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Noise Analysis of Ion Current Through the Open and the Sugar-Induced Closed State of the LamB Channel of *Escherichia coli* Outer Membrane: Evaluation of the Sugar Binding Kinetics to the Channel Interior

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ABSTRACT LamB, a sugar-specific channel of *Escherichia coli* outer membrane was reconstituted into lipid bilayer membranes and the current noise was investigated using fast Fourier transformation. The current noise through the open channels had a rather small spectral density, which was a function of the inverse frequency up to about 100 Hz. The spectral density of the noise of the open LamB channels was a quadratic function of the applied voltage. Its magnitude was not correlated to the number of channels in the lipid bilayer membrane. Upon addition of sugars to the aqueous phase the current decreased in a dose-dependent manner. Simultaneously, the spectral density of the current noise increased drastically, which indicated interaction of the sugars with the binding site inside the channel. The frequency dependence of the spectral density was of Lorentzian type, although the power of its frequency dependence was not identical to –2. Analysis of the power density spectra using a previously proposed simple model (Benz, R., A. Schmid, and G. H. Vos-Scheperkeuter. 1987. *J. Membr. Biol.* 100: 12–29.) allowed the evaluation of the on- and the off-rate constants for the maltopentaose binding to the binding site inside the LamB channels. This means also that the maltopentaose flux through the LamB channel could be estimated by assuming a simple one-site, two-barrier model for the sugar transport from the results of the noise analysis.

INTRODUCTION

The outer membrane of Gram-negative bacteria contains two different routes for the uptake of hydrophilic nutrients (Nikaido and Vaara, 1985; Benz, 1988). They are due to the presence of major classes of proteins, called porins (Nakae, 1976, Benz, 1988). The first route consists of porin pores that form large, water-filled channels. They show only little or no specificity for solutes below a defined molecular mass. Other porins such as LamB (maltoporin, Luckey and Nikaido, 1980; Benz et al., 1986, 1987) and Tsx of *Escherichia coli* (Maier et al., 1988) form solute-specific channels in the outer membrane that contain binding sites. These porins are either constitutive or they are induced in the outer membrane when the organisms grow under special conditions. LamB of various *Enterobacteriaceae* is part of the maltose uptake (the malsystem, Szmelcman and Hofnung, 1975; Bloch and Desaymard, 1985). This system is composed of several different proteins located in the inner membrane, the periplasmic space and the outer membrane. The components of the mal-system are induced in various *Enterobacteriaceae* when the growth media contain maltose or maltooligosaccharides (Szmelcman and Hofnung, 1975).

In recent publications it has been demonstrated that LamB of *E. coli* and *Salmonella typhimurium* form ion-permeable channels when reconstituted into lipid bilayer membranes (Benz et al., 1986, 1987; Dargent et al., 1987; Schülein and Benz, 1990). Sugars bind to LamB (Ferenci et al., 1980) and the binding leads to a block of the channel for the permeation of ions (Benz et al., 1986), 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.

The kinetics of the sugar binding could not be evaluated from the titration experiments since the binding of the sugars was much larger than the diffusion of these molecules through the unstirred layer. Furthermore, it has been demonstrated that the sugar binding was not voltage-dependent (Benz et al., 1987). Thus, it was impossible to measure the kinetics of sugar binding by voltage-clamp experiments. On the other hand, we have recently shown that the presence of long chain malto-oligosaccharides did not simply reduce the single-channel conductance but caused also a dramatic increase of the current noise (Schülein and Benz, 1990). These results have suggested that measurements of the sugar-induced current noise of the LamB-channel may give some insight into the kinetics of sugar binding. In this publication we were able to give a quantitative description of the sugar transport through the LamB-channel of *E. coli* using the analysis of the sugar-induced current noise. The analysis was performed using a treatment proposed previously for the study of the kinetics of nerve channels (Conti and Wanke, 1975; Conti et al., 1980), of gramicidin (Kolb et al., 1975) and of the analysis of ameloride-induced block of frog epithelial sodium channels (Lindemann and Van Driessche, 1977; Van Driessche and Lindemann, 1979).

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The results show that the spectral density of the current noise of the open LamB channels (i.e., without sugar) is governed at low frequencies up to 100 Hz by $1/f$ noise. At higher frequencies it is limited by the noise of the membrane capacitance. The addition of sugar increased the current noise and the spectral density; as a function of the frequency, became of Lorentzian type, although the power of the decay was not exactly -2. Analysis of the density spectra allowed the evaluation of the rate constants of sugar binding to the binding site inside LamB.

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 et al., 1984). Briefly, LamB was isolated by (i) extraction of cell envelopes with sodium dodecyl sulfate (SDS) at 60°C and (ii) release of LamB from the protein-peptidoglycan complex by treatment with 0.4 M NaCl-solution. The crude LamB fraction obtained was further purified over a QAE-Sephadex column run in the presence of Triton X-100. The protein was more than 95% pure, and it was dissolved at a concentration of 1 mg/ml in an aqueous solution containing 10 mM Tris hydrochloride (pH 7.5), 0.25 M NaCl, and 1% (v/v) Triton X-100. The protein was in its trimeric form (Benz et al., 1986).

Lipid bilayer experiments

Black lipid bilayer membranes were formed as described previously (Benz et al., 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 mm^2. Membranes were formed by painting onto the holes a 1% solution of diphytanoyl phosphatidylcholine (Avanti Polar Lipids, Alabaster, AL) in *n*-decane. The aqueous salt solutions (Merck, Darmstadt, Germany) were used unbuffered and had a pH around 6. The LamB was added from the concentrated stock solution either to the aqueous phase bathing a membrane in the black state or immediately before membrane formation, to prevent protein inactivation. The temperature was kept at 25°C throughout.

Noise analysis

The membrane current was measured with a pair of Ag/AgCl electrodes switched in series with a 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 Keithley 610C electrometer to measure the absolute magnitude of the membrane current and to calculate the stability constant for sugar binding (Benz et al., 1987). Occasionally the signal was passed through a Krohn-Hite filter (Model 113340). The amplified AC component of the signal was analyzed with a digital signal analyser (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)). Alternatively, the amplified signal was recorded with a digital oscilloscope (Nicolet 2091). The signal was transferred into the HP computer and analyzed with different time-domain windows and fast Fourier transformation. This was performed to check the accuracy of the Ono Sokki CF 210 during signal analysis.

