J. Membrane Biol. 164, 263–274 (1998)

The Journal of
Membrane
Biology

Study of Sugar Binding to the Sucrose-specific ScrY Channel of Enteric Bacteria Using Current Noise Analysis

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Received: 3 December 1997/Revised: 28 April 1998

Abstract. ScrY, an outer membrane channel of enteric Gram-negative bacteria, which confers to the bacteria the rapid uptake of sucrose through the outer membrane was reconstituted into lipid bilayer membranes and the current noise was investigated in the open and in the carbohydrate-induced closed state of the channel. The open state of the channel exhibited up to about 200 Hz 1/*f*-noise with a rather small spectral density. Upon addition of carbohydrates to the aqueous phase the current through the ScrY channels decreased in a dose-dependent manner. Simultaneously, the spectral density of the current noise increased drastically, which indicated interaction of the carbohydrates with the binding site inside the channel and its reversible block. The frequency dependence of the spectral density was of the Lorentzian type but very often two Lorentzians were observed, from which the slow one may not be related to carbohydrate binding. Analysis of the power density spectra of the second Lorentzian using a previously proposed simple model of carbohydrate binding allowed the evaluation of the on- and the off-rate constants for the carbohydrate association with the binding site inside the ScrY channel and of a mutant (ScrYΔ3-72), in which 70 amino acids at the N-terminus are deleted. The binding of carbohydrates to ScrY was compared to those of the closely related maltoporin channels of *Escherichia coli* and *Salmonella typhimurium* by assuming that only the time constant and spectral density of the high frequency Lorentzian is related to carbohydrate transport.

Key words: Noise analysis — ScrY — LamB — Carbohydrate-binding — Sucrose transport — Lipid bilayer membrane — Outer membrane — Gram-negative bacteria

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Introduction

The cell envelope of Gram-negative bacteria consists of different layers. The inner cytoplasmic membrane contains the respiration chain, proteins for the transport of nutrients and proteins involved in the synthesis of phospholipids, peptidoglycan, and the lipopolysaccharides (Beveridge, 1981; Nikaido & Vaara, 1985). The periplasmic space between the inner and outer membrane is an aqueous compartment iso-osmolar to cytoplasm. It contains the peptidoglycan and a large number of different proteins. The outer membrane is composed of protein, lipid and polysaccharide (Benz, 1988; Nikaido & Vaara, 1985). It represents a strong permeability barrier for hydrophobic compounds (Benz & Bauer, 1988). Translocation of hydrophilic solutes through the outer membrane is catalyzed by a major class of bacterial proteins, called porins, which form water-filled channels. They can be divided into two classes: the general diffusion porins form large channels that allow the nonspecific diffusion of hydrophilic compounds, which means that they sort mainly according to the molecular mass of the solutes (Nikaido & Vaara, 1985; Hancock, 1987; Benz & Bauer, 1988). Besides the constitutively expressed general diffusion porins the outer membrane may contain porins, which are induced under special growth conditions (Szmelcman et al., 1976; Tommassen & Lugtenberg, 1980; Hancock, Poole & Benz, 1982; Bauer et al., 1985). They often form solute-specific pores and contain binding sites for neutral substrates such as carbohydrates (Ferenci et al., 1980; Benz et al., 1986), nucleosides (Maier et al., 1988) and charged solutes such as phosphate (Hancock et al., 1982; Benz &

Hancock, 1982). Many of these specific porins are part of systems, which are composed of different proteins involved in uptake and fermentation of carbohydrates. On example for such a system is the mal-system (Schwartz, 1987), which contains also an outer membrane channel, LamB.

Another one of these specific porins is the sugar-binding ScrY. It has been found during the investigation of the plasmid-encoded metabolic pathway of sucrose in *Salmonella typhimurium.* Sucrose has, without the plasmid pUR400, only a small permeability through the outer membrane (Schmid et al., 1988). Cells containing pUR400 are able to use sucrose as a sole carbon source (Wohlhieter et al., 1975; Schmid, Schupfner & Schmitt, 1982; Schmid et al., 1988, 1991; Hardesty et al., 1987; Hardesty, Ferran & DiRienzo, 1991). ScrY has been cloned and sequenced. Sequence similarities to LamB of *Escherichia coli* and the growth of ScrY containing strains on maltotetraose suggest that it is a porin (Schmid et al., 1988, 1991; Hardesty et al., 1991). The porin function has been shown in electrophysiological measurements with ScrY reconstituted into planar lipid membranes. The single-channel conductance of ScrY is in the range of that observed for general diffusion porins. The ionic current through the channel could be blocked by addition of maltooligosaccharides. This occurs in a dose-dependent manner, which demonstrate that ScrY contains a binding site for sugars (Schülein, Schmid & Benz, 1991). Liposome swelling experiments demonstrate that transport of sucrose through ScrY (Hardesty et al., 1991) is more effective than through LamB, which is a better channel for uptake of maltose (Luckey & Nikaido, 1980).

The crystal structure of LamB has been solved at 3.1 Å resolution (Schirmer et al., 1995) and the residues that are involved in sugar transport have been determined (Dutzler et al., 1996). Comparison of the sequences of LamB and ScrY shows besides large regions of similarity that residues involved in sugar transport are well conserved. Six aromatic residues that line the channel and form a path, which extends from the floor of the vestibule through the constriction to the periplasmic space are nearly conserved in ScrY (Schülein, Andersen & Benz, 1995). The mechanism of sugar-transport through the ScrY-pore seems to be similar to LamB. An obvious difference in the sequence of the two porins is an N-terminal extension of 70 amino acids in the mature form of ScrY that cannot be found in LamB. The influence of this region of the sugar binding is investigated with the deletion mutant of ScrYΔ3-72, which lacks amino acids 3 to 72 from the N-terminal end (Schülein et al., 1995). Using the analysis of the current noise it is possible to evaluate the kinetics of carbohydrate binding to the binding site in LamB (Nekolla, Anderson & Benz, 1994). In recent studies we have investigated the carbohydrate-binding to LamB (Andersen, Jordy & Benz, 1995) and LamB mutants (Jordy, Andersen & Benz, 1996). In this study we investigated the binding kinetics of carbohydrates to ScrY and ScrYΔ3-72. The results are discussed with respect to carbohydrate-binding to LamB and to the 3D-structure of ScrY, which has recently been resolved from X-ray crystallography (Forst et al., 1997).

