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Mechanism of Sugar Transport through the Sugar-Specific LamB Channel of *Escherichia coli* Outer Membrane

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Summary. Lipid bilayer experiments were performed with the sugar-specific LamB (maltoporin) channel of *Escherichia coli* outer membrane. Single-channel analysis of the conductance steps caused by LamB showed that there was a linear relationship between the salt concentration in the aqueous phase and the channel conductance, indicating only small or no binding between the ions and the channel interior. The total or the partial blockage of the ion movement through the LamB channel was not dependent on the ion concentration in the aqueous phase. Both results allowed the investigation of the sugar binding in more detail, and the stability constants of the binding of a large variety of sugars to the binding site inside the channel were calculated from titration experiments of the membrane conductance with the sugars. The channel was highly cation selective, both in the presence and absence of sugars, which may be explained by the existence of carbonyl groups inside the channel. These carbonyl groups may also be involved in the sugar binding via hydrogen bonds. The kinetics of the sugar transport through the LamB channel were estimated relative to maltose by assuming a simple one-site, two-barrier model from the relative rates of permeation taken from M. Luckey and H. Nikaido (*Proc. Natl. Acad. Sci. USA* **77**:165–171 (1980*a*)) and the stability constants for the sugar binding given in this study.

Key Words LamB · maltoporin · lipid bilayer · sugar transport · transport mechanism · membrane channel

Introduction

The outer membrane of gram-negative bacteria acts as a molecular filter which has a defined exclusion limit for the permeation of hydrophilic solutes (*see* Benz, 1985; Nikaido & Vaara, 1985; Hancock, 1987, for recent reviews). These molecular sieving properties are due to a major class of proteins called porins (Nakae, 1976). Many porin pores show only little or no specificity for solutes and sort according to the molecular weight of the solutes. They act as general diffusion pathway for the rapid uptake of nutrients (Benz, 1985; Nikaido & Vaara, 1985). Other porin pores such as LamB (maltoporin) and Tsx of *Escherichia coli* (Luckey & Nikaido, 1980*a*, Benz et al., 1986; Maier et al., 1987) and protein P of *Pseudomonas aeruginosa* (Hancock, Poole & Benz, 1982; Benz & Hancock, 1987) form solute-specific pores and contain binding sites for neutral or anionic solutes. These specific pores are either permanently present in the outer membrane or they are induced if the cells are grown on special growth media.

LamB is part of the maltose uptake system (the *mal*-system) in *Escherichia coli* and other *Enterobacteriaceae* (Smelcman & Hofnung, 1975; Palva, 1978). Mutants lacking this protein are impaired in maltose uptake when the concentration of maltose is below 0.1 mM (Smelcman & Hofnung, 1975). This suggested a high specificity of LamB for sugars (von Meyenburg & Nikaido, 1977). In fact, swelling experiments using reconstituted liposomes showed that the LamB channel exhibited a considerable specificity for the permeation of maltose and maltodextrins over that of other sugars like sucrose and lactose (Luckey & Nikaido, 1980*a*). The permeation of glucose through LamB could be blocked by maltodextrins (Luckey & Nikaido, 1980*b*) and LamB was identified as a binding site for sugars in vivo on the cell surface (Ferenci et al., 1980). Lipid bilayer experiments in the presence of purified LamB resulted in a strong conductance increase caused by the formation of small ion-permeable pores (Benz et al., 1986). The ion permeation through the pores could be completely blocked by the addition of increasing concentrations of maltotriose and an apparent stability constant for the binding of this sugar to the binding site inside the pore could be calculated (Benz et al., 1986).

In this paper we show that there is no competition between ions and sugars for the binding site, i.e., the apparent stability constant as calculated from the titration experiments is identical to the absolute stability constant for the binding between the sugars and the binding site. The structure of the

binding site was studied by the investigation of the binding of a large variety of sugars. Furthermore, we were able to give a quantitative description of the sugar transport through the LamB channel on the basis of a simple one-site two-barrier model by combining the relative rates of permeation of sugars (Luckey & Nikaido, 1980*a*) with the stability constants calculated in this study.

Materials and Methods

Purification of LamB

LamB was isolated from envelopes of maltose-grown cells of *Escherichia coli* TK24, which lacks OmpC, OmpF, and OmpA. Details of the isolation procedure have been described in detail elsewhere (Vos-Scheperkeuter, Hofnung & Witholt, 1984). Briefly, LamB was isolated by (i) extraction of cell envelopes with sodium dodecyl sulfate (SDS) at 60°C and (ii) release of LamB from the protein-peptidoglycan complex by treatment with 0.4 M NaCl solution. The crude LamB fraction obtained was further purified over a QAE-Sephadex column run in the presence of Triton X-100. The protein was more than 95% pure, and it was dissolved at a concentration of 1 mg/ml in an aqueous solution containing 10 mM Tris hydrochloride (pH 7.5), 0.25 M NaCl, and 1% (vol/vol) Triton X-100. The protein was in its trimeric form (Benz et al., 1986).