THEORY

In recent publications the movement of the sugars through the LamB channel has been studied in detail (Benz et al., 1986, 1987; Dargent et al., 1987). It has been demonstrated that the minimum requirements for the description of the sugar transport are given by a simple one-site, two-barrier model (Läuger, 1973; Benz et al., 1987; Benz and Hancock, 1987). This model assumes a binding site for sugar in the center of the channel. The rate constant k_1 describes the jump of the sugars from the aqueous phase (concentration c) across the barrier to the central binding site, whereas the inverse movement is described by the rate constant k_{-1}. We found virtually no indication for an asymmetry of the LamB channel, therefore symmetry of the channel regarding the binding site is assumed.

The stability constant of the binding between a sugar molecule and the binding site is $K = k_1/k_{-1}$. Furthermore, we assume that only one sugar can bind to the binding site at a given time and that no sugar or ion (Benz et al., 1986) can pass the channel if the binding site is occupied by a sugar. This means that a sugar can enter the channel only when the binding site is free. This means that LamB (given by P) is open when no sugar S is bound, and closed when the LamB channel is occupied by a sugar, S, to form the nonconducting sugar-channel complex PS

$$P + S \underset{k_{-1}}{\overset{k_1 c}{\rightleftarrows}} PS \tag{1}$$

The probability, p, that the binding site is occupied by a sugar (identical concentrations on both sides) and the channel does not conduct ions is given by

$$p = \frac{K \cdot c}{1 + K \cdot c} \tag{2}$$

and that it is free and the channel conducts ions is given by

$$1 - p = \frac{1}{1 + K \cdot c} \tag{3}$$

The conductance, $G(c)$ $(= I(c)/V_m)$, of a LamB containing membrane in the presence of a sugar with the stability constant, K, and a sugar concentration, c, is given by the probability that the binding site is free

$$G(c) = \frac{G_{\max}}{1 + K \cdot c} \tag{4}$$

where $G_{\max}$ is the membrane conductance before the start of the sugar addition to the aqueous phase (I_0 is the initial current and $I(c)$ is the current at the sugar concentration c). Eq. 4 may also be written as

$$\frac{G_{\max} - G(c)}{G_{\max}} = \frac{I_0 - I(c)}{I_0} = \frac{K \cdot c}{1 + K \cdot c} \tag{5}$$

which 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.

Assuming small perturbations of the number of closed channels due to microscopic variations of the number of bound sugar molecules, the reaction rate of the second order reaction given in (1) is given by (Verveen and De Felice, 1974)

$$1/\tau = 2\pi \cdot f_c = k_1 \cdot c + k_{-1} \tag{6}$$

Where f_c is the corner frequency of the power density spectrum, $S(f)$, which is given by a "Lorentzian" function (Verveen and De Felice, 1974; De Felice, 1981)

$$S(f) = \frac{S_0}{1 + (f/f_c)^a} \tag{7}$$

a is the slope of the decay of the Lorentzian function (usually close to 2) and S_0 is the plateau value of the power density spectrum at small frequencies. It is given by Verveen and De Felice (1974)

$$S_0 = 4 \cdot N \cdot i^2 \cdot p \cdot (1 - p) \cdot \tau. \tag{8}$$

N is the total number of channels (blocked and unblocked) within the membrane and i is the current through one single open channel. The membrane current, $I(c)$, is given by the number of open channels times the current through one single-channel and the probability that the channel is open

$$I(c) = i \cdot N \cdot (1 - p). \tag{9}$$

Equations 8 and 9 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

$$g = \frac{i}{V_m} = \frac{S_0}{4 \cdot V_m \cdot I(c) \cdot p \cdot \tau}. \tag{10}$$

RESULTS

Current noise of the open LamB channel

The measurements of the current noise were performed in the following way: A membrane was formed of diphytanoyl phosphatidylcholine/*n*-decane in 1 M KCl solution. After the membrane was in the black state its specific conductance was measured to make sure that the membrane did not have an unusual high conductance and produced excess noise due to leaks. The experiments were only continued if the specific membrane conductance was about 0.1 nS/cm^2 or below. Fig. 1 shows the spectral density of the current noise as a function of the frequency taken from a colored lamella (*trace 1*) and two black lipid bilayer membranes (*traces 2* and *3*). The power density spectra of membranes without protein were very similar to those, which have been published previously (Kolb et al., 1975; Sauvé and Bamberg, 1978). The data demonstrate that the spectral density was very small at low frequencies. At high frequencies the noise increased again. This was caused by the intrinsic noise of the preamplifier that produces a frequency-dependent current noise through the membrane capacity C_m. The time resolution of the instrumentation was approximately 10 kHz, which was limited in the experiments of Fig. 1 by the bandwidth of the current amplifier (0.3 ms).

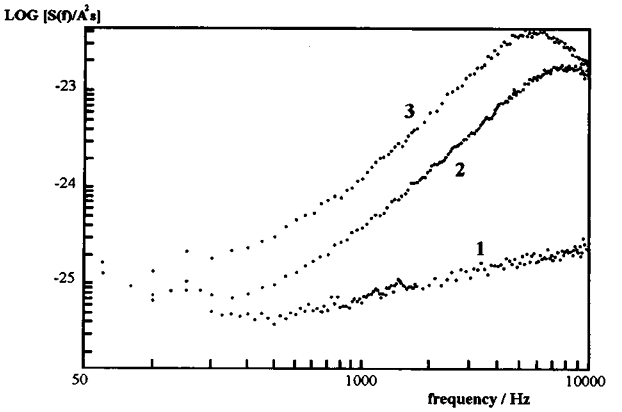

FIGURE 1 Power density spectra of three diphytanoyl phosphatidylcholine/*n*-decane membranes bathed in 1 M KCl. Trace 1 represents the power density spectrum of a colored lamella. Traces 2 and 3 correspond to that of black membranes with slightly different capacities. The bandwidth of the preamplifier was 3 kHz. The applied membrane potential was 20 mV; T = 25°C.

