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Heptose-deficient Mutants of *Escherichia coli* K12 Deficient in Up To Three Major Outer Membrane Proteins

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Summary. Mutants of *Escherichia coli* K12, deficient in up to three major outer membrane proteins *b*, *c* and *d* have been constructed. Mutants that lack the lipopolysaccharide sugar heptose are deficient in protein *b*. All heptose-deficient strains are supersensitive to lysozyme, various antibiotics and detergents. They excrete the periplasmic enzyme ribonuclease I. Mutants deficient in proteins *c* and/or *d* have the same sensitivity towards these compounds as the parent strain. Cells of single, double and triple mutants are all rod-shaped. Electrophoretic analysis of cell envelope proteins indicates that in some mutants the protein deficiency is partially compensated for by increased amounts of one or two of the other major outer membrane proteins. Heptose-deficient strains have an increased amount of 2-keto-3-deoxyoctonate.

Introduction

The cell envelope of *Escherichia coli* and other Enterobacteriaceae is composed of three layers: (i) an outer membrane, containing proteins, phospholipids and LPS [1] (Osborn et al., 1972), (ii) a peptidoglycan layer (Weidel and Pelzer, 1964; Braun et al., 1973), and (iii) a cytoplasmic membrane, containing proteins and phospholipids (Osborn et al., 1972). The peptidoglycan layer is covalently linked to the outer membrane by the lipoprotein described by Braun and Rehn (1969).

Schnaitman (1970a, b) reported that one protein (molecular weight 44,000 daltons) designated as the "major outer membrane protein", accounts for 40 per cent of the cell envelope protein. By using new gel electrophoresis systems the "major outer membrane protein" of *E. coli* K12 can be resolved into two protein bands (Schnaitman, 1974; Henning et al., 1973b). Recently it was reported that the application of an improved gel electrophoresis system enables the resolution of the "major outer membrane protein" of *E. coli* K12 into four distinct protein bands, designated as *a*, *b*, *c* and *d* (Lugtenberg et al., 1975). For a comparison of designations of the major outer membrane proteins, given by various authors, we refer to a previous paper (Lugtenberg et al., 1976). The designation "major outer membrane protein" is somewhat misleading, as it refers only to the 44,000 dalton protein described by Schnaitman (1970b), while the lipoprotein described by Braun and Rehn (1969) is a major outer membrane protein too.

Using the gel electrophoresis system described by Lugtenberg et al. (1975) we have analyzed the protein composition of the cell envelopes of various mutants for which deficiencies in the composition of the major outer membrane proteins have been reported (Ames et al., 1974; Chai and Foulds, 1974; Koplow and Goldfine, 1974; Schnaitman, 1974; Skurray et al., 1974). In a previous paper we have shown that mutants exist which are deficient in one of the proteins *b*, *c* or *d* (Lugtenberg et al., 1976). Various authors have reported that in some mutants which are deficient in one of the major outer membrane proteins, the deficiency is compensated for by increased amounts of another major outer membrane protein (Schnaitman, 1974; Lugtenberg et al., 1976). These results suggest that these proteins might have similar or identical functions. From the existance of single mutants it therefore cannot be concluded that a cell can exist without two or three of these major proteins. In order to answer the question of whether such a cell can exist, we have tried to construct double and triple mutants. Although the major outer membrane proteins contribute about 70 per cent to the outer membrane protein of a wild type cell (Schnaitman, 1970a), the

[1] *Abbreviations:* EDTA, ethylenediaminetetraacetate; KDO, 2-keto-3-deoxyoctonate; LPS, lipopolysaccharide; SDS, sodium dodecyl sulphate; Tris, tris (hydroxymethyl) aminomethane

results described in this paper show that these mutants indeed can be constructed. Some properties of these mutants will be described.

Materials and Methods

Bacterial Strains and Media

The bacterial strains and some of their relevant characteristics are listed in Table 1. The compositions of yeast broth and glucose minimal medium have been described previously (Lugtenberg et al., 1976) except that the glucose concentration in the latter medium was 0.2%. The auxotrophic requirements thiamine and, if necessary, proline, were present in concentrations of 5 and 20 μg per ml respectively.