Lipid Bilayer Experiments

Black lipid bilayer membranes were formed as described previously (Benz et al., 1978). The instrumentation consisted of a Teflon chamber with two aqueous compartments connected by small circular holes. The holes had a surface area of either 1 mm^2 (for the multi-channel measurements) or 0.1 mm^2 (in the case of the single-channel experiments). Membranes were formed across the holes by painting on a 1% solution of diphytanoyl phosphatidylcholine (Avanti Biochemicals, Birmingham, AL) in *n*-decane. The aqueous salt solutions (Merck, Darmstadt, FRG) were used unbuffered and had a pH around 6. The LamB was added from the concentrated stock solution either to the aqueous phase bathing a membrane in the black state or immediately prior to membrane formation, to prevent protein inactivation. The temperature was kept at 25°C throughout.

The membrane current was measured with a pair of calomel electrodes switched in series with a voltage source and an electrometer (Keithley 602). In the case of the single-channel recordings the electrometer was replaced by a current amplifier. The amplifier signal was monitored with a storage oscilloscope and recorded with a tape or a strip-chart recorder. Zero-current membrane potential measurements were performed by establishing a salt gradient across membranes containing 100 to 1000 LamB channels as described earlier (Benz, Janko & Läuger, 1979).

Theory

For the movement of the sugars through the LamB channel it is assumed that the transport can be explained by a simple two-barrier one-site model (Läuger, 1973; Lieb & Stein, 1974; Benz & Hancock, 1987). This model assumes a binding site in the center of the channel. The rate constant k_1 describes the jump of the sugars from the aqueous phase (concentration c) across the barrier to the central binding site, whereas the inverse movement is described by the rate constant k_2. We found no indication for an asymmetry of the LamB channel; therefore, symmetry of the channel with respect to the binding site is assumed.

The stability constant of the binding between a sugar molecule and the binding site is $K = k_1/k_2$. Furthermore, we assume that only one sugar can bind to the binding site at a given time and that no sugar or ion (Benz et al., 1986) can pass the channel if the binding site is occupied by a sugar. This means that a sugar can enter the channel only when the binding site is free. The probability, p, that the binding site is occupied by a sugar (identical concentrations on both sides) and does not conduct ions is given by

$$p = Kc/(1 + Kc) \tag{1}$$

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

$$1 - p = 1/(1 + Kc). \tag{2}$$

The net flux of sugar molecules, ϕ, through the channel under stationary conditions as the result of a concentration gradient $c'' - c'$ across the membrane is given by the net movement of sugar across one barrier

$$\phi = k_1 c''/(1 + K') - k_2 K'/(1 + K') \tag{3}$$

where K' is given by

$$K' = K(c' + c'')/2. \tag{4}$$

In Eq. (3) the rate constants k_1 and k_2 are multiplied by the probabilities that the binding site is free or occupied, respectively. Equation (3) has in the case $c'' = c$, $c' = 0$ the following form

$$\phi = k_1 c/(2 + Kc). \tag{5}$$

The latter form may be used for the quantitative description of the relative rate of permeation as given by Luckey and Nikaido (1980*a*) (*see* Discussion).

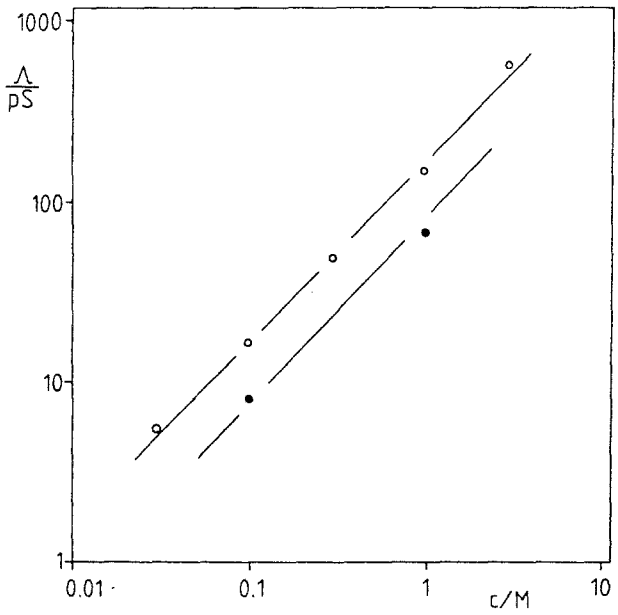

Fig. 1. Average single-channel conductance of LamB as a function of the KCl concentration in the aqueous phase in the absence (open circles) and in the presence of 5 mM maltotriose (filled circles). The membranes were formed from diphytanoyl phosphatidylcholine/*n*-decane. Λ was calculated as the average of at least 78 conductance steps: V_m = 20 mV; T = 25°C

