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Site-Directed Mutagenesis of Tyrosine 118 within the Central Constriction Site of the LamB (Maltoporin) Channel of *Escherichia coli.* II. Effect on Maltose and Maltooligosaccharide Binding Kinetics

Frank Orlik, Christian Andersen, and Roland Benz

Lehrstuhl für Biotechnologie, Theodor-Boveri-Institut (Biozentrum) der Universität Würzburg, Am Hubland, D-97074 Würzburg, Germany

ABSTRACT The 3-D structure of the maltooligosaccharide-specific LamB channel of *Escherichia coli* (also called maltoporin) is known from x-ray crystallography. The central constriction of the channel formed by the external loop 3 is controlled by tyrosine 118. Y118 was replaced by site-directed mutagenesis by 10 other amino acids (alanine (A), isoleucine (I), asparagine (N), serine (S), cysteine (C), aspartic acid (D), arginine (R), histidine (H), phenylalanine (F), and tryptophan (W)) including neutral ones, negatively and positively charged amino acids to study the effect of their size, their hydrophobicity index, and their charge on maltose and maltooligosaccharide binding to LamB. The mutants were reconstituted into lipid bilayer membranes and the stability constants for binding of maltose, maltotriose, maltopentaose, and maltoheptaose to the channel were measured using titration experiments. The mutation of Y118 to any other non-aromatic amino acid led to a substantial decrease of the stability constant of binding by factors between about two and six. The highest effect was observed for the mutant Y118A. Replacement of Y118 by the two other aromatic amino acids, phenylalanine (F) and tryptophan (W), resulted in a substantial increase of the stability constant maximally by a factor of almost 400 for the Y118W mutant. The carbohydrate-induced block of the channel function was used for the study of current noise through the different mutant LamB channels. The analysis of the power density spectra allowed the evaluation of the on- and off-rate constants (k_1 and k_{-1}) of sugar binding. The results suggest that both rate constants were affected by the mutations. For most mutants, with the exception of Y118F and Y118W, k_1 decreased and k_{-1} increased, whereas the opposite was found for the aromatic amino acid mutants. The results suggest that tyrosine 118 has a crucial effect on carbohydrate transport through LamB.

INTRODUCTION

The maltose regulon of *Escherichia coli* encodes for proteins located in the outer membrane, the periplasmic space, the inner membrane, and the cytosol, essential for transport and degradation of maltooligosaccharides (Szmelcman and Hofnung, 1975; Palva, 1978; Schwartz, 1987). Important for the transport across the outer membrane is LamB (maltoporin), composed of three identical polypeptide subunits with a molecular mass of ~45 kDa (421 amino acids) that form 18 antiparallel β-strands (Schirmer et al., 1995). Carbohydrates bind to LamB (Ferenci et al., 1980) and the binding leads to a block of the channel for the permeation of ions (Benz et al., 1986), indicating that the carbohydrate-specific binding site is located in the interior of the channel. In fact, swelling experiments using reconstituted liposomes showed that the LamB channel exhibited a considerable specificity for the permeation of maltose and maltooligosaccharides over that of other carbohydrates such as sucrose and lactose (Luckey and Nikaido, 1980). The protein has been crystallized and its 3-D structure is known from x-ray crystallography (Schirmer et al., 1995). The dimension of the channel in the subunits is limited by external loop L3, which is folded inside the cylinder and restricts it to ~0.6 × 1.0 nm. A number of different amino acid residues are involved in carbohydrate binding, which has been demonstrated in a number of studies (Charbit et al., 1988; Francis et al., 1991; Benz et al., 1992; Klebba et al., 1994; Schirmer et al., 1995; Jordy et al., 1996; Newton et al., 1996; Dutzler et al., 1996). Important are amino acids of the greasy slide (Y6, Y41, W74, W358, and W420) and amino acids that are able to form hydrogen bonds with the carbohydrates (D116, E43, R8, R109, R82, and R33). Involved in carbohydrate binding is also tyrosine 118, which has a central position within the constriction of the channel (see Fig. 1, *A* and *B*).

Carbohydrate binding to the LamB channel has been studied in detail by assuming a symmetrical one-site two-barrier model for sugar transport and titrating the ion current through LamB with increasing concentrations of different carbohydrates (Benz et al., 1987). The results suggest that carbohydrate binding affinity increases with the chain length of the maltooligosaccharides. The kinetics of the sugar binding could not be evaluated from titration experiments because the on-rate of their binding was much higher than the diffusion of these molecules through the unstirred layer. However, we have recently demonstrated that the kinetics of carbohydrate transport can be derived from carbohydrate-induced current noise of the LamB channel (Nekolla et al., 1994; Andersen et al., 1995; Jordy et al., 1996). In this study we give a quantitative description of the effect of replacement of Y118 by a variety of different amino acids on the kinetics of carbohydrate transport through the LamB channel. This analysis is based on binding studies using titration experiments and on the analysis of

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Address reprint requests to Dr. Roland Benz, Lehrstuhl für Biotechnologie, Theodor-Boveri-Institut (Biozentrum) der Universität Würzburg, Am Hubland, D-97074 Würzburg, Germany. Tel.: +49-931-888-4501; Fax: +49-931-888-4509; E-mail: roland.benz@mail.uni-wuerzburg.de.

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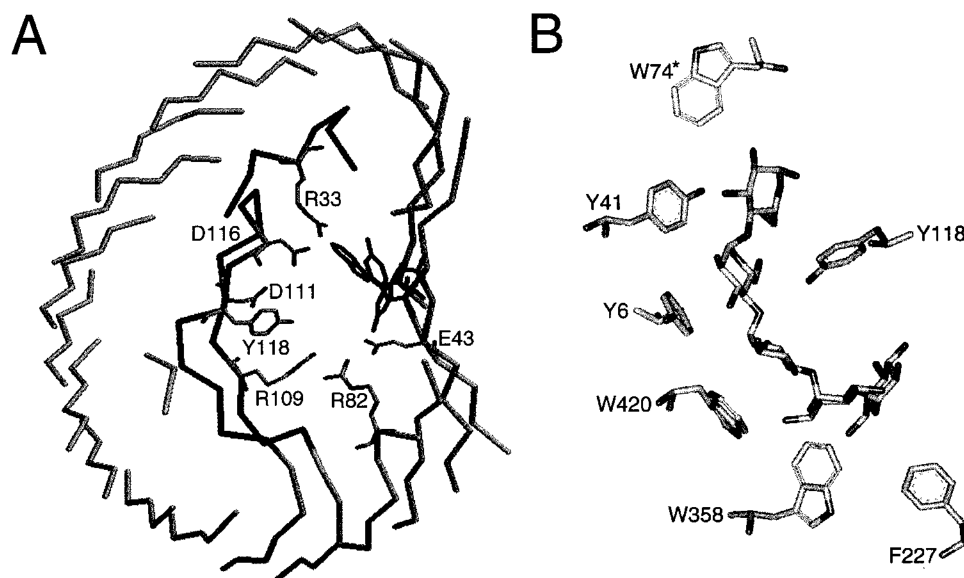

FIGURE 1 (*A*) Cross-section of the *E. coli* LamB monomer (maltoporin). The panel shows loop 3 (*dark gray*) and the amino acid residues (denoted with their numbers from the mature N-terminal end) that are relevant for passage of carbohydrates and ions through the central constriction. The strands of the β-barrel cylinder of the porin are given in light gray. The coordinates of maltoporin were taken from the crystallographic data of Schirmer et al., 1995. (*B*) Position of the greasy slide amino acids with respect to maltopentaose and Y118. The coordinates of maltoporin with respect to maltopentaose binding were taken from the crystallographic data of Dutzler et al. (1996). W74* is contributed from the adjacent subunit.

the carbohydrate-induced current noise. The analysis was performed using a similar treatment proposed previously for the kinetics of nerve channels (Conti and Wanke, 1975; Conti et al., 1980), of gramicidin (Kolb et al., 1975), and of the analysis of ameloride-induced block of frog epithelial sodium channels (Lindemann and Van Driessche, 1977a; Van Driessche and Lindemann, 1979).

