pH-induced Collapse of the Extracellular Loops Closes Escherichia coli Maltoporin and Allows the Study of Asymmetric Sugar Binding*

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LamB (maltoporin) is essential for the uptake of maltose and malto-oligosaccharides across the outer membrane of Escherichia coli. Purified LamB was reconstituted in artificial lipid bilayer membranes forming channels in the permanently open configuration at neutral pH. Almost complete channel closure was observed when the pH on both sides of the membrane was lowered to pH 4. When LamB was added to only one side of the membrane, the cis-side, and the pH was lowered at either side of the membrane, the cis- or the trans-side, the response to pH was asymmetric, suggesting preferential orientation of maltoporin channels and pH-dependent closure of only one side of the channel. In experiments with LamB mutants in which major external loops L4, L6, and/or L9 were deleted, we identified the deleted loops L4 and L6 as the cause of pH-mediated closure. The pH dependence of the LamB channel is consistent with the assumption that it inserts in a preferential orientation into the lipid bilayer. About 70–80% of the reconstituted channels are oriented with the extracellular entrance toward the side to which the protein was added (the cis-side) and with the periplasmic opening on the opposite side (the trans-side). The possibility of closing the channels, which are oriented in the reverse direction by low pH at the trans-side, allowed the deduction of channel asymmetry with respect to carbohydrate binding kinetics. Whereas maltose binding was found to be almost symmetric with respect to the channel orientation, the sucrose and trehalose binding to LamB was asymmetric. The results are discussed in respect to possible physiological function of the pH-dependent closure of maltoporin.

The outer membrane protects Gram-negative bacteria against noxious substances such as bile salts or degrading enzymes like proteinases or lipases (1, 2). Water-filled channels, the so-called porins, allow the passage of solutes through this diffusion barrier. Porins are divided in two classes: general diffusion pores, which sort solutes according to their molecular mass, and specific pores, which have a binding site for specific substrates inside the channel and facilitate the diffusion of these substrates through the outer membrane (3, 4). In the last few years the structure of several porins were solved by x-ray crystallography (5–7). Porins are built of three identical polypeptide subunits (monomers). The common structure of the monomers is a barrel formed by 16 or 18 antiparallel β-strands connected by small turns on the periplasmic side and by long loops on the extracellular side. In all known porin structures loop 3, between β-strands 5 and 6, is folded inside the channel and leads to a decrease in the diameter of the constriction of the channel. The other loops form a protrusion that protects the extracellular entrance of the outer membrane channels.

LamB (maltoporin) from Escherichia coli is a specific porin for malto-oligosaccharides (8, 9). The monomer is composed of 18 β-strands, which means that there are nine extracellular loops. The binding site inside the channel is composed of six aromatic amino acids that line the channel lumen from the extracellular to the periplasmic opening (6). In addition to this so-called “greasy slide” there are several amino acid residues inside the channel that are involved in malto-oligosaccharide binding (10–12). In a previous study we investigated the influence of the extracellular loops on maltoporin function in vivo and in vitro (13). Deletion of the large loops L4 or L6 leads to instability of the protrusion. This instability influences the binding kinetics of malto-oligosaccharides entering the pore from the extracellular side. The deletion of the three major loops (L4, L6, and L9) has no effect on sugar binding, and the mutant shows wild-type characteristics in in vitro experiments. So, the interaction of the loops seems to be crucial for the stability of the protrusion built by loops L4, L6, and L9. General diffusion pores from E. coli outer membrane have been shown susceptible to low pH. In particular, low pH leads to channel closure in the case of OmpF, OmpC, and PhoE (14–17) and in a shift of the voltage dependence (18). Low pH may also be involved in cadaverine-mediated channel block (19). Atomic force microscopy of OmpF at low pH suggests that a conformation change of the loops exposed to the external surface is the reason for channel closure at low pH (20). Here we investigated the influence of low pH on the properties of the maltoporin channel, which had not been studied yet at low pH. We show that pH 4 leads to an almost complete closure of the maltoporin channel and provide evidence that the closure is due to destabilization of the protrusion on the extracellular side. This allows some insight into which direction maltoporin inserts into artificial bilayers and provides a method of studying sugar binding in a system with oriented inserted maltoporin channels in lipid bilayer membranes. The results are discussed in respect to the possible role of maltoporin closure in protecting the organism from drastic changes of the environment.