Materials and Methods

Purification of ScrY, the Deletion Mutant ScrYΔ3-72 and of LamB of *E. coli*

ScrY and ScrYΔ3-72 were isolated from envelopes of cells of *E. coli* KS26, which lacks OmpC, OmpF and LamB. Details of isolation have been described elsewhere (Schülein et al., 1991, 1995). LamB of *Escherichia coli* was purified essentially as has been published previously (Benz, Schmid & Vos-Scheperkeuter, 1987).

Lipid Bilayer Experiments and Noise Analysis

The porins were reconstituted in artificial membranes. The method has been described in detail previously (Benz et al., 1978). The instrumentation consists of a Teflon chamber divided in two compartments. The electrolyte-filled compartments are connected by a small circular hole (diameter 0.5 mm). The membranes were formed by painting a solution of 1% (weight/volume) diphytanoyl phosphatidylcholine in n-decane (Avanti Polar Lipids, Alabaster, AL) onto the hole. After the membrane turned to black a small aliquot of a stock solution of ScrY or ScrYΔ3-72 was added to the aqueous salt solution in the compartments. The membrane current was measured by a pair of calomel electrodes switched in series with a battery-operated voltage source and a current amplifier (Keithley 427 with a four-pole filter). Feedback resistors between 0.01 and 10 GΩ were used in the experiments. The reconstitution of ScrY and ScrYΔ3-72 in the membranes resulted in an increase of the membrane current. The amplified signal was monitored by a strip chart recorder and simultaneously analyzed with a digital signal analyzer (Ono Sokki CF 210), which performed fast Fourier transformation of the current noise. The spectra were composed of 400 points and they were averaged either 128 or 256 times. They were transferred to an IBM-compatible personal computer (PC) for further analyses. Alternatively the output of the current amplifier was connected to an AD-converting card of the PC. The digitized data were analyzed with a home-made fast Fourier transformation program, which yielded identical results as compared to the commercial digital signal analyzer.

Derivation of the Rate Constants from the Frequency-Dependence of the Spectral Density

For the analysis of the current noise we used a simple one-site, two-barrier model (Läuger, 1973; Benz et al., 1987; Benz & Hancock, 1987) with a central binding site inside the channel. The binding of carbohydrates (aqueous concentration c) to the central binding site inside the channel is described by a first order chemical reaction (on-rate constant k_l and off-rate constant k_{-l}. The stability constant of the binding of a carbohydrate to the channel is $K = k_l/k_{-l}$. Furthermore, it is assumed that the ScrY-channel is a single file channel (Benz et al., 1986). This means that ScrY is open when no carbohydrate is bound,

and closed when it is occupied. The conductance, $G(c)$ ($= I(c)/V_m$ with V_m membrane voltage), of a ScrY-containing membrane in the presence of carbohydrate (concentration c), which binds with the stability constant, K, is given by the following equation (Benz et al., 1987):

$$\frac{G_{max} - G(c)}{G_{max}} = \frac{I_0 - I(c)}{I_0} = \frac{K \cdot c}{l + K \cdot c} \tag{1}$$

G_{max} is the membrane conductance before the start of the carbohydrate addition to the aqueous phase (I_0 is the initial current and $I(c)$ is the current at the concentration c). This means that the titration curves can be analyzed using Lineweaver-Burke plots as has been shown in previous publications (Benz et al., 1986, 1987). The half saturation constant, K_S is given by the inverse stability constant l/K.

The measurements of current noise presented here are based on small perturbations of the number of closed channels due to microscopic variations involved in the chemical reaction between carbohydrate and binding site, which can be monitored by current fluctuations. Its reaction rate l/τ is given by (Verveen & DeFelice, 1974; DeFelice, 1981):

$$\frac{l}{\tau} = 2\pi \cdot f_c \cdot k_l \times c + k_{-l} \tag{2}$$

f_c is the corner frequency of the power density spectrum, $S(f)$, given by a ''Lorentzian'' function. Lorentzian spectra correspond to the noise expected for a random switch with different on and off probabilities, which are coupled by a chemical reaction (Verveen & DeFelice, 1974; Conti & Wanke, 1975; DeFelice, 1981):

$$S(f) = S_0/(l + (f/f_c)^2) \tag{3}$$

S_0 is the plateau value of the power density spectrum at small frequencies. It is given by (Verveen & DeFelice, 1974):

$$S_0 = 4 \cdot N \cdot i^2 \cdot p \cdot (l - p) \cdot \tau \tag{4}$$

N is the total number of channels (blocked and unblocked) within the membrane i is the current through one single open channel and p is the probability that the channel is occupied by a sugar (i.e., closed). The membrane current, $I(c)$, is given by the number of open channels multiplied with the current through one single-channel and the probability $l - p$ that the channel is open:

$$I(c) = i \cdot N \cdot (l - p) \tag{5}$$

Eqs. (4) and (5) can be used to calculate the single-channel conductance, g, of the channels from the applied membrane potential V_m and the current through one single channel:

$$g = \frac{i}{V_m} = \frac{S_0}{4 \cdot V_m \cdot I(c) \cdot p \cdot \tau} \tag{6}$$

This means that S_0 is a function of the concentration, c, of carbohydrates in the aqueous phase, as a combination of Eqs. (1), (2) and (6) shows:

$$S_0 = \frac{4 \cdot g \cdot V_m \cdot I_0 \cdot K \cdot c}{k_{-1}\,(l + K \cdot c)^3} \tag{7}$$

Eq. (7) allows the calculation of the single-channel conductance and the stability constant, K, when the rate constant for the off-reaction of the sugar binding is known together with the initial current, I_0.