Results

Single-Channel Conductance

When LamB is added in small quantities (10 to 100 ng/ml) to the aqueous solution bathing a lipid bilayer membrane, the specific membrane conductance increased by several orders of magnitude. The time course of the increase was very similar to that described earlier for other bacterial and mitochondrial porins (Benz & Hancock, 1981; Benz, 1985*a*) and is not described in full detail here. After an initial rapid increase for 15 to 20 min, the membrane conductance increased only at a very slow rate. It has to be noted that the addition of the detergent Triton X-100 alone did not lead to any appreciable increase of the membrane conductance above the specific conductance of the membranes in the absence of LamB (10^{-8} to 10^{-7} S/cm^2).

We have shown in a previous publication (Benz et al., 1986) that the conductance increase caused by LamB is due to the formation of ion-permeable channels which have a much smaller single-channel conductance than the general diffusion porins of the same organism. The transport of ions through LamB could be blocked in part or completely by the addition of the sugar maltotriose. To test whether ions and sugars compete for the binding site inside the channel we measured the single-channel conductance as a function of the KCl-concentration in the aqueous phase. The results are shown in Fig. 1. The single-channel conductance of the LamB channel was a linear function of the KCl concentration in the aqueous phase up to 3 M. In a second set of experimental conditions we studied the influence of maltotriose on the single-channel conductance of LamB in 1 M and in 0.1 M KCl. Whereas at a maltotriose concentration of 50 mM no channels could be resolved within the limits of the conductance resolution of our single-channel instrumentation (about 4 pS), the single-channel conductance at a sugar concentration of 0.5 mM was about half of the value in the absence of maltotiose for both salt concentrations (Fig. 1). These results indicated that sugars and ions do not compete for the binding site inside the LamB channel because there is (i) no apparent binding site inside the channel for potassium or chloride ions and (ii) the salt concentration has little if any influence on the sugar binding. This is consistent with the assumption that the binding constant derived from the titration experiments is the real stability constant.

Table 1. Average single-channel conductance, $\bar{\Lambda}$, of the LamB channel as a function of different 1 M salt solutions[a]

Salt	V_m (mV)	$\bar{\Lambda}$ (pS)	n
LiCl	20	40	147
NaCl	20	85	125
KCl	20	155	255
KCl	100	210	95
NH_4Cl	20	83	82
RbCl	20	120	74
CsCl	20	106	54
CH_3NH_3Cl	20	76	75
$(CH_3)_2NH_2Cl$	20	22	79
$(CH_3)_4NCl$	20	18	89
TrisCl	20	26	92
KCH_3COO (pH 7)	20	135	156

[a] The membranes were formed from 1% diphytanoyl phosphatidylcholine dissolved in *n*-decane; T = 25°C. The pH of the aqueous salt solutions was around 6 unless otherwise indicated. n is the number of events used for the calculation of $\bar{\Lambda}$.

Single-channel experiments were also performed in a variety of salts other than KCl in order to gain some information about the size and the selectivity of the LamB channel. The results are summarized in Table 1. Variation of the cation had a much larger influence on the single-channel conductance than variation of the anion. In fact, there was only little change of the single-channel conductance when the chloride anion was replaced by the acetate anion. On the other hand, a strong decrease of the single-channel conductance was observed