Table 1. Properties of bacterial strains

Strain	Parent	Genetic markers[a] phenotypical properties[b]	Source; references
AB1859		K12, F$^-$, *thi, lacY, galK, mtl, xyl, ara, strA, supE*	CGSC[c]; Schnaitman (1974)
CE1032	AB1859	m-AB1859[d], *pro*[e]; Hpd	This paper
CE1034	AB1859	m-AB1859; Ktr	This paper
CE1035	CE1032	m-CE1032; Hpd, Ktr	This paper
CE1036[f]	AB1859	m-AB1859, *tsx*; Pcd	CGSC; Schnaitman (1974)
CE1037	CE1036	m-CE1036, *pro*; Pcd, Hpd	This paper
CE1038	CE1036	m-CE1036; Pcd, Ktr	This paper
CE1040	CE1038	m-CE1036, *pro*; Pcd, Ktr, Hpd	This paper
CE1041	CE1036	m-CE1036, *tolG*; Pcd, Ktr	J. Foulds
CE1042	CE1041	m-CE1041, *pro*; Pcd, Ktr, Hpd	This paper

[a] The genetic nomenclature is the one used by Taylor and Trotter (1972)

[b] Heptose-deficiency, protein *c*-deficiency and phage K3-resistance are abbreviated as Hpd, Pcd and Ktr respectively

[c] E. coli Genetic Stock Center, Yale University, New Haven, Conn., U.S.A.

[d] Genetic markers of the indicated strain

[e] The requirement for proline and the heptose deficiency were introduced simultaneously by selection for resistance to the bacteriophages T3, T4 and T7

[f] This strain is a derivative of strain AB1621, a bacteriophage T4- and T6-resistant mutant of strain AB1859 (Bachman, 1972). When we tested the strain designated as AB1621, it appeared to be resistant to phage T6, but sensitive to phage T4. We designated this AB1621 derivative strain as CE1036

Isolation of Heptose-deficient Mutants

About 4×10^7 stationary phase cells were mixed with 10^7 plaque forming units of each of the bacteriophages T3, T4 and T7. The suspension was spread as a lawn on a yeast agar plate and incubated at 37° C for 16–40 h. Putative T3-, T4- and T7-resistant clones were purified and checked on plates on sensitivity towards rifampicin (4 μg/ml) and on excretion of the periplasmic enzyme ribonuclease I. The heptose content of cell envelopes of strains which are sensitive to rifampicin and leaky for ribonuclease was determined. The chosen heptose-deficient mutants have less than two per cent of the heptose content of strain AB1859. Heptose-deficient strains formed small colonies on yeast agar plates. The strains in which heptose-deficiency coincided with proline requirement were used in the experiments described in this paper as they probably resulted from a chromosomal deletion and therefore are supposed to be stable mutants (Havekes et al., 1976).

Isolation of Bacteriophage K3-resistant Mutants

About 4×10^7 stationary phase cells were mixed with 10^8 plaque forming units of bacteriophage K3. The suspension was spread as a lawn on a yeast agar plate and incubated for 16 h. K3-resistance in some mutant clones was obviously caused by excessive capsule slime formation. Putative K3-resistant clones with wild type colony morphology were purified and checked on resistance to the phage. About half of the K3-resistant strains were deficient in protein *d*, as was shown by polyacrylamide gel electrophoresis of cell envelopes. Only these K3-resistant strains were used in the experiments described in this paper.

Isolation and Characterization of Cell Envelopes

An overnight culture in yeast broth was diluted 1 in 20 in the same medium. The cells were grown at 37° C under aeration. When the absorbance, measured with an Unicam SP600 spectrophotometer at 660 nm wavelength, was 0.75, the cultures were rapidly chilled in icewater, after which a sample was removed for morphological investigation. For the isolation of cell envelopes cells were harvested, washed with a 0,9% NaCl solution and resuspended in a 50 mM Tris-HCl buffer (pH 8.5) containing 2 mM EDTA. After ultrasonic desintegration of the cells, cell envelopes were isolated by differential centrifugation as described by Lugtenberg et al. (1975).

Protein was determined by the method of Lowry et al. (1951). Total cell protein was determined after ultrasonic treatment of the cells. KDO was determined by the thiobarbituric acid method of Weissbach and Hurwitz (1959) as modified by Osborn (1963). In the case of heptose-containing LPS the measured KDO values were corrected for the fact that only two of the three KDO residues are measured (Dröge et al., 1970). Heptose was determined by the cysteine-H_2SO_4 procedure of Dische (1953) as modified by Osborn (1963). Phospholipids were extracted from cell envelopes as described by Bligh and Dyer (1959). Lipid-phosphate was determined as described by Ames and Dubin (1960). Polyacrylamide gel electrophoresis was performed as described by Lugtenberg et al. (1975).