The variance, var, of current noise may be calculated according to (Lindemann, 1980):

$$\text{var} = S_0/4\tau = S_0\, f_c \pi/2 \tag{8}$$

A combination of Eqs. (2), (7), and (8) yields:

$$\text{var} = \frac{g \cdot V_m \cdot I_0 \cdot c}{(1 + K \cdot c)^2}. \tag{9}$$

Results

Evaluation of the Stability Constants for Carbohydrate Binding with Wild-type ScrY

The stability constants for carbohydrate-binding to ScrY were calculated from titration experiments. An example for this type of measurement is given in Fig. 1. ScrY was added while stirring from a concentrated stock solution to the aqueous phase (concentration about 50 ng/ml) bathing a black lipid bilayer membrane. The corresponding current increase was monitored on a strip chart recorder. After about 30 min most of the conductance increase was over and the titration experiment could start. However, the titration experiments were always combined with noise measurements. Therefore we waited a much longer time until the current was virtually stationary (usually 2 hr; Nekolla et al., 1994) because of the further slow conductance increase, which interfered with the current noise measurements. Titration experiments were performed by the addition of carbohydrates to both sides of the membrane. This led to a decrease of membrane conductance in a dose-dependent manner as is shown in Fig. 1. Simultaneously the current noise increased (Schülein et al., 1991). At a high carbohydrate concentration the current through the membrane almost decreased to zero which means that the channels were totally blocked for ion movement because of sugar binding to the binding site. The stability constant K was evaluated using Lineweaver-Burke plots according to Eq. (1). The results are summarized in Table 2.

Measurement of Current Noise with Wild-type ScrY

Parallel to the titration measurements the current noise was measured and the frequency dependence of the spectral density spectra was obtained from fast Fourier transformation. Before addition of carbohydrates the reference spectrum was taken from the current noise of the open ScrY-channel (curve 1, Fig. 2*A*). At small frequencies up to 200 Hz the spectral density was dependent on l/f. This was typical for open bacterial porin channels as we have demonstrated in previous investigations (Nekolla et al., 1994; Jordy et al., 1996) and has been

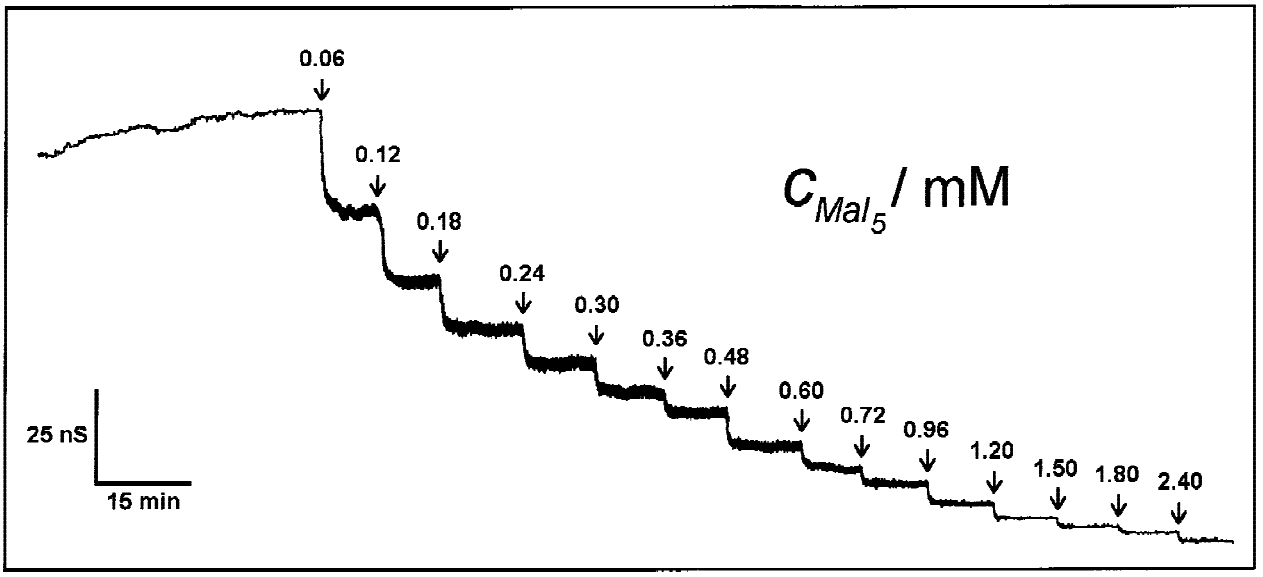

Fig. 1. Titration of membrane conductance induced by ScrY with maltopentaose. The membrane was formed from diphytanoyl phosphatidylcholine/n-decane. The aqueous phase contained 100-ng/ml protein, 1-M KCl, and maltopentaose at the concentrations shown at the top of the figure. The temperature was 25°C and the applied voltage was 20 mV.

discussed in detail recently (Wohnsland & Benz, 1997). The increase of the spectral density at frequencies above 500 Hz was caused by the intrinsic noise of the preamplifier that produces a frequency-dependent current noise through the membrane capacity C_m. It is observed also with membranes without reconstituted ScrY channels. The time resolution of the instrumentation was approximately 10 kHz, which was limited in the experiments of Fig. 2 and similar experiments by the bandwidth of the current amplifier (0.3 msec). The reference spectrum was subtracted from each spectrum taken after the successive addition of carbohydrates in increasing concentration.

Addition of carbohydrates led to a considerable increase of the spectral density of the current noise. Figure 2*A* curve 1 shows the spectral density of a ScrY-containing membrane before carbohydrate addition. Curve 2 shows a spectrum taken after addition maltotriose ($c = 700$ μM; the reference spectrum of curve 1 was subtracted). Surprisingly, the current noise spectrum of the ScrY channel after the addition of carbohydrates could not be fitted to a single Lorentzian function. This result is in contrast to earlier investigations with LamB (maltoporin) of *E. coli* (Nekolla et al., 1994; Anderson et al., 1995) and *S. typhimurium* (Jordy et al., 1996), in which it has been possible to fit the experimental data by one Lorentzian without any exception. This is demonstrated by the noise spectrum of Fig. 2*B*, which shows an experiment with LamB of *E. coli* for comparison

The alternative fit of the ScrY noise spectra with a sum of *1/f* curve and a Lorentzian curve did also not lead to satisfactory results. Therefore we fitted the spectrum of Fig. 2*A* with the sum of two Lorentzians. Curve 5 of Fig. 2*A* corresponds to the sum of the two single Lorentzian functions shown as curves 3 and 4. This curve provided an excellent fit of the experimental results. It yielded two corner frequencies f_c (7.2 and 550 Hz) and two plateau values of the power densities spectra at small frequencies, S_0 (107 and 11.5×10^{-24}A^{2}sec). In the following we distinguish in noise measurement with ScrY between a slow Lorentzian (corner frequencies smaller than 50 Hz) and a fast Lorentzian (corner frequencies above 200 Hz). It is noteworthy that also previously two Lorentzian noise spectra have been observed in the investigation of current noise in ionic channels from eukaryotic cells (Frehland, Hshiko & Malchup, 1983; Matthews, 1986; Attwell et al., 1987; Ho, Duszky & French, 1994; Fischer & Machen, 1994).