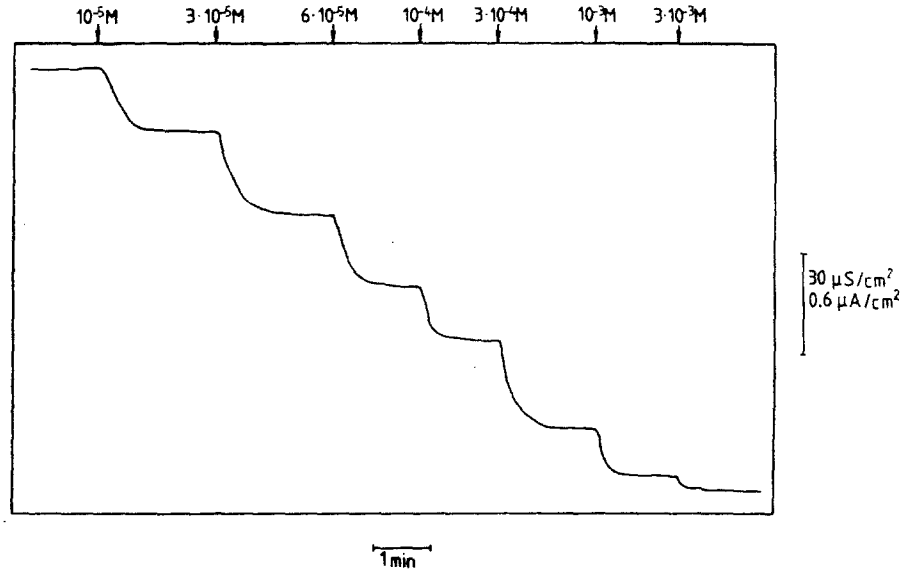

Fig. 2. Titration of LamB-induced membrane conductance with maltopentaose. The membrane was formed from diphytanoyl phosphatidylcholine/*n*-decane. The aqueous phase contained 50 ng-/ml LamB, 1 M KCl, and maltopentaose at the concentrations shown at the top of the figure. The temperature was 25°C and the applied voltage was 20 mV

when potassium was replaced by lithium or by the large cations tetramethylammonium and Tris. These results were consistent with the assumption that the LamB channel is cation selective. The selectivity sequence is K > Rb > Cs > Na > Li, which follows the Eisenman sequence IV (Eisenman, 1965). The potential across the membranes had a considerable influence on the single-channel conductance of LamB. It increased from 155 to 210 pS if V_m was increased from 20 to 100 mV (*see also* below).

Binding of Sugars to LamB

The single-channel experiments described above showed that it is in principle possible to obtain the stability constant for the binding of a sugar to LamB by single-channel recordings. The use of this method was, on the other hand, very time consuming, and, especially at high sugar concentrations (and subsequent small single-channel conductances), it was not very precise. Therefore, the stability constant for the binding of sugars was measured in multi-channel experiments, performed as follows: LamB was added to black lipid bilayer membranes in a concentration of 50 ng/ml. 30 min after the addition of the protein, the rate of conductance increase had slowed down considerably. At this time small amounts of concentrated sugar solutions were added to the aqueous phase to both sides of the membrane, while stirring to allow equilibration. Figure 2 shows an experiment of this type in which increasing concentrations of maltopentaose were added (arrows). The membrane conductance decreased as a function of the maltopentaose concentration. The data of Fig. 2 and of similar experiments were analyzed using the following equations (*see* Theory). The conductance, $\lambda(c)$, of a LamB-containing membrane in the presence of a sugar with the stability constant, K, and a sugar concentration, c, is given by the probability that the binding site is free

$$\lambda(c) = \lambda_{max}/(1 + Kc) \tag{6}$$

where λ_{max} is the membrane conductance before the start of the sugar addition to the aqueous phase. Equation (6) may also be written as

$$[\lambda_{max} - \lambda(c)]/(\lambda_{max} = Kc/(Kc + 1) \tag{7}$$

which means that the titration curves can be analyzed using a Lineweaver-Burke plot as shown in Fig. 3 for the data of Fig. 2. The straight line in Fig. 3 corresponded to a stability constant, K, of 17,000 liter/mol (half saturation constant $K_s = 5.9 \times 10^{-5}$ M). In another set of experimental conditions the experiment was repeated with 0.1 M KCl instead of 1 M KCl (open circles in Fig. 3). The results showed satisfactory agreement with those obtained in 1 M KCl, which was expected on the basis of the single-channel measurements (*see* Fig. 1). Figure 3 shows also the reduced data of an experiment in which the maltopentaose was only added to one side of the LamB-containing membrane. The stability constant, K, of the binding was in this case about 8000 liter/mol which is about half of the value obtained for the addition of the sugar to both sides of the membrane. This result indicated that the binding site inside the LamB channel is accessible from both sides of the membrane and that there exists