Morphology of the Cells

The morphology of the cells was judged with the help of an interference contrast microscope (Leitz Wetzlar Germany, magnification

1,000 times). The cells were photographed on a Kodak Panatomic X136-36 film.

Sensitivity to Bacteriophages, Sodium Taurocholate, SDS, Cristal Violet, Antibiotics and Lysozyme

Sensitivity to bacteriophages was tested with the double layer technique (100 plaque forming units per plate). After incubation for 16 h at 37 °C plaque formation was scored. The phages χ and K3 were obtained from J. Adler and P. Reeves respectively.

Sensitivity to SDS, sodium taurocholate, cristal violet and rifampicin was tested on yeast agar plates containing final concentrations of 1%, 0.15%, 1 µg/ml and 4 µg/ml of these agents respectively. Strains were scored as resistant when cells were able to form colonies after incubation overnight at 37 °C.

In order to test the sensitivity to novobiocin and lysozyme, 0.1 ml of an overnight culture was spread on the surface of a yeast agar plate. After drying, 30 µliters of fresh solutions of novobiocin (1 mg/ml) and lysozyme (1 mg/ml) were applied on the lawn. After incubation overnight at 37 °C the results were scored. A strain was scored as sensitive when the diameter of the zone of no growth was at least one cm.

Leakage of Ribonuclease I

Leakage of ribonuclease I was determined as described by Havekes et al. (1976).

Antibiotics

Novobiocin was obtained from Upjohn Co., Kalamazoo, Michigan, U.S.A. and rifampicin from Lepetit, Amsterdam, The Netherlands.

Results

Stepwise Construction of Mutants Deficient in Outer Membrane Proteins b, c and d

Heptose-deficient strains are known to have deficiencies in their major outer membrane proteins (Ames et al., 1974; Koplow and Goldfine, 1974). Heptose-deficient mutants, isolated from five different parent strains with considerable differences in their content of protein *b*, contain greatly diminished amounts of this protein (*unpublished results* and Havekes et al., 1976). Strain CE1036, originally derived from strain AB1859 (see legend f of Table 1) is deficient in protein *c*. Mutants deficient in protein *d* can be isolated by selection either for resistance to bacteriophage K3 (Skurray et al., 1974; Lugtenberg et al., 1976) or for tolerance to bacteriocin JF246 (Foulds and Barrett, 1973; Chai and Foulds, 1974; Lugtenberg et al., 1975).

The methods mentioned above gave us the possibility to attempt the stepwise construction of mutants deficient in two and three of the major outer membrane proteins *b*, *c* and *d*. As strain CE1036 was the only strain available which is deficient in protein *c*, we used strain AB1859 as the parent strain. A *tolG* mutant of strain CE1036 was isolated for us by J. Foulds. Heptose-deficient mutants and bacteriophage K3-resistant mutants could easily be isolated from all strains. The strains described in this paper are listed in Table 1.

Protein Composition of Cell Envelopes

The protein patterns of envelopes of the strains are shown in Figure 1. Except for minor changes, the cell envelope proteins only differ in their content of the major outer membrane proteins *b*, *c* and *d*. No differences could be detected in the protein profiles of the soluble protein fractions (*not shown*). A quantitative analysis of the content of proteins *a*, *b*, *c* and *d* in envelopes of the various strains, as determined after scanning of the stained gel, is given in Table 2. The relative amount of the major outer membrane proteins present in envelopes of the mutant strains is decreased. Heptose-deficient strains contain a decreased amount of protein *b* (Havekes et al., 1976). This effect is not convincingly shown in Table 2 as the parent strain AB1859 contains only low amounts of protein *b* after growth in yeast broth. However, after growth in minimal medium the relative amount of protein *b* is much higher in strain AB1859 than

Table 2. Content of major outer membrane proteins

Strain	Phenotypical properties	Total major outer membrane protein ($a+b+c+d$) relative to total cell protein[a] (arbitrary units)	Individual major outer membrane proteins relative to total cell protein[b] (arbitrary units)			
			a	*b*	*c*	*d*
AB1859		100	7	5	30	58
CE1032	Hpd	64	5	2	20	37
CE1034	Ktr	58	7	7	41	3
CE1035	Hpd, Ktr	42	5	3	31	3
CE1036	Pcd	87	6	13	4	64
CE1037	Pcd, Hpd	92	10	7	4	71
CE1038	Pcd, Ktr	30	8	13	5	4
CE1040	Pcd, Ktr, Hpd	28	12	4	5	7
CE1041	Pcd, Ktr	55	6	41	5	3
CE1042	Pcd, Ktr, Hpd	23	4	12	3	4

