Transport of Maltodextrins through Maltoporin: A Single-Channel Study

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ABSTRACT Transport of sugars through maltoporin channels reconstituted into planar lipid membranes has traditionally been addressed using multichannel preparations. Here we show that single-channel experiments offer new possibilities to reveal molecular details of the interaction between the sugar and the channel. We analyze time-resolved transient interruptions in the maltoporin ionic current in the presence of differently sized maltodextrins. We find for all studied sugars, from maltotriose to maltoheptaose, that only one sugar molecule is required to completely block one of the pores in the maltoporin trimer. The probability of simultaneous blockage of different pores increases with sugar concentration in a manner that demonstrates their mutual independence. The maltoporin channel is asymmetric and, added from one side only, predominantly inserts in an oriented manner. The asymmetry of the channel structure manifests itself in two ways. First, it is seen as an asymmetrical response to applied voltage at otherwise symmetrical conditions; second, as asymmetrical rates of sugar entry into the channel with asymmetrical (one-sided) sugar addition. Importantly, we find that the sugar residence time in the pore does not depend on which side the sugar is added. This voltage-dependent time is the same for symmetrical, *cis*, or *trans* sugar addition. This observation suggests that once a sugar molecule is captured by the "greasy slide" of the channel, it spends enough time there to "forget" from what entrance it was captured. This also means that the blockage events studied here represent sugar translocation events, and not just binding at and release from the same entrance of the channel.

INTRODUCTION

To maintain homeostasis, cells have to exchange substrates across their walls. In part this is accomplished via pore-forming proteins that span the cell membrane. Gram-negative bacteria like *Escherichia coli* are surrounded by two membranes, an outer and inner membrane. Protein pores of the outer membrane can be divided into two categories: general and solute-specific channels (Nikaido and Vaara, 1985; Benz, 1988; Jap and Walian, 1990; Schulz, 1996; Schirmer, 1998). The general diffusion channels are more abundant and have little specificity for small solutes. They exhibit only a weak selectivity toward cations or anions. The outer membrane also contains solute-specific channels whose population can be boosted under special conditions. At very low substrate concentrations the solute-specific pores with binding structures inside the pore are more effective in transporting the substrate through the cell wall than the general diffusion pores (Nikaido, 1992). One such solute-specific channel is maltoporin (also called LamB) that facilitates transport of maltooligosaccharides (Boos and Shuman, 1998).

Maltoporin was first identified as the receptor for λ-phage in *E. coli* (Randall-Hazelbauer and Schwartz, 1973). It was shown that this membrane protein is induced during growth on maltodextrins and is involved in the maltose transport (Szmelcman and Hofnung, 1975). The maltoporin channels provide a faster uptake of maltooligosaccharides than of other oligosaccharides. Permeation rates measured by the liposome-swelling assay showed that maltoporin discriminates between the transported substrates based both on their size and conformation (Luckey and Nikaido, 1980).

The crystallographic structure of maltoporin reveals its homotrimeric organization (Schirmer et al., 1995). Each monomer is formed by an 18-stranded β-barrel with short turns at the periplasmic side and large irregular loops at the outside of the cell. The third loop, L3, folds inside the β-barrel and forms a constriction at the middle of the channel, giving the pore an hourglass shape. The channel has a slight helical twist following the secondary structure of larger maltodextrins. The structure of sugar-soaked crystals of maltoporin has also been determined (Dutzler et al., 1995). It shows a specific sugar translocation pathway of an aromatic amino acid "greasy-slide" aligned by polar track residues. Sugar residues are in van der Waals contact with the greasy slide via the hydrophobic face of their sugar rings. In addition, there are hydrogen bonds between the sugar hydroxyl groups and the polar track residues.

Maltoporin channels have been extensively studied using planar lipid bilayers, a technique that offers the advantages of a well-controlled environment. When reconstituted into a lipid membrane, maltoporin forms ion-permeable channels (Schindler and Rosenbusch, 1978; Dargent et al., 1987; Benz et al., 1986). Sugars bind to maltoporin (Ferenci et al., 1980; Luckey and Nikaido, 1980) and block the small ion permeation (Benz et al., 1986, 1987), which suggests that the binding site is within the channel's water-filled pore. Sugar titration experiments gave "stability constants" that increased with the length of maltodextrin from $(400\ \mu\mathrm{M})^{-1}$

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for maltriose to $(67\ \mu M)^{-1}$ for maltoheptaose (Benz et al., 1987). A 30-fold increase in the binding strength between maltose and maltotriose, but less than 2-fold increase between tetraose and pentaose, indicate that the binding site extends over three to four glucose residues (Ferenci, 1989), which is in agreement with the more recent crystallographic structure (Dutzler et al., 1995).

To address transport of sugars through maltoporin, Benz and his colleagues have initiated noise analysis (Nekolla et al., 1994) of ion currents through reconstituted maltoporin channels. In addition to the binding constants that are determined from the effect of sugar addition on the average current, noise analysis gives the absolute rates of the sugar-binding reaction.

The equilibrium binding constants derived from noise analysis increase for the maltodextrin series maltotriose (m3) to maltoheptaose (m7) from $(233\ \mu M)^{-1}$, $(123\ \mu M)^{-1}$, $(77\ \mu M)^{-1}$, $(50\ \mu M)^{-1}$ to $(32\ \mu M)^{-1}$ (Andersen et al., 1995), although a significantly smaller value of $(286\ \mu M)^{-1}$ has been reported for maltopentaose (m5) (Winterhalter, 1999). The on-rate constants were found to be $8.4 \cdot 10^6\ (M{\cdot}s)^{-1}$ for maltotriose and remaining approximately invariable at $5.5 \cdot 10^6\ (M{\cdot}s)^{-1}$ for maltotetraose (m4) to meltoheptaose (Andersen et al., 1995). It is interesting to note that maltoheptaose is the longest linear maltooligosaccharide that can be utilized by the cell (Wandersman et al., 1979).

