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Outer Membrane of *Escherichia coli* K-12: Isolation of Mutants with Altered Protein 3A by Using Host Range Mutants of Bacteriophage K3

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A series of mutants has been isolated with alterations to protein 3A of the outer membrane. These mutations map at the previously described *con* locus as shown by cotransduction with *pyrD*. Most of them do not have detectable levels of protein 3A but are thought to have low levels of altered protein. These mutants have been detected by screening *con* mutants, isolated as resistant to bacteriophage K3, for their ability to plaque host range mutants of this bacteriophage. These host range phage mutants have activity spectra on the various *con* mutants that enable the bacterial mutants to be arranged in an order of increasing resistance to the host range phage mutants, from mutants sensitive to all host range phage to those sensitive to only one class. Likewise, the phage can be arranged in an order of increasing ability to plaque on the *con* mutants. Some of the mutants resemble the previously described *con* mutants in being tolerant to colicins K and L, and others resemble them in being highly defective as recipients with the F factor. These properties vary independently, suggesting that protein 3A can be modified to independently affect the three properties of bacteriophage receptor function, involvement in colicin sensitivity, and involvement in conjugation.

There are a number of major proteins in the outer membrane of the cell envelope of *Escherichia coli* K-12. Protein 1 (using the nomenclature of Schnaitman [22]) is normally the most abundant of these proteins. The next most abundant protein is protein 3A, which has been shown to be missing in *con* (*tolG*) mutants (4, 17, 23). Protein 3A has now been shown to function as the receptor for bacteriophage K3 and a number of similar phages (P. A. Manning and P. Reeves, manuscript in preparation). The *con* mutants are also of particular interest because of their defect in recipient ability in conjugation with most F-like plasmid donors (16, 17, 23). Proteins 1 and 3A are followed in abundance by protein 3B and the *tsx* protein. Of these, the *tsx* protein has been shown to function as the receptor for bacteriophage T6 and for colicin K (P. A. Manning, M. Lavoie, and P. Reeves, manuscript in preparation), whereas no function has as yet been found for protein 3B. All of the outer membrane proteins are normally produced in much lower amounts and so cannot really be considered as major proteins.

In this paper we report on the properties of a series of mutants that are resistant to bacteriophage K3 and, in general, have undetectable levels of protein 3A. However, the properties of these mutants suggest that they have an altered protein 3A, which is present in greatly reduced amounts. The mutants are altered in some or all of the properties attributed previously to protein 3A.

MATERIALS AND METHODS

Bacterial strains. All strains were derivatives of *E. coli* K-12 and are listed in Table 1.

Media and culture conditions. Nutrient broth, nutrient agar, and minimal agar, supplemented with the appropriate growth factors and carbon source, were as described previously (24, 25). All cultures were incubated at 37°C.

Bacteriophages. All bacteriophages were from stocks maintained in this laboratory and were described previously (11) with the exception of the host range mutants of bacteriophage K3, isolated in this study as described below.

Bacteriophage sensitivity. The sensitivity of the mutants to the set of 64 bacteriophages described previously (11, 17) was determined by using a multiple-syringe phage applicator (11).

Sensitivity to the host range bacteriophage mutants was measured by efficiency of plating using 2 × 10^7 log-phase bacteria and varying amounts of phage in a 4-ml 0.7% nutrient agar overlay.

Mutant selection. Independently derived, spontaneous bacterial mutants resistant to bacteriophage

TABLE 1. *Bacterial strains*[a]

Strain	Characteristics	Source/reference
P400	F⁻/*thi argE proA thr leu mtl xyl ara galK lacY str supE non* λ⁻	23
P460	*con-1* mutant of P400	23
P407	*tsx* mutant of P400	11
CSH23 (E5014)	F′ *lac*⁺ *proA*⁺,*B*⁺/Δ(*lac pro*)	Cold Spring Harbor
W620	F⁻/*thi pyrD gltA galK str rel* λ⁻	B. Bachmann

[a] All *con* mutants isolated in this study are derivatives of strain P400.

