Available online at www.sciencedirect.com
SCIENCE DIRECT®
Research in Microbiology 155 (2004) 611–616
Research in Microbiology
www.elsevier.com/locate/resmic

Maltoporin: sugar for physics and biology

An Ranquin, Patrick Van Gelder *

Department of Molecular and Cellular Interactions, Free University of Brussels and Flanders Interuniversity Institute for Biotechnology (VIB), Pleinlaan 2, 1050 Brussels, Belgium

Received 4 March 2004; accepted 11 May 2004

Available online 31 May 2004

Abstract

Maltoporin has been studied for over 50 years. This trimeric bacterial outer membrane channel allows permeation of sugars such as maltodextrins. Its structure is described and functional studies resulting in a mechanistic transport model are critically discussed.

Keywords: Maltoporin; Maltodextrin; Transport model

1. Transport through the Gram-negative cell envelope

The double membrane of Gram-negative bacteria poses a formidable barrier for the diffusion of molecules in and out of the cell. The inner membrane is a classical phospholipid bilayer that forms a tight seal around the cytoplasm that is impermeable to ions. There are no "open" channel-forming proteins present, and thus transport requires an active mechanism. This is often provided by nucleoside triphosphate hydrolysis on the cytoplasmic side of the membrane. Due to the poor permeability of charged molecules through the hydrophobic interior of the lipid bilayer, it is possible to create a proton gradient by dedicated proton pumping proteins and thus "energize" the membrane. The resulting protonmotive force can be used to fuel transport events.

The periplasm, an aqueous compartment between the two cell membranes, contains peptidoglycan (PG) and proteins. Peptidoglycan forms a gelatinous network with a mesh size (1.6 to 2.0 nm [32]) that in theory can retain many proteins. This suggests that transported molecules (MW > 30 kDa) are accompanied by proteins which locally hydrolyze the PG or, alternatively, a continuous channel is formed, connecting the two membranes. However, this problem is inappropriately neglected by most researchers (including the authors).

The outer membrane (OM) is an asymmetrical membrane consisting of phospholipids and mostly lipopolysaccharides (LPS) in the inner and outer monolayer, respectively. Transport across the outer membrane is mediated by channel-forming proteins. General porins like OmpF, OmpC and PhoE allow passive diffusion of small hydrophilic solutes with molecular weights up to 600 Da. The flux of solutes through these porins is proportional to the permeability and the concentration gradient between the periplasmic space and the outside medium. If this concentration gradient becomes too small, the flux can only be maintained by increasing the permeability. One possible option for doing this is to increase the number of channels [16]. However, only small increases in flux can be gained using this option: the theory of diffusion tells us [5] that only 1% coverage of the cell surface with channels is sufficient to reach 50% of the flux of a cell that is completely covered with channels. Alternatively, higher fluxes could potentially be obtained by increasing the radius (r) of the channel with the consequence that the channel surface increases with r^2. However, toxins and bile salts would then easily enter the cell and the protective function of the OM would be lost. Therefore, other porins have evolved that facilitate the uptake of certain solutes by the presence of a specific binding site. Examples are LamB, ScrY and Tsx, which are specific for maltodextrins, sucrose and nucleosides respectively. Not all transport problems can be solved by simple diffusion channels and therefore active uptake mechanisms have evolved. In contrast to inner membrane channels, active transport processes across the OM must have a different mechanism because the periplasm does not contain nucleotides or other high energy molecules and the

* Corresponding author.
E-mail address: pvgelder@vub.ac.be (P. Van Gelder).

0923-2508/$ – see front matter
doi:10.1016/j.resmic.2004.05.007

OM cannot be energized. This kind of transport across the OM is mediated by receptors, e.g., FhuA and FepA (Fe^{3+}-siderophore transport). Upon ligand-receptor binding, energy is provided by the inner membrane-based TonB complex that transduces the protonmotive force to "gate" the receptor and transport the ligand. The interested reader is encouraged to read the long-awaited updated review on the bacterial outer membrane by Hiroshi Nikaido [21] for further details.