[a] This value was obtained by combining the values for (i) the percentage of cell envelope protein over total cell protein, and (ii) the contribution of the major outer membrane proteins $a+b+c+d$ to the total cell envelope protein as determined from densitometer tracings of the stained gel. The tracing data are only reproducible when compared on the same gel as they depend on the degree of destaining of the gel. Therefore the values are given in arbitrary units. For the parent strain AB1859 the value is defined as 100, while those of the mutants are given relative to strain AB1859

[b] Values were calculated as described for the previous column

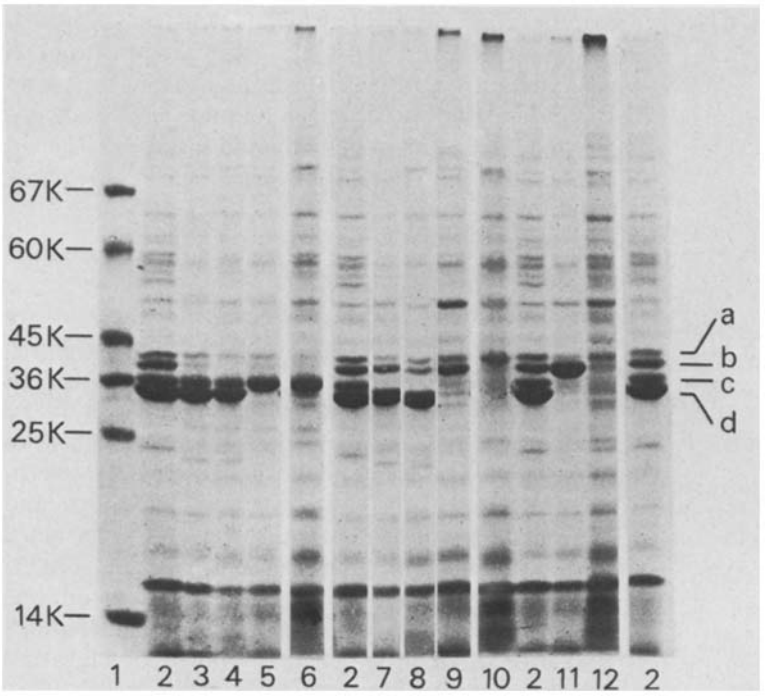

Fig. 1. SDS-polyacrylamide gel electrophoresis of cell envelopes. 1: molecular weight standard proteins. The molecular weights are indicated at the left. The numbers 2 through 12 represent cell envelope proteins of the following strains: 2: JC7620, previously designated as strain PC1349 (Lugtenberg et al., 1976). This sample is repeated several times in order to identify the lacking proteins in the mutants; 3: AB1859; 4: CE1032; 5: CE1034; 6: CE1035; 7: CE1036; 8: CE1037; 9: CE1038; 10: CE1040; 11: CE1041; 12: CE1042

in its heptose-deficient mutant strain CE1032. Strain CE1036 and its derivatives are deficient in protein *c*. The *tolG* strains CE1041 and CE1042 are deficient in protein *d*. Of course this is also the case for the K3-resistant mutants used (see Materials and Methods).

Properties of the Strains

All strains grow well at 30, 37 and 42 °C. The more major outer membrane proteins are missing in a mutant, the slower that mutant strain grows. The strains were tested for their sensitivity towards bacteriophages, SDS, sodium taurocholate, crystal violet, lysozyme and the antibiotics novobiocin and rifampicin. Also the effect of the deficiency in the major outer membrane protein on the excretion of the periplasmic enzyme ribonuclease I was determined. The results are presented in Table 3. Resistance to the bacteriophages T3, T4 and T7 is restricted to the heptose-deficient strains, which were selected for this property. While strain AB1859 is resistant towards P1 and Mu-1, its derivative CE1036 is sensitive to these phages. However, the heptose-deficient mutants of strain CE1036 are resistant, suggesting that the receptor for these phages is masked in strain AB1859, exposed in strain CE1036, but absent or inactive in the heptose-deficient mutants of the latter strain. Strain CE1036 and its derivatives are deficient in protein *c*. Comparison of the genetic markers of strains AB1859 and CE1036 suggests that the deficiency in protein *c* in strain CE1036 corresponds with phage T6 resistance. By analysis of transconjugants of strain CE1036, it was shown that phage T6 resistance and deficiency in protein *c* are caused by mutations in different genes (Verhoef and Lugtenberg, unpublished observations). The phage K3 resistant mutants described in this paper, as well as the *tolG* mutant strains CE1041 and CE1042, are deficient in protein *d* (Fig. 1). It should be noted that the *tolG* mutants are also resistant to bacteriophage K3 (Table 3). Only the heptose-deficient strains are sensitive to SDS, sodium taurocholate, lysozyme, novobiocin and rifampicin and excrete ribonuclease I. These properties have been described earlier for other heptose-deficient mutants (Tamaki et al., 1971; Tamaki and Matsuhashi, 1973; Ames et al., 1974; Havekes et al., 1976).

