JOURNAL OF BACTERIOLOGY, Sept. 1981, p. 1113-1116
0021-9193/81/091113-04$02.00/0

Vol. 147, No. 3

The *tsx* Protein of *Escherichia coli* Can Act as a Pore for Amino Acids

MICHAEL W. HEUZENROEDER AND PETER REEVES*

Department of Microbiology and Immunology, The University of Adelaide, Adelaide, South Australia 5000

Received 10 March 1981/Accepted 30 May 1981

The *tsx* protein is known to be a specific diffusion pathway for nucleosides. The ability of this protein to facilitate the transport of molecules other than nucleosides was examined in strains lacking detectable amounts of porin (*ompB* mutants). The *tsx* protein was shown to promote serine, glycine, and phenylalanine transport and to have no effect on either glucose or arginine transport.

Genetic evidence based on the existence of mutants resistant to both colicin K and bacteriophage T6 of *Escherichia coli* led to the postulate that colicin K and bacteriophage T6 share a common receptor (7), later shown to be a protein (26). It was more recently found that most T6-resistant mutants lack an outer membrane protein of molecular weight around 25,000, which is the product of the *E. coli tsx* gene (13). This protein is able to inactivate both colicin K and phage T6 (14) and is presumably the receptor for both agents. Several outer membrane proteins, in addition to acting as bacteriophage and colicin receptors, have as their primary function facilitating the diffusion of hydrophilic molecules across the permeability barrier of the outer membrane. The diffusion of low-molecular-weight hydrophilic molecules is due to the presence of pores formed by these proteins. *ompB* mutants lack detectable levels of OmpC and OmpF proteins (19). Experiments conducted both in vivo and in vitro show that OmpC and OmpF proteins constitute the major porins of *E. coli* (2, 3, 4, 16, 17, 21).

In addition to these major porins, there exist outer membrane proteins involved in the transport of specific classes of substrate. These include the *tonA* protein for ferrichrome transport (25), the *fepA* (*cbr*) protein for ferrienterochelin transport (8, 18, 24), the *btuB* protein for vitamin B_{12} transport (5), the λ receptor for maltose and maltodextrin transport (20), and the *tsx* protein for nucleoside transport (9, 11). The specificity of these systems has only been investigated in detail for the maltose-maltodextrin systems (6, 10, 12, 22, 23).

In this paper we examine the diffusion of molecules other than nucleosides through the *tsx* pore. This was done by observing the ability of the *tsx* protein to compensate for an *ompB* mutation under conditions of limiting substrate concentration. The *E. coli* K-12 strains used in this study are listed in Table 1. Whole membrane preparations were made from 10 ml of late-log-phase cultures grown in nutrient broth. The cells were washed once in 30 mM Tris-hydrochloric acid, pH 8.1 (10 ml), suspended in 0.2 ml of 20% sucrose containing 30 mM Tris-hydrochloride, pH 8.1, and kept on ice. These cells were then converted to spheroplasts by the addition of 0.02 ml of lysozyme (1 mg/ml) in 0.1 M EDTA (pH 7.3) and incubation on ice for 30 min. The spheroplasted cells were centrifuged (15 min; 15,000 × *g*). The supernatant fluid containing periplasmic proteins was discarded, and the pellet was frozen, thawed, suspended in 3 ml of 3 mM EDTA (pH 7.3), and subjected to sonication for 1 min, the tubes being kept on ice. The sonicated spheroplasts were centrifuged (1 h; 28,000 × *g*). The pellet obtained was the whole membrane preparation. The whole membranes were run on 11 to 20% polyacrylamide gels by the method of Achtman et al. (1). The uptake of radioisotopes was investigated on log-phase cultures (optical density in 1-cm light path at 625 nm = 0.5), grown in nutrient broth. All conditions for uptake are as described previously (10). In all cases, the concentration of isotope was such that outer membrane permeability limited uptake.

P400 and its *tsx*$^+$ derivatives produce *tsx* protein in large quantities (15). It is therefore quite easy to determine from gels whether *tsx* protein is present in this genetic background; however, in strain P1848 and its derivatives, *tsx* protein is only produced in "normal" amounts and is difficult to visualize except after overloading polyacrylamide gels. The membrane protein preparations of strains P1578, P1602, P1930, and P2024 show markedly reduced amounts of

OmpC and OmpF proteins, typical of the pattern expected of *ompB* mutants (19). P1602 and P2024 also lack *tsx* protein in their membrane preparations (data not shown). The ability of both series of strains to take up the nucleosides adenosine and thymidine was investigated to determine whether the mutations carried by both series of strains are consistent with published results. The rate of nucleoside uptake for both series of strains is markedly reduced in *tsx* mutants, thus confirming the work of Hantke (9). The effect of the *ompB* mutation on both thymidine and adenosine uptake is significant, although the *tsx* mutation has the major effect on nucleoside uptake. When *ompB* and *tsx* mutations are present together as in P2024 and P1602, these strains are particularly disadvantaged with respect to nucleoside uptake (data not shown).