2. From LamB to maltoporin

The maltose system of *E. coli* has been studied extensively since the late 1940's. One of the major questions at that time was the mechanism of maltose uptake through the bacterial cell envelope. During that period an impressive amount of knowledge was generated and, as a result, maltoporin is one of the best understood channels today. This essay on maltoporin is not intended to give a detailed overview of the current status of the research, but rather, it will comment on controversies in the light of the assembled knowledge in the field.

Maltoporin was first discovered in 1973 by Schwartz's group [22], as the bacteriophage λ receptor, hence its original name LamB. Adsorption of bacteriophage λ occurs initially through a reversible interaction of the fibertail J protein with LamB followed by irreversible binding of the distal end of the tail tube to the LamB porin. A second step in viral infection is the injection of the λ phage DNA into the cell. This process is not clearly understood. It seems very unlikely that this injection occurs through the LamB pore because the λ DNA molecule is too large to fit the channel. Only two years after the discovery of LamB, Maurice Hofnung's group found that LamB was actually the OM transporter for maltodextrins [25]. Maltoporin is called a specific diffusion channel because of its binding site for maltosaccharides. Mutational studies showed that some mutants that were resistant to bacteriophage λ were not affected in their maltose uptake, suggesting that the binding sites for maltosaccharides and bacteriophage λ are distinct [7]. Further studies showed that bacteriophage λ binds at the cell surface-exposed loops and maltosaccharides bind in the pore itself. Electrophysiological measurements and liposome swelling assays showed that the binding affinity for maltosaccharides increases with increasing chain length. Maltodextrins as large as maltoheptaose, a compound well above the apparent pore size, are transported into the periplasm. In 1980, Thomas Ferenci found that the maltodextrin binding site is accessible in whole cells to macromolecular polysaccharides such as starch [14]. This property permitted the development of affinity-chromatographic and chemotactic techniques for the separation of different mutant cells. This makes maltoporin easy to study and a model system for membrane transport as well as phage adsorption.

3. Structure: a common theme

Even before the first X-ray structures became available, Raman spectroscopy, infrared absorption and circular dichroism measurements showed that maltoporin, like all other outer membrane proteins, is a β-barrel. The amphipathic β-strands with alternating hydrophobic and hydrophilic amino acid residues allow the creation of a hydrophobic face towards the lipids and a hydrophilic interior of the barrel. This common folding pattern is substantially different from inner membrane proteins which contain hydrophobic α-helical transmembrane domains. The reason for this discrepancy is not yet clear. In a recent review, Phil Klebba argues that β-barrels have evolved as a consequence of intimate interactions with LPS in the OM [19]. These tight interactions will therefore enforce the protective properties of the OM towards detergents and bile salts. Indeed, the recently solved structure of FhuA by the group of Wolfram Welte shows a bound LPS molecule [15]. However, besides ionic interactions with charged groups on the lipid A sugars, binding to LPS is mostly mediated via the hydrophobic acyl chains. Similarly, strong interactions with phospholipids have been reported for inner membrane proteins [20]. Thus, there doesn't seem to be a benefit of β-barrel proteins over α-helical proteins for lipid interactions. An alternative explanation that β-barrel structures are extremely resistant to denaturation and thus can provide a protection against bile salts is also questionable, since all membrane proteins are rather stable as long as they are embedded in the lipid bilayer. Denaturation requires a disruption of the lipid phase and, consequently, a complete loss of protection. The most plausible theory comes from Jan Tommassen. He suggests that β-barrel structures are necessary to cross the inner membrane [26]. If the protein contains a hydrophobic α-helical stretch like inner membrane proteins, it would get stuck in the inner membrane due to its highly hydrophobic nature. The amphipatic β-strands are less hydrophobic and can cross the inner membrane.

A breakthrough in maltoporin studies came with the publication of the first high-resolution structure of maltoporin by the groups of Tilman Schirmer and Jürg Rosenbusch in 1995 [23]. The structure revealed the general model of specific channel-forming outer membrane proteins: a β-barrel with 18 antiparallel strands. In contrast, general diffusion pores only have 16 strands. Like the general diffusion porins the functional unit of maltoporin is a trimer with long loops exposed to the cell exterior and short turns exposed to the periplasm. As in other OM porins, a constriction is formed by the inward folded loop L3 but, additionally, loops L1 and L6 further restrict the channel to a diameter of 5 Å. The most striking feature revealed by the X-ray structure is a consecutive stretch of aromatic residues inside the channel arranged along a left-handed helical path which is dubbed the "greasy slide". Several polar residues, the so called polar tracks, are also situated at the constriction site and play an important role in maltose and maltodextrin translocation (Fig. 1).

