytoplasmic membrane shows a similar temperature dependent aggregation of particles, as reported earlier by several authors (for a review see ref. 4).

The fracture faces of strain AB1859, grown under conditions that cause it to be deficient in protein b (Table I), strain CE1034 (lacking protein d) and strain CE1036 (lacking protein c) are similar to those of strain AB1859 shown in Fig. 2. Therefore it can be concluded that the loss of one of the proteins b, c or d does not result in the loss of intramembranous particles.

It should be noted that hardly any fracture faces of the cytoplasmic membrane of strain CE1034 could be found in the presence of glycerol. This result indicates that in this strain the loss of protein d favours fracturing of the outer membrane.

Remarkable differences were found between the parent strain AB1859 and the two mutants CE1032 and CE1042 (Fig. 3). The particle density on the $\breve{OM}$ of the two mutant strains is remarkably reduced. This effect is visible more clearly when quenched from 20°C or lower. On the $\breve{OM}$ large smooth areas are visible between aggregates of particles. Fissures, most likely complementary to the particle aggregates on the $\breve{OM}$, are visible on the $\widehat{OM}$. The $\widehat{OM}$ contains a few particles. The fracture faces in cells of strains CE1032 and CE1042 are predominantly in the outer membrane both in the absence as well as in the presence of glycerol.

The low particle density on the $\breve{OM}$ of the two heptose-deficient mutants prompted us to investigate a series of lipopolysaccharide mutants. The morphology of the parent strain PC0479 and its derivative CE1007 (galactose-deficient lipopolysaccharide) is similar to that of strain AB1859. The heptose-deficient strain CE1023 has the same abnormal morphology as described for strains CE1032 and CE1042. Strain CE 1018 (glucose-deficient lipopolysaccharide) is similar to strain CE1034 (deficient in protein d) in that in the presence of glycerol the fracture faces are predominantly in the outer membrane, while the particle density in the $\breve{OM}$ is normal.

The simultaneous appearance of smooth areas and aggregation of particles in the $\breve{OM}$ was observed in all heptose-deficient mutants (Fig. 3C) and, to a less extent, also in the other strains (Fig. 2D). Van Gool and Nanninga explained the occurrence of smooth areas in a wild type *E. coli* outer membrane as an indication for a third fracture face [5]. However, our

