MUTANTS OF *ESCHERICHIA COLI* K12 LACKING ALL 'MAJOR' PROTEINS OF THE OUTER CELL ENVELOPE MEMBRANE

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1. Introduction

The outer cell envelope membrane of *E. coli*, and very likely of Gram-negative bacteria in general, contains a set of so-called major proteins in a mol. wt range between 33 000 and 38 000 (references see table 1). These proteins and their designations used by several authors are listed in table 1; horizontal lines compare proteins that are likely to be identical.

Proteins I and II* can be produced in rather large quantities, and it has been calculated that about 10^5 copies of each protein are present per cell [4,6]. Also, several lines of evidence strongly indicate that these proteins are orderly arranged in the outer membrane [6,11]. On the basis of these and other observations [12] we had considered the possibility that the proteins in question belong to a self-assembly system which participates in the expression of cellular shape. Recently, a number of mutants have been isolated exhibiting various types of abnormal protein composition of the outer membrane. Such mutants miss one or another major protein [8,10,13] or harbor decreased amounts of several of these proteins [7,14]. Also, it appears that the relative amounts of these proteins can differ substantially depending on growth conditions [10]. All these facts indicated that at least a rather precise, stoichiometric arrangement of the outer membrane proteins in question is not a requirement for an indispensible cellular function.

We wished to clarify the issue especially regarding the determination of cellular shape, and here we describe the isolation and some properties of mutants that miss all of these major outer membrane proteins.

Table 1
Protein designation

Molecular weight	(Reference; *E. coli* strain)						
	(1,2; not specified)	(3; 0111)	(4,5; K12 and B)	(6; B)	(7; K12)	(8; K12)	(9; MX74)
38 000	A_1	1	I[a]	Matrix protein	B		peak 4
	A_2	2	not observed[d]	not observed[d]	not observed[d]		
33 000	B, B*[b]	3(3a + 3b)[c]	II*		C, D[b]	G	peak 6[b] and 7

[a] Consists of two different polypeptide chains or two species of the same chain in *E. coli* K12 (see fig.1) but not in B.
[b] Two conformations of the same protein ('heat-modifiable' protein); this characteristic has also been found with proteins 3 and II*.
[c] Schnaitman [3] reported that protein 3 consists of two different polypeptides 3a and 3b; so far we have no evidence that our II* also consists of two polypeptides, this may well be due to strain differences as it is the case for protein 2.
[d] In agreement with Schnaitman's observation [10] that other *E. coli* strains than 0111 can lack protein 2.

2. Experimental

2.1. *Bacterial strains and growth conditions*

Strains JF404 (HfrH) and its colicin tolerant derivative JF404-2a (8) were from Dr J. Foulds. Strains P400 (an AB1133 derivative) and its colicin tolerant derivatives P530 and P692 [15] were from Dr P. Reeves. Cells were grown in complete medium (Antibiotic Medium No. 3, Difco) at 30°C; selection for phage resistance was performed at 37°C.

2.2. *Cell envelopes and electrophoresis*

Cell envelopes were prepared according to Braun et al. [16]. Envelopes consist of outer membrane, murein, and fragments of the cytoplasmic membrane. The band pattern obtained from such preparations by dodecyl sulfate (SDS) polyacrylamide gel electrophoresis is almost identical to that exhibited by outer membrane isolated according to Osborn et al. [17]. When whole cells were used for electrophoretic analysis they were lyophilized, extracted with chloroform-methanol (2:1), and kept for 5 min in boiling 2% SDS containing 0.05 M mercaptoethanol (5 mg dry cells per ml). Upon centrifugation the protein from the supernatant was precipitated with acetone (final concentration 90%) and lyophilized. SDS-polyacrylamide gel electrophoresis was performed according to Studier [18] as detailed for bacterial membranes by Ames [19].

3. Results

3.1. *Isolation of mutants*

Chai and Foulds [8] have described colicin tolerant (*tolG*) mutants of *E. coli* K12 that miss a major outer membrane protein (protein G). Electrophoretic analysis of one of these mutants (strain JF404-2a) showed that protein G is identical to our protein II*.

From local sewage a number of phages were isolated and tested for growth on strains JF404 (wild type) and JF404-2a. One phage was found that forms plaques on JF404 but not on the *tolG* mutant. The phage produces clear plaques and its electronmicroscopic appearance is similar to phage T2. We have provisionally named it TuII*. Selection of JF404 mutants resistant to this phage was performed and it was found that 16/18 of the resistants lacked protein II*.