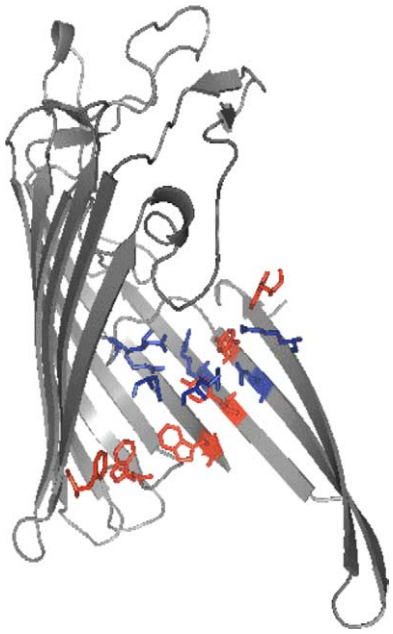

Fig. 1. Cross-section of the maltoporin monomer. The greasy slide residues are shown in red, in the following sequence: Trp^{74} (at the top; contributed from the adjacent subunit), Tyr^{41} Tyr^{6}, Trp^{420}, Trp^{358} and Phe ^{227}. The polar tracks are shown in blue, comprising the residues: Arg^{8}, Arg^{33}, Glu^{43}, Arg^{82}, Arg^{109}, Asp^{111} and Asp116.

4. A model for transport

The group of Tilman Schirmer has made several "snapshots" of a variety of sugars bound to LamB [11,31]. In addition to maltodextrins, LamB can bind and transport melibiose and trehalose. Thus, maltoporin shows a rather promiscuous behavior towards the sugars it transports. A very interesting observation made from the high resolution structures is the binding of sucrose to the channel constriction site. Three strategically placed residues (R109, Y118, D121) hinder transport of the molecule by sterically blocking the fructose ring. Interestingly, another homologous 18 β-stranded protein, ScrY or sucrose porin has altered side chains at analogous positions (N192, D201, F204) [16]. This leads to a larger channel and, consequently, permits permeation of sucrose. The reasons for the different specificities of the two porins for sucrose and maltose were studied by site-directed mutagenesis of the three strategic residues. It was indeed possible to block sucrose transport in ScrY by changing those three amino acids for their LamB homologous [27] and, in the reverse experiment mutated LamB was able to transport sucrose [29]. However, specificity could not be inverted since, in the triple-mutated LamB, maltose was still transported. Therefore, cargo discrimination by LamB is mostly a result of steric hindrance rather than specific interaction inside the channel.

How is sugar transport inside the channel envisaged? Sugar initially binds to the first residue of the greasy slide via van der Waals interactions to the hydrophobic face of the glycosyl ring. Further guided diffusion of the sugar along the greasy slide will bring it deeper into the channel. The greasy slide is not considered as one binding site but rather a collection of individual binding positions. Mutational analysis of greasy slide residues has confirmed this idea and indeed shown that the three central residues have the highest affinity for the sugar [30]. At the channel constriction the sugar residues undergo a gradual degree of dehydration which is compensated for by an increased number of favorable protein/sugar interactions. The presence of the charged side chains (polar tracks) can mimic the lost hydration shell by providing H-bonds to the sugars' hydroxyl groups. The movement of the glycosyl residues to the next binding site on the greasy slide in combination with a rearrangement of hydrogen bonds is called the register shift. Thus, by a mechanism of making and breaking of H-bonds between sugar and channel, the molecule moves in a caterpillar-like fashion through the porin. Possibly, the ability to make H-bonds (number and strength) to compensate for the bound water molecules may be reflected in the transport efficiency of different kinds of sugars. Interestingly, these polar tracks are divided into donor and acceptor lanes along the greasy slide. Molecular dynamics calculations by Stephan Fisher revealed a smooth path for sugar transport with small energy barriers for each register shift [12]. A movie showing sugar transport through maltoporin can be downloaded from http://www.iwr.uni-heidelberg.de/groups/biocomp/fischer/research/maltoporin.html. Recently the role of the polar tracks in sugar transport was experimentally confirmed by the analysis of site-directed mutants [10].

