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Characterization of the nucleoside-binding site inside the Tsx channel of *Escherichia coli* outer membrane

Reconstitution experiments with lipid bilayer membranes

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Reconstitution of purified Tsx protein from *Escherichia coli* into lipid bilayer membranes showed that Tsx formed small ion-permeable channels with a single-channel conductance of 10 pS in 1 M KCl. The dependence of conductance versus salt concentration was linear, suggesting that Tsx has no binding site for ions. Conductance was inhibited by the addition of 20 mM adenosine. Titration of the Tsx-mediated membrane conductance with different solutes including free bases, nucleosides, and deoxynucleosides suggested that the channel contains a binding site for nucleosides but not for sugars or amino acids, and binding increased in the following order: free base, nucleoside, and deoxynucleoside. Among the five nucleosides the stability constant for the binding increased in the order of cytidine, guanosine, uridine, adenosine, and thymidine. Control experiments revealed that the binding of the nucleosides is independent of ion concentration in the aqueous phase, i.e. there was no competition between nucleosides and ions for the binding site inside the channel. The binding of the solutes to the channel interior can be explained by a one-site two-barrier model for the Tsx channel. The advantage of a binding site inside a specific porin for the permeation of solutes is discussed with respect to the properties of a general diffusion pore.

The *tsx* gene of *Escherichia coli* encodes a minor outer-membrane protein (Tsx) that is the receptor for colicin K and bacteriophage T6 [1–3]. Synthesis of Tsx is coregulated with the systems for nucleoside uptake and metabolism, suggesting that it plays an important role in the permeation of nucleosides across the outer membrane [4–6] (and P. Valentin-Hansen, personal communication). Indeed, *E. coli* strains lacking Tsx are impaired in the uptake of all nucleosides, with the notable exception of cytidine and deoxycytidine [5, 7–9]. This Tsx-mediated permeation of nucleosides across the outer membrane is most clearly detected when the exogenously provided nucleosides are present at low (< 1 μM) substrate concentration, i.e. when Tsx is the limiting factor for the overall transport process [14, 17, 18]. At high concentrations of substrate, the Tsx protein becomes dispensable, and the nucleosides diffuse across the outer membrane primarily through OmpF [10–12], a major outer membrane protein that forms general diffusion pores for the permeation of hydrophilic substances through the outer membrane [13–16]. Since *E. coli* can efficiently use exogenous nucleosides as precursors in nucleic acid synthesis and as carbon and nitrogen sources [17, 18], the presence of the Tsx protein seems to be particularly important for efficient scavenging of substrate when the nutrient supply is low.

The finding that *tsx* mutants show reduced uptake of nucleosides has led to the hypothesis that the Tsx protein forms a specific pore for nucleosides [5, 7–9]. In support of the proposed *in vivo* function of Tsx we have recently reported that this protein can form small ion-permeable channels when highly purified Tsx is reconstituted into black lipid membranes [19]. Furthermore, we have shown that the ion permeation through the Tsx pores can be blocked by increasing concentrations of adenosine and to a much smaller extent by the addition of cytidine. We have concluded that the Tsx pore contains a binding site for nucleosides and have proposed that this binding site accounts for the nucleoside specificity of Tsx observed *in vivo* [5, 7–9]. Tsx, therefore, appears to be functionally comparable to the *E. coli* outer membrane protein LamB [20], which is involved in the passage of maltose and maltodextrins across the outer membrane. LamB has been intensively studied by both *in vivo* and *in vitro* approaches and has been characterized as a specific and saturable channel-forming protein [21–29]. Its substrate specificity has been attributed to the presence of a binding site inside the LamB channel. The properties of the substrate-specific channels formed by LamB and Tsx are thus fundamentally different from those formed by the OmpC and OmpF pores, which do not contain such substrate-binding sites and hence function as non-saturable general diffusion porins for hydrophilic substances [13–16].

