

Evaluation of the Rate Constants of Sugar Transport Through Maltoporin (LamB) of *Escherichia coli* from the Sugar-induced Current Noise

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ABSTRACT LamB (maltoporin) of *Escherichia coli* outer membrane was reconstituted into artificial lipid bilayer membranes. The channel contains a binding site for sugars and is blocked for ions when the site is occupied by a sugar. The on and off reactions of sugar binding cause an increase of the noise of the current through the channel. The sugar-induced current noise of maltoporin was used for the evaluation of the sugar-binding kinetics for different sugars of the maltooligosaccharide series and for sucrose. The on rate constant for sugar binding was between 10^6 and 10^7 $M^{-1} \cdot s^{-1}$ for the maltooligosaccharides and corresponds to the movement of the sugars from the aqueous phase to the central binding site. The off rate (corresponding to the release of the sugars from the channel) decreased with increasing number of glucose residues in the maltooligosaccharides from $\sim 2,000$ s^{-1} for maltotriose to 180 s^{-1} for maltoheptaose. The kinetics for sucrose movement was considerably slower. The activation energies of the stability constant and of the rate constants for sugar binding were evaluated from noise experiments at different temperatures. The role of LamB in the transport of maltooligosaccharides across the outer membrane is discussed.

INTRODUCTION

The cell envelope of Gram-negative bacteria consists of different layers. The inner or cytoplasmic membrane contains the respiration chain, proteins for the transport of nutrients and proteins involved in the synthesis of phospholipids, peptidoglycan, and lipopolysaccharides (Beveridge, 1981; Nikaido and Vaara, 1985). The periplasmic space between the membranes is an aqueous compartment isoosmolar to the cytoplasm. It contains the peptidoglycan and a large number of different proteins. The outer membrane is composed of protein, lipid and lipopolysaccharide (Benz,

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1988; Nikaido and Vaara, 1985). It contains only a few major proteins. At least one of them is a constitutive transmembrane protein, called porin, which contains a general diffusion pore with a defined exclusion limit for hydrophilic solutes, which means that molecules with higher molecular masses than the exclusion limit cannot pass the outer membrane (Nikaido and Vaara, 1985; Hancock, 1986; Benz and Bauer, 1988).

Besides the constitutive porins, the outer membrane may contain porins, which are induced under special growth conditions (Szmelcman, Schwartz, Silhavy, and Boos, 1976; Tommassen and Lugtenberg, 1980; Hancock, Poole, and Benz, 1982; Bauer, Benz, Brass, and Boos, 1985). They often form solute-specific pores and contain binding sites for neutral substrates such as sugars (Ferenci, Schwentorat, Ullrich, and Vilmart, 1980; Benz, Schmid, Nakae, and Vos-Scheperkeuter, 1986) and nucleosides (Maier, Bremer, Schmid, and Benz, 1988) or anionic solutes such as phosphate (Hancock et al., 1982; Benz and Hancock, 1987). One of the specific porins is LamB (maltoporin) of *Escherichia coli* and of other *Enterobacteriaceae*, which is part of the mal system induced during growth on maltose and maltooligosaccharides (Szmelcman and Hofnung, 1975; Szmelcman et al., 1976; Bloch and Desaynard, 1985). LamB forms a channel for the permeation of maltose and higher maltooligosaccharides through the outer membrane but has a small permeability for sucrose (Szmelcman and Hofnung, 1975; Luckey and Nikaido, 1980a; Schmid, Ebner, Jahreis, Lengeler, and Titgemeyer, 1991).

LamB of *E. coli* and *Salmonella typhimurium* form ion-permeable channels when reconstituted into lipid bilayer membranes (Benz et al., 1986; Benz, Schmid, and Vos-Scheperkeuter, 1987; Dargent, Rosenbusch, and Pattus, 1987; Schülein and Benz, 1990). Sugars bind to LamB (Ferenci et al., 1980; Luckey and Nikaido, 1980a) and the binding leads to a block of the channel for the permeation of sugars (Luckey and Nikaido, 1980b) and ions (Benz et al., 1986; 1987), which suggests that the sugar-specific binding site is located in the interior of the channel. The structure of the binding site inside the channel has been studied in detail by assuming a one-site two-barrier model for sugar transport and titrating the ion current through LamB with increasing concentrations of different sugars (Benz et al., 1987). The results suggest that the sugar binding site inside the channel has a length of about five glucose residues.

In a recent publication, we have demonstrated that the open LamB channel shows $1/f$ noise, which probably reflects slow opening and closing of channels. This process varies considerably from membrane to membrane and is not correlated to the number of open channels (Nekolla, Andersen, and Benz, 1994), which means that the $1/f$ -noise is not an intrinsic property of the LamB channel. The addition of maltopentaose to the aqueous phase bathing a membrane containing LamB channels leads to a drastic increase of the current noise of the channels. The spectral density of the current noise is of Lorentzian type and the corner frequency could be used for the evaluation of the rate constants for maltopentaose binding (Nekolla et al., 1994). In this study we extended the noise analysis to other sugars of the maltooligosaccharide series and to sucrose. The binding kinetics of the sugars was used for the analysis of sugar transport through the LamB channel.

MATERIALS AND METHODS

Purification of LamB

LamB was isolated from envelopes of maltose-grown cells of *E. coli* TK24, which lacks OmpC, OmpF, and OmpA. Details of the isolation procedure have been described in detail elsewhere (Vos-Scheperkeuter, Hofnung, and Witholt, 1984).