K3 were obtained by plating 2×10^7 log-phase cells with 2×10^8 plaque-forming units of phage in a 4-ml 0.7% nutrient agar overlay. Each mutant was derived by using a culture grown from a separate single colony to ensure that the mutations were of independent origin. The mutants were purified by three single-colony isolations, and 2×10^7 cells were plated with 10^9 plaque-forming units of bacteriophage K3. Plaques occurring at a frequency of less than 10^{-5} were picked and propagated through three single-plaque isolations on the mutant on which they were isolated. These phages are the host range mutants.

Mating procedures. Matings were performed as described previously (16).

Preparation of outer membranes and polyacrylamide gel electrophoresis. Outer membranes were the Triton X-100-insoluble components of the cell envelope prepared by the methods of Schnaitman (22).

Samples for electrophoresis were prepared by the method of Schnaitman (21, 22) and were run under his conditions using both the pH 7.2 buffer system of Maizel (15) and the pH 11.4 buffer system of Bragg and Hou (3). Gels were stained with Coomassie brilliant blue (26), and densitometer tracings were obtained with a Quick Scan Jr. gel scanner (Helena Laboratories Corp.)

Protein estimation. Protein concentrations were determined by using bovine serum albumin as a standard and the method of Schacterle and Pollack (20).

Transduction. P1 phage stocks were prepared as described by Miller (18), using heat-inducible P1 carrying chloramphenicol resistance derived from the R-factor R100. The transduction procedure was that of Pittard (19).

Colicin sensitivity. Colicin sensitivity was determined by the conventional cross-streak plate test (7).

RESULTS

Bacteriophage resistance. Of the 48 independent bacterial mutants isolated, 10 initially gave plaques of host range mutants, and of the others, 9 were shown to be able to plaque the host range phage isolated. All of the mutants were resistant to wild-type bacteriophage K3 and were also resistant to only bacteriophages K4, K5, Ox2, Ox3, Ox4, Ox5, M1, and Ac3 of the 64 phages tested (11). That is, they appeared to be typical *con* mutants (17, 23). However, it was found that there was considerable variation in the ability of the *con* mutants to plaque the host range phage mutants (Table 2). The overall pattern ranges from the ability to plaque all of the host range phage down to the typical *con* mutants (e.g., P460), which are unable to plaque any and on which we have been unable to isolate any host range phage mutants. The data also allow the host range mutants to be arranged in an order of increasing potency, with the wild-type K3 (h^+) being the least potent.

Map position of the mutants. All of the mutations were shown to be linked to *pyrD* by cotransduction into strain W620. The cotransduction frequencies were comparable for all classes of *con* mutation as listed in Table 3.

Colicin resistance. Table 2 also shows variation in the resistance of the mutants to colicin L-JF246.

Recipient ability in conjugation. From Table 2 it can be seen that in the bacterial mutants under study there is a general reduction in recipient ability, although in most cases it remains at a level greater than that observed in mutants such as P460, thought to be totally lacking the protein 3A.

Outer membrane proteins. We obtained *tsx* mutants of all the bacterial mutants, by selecting for resistance to bacteriophage T6, so that it would be easier to analyze the protein 3A content of the mutants (17).

In all cases, except for P1667, no readily detectable protein 3A was found (Fig. 1; P1668 is used as an example). This was determined by measuring the amount of heat-modifiable peak C on Maizel gels using unheated and heated samples. In no case were any extra peaks, which may have corresponded to an altered protein, detected. In the case of P1667, normal amounts of peak C were detected (Fig. 1) which appeared to run in the position identical to that normally occupied by protein 3A. Samples of outer membrane from a *tsx* mutant of strain P1667 were run mixed with equal amounts of outer membrane from the parent strain P400 and its *tsx* derivative, strain P407. There was