These examples show that all the basic features of thermodynamics and kinetics of sugar binding to maltoporin channels can be obtained by noise analysis of multichannel membranes. However, recent progress in single-molecule studies (e.g., Xie and Lu, 1999; Bai et al., 1999; Mehta et al., 1999, Merkel et al., 1999; Bezrukov, 2000; Laughlin et al., 2000) demonstrates that the time-resolved observation of single molecules reveals much finer details than traditional experiments with large ensembles of molecules. The detailed picture of molecular interactions involved in transport phenomena is very helpful for understanding the underlying physics.

Here we present results obtained for single maltoporin channels reconstituted into planar lipid bilayers. We have been able to resolve transient interruptions in the small-ion current through the channel that are induced by single sugar molecules. We show that all sugar molecules, from maltotriose to maltoheptaose, block one of the monomers in the maltoporin trimer on the time scale that is accessible with the time-resolution of reconstitution experiments. For all the sugars the blockage seems to be complete. In the case of maltohexaose, which, together with maltoheptaose, gives the longest blockages, the residual current through a monomer is smaller than 2% of its initial value. By analyzing the probability of blockage events as a function of sugar concentration we establish that blockages of different monomers are mutually independent. The probability of finding the channel at a particular state of blockage is perfectly described by a corresponding binomial distribution for the first-order blocking reaction.

The channel asymmetry at the single-channel level was initially seen as a sign-dependent response to the applied voltage under otherwise symmetric conditions (Bezrukov et al., 2000). It is now explored in experiments with one-sided addition of sugars. We find that the rate constants for sugar entering the channel differ significantly for the *cis* and *trans* sides. They also depend on applied voltage bias. However, once the sugar molecule has found its way into the channel at a given voltage bias, it resides there for the same average time. This result demonstrates that the blocking events we report here are related to sugar interaction with the continuous set of binding sites within the channel, the so-called "greasy slide" of the pore and reflect sugar *translocation* through the channel.

MATERIALS AND METHODS

To form planar lipid bilayers with the lipid monolayer opposition technique (Montal and Mueller, 1972) we used a 0.25% solution of diphytanoylphosphatidylcholine (Avanti Polar Lipids, Inc., Alabaster, AL) in pentane (Burdick and Jackson, Muskegon, MI). A Teflon cell with a 70-μm diameter aperture in the 15-μm-thick Teflon partition and silver chloride electrodes with agarose bridges were described previously (Bezrukov and Vodyanoy, 1993). The total capacitance was 50-60 pF, the film capacitance was close to 25 pF. Small amounts of wild-type maltoporin from a diluted stock solution of 1 mg/ml containing 1% (vol/vol) of OctylPOE from Alexis, Switzerland, were added to the *cis* side of the chamber. The final concentration of the porin in the membrane-bathing solution (1 M KCl, 1 mM $CaCl_2$, and 10 mM Tris buffered to pH 7.4) was ~0.1 pM.

Spontaneous insertion of single maltoporin channels usually happened within minutes after protein addition to the aqueous phase. Both the small-ion conductance and the sugar transport properties demonstrate that in our reconstitution protocol the channel insertion is directional. As we argue based on experiments with bacteriophage binding (Van Gelder et al., 2000; Bezrukov et al., 2000) the *cis* side of the lipid bilayer corresponds to the inner side of the outer bacterial membrane. Our convention is that a plus sign means that the *cis* side of the membrane cell compartment, that is, the side of protein addition, is more positive.

Maltodextrins of various lengths (maltotriose, maltotetraose, maltopentaose, maltohexaose, and maltoheptaose) were purchased from Sigma Chemical Company (St. Louis, MO). All experiments were done at 23°C maintained by a temperature-controlled water bath.

Conductance measurements were performed using an Axopatch 200B amplifier (Axon Instruments, Inc., Foster City, CA) in the voltage clamp mode. Data were filtered by a low-pass 8-pole Butterworth filter (Model 9002, Frequency Devices, Inc., Haverhill, MA) at 15 kHz and recorded simultaneously by a VCR operated in a digital mode and directly saved into the computer memory with a sampling frequency of 50 kHz. Amplitude, probability, and power spectrum analyses were performed using software developed in-house.

RESULTS AND DISCUSSION

Time-resolved events of sugar binding

The effect of sugar molecule length on the binding parameters was studied with maltodextrins of various molecular weights. The sugars had between three and seven glucose