Lipid Bilayer Experiments and Noise Analysis

Black lipid bilayer membranes were formed as described previously (Benz, Janko, Boos, and Läuger, 1978). The instrumentation consisted of a Teflon chamber with two aqueous compartments connected by a small circular hole. The hole had a surface area of 0.5 mm². Membranes were formed by painting onto the hole a 1% solution of diphytanoyl phosphatidylcholine (Avanti Polar Lipids, Inc. Alabaster, AL) in *n*-decane. The aqueous salt solutions (Merck, Darmstadt, Germany) were used unbuffered and had a pH ~ 6. The maltooligosaccharides were obtained from Böhringer (Mannheim, Germany). LamB was reconstituted into the lipid bilayer membranes by adding the concentrated stock solution to the aqueous phase bathing a membrane in the black state.

The membrane current was measured with a pair of calomel electrodes with salt bridges switched in series with a battery operated voltage source and a current amplifier (Keithley 427 with a four-pole filter or a current to voltage converter made using a Burr Brown operational amplifier [three-pole filter]). The feedback resistors of the current amplifier were between 0.01 and 10 GΩ. The amplified signal was monitored with a strip chart recorder. For control purposes the signal was passed through a Krohn-Hite filter (model 113340). The amplified AC-component of the signal was analyzed with a digital signal analyzer (Ono Sokki CF 210), which performed fast Fourier transformation of the current noise. The spectra were composed of either 200 or 400 points and they were averaged either 128 or 256 times. The further analysis of the power density spectra was performed with a Hewlett Packard computer (HP 98561).

RESULTS

Titration of LamB-induced Conductance with Sugars and Evaluation of the Stability Constant of Sugar Binding

LamB was added to black lipid bilayer membranes in a concentration of 50 ng/ml. 30 min after the addition of the protein, the rate of conductance increase caused by reconstitution of LamB into the membrane had slowed down considerably. Then the experiment shown in Fig. 1 started. Small amounts of concentrated maltotetraose solutions were added to the aqueous phase to both sides of the membrane, with stirring to allow equilibration. Fig. 1 demonstrates that the membrane conductance decreased as a function of the maltotetraose concentration. Furthermore, the current noise of the recording on the strip chart recorder increased. The data of Fig. 1 and of similar experiments were analyzed using the following equation derived previously for the block of a one-site two-barrier channel by substrate-binding (Benz et al., 1987).

$$[G_{\max} - G(c)]/G_{\max} = K \cdot c / (K \cdot c + 1). \quad (1)$$

The stability constant for sugar binding, K , is given by the ratio of the on rate k_1 divided by the off rate k_{-1} for sugar-binding to the site inside the channel. G_{\max} is the

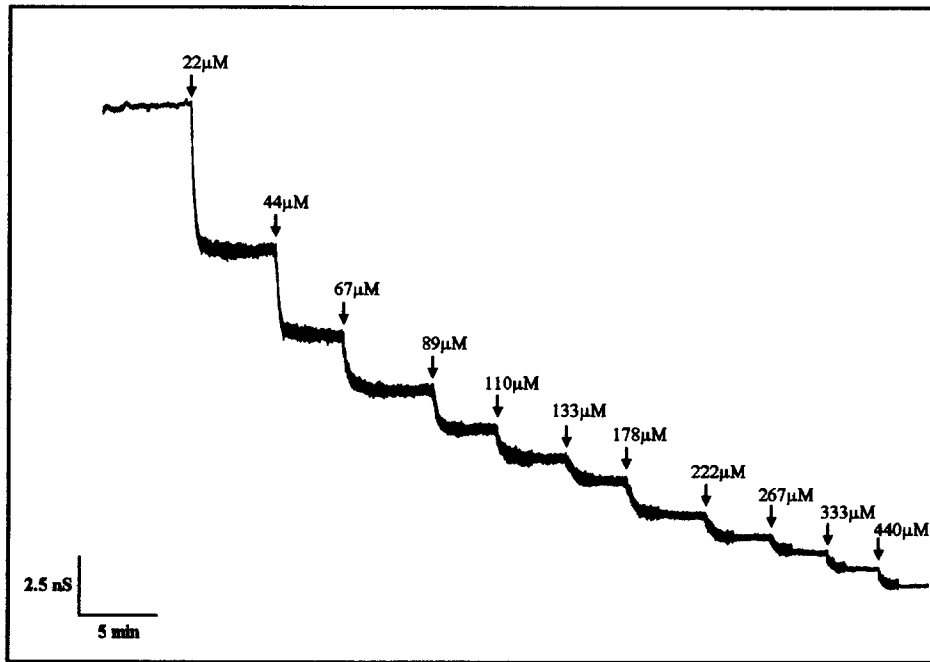


FIGURE 1. Titration of LamB-induced membrane conductance with maltotetraose. The membrane was formed from diphytanoyl phosphatidylcholine/n-decane. The aqueous phase contained 50 ng/ml LamB, 1 M KCl, and maltotetraose at the concentrations shown at the top of the figure. The temperature was 25°C and the applied voltage was 20 mV. For further explanations see text. Note that the current noise increased after the first addition of the sugar.

maximum membrane conductance before the first addition of the sugar. $G(c)$ is the membrane conductance at a given sugar concentration c . Eq. 1 means that the titration curve given in Fig. 1 can be analyzed using a Lineweaver-Burke plot as shown in Fig. 2. The straight line in Fig. 2 corresponded to a stability constant, K , of $15,500 \text{ M}^{-1}$ (half saturation constant $K_S = 64 \text{ } \mu\text{M}$).