TABLE 2. *Properties of the mutants*

Type strain	No. of similar mutants	Genotype[a]	EOP of bacteriophage K3 host range mutants[b]							Resistance to colicin L-JF246[c]	Recipient ability with F′ *lac pro*[d]
			h+	h4	h15 h23 h40	h5	h3 h47	h30	h44	h1	
P400		*con*+									S	1.0
P1668	2	*con-12*	R								S	0.066
P1675	3	*con-19*	R								P	0.050
P1676	2	*con-20*	R								P	0.0004
P1658	2	*con-2*	R								R	ND[e]
P1673	2	*con-17*	R								R	0.012
P1667	1	*con-11*	R	<0.01	<0.1	R	<0.1				S	0.39
P1665	1	*con-9*	R	R	R	<0.01	<0.01		<0.1	<0.1	R	0.016
P1666	2	*con-10*	R	R	R	R	<0.01	<0.1	R	<0.1	S	0.023
P1672	1	*con-16*	R	R	R	R	R	R	<0.1	<0.1	R	0.011
P1662	2	*con-6*	R	R	R	R	R	R	R	<0.1	R	0.009
P1663	1	*con-7*	R	R	R	R	R	R	R	<0.01	R	0.004
P460	29	*con-1*	R	R	R	R	R	R	R	R	R	0.0002

[a] All mutants were shown to map at *con* by cotransduction with *pyrD* (see Table 3).
[b] EOP, Efficiency of plating; R, resistant (EOP $<10^{-6}$).
[c] S, Sensitive; P, partially resistant; R, resistant.
[d] Recipient ability was measured with respect to the number of input donor cells and is expressed as a fraction of the parent strain P400 that gave a mean transfer of 29% for the series. Each result was the mean of at least three matings.
[e] ND, Not detectable.

TABLE 3. *Cotransduction frequencies of the mutants with pyrD*

Strain	Transductants[a]		Cotransduction (%)
	con⁻ *pyrD*+	*pyrD*+	
P1668	4	10	40
P1675	23	42	55
P1676	11	24	46
P1658	19	43	44
P1673	12	25	48
P1667	28	50	56
P1665	10	37	27
P1666	13	38	34
P1672	18	37	49
P1662	17	32	53
P1663	20	43	47
P460	19	43	44

[a] Transductants were scored for *glt*, *str*, and *gal*, as well as for resistance to bacteriophage K3.

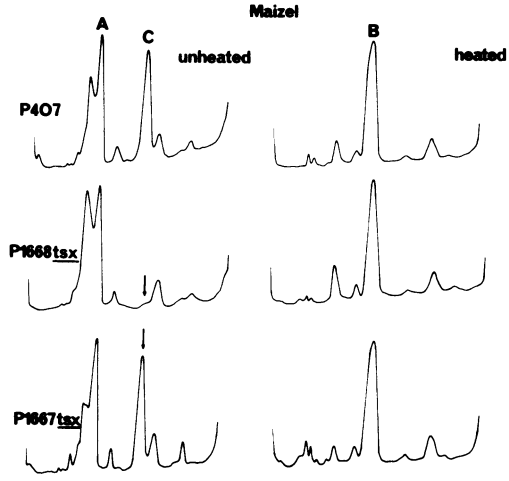

FIG. 1. *Densitometer tracings of sodium dodecyl sulfate-polyacrylamide gels of outer membrane preparations run with the pH 7.2 buffer system of Maizel (15) with unheated and heated samples. tsx mutants were used to simplify the interpretation of the gels (17). Peaks are labeled according to Schnaitman (22).*

no detectable difference in the shape and size of peak C using the mixtures as compared to the strains alone, which indicates that any alteration in protein 3A of P1667 is such as to have no effect on its mobility in sodium dodecyl sulfate-polyacrylamide gel electrophoresis. On Bragg-Hou gels (Fig. 2) a greatly reduced peak 3 was observed in most mutants (P1668 is again used as an example), with the exception of P1667, which has a normal peak 3.