Over the last ten years, the group of Alain Charbit and Maurice Hofnung showed the involvement of external loops in sugar transport. In 1994 they came up with a LamB model that was based on the OmpF structure and consequently the barrel contained only 16 instead of 18 strands [17]. Remarkably, in the absence of high resolution structures, they were able to correctly predict several loops and from their experiments they concluded that LamB had two distinct binding sites, one in the external rim and another in the channel lumen. Although they wrongly predicted that loop L9 was constricting the channel (X-ray structures showed that similar to OmpF, L3 is the constricting loop) evidence was provided that this loop was involved in initial binding steps for maltose and maltodextrins. Further mutational analysis of this loop determined a conserved region, seven residues long, at the start of L9 that was essential for sugar transport [18]. This exposed loop and especially the conserved region has a conspicuously high number of aromatic and charged residues. Site-directed mutagenesis of the seven residues in the conserved motif resulted in the identification of two residues (E374, W376) that might be involved in the first interactions with incoming sugars to guide them with the non-reducing end inside the channel [8].

Finally, they performed a profound deletion study of several surface exposed loops [2]. Most deletions of single loops or a combination of loops (L4, L5, L6, L9) had severe in vivo effects. Except for the combination of L4 and L6,

these deletions showed abolished maltoporin function. The deletions had no effect on the kinetics of sugar binding in in vitro experiments using current fluctuation analysis. Only single deletions of L4 or L6 resulted in reduced sugar uptake from the loop side of the channel. The results might suggest that the exposed loops can serve as a sugar sink to increase local concentrations near the entrance of the channel. However, a collapse of the rim structure cannot be excluded. Until there is structural information about the deletion mutants nothing can yet be concluded. It also remains unclear how transport proceeds from the proposed binding site in the loop region to the first greasy slide residues.

5. Conclusion: return to water?

LamB has been the subject of intensive studies on sugar binding to the central binding sites in the channel. Upon addition of sugar to LamB incorporated in black lipid membranes, a sugar concentration-dependent current decrease was observed. Roland Benz derived affinity constants from these titration experiments and also introduced the current fluctuation or noise analysis technique into the field of bacterial outer membrane channels [1,3,4]. In this model, the sugars are facing a symmetrical energy barrier on both sides of the channel. One starts from the simple assumption that current through an open channel is blocked upon sugar binding at the constriction site and re-established when the sugar leaves the channel (Fig. 2). This leads to a deviation from the main current. A Fourier transform of the autocorrelation function of opening and closing events, sampled at high frequencies, will show a typical power spectrum with a corner frequency (f_c) related to the sugar concentration. Plotting the f_c against the sugar concentration will show a linear relationship with the intercept at the Y-axis giving the k_{off} and the slope the k_{on} of sugar binding (see [1]). This method was further refined by Sergei Bezrukov to allow analysis of single channel events [6]. A dissociation constant for maltose was estimated to be around 10 mM which is surprisingly high. In the periplasmic space, maltose binding protein will accept incoming maltose with an affinity of 100 μM. The inner membrane transporter (MalGFK) has an affinity of 1 μM. Thus an "affinity slide" is created towards the cytoplasm with increasing affinity steps of two orders of magnitude. By using phage λ absorption in combination with sugar transport and current analysis methods, it was shown that the LamB channel inserted in an oriented way into bilayer membranes and moreover that sugar entry from the outside was favored over entry from the periplasmic space [28]. The simplified symmetrical two barrier model thus was rejected and most groups now favor an asymmetrical two barrier model. Curiously, a recent paper described an asymmetrical model but here the barriers have been switched (entry barrier higher than the exit barrier) [9].

