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Outer Membrane Proteins of *Escherichia coli* K-12: Isolation of a Common Receptor Protein for Bacteriophage T6 and Colicin K

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Summary. *tsx* mutants, resistant to T6-like bacteriophages and colicin K, of *Escherichia coli* K-12 lack an outer membrane protein of 26,000 molecular weight. This protein is shown to have receptor activity for both bacteriophage T6 and colicin K. The protein has been purified and its amino acid composition determined. Some *tsx* mutants appear to have an altered receptor protein, as indicated by their ability to plate extended host-range mutants of bacteriophage T6. These mutants are also cotransducible with *proC* and can be arranged in an order of increasing resistance to the host range phages, which appear to have differing degrees of ability to propagate on the *tsx* mutants.

The *tsx* protein was shown to be catabolite repressible both by use of varying growth conditions and *cya* and *crp* mutants.

Introduction

Fredericq (1946) postulated that the reason for mutant bacteria being resistant to some colicins while remaining sensitive to others was loss of specific surface receptors. It is the bindung of the colicin molecule to a specific receptor which constitutes the initial step leading to the killing of a sensitive cell by colicin. Similarly, bacteriophages adsorb to receptors on the cell surface and, following irreversible binding, inject their nucleic acid into the infected host cell. Fredericq observed that bacteria which were sensitive to colicins E, M or K were always sensitive to bacteriophages BF23, T1 or T6 respectively, and conversely that cells which were resistant to one of the colicins or bacteriophages were always resistant to the corresponding bacteriophage or colicin (Fredericq, 1953, 1956). From these observations he suggested that the colicins shared a common receptor with the respective phage.

Sabet and Schnaitman (1973) have purified the receptor for the colicins of type E. The receptor is an outer membrane protein with a molecular weight of 60,000 containing little covalently bound carbohydrate. It has also been shown that phage BF23 uses the same receptor and that this protein also functions as the site for the initial binding of vitamin B-12 to the cell (Di Masi, White, Schnaitman and Bradbeer, 1973; Bradbeer, Woodrow and Khalifah, 1976).

The colicin M receptor has been purified by Braun and his coworkers (Braun, Schaller and Wolff, 1973; Braun and Wolff, 1973) and shown to be the receptor for bacteriophages T1, T5 and $\phi 80$ and also to function as the receptor in the uptake of ferrichrome bound iron (Hantke and Braun, 1975). It is an outer membrane protein which consists of a single polypeptide chain with a molecular weight of 78,000 (Hancock, Hantke, and Braun, 1976).

Sabet and Schnaitman (1971) have shown that the receptor activity for colicin K is found in the Triton X-100 plus EDTA soluble fraction of the outer membrane. It has also been previously suggested that phage T6 uses a protein containing receptor located in the outer lipid-protein layer of the cell wall (Beumer, Beumer-Jockmans, Dirkx and Dekegel, 1966). However, Weltzien and Jesaitis (1971) using cell envelope preparations obtained by treating with various reagents, found some differences in the effects of these agents on colicin K and bacteriophage T6 receptor activities suggesting that they may differ.

In this study we show that the tsx-protein, which we have recently shown to be missing in *tsx* mutants (Manning and Reeves, 1976a), is the receptor for bacteriophage T6 and colicin K, and we also present evidence that these two agents differ in the way they interact with the receptor protein.

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A preliminary report of this work was presented at the 1975 annual meeting of the Australian Biochemical Society (Manning, Lavoie and Reeves, 1975).

Materials and Methods

Bacterial Strains and Culture Conditions. All bacteria were derivatives of *Escherichia coli* K-12 and are listed in Table 1, with the exception of those *tsx* mutants which are described in Table 4. All cultures were grown at 37° C in nutrient broth (Difco). *Cya* and *crp* mutants, defective in adenyl cyclase and cyclic AMP receptor protein respectively, were isolated from strain P400 as mutants defective in catabolite repressible syntheses, (Brickman, Soll and Beckwith, 1973) and distinguished by their response to cyclic AMP.