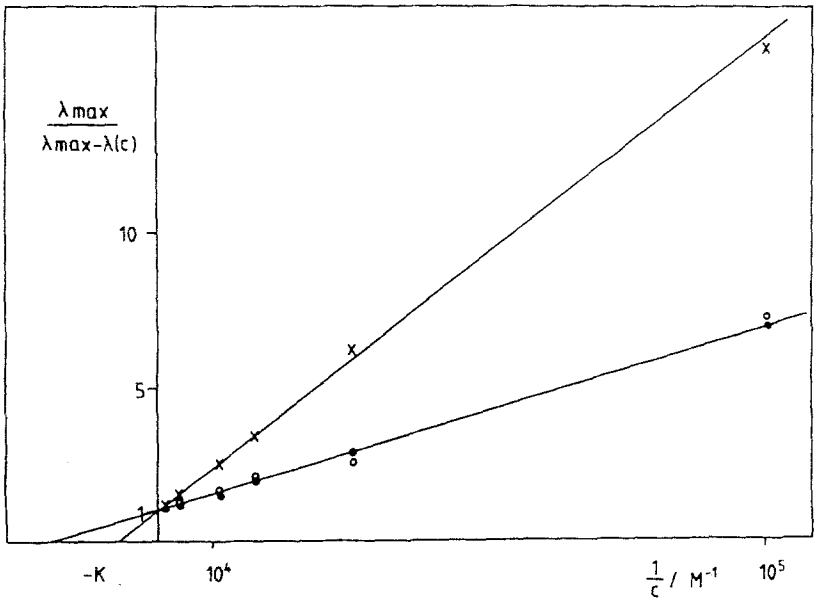

Fig. 3. Lineweaver-Burke plots of the inhibition of LamB-induced membrane conductance by maltopentaose. Three different experiments are shown. Filled circles: experiment of Fig. 2 (1 M KCl), K = 18,000 liter/mol. Open circles: similar experiment as in Fig. 2 but with 0.1 instead of 1 M KCl, K = 17,000 liter/mol. Crosses: experiment similar to Fig. 2, but maltopentaose was added to only one side of the membrane, K = 8,000 liter/mol. For further explanations, *see* text

transmembrane movement of the sugar. It should be noted that the binding of the sugars (i.e., the blockage of the pores) was fully reversible. Removing the maltopentaose and other sugars in experiments similar to that shown in Fig. 2 led to a restoration of the initial membrane conductance (before the addition of maltopentaose).

The stability constant for the binding of a large variety of sugars to the binding site inside the LamB channel was derived from measurements similar to those described above. The results are summarized in Table 2. The stability constant usually increased with the number of residues in a sugar chain. For example in the series glucose, maltose to maltopentaose, the binding constant increased about 1800-fold, whereas there was no further increase between 5 and 7 glucose residues in the maltodextrins. All disaccharides such as maltose, lactose, sucrose and others had stability constants between 7 and 250 liter/mol for the binding to LamB. Sucrose and maltose had approximately the same affinity to LamB. It is interesting to note that the relative rates of permeation of the same sugars derived from the vesicle swelling assay (Luckey & Nikaido, 1980*a*), showed substantial differences. This can be explained by the kinetics of the sugar transport and is discussed in more detail in the Discussion section. The influence of the chirality of the sugars on the binding of the sugars was also investigated in one case. Surprisingly, L-glucose showed a somewhat higher stability constant for the binding than the normal D-glucose. This result is in some contrast to the sugar cotransport system in small intestine which does not bind or transport L-glucose (Crane, 1960; Okada et al., 1977).

Table 2. Stability constants, K, for the binding of different sugars to the LamB channel[a]

Sugar	($K \pm$ SD) (liter/mol)	K_s (mmol/liter)	n
Maltose	100 4.8	10	7
Maltotriose	2,500 380	0.40	6
Maltotetraose	10,000 520	0.10	5
Maltopentaose	17,000 1,600	0.059	7
Maltohexaose	15,000 780	0.067	6
Maltoheptaose	15,000 950	0.067	11
Trehalose	46 3.3	22	3
Lactose	18 1.7	56	4
Sucrose	67 17	15	4
Gentibiose	250 65	4.0	3
Melibiose	180 47	5.5	3
Cellobiose	6.7 0.6	150	3
D-Glucose	9.5 0.8	110	7
L-Glucose	22 2.8	46	3
D-Galactose	24 2.0	42	3
D-Fructose	1.7 0.3	600	3
D-Mannose	6.3 1.1	160	3
Stachyose	20 —	50	2
Raffinose	46 4.2	22	3

[a] The membranes were formed from diphytanoyl phosphatidylcholine dissolved in *n*-decane. The unbuffered aqueous phase (pH around 6) contained 50 ng/ml LamB and 0.1 M or 1 M KCl; T = 25°C; V_m = 20 mV. The stability constant, K is given as the mean of n experiments ± SD. K was calculated from titration experiments similar to that shown in Fig. 2. K_s is the half saturation constant.

Current-Voltage Curves

Current-voltage relationships of membranes containing LamB were measured in the presence and