EXPERIMENTAL PROCEDURES

Isolation and Purification of LamB Wild-type and LamB Mutants

ΔL4, ΔL6, ΔL9v, ΔL4+L6, and ΔL4+L6+L9v—Wild-type LamB of E. coli was purified as published previously (9, 22). The deletion mutants ΔL4, ΔL6, ΔL9v, ΔL4+L6, and ΔL4+L6+L9v affecting loops L4 (resi-
Polar Lipids, Alabaster, AL) in n-decane as described previously (23). The instrumentation consists of a Teflon chamber with two aqueous compartments filled with electrolyte (1 m KCl). The compartments are connected by a small circular hole with a 0.3-mm² surface area across which the membranes were formed. The electrolyte solution (Merck, Darmstadt, Germany) was buffered by Tris/HCl, pH 8.0, and citrate/KOH, pH 4.0, respectively, to a final maximal concentration of 20 mM. To exclude the possibility that the closure of pores by adding citrate is an effect of citrate binding to maltoporin comparable with that observed for maltodextrins and oligosaccharides, we also used phosphate buffer, MES*, or HCl to decrease the pH with the same effect as with citrate (data not shown).

The membrane current was measured with a pair of Ag/AgCl electrodes with salt bridges switched in series with a voltage source and a current amplifier made using a Burr Brown operational amplifier with a three-pole filter. The amplified signal was monitored with a strip chart recorder to measure the absolute magnitude of the membrane current.

Maltoporin or the deletion mutants were added from concentrated stock solutions to both sides or only one side of the membrane. The reconstitution of channels in the black lipid membrane could be monitored on a strip chart recorder by a stepwise increase of the membrane current. The pH of the electrolyte was changed by adding buffer to the same side (cis-side) or the other side (trans-side) with respect to the addition of protein (the cis-side). The temperature was kept at 20 °C and the membrane potential at 20 mV throughout.

Evaluation of the Stability Constants of Carbohydrate Binding under Asymmetric Conditions—Binding of maltose and sucrose to maltoporin were investigated as described in previous publications (24). Binding of the substrate to the binding site inside the channel could be detected because the current through the channel is blocked when the binding site is occupied. Shortly after the membrane turned black, the protein was added to the aqueous compartment (final concentration 100 ng/ml). The membrane conductance increased by the reconstitution of channels. After 20–30 min the increase slowed down. When the conductance was nearly constant the titration experiment started, and carbohydrates were added in defined concentrations to one or both sides of the membrane. Subsequently the membrane conductance decreased in a dose-dependent manner as a result of the channel block for ions because of substrate binding.

In earlier experiments (9, 21) the carbohydrates were added to the aqueous phase on both sides of the membrane. In the present study we expanded the protocol in such a way that carbohydrate was also added to one side of the membrane only, e.g., to either the cis-side (side 1), the side of the addition of LamB, carbohydrate concentration (c) or the trans-side (side 2; the opposite side of the membrane, carbohydrate concentration (c)). In the case where carbohydrate is added to both sides of the membrane in equal concentration (c = c = c) the relative conductance inhibition is given by

\[
\frac{G_{\text{max}} - G(c)}{G_{\text{max}}} = \frac{K' c}{1 + K' c}
\]

where \(G_{\text{max}}\) represents the conductance of a LamB-containing membrane prior to the addition of carbohydrate, \(G(c)\) the conductance in the presence of carbohydrate, and K the stability constant for carbohydrate binding. This means that the titration curves can be analyzed using Lineweaver-Burk plots as shown in previous publications for carbohydrate-specific porins (9, 24). The stability constant, \(K\), for carbohydrate binding to the binding site inside the LamB channel is given by

\[
K = \frac{k_1}{k_1^{-1}} + \frac{k_1^{-1}}{k_2^{-1}} + \frac{k_1}{k_1^{-1}} + \frac{k_2^{-1}}{k_1^{-1}} + \frac{k_2^{-1}}{k_2^{-1}}
\]

and the channels are fully oriented, the relative conductance inhibition is given by

\[
\frac{G_{\text{max}} - G(c)}{G_{\text{max}}} = \frac{K' c}{1 + K' c}
\]