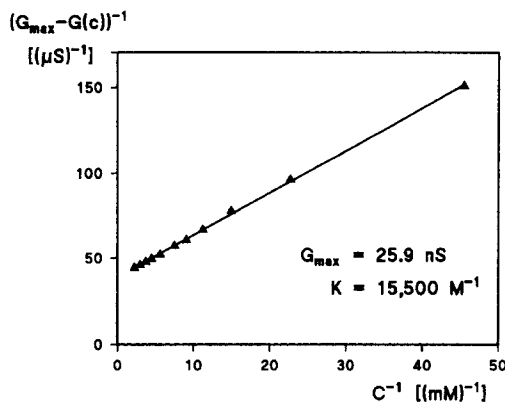


FIGURE 2. Lineweaver-Burke plot of the inhibition of LamB-induced membrane conductance by maltotetraose. The data were taken from Fig. 1 and were analyzed using Eq. 1. The straight line corresponds to a stability constant, K , for maltotetraose-binding of $15,500 \text{ M}^{-1}$.

Evaluation of the Current Noise through the Open and the Sugar-induced Closed State of the LamB Channel

The data of Fig. 1 indicate that the current noise was relatively small before addition of the sugar and showed a dramatic increase after the addition of the different concentrations of the sugar. Fig. 3 shows the power density spectra of the current noise through 690 LamB channels before and after the addition of maltotetraose. The number, N , of channels was calculated from the membrane conductance and the single-channel conductance of LamB in 1M KCl (150 pS). Trace 1 represents the

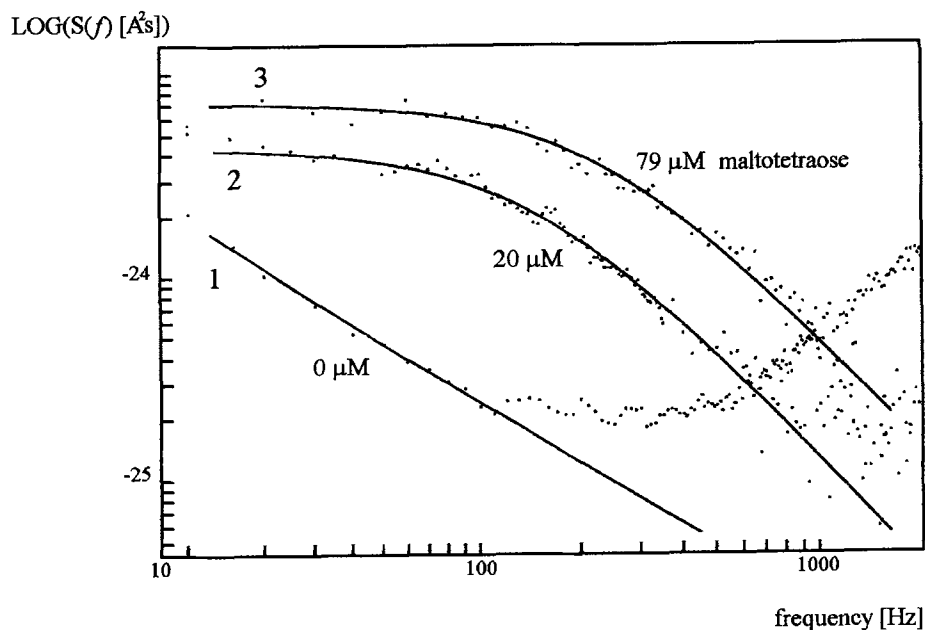


FIGURE 3. Power density spectra of maltotetraose-induced current noise of 690 LamB channels. (Trace 1) Control (1 M KCl). (Trace 2) Aqueous phase contained 20 μM maltotetraose and the power density spectrum of Trace 1 was subtracted ($\tau = 1.1$ ms; $S_0 = 4.3 \times 10^{-24}$ A²s). (Trace 3) Aqueous phase contained 79 μM maltotetraose and the power density spectrum of Trace 1 was subtracted ($\tau = 0.69$ ms; $S_0 = 7.2 \times 10^{-24}$ A²s); $T = 25^\circ\text{C}$; $V_m = 25$ mV. The number of channels, N , was calculated by dividing the membrane conductance through the single-channel conductance of LamB.

control (i.e., the power density spectrum of current noise at 30 mV measured without sugar). This spectrum was taken 60 min after the formation of the membrane made of diphytanoyl phosphatidylcholine/*n*-decane and after the addition of LamB when the current was absolutely stationary. The power spectrum of the current noise of the open LamB channels could be explained by $1/f$ noise at small frequencies, which is typical for the open LamB channels but does not reflect intrinsic properties of the open channels (Nekolla et al., 1994). At higher frequencies, the power density was first stationary and started then to increase in the range between 200 and 500 Hz.

This increase of the power density was caused by the preamplifier and the membrane capacitance, which could easily be demonstrated by the measurement of the current noise of dummy circuits containing an appropriate capacitor.

Then we added the maltotetraose in increasing concentration to the aqueous phase with stirring to allow equilibration (similarly as above in the case of the titration experiments). The power density spectrum was measured again a few minutes after the current decayed to its new value and became stationary, which indicated complete equilibration of the sugar within the aqueous phase and with the LamB channels. Trace 2 of Fig. 3 shows a power density spectrum taken at 20 μM maltotetraose from which the control was subtracted. A spectrum taken after another few minutes did not show any systematic variation compared to spectrum taken before. In further experiments the concentration of the maltotetraose was increased in defined steps. At another concentration of maltotetraose ($c = 79 \mu\text{M}$) the power density spectrum corresponded to that of trace 3 in Fig. 3 (trace 1 was again subtracted).