For the preparation of cell envelopes, bacteria were grown with aeration, in 1 litre amounts of nutrient broth in 5 litre flasks until a density of $7–8 \times 10^8$ cells/ml was reached corresponding to two thirds the maximum growth yield in the medium. The cells were then harvested by centrifugation at $5000 \times g$ for 15 min at 4° C. For large scale preparations bacteria were grown in 75 litre amounts in a fermenter and harvested by centrifugation in a Veronesi KLE160 continuous flow centrifuge (Veronesi, Separatori s.a.s. Bologna, Italy).

The effect of various growth media on the production of the tsx-protein was determined by growing the bacteria in the following media: nutrient broth (Difco 0003), Luria broth (Miller, 1972) plus 10 mM glucose; M9 minimal media (Davies and Mingioli, 1951) containing the required amino acids (100 µg/ml) with either 10 mM succinate, 10 mM lactate, 10 mM glucose, 1% glycerol or 1% lactose as carbon source.

Transduction. P1 transduction, using *tsx* mutant strains as donors and strain χ342 as recipient, was as described previously (Manning, Puspurs and Reeves, 1976).

Table 1. Bacterial strains

Strain	Characteristics	Source or reference
P400	F⁻/*thr, leu, argE, proA, thi, mtl, xyl, ara, galK, lacY, rpsL, supE*, λ⁻	Hancock and Reeves (1975)
P407	*tsx-200* mutant of P400	Hancock and Reeves (1975)
P417	*tonA-201* mutant of P400	
P460	*ompA-1* mutant of P400	Hancock and Reeves (1975)
P1677	*tonA-204* mutant of P400	This study
P1694	*his* derivative of P400	This study
K12	F⁺/prototroph λ⁺	—
P1773	*tsx-202* of K12	[b]
χ342	HfrC/*proC, metB, relA*, λ⁻	C.G.S.C.[a]
CA8000	Hfr/*thi, relA1*, λ⁻	J. Beckwith
CA8306	Hfr/*thi, cya-854*	J. Beckwith
1–23	Hfr/*thi, crp*	J. Beckwith

[a] C.G.S.C. = *Escherichia coli* Genetic Stock Center, Yale University, Conn. via. Dr. Barbara Bachmann

[b] Isolated as resistant to bacteriophage T6 (Manning, Pugsley and Reeves, J. Mol. Biol., in press)

Bacteriophages. Bacteriophages T6, H1, H3, H8, K9, K18, K31 and Ox1 were from stocks maintained in this laboratory and have been described previously (Hancock and Reeves, 1975). Extended host range mutants of bacteriophage T6 were isolated as described below.

Colicin K. Colicin K was the gift of A.P. Pugsley and was obtained from *E. coli* K235.

Bacteriophage Adsorption and Neutralization. Bacteriophage adsorption to bacteria was measured as previously described (Manning and Reeves, 1976b). Bacteriophage receptor was assayed by neutralization of bacteriophage and assays were carried out by mixing 0.1 ml volumes of serial dilutions of the cell envelope preparation or fraction (in 0.1 M sodium phosphate buffer, pH 7.2) with 0.1 ml of nutrient broth containing 10^3 p.f.u. of the bacteriophage and incubating for 3 hours with gentle shaking. Bacteria of the indicator strain (0.1 ml, strain P417, 2×10^8 log phase cells/ml in nutrient broth) was added and incubation continued for a further 15 min. 4 ml of molten 0.7% nutrient agar was added and the total 4.3 ml poured as an overlay on a nutrient agar plate, incubated overnight and scored for plaque forming units. Buffer plus phage plus indicator bacteria was used as control.

Colicin Receptor Assays. Colicin receptor was assayed by colicin neutralization and experiments were carried out by two (similar) methods. In the first method (Fig. 1) 0.1 ml volumes of the cell envelope preparations (in 0.1 M sodium phosphate buffer, pH 7.2) were incubated with 0.1 ml of colicin (containing 10^9 lethal units) for 30 min at 37° C. After this time 0.1 ml of a culture of indicator bacteria (strain P417, 10^9 log phase cells per ml in nutrient broth) was added and incubation continued for a further 30 min. 0.1 ml of this mixture or of a 10^{-2}, 10^{-4}, 10^{-5} dilution were added to 4 ml of molten 0.7% nutrient agar plate, incubated overnight at 37° C and scored for colony forming units. The amount of residual colicin and hence colicin adsorbed is calculated on the assumption of single hit killing kinetics (Reeves, 1965).