After the control experiment, LamB was added to the aqueous phases on both sides of the membrane in a final concentration of 10–100 ng/ml while stirring to allow equilibration. As has been described previously in detail (Benz et al., 1978) the membrane conductance started to increase after a lag time of a few minutes due to slow aqueous diffusion of the protein through unstirred layers. The conductance increase was caused by the formation of small ion permeable channels (Benz et al., 1986). 30 min after addition of the porin the conductance tended to be constant, and binding studies with sugar could be performed without much error. An additional increase of 10% could be tolerated in these experiments (Benz et al., 1987). However, noise experiments could still not be performed after that time, since the current noise was very critically influenced by small changes of the membrane conductance. Therefore we waited for 40–70 min until the conductance increase had slowed down completely and a virtually stationary conductance level was reached.

Fig. 2 shows the power density spectra of the current noise of four different membranes containing 610 (1), 2480 (2), 1770 (3), and 240 LamB (4) channels, respectively. The number of channel was calculated from their single-channel conductance in single-channel experiments (150 pS; Benz et al. (1986)). The membrane potential was 25 mV in all cases. The power density in the frequency range between 1 and approximately 100 Hz was a function of $1/f$. Above that frequency the power density was first more or less constant and then started 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.

The experiments shown in Fig. 2 suggested that the $1/f$ noise component created by the passage of current through the reconstituted LamB channels was not proportional to the number of channels since the ratio S(1 Hz)/N showed considerable variations. The same may be seen from the data of

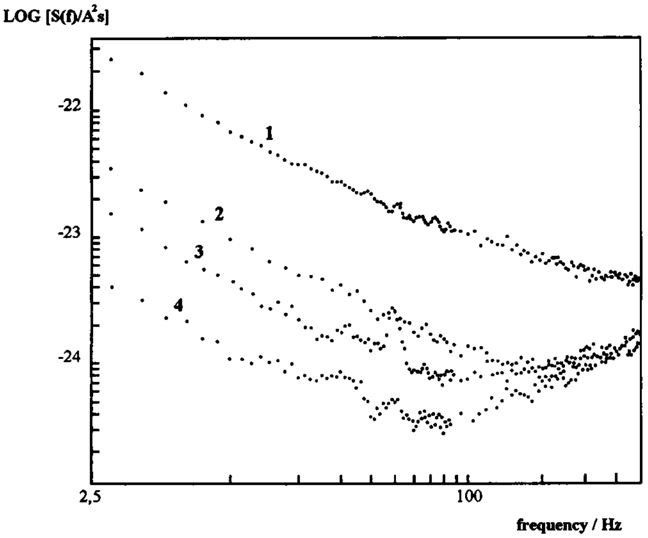

FIGURE 2 Power density spectra of diphytanoyl phosphatidylcholine/*n*-decane membranes containing (*1*) 610 LamB channels, (*2*) 2480 LamB channels, (*3*) 1770 LamB channels, and (*4*) 240 LamB channels. The number of channels were calculated from the membrane conductance and the single-channel conductance of LamB in 1 M KCl (150 pS). The aqueous phase contained 1 M KCl. The applied membrane potential was 25 mV; $T = 25°C$. Note that the amplitudes of the power density spectra were not correlated to the number of open channels.

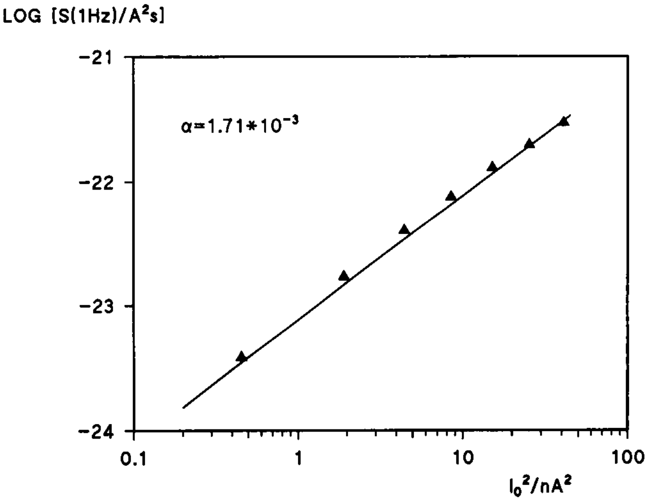

FIGURE 3 Voltage dependence of the 1/*f* current noise through the open LamB channels. Power density spectra of the current noise were measured on a diphytanoyl phosphatidylcholine/*n*-decane membranes containing $N = 225$ LamB channels for different membrane potentials ranging from 20 to 140 mV, and the power density at 1 Hz (S(1 Hz)) was plotted as a function of I_0^2. Note that the data corresponds to a quadratic function of S(1 Hz) on V_m. The aqueous phase contained 1 M KCl. $\alpha = [N \cdot f \cdot S(f)]/I_0^2$ is the fit parameter of the 1/*f* noise using Hooge's formula (Hooge, 1969; Hooge and Gaal, 1971).

Table 1, which gives the number of reconstituted channels, N, the power density of the 1/*f* component, S(1 Hz), at a frequency of 1 Hz, the ratio S(1 Hz)/N, and the parameter α derived from Hooge's formula for eight different membranes (Hooge, 1969; Hooge and Gaal, 1971; see also Discussion).

Voltage dependence of the 1/*f* noise

In additional experiments we studied the voltage-dependence of the 1/*f* noise. Fig. 3 shows the spectral intensity at 1 Hz of the current noise of 225 LamB channels as a function of I_0^2. The number of channels was calculated from the single-channel conductance in 1 M KCl (150 pS; Benz et al. (1986)). The amplitude of the 1/*f* component at 1 Hz was a linear function of I_0^2 (corresponding to a quadratic function of the applied voltage V_m). A similar dependence of the amplitude of the 1/*f* noise has also been found in different other systems (Sibenga and Verveen, 1971; Sauvé and Bamberg, 1978). It is noteworthy that a similar dependence of the 1/*f* noise on the membrane potential was observed for all membranes investigated in this study.