The power density spectra of the current noise (i.e., the dependence of the power density of the current noise on the frequency f) in the presence of sugar corresponded to that of Lorentzian type expected for a random switch with different on and off probabilities, which are coupled by a chemical reaction (Verveen and De Felice, 1974; Conti and Wanke, 1975; DeFelice, 1981):

$$S(f) = S_0 / (1 + (f/f_c)^a). \quad (2)$$

S_0 is the plateau value of the power density spectrum at small frequencies, f_c is the corner frequency, at which the plateau value of the power density decays to half of its value and a (usually close to 2) is the slope of decay of the power density. Traces 2 and 3 of Fig. 3 could be fitted to Eq. 2 by using a slope of $a = 1.8$ for the decay of the Lorentzian function and corner frequencies, f_c , of 145 and 231 Hz. This means that the power density spectra of the sugar-induced current noise did not follow a perfect Lorentzian, for which in principle, a slope of -2 would be required. Nevertheless, we use here the term "Lorentzian" in analogy to similar studies on amelioride-induced block of sodium channels in frog skin, in which the slope of the Lorentzian was similar than described here (Lindemann and van Driessche, 1977*a,b*; Lindemann, 1980).

Evaluation of the Rate Constants for Sugar Binding to the LamB Channel and of the Single-Channel Conductance

The corner frequencies, f_c , of the Lorentzians could be used to calculate the on- and off-rate constant for sugar binding to the binding-site inside the LamB channel according to:

$$\frac{1}{\tau} = 2\pi \cdot f_c = k_1 c + k_{-1}. \quad (3)$$

$1/\tau$ is reaction rate of the second-order reaction between sugar and binding site. This means that the corner frequencies should increase with increasing sugar concentration as it was the case for the experiments shown in Fig. 3. Fig. 4 shows the fit of the corner frequencies, f_c , of the experiments shown in Fig. 3 and of other maltotetraose concentrations (data not shown) to Eq. 3. The rate constants for the binding of

maltotetraose to the LamB channel were $k_1 = 8.89 \times 10^6 \text{ M}^{-1} \cdot \text{s}^{-1}$ and $k_{-1} = 757 \text{ s}^{-1}$. This corresponds to a stability constant, K , for the binding of maltotetraose to the binding site inside the LamB channel of $12,000 \text{ M}^{-1}$. This value agreed reasonably well with the results of the titration experiments, which have been measured previously ($10,000 \text{ M}^{-1}$; Benz et al., 1987) and in this study.

Table I shows the results of maltotetraose-induced current noise taken from eight different membranes. The data of Table I demonstrate that the on- and off-rate constants of maltotetraose binding were fairly independent from the experimental conditions, in particular from the number of reconstituted LamB channels. Similarly, the stability constants, $K = k_1/k_{-1}$ for sugar binding agreed within a factor of about two to three with one another and with the stability constants derived from the titration experiments described above and which have been measured in earlier investigations (Benz et al., 1986, 1987).

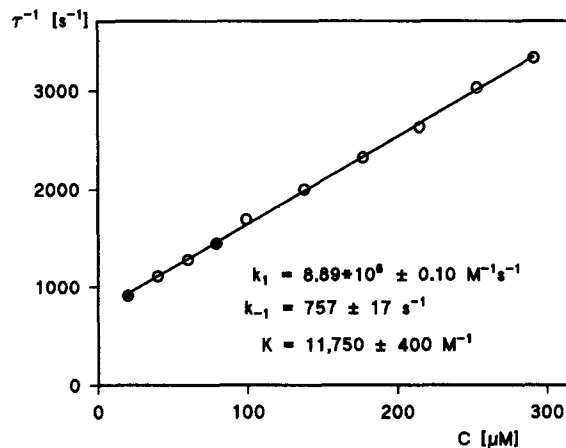


FIGURE 4. Dependence of $2\pi f_c = 1/\tau$ on the maltotetraose concentration in the aqueous phase. The data were derived from the fit of the power density spectra with Lorentzians similar to those given in Fig. 3 (closed circles; 20 and 79 μM) and for other maltotetraose concentrations ranging between 40 and 291 μM (open circles). The aqueous phase contained 1 M KCl. The applied membrane potential was 30 mV; $T = 25^\circ\text{C}$.

The power density, S_0 at very small frequencies allows the calculation of the single-channel conductance, g , of the LamB channel by using Eq. 4 (Verveen and De Felice, 1974; Conti and Wanke, 1975; DeFelice, 1981).

$$S_0 = 4 \cdot g \cdot V_m \cdot I(c) \cdot p \cdot \tau \quad (4)$$

V_m is the membrane potential, $p = K \cdot c / (1 + K \cdot c)$ is the probability that the channel is occupied by a sugar and $I(c) = I_{\max} / (1 + K \cdot c)$ is the current at a given sugar concentration. The introduction of the expressions for p , $I(c)$ and τ leads to Eq. 5 (Nekolla et al., 1994).

$$S_0 = \frac{4 \cdot g \cdot V_m \cdot I_{\max} \cdot K \cdot c}{k_{-1} (1 + K \cdot c)^3} \quad (5)$$

Table I contains the single-channel conductance of the LamB channel calculated from the power density at low frequency of the individual experiments. Since we obtained for all sugar concentrations in a given set of experimental conditions power density spectra, we plotted S_0 as a function of the sugar concentration in the aqueous

phase and fitted it to Eq. 5. The fit parameters were the stability constant K^* and the single-channel conductance g . The off-rate constant k_{-1} was set to the value derived from the corner frequencies. An example for this procedure is given in Fig. 5. It is noteworthy, that we obtained also reasonable fits for the other experiments. Similarly as in a previous study for maltopentaose the single-channel conductance of LamB derived from the noise measurements was smaller than that derived from single-channel experiments (Benz et al., 1986). The reason for this is that the isolated and purified LamB is composed of three identical polypeptide chains, which contain probably each a channel. These three channels cannot be separated in single-channel experiments but we have a good indication that they bind the sugars independently, which means that it is possible to separate them in noise experiments.