Investigation of the Tsx-mediated translocation of nucleosides across the outer membrane has revealed a number of remarkable properties of Tsx. Not only does Tsx-dependent uptake differ for various nucleosides, but the Tsx channel apparently discriminates between the closely structurally related pyrimidine nucleosides, cytidine and thymidine. Uptake of thymidine is strongly reduced in *tsx* mutants, while cytidine uptake is not affected [5, 7–9]. Furthermore, the uptake of deoxynucleosides is more strongly dependent on Tsx than that

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of the corresponding nucleosides [5], while Tsx plays no role in the uptake of the free bases or in the permeation of nucleoside monophosphates *in vivo* [5, 10–12]. Like the LamB channel [30], the Tsx pore also allows the diffusion of small molecules unrelated to its specific substrates. This non-specific element of the Tsx channel can be detected in *ompB* mutants that are severely deficient in the synthesis of the major general porins OmpC and OmpF [31]. We report here a detailed *in vitro* characterization of the nucleoside-specific Tsx channel and show that several different nucleosides interact with the binding site inside the channel. Control experiments revealed that the apparent stability constants as calculated from the titration experiments of the Tsx-mediated membrane conductance are the absolute stability constants because ions and nucleosides did not compete for the binding site.

MATERIALS AND METHODS

Purification of Tsx

The *E. coli* strain CH8 was used for the purification of the Tsx protein. This strain is a derivative of the Tsx-overproducing strain P400 [3, 32] and lacks, or is severely deficient in, the major outer-membrane proteins OmpA, OmpC, OmpF, and LamB [19]. Tsx protein was isolated from the outer-membrane fraction of strain CH8 as described by Maier et al. [19]. The total yield was approximately 1 mg Tsx protein from 4 l cell culture in the mid-log phase [33]. Tsx purity was assessed by SDS gel electrophoresis [34]. The Tsx preparation appeared to be homogeneous and free from other contaminating polypeptides as judged from SDS/polyacrylamide gels stained with Coomassie brilliant blue [35] and silver nitrate stain [36].

Lipid bilayer experiments

Black lipid bilayer membranes were formed as described previously [38] by painting onto circular holes (surface area 0.1–1 mm^2) a 1% solution of diphytanoylglycerophosphocholine (Avanti Biochemicals, Birmingham, AL, USA) in *n*-decane. The aqueous salt solutions (Merck, Darmstadt, FRG) were used unbuffered and had a pH of approximately 6. Free bases, nucleosides, and deoxynucleosides were purchased from Sigma (St Louis, MO, USA). (Note that 5′-deoxythymidine is a dideoxynucleoside since thymidine already lacks the hydroxyl group at the 2′ position.) The Tsx protein was added from the concentrated stock solution either to the aqueous phase bathing a membrane in the black state or immediately prior to membrane formation. The temperature was maintained at 25 °C throughout.

The membrane current was measured with a pair of calomel electrodes switched in series with a voltage source and an electrometer (Keithley 602). In the case of the single-channel recordings, the electrometer was replaced by a current amplifier. The amplified signal was monitored with a storage oscilloscope and recorded with a tape or a strip chart recorder. Zero-current membrane potential measurements were performed by establishing a salt gradient across membranes containing 100–1000 Tsx channels as described earlier [39].

RESULTS

Single-channel analysis

When Tsx was added in small quantities (10–100 ng/ml) to the aqueous solution bathing a lipid bilayer membrane, the specific membrane conductance increased by several orders of magnitude. The time course of the increase was very similar to that described earlier for other bacterial and mitochondrial porins [38, 40, 41]. After an initial rapid increase for 15–20 min, the membrane conductance increased at only a very slow rate. This conductance increase is caused by the formation of ion-permeable channels. Fig. 1 shows a single-channel recording observed with a diphytanoyl glycerophosphocholine/*n*-decane membrane in the presence of 10 ng/ml Tsx protein. The average single-channel conductance of Tsx in 1 M KCl was about 10 pS (see Fig. 2), which is much smaller than that of the general diffusion porins OmpC and OmpF (1900 pS) and of the sugar-specific LamB channel (160 pS) under the same experimental conditions [29, 42, 43].

The transport of ions through Tsx could be blocked in part or completely by the addition of adenosine [19]; an apparent binding constant could be estimated from these experiments. To test whether ions bind to Tsx and whether ions and nucleosides compete for the binding site inside the channel, we measured the single-channel conductance as a function of the KCl concentration in the aqueous phase. Because of the extremely small conductance of the Tsx channel this could only be done in the small range of 0.1–3 M. The results are shown in Table 1. The single-channel conductance of the Tsx channel was a linear function of the KCl concentration in this range. We also studied the influence of adenosine on the single-channel conductance of Tsx in 1 M KCl. At a concentration of 20 mM adenosine no channels could be resolved within the limits of the conductance resolution of our single-channel instrumentation (about 1 pS); however, at a concentration of 0.5 mM adenosine (corresponding to the half-saturation constant [19]), the single-channel conductance was about half of that seen in the absence of adenosine. This result suggests that nucleosides do not compete with ions for the binding site inside the Tsx channel. This is consistent with the assumption that ions and adenosine do not compete for the binding site and that the apparent stability constant derived from the titration experiments [19] is the real stability constant.