The second method (Table 2, Fig. 3) was basically the same as above except that a series of 2 fold dilutions of cell envelope material were used and only 10^3 log phase cells of indicator bacteria were added. The 4 ml of 0.7% nutrient agar was also added directly to the assay tube before pouring as an overlay. The colicin level used gave about 90% kill of the indicator bacteria in control experiments. Because of the low level of bacterial cells, colicin adsorption may not go to completion at the end point and the assay gives only a measure of the relative amount of receptor present.

Colicin Adsorption to Bacterial Cells. Colicin adsorption was measured by determining the amount of colicin which had not adsorbed to the cells under test, by the ability of the unadsorbed colicin to kill an indicator strain. To 1.0 ml of a culture of the bacterial strain being tested (2×10^8 log phase cells/ml) was added 0.1 ml of colicin and 0.1 ml of chloramphenicol (200 µg/ml) and the whole incubated at 37° C. At intervals, 0.1 ml was removed and added to 0.1 ml of a culture of the indicator strain (JC6256/R538Fdrd1; 2×10^8 log phase cells per ml) in the presence of 20 µg/ml of chloramphenicol and incubated for 15 min at 37° C. This mixture was then diluted and plated out on nutrient agar plates containing chloramphenicol (20 µg/ml), incubated overnight at 37° C and scored for colony forming units. The indicator strain is rendered resistant to chloramphenicol due to the presence of the R-factor R538-*drd1*. The strains being tested are all sensitive to this antibiotic.

Isolation of tsx Mutants with Altered tsx-Protein Using Bacteriophage T6 and Isolation of Extended Host Range Mutants of Bacteriophage T6. Independently derived spontaneous bacterial mutants, resistant to bacteriophage T6 and independent extended host range

bacteriophage mutants were isolated using a previously described technique (Manning, Puspurs and Reeves, 1976).

Colicin Sensitivity. Sensitivity to all the colicins previously used was determined using the conventional cross-streak plate test (Davies and Reeves, 1975).

Cell Envelope Preparations. Cells were broken in a French pressure cell (Aminco, Silver Spring, Maryland) at 12,000 p.s.i. and the outer membrane prepared as triton insoluble wall (TIW) by solubilising and removing the cytoplasmic membrane with Triton X-100 using the precedures of Schnaitman (1971, 1974). Triton X-100 was removed from the various fractions by precipitating the protein with 2 volumes of cold 95% ethanol and allowing the precipitate to form overnight at −15° C. The precipitates were then spun down at $5000 \times g$ for 30 min and washed with cold ethanol to ensure complete removal of the Triton X-100.

Lipopolysaccharide (LPS) Extraction. LPS was extracted from the cells using the method of Galanos, Lüderitz and Westphal (1969).

SDS-Polyacrylamide Gel Electrophoresis. Samples were prepared according to Schnaitman (1973, 1974) and run under his conditions using both the pH 7.2 buffer system of Maizel (1966) and the pH 11.4 buffer system of Bragg and Hou (1972). Gels were 0.5 cm × 10 cm and were loaded with 50 µg of protein.

Gels were stained with Coomassie brilliant blue (Swank and Munkies, 1971) and densitometer tracings were obtained using a Quick Scan Jnr. gel scanner (Helena Laboratories Corp., Beaumont, Texas) and protein nomenclature in general follows Schnaitman (1974).

Protein Estimation. Two methods were employed for estimating the concentration of protein in samples. For protein solutions in water or non-Triton containing buffers, the method of Schacterle and Pollack (1973) was used. For samples containing Triton X-100, the method of Wang and Smith (1975) was used. Bovine serum albumin was used as standard in both cases.

LPS Estimation. Keto-deoxyoctonate (KDO) was taken as a measure of the amount of LPS present. This was determined using the method described by Keleti and Lederer (1974).

Column Chromatography. Chromatography of outer membrane proteins solubilised in Triton-EDTA on DEAE-cellulose (Whatman DE-52) using Tris-Triton-EDTA (TTE) buffer pH 7.2 was as described by Schnaitman (1974). Chromatography on QAE-Sephadex in TTE buffer was carried out similarly except that the buffer was pH 8.0. Gel filtration on Sephadex G-200 in the presence of SDS was as previously described (Schnaitman, 1974).