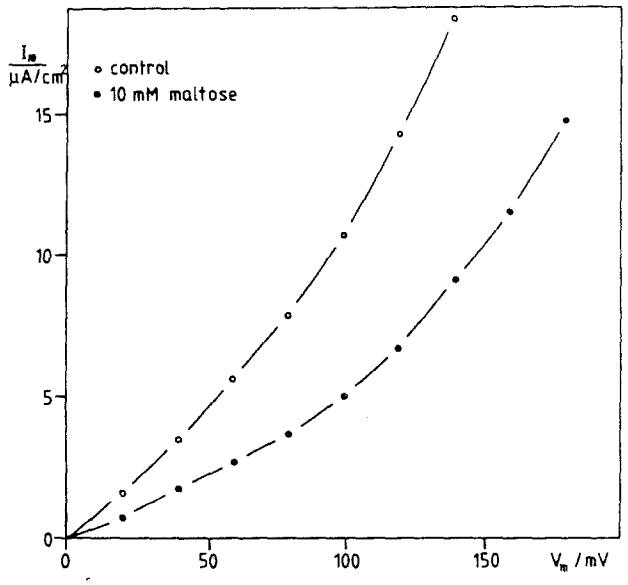

Fig. 4. Current-voltage curves of a diphytanoyl phosphatidylcholine/*n*-decane membrane in the presence of LamB before (open circles) and after (filled circles) the addition of 10 mM maltose to the aqueous phase on both sides of the membrane. The aqueous phase contained 1 M KCl and 50 ng/ml LamB. T = 25°C

the absence of sugars to determine if the membrane potential has any influence on the sugar binding. Figure 4 shows two experiments of this type. The first experiment was performed in 1 M KCl. A superlinear current-voltage curve was observed. Then 10 mM maltose was added to the aqueous phase on both sides of the membrane, and the experiment was repeated. The current was approximately half of the current in the absence of the sugar at all membrane potentials up to 150 mV. This result indicated that the binding of the sugar to the binding site was not influenced by an external electrical field. Similar results were also obtained for other sugars such as fructose, glucose, maltose, and maltopentaose.

Zero-Current Membrane Potentials

The ion selectivity of the LamB channel was investigated by measuring the membrane potential under zero current conditions. After the incorporation of about 100 to 1000 LamB channels into the membranes, the salt concentration on one side of the membrane was raised tenfold to 100 mM and the zero-current potential was measured 5 min after the gradient was established. The results are summarized in Table 3. For all four salts used in these experiments the more dilute side (10 mM) was always positive, which indicated preferential movement of the cations through the LamB channel, i.e.,

Table 3. Zero-current membrane potentials, V_m, of membranes from diphytanoyl phosphatidylcholine/*n*-decane in the presence of LamB measured for a 10-fold gradient of different salts[a]

Salt	V_m (mV)	P_c/P_a
KCL	51	28
KCL, 0.5 M maltotriose	52	30
LiCl	44	12
KCH_3COO (pH 7)	55	50
TrisCl	37	7.3

[a] V_m is defined as the difference between the potential on the dilute side (10 mM) and the potential at the concentrated side (100 mM). The pH of the aqueous salt solutions was around 6 unless otherwise indicated; T = 25°C. P_c/P_a was calculated from the Goldman-Hodgkin-Katz equation from at least four individual experiments (Benz et al., 1979).

the channel is cation selective. The sugar maltotriose had at a concentration of 0.5 mM no detectable influence on the ion selectivity. This result indicated again that the properties of the LamB channel are not influenced by the sugar if no sugar is bound to the binding site. The zero-current membrane potentials ranged between 37 and 55 mV. Analysis of the data of Table 3 using the Goldman-Hodgkin-Katz equation (Benz et al., 1979) suggested that anions also have a certain permeability through the channel because the ratios of the permeabilities P_c for cations and P_a for anions ranged between 7 and 35. However, it has to be noted that the large (organic) Tris cation has a much larger permeability through the channel than the small and highly mobile chloride anion. This result indicated that the LamB channel has (different from the general diffusion porins of *E. coli*) a high selectivity for cations. The ions do not move through this channel in a manner similar to the way they move in the aqueous phase. This is consistent with the results of the single-channel experiments (*see* above).

Discussion

In this study we have confirmed the idea that LamB (or maltoporin) has a sugar-specific binding site and thereby forms a selective channel (Smelcman & Hofnung, 1975; Luckey & Nikaido, 1980*a*,*b*; Ferenci et al., 1980; Benz et al., 1986). We investigated the binding of the sugars to the binding site inside the LamB channel indirectly by measuring the ion movement through the channel as a function of the sugar concentration. It was not clear at the beginning if we were measuring the real stability constant of the binding, because of the possible competition between sugars and ions. The following

control experiments were therefore performed. We measured the dependence of the single-channel conductance of the LamB channel on the KCl-concentration. Even at very high salt concentrations no saturation was observed. Secondly, we investigated whether the ion concentration had any influence on the apparent stability constant of the sugar binding. We found no indication whatsoever for any competition between sugar and ions, and therefore feel very confident that the stability constants derived from the titration experiments are equal to the stability constants in the absence of ions. We would like to note that the binding constants derived here show some qualitative agreement with those from other in vivo and in vitro studies (Ferenci et al., 1980; Luckey & Nikaido, 1980*b*), although similar problems (i.e., the possible interaction between sugars and the probe molecules) apply equally to these investigations.