Binding of nucleosides to Tsx

The single-channel experiments described above showed that the nucleoside binding could be studied in single-channel recordings. The use of this method, however, was limited by the small single-channel conductance of the Tsx channel. Therefore, the stability constant for the binding of nucleosides was derived from multichannel experiments performed as follows. Tsx was added to black lipid bilayer membranes in a concentration of 50–100 ng/ml; 30 min later, the rate of conductance increase had slowed considerably. At this time, small amounts of concentrated nucleoside solutions were added to the aqueous phase to both sides of the membrane, with stirring to allow equilibration. Table 2 shows an experiment of this type in which increasing concentrations of deoxyadenosine were added to a Tsx-containing membrane. The membrane conductance decreased as a function of the deoxyadenosine concentration. The data of Table 2 and of similar experiments with other free bases, nucleosides and deoxynucleosides were analysed using the following theoretical considerations.

For the movement of the nucleosides through the Tsx channel, it is assumed that the transport can be explained by a simple two-barrier one-site model used to explain the transport of sugars and ions through specific porins [29, 37].

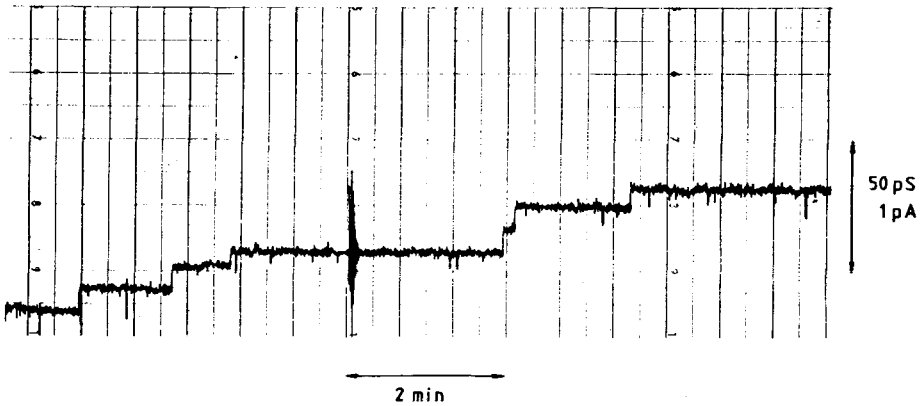

Fig. 1. *Single-channel recording of a diphytanoylglycerophosphocholine membrane in the presence of 10 ng/ml Tsx protein.* The aqueous phase contained 1 M KCl. The applied voltage was 20 mV; the temperature was 25 °C

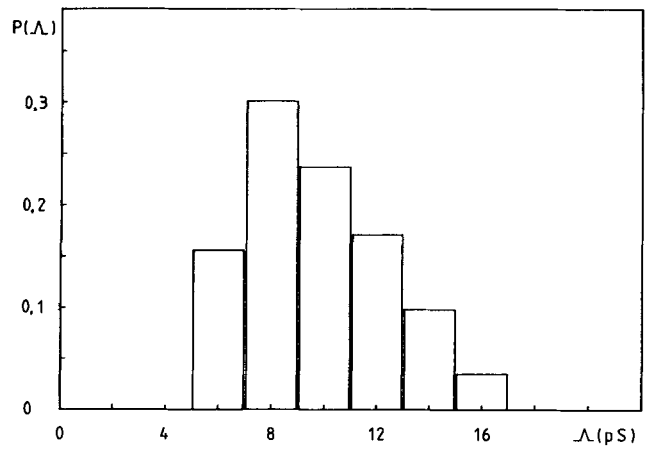

Fig. 2. *Histogram of the conductance steps observed with diphytanoylglycerophosphocholine membranes in the presence of Tsx protein.* The average single-channel conductance was about 10 pS for 122 steps. The aqueous phase contained 1 M KCl, the temperature was 25 °C (the applied voltage was 20 mV)