Amino Acid Analysis. Amino acid composition was determined by Dr. M. Calder of the Department of Biochemistry, University of Adelaide.

Results

Resistance to Bacteriophages and Colicins. tsx mutants are resistant to only a group of eight bacteriophages (T6, H1, H3, H8, K9, K18, K31 and Ox1) and to colicin K (Hancock and Reeves, 1975; Davies and Reeves, 1975; Hancock, Davies and Reeves, 1976). The resistance is thought to be due to loss of a common receptor (Fredericq, 1953) and as expected strain P407, a *tsx* mutant fully resistant to colicin K, bacteriophage T6 and its host range mutants (see below), was unable to bind either colicin K or phage T6 (unpublished data).

Receptor Activity of Cell Envelope Fractions. As previously described (Manning and Reeves, 1976a) *tsx* mu-

Table 2. Neutralization of bacteriophage by cell envelope fractions

Bacteriophage	Envelope fraction[a]							
	WM		TIW		CM		LPS	
	P400	P407	P400	P407	P400	P407	P400	P407
T6	10	>1000	1	>1000	1000	>1000	>1000	>1000
H1	100	>1000	10–1	>1000	>1000	>1000	>1000	>1000
H3	100–10	>1000	1	>1000	>1000	>1000	>1000	>1000
H8	100	>1000	10	>1000	>1000	>1000	>1000	>1000
K9	10	>1000	1	>1000	1000	>1000	>1000	>1000
K18	10	>1000	1	>1000	>1000	>1000	>1000	>1000
K31	10	>1000	1	>1000	1000	>1000	>1000	>1000
Ox1	1000–100	>1000	10	>1000	>1000	>1000	>1000	>1000
Colicin K	10	>1000	10-1	>1000	>1000	>1000	>1000	>1000

Numbers represent the smallest amount of cell envelope material (in µg/ml of contained protein (for WM, TIW and CM) or total dry weight (for LPS) that gave 50% neutralization of the bacteriophages or survival of the indicator strain (with colicin). Concentrations of 1000, 100, 10, 1, 0.1 ane 0 µg/ml were used

>1000 indicates that less than 50% neutralization was obtained with the maximum amount of material used, or for the colicin receptor assay (method 2), 50% survival of the indicator bacteria

[a] Abbreviations: WM = whole membrane or cell envelope; TIW = outer membrane or Triton X-100 insoluble fraction of the cell envelope; CM = cytoplasmic membrane components or Triton X-100 soluble fraction of the cell envelope. LPS = lipopolysaccharide

