PHAGE T6 – COLICIN K RECEPTOR AND NUCLEOSIDE TRANSPORT IN *ESCHERICHIA COLI*

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1. Introduction

The outer membrane of *E. coli* creates a barrier for hydrophilic substrates with a molecular weight above 700 daltons [1]. Receptor proteins for phages and colicins have been found to facilitate the passage of certain substrates across the outer membrane. It is known that the following groups of agents each use a single outer membrane protein: phages T1, T5, colicin M and the iron chelate ferrichrome (*ton*A) [2], colicins B, I, V and enterochelin (*feu*A, *feu*B) [3], phage BF23, the E colicins and vitamin B12 (*bfe*) [4], and phage λ and maltose (*lam*B) [5]. It is reasonable to assume that additional outer membrane proteins originally detected as phage and colicin receptors are involved in the uptake of substrates for which the outer membrane would otherwise form a permeability barrier.

The receptor for phage T6 and colicin K has been shown to be a protein [6,7]. The cross-resistance to phage T6 and colicin K in *tsx* mutants selected using either of the killing agents indicated a common receptor. However the stability of the colicin K receptor activity towards enzymes and chemical reagents differed from the stability of the T6 receptor activity indicating that they required different chemical groupings [7]. In this paper evidence is provided that the receptor protein for phage T6 and colicin K is a constituent of a 'channel' through the outer membrane because the uptake of nucleosides is severely impaired in *tsx* mutants which lack an outer membrane protein with a mol. wt of 25 000.

2. Materials and methods

2.1. *Strains and selection of mutants*

The following strains were used: *E. coli* K-12 HfrH *thi* from which the T6 resistant mutants HX1 and HX2 were isolated. *E. coli* B_{s-1} (Hill) *met* and its T6 resistant derivatives BX5, BX8 and BX16; *E. coli* ML 308–225 and the T6 resistant mutants ML1, ML2 and ML3. The spontaneous T6 resistant mutants indicated above were isolated by mixing a concentrated suspension of bacteria with 5–10 phages per cell in TY medium. After 30 min incubation at 37°C to allow phage adsorption, the mixture was plated on TY plates. Colonies were purified and retested for T6 resistance.

The colicin K producing strain K49 was obtained from P. Fredericq. Cells harvested in the late logarithmic phase were sonified and cell debris spun down. The colicin K in the supernatant gave a clear spot at a dilution of 1 : 1024 on a plate seeded with *E. coli* HfrH.

2.2. *Culture conditions*

Cells were normally grown in TY medium (10 g bacto tryptone, 5 g yeast extract and 5 g NaCl/l). M9 minimal medium contained per litre, 6 g Na_2HPO_4, 3 g KH_2PO_4, 0.5 g NaCl, 0.5 g NH_4Cl, 1 mM $MgSO_4$, 0.4% glucose and the appropriate amino acids (20 μg per ml). M9 salts consisted only of the salt mixture. For membrane separation and uptake studies cells were grown on a low phosphate medium [8] containing 0.12 M Tris (hydroxymethyl)–aminomethane,

0.08 M NaCl, 1 mM $MgSO_4$, 0.5% bacto peptone and 0.6% glycerol, (pH adjusted to 7.5 with HCl).

2.3. *Uptake studies*

From a fresh overnight culture, cells were grown on low phosphate medium to an optical density of 0.3 at 578 nm, spun down and washed once with M9 salts and resuspended in the same salt solution to an E 578 of 0.25, which corresponds to 95 μg dry weight per ml. Two hundred μl of cells were incubated for 2 min at 25°C and 10 μl (20 pmol) of the tritium labeled nucleoside added. The nucleosides had a specific activity ranging from 17–27 Ci/mmol and were obtained from Amersham Buchler.

Uptake was stopped after the appropriate time by dilution with 2 ml of M9 salts and immediate filtration on Sartorius cellulose nitrate membrane filters (pore size 0.45 μm) after which the cells were washed with 2 ml of M9 salts. Filters were dried at 70°C and radioactivity was measured after adding 5 ml toluene-based scintillation fluid in the liquid scintillation counter Mark II from Nuclear Chicago Corp.

Membrane separation was done as described by Osborn [9]. Electrophoresis was performed by the method of Lugtenberg et al. [10] using a 15% acrylamide slab-gel.

3. Results

T6 resistant (*tsx*) mutants were isolated in three different *E. coli* strains (K-12, B and ML) and nucleoside transport was compared in the wild type and the mutants. Figure 1A shows a typical uptake experiment with *E. coli* HfrH and its *tsx* derivative HX1. Adenosine and deoxyadenosine uptake were both strongly reduced in the *tsx* mutant. This reduction was even more pronounced in ML1, the *tsx* mutant of ML 308-225 (fig.1B). Similar curves were obtained with *E. coli* B and its derivatives (data not shown). The T6 receptor seems not to be specific for one nucleoside since there was also a drastic reduction in the uptake of both uridine and thymidine into *tsx* mutants (fig.1C and D). The other strains were also tested and yielded very similar results. The differences in the uptake of deoxyuridine, however, were not as remarkable as they were with the other nucleosides tested. The lowered uptake for adenosine, deoxy-