Table 1. *Single-channel conductance, Λ, of Tsx in different salt solutions*
The membranes were formed from diphytanoylglycerophosphocholine/*n*-decane and the single-channel conductance was calculated from recordings similar to that given in Fig. 1 and by averaging at least 100 single events. The applied membrane potential was 20 mV; the temperature was 25 °C

Aqueous salt solution	Λ
	pS
0.1 M KCl	1.5
0.3 M KCl	3.4
1 M KCl	10
1 M KCl, 0.5 mM adenosine	5.6
3 M KCl	28

Table 2. *Inhibition of Tsx-mediated membrane conductance with increasing concentrations of deoxyadenosine*
The membrane was formed from diphytanoylglycerophosphocholine/*n*-decane. The aqueous phase contained 1 M KCl and 100 ng/ml Tsx protein. The addition of deoxyadenosine started 30 min after membrane formation when the membrane conductance became stationary. The applied voltage was 50 mV; temperature = 25 °C. For the calculation of K it was assumed that the specific membrane conductance at 10 mM deoxyadenosine corresponded to the conductance of the bare membrane (in the absence of Tsx)

c	λ	Decrease	
		absolute	relative
mM	$\mu S\ cm^{-2}$		%
0	21.00	0	0
0.05	15.50	5.50	26
0.10	11.60	9.40	45
0.20	8.30	12.70	61
0.50	3.50	17.50	84
0.89	3.10	17.90	86
1.30	1.95	19.05	92
3.20	1.10	19.90	96
5.00	0.96	20.04	96
10.00	0.21	20.79	100

This model assumes a binding site in the center of the channel. The rate constant k_1 describes the jump of the solutes from the aqueous phase (concentration c) across the barrier to the central binding site, while the inverse movement is described by the rate constant k_2. We found no indication for an asymmetry of the Tsx channel; therefore, symmetry of the channel with respect to the binding site is assumed. The stability constant of the binding between a nucleoside and the binding site is given by the ratio $K = k_1/k_2$ (the half-saturation constant is $K_s = 1/K$). Furthermore, it is assumed that the transport of solutes through Tsx is a single-file transport [29]. This means that a nucleoside or an ion can enter the channel only when the binding site is free. The probability, P, that the binding site is occupied by a nucleoside (identical concentrations c on both sides) and does not conduct ions is given by:

$$P = Kc/(1 + Kc)\,, \tag{1}$$

while the probability that it is free and the channel conducts ions is given by:

$$1 - P = 1/(1 + Kc)\,. \tag{2}$$

The conductance, $\lambda(c)$, of a Tsx-containing membrane in the presence of a nucleoside (concentration c on both sides of the

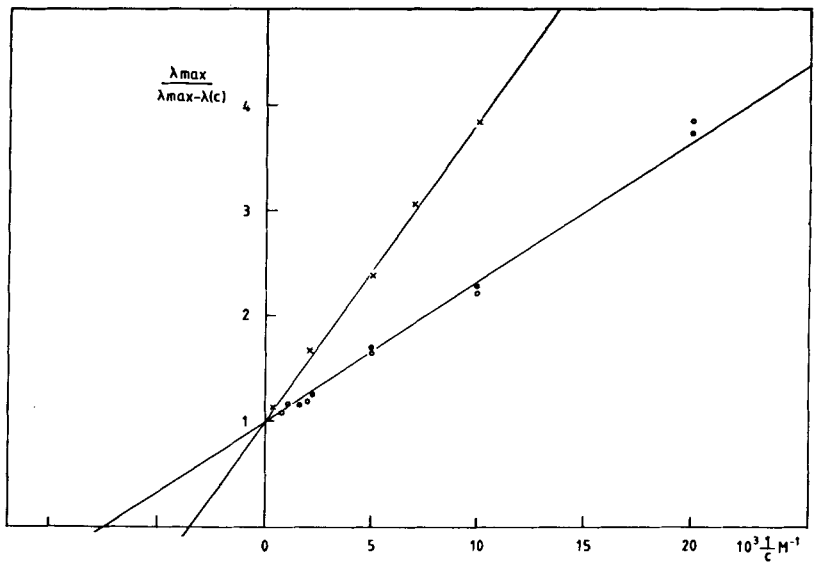

Fig. 3. *Lineweaver-Burk plot of the data given in Table 2 (●) and similar experiments with 0.1 M KCl (○) or in which deoxyadenosine was only added to only one side of the membrane (×, 1 M KCl).* The half-saturation constant for the binding of deoxyadenosine to the binding site inside the Tsx channel was calculated using the assumption that the channel does not conduct ions when it is occupied by a deoxynucleoside