In contrast, when the carbohydrate is added to only one side of the membrane (c = c = 0) and the channels are fully oriented, the relative conductance inhibition is given by

\[
\frac{G_{\text{max}} - G(c)}{G_{\text{max}}} = \frac{K' c}{1 + K' c}
\]

or, depending on the orientation of the channels, by

\[
\frac{G_{\text{max}} - G(c)}{G_{\text{max}}} = \frac{K' c}{1 + K' c}
\]

and

\[
G_{\text{max}} - G(c) = \frac{k_1 c}{1 + k_1 c}
\]

Note that \(K'\) and \(K''\) cannot be compared with the stability constant \(K\) given in Equation 2, as they do not represent the same stable conformational state. \(K'\) reflects the ratio of the on-rates of the binding processes from the two different sides (i.e., \(K'/K'' = k_1/k_2\)).

RESULTS

Maltoporin Channels Close Reversibly at Low pH—For the study of the pH dependence of maltoporin, purified LamB was added to both sides of the membrane bathed in an aqueous potassium chloride solution buffered with 2 mM Tris-HCl, pH 8. The membrane conductance increased in a stepwise fashion caused by reconstitution of maltoporin into the membrane. When the reconstitution process slowed down and the membrane conductance became stationary, the pH of the electrolyte solution on both sides of the membrane was decreased in steps by adding sodium citrate, pH 4.0 (Fig. 1). The resulting pH was determined by measuring the pH of a 50-μl sample of the aqueous compartments using a pH electrode. Lowering the pH to 4.73 by adding the buffer had virtually no influence on membrane conductance. At pH 4.63 the membrane conductance started to decrease. A further decrease of the pH to 4.51 and 4.40 and then to 4.28 continued this process, and the membrane conductance went down to a constant level of about 5% of its initial value at pH 4.02, which could not be reduced further at even lower pH. The same influence on LamB-mediated membrane conductance was also observed when other acids and buffers, such as MES and phosphate and hydrochloric acid instead of citrate, were used to decrease the pH. This excludes the possibility that the decrease of the conductance is caused by a block of the channels by the binding of citrate to the binding side inside the channel similar to that observed for maltodextrins and oligosaccharides (9). Taken together the decrease in membrane conductance was definitely caused by low pH. The inset in Fig. 1 shows the fraction of open channels as a function of pH. It is noteworthy that the experimental data could not be explained by the Henderson-Hasselbalch equation, which suggests that not a single amino acid is responsible for channel closure. It seems, moreover, that it is a cooperative process.
gested also that a conformational change is responsible for the reversible closure of the pores at pH levels lower than 4.7.

Only One Side of the Channel Is Blocked by Low pH—In the next set of experiments, LamB was added to both sides of the membrane, but the pH was first lowered to pH 4.0 on one side of the membrane and then on the other to the same pH (Fig. 2a). When the pH was decreased on one side to 4.0 (leftmost arrow), membrane conductance first increased somewhat, probably because of the increased channel conductance due to increased concentration of protons. Then it diminished to about 45% of its initial value. A further decrease was observed when pH was also lowered on the other side to 4.0 (second arrow from left). In agreement with the results described above, the final conductance reached values of only about 5–10% of the initial membrane conductance when both sides of the membrane had a pH of 4.0. Similar to the description above, the pH-mediated closure was reversible when pH was increased again to 6, first on the trans- and then on the cis-side (two rightmost arrows).