MATERIALS AND METHODS

Materials

Diphytanoyl phosphatidylcholine (DiphPC) was obtained from Avanti Polar Lipids (Alabaster, AL). KCl was analytical grade (Merck, Darmstadt, Germany). Maltose, maltotriose, maltopentaose and maltoheptaose were purchased from Seikagaku America (Falmouth, MA). Ultrapure water was obtained by passing deionized water through Milli-Q equipment (Millipore, Bedford, MA).

Construction and purification of LamB mutants

Expression plasmids containing the genes of the LamB mutants Y118C (pAM2420), Y118N (pAM2421), Y118F (pAM2422), Y118H (pAM2423), Y118S (pAM2424), and Y118I (pAM2425) were kind gifts of Dr. Tom Ferenci, University of Sidney, Australia (Ferenci and Lee, 1982; Clune et al., 1984). The mutants Y118D, Y118R, Y118A, and Y118W were constructed according to standard genetic manipulations using the QuikChange site-directed mutagenesis kit.

Plasmid pAM117 encoding wild-type LamB (Heine et al., 1988) was used as a template for in vitro site-directed mutagenesis. For each mutant two synthetic oligonucleotide primers were designed (purchased from Carl Roth, Karlsruhe, Germany), each complementary to opposite strands of the plasmid and containing the desired mutation. The oligonucleotide primers were extended during temperature cycling by using *Pfu-turbo* DNA polymerase. Incorporation of the primers generates a mutated plasmid containing staggered nicks. After temperature cycling the product was treated with *Dpn*I. The *Dpn*I endonuclease (target sequence: 5′-Gm^{6}ATC-3′) is specific for methylated and hemimethylated DNA and is used to digest the parental DNA template. This led to the selection of the mutation-containing synthesized DNA. The nicked vector DNA was then transformed into XL1-Blue supercompetent cells, where the nicks in the mutated plasmid were repaired. All mutated plasmids were controlled by DNA sequencing.

The LamB mutants Y118A, Y118I, Y118N, Y118S, Y118H, Y118C, Y118D, Y118R, and Y118W were obtained and purified as has been described by Orlik et al. (2002) with the exception of the mutant Y118W, which had to be dialyzed against 10 mM Tris, pH 8, overnight at 4°C to remove the 20% maltose from the elution buffer of the starch affinity column (Ferenci and Lee, 1982). Otherwise, the maltose interfered with the titration experiments with the carbohydrates because of its high stability constant for binding to the Y118W mutant.

Growth experiments with the strains containing LamB or the LamB mutants

The growth experiments with the LamB mutant strains were performed in M9 minimal medium containing 6.4% Na_2HPO_4, 1.5% KH_2PO_4, 0.25% NaCl, 0.5% NH_4Cl (all wt/vol), 2 mM $MgSO_4$, and 0.1 mM $CaCl_2$. The salt solution was autoclaved and then supplemented with sterile-filtered 0.4% maltose (11.7 mM) or 0.4% maltopentaose (4.8 mM), 5 mg/l thiamine, and 50 mg/l ampicillin. The wild-type LamB gene and all the mutated alleles were expressed from pAM117-derived plasmids in KS26, a strain that lacks most of the outer porins (Schülein et al., 1995; Orlik et al., 2002). It is noteworthy that the LamB and the LamB mutant genes are not under the control of an inducible promoter and have an expression rate of ~40% of the level of fully induced, wild-type, chromosomally encoded protein (Heine et al., 1988).

Lipid bilayer experiments

Black lipid bilayer membranes were formed as described previously (Benz et al., 1978). The instrumentation consisted of a Teflon chamber with two aqueous compartments connected by a small circular hole with a surface area of ~0.3 mm^2. Membranes were formed across the holes by painting on a 1% solution of DiphPC (Avanti Polar Lipids) in n-decane. The 1 M KCl solutions were used unbuffered and had a pH of ~6. Control experiments revealed that the pH was stable during the time course of the experiments. The LamB mutants were added from the concentrated stock solution either to the aqueous phase bathing a membrane in the black state or immediately before membrane formation. The temperature was kept at 20°C throughout.

Titration experiments

Wild-type LamB can be blocked for ion transport when a carbohydrate is bound to the binding site inside the channel (Benz et al., 1986). We studied whether the mutant channels also possess a binding site for carbohydrates. These measurements were performed with multi-channel experiments. The LamB mutants were added to black DiphPC/n-decane membranes at concentration of ~500 ng/ml. Thirty minutes after the addition of the proteins, the rate of conductance increase had slowed considerably. At that time small amounts of concentrated solutions of carbohydrates were added to the aqueous phase to both sides of the membrane, with stirring to allow equilibration. In these experiments we observed a strong dose-dependent decrease of the membrane conductance. The conductance data of the titration experiments were analyzed using the following equations used earlier for the carbohydrate-induced block of wild-type LamB (Benz et al., 1986, 1987). The conductance, $G(c)$, of a LamB mutant channel in the presence of a carbohydrate with the stability constant, K (half-saturation constant K_S) and the carbohydrate concentration, c, is given by the maximum conductance (without carbohydrate), G_{max} times the probability that the binding site is free:

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

Equation 1 may also be written as:

$$\frac{(G_{max} - G(c))}{G_{max}} = \frac{K \cdot c}{(K \cdot c + 1)} \tag{2}$$

which means that the conductance as a function of the carbohydrate concentration can be analyzed using Lineweaver-Burke plots.

Noise analysis

The membrane current was measured by a pair of silver/silver chloride electrodes switched in series with a battery-operated voltage source and a current amplifier (Keithley 427 with a four-pole filter or a home-made operational amplifier with a three-pole filter). Feedback resistors between 0.01 and 10 GΩ were used in the experiments. The reconstitution of the LamB mutants in the membranes resulted in an increase of the membrane current. The amplified signal was monitored by a strip chart recorder and simultaneously fed through a low-pass filter (four-pole Butterworth low-pass filter) into an AD-converting card of an IBM-compatible PC. The digitized data were analyzed with a home-made fast-Fourier transformation program, which yielded identical results to a commercial digital signal analyzer (Ono Sokki CF 210). The spectra were composed of 400 points and they were averaged either 128 or 256 times. The spectra were analyzed using commercial graphic programs. For the derivation of the rate constants of carbohydrate binding they were fitted to Eq. A2.

RESULTS

Growth experiments with KS26 strains containing wild-type LamB

In a previous study we could demonstrate that the LamB mutants investigated here formed ion-permeable channels when reconstituted in lipid bilayer membranes (Orlik et al., 2002). To reveal the functional integrity of the LamB mutant channels and to demonstrate their ability to transport maltose and maltooligosaccharides we performed growth experiments with all LamB mutant strains on synthetic M9 medium supplemented with 5 mg/l thiamine and either 0.4% maltose or maltopentaose. For these investigations the plasmid pAM117 carrying the genes for wild-type LamB and the different LamB mutants were transferred into the KS26 strain that lacks most outer membrane porins (Schülein et al., 1995). Ten microliters of an overnight culture grown in LB media was added to 10 ml M9 minimal medium, and the growth was followed over at least one day.

The growth curves showed a substantial difference between the growth of the porin-deficient strain KS26 and KS26 transfected with the plasmids encoding for wild-type LamB and the LamB mutants. The strain KS26 showed only very slow growth on maltose. Growth was substantially induced when the cells contained the plasmid encoding for wild-type LamB. However, the growth of the strains containing the LamB mutants was virtually the same as that of the strain containing the plasmid for wild-type LamB. Similarly, KS26 showed absolutely no growth on maltopentaose, but the strains that expressed wild-type LamB and the LamB mutants showed approximately the same growth rate, which was the same as with maltose in the growth medium. This result indicated that the LamB mutants were able to transport the carbohydrates maltose and maltopentaose, i.e., the mutants were functional. Furthermore, it seems that the outer membrane was not rate-limiting under the conditions used here because 0.4% maltopentaose used in the growth experiments corresponds to a concentration of 4.8 mM maltopentaose, which is always above the half-saturation constant of the LamB mutants used in this study (see also Discussion).