The amounts of the various cell envelope components relative to total cell protein was determined

Table 3. Properties of the strains[a]

Strain	Phenotypical properties	Sensitivity to bacteriophages				Sensitivity to SDS, sodium taurocholate, lysozyme, novobiocin and rifampicin	Excretion of ribonuclease I[b]
		T3, T4, T7	P1, Mu-1	T6	K3		
AB1859		S	R	S	S	R	−
CE1032	Hpd	R	R	S	S	S	+
CE1034	Ktr	S	R	S	R	R	−
CE1035	Hpd, Ktr	R	R	S	R	S	+
CE1036	Pcd	S	S	R	S	R	−
CE1037	Pcd, Hpd	R	R	R	S	S	+
CE1038	Pcd, Ktr	S	S	R	R	R	−
CE1040	Pcd, Ktr, Hpd	R	R	R	R	S	+
CE1041	Pcd, Ktr	S	S	R	R	R	−
CE1042	Pcd, Ktr, Hpd	R	R	R	R	S	+

All strains are resistant towards the bacteriophages T2, C21 and χ and to crystal violet. They are all sensitive to the bacteriophages T5 and λ_{vir}

[a] S, sensitive, R, resistant

[b] + and −. excretion and no excretion respectively

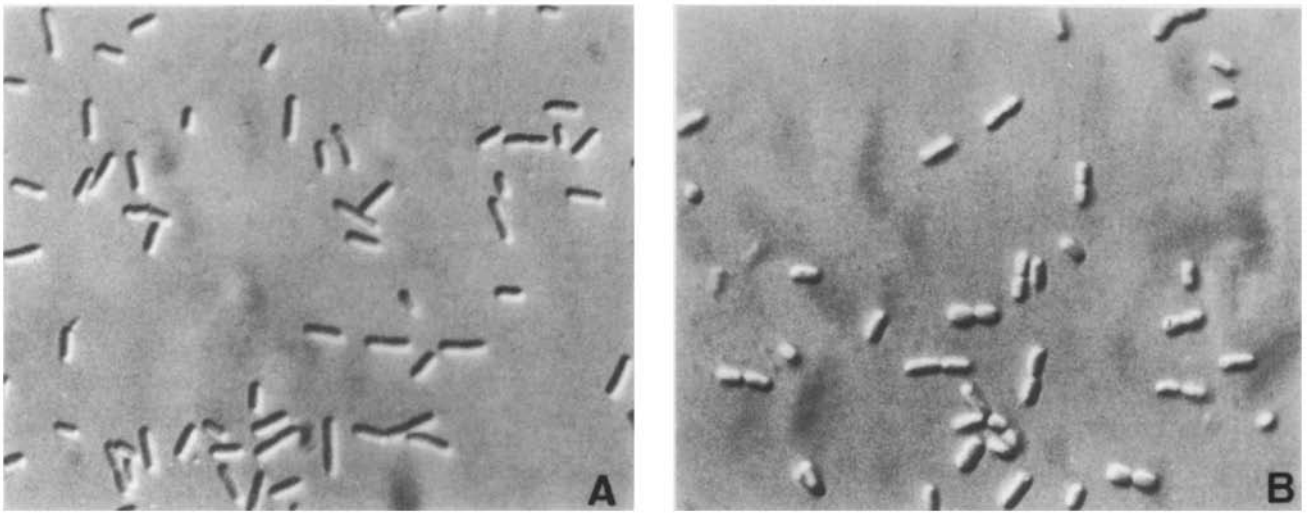

Fig. 2A and B. Morphology of cells of the parent strain AB1859 **A** and of the triple mutant strain CE1040 **B**

for all strains. No significant differences were measured between the mutants and the parent strain with respect to their ratios of cell envelope protein over total cell protein and of phospholipid over total cell protein. Compared with their parent strains all heptose-deficient strains have an increase in the ratio of KDO over total cell protein of approximately 50 per cent.

