Mol Gen Genet (1990) 221:491–494

MGG
© Springer-Verlag 1990

Analysis of a mutated phage T6 receptor protein of *Escherichia coli* K12

Christl Maier, Anke Middendorf, and Erhard Bremer

Department of Biology, University of Konstanz, P.O. Box 5560, D-7750 Konstanz, Federal Republic of Germany

Summary. The *tsx-206* allele encodes an altered Tsx protein, Tsx-206, that can no longer function as the T6 receptor. We show here that this allele also confers resistance to the Tsx-specific phages H1, H3, H8, K9, K18 and Ox1 but not to colicin K. The Tsx-206 protein still mediates the efficient permeation of deoxyadenosine across the outer membrane at low substrate concentration. A host-range mutant of phage T6, T6h3.1, was isolated which can use both the Tsx-206 and the Tsx wild-type protein as its receptor. Cloning and DNA sequence analysis of the *tsx-206* allele showed that the phage resistant phenotype was associated with an Asn to Tyr substitution at position 254 of the 272-residue Tsx protein.

Key words: Tsx – Outer membrane – Colicin K – Bacteriophages – Nucleoside uptake

The *tsx* gene from *Escherichia coli* encodes a minor outer membrane protein (Tsx), which is involved in the permeation of nucleosides across this permeability barrier at low (<1 μM) substrate concentration (Hantke 1976; Munch-Petersen et al. 1979; Krieger-Brauer and Braun 1980). Its dual function as a nucleoside-specific channel (Maier et al. 1988) and a receptor protein for colicin K and bacteriophage T6 (Manning and Reeves 1978) indicates that Tsx is a trans-membrane protein, with some of its segments exposed at the cell surface. Which of these segments are involved in colicin K and bacteriophage binding is unknown. Manning and Reeves (1978) analysed several independent T6^r mutants and found that most (49/50) produced no or greatly reduced amounts of Tsx protein. Only one mutant, strain P1744 (*tsx-206*) (Table 1), synthesized the normal amount of Tsx (Fig. 1) yet was completely resistant to phage T6 (Table 2). We found that strain P1744 differs from its parent strain P400 in that it lacks the OmpA protein (Fig. 1).

Strain P1744 was originally isolated on the basis of resistance to phage T6. We tested its sensitivity to the other known Tsx-specific bacteriophages H1, H3, H8, K9, K18, and Ox1 (Hancock and Reeves 1975) and found it to be entirely resistant. This phenotype was not due to the absence of OmpA in strain P1744 since an OmpA$^+$ derivative of P1744, strain CH14 (Table 2), was equally resistant to all seven Tsx-specific phages. After mutagenesis (Silhavy et al. 1984) in the *mutD5* mutator strain RM1036 (Bremer et al. 1988), we obtained a mutant of phage T6, T6h3.1, that plated with equal efficiency on strains P400 (*tsx*$^+$) and P1744 (*tsx-206*) but not on derivatives of these strains lacking the Tsx$^+$ and Tsx-206 protein, respectively (Table 2). This host-range mutant of T6 proved to be useful in analysing the cloned *tsx-206* gene (see below), since it allowed us to differentiate readily between strains synthesizing either the Tsx$^+$ (T6^{s}T6h3.1^s) or the Tsx-206 (T6^{r}T6h3.1^s) proteins. Manning and Reeves (1978) reported that the *tsx-206* mutation resulted not only in the T6^r phenotype of strain P1744 but also in its cross-resistance to colicin K. We also found that strain P1744 was not killed by the colicin (Table 2), but this phenotype is expected since OmpA$^-$ strains are tolerant to colicin K (Krieger-Brauer and Braun 1980). The OmpA$^+$ derivative of strain P1744, CH14, was as sensitive to colicin K as the *tsx*$^+$ strain P400 (Table 2). Thus, our data suggest that the *tsx-206* mutation alters the properties of the Tsx-206 protein as phage receptor but not as colicin K receptor.