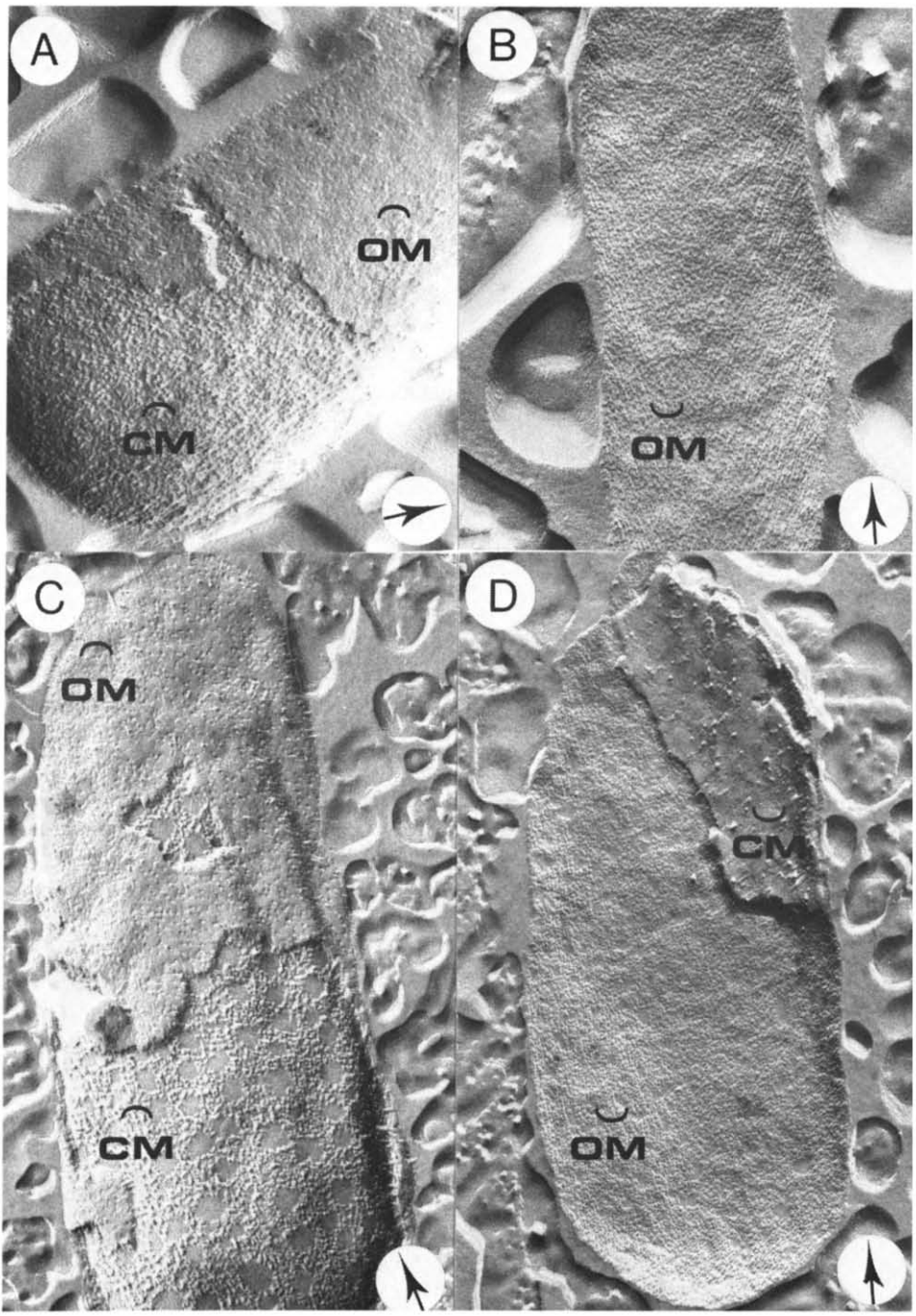

Fig. 2. Fracture faces of *Escherichia coli* K12 strain AB1859 quenched from 37°C (A and B) and from 20°C (C and D). $\widehat{OM}$ and $\breve{OM}$ are convex (inner) fracture faces and concave (outer) fracture faces of the outer membrane respectively. $\widehat{CM}$ and $\breve{CM}$ are the convex fracture face and concave fracture face of the cytoplasmic membrane respectively. (× 60 000).