membrane) with the stability constant, K, is given by the probability that the binding site is free:

$$\lambda(c) = \lambda_{max}/(1 + Kc)\,, \qquad (3)$$

where λ_{max} is the membrane conductance before the start of the nucleoside addition. Eqn (3) may also be written as:

$$[\lambda_{max} - \lambda(c)]/\lambda_{max} = Kc/(1 + Kc)\,, \qquad (4)$$

which means that the titration curves can be analysed using a Lineweaver-Burk plot as shown in Fig. 3 for the data of Table 2. The straight line in Fig. 3 corresponds to a stability constant, K, of 7500 M^{-1} (half-saturation constant K_s = 0.13 mM). In another set of experimental conditions, the experiment was repeated with 0.1 M KCl instead of 1 M KCl (open circles in Fig. 3). The results showed satisfactory agreement with those obtained in 1 M KCl. Fig. 3 also shows the deduced data of an experiment in which deoxyadenosine was added to only one side of the Tsx-containing membrane. Using Eqn (4) the stability constant, K, of the binding was in this case about 3600 M^{-1}, which is about half of the value obtained for the addition of the deoxynucleoside to both sides of the membrane. This is easy to understand on the basis of the theory given above. When nucleosides are added to only one side of the membrane, Eqn (1) has the form (concentration c at one side and 0 at the other side):

$$P = Kc/(2 + Kc)\,, \qquad (5)$$

subsequently Eqn (4) reads:

$$[\lambda_{max} - \lambda(c)]/\lambda_{max} = Kc/(2 + Kc)\,. \qquad (6)$$

The use of Eqn (6) to fit the data given in Fig. 3 for the addition to only one side of the membrane yielded a value of 7200 M^{-1} for K. This was in close agreement to the data obtained for the addition to both sides which means that the data can appropriately by explained by the one-site two-barrier model.

The stability constant for the binding of the five different nucleosides and their derivatives to the binding site inside the Tsx channel was derived from measurements similar to those described above. The results are summarized in Table 3. The binding constant was largest for 5′-deoxythymidine and deoxyuracil and was lowest for deoxycytidine. Deoxynucleosides generally had much larger binding constants than the corresponding nucleosides, which, in turn, had larger binding constants than the corresponding free bases. The binding of cytosine and guanine could not be measured in our experimental approach partially because of solubility problems. On the other hand, we did not detect any conductance decrease in the presence of these compounds in concentrations up to 20 mM, which means that their binding constants are below

Table 3. *Stability constants, K, for the binding of free bases, nucleosides, and deoxynucleosides to the Tsx channel*
The half-saturation constant $K_s = 1/K$. K was calculated from titration experiments similar to that given in Table 2 and Fig. 3. n.d. means not detectable (see text)

Compound	K	K_s
	M^{-1}	mM
Purines		
Adenine	500	2.0
Adenosine	2000	0.50
Deoxyadenosine	7100	0.14
Guanine	n.d.	n.d.
Guanosine	1000	1.0
Deoxyguanosine	3100	0.32
Pyrimidines		
Cytosine	n.d.	n.d.
Cytidine	46	22
Deoxycytidine	100	10
Thymine	170	5.8
Thymidine	5000	0.20
5′-Deoxythymidine	20000	0.050
Uracil	50	20
Uridine	1900	0.54
Deoxyuridine	19000	0.053