Calculation of the Rate Constants for Carbohydrate Transport

The corner frequencies and the initial values of the two Lorentzians were investigated for a given carbohydrate as a function of its concentration. We found that the parameters of the slow Lorentzian were more or less independent on the carbohydrate concentration. In some experiments it was rather difficult to separate the slow Lorentzian from the fast one because amplitudes and corner frequencies of both processes were too close to each other. Another problem was that the number of points in the power density spectra were smaller at low

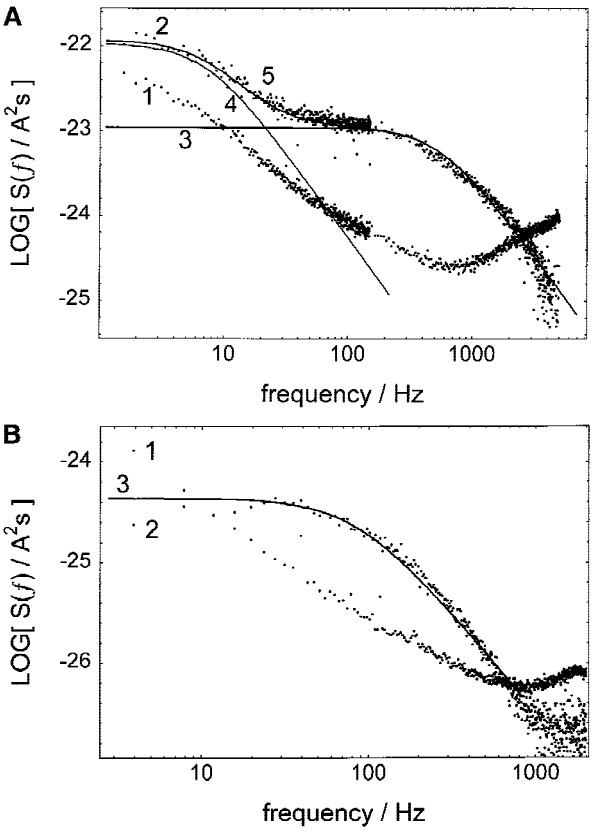

Fig. 2. (*A*) Power density spectra of maltotriose-induced current noise of 1911 ScrY channels. Trace 1 shows the control (1-M KCl). Trace 2: the aqueous phase contained 700-μM maltotriose and the power density spectrum of Trace 1 was subtracted. Trace 3 and 4: single Lorentzian function (f_c = 7.2 Hz, $S_0 = 107 \times 10^{-24}$ A^2*sec*; f_c = 550 Hz, $S_0 = 11.7 \times 10^{-24}$ A^2*sec*). Trace 5: sum of trace 3 and 4. (*B*) Power density spectra of maltoheptaose-induced current noise of 413 LamB-channels. Trace 1 shows the control (1-M KCl). Trace 2: the aqueous phase contained 33.9-μM maltoheptaose and the power density spectrum of Trace 1 was subtracted. Trace 3 shows a fit with a single Lorentzian function (f_c = 86.2 Hz, $S_0 = 0.44 \times 10^{-24}$ A^2*sec*) T = 25°C; V_m = 20 mV.

frequencies, which made it more difficult to fit the curves in this region. Table 1 shows the parameters of an experiment with maltotriose. The corner frequency of the slow Lorentzian seems to be independent of sugar concentration. On the other hand, the corner frequency of the fast Lorentzian showed a linear increase for increasing concentration of maltotriose as has previously been observed for LamB of *E. coli* (Nekolla et al., 1994). Thus we used the corner frequency of the fast Lorentzian for the fit with Eq. (2) (*see* Fig. 3 and Discussion). This fit yielded the on- and off-rate constants, k_1 and k_{-1}, for carbohydrate binding to the binding site inside the ScrY channel, similar to the case of the LamB-channels from *E. coli* and *S. typhimurium* (Nekolla et al., 1994; Andersen et al., 1995; Jordy et al., 1996).

Table 1 shows the dependence of the corner frequencies and the corresponding amplitudes of a titration experiment with ScrY on the maltotriose concentration. The values for S_0 increased and had a maximum at the maltotriose concentration that corresponded approximately to the half saturation concentration determined in the corresponding titration experiment. At a higher car-

Table 1. Parameters of the fit of the power density spectra of the maltotriose-induced block of wild-type ScrY channels with the sum of two Lorentzians[a]

c/mM	*I*/nA	Low frequency component		High frequency component	
		$S_0/10^{-24}$A^2*sec*	f_c/Hz	$S_0/10^{-24}$A^2*sec*	f_c/Hz
0.00	26.75				
0.04	26.50	22.1	5.1	1.54	402
0.10	25.35	25.0	10.4	6.21	433
0.20	24.25	44.6	9.3	8.70	472
0.40	22.5	84.3	8.0	11.1	500
0.70	20.15	107	7.2	11.7	550
0.99	17.90	163	6.2	12.5	575
1.38	16.00	141	7.7	9.73	594
1.96	13.50	120	8.8	6.53	725
2.91	10.75	119	7.8	4.12	873
4.76	7.85	87.3	7.0	1.99	*nd*
6.54	6.25	68.4	5.9	1.18	*nd*
9.09	5.20	45.0	5.3	0.73	*nd*

[a] The membrane was formed from diphytanoyl phosphatidylcholine/n-decane. The aqueous phase contained 1-M KCl and 50-ng/ml ScrY wild type. The membrane potential was 20 mV; T = 25°C. The spectra are fitted by the sum of two Lorentzians. The background noise is subtracted in all cases. *nd* means not detectable cause the corner frequency is in the range of the background noise.