TABLE 1. *Bacterial strains*[a]

Strain	Genotype
P400	*thi-1 argE3 proA2 leu-6 thr-1 xyl-5 ara-14 galK2 lacY1 rpsL31 supE44 non-9*
P407	*tsx-200* derivative of P400
P433	*tsx-201* derivative of P400
P1578	*ompB105* derivative of P400
P1602	*tsx-203* derivative of P1578
P1848	*tonA208* derivative of W1485
P1926	*tsx-10* derivative of P1848
P1930	*ompB106* derivative of P1848
P2024	*tsx-232* derivative of P1930

[a] P400 and its derivatives produce *tsx* protein in large amounts relative to most *E. coli* K-12 strains. All strains are part of our laboratory collection strains.

The uptake of [^{14}C]serine for strains derived from P400 is presented in Fig. 1A. These results indicate that for serine uptake, the *tsx* protein

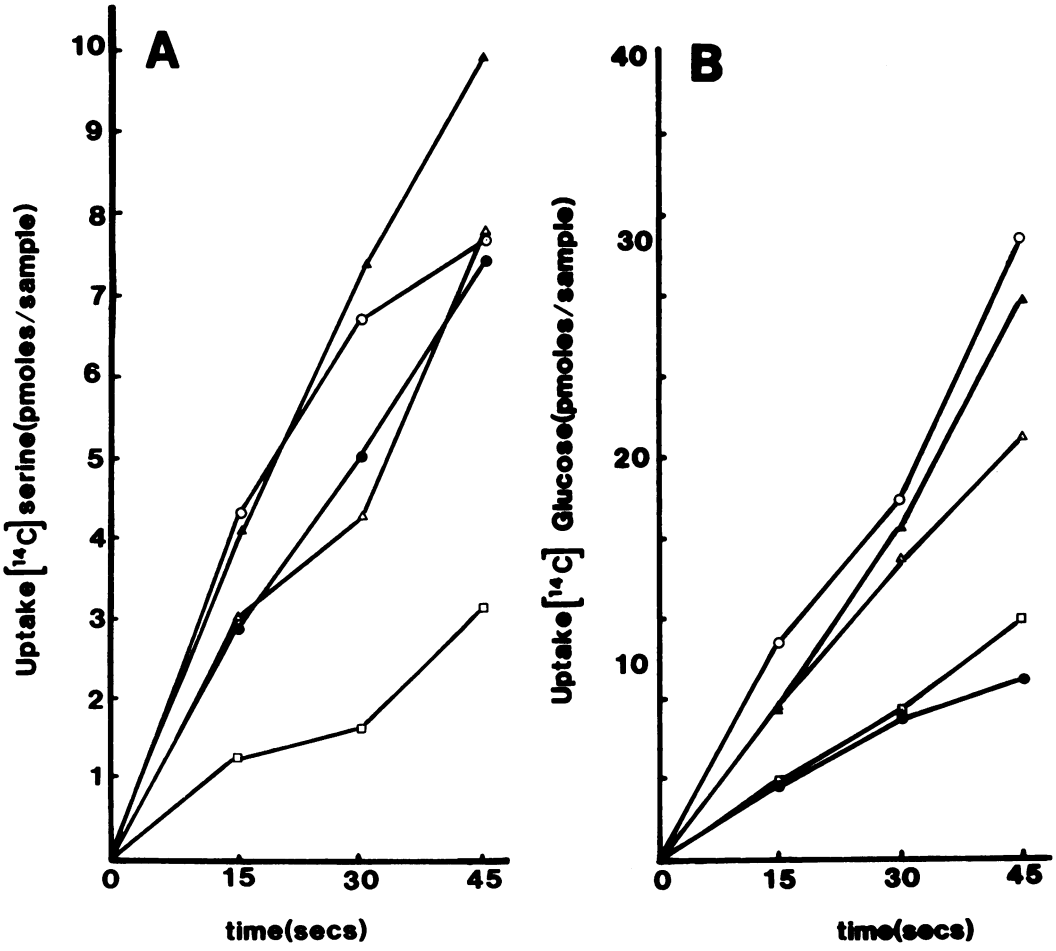

FIG. 1. *Uptake of (A) 0.9 μM [^{14}C]serine at 37°C and (B) 0.83 μM [^{14}C]glucose. Symbols: △, P400; ▲, P407; ○, P433; ●, P1578; □, P1602.*

can compensate for the lack of major porins in the *ompB* mutant P1578. The three *ompB*$^+$ strains P400, P407, and P433 showed very similar initial uptake rates, as we would predict for cells still retaining the major porins. The *ompB* strain P1578, although lacking porins, still had a large amount of *tsx* protein in the outer membrane. This strain had only a slightly lower uptake rate for serine than did either P400, P407, or P433. The double mutant P1602, however, appeared to be considerably disadvantaged in its uptake of serine, indicating lack of diffusion pathways across the outer membrane and significant diffusion through the *tsx* pore in P1578. Fig. 1B depicts the uptake of [^{14}C]glucose for P400 and its derivatives. In comparison with the effect of *tsx* protein on serine uptake, there appeared to be no effect of *tsx* protein on glucose uptake; P1578 appeared to have no advantage over P1602 in uptake ability for glucose.