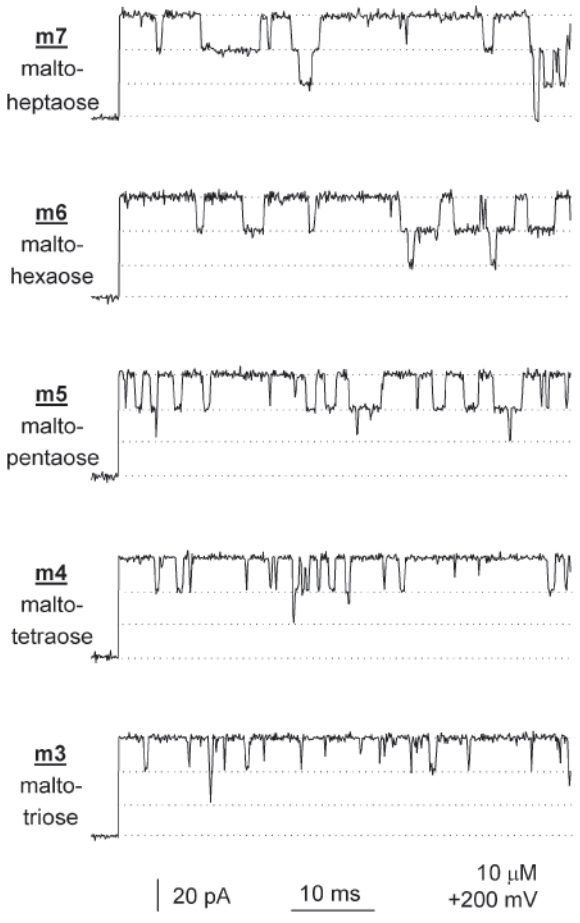

FIGURE 1 Current recordings of single maltoporin channels in solutions of maltodextrins with various lengths shown in the decreasing length order from maltoheptaose (m7), maltohexaose (m6), maltopentaose (m5), maltotetraose (m4), to maltotriose (m3). A one-third decrease in the current corresponds to time-resolved reversible binding of one sugar molecule to one of the subunits of the maltoporin trimer. For shorter sugars the blockage of the small-ion current lasts for a shorter time. Applied transmembrane voltage was +200 mV (positive from the side of protein addition), and sugar concentration was 10 μM for all maltodextrins. Time averaging used for plots here and in other recordings below was set to 0.1 ms.

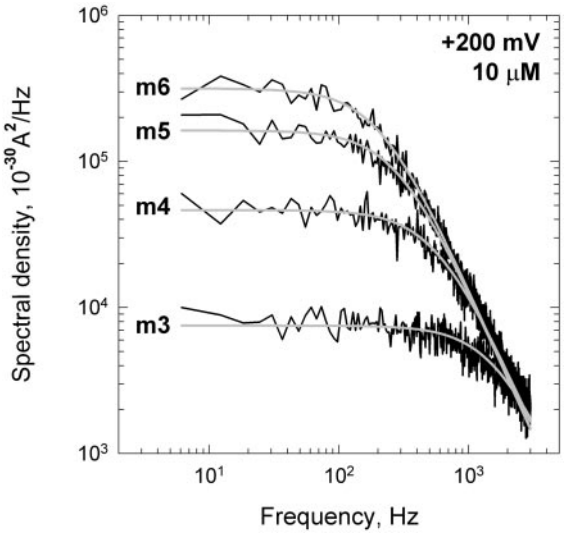

FIGURE 2 Power spectral densities of the fluctuations in the current caused by sugar blockings of single channels. The Lorentzian spectra (smooth lines through the data) show that the sugar binding can be described by a simple two-state Markovian process, where both the amplitude and the characteristic corner frequency, f_c, depend on the length of the sugar. Data for maltoheptaose (m7) are not shown because they almost coincide with those for maltohexaose (m6).

residues: maltotriose (m3), maltotetraose (m4), maltopentaose (m5), maltohexaose (m6), and maltoheptaose (m7). Maltoheptaose is the largest linear maltooligosaccharide that is utilized by the cell (Wandersman et al., 1979). We reconstituted maltoporin channels into membranes formed in sugar-containing solution and into membranes formed in sugar-free solutions with sugar added after channel insertion. Both protocols gave undistinguishable results.

Fig. 1 shows the current recordings from single channels for all the studied maltodextrins. The total ion current through one maltoporin trimer free of sugars is ~60 pA for an applied voltage of +200 mV (our convention is that a plus sign means that the side of protein addition is more positive). Transient downward current steps with the amplitude of one-third of the total initial current through the channel correspond to time-resolved binding events. It is seen that they are reversible and, as shown below, each of them is caused by a single sugar molecule entering the aqueous pore of one subunit of the maltoporin trimer. For longer sugars, such as maltoheptaose, double (or even triple) blockages decreasing the current by two (or even three) thirds can be seen. Shorter sugars block the current during a shorter time, but the amplitude is the same, corresponding to a total blockage of one maltoporin subunit. Analysis of the blocked states in the presence of maltohexaose shows that the residual current per monomer is zero within the accuracy of our measurements (0 ± 0.4 pA). Therefore, the blockage of small-ion current through a monomer is at least 98% complete.

Results of spectral analysis of sugar-induced current fluctuations shown in Fig. 2 demonstrate good fit by Lorentzian power spectra (smooth solid lines through the experimentally measured curves). The spectrum for maltoheptaose almost coincides with that for maltohexaose, and is omitted for the sake of clarity. Lorentzian spectra are usually associated with two-state Markovian processes, therefore the binding process can be approximated by such a process, one state being a pore occupied by a sugar molecule and the other state corresponding to an empty pore. The Lorentzian shapes of the spectra are also suggestive of independence of binding to different monomers.

The low-frequency spectral density is highest for maltohexaose (m6) (and maltoheptaose (m7), not shown here)

and decreases with decreasing sugar length, while the corner frequency of the fitted Lorentzians, f_c, increases. The sugar residence time, τ_r, equals the average time of blockage and, for a simple first-order binding reaction, can be found as (e.g., DeFelice, 1981):

$$\tau_r = \frac{1}{2\pi f_c(1-p)}, \quad (1)$$

where p is the probability of finding a given pore in the blocked state. In the case of independent binding this probability equals $1 - \langle I\rangle/I_{max}$, where $\langle I\rangle$ is the average current through the channel in the presence of sugar and I_{max} is the current in sugar-free solution. One of the advantages of single-channel experiments is that both $\langle I\rangle$ and I_{max} can be obtained from the same current track. In the presence of sugar the current fragments that correspond to the fully open unblocked channel give I_{max}.