TABLE 1 Parameters of the 1/*f* current noise through open LamB channels

Experiment	I/(nA)	N	$\frac{S(1\ \text{Hz})}{10^{-23}A^2\ \text{s}}$	$\frac{S(1\ \text{Hz})}{N/10^{-26}A^2\ \text{s}}$	α
M42Aug24	2.8	747	90	120	0.0858
M26Sep1	6.0	1600	80	50	0.0356
M51Sep1	1.7	453	13	29	0.0204
M41Sep2	2.25	600	80	130	0.0948
M53Sep2	1.7	453	2.4	5.3	0.0038
M22Sep3	1.0	267	1.8	6.7	0.0048
M1Sep6	9.35	2480	11	4.4	0.0031
M36Sep6	6.75	1800	5	2.8	0.0020

The membranes were formed from diphytanoyl phosphatidylcholine/*n*-decane. The aqueous phase contained 1 M KCl and between 10 and 100 ng/ml LamB from *E. coli* outer membrane. The membrane potential was 25 mV; $T = 20°C$. I_0 is the current through the open channels, and N is their number calculated with a single-channel conductance of 150 pS (Benz et al., 1986). S (1 Hz) is the power density of the 1/*f* noise at 1 Hz and $\alpha = (N \cdot f \cdot S(f))/I_0^2$ is the fit parameter of the 1/*f* noise using Hooge's formula (Hooge, 1969).

Current noise in the presence of maltopentaose

The ion permeation through the LamB channels could be completely blocked by the addition of increasing concentrations of maltooligosaccharides and other sugars (Benz et al., 1987). Apparent stability constants for the binding of the sugar to the binding site inside the channels could be calculated. Interestingly, the addition of maltopentaose had a dramatic influence on the current noise of the channels. Fig. 4 *A* shows such an experiment. Trace 1 represents the control (i.e., the power density spectrum of current noise at 25 mV measured without maltopentaose). Then maltopentaose was added in a concentration of 20 μM and the power density spectrum was measured 2 min after the current was stationary, which indicated complete equilibration of the sugar within the aqueous phase (*trace 2*). Power density spectra taken after different time intervals did not show any systematic variations compared with trace 2. In further experiments the maltopentaose concentration was increased in defined steps. At another concentration of maltopentaose ($c = 78$ μM) the power density spectrum corresponded to trace 3.

The power density spectra of traces 2 and 3 are of the Lorentzian type expected for a random switch with unequal

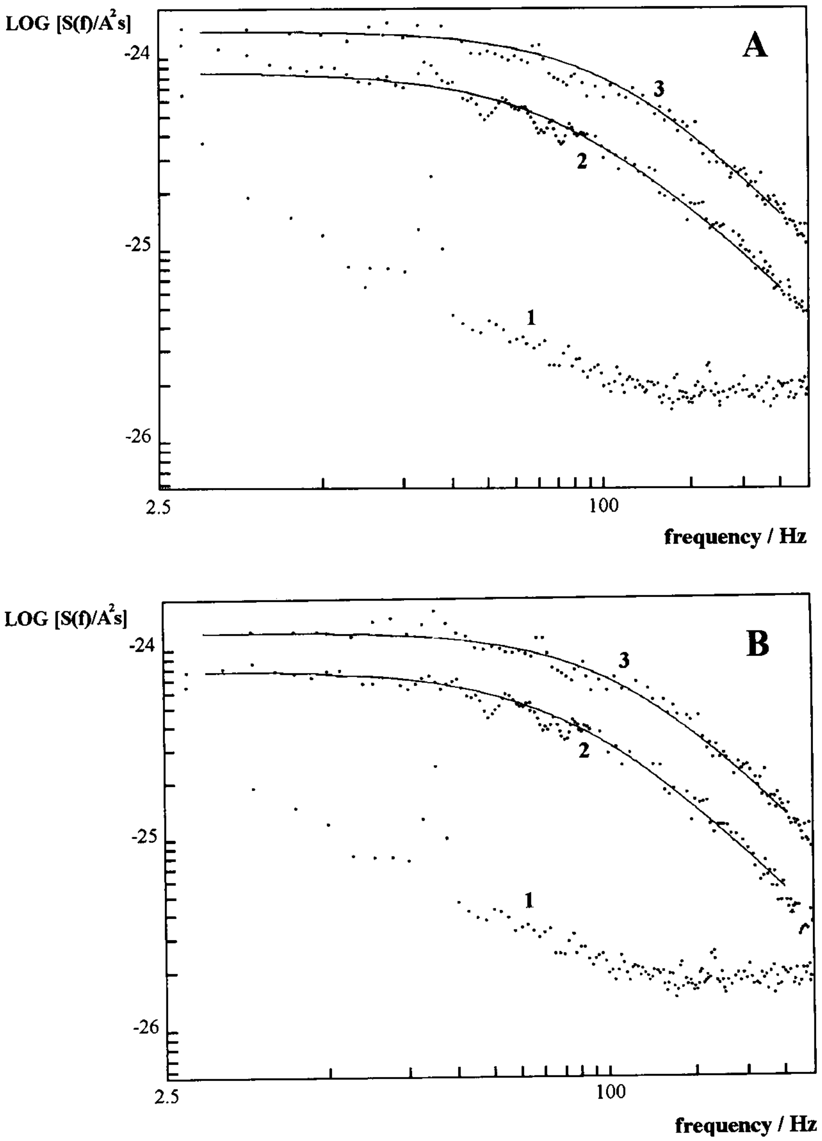

FIGURE 4 (*A*) Power density spectra of maltopentaose-induced current noise of 473 LamB channels. *Trace 1* shows the control (1 M KCl). *Trace 2:* the aqueous phase contained 20 μM maltopentaose (τ = 2 ms; S_0 = 8.5 × 10^{-25}) and trace 3, 78 μM maltopentaose (τ = 1.35 ms; S_0 = 1.4 × 10^{-24}); T = 25°C; V_m = 25 mV. Note that current noise of the LamB channels in the absence of maltopentaose was not substracted. (*B*) Power density spectra of maltopentaose-induced current noise of 473 LamB channels. *Trace 1* shows the control (1 M KCl). *Trace 2:* the aqueous phase contained 20 μM maltopentaose and the power density spectrum of *Trace 1* was substracted (τ = 2 ms; S_0 = 7.9 × 10^{-25}). *Trace 3:* the aqueous phase contained 78 μM maltopentaose and the power density spectrum of Trace 1 was substracted (τ = 1.35 ms; S_0 = 1.25 × 10^{-24}); T = 25°C; V_m = 25 mV.