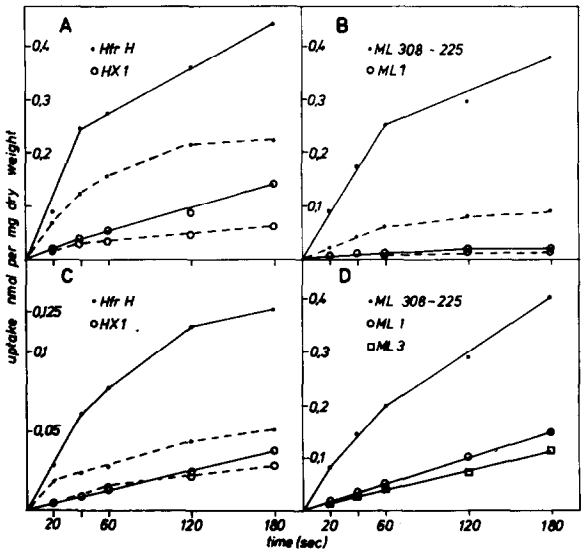

Fig.1A and B. Uptake of (——) [^{3}H]adenosine and (- - - -) [^{3}H]deoxyadenosine; C: Uptake of (——) [^{3}H]uridine and (- - - -) [^{3}H]deoxyuridine; D: Uptake of thymidine; into wild type cells of *E. coli* HfrH, ML 308-225 and the T6 resistant derivatives H1, ML1, ML3. All nucleosides were provided at a concentration of 0.095 μM.

adenosine and uridine in the *tsx* mutant compared to the wild type was not apparent in cells grown on M9 minimal medium in the presence of glucose. The uptake systems of these nucleosides are known to be catabolite repressible as are the other genes under the control of *cyt*R and *deo*R [11]. Thymidine uptake was not repressed by glucose [12]. Poor induction of the transport system may also account for the low deoxyuridine uptake (fig.1C) resulting in a small difference between wild type and the *tsx* mutants. In conclusion the uptake of the ribonucleosides adenosine and uridine and of the deoxynucleosides thymidine and deoxyadenosine were strongly reduced in the *tsx* mutant. This lowered uptake became not apparent with deoxyuridine.

For the characterisation of the T6 resistant strains the outer membranes of wild type and mutant were isolated by sucrose gradient centrifugation [9]. The outer membranes were washed with water and applied to a discontinuous slab-gel electrophoresis (fig.2). It could be seen that all *tsx* mutants tested had lost one protein with a mol. wt of 25 000 daltons (same position as chymotrypsinogen). Reeves and coworkers

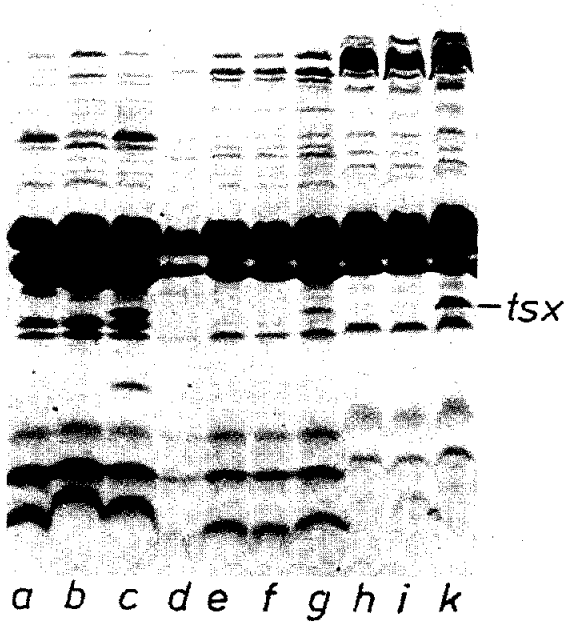

Fig.2. SDS polyacrylamide gel electrophoresis of outer membrane proteins of the *E. coli* K12 strains HX2 (a), HX1 (b), HfrH (c), the *E. coli* B strains BX16 (d), BX8 (e), BX5 (f), B (g), and the *E. coli* ML strains ML2 (h), ML1 (i) and ML308-225 (k). *tsx* marks the position of the T6 receptor.

[14] have already found that in *tsx* mutants a protein is missing in that region of the gel. Growth with thymidine seemed to result at best in a very small increase of the protein band. No inner membrane changes were observed. Since phage T6 shares the receptor with colicin K the sensitivity of the different strains to colicin K was tested. *E. coli* ML 308-225 was only sensitive to very high concentrations of colicin K while the *tsx* mutants ML1, ML2 and ML3 were fully resistant. *E. coli* K-12 and B were sensitive up to a dilution of 1 : 1024 while their *tsx* derivatives were sensitive only to dilutions of 1 : 64 (HX1, HX2) or 1 : 128 (BX5, BX8) of the colicin K solution.

4. Discussion

It is shown in this paper that *tsx* mutants lacked a protein of 25 000 daltons in the outer membrane. Concomitantly the uptake capacities of the mutant cells for the four nucleosides tested were significantly reduced and it may be assumed that other nucleosides not tested might also be affected. McKeown et al. [12]. Recently described a new gene *nup* responsible for nucleoside uptake. This *nup* mutation reduced transport but not metabolism. It affected the uptake of ribo- and deoxy-, pyrimidine and purine nucleosides. This low specificity argues in favor of a 'pore' function rather than a permease. In addition, In addition, the *nup* is located in the 10 min region of the *E. coli* genetic map (*tsx* maps at 9 min). This evidence suggests that *nup* and *tsx* are identical.

The *tsx* receptor is under catabolite repression control since mutants unable to make cyclic AMP and cyclic AMP receptor protein are resistant to T6 on TY plates [13].

The T6 receptor protein seems to be a specific gate through the outer membrane which permits rapid entry of nucleosides. This is relevant to an understanding of the function of the outer membrane. Nucleosides have a molecular weight ranging from 230–280. They should have free access to active transport systems in the cytoplasmic membrane if the outer membrane constituted only a nonspecific sieve excluding molecules above 600–900 daltons. The presence of the T6 receptor and the λ receptor which enhances maltose (mol. wt 342) transport shows that the outer membrane contains gates specific for certain substrate groups. These proteins are required for effective uptake but even in the absence of such gate-proteins low uptake of substrate occurs which in most cases is sufficient for cell growth.

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