TABLE I
Parameters of Maltotetraose-induced Transport Noise in LamB of *Escherichia coli*^a

Experiment	N	$k_1/10^6 \text{ M}^{-1}\text{s}^{-1}$	k_{-1}/s^{-1}	K/M^{-1}	g/pS	K^*/M^{-1}
M1Dec16	510	5.07 ± 0.18	450 ± 35	11270 ± 1280	57.4 ± 4.2	7690 ± 740
M41Sep4	3500	4.80 ± 0.17	717 ± 42	6690 ± 630	46.1 ± 3.9	4800 ± 670
M1Sep5	1090	4.19 ± 0.35	823 ± 59	5080 ± 790	119 ± 4	5480 ± 370
M17Sep5	1180	6.24 ± 0.30	681 ± 33	9170 ± 890	86.7 ± 2.9	6960 ± 530
M33Sep4	10380	3.59 ± 0.37	873 ± 80	4120 ± 800	88.8 ± 4.5	4880 ± 450
M1Dec12	1460	6.85 ± 0.24	877 ± 38	7810 ± 610	157 ± 4	5860 ± 290
M54Dec12	590	8.77 ± 0.38	970 ± 68	9040 ± 1030	130 ± 17	10360 ± 1560
M13Dec5	650	8.89 ± 0.10	757 ± 17	11750 ± 400	92.8 ± 3.5	9190 ± 480
Mean value \pm SD		6.05 ± 2.01	769 ± 160	8100 ± 2800	97.2 ± 36.9	6900 ± 2100

^aThe membranes were formed from diphytanoyl phosphatidylcholine/*n*-decane. The aqueous phase contained 1 M KCl and between 10 and 100 ng/ml LamB; $T = 25^\circ\text{C}$. k_1 and k_{-1} were derived from a fit of the corner frequencies as a function of the maltotetraose concentration (compare Eq. 3). N is the number of channels calculated from the membrane conductance and the single-channel conductance of LamB in 1 M KCl (150 pS). K is the stability constant for maltopentaose binding derived from the ratio k_1/k_{-1} . The stability constant K^* and the single-channel conductance g were derived from a two parameter fit of S_0 according to Eq. 5. The data represent mean \pm SD from least squares fits of S_0 as a function of the sugar concentration according to Eq. 5 and the last row represents mean \pm SD of all experiments.

Rate Constant for the Binding of Different Maltooligosaccharides and of Sucrose to the LamB Channel

In further experiments we investigated the current noise of the LamB-channel induced by other sugars from the maltooligosaccharide series. Only for maltooligosaccharides with four to seven glucose residues it was possible to derive the corner frequency of the Lorentzians with sufficient accuracy. For glucose, maltose, and maltotriose, the corner frequency was shifted to that part of the frequency range, in which the power density was dominated by the preamplifier and the membrane capacitance. The results of the experiments with the other sugars allowed the evaluation of the on- and off-rate constants for their binding to the LamB channel (see Table II). The off rate for sugar binding decreased with increasing number of glucose residues. This means that the off rates for glucose, maltose and maltotriose

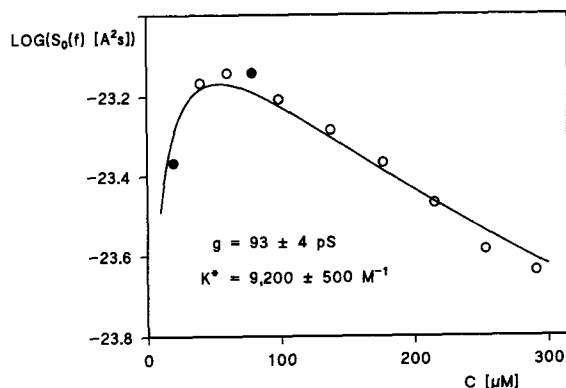


FIGURE 5. Fit of S_0 derived from Lorentzians at different maltotetraose concentrations with Eq. 5 by assuming $k_{-1} = 757 \text{ s}^{-1}$. The solid line was drawn according to Eq. 5 by using $g = 93 \text{ pS}$ and $K^* = 9,200 \text{ M}^{-1}$.

are probably $> 1,000 \text{ s}^{-1}$. The on rate was $\sim 10^7 \text{ M}^{-1} \cdot \text{s}^{-1}$ and is approximately constant for the maltooligosaccharides investigated here.

Sucrose and maltose have approximately the same affinity to LamB (Benz et al., 1987). It is interesting that the relative rates of permeation of the same sugars derived from the vesicle swelling assay (Luckey and Nikaido, 1980a), showed substantial differences. This means probably that the binding of both sugars differs considerably in the on and off rates but their ratio, i.e., the stability constant is very similar. We investigated the sucrose-induced current noise of the LamB channels. Surprisingly, we were able to measure the corner frequencies as a function of the sucrose concentration in the aqueous phase. The on- and off-rate constants for sucrose binding were considerably smaller than the corresponding rate constants for maltotetraose, which suggests that the transport of sucrose through the LamB channel is slow (see Table II).