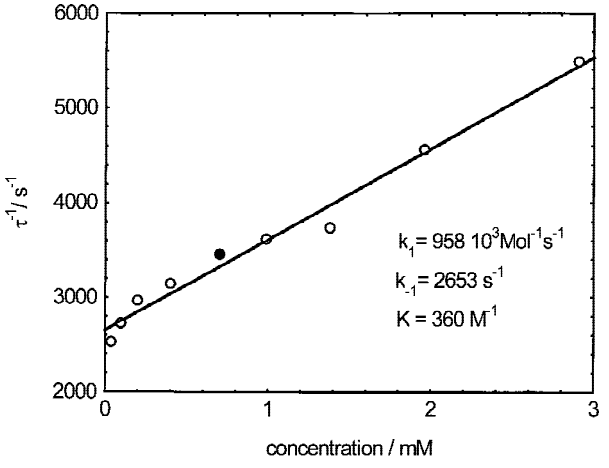

Fig. 3. Dependence of $2\pi f_c = 1/\tau$ on the maltotriose concentration in the aqueous phase. The data were derived from the fit of the power density spectra with Lorentzians similar to those given in Fig. 2 (closed circle; 0.7 mM) and for other maltotriose concentrations ranging between 0.04- and 3-mM (open circles). The aqueous phase contained 1-M KCl and between 10- and 100-ng/ml ScrY. The applied membrane potential was 20 mV; T = 25°C.

Table 2. Parameters of carbohydrate-induced transport noise in wild-type ScrY and the deletion mutant ScrYΔ3-72[a]

Carbohydrate		ScrY		ScrYΔ3-72	
		Titration	Noise	Titration	Noise
Maltose	K (M^{-1})	150	166	160	*nm*
	k_l ($10^6 M^{-1}sec^{-1}$)		0.43		*nm*
	k_{-l} (sec^{-1})		4800		*nm*
Maltotriose	K (M^{-1})	550	720	810	840
	k_l ($10^6 M^{-1}sec^{-1}$)		1.5		5.5
	k_{-l} (sec^{-1})		2100		6100
Maltotetraose	K (M^{-1})	910	1700	1800	1500
	k_l ($10^6 M^{-1}sec^{-1}$)		4.0		5.9
	k_{-l} (sec^{-1})		2800		4000
Maltopentaose	K (M^{-1})	3300	1500	5500	2400
	k_l ($10^6 M^{-1}sec^{-1}$)		2.7		3.9
	k_{-l} (sec^{-1})		1900		1800
Maltohexaose	K (M^{-1})	4800	2800	6300	3100
	k_l ($10^6 M^{-1}sec^{-1}$)		3.2		3.1
	k_{-l} (sec^{-1})		1200		1000
Maltoheptaose	K (M^{-1})	4800	1900	4500	1800
	k_l ($10^6 M^{-1}sec^{-1}$)		1.6		1.6
	k_{-l} (sec^{-1})		840		910

[a] The membranes were formed from diphytanoyl phosphatidylcholine/n-decane. The aqueous phase contained 1-M KCl and between 10- and 100-ng/ml ScrY or its deletion mutant. k_l and k_{-l} were derived from a fit of the corner frequencies of the fast Lorentzians as a function of the carbohydrate concentrations (*compare* Eq. (2)). K is the stability constant for carbohydrate binding derived either from the titration experiments or calculated from the ratio of the on- and off-rate constant derived from noise experiments. The data represent the mean of all experiments with the same carbohydrate. *nm* means not measured. The standard deviations of the constants were in between 10% and 20% of the mean values.

bohydrate concentration the amplitude decreased and the corner frequency of the fast Lorentzian increased with carbohydrate concentration. The corner frequency of the slow Lorentzian differed somewhat from experiment to experiment but it seemed to be independent of the sugar concentration. The power density of the slow process increased first with increasing maltotriose concentration and then decreased.

The results for the on- and off-rate constants for the binding of the malto-oligosaccharide series to ScrY are listed in Table 2. In contrast to LamB we observed an increase of the spectral density when maltose was added to ScrY-containing membranes. This made it possible to measure the on- and off-rates of maltose binding to ScrY. Sucrose binding was probably much faster and it was not possible to evaluate the rate constants because we did not observe any increase of the spectral density upon addition of sucrose to the aqueous phase.

The Deletion Mutant ScrYΔ3-72 Did Not Show Different Current Noise Spectra

We have also studied the sugar-induced noise of the deletion mutant ScrYΔ3-72, which lacks 70 amino acid residues in the N-terminus region. These residues are not conserved in maltoporin. The deletion did not effect the noise spectra to any appreciable extent. As in the case of ScrY the spectra could be fitted to the sum of two Lorentzians, from which the fast one was used for the evaluation of the on- and off-rate constants listed also in Table 2.

Discussion

The Spectra of Current Noise Through ScrY Contain Two Lorentzians

In this study we investigated the current noise through the sucrose-specific ScrY-channel. The open channel noise was of *l*/*f*-type as is discussed in detail in a recent publication (Wohnsland & Benz, 1997). Surprisingly, the carbohydrate-induced current noise contained two Lorentzians, which was not expected when we consider the results obtained with the closely related LamB channels of *E. coli* and *S. typhimurium* (Nekolla et al., 1994; Andersen et al., 1995; Jordy et al., 1996). Experiments with different carbohydrates clearly demonstrated that

the spectra were composed of two parts and it was impossible to fit them with a single Lorentzian curve. In a large number of experiments it was possible to study the two Lorentzians as a function of carbohydrate concentration. The corner frequency of the fast process (f_c > 200 Hz) was dependent on the sugar concentration and increased with increasing carbohydrate concentrations to higher frequencies. Similarly, the initial power density was also a defined function of the carbohydrate concentration, which could be fitted to the usual formalism with high accuracy. We assumed therefore that this part of the spectra is analogous to those obtained previously for LamB (Nekolla et al., 1994), and reflects the kinetics of carbohydrate binding.