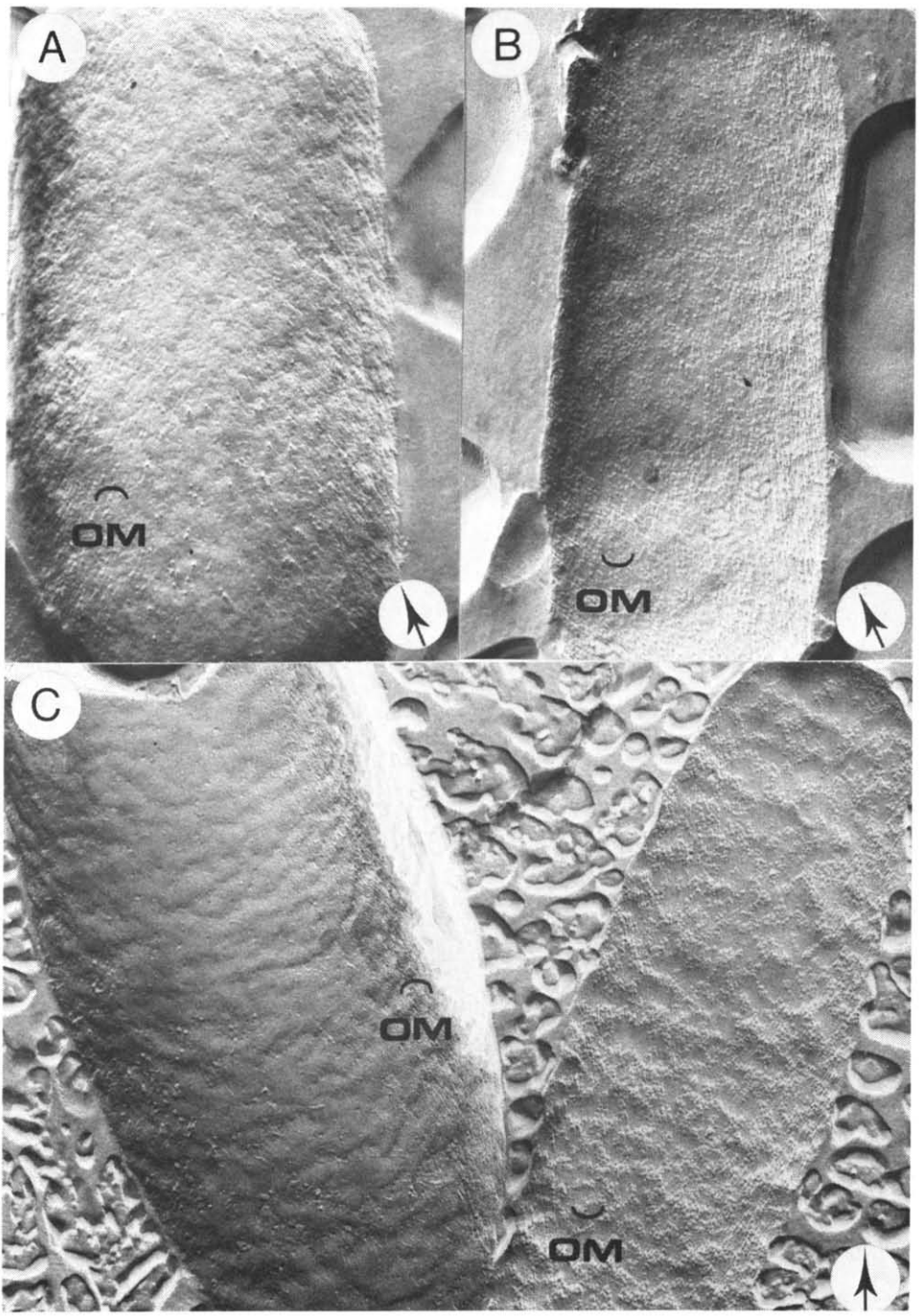

Fig. 3. Fracture faces of the outer membranes of *Escherichia coli* K12 strain CE1032 quenched from 37°C (A and B) and from 20°C (C). Similar fracture faces are obtained with strains CE1042 and CE1023. Abbreviations as in Fig. 2. (× 60 000).

observation, that smooth areas are absent when the cells are quenched from 37°C, makes their explanation unlikely. In our opinion the aggregation of particles should be explained as a reflexion of a lipid phase transition as was described for the cytoplasmic membrane [4]. This explanation is supported by X-ray diffraction studies of Overath et al. [6], which showed that 30 to 40 percent of the outer membrane lipids solidify upon decreasing the temperature. Our freeze etch data are in agreement with their suggestion [6] that the lipids of the outer membrane partially exist as a mono- and/or bilayer.

The particle density on the OM of heptose-deficient strains is decreased (Figs. 3B and 3C). In addition to shorter sugar chains, these mutants are deficient in protein b [1]. The deficiency in protein b is not responsible for the decrease in particle density as normal particle densities were observed in both strain CE1018 (deficient in protein b) as well as in strain AB1859, grown under conditions which cause it to be deficient in protein b. Heptose-deficient mutants have been shown to have an increase of 30 to 40% in their relative amount of 2-keto-3-decxyoctonate, a lipopolysaccharide-marker, in their cell envelopes [1]. Koplow and Goldfine observed that the lipopolysaccharide of heptose-deficient mutants is about equally distributed among cytoplasmic and outer membranes [7], while it is a typical outer membrane component in wild type cells. These two observations indicate that the number of lipopolysaccharide molecules in the outer membrane of heptose-deficient mutants probably is reduced by one third. This reduction in lipopolysaccharide content of the outer membrane might well be related to the reduction in the number of particles as Gilleland et al. observed that a partial extraction of the lipopolysaccharide results in a reduction of the particle density in the OM [8].

We thank C. Verhoef for providing us with some of the lipopolysaccharide mutants and Nelke van Selm for performing lipopolysaccharide-sugar analyses and J. Bijvelt and F. Neys for technical assistance.

References

1 Van Alpen, W., Lugtenberg, B. and Berendsen, W., manuscript in preparation
2 Lugtenberg, B., Meijers, J., van der Hoek, P., Peters, R. and van Alphen, L. (1975) FEBS Lett. 58, 254—258
3 Ververgaert, P.H.J.Th., Elbers, P.F., Luitingh, A.J. and van de Berg, H.J. (1972) Cytobiology 6, 86—96
4 Verkleij, A.J. and Ververgaert, P.H.J.Th. (1975) Annu. Rev. Phys. Chem. 26, 101—122
5 Van Gool, A.P. and Nanninga, N. (1971) J. Bacteriol. 108, 474—481
6 Overath, P., Brenner, M., Gulik-Krzywicki, T., Shechter, E. and Letellier, L. (1975) Biochim. Biophys. Acta 389, 358—369
7 Koplow, J. and Goldfine, H. (1974) J. Bacteriol. 117, 527—543
8 Gilleland, H.E., Stinnett, J.D. and Eagon, R.G. (1974) J. Bacteriol. 117, 302—311