Evaluation of the stability constants for carbohydrate binding with the different LamB mutants

The stability constants for carbohydrate binding to LamB mutants were calculated from titration experiments. An example for this type of measurement is given in Fig. 2 *A*. LamB mutant Y118W was added while stirring from a concentrated stock solution to the aqueous phase (concentration ~500 ng/ml) bathing a black lipid bilayer membrane. The corresponding current increase was monitored on a strip chart recorder. After ~30 min most of the con-

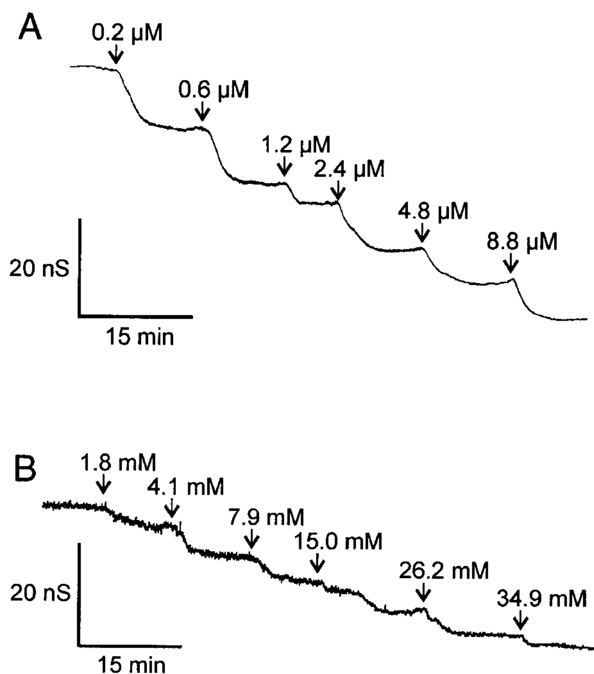

FIGURE 2 Titration of LamB mutant-induced membrane conductance with maltotriose. The membranes were formed from diphytanoyl phosphatidylcholine/n-decane. The aqueous phase contained 500 ng/ml protein, 1 M KCl, and maltotriose at the concentrations shown at the top of the figure. The temperature was 20°C and the applied voltage was 20 mV. (*A*) The experiment was performed using the Y118W mutant. (*B*) The experiment was performed using the Y118A mutant.

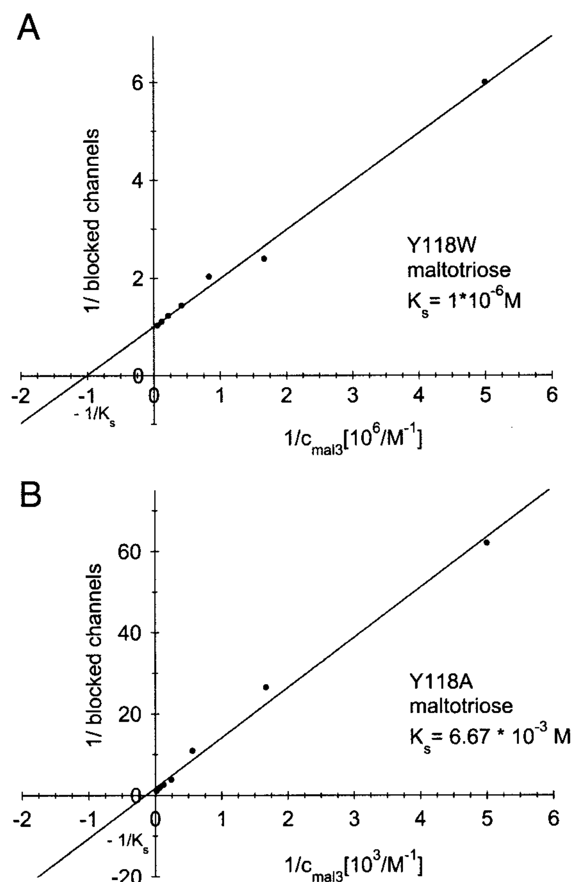

FIGURE 3 Lineweaver-Burke plots of the inhibition of the LamB mutant-induced membrane conductance by maltotriose. The data taken of Fig. 2, *A* and *B* were analyzed using Eq. 2. For further explanations see text. (*A*) The straight line corresponds to a stability constant *K*, for maltotriose binding to the mutant Y118W of *E. coli* of 10^6 l/mol (K_S = 1 μM). (*B*) The straight line corresponds to a stability constant *K* for maltotriose binding to the mutant Y118A of *E. coli* of 148 l/mol (K_S = 6.67 mM).

ductance increase was over and the titration experiments were started by the addition of a concentrated solution of maltotriose to both sides of the membrane. This led to a decrease of membrane conductance in a dose-dependent manner, as is shown in Fig. 2 *A*. At a carbohydrate concentration of 8.8 μM the current through the membrane almost decreased to zero, which means that the LamB mutant Y118W channels were also totally blocked for ions caused by the binding of maltotriose to the binding site. The stability constant for the binding of maltotriose to the binding site inside the Y118W channel was evaluated using Lineweaver-Burke plots according to Eq. 2. An example for the fit of the data of Fig. 2 *A* is given in Fig. 3 *A*. The stability constant for maltotriose binding to Y118W was 10^6 1/M (half-saturation constant 1 μM). This has to be compared with a stability constant of 2800 1/M (half-saturation constant 360 μM) for maltotriose binding to wild-type LamB (Benz et al., 1987).

It is noteworthy that replacement of Y118 by non-aromatic amino acids led to a substantial decrease of the stability constant for maltooligosaccharide binding. Fig. 2 *B* shows an experiment where a membrane containing the mutant Y118A was also titrated with maltotriose. A considerably higher carbohydrate concentration up to ~35 mM was needed to block most of the conductance. This is also demonstrated in Fig. 3 *B*, which shows a Lineweaver-Burke plot of the data of the titration experiment with Y118A. The straight line corresponds to a stability constant of 148 1/M (half-saturation constant ~7 mM), which means that it decreased by about a factor of almost 20 as compared to wild-type LamB.

Similar titration experiments were performed with all LamB Y118 mutants and some of the four different carbohydrates used in this study. The results for *K* and the half-saturation constant, K_S (=1/*K*), are listed in Table 1 together with the stability constants derived from titration experiments with wild-type LamB and the Y118F mutant (Benz et al., 1987; Jordy et al., 1996). The results suggest that the affinity of maltooligosaccharide binding decreased for all mutants tested in this study with the exception of Y118W. The smallest binding affinity was found for the Y118A mutant. The stability constant for the binding of the three maltooligosaccharides, maltotriose, maltopentaose, and maltoheptaose was approximately a factor of 20 smaller

TABLE 1 Stability constants of maltooligosaccharide binding to different LamB mutant channels as derived from titration experiments similar to that shown in Fig. 2 by using Lineweaver-Burke plots (Fig. 3)

	Maltose	Maltotriose	Maltopentaose	Maltoheptaose
Mutant		K/M^{-1}		
Wild type*	110	2,800	14,000	17,000
Y118A		130	540	720
Y118I		205	2,700	2,800
Y118N		510	3,500	4,940
Y118S		940	1,000	1,300
Y118H		490	1,300	3,050
Y118C		340	710	2,860
Y118D		620	2,400	3,530
Y118R		910	1,130	3,750
Y118W	26,000	1,000,000	3,100,000	4,000,000
Y118F†	2,200	60,000	110,000	190,000

The data represent means of at least three individual titration experiments. The standard deviation was typically <10% of the mean value. The results of previous titration experiments are given for comparison.
*Taken from Benz et al., 1986.
†Taken from Jordy et al., 1996.

than wild type and about a factor of 200 and 6000 smaller as compared to the Y118F and the Y118W mutants, respectively. The maltooligosaccharide binding was also smaller for the other mutants (see Table 1). However, the effect was less pronounced for these mutants and ranged between about twofold and about sixfold. The stability constant for binding increased for all mutants, with the number of glucose residues increasing from three to seven. For the Y118S mutant the stability constants increased only slightly for the three maltooligosaccharides.