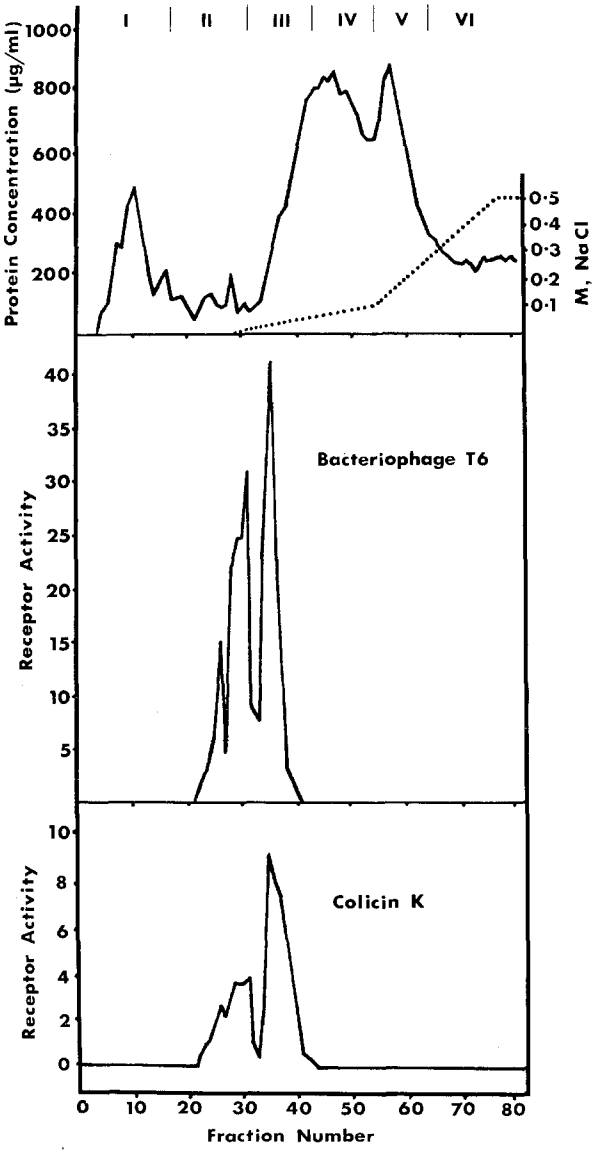

Fig. 1. DEAE-cellulose chromatography in TTE buffer pH 7.2 of the Triton plus EDTA soluble component (TES) of the outer membrane of strain P1677 (*ompA-1*, *tonA-204*). 650 mg of protein was applied to a 2.5 × 50 cm column. This material was obtained from 50 litres of bacteria grown in a fermenter under vigorous aeration at 37° C to a density of 7–8 × 10^8 cells/ml. 25 ml fractions were collected from the column and 2 ml of each was taken and ethanol precipitated for phage and colicin receptor assays. Phage receptor activity is expressed as the reciprocal of the dilution required to give 50% neutralization of the bacteriophages under the assay conditions. Colicin receptor activity (1st method) is expressed as the number of lethal units adsorbed per 0.1 ml sample (× 10^{-8})

tants lack an outer membrane protein (the tsx-protein) of molecular weight 26,000 (previously given in error as 32,000). If we examine the different fractions of the cell envelope for their ability to neutralize colicin K and the T6-like bacteriophages, then it can be seen that the outer membrane has the highest specific activity (Table 2). The whole membrane, triton insoluble wall and cytoplasmic membrane fractions contain LPS, but pure LPS, which is free of protein, is not active. Thus the tsx protein appears to contain the specificity for receptor activity.

Purification of the Receptor Activity for Bacteriophage T6 and Colicin K. It was known from previous studies that the major outer membrane protein 3A cochromatographs on DEAE-cellulose with the tsx-protein (Manning and Reeves, 1976b). We therefore employed an *ompA* mutant (lacking protein 3A; Manning and Reeves, 1976b) in our purification.

From Figure 1 it can be seen, that when the Triton-EDTA soluble component of the outer membrane is chromatographed on DEAE-cellulose, the receptor activities for both bacteriophage T6 and colicin K copurify. The Triton plus EDTA insoluble component of the outer membrane contains negligible receptor activity (unpublished data). From previous studies, the fractions containing the receptor activity were known to correspond to those containing the tsx-protein and this is confirmed by analysis of a number of pooled fractions (Fig. 2). Rechromatography of the pooled receptor fraction on DEAE-cellulose or on QAE-Sephadex, did not give electrophoretically pure tsx protein. However, it was possible to obtain near pure protein by chromatography on QAE-Sephadex using 0.005M NaCl in the buffer (Fig. 3), but only a fraction of the total tsx-protein was eluted under these conditions. However, it can be seen that both bacteriophage T6 and colicin K receptor activities cochromatograph with the tsx protein (Fig. 4). All material which showed receptor activity also contained LPS as measured by KDO analysis. The only method by which we have obtained tsx protein free of LPS was by chromatography of the receptor fraction from DEAE-cellulose, on Sephadex-G200 in the presence of SDS. Treatment with SDS appears to irreversibly denature the tsx protein and neutralising activity was not recoverable. Thus we have been unsuccessful in obtaining electrophoretically pure active protein.

Purification of the tsx protein on Sephadex G200 in the presence of SDS enabled us to obtain a pure protein for amino acid analysis. The results of this analysis are shown in Table 3.

