OUTER MEMBRANE OF *ESCHERICHIA COLI* K-12: *TSX* MUTANTS (RESISTANT TO BACTERIOPHAGE T6 AND COLICIN K) LACK AN OUTER MEMBRANE PROTEIN.

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Summary

Tsx mutants of *Escherichia coli* are fully resistant to a set of T6-like bacteriophage and are resistant to colicin K. We demonstrate that these mutants are missing an outer membrane protein (the tsx-protein) of molecular weight 32,000 as measured by SDS-polyacrylamide gel electrophoresis. *Tsx* mutants are receptor mutants which are unable to absorb either the bacteriophages or the colicin and the loss of receptor function can be demonstrated using outer membrane preparations.

We suggest that the tsx-protein is the receptor for both the bacteriophage and colicin.

Introduction

Cross resistance studies between bacteriophage T6 and colicin K have suggested that these two agents share a common receptor (1). In a recent study on bacteriophage and colicin resistance, it was found that the classical *tsx* mutants, which map at 11 min. on the chromosome of *E.coli* K-12 (2), were resistant only to a group of 8 T6-like bacteriophages (3) and to colicin K (4).

Sabet and Schnaitman (5) have shown that colicin K receptor lies in the outer membrane and Weltzien and Jesaitis (6) have shown that it is protein in nature

and that receptor activity is unable to be detected in membrane extracts of *tsx* mutants.

In this communication we show that *tsx* mutants are missing an outer membrane protein which we can readily detect on SDS-polyacrylamide gels.

Materials and Methods

The bacterial strains used are listed in table 1 and all cultures were grown in nutrient broth at 37°C.

TABLE 1.

Bacterial strains

(all derivatives of E.coli K-12)

Strain	Characteristics	Reference
P400	F⁻/*thr argE proA thi leu mtl xyl ara galK lacY str supE* λ	12.
P407	*tsx* mutant of P400	3.
P460	*con* mutant of P400	12.
P1731	*tsx* mutant of P460	This paper

Outer membranes were prepared as previously described (7) using the methods of Schnaitman (8) to obtain the Triton X-100 insoluble component of the cell envelope. Sample preparation and acrylamide gel electrophoresis methods have been described before (8, 9) and are essentially the pH7.2 system of Maizel (10) and the pH 11.4 system of Braggand Hou (11).

Bacteriophage T6 was from stocks maintained in this laboratory (2) and colicin K was prepared by induction as described elsewhere (7).

Phage neutralization experiments were carried out by incubating 0.1 ml volumes of dilutions of the outer membrane preparations (in phosphate

buffer 0.1M, pH7.2) with 0.1 ml of nutrient broth containing 10^3 pfu of bacteriophage T6 for 3 hours, after which 0.1 ml of a culture of indicator bacteria (strain P400, 2 x 10^8 cells/ml in nutrient broth) was added and incubation continued for a further 15 minutes. 4 ml of molten 0.7% nutrient agar was added and the whole poured as an overlay on a nutrient agar plate, incubated overnight and scored for plaque forming units.

The amount of colicin added was such that 20% survival of indicator bacteria was obtained under the assay conditions with 0.1 ml buffer substituting for the membrane preparations.

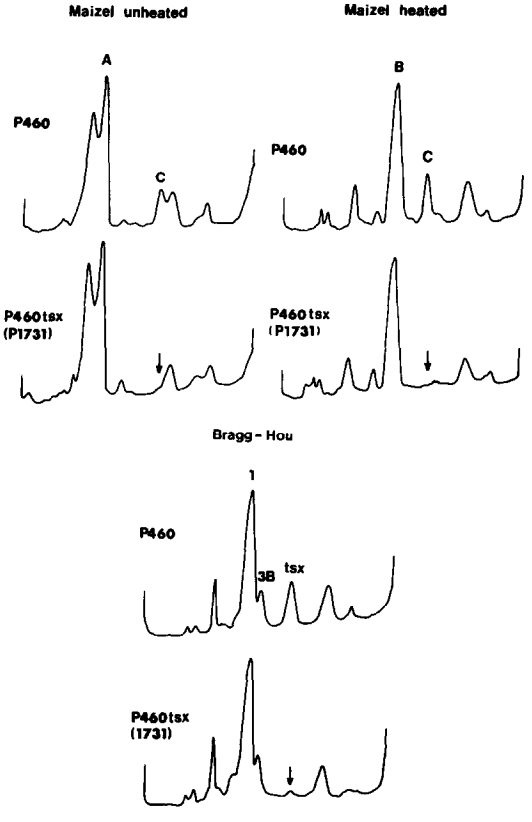

FIGURE 1.

Densitometer tracings of outer membranes of strains P460 (con) and its tsx mutant, P1731, run on SDS-polyacrylamide gels using the pH7.2 buffer system of Maizel (10) with both unheated and heated samples, and using the pH 11.4 system of Bragg and Hou (11) with heated samples. Peaks are labelled according to Schnaitman (8).

Results and Discussion

In our analysis of the outer membrane proteins of tsx mutants we have used tsx, con and tsx con double mutants of the parent strain P400. We present results obtained by using a con mutant (7, 12) as this enables an easier interpretation of the acrylamide gels in the absence of protein 3A (7, 8).

Thus in Figure 1 it can be seen that there is no peak C detectable when using unheated or heated samples on Maizel gels of strain P1731, a tsx con mutant, when compared with the con mutant, P460. If the heated samples are run on Bragg-Hou gels, then again there is a peak missing in the tsx con mutant when compared with a con mutant. In all three cases the protein peak missing in the tsx mutants is in a position corresponding to a protein of molecular weight 32000. (The standard proteins used were phosphorylase A, transferrin, bovine-serum-albumin, ovalbumin and carbonic anhydrase.)

If wild type (P400) and a tsx mutant is compared, then the protein peak can be shown to be present in P400 and absent in tsx mutants, using heated samples on Maizel or Bragg-Hou gels. However, protein 3A masks the protein if unheated samples are used.

We have called the protein missing in tsx mutants, the tsx-protein: it accounts in P400 for about 8% of the outer membrane proteins (as measured by comparing the areas of the peaks on the densitometer trace of the polyacrylamide gels). The tsx-protein is thus present in much greater amounts than the receptors for bacteriophages T5 (13), λ(14) and BF23 (15) which are normally undetectable on SDS-polyacrylamide gels of the whole outer membrane. However, under our normal laboratory growth conditions, no defect has so far been detected in tsx mutants other than the bacteriophage and colicin resistance. The function of this relatively major protein is not known.

The absence of the tsx-protein corresponds with the loss of neutralizing

TABLE 2.

Neutralization of bacteriophage T6 and colicin K by outer membrane

Strain	Amount required under the test conditions for 50% neutralization	
	Bacteriophage T6	Colicin K
P400	1µg	5 µg
P407	>100	>100

(a) 50% of the 10^3pfu of bacteriophage were neutralized in the test as described in the materials and methods.

(b) Only 50% of the indicator bacteria were killed by the residual colicin in the test as described.

activity for bacteriophage T6 and colicin K as previously shown by Weltzien and Jesaitis (6) and confirmed for our mutants as shown in table 2.

We are presently purifying the tsx-protein which we believe to be the receptor for both the bacteriophage T6 and colicin K.

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