TABLE II
Parameters of Sugar-induced Transport Noise in LamB of *Escherichia coli*^a

Sugar	n	$k_1/10^5 \text{ M}^{-1}\text{s}^{-1}$	k_{-1}/s^{-1}	K/M^{-1}	g/pS	K^*/M^{-1}
Sucrose	4	6.4 ± 3.8	39 ± 15	165 ± 162		
Maltotriose	10	8400	1950	4300		
Maltotetraose	9	6100 ± 2000	769 ± 160	8100 ± 2800	97.2 ± 36.9	6900 ± 2100
Maltopentaose	6	5300 ± 2000	417 ± 139	13000 ± 9000	77.7 ± 34.2	16000 ± 5000
Maltohexaose	8	4800 ± 600	238 ± 66	20000 ± 8000	70.8 ± 16.2	18000 ± 5000
Maltoheptaose	5	5600 ± 2400	179 ± 72	31000 ± 26000	97.5 ± 68.2	20000 ± 4000

^aThe membranes were formed from diphytanoyl phosphatidylcholine/*n*-decane. The aqueous phase contained 1 M KCl and between 10 and 100 ng/ml LamB; $T = 25^\circ\text{C}$. k_1 and k_{-1} were derived from a fit of the corner frequencies as a function of the sugar concentrations (compare Eq. 3). K is the stability constant for maltopentaose binding derived from the ratio k_1/k_{-1} . The stability constant K^* and the single-channel conductance g were derived from a two parameter fit of S_0 according to Eq. 5. The maltotriose values were derived from the extrapolation of an Arrhenius plot to 25°C (see text). The data represent mean \pm SD of n experiments with the same sugar.

TABLE III
Comparison of Transport Parameters for Different Sugars through LamB^a

Sugar	Φ^*/s^{-1}	Φ_{\dagger}^*/s^{-1}	K/M^{-1}	$k_1/10^3 M^{-1} s^{-1}$	k_{-1}/s^{-1}
Maltose	7150		100§	1100	11000
Maltotriose	4720	1930	4300°	8400	2000
Maltotetraose	1360	748	8100°	6100	770
Maltopentaose		415	13000°	5300	420
Maltohexaose		237	20000°	4800	240
Maltoheptaose	179	179	32000°	5600	180
Trehalose	5430		46§	520	11300
Lactose	643		18§	44	2400
Sucrose	179	29.8	165°	6.4	39
Gentiobiose	3000		250§	900	3600
Melibiose	2360		180§	540	3000
Cellobiose	929		6.7§	53	7900
D-Glucose	20700		9.5§	1200	130000
D-Galactose	16100		24§	1200	50000
D-Fructose	9650		1.7§	500	290000
D-Mannose	11400		6.3§	640	100000

^aThe flux Φ_{\dagger}^* was calculated by using Eq. 9 ($c'' = 40$ mM, $c' = 0$ mM) and the rate constants of Table II. Φ^* was estimated on the basis of the relative rates of permeation of the different sugars taken from Luckey and Nikaido (1980a) and assuming that the maltoheptaose flux under their conditions was $179 s^{-1}$. k_1 was calculated from Eq. 9 by using the stability constants given in the Table. k_{-1} was derived from k_1/K . § K was taken from Benz et al., (1987). ° K was taken from Table II.

Temperature Dependence of Maltotetraose and Maltotriose Binding to the LamB Channel

The binding kinetics of maltotriose could not be measured at room temperature because of the problems described above. However, it was possible to derive from the Lorentzians the corner frequencies for maltotriose binding at small temperature (see Table III). To get some idea about the rate constant of maltotriose-binding to the LamB channel at room temperature, we used the following experimental approach: the rate constants of maltotetraose binding were measured in the temperature range between 0 and 40°C. Then we plotted the on- and off-rate constants of maltotetraose

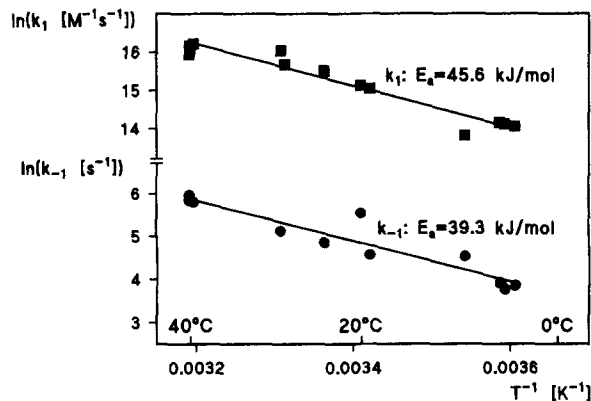


FIGURE 6. Temperature dependence of maltotetraose binding to the lamB channel. k_1 and k_{-1} were plotted as functions of the inverse temperature in Arrhenius plots. The activation energy of the on-rate constant was 45.6 kJ/mol (11 kcal/mol) and that of the off-rate constant 39.3 kJ/mol (9.4 kcal/mol).

binding as a function of the inverse temperature in an Arrhenius plot (see Fig. 6) and calculated the activation energies of these rate constants. We obtained 46 kJ/mol (corresponding to 11 kcal/mol) for the rate constant k_1 and 39 kJ/mol (9.4 kcal/mol) for k_{-1} . This means that the activation energy of the stability constant for maltotriose binding was rather small as compared to that of the individual on- and off-rate constants. The activation energies of the two rate constants were compared with the corresponding energies of maltotriose binding between 0 and 12°C and because they were essentially very similar (i.e., 31.9 kJ/mol for k_1 and 44.6 kJ/mol for k_{-1}), it was possible to give an estimate for the rate constants for maltotriose binding at room temperature (see Table II).