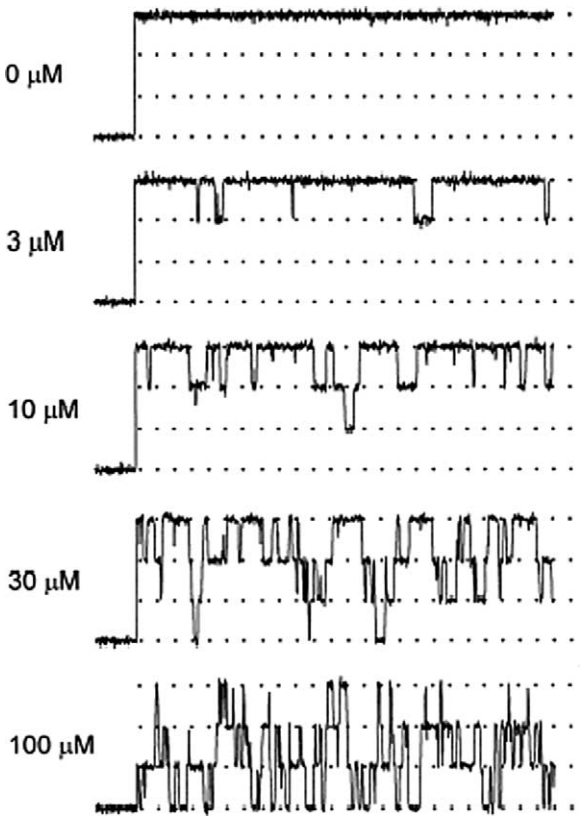

Fig. 2. Single channel measurements of maltoporin reconstituted in black lipid membranes. Sugar concentrations of maltohexaose are indicated. The lower dotted line represents the zero current level, the next lines are the monomer, dimer and trimer current levels, respectively. (This figure is provided by S. Bezrukov.)

Recent years have witnessed the development of an intimate collaboration between biology and physics which is without doubt an evolution that should be encouraged. However, despite some remarkable successes, it also painfully shows that the principles of physics, derived from extremely simple systems, can fail when applied to complex biological matter. In several recent papers [9,30], kinetic parameters for sugar transport were determined by a combination of site-directed mutagenesis and current fluctuation analysis. Analysis of single greasy slide mutants at the periplasmic side of the channel and single-sided sugar addition at the loop-side resulted in the expected decrease in k_{off} values. However, counter-intuitively also, k_{on} values were affected (Fig. 3) [9,30]. In a recent paper from Gerhard Schwarz [24], the voltage dependency of sugar translocation was studied in greater detail. In this paper they discuss the apparent weakening of the binding affinity at higher positive potentials. He argues that the transport anomalies observed could be due to rapidly occurring conformational changes in the channel which inhibit binding of the sugar molecule. Until now only two LamB mutant structures have been determined to 3.2 and 3.5 resolution [29,30]. The structures showed no gross changes in the side chain positions but small perturbations cannot be ruled out. Recently, site-

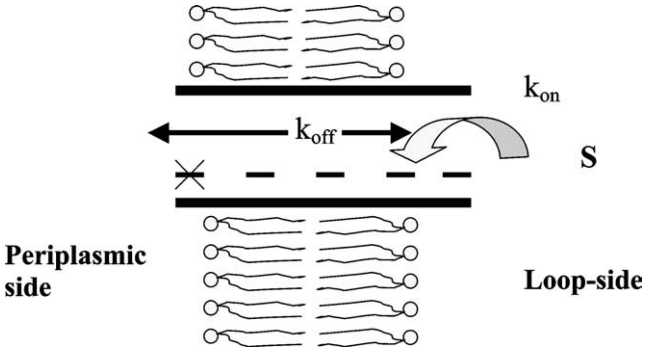

Fig. 3. Schematic drawing of a single-sided sugar addition experiment. Sugar (S) is added at the loop-side of the channel (in vivo this corresponds to the outside medium). Current fluctuation analysis can detect the k_{on} from the loop side. Once the sugar is bound to the central binding site, it can leave the porin in two directions (length of the arrow is no indication of k_{off} values). Thus, k_{off} values of both exits are measured. The dashed line represents the greasy slide residues, the cross is the mutated greasy slide residue.

directed spin labelling and electron paramagnetic resonance measurements on BtuB, the vitamin B12 receptor, showed increased backbone dynamics of the β-barrel strands towards the periplasmic space [13]. Although the lipid-facing residues were far more flexible, possible changes in the dynamics by the introduced mutations is still possible. However, changes introduced in the channel interior might also disrupt strongly bound water molecules, thereby changing the dielectric constant inside the porin and having effects that reach beyond the mutated spot. This may have an influence on the (de)hydration abilities of incoming or leaving sugar molecules. Voltage effects attributed to specific reaction dipole moments in the membrane might locally change the protonation of amino acids. Also, in other channel systems such as the K^+ channel, solvation effects turn out to be crucial for specificity and/or transport of water soluble molecules [33]. Maybe we should regard channels as water-modulating, hydration-compensating devices for molecule transport?