We propose here a one-site, two-barrier model for the mechanism of sugar transport through the LamB channel. This model is supported by the experimental observation that the binding of the sugars to the site can be explained by simple 1 : 1 kinetics even at very high sugar concentrations. We did not observe any indication for the binding of two sugar molecules at the same time to the binding site. This is despite the fact that the increase of stability constant with maltodextrin length suggests that the binding site can accommodate up to 5 glucose units. It is interesting that there is some evidence for a conformational change induced by the binding, which prevents multiple binding. We are therefore convinced that the simple model provides a good description of the sugar transport through the LamB channel and of the blockage of the ion movement by the binding of sugar, although the experimental results could also be explained by more complicated models.

The stability constants for the binding of the sugars with two residues were all close to 100 liter/mol (except for cellobiose and lactose). Thus sucrose and maltose had very similar stability constants (67 and 100 liter/mol, respectively) despite a difference in the relative rates of permeation by a factor of 40 (Table 4, Luckey & Nikaido, 1980*a*). This discrepancy may be explained by differences between the transport kinetics of these sugars which are predicted by the equations given in the Theory section. Substituting the experimental conditions of Luckey & Nikaido (1980*a*) ($c'' = 40$ mM, $c' = 0$) Eq. (5) gives

$$\phi = k_1\ 40\ \text{mM}/(2 + K\ 40\ \text{mM}) \tag{8}$$

so that k_1 is given by

Table 4. Rate constants for sugar transport through the LamB channel calculated relative to the movement of maltose[a]

Sugar	ϕ (1/sec)	K (liter/mol)	k_1 (liter/mol)	k_2 (1/sec)
Maltose	100	100	15,000	150
Maltotriose	66	2,500	170,000	68
Maltotetraose	19	10,000	190,000	19
Maltoheptaose	2.5	15,000	38,000	2.5
Trehalose	76	46	7,300	160
Lactose	9	18	610	34
Sucrose	2.5	67	290	4.3
Gentibiose	42	250	13,000	52
Melibiose	33	180	7,600	42
Cellobiose	13	6.7	740	110
D-Glucose	290	9.5	17,000	1,800
D-Galactose	225	24	17,000	710
D-Fructose	135	1.7	7,000	4,100
D-Mannose	160	6.3	34,000	5,300

[a] The rate constants were calculated assuming that the flux of maltose through the LamB channel under the conditions of the experiment of Luckey & Nikaido (1980*a*) [*compare* Eq. (9)] is 100 1/sec and that the flux of the other sugars relative to maltose is given by the relative rate of permeation (Luckey & Nikaido, 1980*a*). The stability constants, K, were taken from Table 3. k_2 was calculated from k_1/K.

$$k_1 = \phi\ (50\ \text{liter/mol} + K) \tag{9}$$

Equation (9) can be used for a more quantitative description of the kinetics of sugar transport through LamB. If we assume that the flux of maltose under the conditions of Luckey and Nikaido (1980*a*) is 100 sec^{-1}, k_1 is calculated to be 15,000 liter/mole sec for maltose, whereas it is only 290 liter/mole sec in the case of sucrose. The rate constants of the off process, k_2 differ greatly between the two sugars (150 and 4.3 sec^{-1}, respectively). Similar considerations apply to the transport of the other carbohydrates. The transport rate constants relative to that of maltose are summarized in Table 4. The sugars of the maltodextrin series have the largest association constants, whereas the dissociation constants, k_2, are largest for the monosaccharides.

Our analysis also shows that the LamB channel is saturated by 40 mM concentration of those sugars with a large affinity for the binding site [i.e., the conditions of Luckey & Nikaido (1980*a*)]. A further increase of the sugar concentration on one side of the channel cannot increase the flux. In this case, the maximum flux is given by k_2, i.e., the maximum turnover number of a one-site two-barrier channel. The maximum permeability of such a channel is given by $k_1/2$. This can also be seen from Fig. 5 which shows the dependence of the flux of maltose

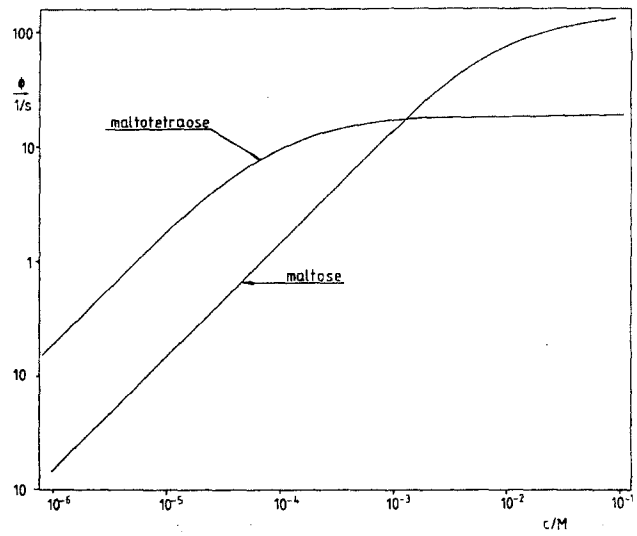

Fig. 5. Flux of maltose (open circles) and maltotetraose (filled circles) through LamB as a function of the concentration c of the corresponding sugar on one side of the channel. The concentration on the other side was set to zero. The flux was calculated from Eq. (5) by using the stability constants given in Table 2 and k_1 given in Table 4. Note that the flux of maltotetraose is at small sugar concentrations (which corresponds to the in vivo situation) higher than the flux of maltose