The average time between successive blockages (of the same monomer), τ, decreases with the increasing sugar concentration, $[C]$, and is given by

$$\tau = \frac{1}{2\pi f_c p}. \quad (2)$$

These times represent "off" and "on" (per monomer) rate constants, correspondingly, so that $k_{off} = 1/\tau_r$, $k_{on} = 1/([C]\tau)$, and the equilibrium binding constant is $K = k_{on}/k_{off}$. Indeed, the equilibrium binding constant can also be directly determined from the average sugar-induced reduction of channel small-ion current by

$$K = \frac{p}{(1-p)[C]}. \quad (3)$$

That is, the sugar concentration corresponding to twofold current reduction ($p = 1/2$) equals the inverse of the equilibrium binding constant.

The average time between successive blockages (per monomer) for different concentrations of maltohexaose is shown in Fig. 3 *A*. It decreases by approximately two orders of magnitude as sugar concentration is increased from 3 μM to 300 μM. Fig. 3 *B* demonstrates the voltage-dependence of sugar residence time, τ_r, for different sugar concentrations. The residence time does not depend on sugar concentration. These observations agree well with the first-order reaction assumed for sugar binding.

Fig. 3 *B* also shows an asymmetry in the channel behavior. In agreement with our earlier observations for 10 μM maltohexaose (Bezrukov et al., 2000) the residence time changes from 0.3 ms at −200 mV to 0.9 ms at +200 mV, with a maximum value of 1.2 ms around +70 mV.

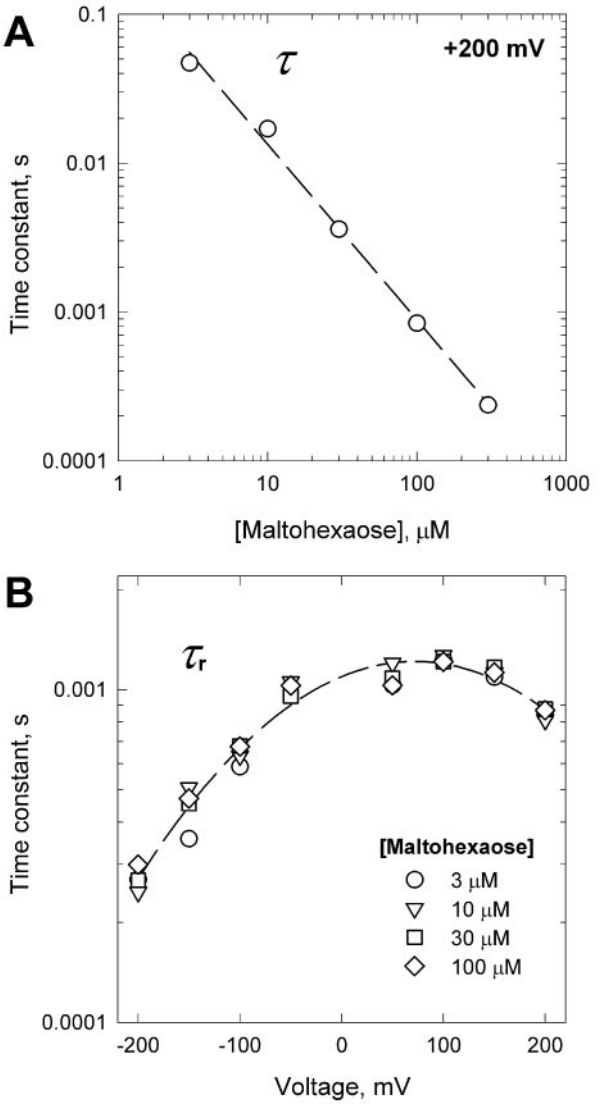

FIGURE 3 (*A*) The average time, τ, between successive blockages (per monomer) for different concentrations of maltohexaose (m6). The decrease in τ is approximately proportional to inverse sugar concentration. (*B*) The sugar residence time, τ_r, does not depend on sugar concentration, but changes with the applied voltage from 0.3 ms for −200 mV to 0.9 ms for +200 mV. This asymmetry in sugar binding is found for all the studied sugars.

Channel asymmetry: sensitivity to the sign of voltage bias

The maltoporin channel structure is highly asymmetric (Schirmer et al., 1995; Dutzler et al., 1995). It is not surprising that this asymmetry manifests itself in sign-dependent response to the voltage bias at otherwise symmetrical conditions (salts, sugars, membrane composition).

Fig. 4 shows conductances of the three states calculated from the current recordings of the type that are shown in Fig. 1 for maltohexaose. It is seen that all states, i.e., fully open, singly blocked, and doubly blocked, exhibit similar behavior. Their conductances increase with the voltage for both negative and positive polarities, so that channel *I-V* curves are superlinear. At +200 mV channel conductance, *I*/*V*, is about two times higher than

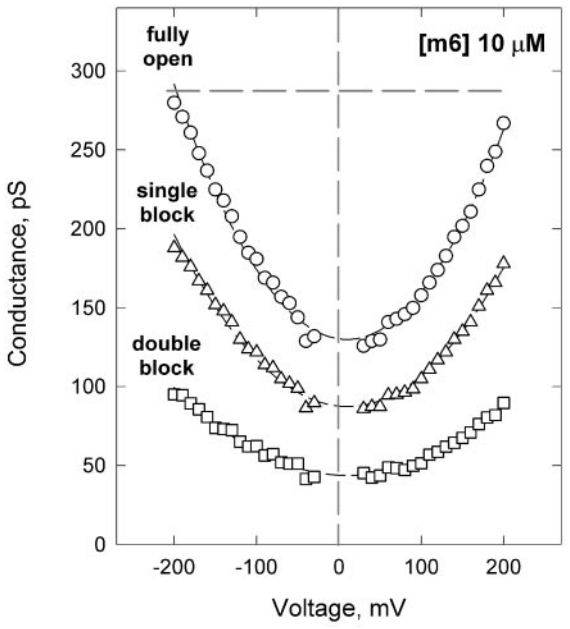

FIGURE 4 Conductance for different blockage states of the maltoporin channel as a function of applied voltage. The channel is asymmetric with lower conductance states for positive voltages, applied from the side of protein addition. Maltohexaose concentration was 10 μM.