Measurement of current noise with the Y118 mutants

Parallel to the titration measurements the frequency-dependence of the spectral density spectra was measured using fast-Fourier transformation of the current noise. For the measurement of current noise absolutely stationary conditions are needed (Nekolla et al., 1994; Andersen et al., 1995). This means that the time between formation of the membrane and the start of the measurements had to be considerably longer (~2 h) as compared to the titration experiments (~30 min) to reach stationary conditions. The reference spectrum was taken before addition of carbohydrates to obtain the current noise of the open LamB mutant channels, which exhibit $1/f$ noise in the frequency range between 1 and 50 Hz (Nekolla et al., 1994; Wohnsland and Benz, 1997). An example is given in Fig. 4 *A* for the measurement of current noise of ~560 Y118W mutant channels without maltotriose (*trace* 1, 0 μM). At small frequencies up to ~100 Hz the spectral density was dependent on $1/f$, which is typical for open bacterial porin channels. This we have demonstrated in a number of previous

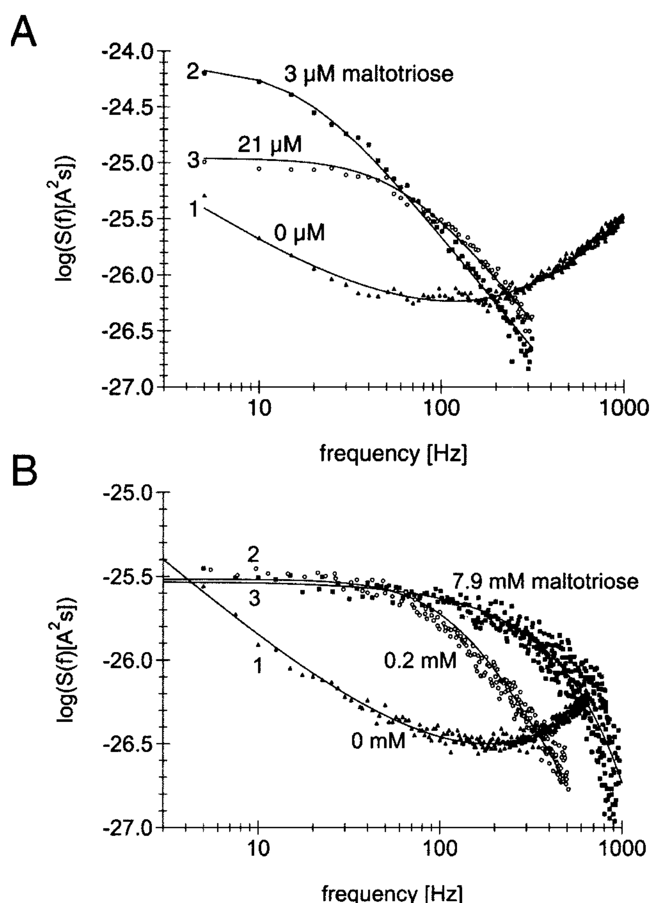

FIGURE 4 (*A*) Power density spectra of maltotriose-induced current noise of ~560 LamB mutant Y118W channels. *Trace 1:* control (1 M KCl). *Trace 2:* the aqueous phase contained 3 μM maltotriose and the power density spectrum of trace 1 was subtracted (τ = 9.07 ms; $S_0 = 0.72 \cdot 10^{-24}$ A^2 s). *Trace 3:* the aqueous phase contained 21 μM maltotriose and the power density spectrum of trace 1 was subtracted (τ = 2.65 ms; $S_0 = 0.11 \cdot 10^{-24}$ A^2 s); T = 20°C; V_m = 20 mV. (*B*) Power density spectra of maltotriose-induced current noise of 220 LamB mutant Y118A channels. *Trace 1:* control (1 M KCl). *Trace 2:* the aqueous phase contained 0.2 mM maltotriose and the power density spectrum of trace 1 was subtracted (τ = 1.9 ms; $S_0 = 0.03 \cdot 10^{-24}$ A^2 s). *Trace 3:* the aqueous phase contained 7.9 mM maltotriose and the power density spectrum of trace 1 was subtracted (τ = 0.52 ms; $S_0 = 0.02 \cdot 10^{-24}$ A^2 s); T = 20°C; V_m = 20 mV.

investigations (Nekolla et al., 1994; Jordy et al., 1996; Wohnsland and Benz, 1997). The increase of the spectral density at frequencies above ~200 Hz was caused by the intrinsic noise of the preamplifier that produces a frequency-dependent current noise through the membrane capacity C_m. It is observed also with membranes without reconstituted LamB channels. The time resolution of the instrumentation was ~10 kHz, which was limited in the experiments of Fig. 4 *A* and similar experiments by the bandwidth of the current amplifier and a low-pass filter (0.3 ms). The reference spectrum was subtracted from each spectrum taken after the successive addition of carbohydrates in increasing concentration, which led to a considerable increase of the

spectral density of the current noise as Fig. 4 *A* clearly indicates.

Fig. 4 *A*, *trace* 2 shows a spectrum taken after addition of maltotriose (c = 3 μM; the reference spectrum of *curve* 1 was subtracted) to the membrane containing ~560 LamB Y118W channels. The current noise spectrum of the LamB mutant channel Y118W after the addition of carbohydrates could be fitted to a single Lorentzian function (see Fig. 4 *A*, *trace* 2). This result agrees well with earlier investigations with the LamB (maltoporin) wild-type of *E. coli* (Nekolla et al., 1994; Andersen et al., 1995) and *Salmonella typhimurium* (Jordy et al., 1996), where also one single Lorentzian has been observed without any exception. A spectrum taken a few minutes later did not show any systematic variation compared with the spectrum taken before. In further experiments the concentration of maltotriose was increased in defined steps. At another concentration of maltotriose (c = 21 μM) the power density spectrum corresponded to that of *trace* 3 in Fig. 4 *A*, which could also be fitted to a single Lorentzian. Such a type of noise is expected for a random switch with different on- and off-probabilities and can be fitted to Eq. A2 with sufficient accuracy (Verveen and De Felice, 1974; Conti and Wanke, 1975; De Felice, 1981).

Fig. 4 *B* shows a similar experiment with the mutant Y118A. Again the power density spectra of the current noise could be fitted to single Lorentzians after the subtraction of the reference spectrum. However, it is clear from the spectra of Fig. 4 *B* that the kinetics of maltotriose binding to the Y118A mutant is much faster than to the Y118W mutant. This reduces the plateau value S_0 of the power density spectra and also increases the corner frequency (Nekolla et al., 1994), and is the reason why for wild-type and many of the mutants the kinetics of maltose- and maltotriose-induced current noise could not be evaluated.

The corner frequencies, f_c, of the Lorentzians are dependent on the on- and off-rate constants, k_1 and k_{-1}, for carbohydrate binding to the binding site inside the mutant LamB channels according to Eqs. A1 and A2. This means that the f_c values should increase with increasing carbohydrate concentration. This was the case for all noise measurements, including the experiments shown in Fig. 4, *A* and *B*. The reaction rate $1/\tau$ was plotted as a function of the carbohydrate concentration in the aqueous phase. Fig. 5, *A* and *B* show the fit of the corner frequencies of the experiments shown in Fig. 4, *A* and *B* and of other maltotriose concentrations (data not shown) to Eq. A2. The rate constants for the binding of maltotriose to the LamB Y118W channel were $k_1 = 26 \cdot 10^6\ M^{-1}\ s^{-1}$ and k_{-1} = 35 1/s. This corresponds to a stability constant, K, for the binding of maltotriose to the binding site inside the LamB Y118W channel of 743,000 1/M, which agrees quite well with the stability constant for the same system derived from the titration experiments (see Table 1). We repeated these experiments several times and yielded similar results for both the rate constants of maltotriose binding and for the stability constants (see Table 2). Similarly, we were able to calculate the rate constants for maltotriose binding to the Y118A mutant channel from the concentration dependence of the corner frequency. They were $k_1 = 0.23 \cdot 10^6\ M^{-1}\ s^{-1}$ and k_{-1} = 2300 1/s, which corresponds to a stability constant, K, for the binding of maltotriose to the binding site inside the LamB Y118A channel of 100 1/M. It is noteworthy that Hilty and Winterhalter (2001) also studied maltotriose transport through the Y118A mutant using the fluctuation