Acknowledgement

The authors admit that the references herein are far from complete due to space limitations of the mini-review format. We thank Dr. John Barlow for critical reading of the manuscript.

References

[1] C. Andersen, M. Jordy, R. Benz, Evaluation of the rate constants of sugar transport through maltoporin (LamB) of *Escherichia coli* from the sugar-induced current noise, J. Gen. Physiol. 105 (1995) 385–401.

[2] C. Andersen, C. Bachmeyer, H. Tauber, R. Benz, J. Wang, V. Michel, S.M. Newton, M. Hofnung, A. Charbit, In vivo and in vitro studies of major surface loop deletion mutants of the *Escherichia coli* K-12 maltoporin: Contribution to maltose and maltooligosaccharide transport and binding, Mol. Microbiol. 32 (1999) 851–867.

[3] R. Benz, A. Schmid, G.H. Vos-Scheperkeuter, Mechanism of sugar transport through the sugar-specific LamB channel of *Escherichia coli* outer membrane, J. Membrane Biol. 100 (1987) 21–29.

[4] R. Benz, H.A. Kolb, P. Lauger, G. Stark, Ion carriers in planar bilayers: Relaxation techniques and noise analysis, Methods Enzymol. 171 (1989) 274–286.

[5] H.C. Berg, Random Walks in Biology, Princeton University Press, Princeton, NJ, 1993.

[6] S.M. Bezrukov, L. Kullman, M. Winterhalter, Probing sugar translocation through maltoporin at the single channel level, FEBS Lett. 476 (2000) 224–228.

[7] A. Charbit, K. Gehring, H. Nikaido, T. Ferenci, M. Hofnung, Maltose transport and starch binding in phage-resistant point mutants of maltoporin, J. Mol. Biol. 201 (1988) 487–496.

[8] A. Charbit, J. Wang, V. Michel, M. Hofnung, A cluster of charged and aromatic residues in the C-terminal portion of maltoporin participates in sugar binding and uptake, Mol. Gen. Genet. 260 (1998) 185–192.

[9] C. Danelon, T. Brando, M. Winterhalter, Probing the orientation of reconstituted maltoporin channels at the single-protein level, J. Biol. Chem. 278 (2003) 35542–35551.

[10] F. Dumas, R. Koebnik, M. Winterhalter, P. Van Gelder, Sugar transport through maltoporin of *Escherichia coli*: Role of the polar tracks, J. Biol. Chem. 275 (2000) 19747–19751.

[11] R. Dutzler, Y.-F. Wang, P.J. Rizkallah, J.P. Rosenbusch, T. Schirmer, Crystal structures of various maltooligosaccharides bound to maltoporin reveal a specific sugar translocation pathway, Structure 4 (1996) 127–134.

[12] R. Dutzler, T. Schirmer, M. Karplus, S. Fischer, Translocation mechanism of long sugar chains across the maltoporin membrane channel, Structure 10 (2002) 1273–1284.

[13] G.E. Fanucci, N. Cadieux, C.A. Piedmont, R.J. Kadner, D.S. Cafiso, Structure and dynamics of the beta-barrel of the membrane transporter BtuB by site-directed spin labeling, Biochemistry 41 (2002) 11543–11551.

[14] T. Ferenci, M. Schwentorat, S. Ullrich, J. Vilmart, Lambda receptor in the outer membrane of *Escherichia coli* as a binding protein for maltodextrins and starch polysaccharides, J. Bacteriol. 142 (1980) 521–526.

[15] A.D. Ferguson, E. Hofmann, J.W. Coulton, K. Diederichs, W. Welte, Siderophore-mediated iron transport: Crystal structure of FhuA with bound lipopolysaccharide, Science 282 (1998) 2215–2220.