DISCUSSION

In this study we isolated a range of mutants at the *con* locus that are able to plaque a series of host range mutants of bacteriophage K3 to different degrees. All of the mutants map at *con* (*tolG*) at 21.5 min on the *E. coli* K-12 linkage map (27) as shown by cotransduction with *pyrD*, and Henning et al. (12, 13) recently showed that this gene is likely to be the structural gene for protein 3A.

All of the mutant classes studied, representing about 40% of the *con* mutants, are able to plaque at least one of the host range phages. Since it is known that the wild-type phage K3 uses protein 3A as its receptor (17, 23; Manning

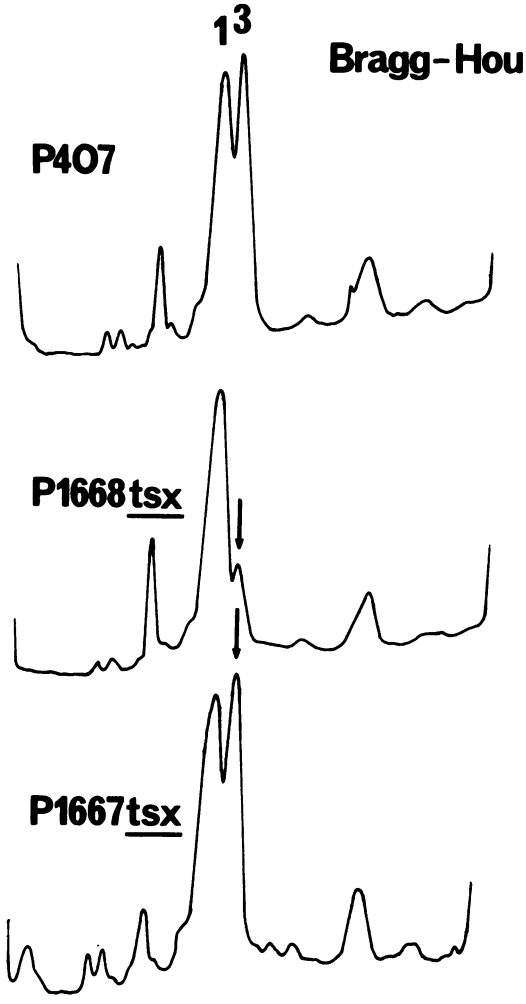

FIG. 2. *Densitometer tracings of sodium dodecyl sulfate-polyacrylamide gels of outer membrane preparations run with the pH 11.4 buffer system of Bragg and Hou (3) with heated samples. Peaks are labeled according to Schnaitman (22).*

and Reeves, manuscript in preparation), we suggest that all of these *con* mutants have some protein 3A present, although at undetectable levels except in strain P1667. It can be seen from Table 2 that, with some exceptions, the activity spectra of the K3h$^-$ mutants allow the *con* mutants to be arranged in an order of increasing phage resistance and the K3h$^-$ mutants in an order of increasing potency. This suggests that for both the bacterial and phage mutant groups the alterations are of one type but vary in degree.

We suggest that the different groups of *con* mutants differ in the amount of protein 3A present and that the bacteriophages differ in the amount of receptor that must be present for them to be able to form plaques.

The structure of all the T-even bacteriophages is similar (5, 6) and so presumably is the process by which they infect the cell (1). Goldberg and co-workers (2, 8, 9) have postulated that, for bacteriophage T4, the six tail fibers each interact with a receptor molecule and that only when all have interacted can the tail pins on the base plate of the phage go on to interact with their receptor. The nucleic acid of the infecting phage can then be injected. Bacteriophage K3 is morphologically similar to other T-even phages (14; Manning and Reeves, unpublished data). We propose that bacteriophage K3h$^-$ mutants are able to plaque on bacteria with greatly reduced levels of the protein 3A receptor, perhaps because the tail pins can be activated after less than six tail fiber-receptor interactions.