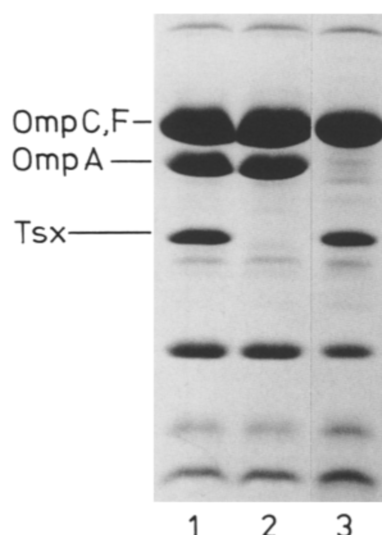

Fig. 1. SDS-polyacrylamide electrophoresis of outer membrane proteins. Outer membranes of strain P400 (*tsx*$^+$; lane 1), strain P407 (*tsx-200*; lane 2) and strain P1744 (*tsx-206*; lane 3) were isolated by sucrose gradient centrifugation and the outer membrane proteins were electrophoretically separated on a 12% SDS-polyacrylamide gel as described by Maier et al. (1988)

Offprint requests to: E. Bremer

To test whether the *tsx-206* mutation influenced the function of the Tsx-206 protein as a nucleoside-specific channel, we measured the initial uptake of deoxyadenosine and deoxycytosine at low substrate concentration in a set of strains that differed only in their *tsx* allele. For these experiments we used the OmpA$^+$ derivative of P1744, strain CH14, since OmpA$^-$ strains are known to show reduced nucleoside uptake (Krieger-Brauer and Braun 1980). Deoxyadenosine is strongly dependent on Tsx for permeation across the outer membrane; consequently its uptake is a sensitive test of the functional integrity of this polypeptide. The uptake of deoxycytosine was measured as a control since its permeation across the outer membrane is indepen-

Table 1. Bacterial strains

Strain	Description	Origin/Reference
P400	F$^-$ *thi-1 argE3 proA2 leu-6 thr-1 xyl-5 ara-14 galK2 lacY1 rpsL31 supE44 non-9*	Manning and Reeves (1978)
P407	P400 *tsx-200*	Manning and Reeves (1978)
P1744	P400 *ompA tsx-206*	Manning and Reeves (1978)
MC4100	F$^-$ *araD139 Δ(argF-lac)U169 rpsL150 relA1 deoC1 ptsF25 rbsR flbB5301*	Casadaban (1976)
HSK42	MC4100 *polA1*	Saarilahti and Palva (1985)
BRE2000	MC4100 *tsx*::Tn*10*	E. Bremer
BRE2101	F$^-$ *metB ilv rpsL Δ(argF-lac)U169 phoR*::Tn*10*	Bremer et al. (1988)
BRE2415	MC4100 *zba*::Tn*10*	E. Bremer
BRE2417[a]	HSK42 *tsx-206 phoR*::Tn*10*	This study

All strains are derived from *Escherichia coli* K12. The gene symbols are according to Bachmann (1983)

[a] The *phoR*::Tn*10* insertion from strain BRE2101 was transduced with phage P1*vir* (Miller 1972) into strain P1744. A P1*vir* lysate was grown on one of the *tsx-206 phoR*::Tn*10* transductants and used to transduce both alleles into strain HSK42. Transductants synthesizing the Tsx-206 protein were identified by their T6^r T6h3.1^s phenotype

Table 2. Sensitivity of strains to bacteriophages and colicin K

Strain	Relevant genotype		Sensitivity to bacteriophages and colicin K		
			T6	T6h3.1	Col K
P400	*tsx*$^+$	*ompA*$^+$	S	S	16
P407	*tsx-200*	*ompA*$^+$	R	R	0
P1744	*tsx-206*	*ompA*$^-$	R	S	0
CH13[a]	*tsx*::Tn*10*	*ompA*$^-$	R	R	0
CH14[b]	*tsx-206*	*ompA*$^+$	R	S	16

R, resistance; S, sensitivity

The colicin K titre is given as the reciprocal of the dilution that still gave complete killing. A crude preparation of colicin K was prepared from strain K49 (Hantke 1976) as described by Krieger-Brauer and Braun (1980)

[a] Strain CH13 is a derivative of strain P1744 into which a *tsx*::Tn*10* insertion was introduced by P1*vir*-mediated transduction using a phage lysate grown on strain BRE2000 (Table 1)

[b] Strain CH14 is a derivative of strain P1744 which was made *ompA*$^+$ by P1*vir*-mediated transduction using a donor strain, strain BRE2415, which carries the *zba*::Tn*10* insertion linked to the *ompA*$^+$ gene (Table 1)