Morphology

Exponentially growing cells of each strain were directly examined with an interference contrast microscope. Some cells looked like spheres. Upon longer observation of those cells it became clear that this was caused by orientation of rods parallel to the direction of the light. For all strains all cells observed were rod shaped. Figure 2 shows photographs of cells of the parent strain AB1859 and of the triple mutant CE1040. It should be noted that the cells of most mutants are somewhat shorter and thicker and more irregularly shaped than those of the parent strain AB1859.

Discussion

The results described in this paper show that the major outer membrane proteins *b*, *c* and *d* can be

removed by mutation (Fig. 1 and Table 2). These three proteins are not essential for the viability of the cell, although heptose-deficient strains are weaker in several aspects (Table 3). Our observation that even the triple mutants are rod-shaped rules out the possibility, previously suggested by Henning and coworkers (Henning et al., 1973a, b; Haller and Henning, 1974), that the shape of *E. coli* is determined by these major outer membrane proteins. Recently Henning and Haller (1975) came to the same conclusion. Starting with the colicin tolerant mutant strains P530 and P692 (Davies and Reeves, 1975a), which are deficient in protein I (our proteins *b* plus *c*), they constructed double mutants by selection for resistance to bacteriophage TuII*. These double mutants P530*tut*1cII and P692*tut*2eI, deficient in proteins I and II*, are still rod-shaped (Henning and Haller, 1975).

Although the defects in outer membrane proteins of the double mutants of Henning and Haller (1975) and our triple mutants (Fig. 1) are very similar, there are two important differences between the two types of mutants. (i) In strains P530 and P692 both proteins *b* and *c* were removed in one step, whereas in our mutants two mutation steps were required to remove these proteins. We have no explanation for this apparent contradiction. A possible explanation might be that in an *E. coli* wild type strain protein *b* can be converted into protein *c* or vice versa, while in strain CE1036 this is not the case. (ii) A second difference between the double mutants of Henning and Haller (1975) and our triple mutants was observed in the LPS sugar composition. Whereas the LPS of our triple mutants lacks heptose, glucose and galactose, that of a mutant strain of Henning and Haller (1975), deficient in proteins I and II*, has a normal sugar composition (B. Lugtenberg and N. v. Selm, unpublished results). The two double mutants are resistant towards antibiotics and other chemicals (Henning and Haller, 1975), while our triple mutants are sensitive (Table 3). This difference in properties is most likely caused by the different LPS composition of the strains. In all probability LPS is very essential for the structural integrity of the outer membrane and therefore for its permeability properties.

The results presented in Figure 1 and Table 2 show that the loss of protein is only partially compensated for by increased amounts of other major outer membrane proteins (see strains CE1032 and CE1034 in Table 2). The triple mutants CE1040 and CE1042 have lost about 75 per cent of the "major outer membrane protein". It is surprizing that a cell can exist with only that small amount of major outer membrane proteins, as these proteins constitute about 40 per cent of the cell envelope protein in a wild type cell (Schnaitman, 1970a, b). There obviously is no compensation by increased amounts of one or more other proteins in the cell envelope (Fig. 1). However protein assays show that the ratio of cell envelope protein over total cell protein of the triple mutants was not significantly different from that of the parent strain AB1859. So far no evidence could be found that there is compensation by cell envelope proteins which do not enter the gel or run faster than the tracking dye. The different results obtained from protein assays and from the gel pattern can formally be explained by assuming that the loss of major outer membrane proteins is compensated for by increased amounts of most other stained envelope proteins to about the same extent. We feel however that this explanation requires a highly unlikely regulatory mechanism. More experiments. preferentially with isolated outer membranes, are necessary to clarify this problem.

Schnaitman (1974) described that the original strain AB1621 is lacking protein 1 (our proteins *b* plus *c*). This strain is bacteriophage T4-resistant, whereas strain CE1036, a derivative of strain AB1621 (see legend f of Table 1) is T4-sensitive and lacks only protein *c* (Fig. 1). Therefore the reappearance of protein *b* coincides with that of T4 sensitivity. The results described in this paper show that the reversion of strain AB1621 to CE1036 can be explained by assuming that strain AB1621 contained two mutations from which one caused the lack of protein *c* and another one caused bacteriophage T4 resistance. As LPS is the receptor for phage T4 (Wilson et al., 1970), T4 resistance can be caused by a mutation which leads to heptose-deficiency, which in turn causes the lack of protein *b* (Havekes et al., 1976). Strain CE1036 then is a revertant with normal LPS, which therefore contains protein *b* and is sensitive to T4. Strain AB1621 then corresponds to strain CE1037.