At lower frequencies (f_c < 50 Hz) the ScrY-spectra contained another Lorentzian, which is definitely also caused by the addition of carbohydrates because it was not observed without them in control experiments (*see* Fig. 2*A*). We tried to fit the spectral density as a function of the frequency by a sum of a l/f noise combined with a Lorentzian function since it could be possible that the presence of carbohydrates changed the property of the channel in such a way that the l/f component of the open channel increased. However, best fit was always obtained when we fitted the whole spectra with the sum of two Lorentzian functions. It is noteworthy, that the fit of the experimental data became difficult when the corner frequencies of the two Lorentzians were similar. In this case it was sometimes rather difficult to distinguish between the two components. In particular, when we used long chain malto-oligosaccharides, the corner frequencies of the fast and slow Lorentzians were close together in contrast to the use of maltotriose where they could easily be distinguished (*see* Table 1).

It is noteworthy that two Lorentzians have also been observed for the study of other membrane channels. One example is the cystic fibrosis transmembrane channel (CFTC), an epithelial AMP-regulated chloride channel. The forskolin-activated channels show two gating modes, which lead to a current noise spectrum of a double Lorentzian type (Fischer & Machen, 1994). The fast gating process is dependent on voltage while the slow gating process shows no dependence. Another system with double Lorentzian noise spectra is the apical sodium channel in frog skin. The channels are competitively blocked by amiloride and by sodium ions (Frehland et al., 1983). The blockage in frog skin and toad bladder is sensitive to Ca^{2+} ions that also induces several Lorentzians (Van Driessche et al., 1991). For the sucrose-specific porin we can exclude a similar cause of the double Lorentzian because the channel does not contain a binding site for ions, which means that carbohydrate and ions do not compete for the binding site (Schülein et al., 1991).

When we accept the idea of the double Lorentzian fit of the noise spectra observed in the presence of carbohydrates with ScrY the question arises for the reason of the second Lorentzian because only one is sufficient to describe the carbohydrate-induced block in the closely related LamB-channel. There exist several possibilities for a second current modulating process but in total we have not a striking explanation for this observation. The first possibility is a second carbohydrate-binding site inside the ScrY channel, corresponding to a second gating process. Such a binding site has not been discovered in the 3D-crystals of ScrY (Forst et al., 1997). Furthermore, such a possibility appears to be extremely unlikely because of several reasons. Firstly, such a binding process should be dependent on the carbohydrate concentration, which we did not observe. Secondly, the kinetics of the process causing the slow Lorentzian is very slow corresponding to a slow binding, which means that it has nothing to do with carbohydrate transport since this occurs very efficiently through ScrY (Schmid et al., 1991). The third possibility is that the additional N-terminal extension of ScrY as compared with LamB forms a binding site. Noise measurements with the deletion mutant ScrYΔ3-72, in which the N-terminal region is deleted showed also a double Lorentzian style of the noise spectra. This means that also this region is not responsible for the additional component in the noise spectra of the sucrose-specific porin.

Another possibility for the slow Lorentzian is that the sucrose-specific porin channel is asymmetric regarding the kinetics of carbohydrate binding because it is known that malto-oligosaccharides bind to LamB from outside with the nonreducing end in advance (Dutzler et al., 1996). Then two Lorentzians could be expected to be dependent on whether the carbohydrate enters the channel from the extracellular or from the periplasmic side. To test this possibility we performed experiments where we added the protein only at one side of the membrane, expecting that the incorporation of the channel into the membrane is not random. The carbohydrates were added to only one side of the membrane (either to the same side as the protein—*cis*, or to the other side—*trans*). The current noise spectra of these experiments were also of the double Lorentzian style and were very similar (*data not shown*), which means that either there does not exist any asymmetry of the channel or the incorporation of the channel is a random event even if the protein is added to one side of the membrane only. The spectral density of current noise does not show Lorentzians for two state channels only, i.e., open and closed channels. Lorentzian noise may also be caused by small structural changes, 'channel-breathing' as it has been discussed for ion channels elsewhere (Sigworth, 1985). This means that the ScrY channel may undergo slow structural changes, which also can create Lorentzian type of noise. In this case we had to assume that the channel breathing is

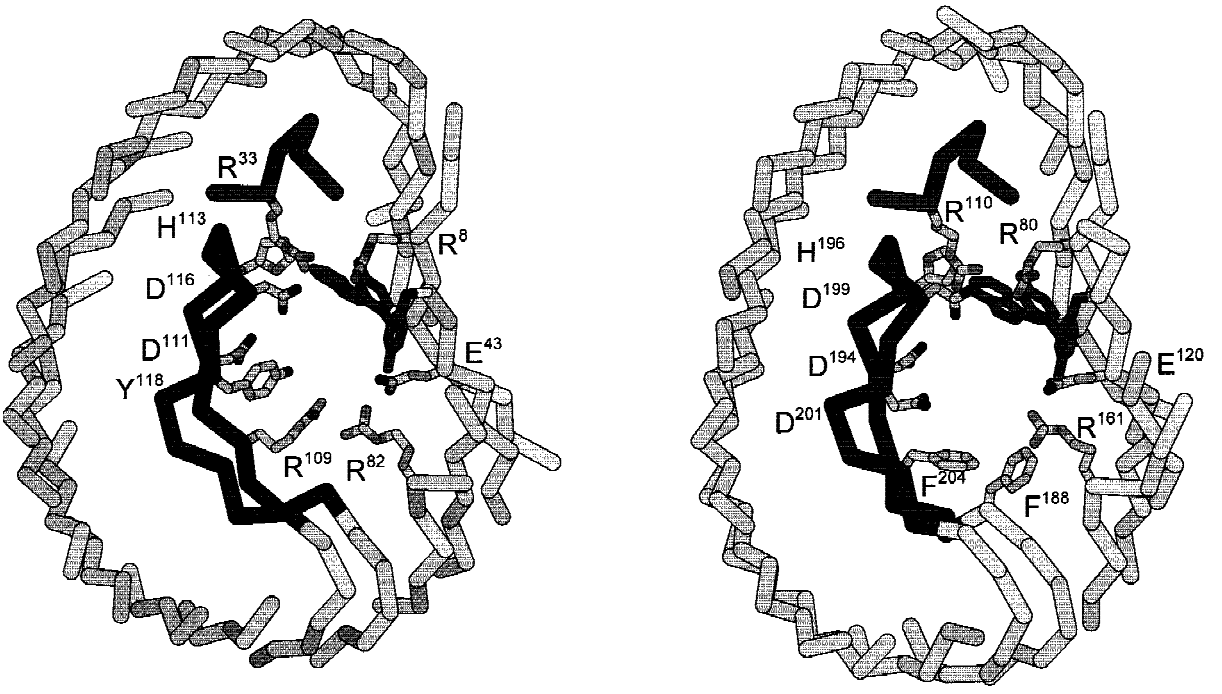

Fig. 4. Comparison of the cross section of LamB (on the left) and ScrY (on the right) at the level of the third loop. Residues which are point to the channel lumen are shown in detail. The crystal data derived from Schirmer et al. (1995) and Forst et al. (1997).

induced by carbohydrate binding. Such a possibility cannot be excluded on the basis of our experimental results.