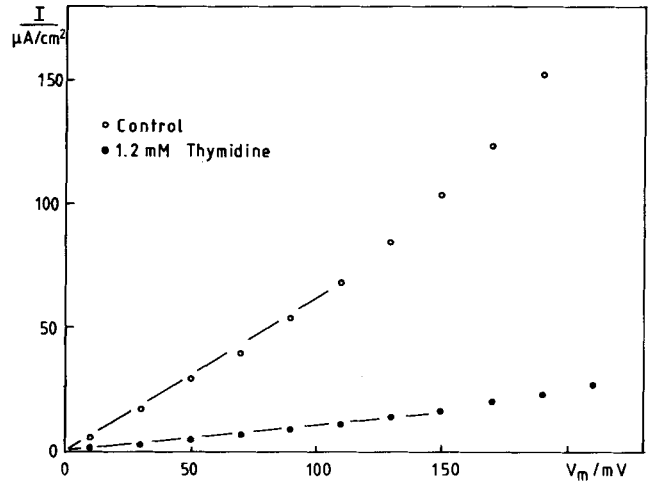

Fig. 4. *Current-voltage curves of a diphytanoylglycerophosphocholine/n-decane membrane in the presence of Tsx before (○) and after (●) the addition of 1.2 mM thymidine to the aqueous phase on both sides of the membrane.* The aqueous phase contained 1 M KCl and 100 ng/ml Tsx. Temperature = 25°C

$10\ M^{-1}$. It has been shown that Tsx could act *in vivo* as a channel for amino acids [31]. However, in titration experiments similar to those described above for nucleosides, amino acids such as glycine, alanine, and serine in concentrations up to 0.1 M were not able to influence ion movement through the Tsx channel. This result is consistent with the assumption that amino acids do not bind to the binding site or that the stability constant for the binding is below $1\ M^{-1}$. Similar results were found in titration experiments with different sugars, because arabinose, fructose, glucose and maltose did not influence the Tsx-mediated membrane conductance in concentrations up to 50 mM.

Influence of membrane potential on Tsx

Current voltage relationships of membranes containing Tsx were measured in the absence and in the presence of nucleobases, nucleosides and deoxynucleosides to determine if the membrane potential had any influence on the lifetime of the Tsx channels or on the binding of the purines and the pyrimidines to the binding site. Fig. 4 shows two experiments of this type. The first experiment was performed with a diphytanoylglycerophosphocholine membrane in which Tsx was reconstituted. The aqueous phase contained only 1 M KCl (control). The current/voltage curve was linear up to 120 mV and supralinear above this voltage. Then 1.2 mM thymidine was added to the aqueous phase on both sides of the same membrane and the experiment was repeated. The current was approximately 15% of the current in the absence of the pyrimidine at all membrane potentials up to 190 mV which was in good agreement with the conductance decrease expected from the titration experiments (see above). This result indicated that the Tsx channel is not voltage-gated and that the binding of the pyrimidine deoxynucleoside thymidine to the binding site was not influenced by an external electrical field. Similar results were also obtained for other purine and pyrimidine nucleosides.

Zero-current membrane potentials

The ion selectivity of the Tsx channel was investigated by measuring the membrane potential under zero-current conditions. After the incorporation of about 100 – 1000 Tsx channels into the membranes, the salt concentration on one side of the membrane was raised tenfold to 100 mM and the zero-current potential was measured 5 min after the gradient was established. The results are summarized in Table 4. For all three salts used in these experiments, the more dilute side (10 mM) was always positive which indicated preferential movement of the cations through the Tsx channel (i.e. the channel is cation-selective). The nucleoside adenosine at a concentration of 0.5 mM had no detectable influence on the ion selectivity. This result indicated again that the properties of the Tsx channel are not influenced by the nucleoside if no nucleoside is bound to the binding site and the ions can permeate freely. The zero-current membrane potentials ranged over 11 – 38 mV. Analysis of the data of Table 4 using the Goldman-Hodgkin-Katz equation [39] suggested that anions also have a certain permeability through the channel because the ratios of the permeabilities P_c for cations to P_a for anions ranged over 1.7 – 7.8. It was smaller for LiCl than for potassium acetate. This result indicated that the ions move through the channel according to their mobility sequence in the aqueous phase. On the other hand, it is obvious from the data presented here that Tsx does not form a wide aqueous channel as do the general diffusion pores OmpC and OmpF.