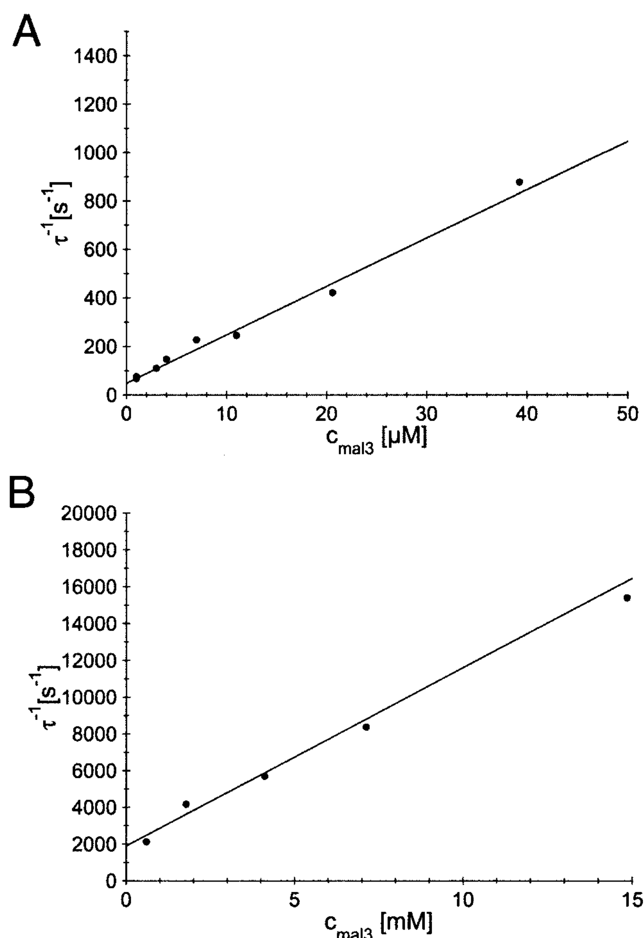

FIGURE 5 (*A*) Dependence of the inverse reaction rate $2\pi f_c = 1/\tau$ of the maltotriose-induced current noise of the LamB mutant Y118W on the maltotriose concentration in the aqueous phase. The data were derived from the fit of the power density spectra with Lorentzians similar to those given in Fig. 4 *A*. The aqueous phase contained 1 M KCl and ~500 ng/ml LamB mutant Y118W. The straight line corresponds to $k_1 = 23 \cdot 10^6\ M^{-1}\ s^{-1}$ and to k_{-1} = 40 1/s. The applied membrane potential was 20 mV; T = 20°C. (*B*) Dependence of the inverse reaction rate $2\pi f_c = 1/\tau$ of the maltotriose-induced current noise of the LamB mutant Y118A on the maltotriose concentration in the aqueous phase. The data were derived from the fit of the power density spectra with Lorentzians similar to those given in Fig. 4 *B*. The aqueous phase contained 1 M KCl and ~500 ng/ml LamB mutant Y118A. The straight line corresponds to $k_1 = 0.29 \cdot 10^6\ M^{-1}\ s^{-1}$ and to k_{-1} = 1860 1/s. The applied membrane potential was 20 mV; T = 20°C.

TABLE 2 Parameters of maltose- and maltooligosaccharide-induced transport noise in different LamB mutants of *E. coli*

Mutant	$k_1/10^6$ M^{-1}/s^{-1}	k_{-1}/s^{-1}	$K/10^3$ M^{-1}
Maltose			
Wild type*	0.8	8000	0.1
Y118W	0.48	20	24
Y118F†	0.18	120	1.5
Maltotriose			
Wild type*	8.4	1950	4.3
Y118A	0.23	2300	0.13
	(0.1)	(6000)	(0.017)
Y118S	3.4	4500	1.2
Y118I	1.2	2200	0.75
Y118W	26	35	743
Y118F†	20	76	290
Maltopentaose			
Wild type*	5.3	420	13
Y118A	1.5	970	1.8
Y118S	4.9	4700	1.5
Y118I	2.4	3100	0.84
Y118N	4.9	2300	3.4
Y118H	5.0	4700	1.2
Y118C	0.93	2900	0.33
Y118D	5.8	2900	1.9
Y118R	1.7	1600	1.1
Y118W	16.5	9	1800
Y118F†	15.5	34	460
Maltoheptaose			
Wild type*	5.6	180	31
Y118A	0.77	660	1.2
Y118S	6.2	3100	2.1
Y118I	2.2	1700	1.4
Y118N	6.1	1600	4.0
Y118H	6.1	2100	3.0
Y118C	3.0	1600	2.0
Y118D	6.9	2300	3.0
Y118R	1.2	530	2.8
Y118W	14.9	2.6	5600
Y118F†	7.0	22	310

The membranes were formed from diphytanoyl phosphatidylcholine/n-decane. The aqueous phase contained 1 M KCl and ~500 ng/ml LamB mutants. k_1 and k_{-1} were derived from a fit of the corner frequencies as a function of the maltose concentration (compare Eq. A3). K is the stability constant for maltose binding derived from the ratio k_1/k_{-1}. The data represent the mean of at least three individual experiments. The standard deviation of the mean value was always <20% of the mean. The parameters for *wild-type LamB (Andersen et al., 1995) and the †Y118F mutant (Jordy et al., 1996) are given for comparison. The numbers in parentheses are those derived by Hilty and Winterhalter (2001) for the same system (mutant Y118A and maltotriose).

analysis. Their results are given in parentheses in Table 2. They differ considerably from our data, which is also the case for the stability constant, K, for binding of maltotriose.

A comparison of Tables 1 and 2 suggests that we found good agreement with the stability constant for the same system derived from the titration experiments and the fluctuation analysis. The rate constants describing the movement of maltotriose to and from the binding site inside the mutant channels are not identical to the vectorial flux through the channel. It is possible, however, to calculate the vectorial flux of carbohydrates through the channels from the rate constants by using the one-site two-barrier model (see Discussion).

Table 2 shows the results of current noise measurement performed with two tyrosine 118 mutants of LamB (Y118F and Y118W) and maltose. The results for wild-type LamB (Andersen et al., 1995) are given for comparison. The kinetic data were derived from measurements similar to those shown in Fig. 4. It is noteworthy that the on- and off-rate constants of carbohydrate binding were fairly independent from the experimental conditions including the number of reconstituted channels. Similarly, the stability constants $K = k_1/k_{-1}$ for maltose binding agreed within less of a factor of 2 with one another and with the stability constants derived from the titration experiments described above for the Y118W mutant (see Table 1). Interestingly, we observed a major influence of the mutation of Y118 on the kinetics of maltose binding (Table 2). In particular, the off-rate decreased from wild-type LamB over Y118F and Y118W by a factor of 400. Similar results were obtained for the kinetics of maltotriose binding to wild-type LamB and some of the mutants (Table 2). Again, a substantial influence on the off-rate constant was found for the replacement of Y118 by other aromatic amino acids. The effect of alanine was mostly on the on-rate constant, which decreased by a factor of almost 40, whereas the off-rate constant remained essentially unaffected. In the case of the serine mutant both rate constants increased, but the effect on the off-rate was more substantial (a factor of ~6). The kinetics of maltotriose binding could not be measured for the other mutants because of the small spectral density S_0 of the current noise in these cases.