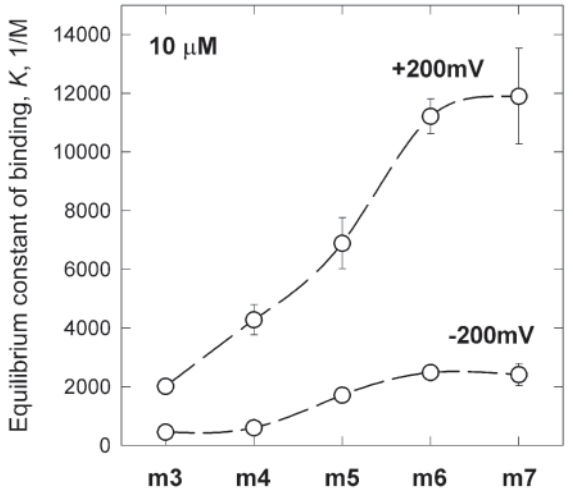

FIGURE 5 Equilibrium binding constant, K, depends on the sugar length and the applied voltage. Maltoheptaose has about a six-time larger binding constant than maltotriose, irrespective of the sign of the voltage. For all sugars the voltage asymmetry is pronounced, with about a four-to-six time difference in the binding constant between positive and negative applied voltages.

at zero voltage. Channel differential conductance, dI/dV, differs by ~3.5–4 times at these voltages. Channel conductance is also sensitive to the voltage sign. In the measured voltage range from −200 mV to +200 mV the channel exhibits asymmetric voltage dependence with ~10% lower small-ion conductance levels for positive voltages. This asymmetry was reproduced in all our experiments (~500 single channel insertions) and served as a test of directional channel insertion.

Fig. 5 presents the equilibrium binding constant, K, for +200 mV and −200 mV. Here the asymmetry of voltage dependence is much larger than in the case of the small-ion conductance. Instead of a 10% difference, the ratio of $K_{+200\text{mV}}$ to $K_{-200\text{mV}}$ is ~4–6 for all studied maltodextrins. The equilibrium binding constant increases with the sugar length from 2000 M^{-1} for maltotriose (m3) up to 12,000 M^{-1} for maltoheptaose (m7) for a positive applied voltage of +200 mV.

The earlier reported K values (Dargent et al., 1987; Benz et al., 1987; Andersen et al., 1995, 1999; Winterhalter, 1999) exhibit similar qualitative dependence on the sugar length. They show an increase in K for longer sugars, but the spread in the previously reported data is large. Although the earlier reported data were obtained in multichannel experiments using different methods, the large spread in the equilibrium binding constants for the same sugar might be partially explained by the pronounced asymmetrical voltage-dependence reported here.

Sugar binding to different monomers is independent

The results obtained above are based on the assumption that the events of sugar binding to the different monomers of the maltoporin trimer are mutually independent. Time-resolved observation of blockage events permits us to probe binding independence with high quantitative accuracy. To approach this problem we performed measurements on the same single channel whose conductance was continuously monitored while the maltohexaose concentration was increased stepwise from 0 μM to 100 μM. Results are shown in Fig. 6 where, for convenience of comparison, the current data are expressed as conductances for +200 mV and −200 mV.

The first conductance track measured in the absence of sugar characterizes the ion current through the completely open maltoporin trimer. The previously discussed conductance asymmetry is again seen as a larger conductance for the negative voltage. When maltohexaose is added to a 3 μM concentration there appear to be some rare blockings to two-thirds of the maximum conductance level. They correspond to the reversible binding of one maltohexaose molecule to one pore within the maltoporin channel. A similar behavior is seen for both positive and negative voltages, although statistical analysis shows that the blockage events are more frequent for positive voltage.

For higher sugar concentrations the frequency of blocking events increases, so that multiple blockages that decrease conductance to one-third of its maximum or even to zero are seen with increased probability. The difference between positive and negative voltages is now obvious. For the positive voltage and the highest maltohexaose concen-