In addition to the enhancement of serine uptake by the *tsx* protein, this effect is also apparent for glycine and phenylalanine to varying degrees. The ability of *tsx* protein to enhance serine and glycine uptake is not confined to P400 and derivatives, as the effect also appears in P1848 and its derivatives which produce normal amounts of *tsx* protein (Table 2).

It can also be seen that arginine, like glucose, appears to be unaffected by *tsx* protein in diffusion across the outer membrane. It is also noteworthy that the *tsx* mutants showed an improvement in uptake ability for some substrates. This effect appears to be dependent upon the genetic background.

The data obtained for the *tsx* protein resemble data obtained in earlier experiments for the λ receptor, which also allows nonspecific permeation of some but not all low-molecular-weight compounds (12, 22): the *tsx* data are best considered in the context of what is known of λ receptor pore function, since in this case there is a considerable volume of evidence relating to its specificity, and it is possible to put forward an overall hypothesis for the specificity of this pore.

TABLE 2. *Rates of uptake of labeled substrates*[a]

Strain	Relevant genotype	Uptake of:			
		Serine (0.9 μM)	Glycine (2.3 μM)	Phenylalanine (0.1 μM)	Arginine (0.15 μM)
P400	Wild type	100 (7.6)[b]	100 (10.5)	100 (0.4)	100 (1.1)
P407	*tsx*	120	74	132	ND
P433	*tsx*	93	64	123	100
P1578	*ompB*	88	62	33	50
P1602	*ompB tsx*	35	41	15	54
P1848	Wild type	100 (33)	100 (7.2)		
P1926	*tsx*	115	108		
P1930	*ompB*	68	115		
P2024	*ompB tsx*	23	79		

[a] Calculated for first 45 s and given as percentage of uptake rate of appropriate wild-type strain. ND, Not determined.

[b] Uptake rate in picomoles per minute in wild-type sample.

The maltose-maltodextrin and nucleoside permeation pathways share similar features. The dependence of nucleoside and maltose uptake upon the specific outer membrane protein applies only at low substrate concentrations; also, there is no competitive inhibition of the adsorption of the specific bacteriophage by the substrates in either case, indicating that no strong substrate binding occurs at the level of the receptor.

It has also been shown that both outer membrane proteins are coregulated with the various components of their respective substrate transport and metabolic pathways (11, 20).

In this study, we show that the *tsx* protein will enable permeation of serine, glycine, and phenylalanine in addition to nucleosides, and this suggests that, like the λ receptor, this protein forms a hydrophilic pore with relatively broad specificity. This degree of nonspecificity would be unexpected if permeation had an enzyme-like binding as an essential first step by active means. The *tsx* protein appears to have little or no effect in alleviating an *ompB* defect for glucose or arginine. This may mean that the internal properties of the *tsx* pore restrict permeation of some materials.

In the case of nucleoside transport, there is no evidence for the existence of a periplasmic binding protein, and further work will be required to determine how closely the *tsx* protein fits the λ receptor model.

We thank P. A. Manning for his many useful discussions during this work and his expertise in the running of acrylamide gels.

LITERATURE CITED

1. **Achtman, M., S. Schwuchow, R. Helmuth, G. Morelli, and P. A. Manning.** 1978. Cell-cell interactions in conjugating *Escherichia coli*: Con$^-$ mutants and stabilization of mating aggregates. Mol. Gen. Genet. **164:** 171-183.
2. **Bavoil, P., H. Nikaido, and K. von Meyenberg.** 1977. Pleiotropic transport mutants of *Escherichia coli* lack porin, a major outer membrane protein. Mol. Gen. Genet. **158:**23-33.
3. **Beacham, I. R., D. Haas, and E. Yagil.** 1977. Mutants of *Escherichia coli* "cryptic" for certain periplasmic enzymes: evidence for an alteration of the outer membrane. J. Bacteriol. **129:**1034-1044.
4. **Beacham, I. R., R. Kahana, L. Levy, and E. Yagil.** 1973. Mutants of *Escherichia coli* K-12 "cryptic" or deficient in 5'-nucleotidase (uridine diphosphate-sugar hydrolase) and 3'-nucleotidase (cyclic phosphodiesterase) activity. J. Bacteriol. **116:**957-964.
5. **Di Masi, D. R., J. C. White, C. A. Schnairman, and C. Bradbeer.** 1973. Transport of vitamin B12 in *Escherichia coli*: common receptor sites for vitamin B12 and

the E colicins on the outer membrane of the cell envelope. J. Bacteriol. **115**:506–513.