on and off probabilities (Verveen and De Felice, 1974; Conti and Wanke, 1975; De Felice, 1981). Both could be fitted to Eq. 7. However, the exponent, a, of the Lorentzian was approximately 1.6 in the experiments given in Fig. 4 *A*, while a real "Lorentzian" requires an exponent of two. It is noteworthy that similar exponents have been found also in other studies (Lindemann and Van Driessche, 1977). The use of real "Lorentzians" (a = 2) had no influence on S_0 and changed the corner frequency, f_c, only to a very small degree. Fig. 4 *B* shows the same experiments but in this case the background noise given by trace 1 was substracted from the original records of the power density (*traces 2* and *3* of Fig. 4 *A* minus *trace 1*). A comparison of traces 1 and 2 with those of Fig. 4 *B* shows that the background noise of the open LamB channels had a rather small influence on the shape of the power density spectra. The corner frequency, f_c, was in all cases independent on the correction. The power density at low frequencies, S_0, was somewhat more sensitive to the correction of the Lorentzian and increased slightly when the $1/f$ noise it was not substracted.

Evaluation of the rate constants of maltopentaose binding

The corner frequencies, f_c, of the Lorentzians increased with increasing sugar concentration. f_c of the experiments shown in Fig. 4 and of other maltopentaose concentrations (see Table 2) could reasonably well be fitted to Eq. 6 as it is shown in Fig. 5. The rate constants for the binding of maltopentaose to the LamB channel were k_1 = 3.44 × 10^6 L/(mols) and k_{-1} = 475 1/s. This corresponds to a stability constant, K, for the binding of maltopentaose to the binding site inside the LamB channel of 7,240 1/M. This value agreed reasonably well with the results of the titration experiments, which have been measured previously (17,000 1/M; Benz et al. (1987)). These results indicate that the kinetics of the transport of uncharged molecules through a porin channel, which contains a binding site for these molecules, can be studied by the measurement of the ion transport.

Fig. 4 demonstrates that the background noise of the open LamB channels did not influence the Lorentzians very much.

TABLE 2 Parameters of the fit of the power density spectra of the maltopentaose-induced block of LamB channels with Lorentzians

Experiment	$C/\mu M$	I/nA	S_0/A^2s	τ/ms
M50AugE	0	1.42		
M51AugE	20	1.25	7.9E-25	2.00
M52AugE	39	1.12	1.3E-24	1.60
M53AugE	59	1.01	1.4E-24	1.45
M54AugE	78	0.93	1.25E-24	1.35
M55AugE	97	0.85	1.3E-24	1.20
M56AugE	113	0.80	1.3E-24	1.10
M57AugE	140	0.72	1.2E-24	1.10
M58AugE	170	0.56	8.6E-25	0.95

The membrane was formed from diphytanoyl phosphatidylcholine/*n*-decane. The aqueous phase contained 1 M KCl and 50 ng/ml LamB. The membrane potential was 25 mV; T = 20°C. The data were taken from experiments M50–58AugE (measured from the same membrane as in Fig. 4) by using Eq. 7 for the fit of the Lorentzians. The $1/f$ background noise was substracted in all cases.

Thus, it was not necessary to substract the background noise in our experiments since it influenced only S_0 to some extent. On the other hand, the subtraction of the background noise allowed a somewhat better time resolution, in particular at high frequencies, because the contribution of the membrane capacitance to the Lorentzians was omitted or decreased.

It is noteworthy that the kinetics of sugar binding to the LamB channels was fairly independent of the single membrane. Table 3 shows the results of noise measurements taken from six different membranes. Despite large variations in the number of channels in a single membrane we observed on- and off-rates for the sugar binding which range more and less within a factor of two from the mean values. Similarly, the stability constants as calculated from the corner frequencies for the binding of maltopentaose to LamB were also similar for the individual experiments and showed considerable agreement to that, which has been found previously for titration experiments (Benz et al., 1987).

Estimation of the single-channel conductance of the LamB channel from the current noise

The analysis of the current noise allows also the estimation of the single-channel conductance, g, from the plateau value, S_0, of the power density at low frequencies according to Eq. 10. Table 2 shows the dependence of S_0 on the maltopentaose concentration taken from the series of experiments of Fig. 4. The data for S_0 could be fitted to Eq. 11, which was derived from a combination of Eqs. 4, 6, and 10

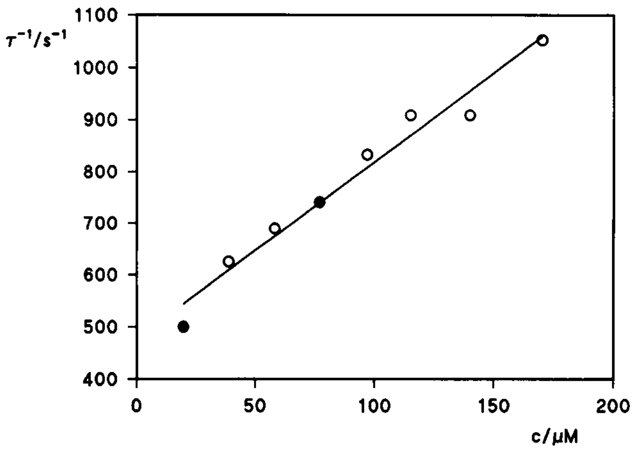

FIGURE 5 Dependence of $2\pi f_c = 1/\tau$ on the maltopentaose concentration in the aqueous phase. The data were derived from the fit of the power density spectra with Lorentzians similar to those given in Fig. 4 *B* (●, 20 and 78 μM) and for other maltopentaose concentrations ranging between 39 and 170 μM (○, see Table 2). The aqueous phase contained 1 M KCl. The applied membrane potential was 25 mV; T = 25°C.

$$S_0 = \frac{4 \cdot g \cdot V_m \cdot I_0 \cdot K \cdot c}{k_{-1}(1 + K \cdot c)^3}. \tag{11}$$