DISCUSSION

The Kinetics of Sugar Binding to Malto porin (LamB) Can Be Derived from the Analysis of the Current Noise

In this study we investigated the sugar-induced current noise of malto porin. This noise has two different aspects. The current noise of the open malto porin channels could be explained by $1/f$ noise up to a frequency of ~ 100 Hz. However, we have shown in a recent study of the $1/f$ noise of the open LamB channel that the information derived from the $1/f$ noise on the channel properties is rather small (Nekolla et al., 1994). The reason is that the $1/f$ noise is probably caused by slow closing and opening kinetics of LamB channels. Because these changes vary from membrane to membrane, they probably contain no information on ion transport through the open channel itself. The power spectra of the current noise in the presence of sugars are governed by some type of Lorentzian because the binding of sugar to the binding site inside the channel (and the block of ion movement through the channel) is controlled by a chemical reaction. The slope of the Lorentzian was not -2 , which would be expected for a real Lorentzian. So far it is not clear why the slope was smaller than expected. It could be caused by a multistate binding site for sugars. However, the slope of the Lorentzian was not dependent on the sugar concentration. Furthermore, the slope (usually between -1.7 and -1.9) was also not much dependent on the type of the sugar. These results argue against a multistate model, although this cannot be excluded. Although the slope of our curve fits was not -2 , we used the term here because of the close analogy to the real Lorentzians observed with gramicidin channels (Kolb, Lauger, and Bamberg, 1975; Kolb and Bamberg, 1977). It is noteworthy also that for the fit of the amiloride-induced current noise of the sodium channels in frog skin, a slope of -1.8 instead of -2 has been observed (Lindemann and van Driessche, 1977a,b; Lindemann, 1980; van Driessche and Lindemann, 1979).

We used here a one-site, two-barrier model for the theoretical treatment of sugar transport through the LamB channel. This model is supported by the experimental observation that the binding of the sugars to the site can be explained by simple 1:1 kinetics even at very high sugar concentrations. We did not observe any indication for the binding of two sugar molecules at the same time to the binding site in both, the titration experiments and in the noise analysis. Furthermore, we did not find any

indication for an asymmetric channel. We are therefore convinced that the simple model provides a good description of the sugar transport through the LamB channel and of the blockage of the ion movement by the binding of sugar, although clearly the experimental results could also be explained by more complicated models, such as a two-site, three-barrier model, if we assumed single-sugar occupancy of the channel.

Our theoretical approach allowed the evaluation of the on- and off-rate constants of a variety of sugars to the binding-site inside the channel. In particular, we were able to calculate the rate constants for the maltooligosaccharide series with three to seven glucose residues. The evaluation of the rate constants of sugar-binding means also that we know the rate-constant for sugar transport. The on rate corresponds to the transport rate from the aqueous phase to the central binding site and the off rate for the rate constant of the inverse movement. The on-rate constant for sugar binding for the maltooligosaccharide series was between 10^6 and $10^7 \text{ M}^{-1} \cdot \text{s}^{-1}$. It is in all cases considerably smaller than the rate of diffusion-controlled reaction processes (Eigen, Kruse, Maass, and De Maeyer, 1964). This means that the rearrangement of groups attached to the protein is probably involved in sugar binding, an assumption, which is supported by the activation energy ($\sim 40 \text{ kJ/mol}$) of the on-rate constant. This was considerably above that of diffusion-controlled reaction processes.

The LamB trimers contain probably three channels. This has been derived on the basis of equilibrium dialysis (Gehring, Cheng, Nikaido, and Jap, 1991) and of LamB-mutants (Ferenci and Lee, 1989). The single-channel conductances as calculated from the power density at very small frequencies suggest the same although the evaluation of the single-channel conductance from S_0 does probably not lead to absolutely precise numbers. This is because g is the least precise number of all parameters derived from the analysis of noise experiments and the errors of all other parameters contribute to the estimation of g (see Eq. 5). In any case, the single-channel conductance derived from single-channel recordings is 150 pS at 1 M KCl (Benz et al., 1986), while the single-channel conductance derived from the noise measurement was in most cases below that value. This means that our results are not inconsistent with the assumption that a LamB trimer contains three individual channels with three binding sites.

The binding of the sugars to the binding site inside the channels is stabilized probably by hydrogen bonds between OH groups from the sugars and carbonyls from the protein. This is very likely because we have shown in a previous study that the channel is selective for cations but does not contain a binding site for ions (Benz et al., 1987). The binding site for sugars has been suggested to be five glucose residues long. The off-rate constant is probably highest for glucose and decreases down to maltoheptaose. This result may also indicate the number of groups involved in sugar binding. On the other hand, it is also possible that long chain maltooligosaccharides move forth and back within the channel several times before they can finally leave the channel to one side. It is noteworthy that starch associates almost irreversibly with the LamB channel (Ferenci et al., 1980), which supports our view of the sugar transport through the LamB channel.

Turnover of Sugars across the LamB Channel and Physiological Role of the Binding Site

The net flux of sugar 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 sugar across one barrier of the two identical potential energy barriers (Benz et al., 1987):

$$\Phi = k_1 \cdot c'' / (1 + K') - k_{-1} \cdot K' / (1 + K'). \quad (6)$$

K' is given by:

$$K' = K \cdot (c' + c'') / 2. \quad (7)$$

In Eq. 6, the rate constants k_1 and k_{-1} are multiplied by the probabilities that the binding site is free or occupied, respectively. Eq. 6 has in the case $c'' = c$, $c' = 0$ the following form:

$$\Phi = k_1 \cdot c / (2 + K \cdot c). \quad (8)$$

The latter form may be used to calculate the flux of a given sugar through one of the three channels in a LamB trimer under the conditions mentioned above. The maximum permeability of the channel for a sugar with an on rate k_1 for its binding to LamB is $k_1/2$, which is obtained at very small sugar concentrations ($c \leq 10 \mu\text{M}$). The flux strongly saturates at high sugar concentrations because the half saturation constant for the sugar flux is $K_S = 1/K$. The maximum turnover of the different sugars through LamB is reached at very high sugar concentration on one side of the membrane and is given by k_{-1} . This means that the flux through LamB is limited at high maltooligosaccharide concentration. It is noteworthy, however, that this is not a serious restriction because the concentration of substrates is small under physiological conditions. For the effective scavenging of nutrients at very small concentrations it seems to be more important to have a high permeability (i.e., a high k_1), which is indeed given for the transport of maltooligosaccharides through the LamB channel.