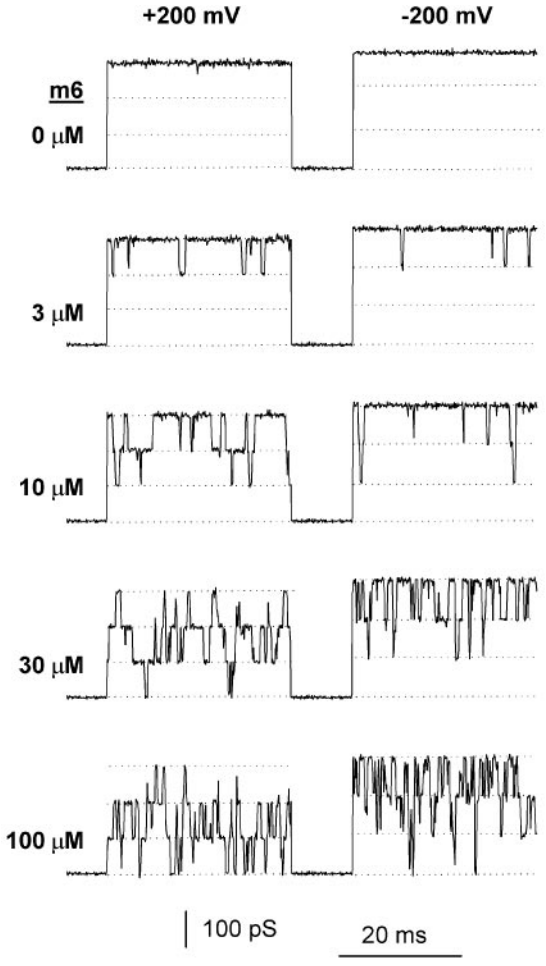

FIGURE 6 Conductance of the same single maltoporin channel at increasing maltohexaose (m6) concentration at positive and negative bias voltages. At low sugar concentrations blocking of one trimer subunit is seen as a decrease of the conductance by one-third. At larger sugar concentrations simultaneous binding of two (or three) sugar molecules to two (or three) subunits is seen as a conductance drop by two-thirds (or to zero conductance). Conductances are higher for negative voltages.

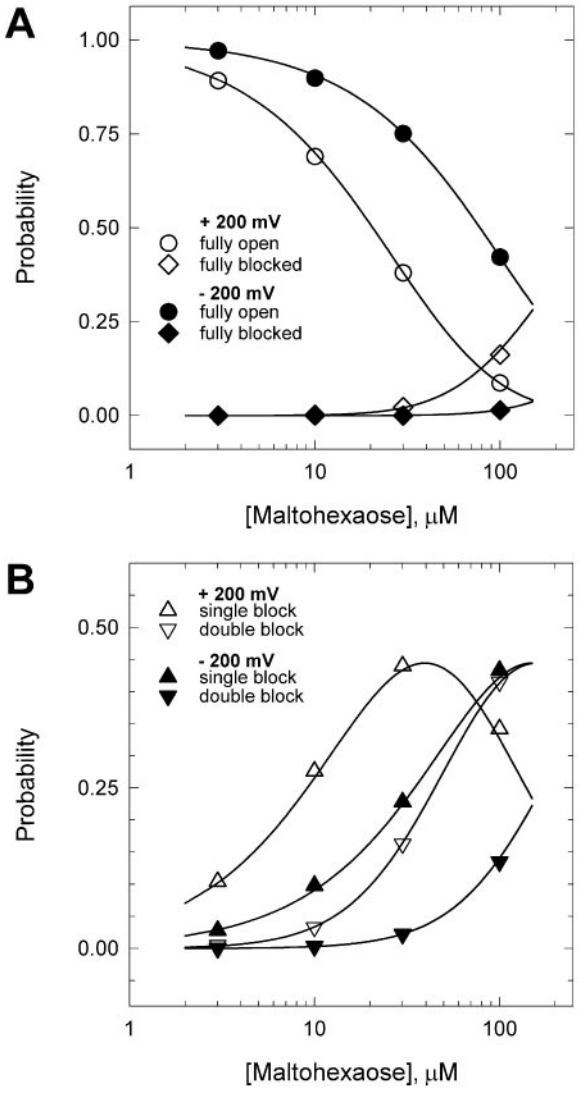

FIGURE 7 Sugar binding to the monomers of the maltoporin trimer is independent, as strongly supported by the binomial fit (*solid lines*) to the experimental blockage probability data (*symbols*). The probabilities were calculated as the ratios of the time the channel spent in a particular state to the total observation time. Data are shown for maltohexaose (m6) for both +200 mV (*open symbols*) and −200 mV (*filled symbols*), and are separated into two displays for clarity: (*A*) fully open and fully closed states; (*B*) singly and doubly blocked states.

tration (100 μM) the maltoporin channel is more often fully closed than fully open, while for the negative voltage the opposite is true.

Fig. 7 demonstrates the probability of finding the maltoporin trimer in a particular blockage state P_k, where k is the number of simultaneously blocked monomers, versus the corresponding binomial distribution

$$P_k = \frac{3!}{k!(3-k)!}\, p^k (1-p)^{3-k}. \qquad (4)$$

Probability p of one given monomer to be blocked is calculated from Eq. 3 and can be written in the form $p = K[C]/(K[C] + 1)$. It is seen that the binomial distributions calculated for $K_{+200\mathrm{mV}} = (79\ \mu\mathrm{M})^{-1}$ and $K_{-200\mathrm{mV}} = (300\ \mu\mathrm{M})^{-1}$ perfectly describe the P_k probabilities for all four conductance levels. This indicates independent binding of sugars to the different monomers of the maltoporin trimer. The data points were calculated from the experiment illustrated in Fig. 6 as the ratios of the time spent by the channel in a particular state to the total observation time. As expected, the probability of finding the channel in the fully open state decreases when the sugar concentration is increased, and the probability of finding the channel in the fully blocked state does the opposite (Fig. 7 *A*). There is a difference between positive and negative voltage. The changes are more pronounced at +200 mV than −200 mV. At +200 mV the probability of finding the channel in the

singly blocked state (Fig. 7 *B*) first grows with sugar concentration. It reaches its maximum at ~40 μM and then decreases, because the probabilities of the doubly and triply blocked states increase and the sum of all probabilities must equal one.

The excellent agreement between empirical probabilities of multiple blockage and theoretical predictions based on independence assumption demonstrates that binding of sugars to different monomers of the maltoporin trimer is *thermodynamically* independent. We did not analyze the rate constants for all the transitions between differently blocked states of the channel to test for the independence; however, the Lorentzian shape of the spectra for sugar-induced conductance fluctuations suggests that the binding is also *kinetically* independent.