Eq. 11 means that the single-channel conductance and the stability constant for the sugar binding can be calculated when the rate constant for the off-reaction of the sugar binding is known together with the initial current, I_0, without sugar. Fig. 6 shows such a double parameter fit of the power densities at low frequencies taken from Table 2 by using Eq. 11. The off rate constant k_{-1} for maltopentaose binding was used from the fit of Fig. 5 (k_{-1} = 475 1/s). The single-channel conductance estimated from the fit in Fig. 6 was approximately 38 pS. Similar single-channel conductances were also derived from other experiments with the LamB-maltopentaose system. The results of six experiments are summarized in Table 3. The single-channel conductances were always smaller than those measured in single-channel experiments (g = 150 pS; Benz et al. (1986)). This result could have to do with the underestimation of the single-

TABLE 3 Parameters of maltopentaose-induced transport noise in LamB of *E. coli*

Experiments	N	$k_1/10^6$ L/(mols)	k_1/s^{-1}	K/L mol^{-1}	g/pS	K^*/L mol^{-1}
M52–59Mar1	768	5.68 ± 0.28	435 ± 14	13,060 ± 1,060	92.9 ± 8.4	21,620 ± 3,780
M17–25SepE	1273	7.75 ± 0.33	290 ± 35	26,720 ± 4,360	59.7 ± 2.9	16,440 ± 970
M7–16SepE	3733	7.29 ± 0.29	242 ± 43	30,120 ± 6,550	47.9 ± 3.1	17,870 ± 1,170
M50–58AugE	473	3.44 ± 0.25	475 ± 25	7,240 ± 910	37.5 ± 1.3	7,300 ± 600
M35–56Sep8	6901	4.92 ± 0.38	630 ± 27	7,810 ± 940	123.0 ± 2.0	13,990 ± 420
M6–25Nov10	65	2.90 ± 0.24	428 ± 35	6,780 ± 1,110	105.0 ± 5.0	18,990 ± 940
Mean ± SD		5.30 ± 1.90	420 ± 140	15,300 ± 10,000	78.0 ± 34.0	15,900 ± 4,900

The membranes were formed from diphytanoyl phosphatidylcholine/*n*-decane. The aqueous phase contained 1 M KCl and between 10 and 100 ng/ml LamB. k_1 and k_{-1} were derived from a fit of the corner frequencies as a function of the maltopentaose concentration (compare Eq. 6). 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. 11. The data represent mean ± SD from the individual fits and the last row represents mean ± SD of all experiments.

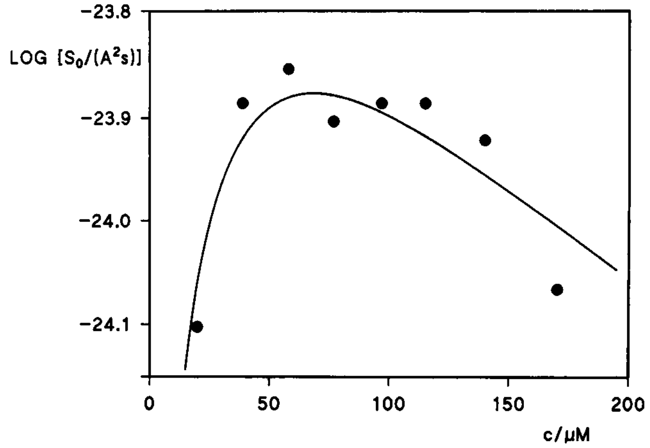

FIGURE 6 Fit of S_0 derived from Lorentzians at different maltopentaose concentrations with Eq. 11 by assuming k_{-1} = 475 1/s. The experimental data were taken from Table 2. The solid line was drawn according to Eq. 11 by using g = 37.5 pS and K^* = 7,300 1/M.

channel conductance, which has occasionally been observed in noise experiments (Kolb and Bamberg, 1977). On the other hand, it is likely that the LamB trimers contain three identical channels (Ferenci and Lee, 1989; Gehring et al., 1991). Table 3 contains also the stability constants, K^*, for maltopentaose binding derived from two parameters fits of S_0 as a function of the maltopentaose concentration similar to that shown in Fig. 6. In most cases we observed a reasonable agreement between the stability constants derived from the corner frequencies and from two parameters fits similarly to that of Fig. 6 (see Table 3).

Voltage dependence of the transport noise

In additional experiments we studied the voltage dependence of the transport noise, i.e., the voltage dependence of the Lorentzians. Fig. 7 shows the power density spectra of a membrane that contains 225 LamB channels for different voltages (0, 20, 40, and 100 mV). The aqueous phase contained 1 M KCl and 26.7 μM maltopentaose. It is noteworthy that the corner frequencies were found to be independent on the voltage. This means that the sugar binding was independent on the membrane potential. This result agrees with previous current-voltage curves, which indicate that the binding of sugars to the binding site inside the LamB channel could not be disturbed by the increase of the membrane potential (Benz et al., 1987). The power density S_0 of the current noise was a linear function of the second power of the membrane voltage as Fig. 8 clearly demonstrates for two different maltopentaose concentrations (26.7 and 80.1 μM).

DISCUSSION

The noise analysis of the current through the LamB channels has several different aspects. The first is the study of the noise of the open channels. They exhibit 1/f noise, which showed considerable variations from membrane to membrane and was not correlated to the number of open channels. This observation makes it rather unlikely that the current through the open channel is responsible for the 1/f noise. Sugars block the channel in an association-dissociation reaction. The power density of the current noise increases, and it is governed by Lorentzians. The analyses of the Lorentzians allow the evaluation of the rate constants and the stability constants for sugar binding. Finally, the single-channel conductance of the LamB monomers can be estimated and compared to that of the single-channel experiments. The evaluation of the rate constants allows also the analysis of substrate transport through the channels and the comparison with the in vivo situation.