Our data allow a comparison of the flux of maltooligosaccharides with that of sucrose through the LamB channel. Maltoporin has been shown to be an extremely bad channel for the transport of sucrose into the cell in vivo (Schmid et al., 1991) despite a similar stability constant for sucrose binding as compared to maltose (67 and 100 M^{-1} , respectively; Benz et al., 1987). This result is easy to understand on the basis of our data. The on- and off-rate constants for sucrose binding to the channel were considerably smaller than the corresponding rate constants of maltotriose, which has a smaller transport rate than maltose otherwise it would have been possible for us to measure the corner frequency in the noise experiments. So far it is an open question why the binding of sucrose is so slow as compared with that of maltose. It is possible that the binding of sucrose requires a major rearrangement of the binding site and/or of the sugar, which slows down both the binding and the release processes.

Comparison with the Relative Rate of Sugar Permeation Derived by Luckey and Nikaido (1980a)

Luckey and Nikaido (1980a) measured the relative rate of permeation of different sugars through the LamB channel by using the liposome-swelling technique. They have found remarkable differences in the rate of permeation of different sugars with respect to maltose and postulated the maltooligosaccharide specificity of maltoporin. It is possible to compare our data with that of Luckey and Nikaido (1980a) on the basis of the following considerations. Substituting the experimental conditions of Luckey and Nikaido (1980a) ($c'' = 40$ mM, $c' = 0$) Eq. 8 has the following form:

$$\Phi = k_1 \cdot (40 \text{ mM}) / [2 + K \cdot (40 \text{ mM})] \quad (9)$$

This means that k_1 is given by:

$$k_1 = \Phi \cdot [(50 \text{ M}^{-1}) + K]. \quad (10)$$

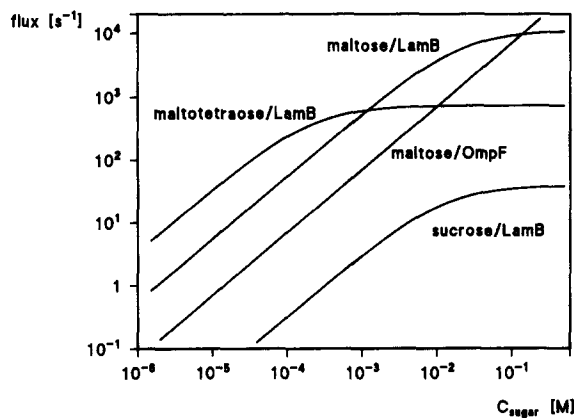


FIGURE 7. Flux of maltose, sucrose and maltotetraose through one single LamB channel as a function of the corresponding sugar concentration on one side of the channel. The concentration on the other side was set to zero. The flux was calculated using Eq. 8 and the rate constants given in Table III. The flux of maltose through OmpF was calculated on the basis on its dimensions (Cowan et al., 1992) and using the Renkin correction factor (Renkin, 1954).

Eq. 10 can be used for a more quantitative description of the sugar transport through LamB using the liposome swelling assay. Maltoheptaose shows a rather slow diffusion in this approach, which means that its limited time resolution (10 s) should not change the boundary conditions too much. If we set the flux of maltoheptaose under the conditions of Luckey and Nikaido (1980a) to the flux, which we would obtain using our rate constants it is possible to calculate from Eq. 10, the corresponding sugar fluxes relative to that of maltoheptaose and the stability constants K , the on and off-rate constants k_1 and k_{-1} for all the sugars used by Luckey and Nikaido (1980a). The transport rate constants of a large number of sugars relative to that of maltoheptaose are summarized in Table III. The data of Table III show that the flux of sugars through the LamB channel is saturated by 40 mM concentration of those sugars with a large affinity for the binding site (i.e., the conditions of Luckey and Nikaido, 1980a). This means that the flux is similar to k_{-1} , which is the turnover number of the sugars through the LamB channel. It is noteworthy that the rate constants show good agreement for long chain maltooligosaccharides but differ

considerably for maltotriose probably because of the limited time resolution of the liposome swelling assay mentioned above. On the other hand, the combination of the results from the noise analysis and the liposome-swelling assay allow the estimation of the rate constants for glucose and maltose, which are not available from the noise analysis alone because of its limited time resolution.

Flux of Sugars through the LamB Channel

The rate constants obtained from the noise measurements and from a combination of our results with those of Luckey and Nikaido (1980a) allow a quantitative evaluation of the sugar flux through LamB. Fig. 7 shows the maximum flux of sucrose, maltose and maltotetraose across LamB calculated on the basis of Eq. 8 under the assumption that the concentration of the sugars on one side (the periplasmic side) is zero. The curves were calculated using the rate constants given in Table III. The flux of maltose through a general diffusion pore was estimated according to the dimensions of OmpF (0.45 nm radius [Cowan, Schirmer, Rummel, Steiert, Gosh, Pauptit, Jansonius, and Rosenbusch, 1992]). It was corrected by using the Renkin correction factor (Renkin, 1954) for the hit of the maltose to the rim of the OmpF channel, which leads to the reflection of the sugar. The flux of sucrose through LamB is ~ 200 times smaller than that of maltose. No wonder that enteric bacteria may contain a plasmid that codes for proteins of an uptake and degradation system for sucrose (Hardesty, Ferran, and DiRienzo, 1991). This plasmid (pUR400) contains also the gene *scrY* that codes for an outer membrane protein, which has a similar function as LamB (Hardesty et al., 1991; Schülein, Schmid, and Benz, 1991). Specific porins have their maximum permeability in the linear range of Fig. 7. The comparison of the different fluxes of Fig. 7 demonstrates again the advantage of a binding site for the maximum scavenging of substrates. It furthermore demonstrates that the flux through a general diffusion pore can exceed that through a specific porin at very high substrate concentration.

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