The stability constants for carbohydrate-binding can also be calculated from the variance of the noise spectra according to Eqs. (8) and (9). However, we were not able to receive reasonable values for K from both the variance of the slow and of the fast Lorentzian (as compared to those of the titration experiments) when we did not fit K together with the single-channel conductance g. When we fitted both K and g the single-channel conductance correlated with the slow process was less than 20 pS and that for the fast process was less than 100 pS. This result could indicate that the slow process does not completely close the channel. On the other hand, the variance of the slow Lorentzian process is always considerably smaller than 20% of that of the fast process (Table 1) and it may probably be reasonable to ignore the slower Lorentzian component in terms of carbohydrate binding.

Comparison of Carbohydrate Binding to ScrY (Sucrose-specific Porin) and LamB (Maltoporin)

LamB and ScrY are porins that are specific for carbohydrates. The solution of the 3D-structure of maltoporin allows the elucidation of the molecular basis for carbohydrate binding (Schirmer et al., 1995; Meyer et al., 1997). Several residues in the channel lumen that are involved in sugar binding via hydrogen bonds have been determined (Dutzler et al., 1996). Furthermore, six aromatic residues, the so called 'greasy-slide,' lining the channel interior from the extracellular site to the periplasmic space seem also to be involved in carbohydrate transport. The sequence homology between maltoporin and sucrose-specific porin makes it possible to suspect that the molecular basis and the mechanism of sugar transport through both carbohydrate-specific porins are very similar (Schülein et al., 1995).

The single-channel conductance of sucrose-specific porin is considerably larger than that of maltoporin (approximately 10 times; Benz et al., 1987; Schülein et al., 1991). This could be supported by the crystal structure of both porins. Recently, Forst et al. (1997) have solved the 3D-structure of ScrY. Figure 4 shows a comparison of the cross section through both porins at the narrowest side in the channel. It is obvious that the central friction in ScrY, which means the narrowest region in the channel is wider, mainly caused by different residues localized on the third loop that is folded inside the channel lumen. These observations are supported by measurements with the LamB mutant Y118S (K. Schmid, C. Andersen, T. Ferenci and R. Benz, *unpublished results*). This tyrosine residue is localized on the third loop of the LamB-channel and we have already shown that it influences sugar binding (Jordy et al., 1996). The mutant Y118S has a 10-fold higher single-channel conductance

Table 3. Comparison of transport parameters for different carbohydrates through ScrY and LamB from *Salmonella typhimurium* and *Escherichia coli*

Carbohydrate		ScrY		LamB *E. coli*[a]		LamB *S. typh.*[b]	
		Titration	Noise	Titration	Noise	Titration	Noise
Sucrose	K (M^{-1})	20[c]			80	59[d]	
	k_l ($10^6 M^{-1} sec^{-1}$)		0.36[e]		0.004		
	k_{-l} (sec^{-1})		17800[e]		50		
Maltose	K (M^{-1})	150	166	110		150	81
	k_l ($10^6 M^{-1} sec^{-1}$)		0.43		0.8		0.09
	k_{-l} (sec^{-1})		4800		8000		1060
Maltotriose	K (M^{-1})	550	720	2800	4300	4800	2170
	k_l ($10^6 M^{-1} sec^{-1}$)		1.5		8.4		2.6
	k_{-l} (sec^{-1})		2100		1950		1420
Maltotetraose	K (M^{-1})	910	1700	9000	8100	7700	12900
	k_l ($10^6 M^{-1} sec^{-1}$)		4.0		6.1		4.4
	k_{-l} (sec^{-1})		2800		769		343
Maltopentaose	K (M^{-1})	3300	1500	14000	13000	13000	9350
	k_l ($10^6 M^{-1} sec^{-1}$)		2.7		5.3		4.1
	k_{-l} (sec^{-1})		1900		417		447
Maltohexaose	K (M^{-1})	4800	2800	16000	20000	14000	14700
	k_l ($10^6 M^{-1} sec^{-1}$)		3.2		4.8		4.1
	k_{-l} (sec^{-1})		1200		238		290
Maltoheptaose	K (M^{-1})	4800	1900	17000	31000	14000	18900
	k_l ($10^6 M^{-1} sec^{-1}$)		1.6		5.6		3.6
	k_{-l} (sec^{-1})		840		179		211

[a] Taken from Andersen et al. (1995); [b] taken from Jordy et al. (1996); [c] taken from Schülein et al. (1995); [d] taken from Schülein & Benz (1990); [e] taken from Table 4 (the data not derived from noise measurements).

than maltoporin, which means that it is in the range of that of the sucrose-specific porin. Thus it may be that in ScrY the carbohydrate molecules can also bind in opposite orientation, with the nonreducing end directed to the extracellular surface. The wider central friction of the channel is also the reason for the fast sucrose transport through ScrY. The tilted sucrose molecule is able to pass rapidly (*see also below*) through the constriction zone because we did not observe any indication for the generation of sucrose-mediated current noise.