The 1/*f* noise of the open LamB channels

The porin channels including LamB from the outer membrane of Gram-negative bacteria have long lifetimes of more

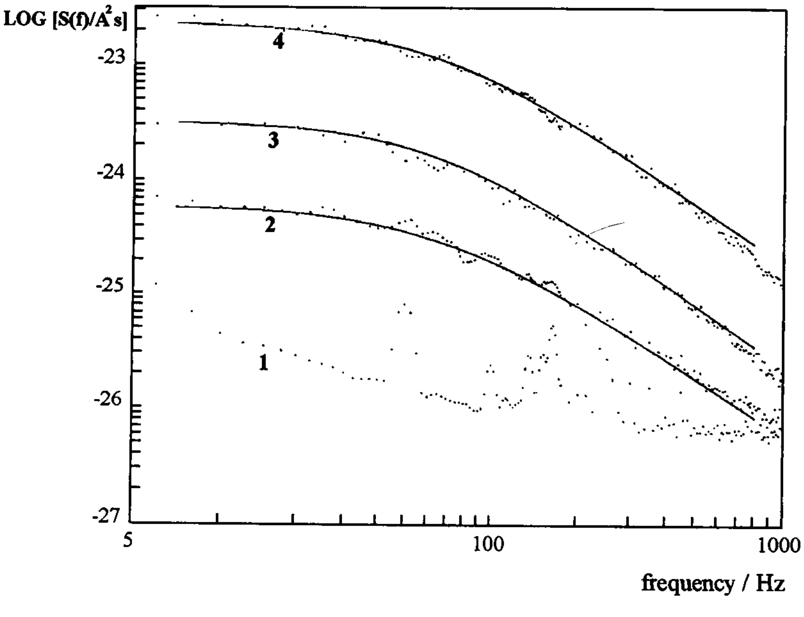

FIGURE 7 Voltage dependence of the maltopentaose-induced transport noise in LamB channels. Power density spectra of the current noise were measured on a diphytanoyl phosphatidylcholine/n-decane membranes containing 225 LamB channels for four different membrane potentials (0, 20, 40, and 100 mV; *Traces 1, 2, 3, 4*). Note that the Lorentzian could be fitted to Eq. 7 by using the same corner frequencies (2.4 ms) but different S_0 (*trace 2*, 6.0 × 10^{-25}; *trace 3*, 3.2 × 10^{-24}; *trace 4*, 2.3 × 10^{-23}). The aqueous phase contained 1 M KCl and 26.7 μM maltopentaose.

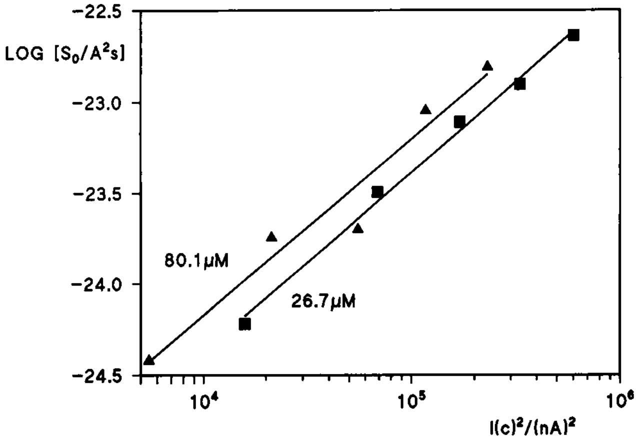

FIGURE 8 Voltage dependence of the maltopentaose-induced transport noise through LamB channels. Power density spectra of the current noise were measured on a diphytanoyl phosphatidylcholine/*n*-decane membranes containing 225 LamB channels for different membrane potentials ranging from 20 to 100 mV and the power density S_0 was plotted as a function of $I(c)^2$. Note that the data corresponds to a quadratic function of S_0 on V_m. The aqueous phase contained 1 M KCl and two different maltopentaose concentrations (26.7 and 80.1 μM).

than several minutes in reconstituted systems (Benz et al., 1978, 1986). This means that these channels do not exhibit the typical opening and closing characteristics of ion channels from nerve and muscle tissues. The current noise of the open channel should be rather small in this case. Nevertheless, the reconstitution of the LamB channels into lipid bilayer membranes resulted in an increase of the power density of the membrane noise at low frequencies. The power density decreased with increasing frequency and showed the typical $1/f$ dependence, which has also been observed in other systems including the head to head dimerized gramicidin channel. (Sibenga and Verveen, 1972; Conti and Wanke, 1975; Bamberg and Janko, 1977, Sauvé and Bamberg, 1978). For the noise analysis of the open gramicidin channel Hooge's formalism could be used with satisfactory accuracy (Sauvé and Bamberg, 1978). However, it was not possible to fit our data to Hooge's formula (Hooge, 1969; Hooge and Gaal, 1971)

$$S(f) = \frac{\alpha}{N \cdot f} I_0^2, \qquad (12)$$

since the factor α varied considerably from experiment to experiment (N is the number of LamB channels in a membrane; see Table 1). In particular, α varies about 50-fold from the lowest to the highest value. This means probably that the current through the single open LamB channel is not alone responsible for $1/f$ noise observed in our system, and it may be questionable if the current through any open channel is responsible for the $1/f$ noise in these systems, in which $1/f$ noise has been observed. In particular, the dimerised gramicidin-channel shows some fast brief interruptions in the channel current (Bamberg and Janko (1977); similar as the gramicidin A channel (Ring, 1985; Sigworth et al., 1987)), and it has been demonstrated in another study that the occurrence of several corner frequencies in power density spectra of current noise can mimic $1/f$ noise (Sauvé and Szabo, 1985), i.e., it is possible that the noise is caused by the channel flickering and not by the current through the open channel.

This means that we have to look for other reasons to explain the $1/f$ noise in the LamB system. One possible explanation is that the lifetime of the channels is not infinite. In fact, we have shown that porin channels open and close in multichannel systems, i.e., when many channels are reconstituted in lipid bilayer membranes (Benz et al., 1980; Schülein et al., 1991). The reason for this channel closure is not clear so far. The channels may disappear since they meet micro lenses or the torus of the membrane. It is also possible that the channel structure collapses because of other reasons. Simultaneously new channels may open caused by the reconstitution of new LamB trimers into the membrane. The processes of channel closure and opening (the number of channels was virtually constant in our experiments) are probably not correlated to one another on the basis of a chemical reaction. This means that the current noise in the LamB system without the sugar creates $1/f$ noise since events in different time regimes may be involved (Sauvé and Szabo, 1985). If we assume that the slow closure and opening of channels create the $1/f$ noise, the variation of the open channel noise from membrane to membrane is easy to understand. If these considerations would be correct they would also explain the $1/f$ noise, which has been observed in biological and artificial membranes (Sibenga and Verveen, 1972; Dorset and Fishman, 1975; Sauvé and Bamberg, 1978; see also Verveen and De Felice, 1974; and De Felice, 1981, for reviews), i.e. the $1/f$ noise in these system is also caused by slow drift of the number of channels and not by the current trough an individual conductive unit. On the other hand, we cannot completely exclude that the current through the open channels contributes to the $1/f$ noise. If this was correct the parameter α has for the open channel noise a value, which is either close to the smallest α of Table 1, or it is even below that value (i.e., $\alpha \leq 0.002$, or $\alpha \leq 0.00067$ in the case of three channels in a trimer; see below).