The kinetics of maltooligosaccharide binding could be evaluated for all mutants in the case of maltopentaose and maltoheptaose (see Table 2). This is caused by the general decrease of the off-rate constants with the number of glucose residues as compared to the situation for maltose and maltotriose, which leads also to an increase of the time constant of the chemical reaction. A similar effect of the number of glucose residues has been observed for wild-type LamB of *E. coli* (Andersen et al., 1995) and *S. typhimurium* (Jordy et al., 1996). In both cases the off-rate constant, k_{-1}, decreased from maltose to maltoheptaose by factors of 5 to 6. The results of the current noise measurements with all 10 mutants and the maltooligosaccharides maltopentaose and maltoheptaose are summarized in Table 2, respectively. A comparison of the on- and off-rate constants with wild-type LamB reveals again a major influence of Y118 on the kinetics of carbohydrate binding. Both the on- and the off-rate constants were influenced. The highest on-rate and lowest off-rate constants were again obtained for the replacement of Y118 by the two other aromatic amino acids. Otherwise, the on-rate constants of the mutant channel showed some minor effect or they decreased with respect to the LamB wild type, which was highest for Y118C (malto-

TABLE 3 Parameters of maltopentaose-induced transport noise using asymmetric addition of the LamB mutant Y118W to the *cis* side of the membranes and asymmetric addition of maltopentaose

Addition of Maltopentaose	$k_1/10^6$ M^{-1}s^{-1}	k_{-1} / s^{-1}	$K/10^3$ M^{-1}
trans Side	11 ± 1.5	10 ± 0.8	1100
cis Side	7.7 ± 1.5	9.3 ± 1.5	830
Both sides	16.5 ± 2.1	9.0 ± 1.1	1800

For explanations see legend to Table 2 and text. K is defined as k_1/k_{-1}. In case of symmetric conditions it corresponds to the stability constant of carbohydrate binding.

pentaose, fivefold) or for Y118A (maltoheptaose, eightfold). However, much higher effects were observed for the off-rate constants, which increased by factors between 2 and 10 for maltopentaose binding and by factors between 2 and 15 for maltoheptaose.

Is the maltopentaose transport through LamB Y118W mutant asymmetric?

Previous titration experiments with LamB wild-type and loop deletion mutants suggest that carbohydrate transport through the LamB wild type is symmetric concerning the energy potential barriers (Benz et al., 1986, 1987; Andersen et al., 1999). Symmetrical energy barriers for carbohydrate transport through LamB have recently been questioned. Based on liposome-swelling assays and current-fluctuation analysis, Van Gelder et al. (2000) concluded that the periplasmic side of the porin shows a two to threefold higher energy barrier than the extracellular loop-side of the channels. To check a possible asymmetry introduced by the Y118W mutation we performed experiments where the Y118W mutant was only added to one side of the DPHPC/n-decane membranes, the *cis* side. Accordingly, LamB (Van Gelder et al., 2000) or LamB mutants (Andersen et al., 1999) insert preferentially (to ~80%) with the periplasmic side in front into the membrane, i.e., the external side is preferentially exposed to the *cis* side. Using this approach, maltopentaose was added also to only one side of the membrane, either to the *cis* side (same side as the protein) or to the *trans* side (the opposite side) and the kinetic constants were evaluated using the measurement of current noise. The results of the noise experiments are summarized in Table 3. The addition of both Y118W and maltopentaose to the *trans* side resulted on average in an on-rate of $11 \cdot 10^6$ M^{-1} s^{-1} and an off-rate of 10 s^{-1}. When maltopentaose was added to the *cis* side the rate constants were $7.7 \cdot 10^6$ M^{-1} s^{-1} and 9.3 s^{-1}, respectively. This result suggests that there exists little, if any, asymmetry (when the experimental error is considered) for maltopentaose transport across the Y118W mutant.

DISCUSSION

The mutation of tyrosine 118 has a major effect on maltooligosaccharide binding affinity

In the preceding study by Orlik et al. (2002) we demonstrated that tyrosine 118, which controls the central constriction of the LamB channel, has a major effect on ion permeation through LamB of *E. coli*. Its single-channel conductance increases substantially for any other amino acid, with the exception of Y118F and Y118W. The effect on ion conductance is definitely caused by the size of the bulky side chain of tyrosine, phenylalanine, or tryptophan, which limits the channel size. This is easy to understand (see Fig. 1) because the size of the channel is ~0.6 × 1.0 nm for wild-type LamB (Schirmer et al., 1995). The mutation of tyrosine to alanine changes the size to ~1.0 × 1.0 nm. This means that most mutant channels are wider than wild type, which has a major effect on ion conductance.

LamB functions in the outer membrane of enteric bacteria as a channel for carbohydrates, in particular for the uptake of maltooligosaccharides, although it is induced by maltose. In the growth experiments we could demonstrate that this is also the case for the mutants used in this study. All were able to confer to the porin-deficient KS26 strain the growth on maltose and maltopentaose, and the growth rate was very similar to that when the strain KS26 contained a plasmid for the expression of wild-type LamB. This result means that the mutant channels have the same function as wild-type LamB. The central constriction of the channel with tyrosine 118, according to the 3-D structure, plays a major role because it is localized within the center of the channel (Schirmer et al., 1995; Dutzler et al., 1996; Meyer et al., 1997). The increase of channel size caused by the replacement of Y118 by non-aromatic amino acids leads to a drastic decrease of the affinity of the LamB mutant channels toward carbohydrates of the maltose and maltooligosaccharide series, as Table 1 clearly indicates, although Y118 is not directly involved in maltooligosaccharide binding through a hydrogen bond (Dutzler et al., 1996; Meyer et al., 1997). The strongest effect was observed for Y118A, where the stability constants for maltotriose, maltopentaose, and maltoheptaose binding decreased by factors of ~20, 25, and 23, respectively. A strong effect on maltooligosaccharide binding was also observed for the Y118C, Y118H, Y118N, and Y118S mutants. However, the decrease of the stability constant was in these cases much smaller than for the Y118A mutant. In particular, for maltoheptaose binding it decreased by a factor of <13 (Y118S); in general, by a factor of ~6. The stability constant for maltooligosaccharide binding increased for most mutants with increasing numbers of glucose residues, as has been found for wild-type LamB.

The most substantial effect of the mutation was observed for the binding of maltose and maltooligosaccharides when tyrosine was replaced by the other two aromatic amino

acids, phenylalanine (Jordy et al., 1996) and tryptophan (this study). In particular, the Y118W mutant showed a dramatically increased affinity for maltose and maltooligosaccharides, and the stability constants for maltose, maltotriose, maltopentaose, and maltoheptaose increased by factors of 26, 385, 200, and again, ~200, respectively, as compared to wild type. This means that the free energy, ΔG_0, of binding for the long-chain maltooligosaccharides increased by a factor of ~13 kJ/mol as calculated from the expression $\Delta G_0 = R \cdot T \ln K$ when the Y118W mutant is compared to the wild type. The increase of the free energy of the Y118F mutant studied previously by Jordy et al. (1996) was smaller by far (~6 kJ/mol) than observed here for the Y118W mutant, which is basically caused by the small off-rate k_{-1} (see below).

Effect of tyrosine 118 on maltooligosaccharide binding kinetics as derived from the analysis of carbohydrate-induced current noise

Besides the binding affinity, we also studied the effect of the Y118 mutation on the binding kinetics of the maltooligosaccharide binding using the analysis of the current noise. The current noise of LamB of *E. coli* had two different aspects. The open channel showed 1/*f* noise up to frequencies of ~100 Hz. A major part of this noise represents the characteristics of the open porin channels (Wohnsland and Benz, 1997). The rest of the 1/*f* noise is probably caused by slow closing and opening kinetics of LamB channels (Nekolla et al., 1994), which we tried to avoid in the measurement of the current noise reported in this study. The current recordings were only analyzed for current noise when the recordings on the strip chart recorder were absolutely stationary. Furthermore, we controlled the current recordings for the reconstitution of general diffusion pores. This means also that the current through the membranes was very small at high carbohydrate concentrations because the trimeric channels are completely blocked by carbohydrate binding.