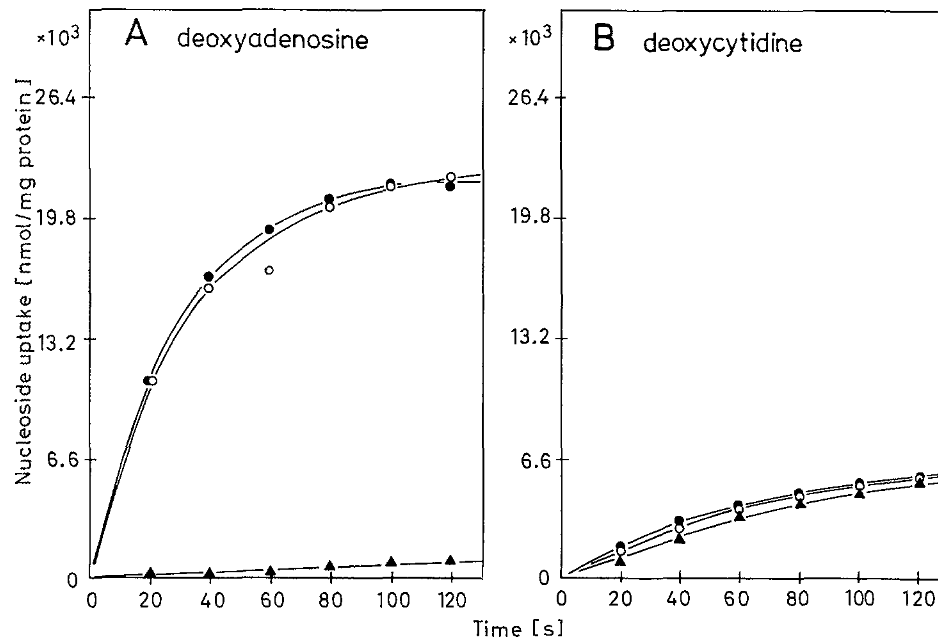

Fig. 2A and B. Uptake of [^{14}C]-deoxyadenosine and [^{14}C]-deoxycytidine into cells at low substrate concentration. The initial deoxynucleoside uptake by glycerol-grown cells was measured as described by Krieger-Brauer and Braun (1980). The final substrate concentrations of 8-[^{14}C]-deoxyadenosine (54.9 mCi/mmol) and 2-[^{14}C]-deoxycytidine (25.2 mCi/mmol; New England Nuclear, Dreieich, FRG) in the transport assay were 0.072 μM and 0.08 μM, respectively. Uptake of radiolabelled substrate is expressed as nmol/mg protein. Protein concentration was estimated from the optical density of the culture as described by Miller (1972). The following strains were used: CH15 (Tsx$^+$; ○); CH16 (Tsx$^-$; ▲) and CH14 (Tsx-206; ●). Strains CH15 and CH16 are derivatives of strains P400 and P407, respectively, and carry the *zba*::Tn*10* insertion used to construct strain CH14, the OmpA$^+$ derivative of P1744