However, this hypothesis does not allow any simple explanation of the resistance of P1667 to K3h5 or P1666 to K3h44. The other properties of P1667 could be explained if the protein 3A of this strain, present in normal amounts, were altered so as to reduce its receptor activity.

If our hypothesis to explain the K3h$^-$ activity spectra is correct, then the *con*$^-$ mutants in Table 2 are arranged in approximate order of decreasing amounts of protein 3A (with the exception of strain P1667). It can be seen that sensitivity to colicin L and reduction in recipient ability do not decrease in the same order. It appears that reducing the level of protein 3A does in itself reduce recipient ability, but some mutants have a level of recipient ability well below that expected from their sensitivity to host range phage. We suggest that, whereas sensitivity of our *con*$^-$ mutants to K3h$^-$ mutants is determined largely by the amount of protein 3A present, the recipient ability and colicin L sensitivity are also affected by alterations to the structure of the proteins.

It thus appears to be possible to independently vary all three properties of the *con* mutants—the ability to plaque the host range phage, sensitivity to colicin L-JF246, and recipient ability in conjugation.

For example, strains P1658 and P1668 have similar abilities to plaque the host range mutants, but P1668 is sensitive to L-JF246 and permits conjugation at 6.6% of normal, whereas P1658 is resistant to the colicin and no detectable conjugation occurs.

Similarly, we can compare P1675 and P1676. Both have similar efficiencies of plating of the host range phage and are partially resistant to the colicin, but P1675 is a much better recipient in conjugation.

If one compares P1672 and P1673, both of

which are resistant to colicin L-JF246 and have similar abilities as recipients in conjugation, it can be seen that they are quite different in their ability to plaque the host range phage.

We conclude, then, that in general those mutants able to plaque K3h$^-$ mutants contain residual amounts of protein 3A and that in some, at least of those mutants, it is an altered protein which is present. In the case of P1667, the alteration is such as to reduce the receptor activity but not the amount of protein in the outer membrane.

It appears that mutations in the structural gene for protein 3A are able to affect both the nature of the protein and the amount which is synthesized, or inserted, in the outer membrane.

ADDENDUM IN PROOF

Since submitting this paper, we have learned of the recent work of Hofnung et al. (Mol. Gen. Genet. 145:207-213, 1976), who reported similar findings with bacteriophage λ and *lamB* mutants of *E. coli* K-12.