On and off rate constants for maltopentaose binding

The noise of the current through the LamB channels increased considerably when maltopentaose was added to the aqueous phase on both sides of the membrane. The $1/f$ noise was shifted to a Lorentzian type power spectrum due to appearance and disappearance of blocked LamB channels. The corner frequency f_c was found to be a function of the maltopentaose concentration. Similar spectra of the current noise have been found for the transitory channels in biological and artificial membranes (Verveen and De Felice, 1974; Conti et al., 1975; Conti and Wanke, 1975; Kolb et al., 1975; Kolb and Bamberg, 1977; De Felice, 1981; Kolb, 1984). The analysis of the current noise is similar to that used previously for the study of epithelial ion transport (Lindemann and Van Driessche, 1977; Van Driessche and Lindemann, 1979; see Lindemann, 1980, for a review). In this case the epithelial sodium channels are also permanently open. The addition of ameloride leads to a reversible block of the sodium channels. The analysis of the current noise in the presence of ameloride allows the evaluation of the rate constants of the ameloride-induced block of the sodium channels, i.e., the binding reaction of ameloride (Van Driessche and Lindemann, 1979). Furthermore, the single-channel conductance of the sodium channels could be calculated from the power density plateau at low frequencies (Van Driessche and Lindemann, 1979; Lindemann, 1980).

In this study the main goal of the noise analysis of the maltopentaose-induced block of the LamB channel is not the single-channel conductance since this is known from the single-channel recordings (Benz et al., 1986; Dargent et al., 1987). The analysis of the current noise allows the calculation of binding kinetics of the sugar to the binding site inside the channel, which cannot be derived from the titration experiments since the movement of the sugar molecules through unstirred layers on the membrane surface is much slower than the binding kinetics. This means that the transport of sugars through the LamB channel can be elucidated by the noise analysis and a quantitative description of sugar transport is possible. This is based on a one-site, two-barrier model for the mechanism of sugar transport through the LamB channel. The assumption of one binding site and two barriers is supported by the experimental observation that the binding of the sugars to the site can be explained by simple 1:1 kinetics in the noise experiments 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, i.e., the occurrence of two Lorentzians. We are therefore convinced that the simple model provides a good description of maltopentaose transport through the LamB channel and of the blockage of the ion movement by the binding of sugar, although also more complicated models may also explain the experimental results.

Maltopentaose transport

The absolute rates for the sugar transport through LamB were not known. Only Luckey and Nikaido (1980) have been able to give some qualitative numbers of the sugar flux relative to maltose based on the liposome-swelling assay (Luckey and Nikaido, 1980). The on-rate of maltopentaose movement through the LamB channel was approximately 5×10^6 L/(mols), which means that it is about 1000-fold smaller than that of diffusion-controlled reaction processes (Eigen et al., 1964). The off-rate was about 400 1/s and represents the rate limiting step of sugar movement. The knowledge of k_1 and k_{-1} allows the estimation of the net flux of maltopentaose molecules, ϕ, through the channel under stationary conditions as the result of a concentration gradient $c'' - c'$ across the membrane (Benz et al., 1987)

$$\phi = \frac{k_1 \cdot c''}{1 + K'} - \frac{k_{-1} \cdot K'}{1 + K'}, \tag{13}$$

where K' is given by

$$K' = K(c' + c'')/2. \tag{14}$$

In Eq. 13 the rate constants k_1 and k_{-1} are multiplied by the probabilities that the binding site is free or occupied, respectively. Equation 13 has in the case $c'' = c$, $c' = 0$ the form

$$\phi = \frac{k_1 \cdot c}{2 + K \cdot c}. \tag{15}$$

The latter form may be used to calculate the maltopentaose flux under the conditions mentioned above. The maximum permeability of the channel for maltopentaose is $k_1/2$, which is obtained at very small sugar concentrations ($c \leq 10$ μM). The flux strongly saturates at high sugar concentrations since the half saturation constant for the sugar flux is about 1/15,000 L/(mols) = 67 μM. The maximum turnover of maltopentaose through LamB is reached at very large sugar concentration on one side of the membrane and is given by $k_{-1} = 420$ 1/s. It is noteworthy that the latter number is less important under *in vivo* conditions since the bacteria live normally under very dilute substrate concentrations.

The single channel conductance derived from noise measurement is smaller than that obtained from single-channel records

The mean single-channel conductance, g, derived from the noise measurements was 78 pS. This is considerable below the value measured in single-channel experiments ($g = 150$ pS (Benz et al., 1986)). There exist several explanations for the possible underestimation of g. First Eq. 8 that is used for the fit of the plateau value of S_0 is only valid for an exponent of $a = -2$ in the Lorentzian function, although the use of exponents around -1.6 did not change S_0 and g very much. Second, 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. 11). On the other hand, it has been argued (Kolb and Bamberg, 1977; Kolb, 1980) that the analysis of current noise within channels tends to underestimate the single-channel conductance. However, the most convincing reason for the small value of g is probably caused by the architecture of LamB in *E. coli* outer membrane. LamB is organized as trimers in the outer membrane (Palva and Westermann, 1979) and one trimer contains three channels surrounded by a β-sheet structure (Clement and Hofnung, 1981; Ferenci et al., 1988; Ferenci and Lee, 1989) and three binding sites for sugars (Gehring et al., 1991). Thus, it is very likely that in the single-channel records three channels are measured simultaneously ($g = 150$ pS), while the noise analysis recognises the single conductive unit ($g = 50$ pS). The crystallization and the three-dimensional reconstitution of LamB (Lepault et al., 1988) is unfortunately not such far that this open question can be decided.

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