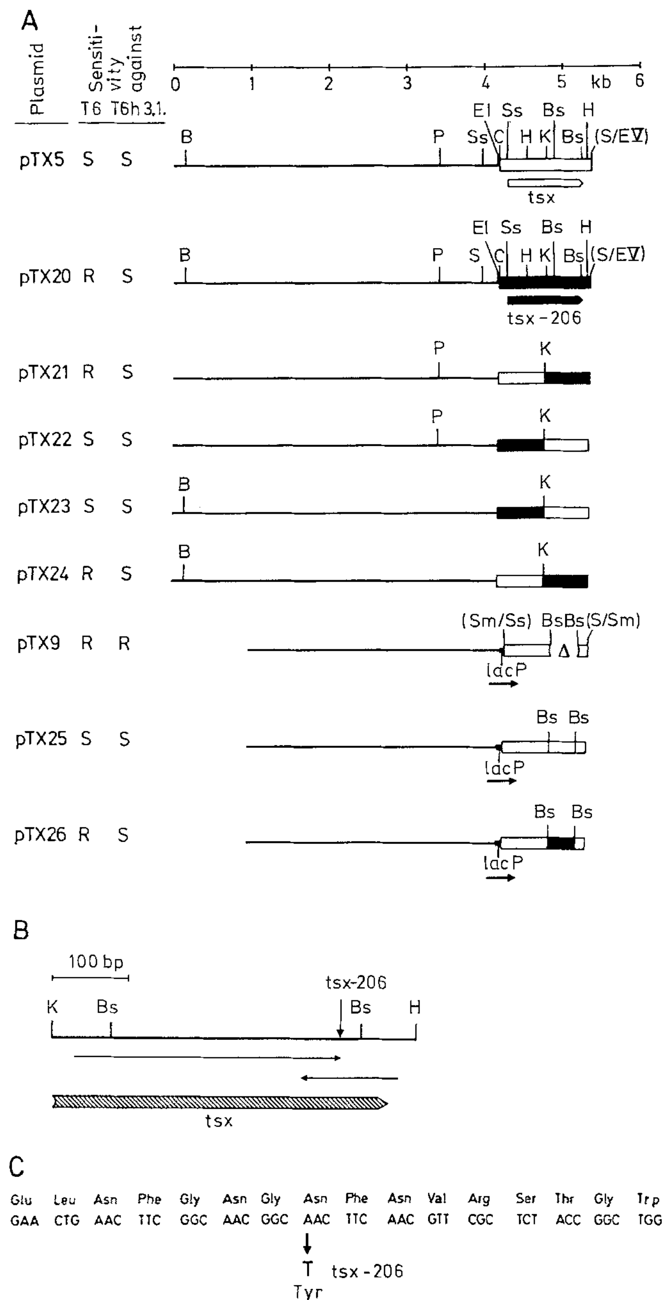

Fig. 3A–C. Mapping and DNA sequence analysis of the *tsx-206* mutation. **A** Plasmid pTX5 carries the wild-type tsx^+ gene; plasmid pTX20 is a derivative of pTX5 into which the *tsx-206* mutation was recombined in vivo by homologous recombination. Plasmids pTX21, pTX22, pTX23 and pTX24 carry hybrid $tsx^+/tsx\text{-}206$ genes which were constructed by the reciprocal exchange of restriction fragments from plasmids pTX5 (tsx^+) and pTX20 (*tsx-206*). Plasmids pTX21 and pTX22 were constructed by exchanging a 1.37 kb *Pst*I-*Kpn*I fragment and plasmids pTX23 and pTX24 were isolated after the exchange of a 0.92 kb *Kpn*I-*Bam*HI fragment. All these plasmids are derivatives of pBR322. Plasmid pTX9 is a derivative of the expression vector pHG329 (Stewart et al. 1986) and carries a partially deleted *tsx* gene (*ΔBst*EII-*Bst*EII) under *lacPO* control; pTX25 and pTX26 are derivatives of pTX9 into which the 305 bp *Bst*EII fragments from pTX5 (tsx^+) and pTX20 (*tsx-206*), respectively, were inserted. The vector sequences are indicated by the *thin line*, those of the chromosomal segments are shown as *open* (tsx^+) or *closed* (*tsx-206*) *boxes*. The extent and direction of the coding regions for the tsx^+ and *tsx-206* genes are indicated by *arrows*. Restriction site abbreviations: B, *Bam*HI; P, *Pst*I; Ss, *Ssp*I; EI, *Eco*RI; C, *Cla*I; H, *Hpa*I; K, *Kpn*I; Bs, *Bst*EII; S, *Stu*I; E, *Eco*RV; Sm, *Sma*I. The restriction sites shown in *brackets* were destroyed during the blunt end ligation of restriction fragments. Sensitivity to phages and colicin K were determined after transforming the plasmids into the Tsx^- strain P407; S = sensitivity; R = resistance. **B** DNA sequencing strategy. A restriction map is shown of the 474 bp *Kpn*I-*Hpa*I fragment which was cloned into the M13mp18 and M13mp19 phages; the 3′ end of the *tsx* coding region is indicated by the *shaded arrow*. The extent of the DNA sequence determined is indicated by the *thin arrows* and the position of the *tsx-206* mutation is marked. **C** Nucleotide sequence of the *tsx-206* mutation. Part of the *tsx* sequence along with the deduced amino acid sequence of the Tsx protein (Bremer et al.; manuscript in preparation) is shown. The *tsx-206* mutation is an A to T exchange resulting in the replacement of Asn^{254} by Tyr

dent of Tsx (Hantke 1976; Munch-Petersen et al. 1979; Krieger-Brauer and Braun 1980). The Tsx^+ and Tsx^- strains CH15 and CH16, respectively, showed the expected strong dependence on the Tsx protein for deoxyadenosine uptake (Fig. 2A). Strain CH14, which synthesized the Tsx-206 protein, behaved like the Tsx^+ strain CH15 (Fig. 2A, B), demonstrating that the mutant Tsx-206 protein still functions as an efficient deoxyadenosine-specific channel in vivo.