Table 4. *Zero-current membrane potentials, V_m, of membranes from diphytanoylglycerophosphocholine/n-decane in the presence of Tsx measured for a 10-fold gradient of different salts*

V_m is defined as the difference between the potential on the dilute side (10 mM) and the potential at the concentrated side (100 mM). The pH of the aqueous salt solutions was approximately 6 unless otherwise indicated; the temperature was 25°C. P_c/P_a was calculated from the Goldman-Hodgkin-Katz equation from at least four individual experiments [39]

Salt	V_m	P_c/P_a
	mV	
KCl	28	4.2
LiCl	11	1.7
Potassium acetate (pH 7)	38	7.8

DISCUSSION

We have recently shown that the Tsx protein of *E. coli* forms a small ion-permeable channel that contains a binding site for nucleosides [19]. Because this binding site apparently accounts for the substrate specificity of Tsx observed *in vivo* [5, 7 – 9], we have characterized it in detail by *in vitro* reconstitution of Tsx into lipid bilayer membranes. Analysis of the binding site was carried out by titrating the ion flux through the Tsx channel with increasing concentrations of nucleosides. The titration experiments were fitted using a two-barrier, one-site model for the movement of nucleosides through Tsx. It has to be noted that more complicated models such as a three-barrier, two-site model could also explain the permeation of nucleosides through the Tsx channel. However, we did not observe any indication for a binding of more than one molecule at the same time to the channel because of the straight line in the Lineweaver-Burk plot. Furthermore, the addition of deoxyadenosine (as well as other nucleosides; unpublished data) to only one side of the membrane resulted in a stability constant by using Eqn (4) that was half of that seen when the

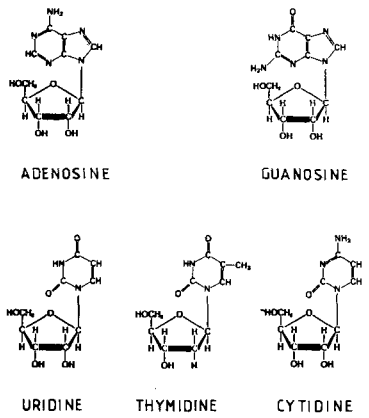

Fig. 5. *Structure of the nucleosides used in this study*

compounds were added to both sides of the membrane. Using Eqn (6) both stability constants were found to be identical.

The results of our *in vitro* determinations for nucleoside binding to a site within the Tsx channel are in close agreement with *in vivo* measurements of nucleoside permeation through Tsx [5, 7–12]. Both types of data demonstrate that the specificity of Tsx decreases in the order deoxynucleosides > nucleosides > free bases. Only cytidine and deoxycytidine deviate from this pattern: both exhibited weak binding in the *in vitro* assay and both permeate the *E. coli* outer membrane independently of Tsx [5, 9]. Among the deoxynucleosides we observed stronger binding of the pyrimidines (5′-deoxythymidine and deoxyuridine) than of the purines (deoxyadenosine and deoxyguanosine). However, among the nucleosides the difference between the purines and pyrimidines is less obvious (Table 3). With respect to the free bases, some binding was detected *in vitro*, but apparently this is not of great physiological importance since their permeation across the outer membrane at low substrate concentration was not decreased in strains lacking Tsx [5]. It has been previously reported that certain amino acids can diffuse through Tsx *in vivo* [31]. Our results with the *in vitro* system show that such permeation is non-specific because the amino acids and sugars tested (unlike the nucleosides) did not compete with the ion flow through the Tsx channel, i.e. there is no binding site for them inside the channel.

In the case of LamB, the other substrate-specific outer-membrane channel of *E. coli*, there is an apparent relationship between binding affinity and the structure of the substrate, i.e. the binding affinity increases with increasing chain length of the malto-oligosaccharide [24, 26, 27, 29]. We looked for a similar relationship among the Tsx substrates. The addition of a ribofuranose to the free bases (see Fig. 5) increased the affinity of the binding site. This increase could in principle be caused by the formation of hydrogen bonds. However, the removal of the hydroxyl group from the 2′ position of the sugar (i.e. the deoxynucleosides) resulted in an even larger stability constant, making it rather unlikely that the formation of hydrogen bonds between the substrate and the binding site is the main reason for the increase in the stability constants. Furthermore, we found no obvious structural differences that could account for the severely decreased affinity of Tsx for cytidine and deoxycytidine compared to the other pyrimidines tested in the *in vitro* system.

In 1 M KCl, the single-channel conductance of Tsx (10 pS) is considerably smaller than that of the sugar-specific LamB channel (160 pS [27, 43]) and much smaller than that of the general diffusion pore OmpC (1900 pS [42]), although the molecular mass of the molecules that can penetrate the different channels is of the same order of magnitude. This might indicate that the interior of the Tsx channel is much more restrictive than that of the OmpF pore and that it undergoes conformational changes when a substrate molecule is bound.