Davies and Reeves [15] have isolated colicin tolerant mutants of *E. coli* K12 that also miss a major outer membrane protein. Electrophoretic analysis of two of these mutants (P530 and P692) showed that the band missing is identical to our protein I. Both mutants are sensitive to TuII*. Consequently, selection of resistance to TuII* was performed in these mutants. To date 8 such resistants have been analyzed.

3.2. *Properties of P530 and P692 resistant to TuII**

Cell envelopes were prepared from these TuII* resistant mutants and analyzed electrophoretically. Fig.1 shows that they now lack both proteins I and II*, i.e., none of the major proteins is left in the outer membrane Cell envelope preparation involves only shaking of cells with glass beads and subsequent centrifugation, and it appeared unlikely that by virtue of a mutationally altered outer membrane protein II* would be lost preferentially and quantitatively during this procedure. We have nevertheless tried to electrophoretically identify proteins I and II* in SDS extracts of whole cells. This is in fact possible, and fig.1 shows that also in such extracts of the mutants both proteins are missing.

To our surprise, these mutants did not show any obvious defects in vivo. Like the parent strains they are resistant to detergents (SDS, deoxycholate, Triton X-100), EDTA, and a number of antibiotics (Novobiocin, Actinomycin, Bacitracin, Erythromycin, Daunomycin, Distamycin). They are sensitive to phages T2, T3, T4, T5, T6, T7, λ_{vir}, ϕ80, P1, and to colicin E3, i.e., even the uptake of the 60 000 daltons [20] colicin does not appear to be impaired. The mutants grow well in the medium used, they are mobile, and exponentially growing are indistinguishable morphologically from the parent strains. In the presence of mitomycin C normal filament formation occurs, and cell envelopes prepared from such filaments also proved to miss proteins I and II*. Finally, ultrathin-sectioned mutant cells did not exhibit any qualitative alteration of the outer cell envelope membrane.

4. Discussion

The commonly used designation 'major' proteins for the proteins discussed here is somewhat misleading in that the 10 000 daltons lipoprotein studied in detail

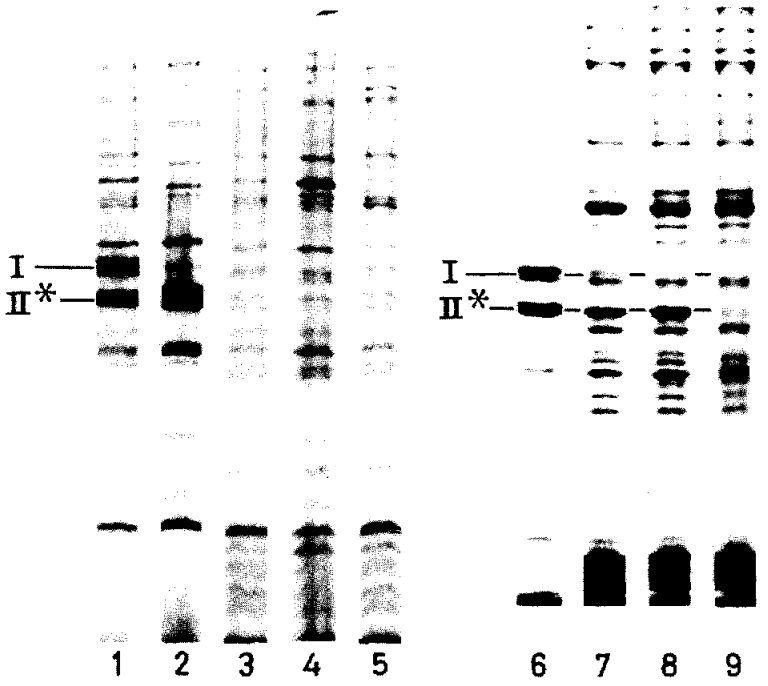

Fig. 1. SDS-Electrophoreses of envelopes and total cellular protein. 1–6, envelopes; 7–9 whole cells; 1, wild type; 2, P692; 3–5, three different TuII* resistant mutants of P692; 6,7, wild type; 8, P692; 9, TuII* resistant mutant of P692. Photographs 6–9 were exposed shorter than 1–5 for a clearer presentation of missing bands, and many minor bands are not visible in 6–9. Both bands at I in 1 and 6 belong to protein I (see table 1). Each sample represents about 50 μg dry material. The other 5 TuII* resistant mutants of P692 and P530 mentioned in the text exhibit similar patterns as those shown for samples 3–5 and 9.

by Braun and collaborators [21] clearly also constitutes a major outer membrane protein. The mutants described here do not lack the lipoprotein.