Protein was added to both sides of the membrane in the experiments described above, and the decrease was about 45% of the initial membrane conductance when the pH on one side was lowered. This behavior was different when maltoporin was added to only one side of the membrane (the cis-side, Fig. 2b). Lowering the pH on the trans-side to 4.1 reduced the conductance by about 20–30% of its initial value (left side of Fig. 2b) compared with a reduction of 70–80% in the same experiments when the pH was decreased on the cis-side to pH 4.1 (right side of Fig. 2b). Again, in both cases the residual membrane conductance could be reduced to about 5–10% of the initial level also when both compartments were acidified. These experiments demonstrate that acidifying only one side of the membrane is sufficient to induce channel closure. The difference between the experiments in which the protein was added to one or both sides of the membrane can only be explained when it is assumed that maltoporin does not insert randomly but in a preferred orientation into the membranes. Low pH at the cis-side induces the closure of the pores inserted in the preferred orientation. The orientation of maltoporin when it is added to one of the membrane is about 70–80%, which is in agreement with the results of the experiment described above and also with those of a previous study (13).

The Channel Monomers in a LamB Trimer Close Independently—To detect the pH-induced maltoporin closure on the single-channel level, we added only a small amount of wild-type protein (final concentration about 5 ng/ml) to one side of a membrane. After reconstitution of only one channel into the membrane, the pH was lowered at the cis-side to 4 (left arrow in Fig. 3). First, an increase of the conductance could be observed as seen in the multi-channel experiments, probably caused by the higher single-channel conductance of LamB at an increased proton concentration (pH 4). Subsequently the conductance decreased to almost zero in three distinct steps of the same size (about 65 picosiemens; see Fig. 3). In the next step the pH was also lowered to 4 on the trans-side (middle arrow), which had no further effect on conductance. Then, the pH on the cis-side was neutralized by adding Tris/HCl (right arrow in Fig. 3). As a consequence the channel opened again in three distinct steps and had the same conductance as before the pH was lowered on the cis-side (150 picosiemens). Neutralizing the trans-side also had no effect on the channel conductance (data not shown). This experiment demonstrates that the three monomers within a LamB trimer close and open independently from one another as a reaction to pH change in the cis-compartment.

Extracellular Loops L4 and L6 Are Responsible for pH-dependent Closure—In a previous study (13) we demonstrated that certain LamB loop mutants also showed preferential orientation and asymmetric carbohydrate binding when added to only one side of the membrane. Looking for the basis of the pH-induced channel closure of LamB wild type, we tested maltoporin mutants in which the three major external loops, L4, L6, and L9, are expressed in a LamB-deficient E. coli strain. They can be purified from the outer membrane and form channels when reconstituted in black lipid membranes (13). Each mutant protein was added to both sides of the membrane, and pH dependence was tested in the same way as described above for LamB wild type. The mutant ΔL4L6L9, in which all three major loops were deleted, showed no conductance decrease after the pH was lowered to 4.0 on the cis- and the trans-sides (Fig. 4). This was clear evidence that the pH-induced closure of LamB wild type is not the result of conformational changes inside the channel lumen but has its origin in the extracellular loops, which become unstable under acidic conditions and collapse into the channel lumen and block it for the permeation of ions. Further experiments with the single-loop deletion mutants ΔL4, ΔL6, and
were performed to examine which loops are involved in the closing event. The mutants \( \Delta L4 \) and \( \Delta L6 \) as well as the combination \( \Delta L4 + \Delta L6 \) showed no pH-induced closure, whereas acidic pH led to the closure of the mutant \( \Delta L9v \). We therefore concluded that the two major loops, L4 and L6, are responsible for the closure.

Combining the knowledge that lowering the pH at the cis-side had a greater effect when LamB wild type was added to only one side (the cis-side; see above) and that the extracellular loops are the origin of the pH-induced closure, we concluded that the preferred orientation of the LamB trimers in artificial lipid bilayers is with the periplasmic end first. The extracellular loops point toward the cis-side and lead to almost complete channel closure when pH is decreased at this side to pH 4.