Table 3 contains the on- and off-rate constants of carbohydrate binding to the sucrose-specific porin in comparison to those of the maltoporins of *E. coli* and *S. typhimurium.* It is obvious that the kinetics of carbohydrate transport is very similar for both types of channels with the exception of sucrose. Sucrose shows a slow binding kinetics to LamB, whereas it binds rapidly to ScrY with much higher on- and off-rate constants. The highest value for the on-rate (which corresponds to the maximum permeability of these channels) in ScrY is reached for maltotetraose. The on-rate for maltose binding is an order of magnitude smaller. For longer carbohydrates the on-rate constant decreases. This could be caused by sterical hindrance by the binding to the site. The comparison of the absolute value shows that the on-rate of the sugar binding is higher in maltoporin. Similar as in maltoporin the off-rate constants (which correspond to the maximum turnover number of these channels) for carbohydrate-binding decrease with increasing sugar length. This is easy to understand because longer carbohydrates can move several times back and forward in the channel before they can finally leave the binding site. The major difference between the two carbohydrate-specific porins consists in the higher off-rates of carbohydrate binding in the case of the sucrose-specific porin. In maltoporin the values range between 180 sec^{-1} and 2,000 sec^{-1} for the different carbohydrates while they are in the range of 840 to 4800 sec^{-1} for ScrY. This means that the time during which the carbohydrate is bound to the binding site is much shorter in the sucrose-specific porin than in maltoporin. This could again be explained by the larger constriction zone of the sucrose channel.

Comparison of the Sugar Binding of ScrY Wild-type and the Deletion Mutant ScrYΔ3-72

A comparison of the sugar binding constants of ScrY wild-type and deletion mutant ScrYΔ3-72 is given in Table 2. It contains the on- and off-rate constants and the stability constants from noise measurements, and the stability constants that were evaluated by titration experiments. The comparison of the results of ScrY wild-type with those of the deletion mutant shows that there does not exist any significant difference for carbohydrate binding with the exception of maltotriose binding. This

Table 4. Rate constants for carbohydrate transport through the ScrY channel calculated relative to the relative rate of permeation of sucrose[a]

Carbohydrate	Relative rate of permeation (Hardesty et al., 1991)	Flux through one single ScrY (sec^{-1})	K (M^{-1})	k_l (10^3 $M^{-1}sec^{-1}$)	k_{-l} (sec^{-1})
Maltoheptaose	27	800	1900 (this work)	1600 (this work)	840 (this work)
DL-Arabinose	109	3233	9.1	350	38800
D-Glucose	91	2700	8.3	290	35200
D-Fructose	149	4420	1.9	450	237000
Sucrose	100	2970	20	360	17800
Maltose	33	980	150	245 (430)	1630 (4800)
Lactose	33	980	17	115	6700
Raffinose	19	560	640	414	648

[a] The on-rate constants, k_l, were calculated from Eq. (9) using the flux of maltoheptaose (800 sec^{-1}) through the ScrY channel under the conditions of the experiment of Hardesty et al. (1991) (*compare* Eq. (8)). For the calculation of the rate constants for the individual carbohydrate it was assumed that its flux through ScrY relative to that of maltoheptaose is given by the relative rates of permeation (Hardesty et al., 1991). The stability constants, K, were taken from titration experiments of Schülein et al. (1991) with the exception of maltoheptaose (this study), k_{-l} was calculated from k_l/K. In the case of maltose the numbers in brackets correspond to those obtained from noise measurements (*compare* Table 1).

is easy to understand on the basis of the secondary structure model (Schülein et al., 1995). The N-terminal 70 amino acids long extension of the sucrose-specific porin in comparison to maltoporin is located in the periplasmic space. This means that it does not represent part of the channel itself. Furthermore it has no influence on carbohydrate binding to the site, which is located inside the channel. Differences in sugar transport that are observed in in vivo experiments (Schülein et al., 1995) must be due to changed transport or binding properties within the periplasmic space.

Comparison with the Relative Rate of Carbohydrate Permeation Derived by Hardesty et al. (1991)

Hardesty et al. (1991) measured the relative rate of permeation of different carbohydrates through liposomes-containing outer membrane protein of a ScrY-producing *E. coli* strain by using the liposome swelling technique (Nikaido & Rosenberg, 1981). Although the protein contained probably also other outer membrane porins despite ScrY they have found remarkable differences in the rate of permeation of different carbohydrates with respect to sucrose and demonstrated the sucrose specificity of ScrY. It is possible to compare our data with those of Hardesty et al. (1991) using the following considerations. Substituting the experimental conditions of Hardesty et al. (1991) ($c'' = 20$ mM, $c' = 0$) the flux through ScrY is given by (Andersen et al., 1994):

$$\Phi = k_l \cdot (20\ mM)/(2 + K \cdot (20\ mM)) \qquad (10)$$

This means that k_l is given by:

$$k_l = \Phi \cdot ((100\ M^{-1}) + K) \qquad (11)$$

Eq. (11) can be used for a more quantitative description of the sugar transport through ScrY using the liposome-swelling assay. Maltoheptaose shows a rather slow diffusion in this approach. The flux, Φ, of maltoheptaose under the conditions of Hardesty et al. (1991) is estimated from Eq. (10) by using the rate constants from noise measurements to be 800 sec^{-1}. This number is now used to calculate the flux of other carbohydrates through ScrY from the relative rates of permeation of Hardesty et al. (1991) (column 1 of Table 4) and receive the data of column 2 of the same table. It is possible to calculate according to Eq. (11) by using the corresponding carbohydrate fluxes and the stability constants K (column 3), the on- and off-rate constants k_l and k_{-l} for a large number of carbohydrates given in columns 4 and 5 of Table 4. The data of Table 4 demonstrate that the flux of certain carbohydrates through the sucrose-specific porin is saturated at 20-mM concentration of those carbohydrates with a large affinity for the binding site. This means that the flux is similar to k_{-l}, which is the turnover number (i.e., the maximum flux) of the carbohydrates through the ScrY channel (Jordy et al., 1996). This is given for maltoheptaose and for raffinose. It is noteworthy, that the rate constants show reasonable agreement for maltose, where the noise measurements allowed also the derivation of the rate constants for its binding to the ScrY-channel (given in brackets). The combination of the results from the noise analysis and

the liposome-swelling assay allow the estimation of the rate constants for a variety of carbohydrates including sucrose, which are not available from the noise analysis alone because of its limited time resolution. The comparison of the parameters of maltose and sucrose transport demonstrates that the on-rates are comparable, whereas the off-rate of sucrose binding to ScrY is 10 times higher than that for maltose, which represents a considerable advantage for the velocity of sucrose permeation through ScrY.

The authors would like to thank Angela Schmid for many helpful discussions and Wolfram Welte for the opportunity to use the 3D-structure of ScrY prior to publication. This work was supported by the Deutsche Forschungsgemeinschaft (SFB 176/B9) and the Fonds der Chemischen Industrie.

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