Channel asymmetry: Sensitivity to the side of sugar addition

So far we have demonstrated examples of channel asymmetry that was induced (or probed) by the sign of the applied voltage bias. We have shown that the channel responds differently to positive and negative potentials both in small-ion conduction and in sugar binding. The voltage-induced asymmetry in ion conductance is small: comparing results at $\pm$200 mV we conclude that the positive potential increases channel conductance by ~10% less than the negative one (Fig. 4). At the same time (literally, within the same experiment), the positive potential increases the equilibrium sugar-binding constant by ~4–6 times (Fig. 5).

The origin of this voltage-induced asymmetry is not clear at the moment. The channel structure is notably asymmetric (Schirmer et al. 1995; Dutzler et al., 1995), but an asymmetric structure itself does not necessarily mean measurable asymmetry in transport properties. Indeed, given the present state of understanding transport phenomena, all the fine crystallographic structure details do not allow an a priori prediction of the magnitude of such asymmetry, or even of its sign (e.g., Schirmer and Phale, 1999). Many effects are shown to be able to contribute to the voltage-dependence (Tikhonov and Magazanik, 1998). We are tempted to think that the applied electric field, which reaches 4×10^5 V/cm for 200 mV, interacts with the maltoporin protein, changing its geometry in a sign-dependent manner. The "net change" in geometry is reflected by the change in channel conductance with a small asymmetry. The interaction between sugar molecules and the pore is changed more dramatically by the applied field and the observed asymmetry is much higher, probably pointing to the importance of the exact fit between the sugar molecule and the elements of the "greasy slide" (Meyer and Schulz, 1997; Hilty and Winterhalter, 2001).

However, an important question is whether the transport properties of maltoporin are really asymmetric or whether this asymmetry is voltage-induced. To approach this problem we carried out a series of measurements with one-sided sugar addition. We performed careful perfusions of the solutions at the both sides of the membrane (10 times the cell volume) and continuously monitored channel conductance. Table 1 gives the protocol of solution perfusion specifying experimental procedures and maltohexaose concentrations in the *cis* and *trans* cell compartments. By following this protocol it was possible to perform four experiments on the same maltoporin channel. Using the same channel helped us to minimize the obvious consequences of the statistical spread in the channel properties. Although even the same channel shows certain time-dependent variations in its transport properties (Bezrukov and Winterhalter, 2000), they are usually much smaller than channel-to-channel variations (Kullman et al., 2001).

TABLE 1 Protocol of the asymmetric solution experiments

cis (m6)	*trans* (m6)	Experimental Procedures
0 μM	0μM	Insertion of the channel
10 μM	0μM	Addition of sugar to the *cis* side
0 μM	0μM	Perfusion of the *cis* side
0 μM	10μM	Addition of sugar to the *trans* side

Current recordings for 10 μM (*cis*)/0 μM (*trans*) and 0 μM (*cis*)/10 μM (*trans*) are shown in Fig. 8. As in the case of the two-sided sugar addition (Figs. 1 and 6), the time-resolved blockages are seen as transient interruptions in the current tracks. The amplitudes of the blockage are the same, the average durations of the blockage seem to be close, but the blocking events are more frequent at sugar addition to the *trans* side. Thus, the channel is asymmetric and is more accessible to sugar molecules from the *trans* side.

The kinetic parameters of transport for the asymmetric sugar addition, the average sugar residence time, τ_r, and the

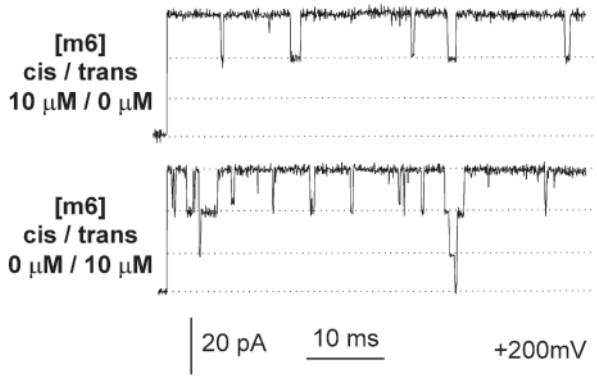

FIGURE 8 Current recordings obtained in experiments with asymmetric sugar addition with 10 μM maltohexaose (m6) solution on one side, and sugar-free buffer solution on the other. To minimize uncertainties that are due to a natural statistical spread in properties from channel to channel, the successive perfusions of the membrane-bathing solutions were performed on the same maltoporin channel. It is seen that sugar addition to the *cis* side causes fewer sugar blockages than sugar addition to the *trans* side.

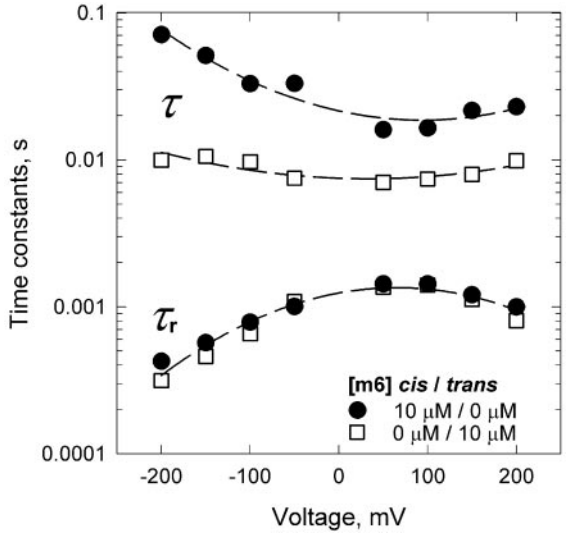

FIGURE 9 The average time, τ, between successive blockages (per monomer) and sugar residence time, τ_r, from the experiments with asymmetric sugar addition (Fig. 8). The time between blockages is smaller at the *trans* addition. The sugar residence time does not depend on whether the sugar solution is on the *cis* or *trans* side, meaning that the same binding site (or, rather, binding zone comprising many adjacent sites) is accessible from both sides.

average time between successive blocking events, τ, are given in Fig. 9.