[16] D. Forst, W. Welte, T. Wacker, K. Diederichs, Structure of the sucrose-specific porin ScrY from *Salmonella typhimurium* and its complex with sucrose, Nat. Struct. Biol. 5 (1998) 37–46.

[17] P.E. Klebba, M. Hofnung, A. Charbit, A model of maltodextrin transport through the sugar-specific porin, LamB, based on deletion analysis, EMBO J. 13 (1994) 4670–4675.

[18] P.E. Klebba, S.M.C. Newton, A. Charbit, V. Michel, D. Perrin, M. Hofnung, Further genetic analysis of the C-terminal external loop region in *Escherichia coli* maltoporin, Res. Microbiol. 148 (1997) 357–387.

[19] P.E. Klebba, Mechanism of maltodextrin transport through LamB, Res. Microbiol. 153 (2002) 417–424.

[20] D. Marsh, Lipid interactions with transmembrane proteins, Cell. Mol. Life Sci. 60 (2003) 1575–1580.

[21] H. Nikaido, Molecular basis of bacterial outer membrane permeability revisited, Microbiol. Mol. Biol. Rev. 67 (2003) 593–656.

[22] L. Randall-Hazelbauer, M. Schwartz, Isolation of the bacteriophage lambda receptor from *Escherichia coli*, J. Bacteriol. 116 (1973) 1436–1446.

[23] T. Schirmer, T.A. Keller, Y.-F. Wang, J.P. Rosenbusch, Structural basis for sugar translocation through maltoporin channels at 3.1 Å resolution, Science 267 (1995) 512–514.

[24] G. Schwarz, C. Danelon, M. Winterhalter, On translocation through a membrane channel via an internal binding site: Kinetics and voltage dependence, Biophys. J. 84 (2003) 2990–2998.
[25] S. Szmelcman, M. Hofnung, Maltose transport in *Escherichia coli* K-12: Involvement of the bacteriophage lambda receptor, J. Bacteriol. 124 (1975) 112–118.
[26] J. Tommassen, Biogenesis and membrane topology of outer membrane proteins in *Escherichia coli*, in: J.A.F. op den Kamp (Ed.), Membrane Biogenesis, in: NATO ASI Series, vol. H16, Springer-Verlag, Berlin/Heidelberg, 1988.
[27] C. Ulmke, J. Kreth, J.W. Lengeler, W. Welte, K. Schmid, Site-directed mutagenesis of loop L3 of sucrose porin ScrY leads to changes in substrate selectivity, J. Bacteriol. 181 (1999) 1920–1923.
[28] P. Van Gelder, F. Dumas, J.P. Rosenbusch, M. Winterhalter, Oriented channels reveal asymmetric energy barriers for sugar translocation through maltoporin of *Escherichia coli*, Eur. J. Biochem. 267 (2000) 79–84.
[29] P. Van Gelder, R. Dutzler, F. Dumas, R. Koebnik, T. Schirmer, Sucrose transport through maltoporin mutants of *Escherichia coli*, Protein Eng. 14 (2001) 943–948.
[30] P. Van Gelder, F. Dumas, I. Bartoldus, N. Saint, A. Prilipov, M. Winterhalter, Y.-F. Wang, A. Philippsen, J.P. Rosenbusch, T. Schirmer, Sugar transport through maltoporin of *Escherichia coli*: Role of the greasy slide, J. Bacteriol. 184 (2002) 2994–2999.
[31] Y.-F. Wang, R. Dutzler, P.J. Rizkallah, J.P. Rosenbusch, T. Schirmer, Channel specificity: Structural basis for sugar discrimination and differential flux rates in maltoporin, J. Mol. Biol. 272 (1997) 56–63.
[32] X. Yao, M. Jericho, D. Pink, T. Beveridge, Thickness and elasticity of Gram-negative murein sacculi measured by atomic force microscopy, J. Bacteriol. 181 (1999) 6856–6875.
[33] Y. Zhou, J.H. Morais-Cabral, A. Kaufman, R. MacKinnon, Chemistry of ion coordination and hydration revealed by a K^+ channel–Fab complex at 2.0 Å resolution, Nature 414 (2001) 43–48.