LITERATURE CITED

1. **Bayer, M. E.** 1968. Adsorption of bacteriophages to adhesions between wall and membrane of *Escherichia coli*. J. Virol. 2:346-356.
2. **Benz, W. C., and E. B. Goldberg.** 1973. Interactions between modified phage T4 particles and spheroplasts. Virology 53:225-235.
3. **Bragg, P. D., and C. Hou.** 1972. Organization of proteins in the native and reformed outer membrane of *Escherichia coli*. Biochim. Biophys. Acta 274:478-488.
4. **Chai, T., and J. Foulds.** 1974. Demonstration of a missing outer membrane protein in tolG mutants of *Escherichia coli*. J. Mol. Biol. 85:465-474.
5. **Cummings, D. J., V. A. Chapman, S. S. DeLong, A. R. Kusy, and K. R. Stone.** 1970. Characterization of T-even bacteriophage substructures. II. Tail plates. J. Virol. 6:545-555.
6. **Cummings, D. J., A. R. Kusy, V. A. Chapman, S. S. DeLong, and K. R. Stone.** 1970. Characterization of T-even bacteriophage structures. I. Tail fibers and tail tubes. J. Virol. 6:534-544.
7. **Davies, J. K., and P. Reeves.** 1975. Genetics of resistance to colicins in *Escherichia coli* K-12: cross-resistance among colicins of group A. J. Bacteriol. 123:102-117.
8. **Dawes, J., and E. B. Goldberg.** 1973. Functions of baseplate components in bacteriophage T4 infection. I. Dihydrofolate reductase and dihydropteroylhexaglutamate. Virology 55:380-390.
9. **Dawes, J., and E. B. Goldberg.** 1973. Functions of baseplate components in bacteriophage T4 infection. II. Products of genes 5, 6, 7, 8, and 10. Virology 55:391-396.
10. **Foulds, J.** 1975. Chromosomal location of the *tolG* locus for tolerance to bacteriocin JF246 in *Escherichia coli* K-12. J. Bacteriol. 117:1354-1355.
11. **Hancock, R. E. W., and P. Reeves.** 1975. Bacteriophage resistance in *Escherichia coli* K-12: general pattern of resistance. J. Bacteriol. 121:983-993.
12. **Henning, U., and I. Haller.** 1975. Mutants of *Escherichia coli* K-12 lacking all 'major' proteins of the outer cell envelope membrane. FEBS Lett. 55:161-164.
13. **Henning, U., I. Hindennach, and I. Haller.** 1976. The major proteins of the *Escherichia coli* outer cell envelope membrane: evidence for the structural gene of protein II*. FEBS Lett. 61:46-48.
14. **Krzywy, T., A. Kucharewicz-Krukowska, and S. Slopek.** 1972. Morphology of bacteriophages of E. Hammerstrom's set for typing *Shigella sonnei*. Arch. Immunol. Ther. Exp. 20:73-83.
15. **Maizel, J. V., Jr.** 1966. Acrylamide gel electrophoresis by mechanical fractionation: radioactive adenovirus proteins. Science 151:988-990.
16. **Manning, P. A., and P. Reeves.** 1975. Recipient ability of bacteriophage-resistant mutants of *Escherichia coli* K-12. J. Bacteriol. 124:576-577.
17. **Manning, P. A., and P. Reeves.** 1976. Outer membrane of *Escherichia coli* K-12: differentiation of proteins 3A and 3B on acrylamide gels and further characterization of *con* (*tolG*) mutants. J. Bacteriol. 127:1070-1079.
18. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, New York.
19. **Pittard, J.** 1965. Effect of integrated sex factor on transduction of chromosomal genes in *Escherichia coli*. J. Bacteriol. 89:680-686.
20. **Schacterle, G. R., and R. L. Pollack.** 1973. A simplified method for the quantitative assay of small amounts of protein in biologic material. Anal. Bicohem. 51:654-655.
21. **Schnaitman, C. A.** 1973. Outer membrane proteins of *Escherichia coli*. I. Effect of preparative conditions on the migration of protein in polyacrylamide gels. Arch. Biochem. Biophys. 157:541-552.
22. **Schnaitman, C. A.** 1974. Outer membrane proteins of *Escherichia coli*. III. Evidence that the major protein of *Escherichia coli* O111 outer membranes consists of four distinct polypeptide species. J. Bacteriol. 118:442-453.
23. **Skurray, R. A., R. E. W. Hancock, and P. Reeves.** 1974. Con$^-$ mutants: class of mutants in *Escherichia coli* K-12 lacking a major cell wall protein and defective in conjugation and adsorption of a bacteriophage. J. Bacteriol. 119:726-735.
24. **Skurray, R. A., and P. Reeves.** 1973. Physiology of *Escherichia coli* K-12 during conjugation: altered recipient cell functions associated with lethal zygosis. J. Bacteriol. 114:11-17.
25. **Skurray, R. A., and P. Reeves.** 1973. Characterization of lethal zygosis associated with conjugation in *Escherichia coli* K-12. J. Bacteriol. 113:58-70.
26. **Swank, R. T., and K. D. Munkres.** 1971. Molecular weights of oligopeptides by electrophoresis in polyacrylamide gels with sodium dodecyl sulphate. Anal. Biochem. 39:462.
27. **Taylor, A. L., and C. D. Trotter.** 1972. Linkage map of *Escherichia coli* strain K-12. Bacteriol. Rev. 36:504-524.