Our first observation is that there is a significant difference in the average time between successive blocking events in these two cases. This time is smaller in the case of sugar in the *trans* compartment. This result statistically confirms our conjecture based on visual examination of Fig. 8: the "on" rate of sugar binding is higher at the *trans* side. Parameters depend on the applied bias, but the difference favoring the *trans* opening of the channel persists for all the voltages used.

Our second observation is that the average sugar residence time does not depend on the way of sugar addition. Again, as in the case with the average time between successive blockages, the residence time is voltage-dependent. However, within the accuracy of our experiment, it is the same at the *cis*, *trans*, and symmetric sugar addition. We conclude that sugar molecules entering the protein channel from either side reach the same binding region. Moreover, sugar molecules spend enough time in the binding region, "greasy slide," to lose the memory about the way they arrived here. This means that the binding events we report here reflect sugar *translocation* events and not just binding from and release to the same side.

Indeed, the number of events corresponding to sugar molecules crossing the channel is always smaller than the total number of observed events. It is clear that in the case of a perfectly symmetric channel the ratio between successful translocations and the total number of events that include sugar returns to the same side would be equal to one-half; it is also easy to understand that under otherwise symmetric conditions channel asymmetry further reduces this ratio. Using arguments of the detailed balance principle and neglecting all possible interactions between sugar molecules except those within the framework of the simple first-order reaction, for symmetric sugar application we obtain:

$$\frac{\text{Translocation events}}{\text{Total blockages}} = \frac{2k_{on}^{cis}k_{on}^{trans}}{(k_{on}^{cis} + k_{on}^{trans})^2}, \qquad (5)$$

where k_{on}^{cis} and k_{on}^{trans} are *cis*- and *trans*-side "on" rate constants, correspondingly. For the 2.5-fold to 7-fold difference in the "on" rates that follows from Fig. 9, this ratio changes between 0.41 and 0.22. Thus, depending on the voltage applied to the channel, every second to every fifth event (on average) represents a translocation event at the symmetric sugar application.

In the present study, to assess characteristic times of the blockage reaction, we have used spectral analysis of single-channel ion currents. Indeed, the quality of our recordings also permits us to calculate these parameters directly by averaging times of appropriate events within a particular trace. For example, for the 20-s recording of the channel current in the presence of 3 μM maltohexaose at +200 mV (a small fragment of this recording is shown in Fig. 6) direct averaging of sugar residence times gives (0.88 $\pm$ 0.02) ms; this time should be compared to (0.90 $\pm$ 0.03) ms derived from noise analysis. Similar good accord between results obtained by these two methods has been found in all other cases that we examined. These tests demonstrate the fine accuracy of our noise analysis procedures involving high-quality Lorentzian shape power spectra (Fig. 2) and the unambiguous two-state Markovian model for their interpretation.

CONCLUSIONS

We have studied single maltoporin channels reconstituted into planar lipid bilayers bathed in 1 M KCl in the presence of differently sized oligosaccharides.

Generally, we show that reversible binding of a single sugar molecule to a maltoporin channel can be time-resolved for the maltodextrin series with oligosaccharide lengths from three to seven. All of the studied sugars (maltotriose, maltotetraose, maltopentaose, maltohexaose, and maltoheptaose) induce complete blockage of the small-ion current through a monomer in the maltoporin trimer. For maltohexaose, the transient blockage of monomer's conductance is at least 98% effective.

Specifically, we find:

1. With one-sided maltoporin addition, channel insertion is directional. Asymmetry in the voltage-dependence of the small-ion conductance provides a quick test for channel orientation. The channel is ~10% less conductive at

+200 mV (positive at the side of protein addition) than at the same voltage with opposite polarity;
2. Channel *I-V* curves are markedly superlinear for both positive and negative potentials. At 200 mV channel differential conductance, dI/dV, is ~3.5–4 times higher than its zero voltage extrapolation;
3. The blockage is satisfactorily described by a simple first-order binding reaction in which one sugar blocks one monomer of the trimer. The "on" rate is approximately proportional to the sugar concentration, and the "off" rate is independent of the sugar concentration;
4. The blockage of different monomers within a trimer is mutually independent, at least thermodynamically. Empirical probabilities for the simultaneous monomer blockage are in perfect agreement with a binomial distribution that uses only one adjustable parameter;
5. Asymmetry in the channel structure is readily manifested as sensitivity to the sign of the voltage bias under otherwise symmetrical conditions. In addition to small asymmetry in small-ion conduction, the equilibrium binding constant at +200 mV is 4 to 6 times higher than at −200 mV for all the sugars studied;
6. More interestingly, channel asymmetry is also seen in experiments with one-sided addition of sugars. At voltage biases in the range from −200 mV to +200 mV, the "on" rates of sugar binding are always higher when sugar enters the channel from its *trans* opening;
7. One-sided sugar additions reveal that, independently of which side a sugar molecule enters the channel, it spends the same average time there. Importantly, this finding demonstrates that the binding events discussed above (and extensively studied by other authors in multichannel preparations) relate to sugar *translocation* through the channel rather than to the reversible sugar association with different sites at the *cis* and *trans* channel openings.

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