Extended Host Range Mutants of Bacteriophage T6 and Mutants with an Altered tsx Protein. We have obtained a series of *tsx* mutants and also extended host range mutants of bacteriophage T6. These *tsx* mutants can be placed in an order of increasing resis-

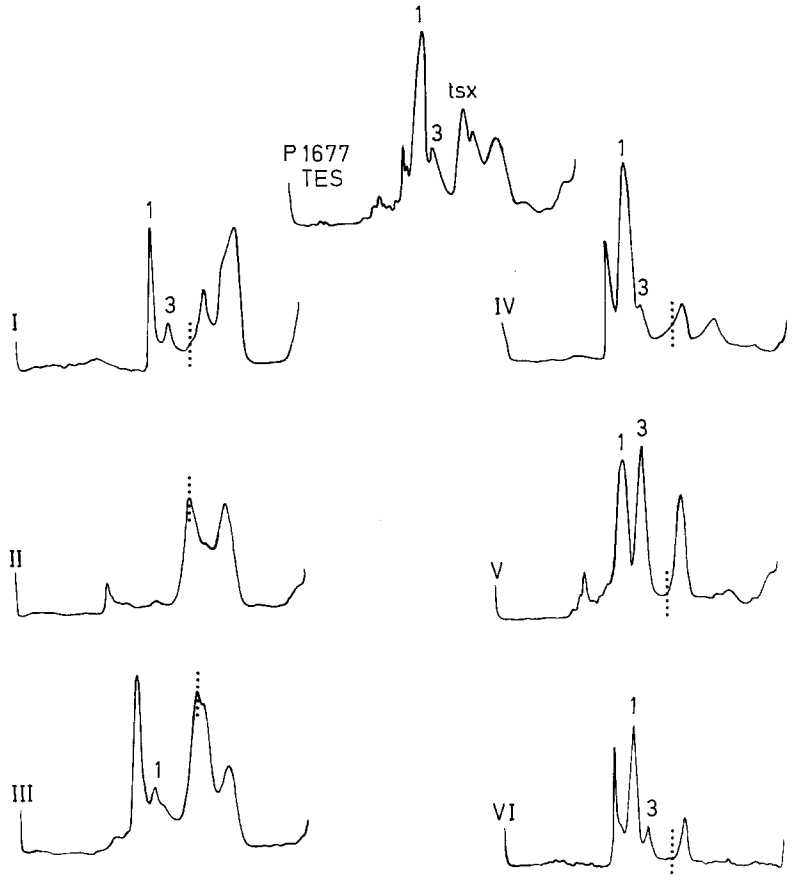

Fig. 2. Protein composition of the fractions of the DEAE-cellulose column in Figure/and pooled as shown. The proteins were analyzed on sodium dodecyl sulphate polyacrylamide gels using the pH 11.4 buffer system of Bragg and Hou (1972). The dotted lines indicate the position of the tsx protein and proteins 1 and 3 (3A and 3B) are indicated

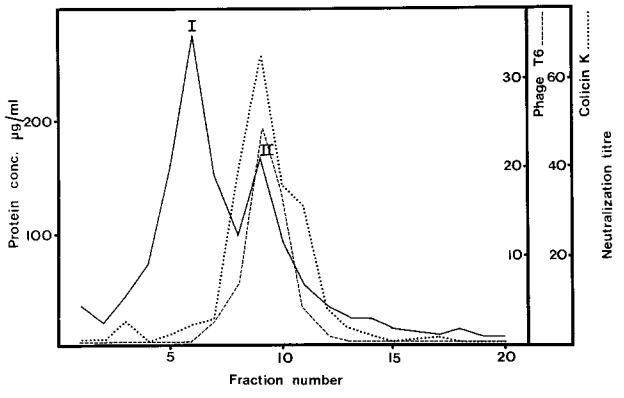

Fig. 3. QAE-Sephadex chromatography in TTE buffer (pH 8.0 containing 0.005 M NaCl) of the TES component of the outer membrane of P460 (*ompA-1*). 105 mg of protein was applied to a 2.0 × 20 cm column. This was obtained from 10 litres of bacteria grown in 1 litre amounts with vigorous aeration at 37° C to a density of $7–8 \times 10^8$ cells/ml. 5 ml fractions were collected from the column and 1 ml of each was taken and ethanol precipitated for phage and colicin neutralizations. Only that part of the column prior to the addition of the salt gradient is shown. Phage receptor activity is expressed as the reciprocal of the dilution required to give 50% neutralization of bacteriophages under the assay conditions. Colicin receptor activity is expressed as the reciprocal of the dilution required to give 50% survival of the indicator strain in the assay