The other aspect is that the power spectra of the current noise of the LamB mutant channels showed Lorentzian type of noise in the presence of carbohydrates in the aqueous phase because of the block of the channel for ion movement, which is controlled by a chemical reaction between carbohydrates from the aqueous phase and the binding site (Nekolla et al., 1994; Andersen et al., 1995). It is noteworthy that the power density spectra of the amiloride-induced current noise of the sodium channels in frog skin, which represents a similar system as investigated here, also shows Lorentzian type of noise (Lindemann and Van Driessche, 1977a, b; Lindemann, 1980; Van Driessche and Lindemann, 1979). Similar to previous studies, here we used a one-site, two-barrier model for the analysis of carbohydrate binding to the different LamB mutant channels (Nekolla et al., 1994; Andersen et al., 1995). This model provides a good explanation for the experimental data derived here from experiments with the LamB mutants. This means that the one-site, two-barrier model allowed the evaluation of the on- and off-rate constants for the binding of maltose, maltotriose, maltopentaose, and maltoheptaose to the central binding site inside the channel of a variety of Y118 LamB mutants. For other carbohydrates and also for some of the mutants it was impossible to derive the rate constants from the noise measurements because the corner frequency could not be obtained from the current noise, i.e., the binding kinetics was too fast or the spectral density was too small. We furthermore assumed that the binding of maltose and the maltooligosaccharides was symmetrical with respect to the sidedness of the LamB channel, i.e., the on- and off-rate constants were the same from both sides. This has recently been questioned (Van Gelder et al., 2000). However, neither in this study nor in other investigations did we find any indication for channel asymmetry concerning maltose and maltooligosaccharide binding (Andersen, Orlik, and Benz, unpublished results).

The results of our analysis of the current noise suggest, in general, that both the on-rates and the off-rates are influenced by the mutation of Y118. The degree of change varied somewhat for the three maltooligosaccharides. For maltotriose binding k_1 varied ~100-fold between the smallest on-rate (Y118A, $k_1 = 2.3 \cdot 10^5\ M^{-1}\ s^{-1}$) to the highest one (Y118W, $k_1 = 2.5 \cdot 10^7\ M^{-1}\ s^{-1}$). This results suggest that the on-rates are ~100-fold smaller than those of diffusion-controlled reaction processes (Eigen et al., 1964). To explain this smaller on-rate we have to assume that the carbohydrates have to hit the channel many times before they are bound to the binding site. The reason for this process being slower than the diffusion-controlled one is presumably that the carbohydrates can only be bound to LamB and its mutants when they are in a special order with respect to the binding site. In a previous study we could demonstrate that the maltooligosaccharides bind to LamB with the nonreducing end (the anomeric carbon atom in the 4-position of the α-D-glucopyranosyl moiety of the maltooligosaccharides) in front of the surface-exposed side and vice versa for the periplasmic side (Andersen et al., 1999), which agrees with crystallographic studies (Dutzler et al., 1996).

A strong influence of the Y118 mutation was also observed for the off-rates of carbohydrate binding to the LamB mutant channels. However, the off-rates for Y118A, Y118S, and wild type were approximately the same, and only those for Y118W and for Y118F (Jordy et al., 1996) were considerably smaller. This result was previously explained by the increased interaction of the bulky phenylalanine side chains with the carbohydrates as compared with tyrosine, which contains a hydroxy group (Jordy et al., 1996). The experiments presented here with the even more bulky tryptophan side chain support this assumption, and the off-rate constants for the binding of all carbohydrates are even smaller in this case. In particular, the change may increase

the hydrophobicity inside the central fraction of the channel and/or may restrict the space inside the channel, which means that the interaction between the apolar side of the carbohydrates and the channel interior increases and slows down the movement of the maltooligosaccharides through the central constriction of the channel, thus reducing the off-rate constant of carbohydrate binding.

The effect of the mutation on the on-rate of maltopentaose binding was less pronounced and it changed only by factors between ~3 and 20. Most of the on-rate constants were within $1 \cdot 10^6$ and $5 \cdot 10^6$ M^{-1} s^{-1}, with the exception of the Y118F (Jordy et al., 1996) and the Y118W mutants, which again had the highest on-rate, presumably caused by the increased interaction between the bulky aromatic side chains and the maltooligosaccharides. The differences between the off-rates were more substantial. Interestingly, all mutants with the exception of Y118F and of Y118W had higher off-rates, suggesting that the replacement of the aromatic side chains of tyrosine, phenylalanine, and tryptophan by others facilitated the release of the carbohydrates from the binding site. Similar effects were found for the kinetics of maltoheptaose binding: the on-rates differed only by less than a factor of 10 and were in some cases somewhat smaller than for maltopentaose binding, whereas the effect on the off-rates was again more substantial. This result indicates that the replacement of Y118 by non-aromatic amino acids generally influenced the off-rates more than the on-rates of carbohydrate binding. It is noteworthy that in the sucrose-specific ScrY channel, a natural mutant of LamB, the aromatic amino acid tyrosine is replaced by aspartic acid (Forst et al., 1997), which also increases the off-rate of maltooligosaccharide binding (Andersen et al., 1998). The effect of the aromatic residues on carbohydrate binding may be explained by the following: the Y118 points to the flat (hydrophobic) side of the carbohydrates according to the crystal data (Dutzler et al., 1996). The interaction between carbohydrate and amino acid residue increases in the series Y, F, and W, thus suggesting that size and hydrophobicity reduce the velocity of the carbohydrates within the channel, which leads to a decrease of k_{-1} and an increase of K.

The results presented here and in a previous study (Jordy et al., 1996) indicate that the maximum interaction between the binding site and the sugar is already given for three glucose residues, i.e., the binding site inside the LamB channel appears to be three glucose residues long in the case of the maltooligosaccharides. However, it is also possible that more sites are involved that may play a possible dynamic role. The binding between carbohydrates and the binding site occurs via hydrogen bonds (Dutzler et al., 1996; Jordy et al., 1996; Meyer et al., 1997). The off-rate constants of binding to wild type and the mutants tended to decrease in the series maltotriose to maltoheptaose, which is also easy to understand on the basis of a three-glucose-long binding site. When a maltopentaose molecule binds to the site only three glucose residues are in contact with it (Dutzler et al., 1996; Meyer et al., 1997). The movement of the maltopentaose by one glucose unit could still result in maximum binding affinity, and the maltopentaose may move forth and back several times within the channel before it can finally leave the channel to one side. This process may lower the desorption reaction considerably, in particular when the long-chain maltooligosaccharides have even more glucose residues, as is the case for maltoheptaose. It is noteworthy that starch associates almost irreversibly with the LamB channel (Ferenci et al., 1980), which supports our view of the mechanism of carbohydrate transport through the LamB channel.

Implication of Y118 mutations for the carbohydrate transport in vivo

The porin-deficient KS26 strain has a small growth rate on maltose as sole carbon source and did not grow on the M9 minimal medium containing maltopentaose. The expression plasmids containing the genes for wild-type LamB and its mutants confer to strain KS26 the possibility to grow on maltose and maltopentaose as sole carbon sources with approximately the same rate for both carbohydrates, but a much higher rate compared with the growth of KS26. These experiments suggest that maltose and maltooligosaccharides can pass through the mutant channels, which means that the mutant channels are functional. This allows calculation of the flux of carbohydrates through the channel using the one-site two-barrier model and the kinetics of carbohydrate binding. Despite a recent study, which has suggested that the LamB channel is asymmetric with respect to carbohydrate binding from both sides of the channel (Van Gelder et al., 2000), we never found any asymmetry in carbohydrate binding of LamB wild type (Benz et al., 1986, 1987; Andersen et al., 1999) and only a small asymmetry, if any, for carbohydrate binding kinetics to the Y118W mutant. This means that we can assume a symmetrical channel with sufficient accuracy. 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 of the two identical potential energy barriers (Benz et al., 1987):

$$\Phi = k_1 \cdot c'(1 + K') - k_{-1} \cdot K'/(1 + K') \qquad (3)$$

K' is under symmetrical conditions given by:

$$K' = K \cdot (c' + c'')/2 \qquad (4)$$

In Eq. 3 the rate constants k_1 and k_{-1} are multiplied by the probabilities that the binding site is free or occupied, respectively. Equation 3 has in the case $c'' = c$, $c' = 0$, when

carbohydrates are only present on one side of the channel, the following form:

$$\Phi = k_1 \cdot c/(2 + K \cdot c) \quad (5)$$

The latter form may be used to calculate the flux of a given carbohydrate through one of the three channels in a LamB trimer under the assumption of a symmetrical channel. Equation 5 suggests that the maximum permeability of the channel for a carbohydrate with an on-rate k_1 for its binding to the binding site is $k_{-1}/2$, which is obtained for wild-type LamB at a very small carbohydrate concentration ($c \leq 10$ μM). The flux strongly saturates at high carbohydrate concentration as the half-saturation constant for the sugar flux is $K_S = 1/K$. The maximum turnover number of the channel (similar to the maximum turnover number of an enzyme that is saturated by substrate) is reached at high carbohydrate concentration on one side of the membrane and is given by k_{-1}. This means that the flux through LamB is limited at high maltose and maltooligosaccharide concentration. It is noteworthy, however, that this is not a serious restriction because the concentration of substrates is normally small under physiological conditions. For the effective scavenging of nutrients at very small concentrations it seems to be more important to have a high permeability (i.e., a high k_1), which is indeed given for the transport of maltooligosaccharides through the wild-type LamB channel (Andersen et al., 1995).