**Evaluation of Carbohydrate Binding Constants under Asymmetric Conditions**—The investigation of carbohydrate binding to LamB under asymmetric conditions has been a problem because the orientation of maltoporin in artificial membranes was not known. Furthermore it has been a matter of debate as to whether the channel is asymmetric with respect to carbohydrate binding and transport (9, 12, 25, 26). The pH-induced block of reconstituted maltoporin channels offers an elegant way to manipulate the reconstituted channels, in a way that all...
pH-induced Closure of Maltoporin from E. coli

FIG. 5. Determination of the apparent maltose binding constant to maltoporin under asymmetric conditions. The membrane was formed from diphytanoyl phosphatidylcholine/n-decane. The aqueous phase contained 1 M KCl buffered with 2 mM Tris/HCl, pH 8, and stastis to maltoporin under asymmetric conditions. Assuming in set shows a fit of the conductance decrease using Equation 4 and asuming $K = 90 \text{ M}^{-1}$.

open channels have the same orientation, whereas the channels in the other orientation are closed. It allowed a detailed investigation of carbohydrate binding under asymmetric conditions. The asymmetric binding constants $K'$ and $K$ and the stability constant $K$ (see “Experimental Procedures”) were derived by titration experiments in which the ion flux through the channels is blocked when carbohydrate molecules are bound to the binding site inside the channel. To exclude the possibility that the pH change influences carbohydrate binding, we used LamB/L6+L9v as a control. Its carbohydrate binding characteristics were very similar to that of LamB wild type (9, 13). Because the mutant channels do not close under acidic conditions (see above), it was possible to determine the carbohydrate binding constants at pH 4.0. The binding constant of maltose added to both sides is almost identical ($K_{\text{neutral}} = 200 \text{ M}^{-1}; K_{\text{acidic}} = 220 \text{ M}^{-1}$) to that measured at neutral conditions, demonstrating that the carbohydrate binding is not affected by pH.

The asymmetric carbohydrate-binding experiments were performed in a manner similar to that described above for the experiments in which the pH was lowered. The protein was added to one compartment (the cis-side), and the trans-compartment was acidified to close the channels oriented with their extracellular side (the side of the loops) toward the trans-compartment (Fig. 5). This means that the membrane conductance is due to maltoporin channels in which the extracellular side points exclusively toward the cis-side. We determined the asymmetric binding constants of maltose, sucrose, and trehalose added either from the cis-side (extracellular side) or the trans-side (periplasmic side). Table I shows that the binding of maltose is 1.5-fold better if it is added from the cis-side (the external side; $K' = 170 \pm 20 \text{ M}^{-1}$) than from the trans-side (the periplasmic side) of the channel ($K'' = 110 \pm 20 \text{ M}^{-1}$). This result suggests that the asymmetry of the LamB channel is indeed very small. For sucrose the asymmetric binding constants $K'$ and $K''$ differed more substantially ($K' = 130 \pm 30 \text{ M}^{-1}$ and $K'' = 6 \pm 4 \text{ M}^{-1}$). This means that for sucrose the difference between cis and trans addition is more than 20-fold. For trehalose the asymmetric binding constants were also significantly different. Binding from the extracellular site is 3-fold better than from the periplasmic side ($K' = 42 \pm 2 \text{ M}^{-1}$, $K'' = 13 \pm 2 \text{ M}^{-1}$). A comparison with earlier data in which protein and carbohydrates were added to both sides of the membranes shows satisfactory agreement with the present data (9). It is noteworthy that it is impossible without the pH-induced closure of one population of channels to determine the asymmetric binding constants, because the conductance decrease in titration experiments is always caused by channels blocked by substrate entering from their extracellular or periplasmic side.