and maltotetraose as a function of the sugar concentration, c, on one side of the LamB channel, while the concentration on the other side is zero [*compare* Eq. (5)]. The maximum flux for both sugars is given by the individual rate constants k_2, whereas the flux in the linear range (at small c) is given by the rate constants $k_1c/2$. It is interesting to note that the flux of maltotetraose through the LamB channel is at small substrate concentrations (which corresponds to the in vivo situation) much higher than the flux of maltose. This result demonstrates how a binding site inside a channel leads to a faster transport than given by a general diffusion pore (Benz et al., 1986).

We would like to stress the point that the rate constants given in Table 4 are only valid with respect to the flux of maltose. If the flux, ϕ, is in the experiment of Luckey & Nikaido (1980*a*) a certain factor f larger (or smaller) than the assumed 100 sec^{-1} then all rate constants of Table 4 (including those for maltose) have to be multiplied by this factor leaving K unchanged. In fact, the preliminary noise analysis of the blockage of the ion current through LamB by maltoheptaose showed that the maltose flux under the conditions of Luckey and Nikaido (1980*a*) is most likely on the order of 10^4 sec^{-1} (R. Benz, *unpublished results*). This would mean that the rate constants given in Table 4 are approximately 1% of the real value, i.e., the factor f is 100.

The LamB channel was found to be cation selective for all salts tested in this study. Cation selectivity can be explained by the existence of negatively charged groups inside the channel forming a binding site (Latorre & Miller, 1983; Benz, Tokunaga & Nakae, 1984) or by the existence of carbonyl groups such as the well-studied gramicidin A channel (Finkelstein & Andersen, 1981). The results presented here make it rather unlikely that charges are involved in the selectivity of the LamB channel. Thus we have to assume that the selectivity is caused by the presence of carbonyls inside the channel. The presence of carbonyls inside the channel would also be consistent with the existence of a binding site for sugars because it is known that the hydroxyl groups of sugars form hydrogen bonds with carbonyls (Crane, 1960). The number of hydrogen bonds involved in the binding are most likely responsible for the stability of the sugar-channel complex, as has been discussed for the sodium-driven cotransport of sugar in small intestine (Crane, 1960; Kimmich, 1973).

The large differences of the rate constants for different sugars cannot be understood on the basis of a simple static model of the LamB channel. Maltose and lactose differ only in a few groups. The small change of the structure has already a dramatic influence on the rate constants which decrease in the case of lactose by a factor of about 30 (k_1) and 40 (k_2) as compared with maltose. This seems to be caused by a conformation change of part of the channel caused by the binding. Rapid translocation of the sugars through the channel may be obtained if these conformation changes can be kept minimal, i.e., if the sugars diffuse simply along the binding site with small or no conformation change of the groups involved in the binding process.

The investigation of the LamB channel using different sugars allows us to deduce a rough steric model of the binding site. The stability constant for the binding of the maltodextrins increased with their length, but saturated after five residues. This result makes it very likely that the binding site has approximately a length of five glucose molecules, corresponding to a maximum length of 3 nm. Long maltodextrins and amylose (starch) form helices with approximately 10 to 12 glucose residues per turn. Thus, it is very likely that the LamB channel is not a straight cylinder but fits the curved maltodextrins. Furthermore, it is obvious from the data presented here that the channel is not wide, otherwise we could not understand the loss of ion permeability when a single sugar molecule was bound. The maltodextrins obviously permeate the channel in a single file. From the dimensions of maltopentaose and independently from the selectivity sequence for ions we may conclude that the channel is only approximately 0.7 nm wide, whereas the diameter of the general diffusion pores of *E. coli* outer mem-

brane is on the order of 1.1 nm (Nikaido & Rosenberg, 1983; Benz, Schmid & Hancock, 1985*b*). Further studies on mutant LamB (Ferenci & Lee, 1982; Nakae, Ishii & Ferenci, 1986) and the investigation of the primary sequence of the mutants (Heine, Kyngdon & Ferenci, 1987) may give further insight into the three-dimensional structure of the binding site and may help to decide the question of which amino acids are involved in the binding of the sugars.

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