6. **Ferenci, T., M. Schwentorat, S. Ullrich, and J. Vilmart.** 1980. Lambda receptor in the outer membrane of *Escherichia coli* as a binding protein for maltodextrins and starch polysaccharides. J. Bacteriol. **142**:521–526.
7. **Fredericq, P.** 1949. Sur la resistance croisee entre colicine E et bacteriophage II. C.R. Soc. Biol. **143**:1011–1013.
8. **Hancock, R. E. W., K. Hantke, and V. Braun.** 1976. Iron transport in *Escherichia coli* K-12: involvement of the colicin B receptor and of a citrate-inducible protein. J. Bacteriol. **127**:1370–1375.
9. **Hankte, K.** 1976. Phage T6-colicin K receptor and nucleoside transport in *Escherichia coli*. FEBS Lett. **70**: 109–112.
10. **Heuzenroeder, M. W., and P. Reeves.** 1980. Periplasmic maltose-binding protein confers specificity on the outer membrane maltose pore of *Escherichia coli*. J. Bacteriol. **141**:431–435.
11. **Krieger-Brauer, H. J., and V. Braun.** 1980. Functions related to the receptor protein specified by the *tsx* gene of *Escherichia coli*. Arch. Microbiol. **124**:233–242.
12. **Luckey, M., and H. Nikaido.** 1980. Specificity of diffusion channels produced by λ phage receptor protein in *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. **77**:167–171.
13. **Manning, P. A., and P. Reeves.** 1976. Outer membrane of *Escherichia coli* K12: *tsx* mutants (resistant to bacteriophage T6 and colicin K) lack an outer membrane protein. Biochem. Biophys. Res. Commun. **71**:446–471.
14. **Manning, P. A., and P. Reeves.** 1978. Outer membrane proteins of *Escherichia coli* K12: isolation of a common receptor for bacteriophage T6 and colicin K. Mol. Gen. Genet. **158**:279–286.
15. **Manning, P. A., A. P. Pugsley, and P. Reeves.** 1977. Defective growth functions in mutants of *Escherichia coli* K12 lacking a major outer membrane protein. J. Mol. Biol. **116**:285–300.
16. **Nakae, T.** 1976. Outer membrane of *Salmonella*: isolation of protein complex that produces transmembrane channels. J. Biol. Chem. **251**:2176–2178.
17. **Nakae, T.** 1976. Identification of the outer membrane protein of *E. coli* that produces transmembrane channels in reconstituted vesicle membrane. Biochem. Biophys. Res. Commun. **71**:877–884.
18. **Pugsley, A. P., and P. Reeves.** 1977. The role of colicin receptors in the uptake of ferrienterochelin by *Escherichia coli* K12. Biochem. Biophys. Res. Commun. **74**: 903–911.
19. **Sarma, V., and P. Reeves.** 1977. Genetic locus (*ompB*) affecting a major outer membrane protein in *Escherichia coli* K-12. J. Bacteriol. **132**:23–27.
20. **Szmelcman, S., and M. Hofnung.** 1975. Maltose transport in *Escherichia coli* K-12: involvement of the bacteriophage lambda receptor. J. Bacteriol. **124**:112–118.
21. **Von Meyenberg, K.** 1971. Transport limited growth rates in a mutant of *Escherichia coli*. J. Bacteriol. **107**:878–888.
22. **Von Meyenberg, K., and H. Nikaido.** 1977. Outer membrane of gram-negative bacteria. XVII. Specificity of transport process catalysed by the λ-receptor protein in *Escherichia coli*. Biochem. Biophys. Res. Commun. **78**: 1100–1107.
23. **Wandersman, C., M. Schwartz, and T. Ferenci.** 1979. *Escherichia coli* mutants impaired in maltodextrin transport. J. Bacteriol. **140**:1–13.
24. **Wayne, R., K. Frick, and J. B. Neilands.** 1976. Siderophore protection against colicins M, B, V, and Ia in *Escherichia coli*. J. Bacteriol. **126**:7–12.
25. **Wayne, R., and J. B. Neilands.** 1975. Evidence for common binding sites for ferrichrome compounds and bacteriophage φ80 in the cell envelope of *Escherichia coli*. J. Bacteriol. **121**:497–503.
26. **Weltzien, H. U., and M. A. Jesaitis.** 1971. The nature of the colicin K receptor of *Escherichia coli* Cullen. J. Exp. Med. **133**:534–553.