A mutation which causes both K3 resistance and conjugation deficiency is located at either 14.4 or 14.9 minutes (Davies and Reeves, 1975b) on the genetic map of Taylor and Trotter (1972). The gene (tut), which determines resistance towards bacteriophage TuII* maps at about 21.5 min (Henning et al., 1976). The *tolG* mutation also maps at this position (Foulds, 1974). Resistance towards bacteriophages TuII* and K3, as well as the *tolG* mutation can cause deficiency in protein *d*. We have shown that *tolG* mutants are also resistant towards bacteriophage K3 (Table 3). Taken these data together it is very likely that the *tut* gene is identical to the *tolG* gene. Henning et al. (1976) mention that isolated protein *d* is the receptor for phage TuII*. It has been hypothesized that this protein is the receptor for conjugation (Skurray et al., 1974; Havekes et al., 1976). The most

likely explanation for the finding that many K3-resistant mutants are deficient in protein *d* is that this protein is also the receptor for phage K3.

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References

Ames, B.N., Dubin, D.T.: The role of polyamines in the neutralization of bacteriophage deoxyribonucleic acid. J. biol. Chem. **235**, 769–775 (1960)

Ames, G.F., Spudich, E.N., Nikaido, H.: Protein composition of the outer membrane of *Salmonella typhimurium:* Effect of lipopolysaccharide mutations. J. Bact. **117**, 406–416 (1974)

Bachman, B.J.: Pedigrees of some mutant strains of *Escherichia coli* K-12. Bact. Rev. **36**, 525–557 (1972)

Bligh, E.G., Dyer, W.J.: A rapid method of total lipid extraction and purification. Canad. J. Biochem. Physiol. **37**, 911–917 (1959)

Braun, V., Gnirke, H., Henning, U., Rehn, K.: Model for the structure of the shape-maintaining layer of the *Escherichia coli* cell envelope. J. Bact. **114**, 1264–1270 (1973)

Braun, V., Rehn, K.: Chemical characterization, spatial distribution and function of a lipoprotein (murein-lipoprotein) of the *E. coli* cell wall. Europ. J. Biochem. **10**, 426–438 (1969)

Chai, T., Foulds, J.: Demonstration of a missing outer membrane protein in *tolG* mutants of *Escherichia coli.* J. molec. Biol. **85**, 465–474 (1974)

Davies, J.K., Reeves, P.: Genetics of resistance to colicins in *Escherichia coli* K-12: cross-resistance among colicins of group A.J. Bact. **123**, 102–117 (1975a)

Davies, J.K., Reeves, P.: Colicin tolerance and map location of conjugation-deficient mutants. J. Bact. **123**, 372–373 (1975b)

Dische, Z.: Qualitative and quantitative colorimetric determination of heptoses. J. biol. Chem. **204**, 983–997 (1953)

Dröge, W., Lehmann, V., Lüderitz, O., Westphal, O.: Structural investigations on the 2-keto-3-deoxyoctonate region of lipopolysaccharides. Europ. J. Biochem. **14**, 175–184 (1970)

Foulds, J.: Chromosomal location of the *tolG* locus for tolerance to bacteriocin JF246 in *Escherichia coli* K-12. J. Bact. **117**, 1354–1355 (1974)

Foulds, J., Barrett, C.: Characterization of *Escherichia coli* mutants tolerant to bacteriocin JF246: two new classes of tolerant mutants. J. Bact. **116**, 885–892 (1973)

Haller, I., Henning, U.: Cell envelope and shape of *Escherichia coli* K-12. Crosslinking with dimethylimidoesters of the whole cell wall. Proc. nat. Acad. Sci. (Wash.) **71**, 2018–2021 (1974)

Havekes, L.M., Lugtenberg, B.J.J., Hoekstra, W.P.M.: Conjugation deficient *E. coli* K12 mutants with heptose-less lipopolysaccharide. Molec. gen. Genet. **146**, 43–50 (1976)

Henning, U., Haller, I.: Mutants of *Escherichia coli* K12 lacking all 'major' proteins of the outer cell envelope membrane. FEBS Letters **55**, 161–164 (1975)