**DISCUSSION**

Low pH Closes the Maltoporin Channel in an Asymmetric Manner—Low pH has a substantial influence on the properties of maltoporin when it is reconstituted into lipid bilayer membranes. Starting with a pH of about 4.6, the channel closes almost completely for the transport of ions when the aqueous pH is lowered to pH 4.0. The channel closure is reversible; they open again when the pH is increased to pH 6. This means that the channel is not removed from the membrane and not irreversibly destroyed by low pH. It is noteworthy also that general diffusion pores such as OmpF, OmpC, and the phosphate starvation-inducible PhoE close reversibly at low pH (14–17). A similar effect has also been demonstrated for RafY and TolC with a more specific function (27, 28). This channel closure has been explained in terms of protection of enteric bacteria against low pH environment when they pass through the digestive tract. Eliminating the proton influx in the periplasmic space could protect the bacterial cell from damaging the cytoplasmic membrane and from a breakdown of metabolic processes. It is known that acidic conditions lead to changes in the expression of outer membrane proteins (15, 29). Also other responses of the bacterial cells to acidic stress occur at transcriptional or translational level (30), which means that it takes some time until the cell is adapted to the acidic conditions. The pH-induced closure of the outer membrane pores may provide an immediate response to acidic stress and helps the cell to survive sudden changes of the pH in the environment.

A similar mechanism for LamB makes sense because it is necessary that all outer membrane channels close when the organism is taken to low pH for some time; otherwise the protection against low pH would not be complete. Our experimental results suggest that the LamB channel closes in an asymmetric manner. When the protein is added to the cis-side of the membrane and the pH is lowered at the same side to pH 4, about 70–80% of the channels close. Similarly, 20–30% of the channels close when the pH is lowered to 4 at the trans-side. It is important to note that the membrane conductance drops almost to zero when both sides of the membrane are acidified. These results are consistent with the assumption that the maltoporin channel has a preferential but not full orientation when the protein is added to only one side of the
Our results show that the mutant nel Closure membrane. All data presented here are consistent with the channel lumen, resulting in the closure of the channel. This figure was made using WebLab ViewerPro (Accelrys). Protein Data Bank code, 1MAL.

In acidic conditions these interactions may be weakened followed by the destabilization of the loops. This can lead to the collapse of the loops into the channel lumen, leading to an almost complete blockage of the channel for ions and carbohydrates. This is in good agreement with experiments performed with the general diffusion pore OmpF from E. coli (20). Here, it was visualized by atomic force microscopy that the protrusion built by the major extracellular loops of OmpF becomes unstable at low pH and shows a conformation change. In our previous work in which we studied the properties of loop deletion mutants of maltoporin, we showed that under neutral conditions, mutants ΔL4 or ΔL6 as well as the combination ΔL4+L6 were also pH-insensitive, whereas ΔL9 were significantly reduced protonation potential (more than 100 mV) is needed for successful reconstitution of maltoporin (25, 31). In these studies it has been shown that the surface-exposed side moves through the lipid bilayer membranes based on phage lambda and carbohydrate binding. The difference from the experimental data described here is not clear. It has to be mentioned, however, that in previous studies using solvent-depleted membranes a considerable membrane potential (more than 100 mV) is needed for successful reconstitution of LamB. It has to be noted that insertion in vivo does not occur in the preferred orientation we found here in vitro but in the opposite direction. The mechanism as to how proteins are inserted into the outer membrane is still not understood (32) but may need special uptake machinery for correct orientation of the maltoporin trimers pointing with the loop region to the cell surface. The machinery may also provide the high energy needed to move the hydrophilic loops through the hydrophobic permeability barrier within the outer membrane.