Although the binding site within the Tsx channel exhibits different affinities for the various nucleosides, it must be noted that the affinity of the binding is a prerequisite but it is not sufficient for the rapid permeation of molecules through a specific channel. This is easy to understand on the basis of our model for the Tsx channel. The stability constant, K, for the binding is given as the ratio of the on (k_1) and the off (k_2) rate constants. Consequently, high on and off rate constants result in the same stability for binding as do small rate constants if they have the same ratio. Only the first case leads to a rapid movement of the molecules through the channel. This situation has been demonstrated for the sugar-specific LamB channel, which exhibits approximately the same affinity for several disaccharides but allows permeation of maltose at a considerably higher speed than the others [23, 29].

The reconstitution experiments reported here provide strong evidence that Tsx forms a specific channel for nucleosides. So far, besides Tsx, only two other substrate-specific outer membrane channels (porine) have been characterized in some detail [23, 24, 27, 29, 37, 43, 44]. Protein P of the *Pseudomonas aeruginosa* outer membrane forms anion-selective channels with a small selectivity filter, whereas LamB of *E. coli* is sugar-specific and can be blocked for the permeation of ions and glucose by larger oligosaccharides of the maltose series. The Tsx channel has similar properties. The permeation of ions through the channel could be blocked by the addition of the nucleosides. Protein P of *P. aeruginosa* showed a concentration-dependent saturation of the single-channel conductance [37, 44]. A similar saturation of the flux of nucleosides through the Tsx channel may also be expected for increasing concentrations of nucleosides. This can be shown by the following theoretical considerations. The net flux of nucleoside molecules, ϕ, through the channel under stationary conditions as the result of a concentration gradient $c'' - c'$ across the membrane is given by the net movement of molecules across one barrier of the two-site one-barrier channel:

$$\phi = k_1 c''/(1 + K') - k_2 K'/(1 + K'), \qquad (7)$$

where K' is given by:

$$K' = K(c' + c'')/2. \qquad (8)$$

In Eqn (7) the rate constants k_1 and k_2 are multiplied by the probabilities that the binding site is free or occupied, respectively (see Eqns 1 and 2). Eqn (7) has in the case $c'' = c$, $c' = 0$, the following form:

$$\phi = \phi_{max} Kc/(2 + Kc), \qquad (9)$$

where $\phi_{max} = k_2$ is the maximum flux at very high substrate concentrations and $K = 1/K_m$ is the stability constant for the binding of adenosine to the binding site. The flux saturates at high substrate concentrations as shown in Fig. 6 for adenosine. In contrast, the flux of adenosine through a general diffusion pore is linearly dependent on the concentration (Fig. 6). This indicates that the relative permeation of substrate through both types of pores is concentration-dependent. Fig. 6 clearly shows that the flux of adenosine through a general diffusion pore could exceed that through the specific Tsx channel even if at a substrate concentration of 0.1 mM

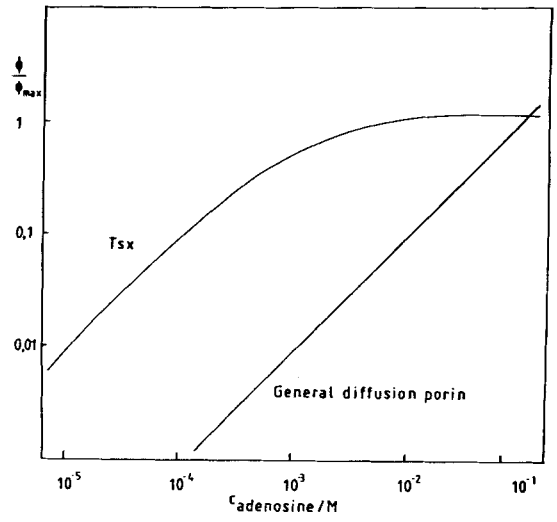

Fig. 6. *Flow of adenosine through Tsx (calculated according to Eqn 9) and through a general diffusion pore as a function of the nucleoside concentration on one side of the membrane.* (The concentration on the other side is set to zero.) The flow through Tsx is given relative to the maximum flux ϕ_{max}. For the flux of adenosine through the general diffusion pore, it was assumed that at 0.1 mM it was only 1% of the flux through Tsx

was only 1% of the flux through Tsx. These theoretical considerations are consistent with the *in vivo* situation, where the general diffusion pores become rate-limiting at small adenosine concentration [5].

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