tance to host range mutants, and the phage mutants in an order of increasing potency (Table 4). All mutants map at *tsx* as shown by P1 cotransduction with *proC* (Table 5; Bachmann, Low and Taylor, 1976) and all the mutants isolated with the exception of strain P1744 had no detectable tsx protein on SDS polyacrylamide gel electrophoresis. The *tsx* mutants varied in their sensitivity to colicin K, suggesting that bacteriophage T6 and the colicin require different amounts of, or different regions of the common receptor molecule. The tsx protein in strain P1744 moved identically to that of its parent strain upon electrophoresis, however, outer membrane prepared from this mutant was unable to neutralize bacteriophage T6 or colicin K: no neutralization occurred with material at a concentration of 1 mg per ml contained protein. Even the extended host range mutant ehr 7 of bacteriophage T6, which strain P1744 was able to plaque efficiently, was not neutralized by the same amount of material. It was also not possible to detect *any receptor activity in the fractions containing tsx* protein from a DEAE-cellulose column of the TES component of the outer membrane of P1744 which appeared otherwise identical to those obtained before.

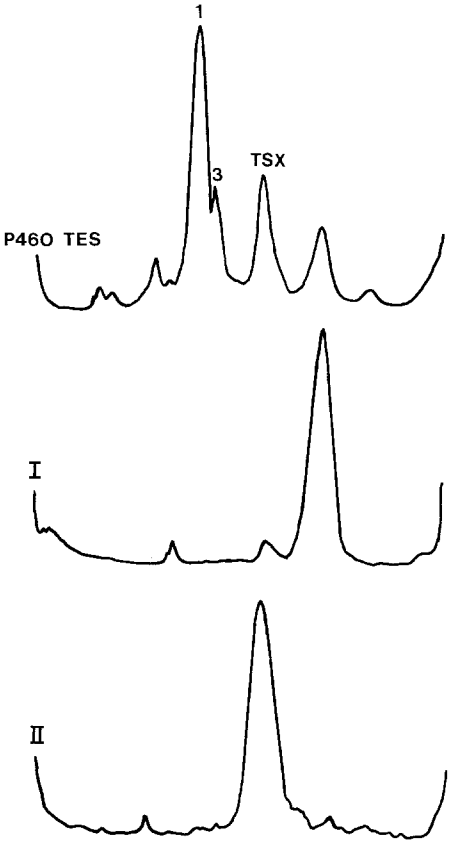

Fig. 4. Protein composition of peaks I and II corresponding to fractions 6 and 9 of the QAE-Sephadex column shown in Figure 3. The proteins were analyzed on sodium dodecyl sulphate polyacrylamide gels using the pH 11.4 buffer system of Bragg and Hou (1972). Proteins 1, 3 (3A and 3B) and tsx are indicated

Thus, although the protein eluted at the same salt concentration as the protein of P1694, we believe that P1744 (*tsx-206*) contains an altered tsx protein.

Effect of Various Mutations and Growth Conditions on the Amount of tsx Protein. We have compared the *tsx* mutants P407 and P1773 with their parent strains, P400 and K12 respectively, under a wide range of growth conditions both in this study and elsewhere (Manning, Pugsley and Reeves, J. Mol. Biol., in press). The growth rate of *tsx* mutants was indistinguishable from that of the respective parent using glucose, maltose, succinate, lactate or glycerol as sole carbon source, nutrient broth or Luria broth plus glucose. However, these different media did affect the amount of tsx protein present in strain P400. Maximum amounts of the protein were present when cells were grown in nutrient broth, but glucose severely repressed the amount produced. The other carbon sources permitted the production of levels of

Table 3. Amino acid analysis of tsx protein purified by Sephadex G200 chromatography in the presence of SDS of the receptor fraction obtained by DEAE-cellulose chromatography

Amino acid	% of total amino acid content (M)
Aspartic acid	12.49
Threonine	5.12
Serine	6.30
Glutamic acid	9.18
Proline	3.52
Glycine	9.71
Alanine	8.43
Valine	5.34
Methionine	0.85
Isoleucine	3.84
Leucine	6.95
Tyrosine	4.16
Phenylalanine	4.48
Tryptophane	not determined
Lysine	10.78
Histidine	1.49
Arginine	5.02
Cysteic acid	1.17
Half cystein	0