Which Amino Acids Might Be Involved in Channel Closure?—The three-dimensional structure of maltoporin is known from crystallographic studies (6). Our experiments show that loops L4 and L6 are involved in pH-dependent channel closure, which occurs at pH lower than 4.6, suggesting that protonation of aspartate or glutamate residues could play a role in this process. Looking for such residues within loops L4 and L6, we found some that form hydrogen bonds and are most likely responsible for maintaining their structure (Fig. 6). Asp-170 and Asp-237 are located at the base of loop L4 and loop L6, respectively; Glu-148 connects L4 with L6 via two hydrogen bonds, and the two residues, Asp-255 and Glu-257 at the tip of loop L6, interact with the residues of loop L9. It is possible that weakening all of these interactions by lowering the pH leads to the destabilization of the protrusion followed by loops L4 and L6 collapsing into the channel lumen. When we investigated the closing events on the single-channel level, we found that
the monomers do not close simultaneously but independently. A similar behavior was observed with RaY, a pore-forming protein, which is part of the raf operon of E. coli plasmid pRSD2 and enables the uptake of raffinose across the outer membrane. Here, the trimeric nature of the outer membrane channel could be visualized at pH 6, where the monomers switched reversibly into closed configuration (27).

*pH*-dependent Closure of LamB Allows Evaluation of Asymmetric Binding of Carbohydrates to the Binding Site—For the *in vitro* study of carbohydrate binding to maltoporin the *pH*-induced closure represents a useful method to determine the possible asymmetric binding of carbohydrates. It was used here to eliminate one population of channels, which means that all remaining open channels have the same orientation and allow the determination of the apparent stability constants for carbohydrate binding from the extracellular (K′ = k′/k′−1 + k′−1) and the periplasmic side (K′ = k′/k′−1 + k′−1) as pointed out under “Experimental Procedures.” In separate control experiments studying carbohydrate binding to LamBΔL4+L6+L9v under neutral and acidic conditions, we demonstrated that the pH level had no influence on carbohydrate binding between pH 4 and 7. The results show that the apparent stability constant for maltose binding is 1.5-fold greater when maltose enters the channel from the extracellular side as compared with the apparent stability constant of maltose binding when it enters from the periplasmic side. This means that there is a slight asymmetry for maltose binding to LamB from both sides (i.e. k′/k′−1 = 1.5), which is in agreement with our previous studies of maltoporin asymmetry (9, 12). It is, however, again in some contrast with other studies, in which a higher asymmetry and a higher on-rate from the periplasmic side has been observed (25, 31).

*In vivo* experiments have demonstrated that LamB cannot provide enough sucrose transport across the outer membrane to allow growth on sucrose as sole carbon source (33). Here the asymmetric binding studies reveal an interesting asymmetry for the binding properties of sucrose. The apparent binding constant for sucrose binding from the extracellular side (K′) is almost as high as for maltose (K′ = 130 ± 20 μM−1), whereas K′ from the periplasmic side is more than 20-fold smaller (6 ± 4 μM−1). This difference can be explained by the structure of the sucrose molecule, which is bent in contrast to the linear maltose. The crystal structure of the maltoporin-sucrose complex shows that the glucosyl moiety of sucrose is partially inserted in the channel constriction. The nonreducing end of the glucosyl moiety points to the periplasmic side similar to the case for the bound malto-oligosaccharide molecule (see Fig. 7 and Ref. 34). It is possible that a sucrose molecule can access only the position between the aromatic residues Tyr-6, Tyr-41, and Trp-74 of the greasy slide and Tyr-118, located at the inwardly folded loop 3. The position of its glucose residue is similar to a glucose residue of bound maltose. It is only possible that sucrose assumes this position if it enters the maltoporin from the extracellular side. Steric hindrance makes it impossible to assume this position if sucrose enters from the periplasmic side. The crystal data are taken from Wang et al. (34). The figure was made using WebLab ViewerPro (Accelrys). Protein Data Bank code, 1AF6.

![Fig. 7. Longitudinal section through the maltoporin channel.](image)

A sucrose molecule is bound at the constriction site between the aromatic residues Tyr-6, Tyr-41, and Trp-74 of the greasy slide and Tyr-118, located at the inwardly folded loop 3. The position of its glucose residue is similar to a glucose residue of bound maltose. It is only possible that sucrose assumes this position if it enters the maltoporin from the extracellular side. Steric hindrance makes it impossible to assume this position if sucrose enters from the periplasmic side. The crystal data are taken from Wang et al. (34). The figure was made using WebLab ViewerPro (Accelrys). Protein Data Bank code, 1AF6.

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