Henning, U., Hindennach, I., Haller, I.: The major proteins of the *Escherichia coli* outer cell envelope membrane: evidence for the structural gene of protein II*. FEBS Letters **61**, 46–48 (1976)

Henning, U., Höhn, B., Sonntag, I.: Cell envelope and shape of *Escherichia coli* K12. Europ. J. Biochem. **39**, 27–36 (1973b)

Henning, U., Rehn, K., Hoehn, B.: Cell envelope and shape of *Escherichia coli* K12. Proc. nat. Acad. Sci. (Wash.) **70**, 2033–2036 (1973a)

Koplow, J., Goldfine, H.: Alterations in the outer membrane of the cell envelope of heptose-deficient mutants of *Escherichia coli.* J. Bact. **117**, 527–543 (1974)

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J.: Protein measurement with the Folin phenol reagent. J. biol. Chem. **193**, 265–275 (1951)

Lugtenberg, B., Meijers, J., Peters, R., van der Hoek, P., van Alphen, L.: Electrophoretic resolution of the "major outer membrane protein" of *Escherichia coli* K12 into four bands. FEBS Letters **58**, 254–258 (1975)

Lugtenberg, B., Peters, R., Bernheimer, H., Berendsen, W.: Influence of cultural conditions and mutations on the composition of the outer membrane proteins of *Escherichia coli.* Molec. gen. Genet. **147**, 251–262 (1976)

Osborn, M.J.: Studies on the gram-negative cell wall, I. Evidence for the role of 2-keto-3-deoxyoctonate in the lipopolysaccharide of *Salmonella typhimurium.* Proc. nat. Acad. Sci. (Wash.) **50**, 499–506 (1963)

Osborn, M.J., Gander, J.E., Parisi, E., Carson, J.: Mechanism of assembly of the outer membrane of *Salmonella typhimurium.* J. biol. Chem. **247**, 3962–3972 (1972)

Schnaitman, C.A.: Examination of the protein composition of the cell envelope of *Escherichia coli* by polyacrylamide gel electrophoresis. J. Bact. **104**, 882–889 (1970a)

Schnaitman, C.A.: Protein composition of the cell wall and cytoplasmic membrane of *Escherichia coli.* J. Bact. **104**, 890–901 (1970b)

Schnaitman, C.A.: Outer membrane proteins of *Escherichia coli.* IV. Differences in outer membrane proteins due to strain and cultural differences. J. Bact. **118**, 454–464 (1974)

Skurray, R.A., Hancock, R.E.W., Reeves, P.: Con$^-$ mutants: class of mutants in *Escherichia coli* K-12 lacking a major cell wall protein and defective in conjugation and absorption of a bacteriophage. J. Bact. **119**, 726–735 (1974)

Tamaki, S., Matsuhashi, M.: Increase in sensitivity to antibiotics and lysozyme on deletion of lipopolysaccharides in *Escherichia coli* strains. J. Bact. **114**, 453–454 (1973)

Tamaki, S., Sato, T., Matsuhashi, M.: Role of lipopolysaccharides in antibiotic resistance and bacteriophage adsorption of *Escherichia coli* K-12. J. Bact. **105**, 968–975 (1971)

Taylor, A.L., Trotter, C.D.: Linkage map of *Escherichia coli* strain K-12. Bact. Rev. **36**, 504–524 (1972)

Weidel, W., Pelzer, H.: Bagshaped macromolecules, a new outlook on bacterial cell walls. Advanc. Enzymol. **26**, 193–232 (1964)

Weissbach, A., Hurwitz, J.: The formation of 2-keto-3-deoxyheptonic acid in extracts of *Escherichia coli* B. I. Identification. J. biol. Chem. **234**, 705–709 (1959)

Wilson, J.H., Luftig, R.B., Wood, W.B.: Interaction of bacteriophage T4 tail fiber components with a lipopolysaccharide fraction from *Escherichia coli.* J. molec. Biol. **51**, 423–434 (1970)

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Note Added in Proof

In contrast to our finding Smit et al. [J. Bact. **124**, 942–958 (1975)] measured an increased amount of phospholipids in the outer membrane of heptosedeficient mutants of *Salmonella typhimurium.* Recent observations showed that the phospholipids of heptose-deficient mutants derived of strain AB1859 are partly degraded by an endogenous phospholipase to lysophospholipids (L. van Alphen et al., unpublished observations). As this degradation product is not extracted with the method used, our data for phospholipid in heptose-deficient mutants are probably too low.