Table 4. Sensitivity of the mutants to colicin K, and their efficiency in plaquing the extended host range mutants of bacteriophage T6

Strain	Tsx allele	No. of similar mutants	Response[a] to colicin K	E.O.P.[c] of extended host range mutants				
				T6 (ehr$^+$)[b]	ehr7	ehr18	ehr11	ehr13
P1694	*tsx*$^+$	—	s	s	s	s	s	s
P1814	*tsx-218*	3	Sl	R[c]	10^{-4}	s	s	s
P1809	*tsx-213*	2	s	R	R	10^{-3}	s	s
P1808	*tsx-212*	2	s	R	R	10^{-1} 10^{-2}	10^{-1}	s
P1744	*tsx-206*	1	R	R	s	R	R	R
P1807	*tsx-211*	1	Sl	R	R	R	R	R
P1816	*tsx-220*	41	R	R	R	R	R	R

[a] s = sensitive; Sl = slight resistance; R = resistant; s < Sl < R
[b] ehr = extended host range
[c] EOP = efficiency of plating; R = EOP < 10^{-6}

Table 5. Mapping of the mutants by cotransduction of *tsx* with proC

Strain	Transductants[a]		Cotransduction %
	tsx proC$^+$	(*tsx*+*tsx*$^+$) *proc*$^+$	
P1814	28	56	50
P1809	28	54	52
P1808	34	59	58
P1744	27	55	49
P1807	30	55	55
P1816	31	60	52

[a] Transductants were scored for *met*, sensitivity to colicins K, A, E2, E3 and L and to bacteriophage T6. At least one of each class of transductants was examined for the presence of the tsx protein in its outer membrane. All properties of the *tsx* transductants were the same as for the corresponding *tsx* mutants

tsx protein approaching that observed in nutrient broth.

These results suggest that synthesis of the tsx protein is catabolite repressible and this was further tested using mutants defective in the synthesis of such proteins. Strains 1–23 and CA8306 (*crp* and *cya* mutants respectively) did not produce detectable levels of tsx protein. However, the parent strain, CA8000 did not produce very large amounts of tsx protein and therefore *cya* and *crp* mutants were made in strain P400 which produces much more tsx protein than most K12 derivatives. In these *cya* and *crp* mutants of strain P400, the outer membrane protein profile showed very little tsx protein, as for glucose grown cells of strain P400, again demonstrating the catabolite repressible nature of the tsx protein. A comparable result was obtained by Kumar (1976) who showed that *cya* and *crp* mutants adorbed less bacteriophage T6 than a wild type strain.

Discussion

We have demonstrated that the receptor activity for bacteriophage T6 and colicin K in the cell envelope of *E. coli* K-12 can be accounted for by an outer membrane protein of 26,000 molecular weight, which is absent or altered in *tsx* mutants. We believe this protein is the receptor. One mutant, strain P1744, produces a tsx protein unable to function as the receptor: this production of an altered protein by a *tsx* mutant indicates that *tsx* is the structural gene for the tsx protein.

We have also demonstrated that synthesis of the tsx protein is catabolite repressible, and this is compatible with the recently demonstrated role of the *tsx* gene in the uptake of nucleosides (Hankte, 1976) which are known to be readily utilised by *E. coli* as a carbon source.

As with bacteriophages K3 (Manning, Puspurs and Reeves, 1976) and λ (Hoffnung, Jezierska and Braun-Breton, 1976) host range mutants of bacteriophage T6 can be arranged in an order of increasing potency on the range of *tsx* mutants able to plaque host range mutants at all. As for K3 and protein 3A, we assume that host range mutants of T6 are able to plaque only on *tsx* mutants which have an altered protein; *tsx* mutants with no functional protein at all will be included in the class on which no host range mutants can plaque. However, as for *ompA* mutants (Manning, Puspurs and Reeves, 1976), we again find that in general *tsx* mutants able to plaque host range phage do not produce detectable levels of the tsx protein. We nonetheless believe that each of these mutants has sub-detectable levels of tsx protein and that the range in ability to plaque host range mutants is due to variation in the amount of protein inserted into the outer membrane. However, as for the *ompA* mutants described earlier (Manning, Puspurs and Reeves, 1976) we are at present unable to demonstrate this directly.

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