In any case, we have shown that proteins I, II* and the lipoprotein are very densely packed in the outer membrane of *E. coli* K12 [11]; in fact, there is hardly enough space provided by this membrane to accomodate the total mass of these proteins [4]. Schnaitman [10] described a mutant lacking protein 1 (our I, see table 1), and this mutant produced increased amounts of protein 3 (containing our II*, see table 1). We have observed a similar phenomenon in the *tolG* mutant [8] missing protein II*, there is more protein I than in the corresponding wild type. One could therefore have been led to suspect that lack of one major protein is compensated for by increased production of another one, possibly in order to maintain integrity of the outer membrane. Doubts to this view came from the studies of Koplow and Goldfine [7] as well as from those of Ames [14]; lipopolysaccharide defective mutants of *E. coli* K12 and *Salmonella typhimurium* showed rather significant deficiencies of all outer membrane proteins in the 38 000–33 000 daltons range. The former authors showed that in the outer membrane of such a mutant the phospholipid-to-protein ratio had increased 2.4-fold. We have shown here that all these proteins can be missing without that any other single protein species would increase visibly, and it remains to be seen if in this case as in that of Koplow and Goldfine the space available is 'filled up' with phospholipid.

The flexibility of the architecture of the outer membrane is truly astonishing, and even more so since no gross functional defect has become apparent in our mutants. It has finally also become obvious that an assembly of the proteins in question is not required for the cell to express cylindrical shape.

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References

[1] Bragg, P. D. and Hou, C. (1972) Biochem. Biophys. Acta 274, 478–488.
[2] Reithmeier, R. A. F. and Bragg, P. D. (1974) FEBS Lett. 41, 195–198.
[3] Schnaitman, C. A. (1974) J. Bacteriol. 118, 442–453.
[4] Henning, U., Höhn, B. and Sonntag, I. (1973) Eur. J. Biochem. 39, 27–36.
[5] Garten, W. and Henning, U. (1974) Eur. J. Biochem. 47, 343–352.
[6] Rosenbusch, J. (1974) J. Biol. Chem. 249, 8019–8029.
[7] Koplow, J. and Goldfine, H. (1974) J. Bacteriol. 117, 527–543.
[8] Chai, T. and Foulds, J. (1974) J. Mol. Biol. 85, 465–474.
[9] Inouye, M. and Yee, M.-L. (1973) J. Bacteriol. 113, 304–312.
[10] Schnaitman, C. A. (1974) J. Bacteriol. 118, 454–464.
[11] Haller, I. and Henning, U. (1974) Proc. Natl. Acad. Sci. USA 71, 2018–2021.
[12] Henning, U., Rehn, K. and Höhn, B. (1973) Proc. Natl. Acad. Sci. USA 70, 2033–2036.
[13] Skurray, R. A., Hancock, R. E. W. and Reeves, P. (1974) J. Bacteriol. 119, 726–735.
[14] Ames, F. G.-L., Spudich, E. N. and Nikaido, H. (1974) J. Bacteriol. 117, 406–416.
[15] Davies, J. K. and Reeves, P. (1975) J. Bacteriol., in press
[16] Braun, V., Gnirke, H., Henning, U. and Rehn, K. (1973) J. Bacteriol. 114, 1264–1270.
[17] Osborn, M. J., Gander, J. E., Parisi, E. and Carson, J. (1972) J. Biol. Chem. 247, 3962–3972.
[18] Studier, F. W. (1973) J. Mol. Biol. 79, 237–248.
[19] Ames, G. F.-L. (1974) J. Biol. Chem. 249, 634–644.
[20] Herschmen, H. R. and Helinski, D. R. (1967) J. Biol. Chem. 242, 5360–5368.
[21] Braun, V. and Bosch, V. (1972) Eur. J. Biochem. 28, 51–69.