To characterize the *tsx-206* allele, we transferred this chromosomal mutation by in vivo homologous recombination in a *polA* mutant on to the tsx^+ plasmid pTX5 (Bremer et al.; manuscript in preparation) using a procedure described by Saarilahti and Palva (1985). One of the isolated plasmids carrying the *tsx-206* mutation is pTX20. When this plasmid, of which the restriction map was found to be indistinguishable from its parent tsx^+ plasmid pTX5 (Fig. 3A), is present in the Tsx^- strain P407, it confers the characteristic Tsx-206 phenotypes: the resulting strain is sensitive to colicin K and to phage T6h3.1, but resistant to all other Tsx-specific phages including phage T6. In such strains, the Tsx-206 protein is produced at high levels (data not shown).

Using standard recombinant DNA techniques (Maniatis et al. 1982), we mapped the *tsx-206* mutation to a 305 bp *Bst*EII fragment within the *tsx* coding region by constructing hybrid $tsx^+/tsx\text{-}206$ genes (Fig. 3A). To determine the alteration in the *tsx-206* allele, we cloned the 474 bp *Kpn*I-*Hpa*I fragment that contains the 305 bp *Bst*EII segment (Fig. 3B) from the wild-type and the mutant *tsx* genes into the M13mp18 and M13mp19 phages. We established the entire DNA sequence (Sanger et al. 1977) between the two *Bst*EII sites from both genes in parallel and found a single difference: an AAC codon which encodes the Asn residue at position 254 of the 272-residue Tsx protein (Bremer et al.; manuscript in preparation) is altered to a TAC codon which encodes Tyr (Fig. 3C). Since the Tsx-206 protein still functions as a nucleoside-specific channel in vivo and as colicin K receptor, it appears that the overall topology of the Tsx polypeptide within the outer membrane is not grossly altered by the Asn^{254} to Tyr change. Our results therefore suggest that Asn^{254} plays a crucial role for the function of Tsx as a phage receptor.

Acknowledgements. We thank K. Hantke, T. Palva and P. Reeves for generously providing bacterial strains and W. Boos, I. Rasched and K. Heller for critical reading of the manuscript. This work was supported by a grant from the Deutsche Forschungsgemeinschaft through SFB 156.

References

Bachmann BJ (1983) Linkage map of *Escherichia coli* K-12, edition 7. Microbiol Rev 47:180–230

Bremer E, Gerlach P, Middendorf A (1988) Double negative and positive control of *tsx* expression in *Escherichia coli.* J Bacteriol 170:108–116

Casadaban MJ (1976) Transposition and fusion of the *lac* genes to selected promoters in *Escherichia coli* using bacteriophage lambda and Mu. J Mol Biol 104:541–555

Hancock REW, Reeves P (1975) Bacteriophage resistance in *Escherichia coli* K-12: general pattern of resistance. J Bacteriol 121:983–993

Hantke K (1976) Phage T6-colicin K receptor and nucleoside transport in *Escherichia coli.* FEBS Lett 70:109–112

Krieger-Brauer HJ, Braun V (1980) Functions related to the receptor protein specified by the *tsx* gene of *Escherichia coli.* Arch Microbiol 124:233–242

Maier C, Bremer E, Schmid A, Benz R (1988) Pore-forming activity of the Tsx protein from the outer membrane of *Escherichia coli.* Demonstration of a nucleoside-specific binding site. J Biol Chem 263:2493–2499

Maniatis T, Fritsch EF, Sambrook J (1982) Molecular cloning. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York

Manning PA, Reeves P (1978) Outer membrane proteins of *Escherichia coli* K-12: isolation of a common receptor protein for bacteriophage T6 and colicin K. Mol Gen Genet 158:279–286

Miller JH (1972) Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York

Munch-Petersen A, Mygind B, Nicolaisen A, Pihl NJ (1979) Nucleoside transport in cells and membrane vesicles from *Escherichia coli* K-12. J Biol Chem 254:3730–3737

Saarilahti HT, Palva ET (1985) In vivo transfer of chromosomal mutations onto multicopy plasmids utilizing *polA* strains: cloning of an *ompR2* mutation in *Escherichia coli* K12. FEMS Microbiol Lett 26:27–33

Sanger F, Nicklen S, Coulson AR (1977) DNA sequencing with chain-terminating inhibitors. Proc Natl Acad Sci USA 74:5463–5467

Silhavy TJ, Berman ML, Enquist LW (1984) Experiments with gene fusions. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York

Stewart GBSA, Lubinsky-Mink S, Jackson CG, Cassel A, Kuhn J (1986) pHG165: an pBR322 copy number derivative of pUC8 for cloning and expression. Plasmid 15:172–181

Communicated by J. Lengeler

Received October 17, 1989