Our data allow a comparison of the flux of maltose and maltooligosaccharides through wild type and some of the LamB mutant channels. Fig. 6 *A* shows the maximum flux of maltotriose through a single LamB wild-type, Y118A, and Y118W mutant channel calculated on the basis of Eq. 5 under the assumption that the concentration of the sugars on one side (i.e., the periplasmic side) is zero. The curves were calculated using the rate constants given in Table 2. Fig. 6 *A* shows the substantial effect of a single amino acid mutation within the primary sequence of LamB on the transport of maltotriose. The carbohydrate-specific porins have their maximum permeability in the linear range of the figure. This means Y118W has the highest permeability, followed by wild type and Y118A. This mutant had the highest turnover number of 2300 1/s as compared with wild type (1950 1/s), and it was lowest for the Y118W mutant (40 1/s). The same relation for the wild-type and the two mutants is obtained for maltoheptaose transport (see Fig. 6 *B*). The comparison of the different fluxes again demonstrates the advantage of a binding site for the maximum scavenging of substrates and the role of Y118 within this binding site. The expression of LamB in enteric bacteria is always induced with the expression of the periplasmic maltose binding protein MBP or MalE. MalE is present in the periplasmic space in a concentration in the range of millimolar and transforms this space into a sink for carbohydrates, although it does not modulate LamB channel function (Schwartz, 1987; Brass et al., 1985). This property is an essential part of carbohydrate uptake across the outer membrane because a carbohydrate bound from the cell surface to the binding site inside the LamB channel still has two possibilities with equal probabilities for further movement because the channel is symmetric with respect to its transport properties. It can move back to the cell surface or further on to the periplasmic space. In the latter case it is bound to MBP with a half-saturation constant of considerably less than 10 μM, which means that the carbohydrate is trapped within the periplasmic space, and as long as inner membrane transport functions it is unlikely that the carbohydrate can be lost through the outer membrane.

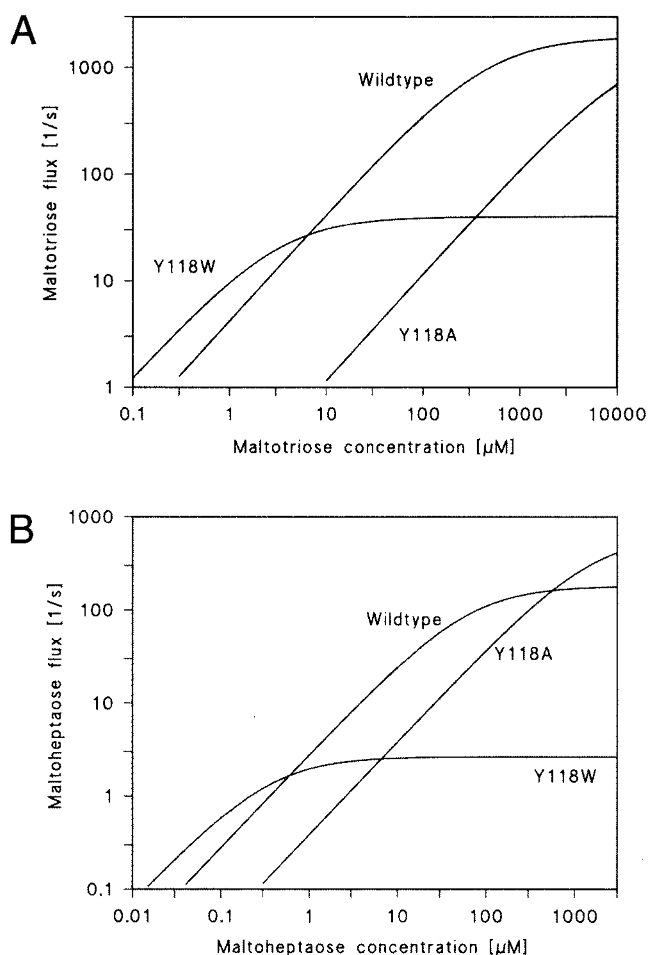

FIGURE 6 (*A*) Flux of maltotriose through one single wild-type LamB, Y118W, and Y118A channel (i.e., through the monomer in a wild-type, Y118W, and Y118A trimer channel) as a function of the maltotriose concentration on one side of the channel. The concentration on the other side was set to zero. The flux was calculated using Eq. 5 and the rate constants given in Table 3. (*B*) Flux of maltopentaose through one single wild-type LamB, Y118W, and Y118A channel (i.e., through the monomer in a wild-type, Y118W, and Y118A trimer channel) as a function of the maltoheptaose concentration on one side of the channel. The concentration on the other side was set to zero. The flux was calculated using Eq. 5 and the rate constants given in Table 2.

The calculation of the maximum flux of carbohydrate molecules through LamB and its mutants also allows a meaningful comparison with the in vivo requirements of maximum growth of *E. coli* cells. The minimum carbon source supply for maximum growth rate is 20 nmol glucose/(min $\times$ 10^9 cells) (Freundlieb et al., 1988). The expression of the LamB mutant Y118W is ~40% of full expression (Heine et al., 1988). This means that a single cell has ~10^4 channels ($3.3 \cdot 10^3$ trimers) in the outer membrane. The maximum flux of maltopentaose through one single Y118W monomer is 9 s^{-1} (i.e., the turnover number), which means that the maximum flux of maltopentaose in 10^9 cells is ~$9 \cdot 10^{13}$ s^{-1} or $3.6 \cdot 10^{15}$ min^{-1}. This number corresponds to a maximum flux of ~6 nmol maltopentaose/(min $\times$ 10^9 cells). The carbon source supply under these condition in the cells expressing the Y118W mutant (with the smallest turnover) is 30 nmol glucose/(min $\times$ 10^9 cells). All other mutant channels have a higher turnover, i.e., a higher supply of the carbon source. This result suggests indeed that the KS26 cells expressing the mutant LamBs can show maximum growth under the conditions of our growth experiments.

APPENDIX

Derivation of the rate constants of carbohydrate binding from the frequency dependence of the spectral density

For the analysis of the current noise we used a simple one-site, two-barrier model (Läuger, 1973; Benz et al., 1987; Benz and Hancock, 1987) with a central binding site inside the channel. The binding of the carbohydrates (aqueous concentration c) to the central binding site inside the channel is described by first-order chemical reactions (on-rate constant k_1 and off-rate constant k_{-1}) from both sides, i.e., the channel is assumed to be symmetrical with respect to carbohydrate binding. The stability constant of the binding of a carbohydrate to the channel is $K = k_1/k_{-1}$. Furthermore, it is assumed that the LamB channel and its mutants represent single-file channels (Benz et al., 1986). This means that the LamB channels are open when no carbohydrate is bound, and closed when they are occupied. The measurements of current noise presented here are based on small perturbations of the number of closed channels due to microscopic variations involved in the chemical reaction between carbohydrate and binding site, which can be monitored by current fluctuations. Its reaction rate $1/\tau$ is given by Verveen and De Felice, 1974; De Felice, 1981:

$$\frac{1}{\tau} = 2\pi \cdot f_c = k_1 \cdot c + k_{-1} \quad \text{(A1)}$$

where f_c is the corner frequency of the power density spectrum, $S(f)$, given by a "Lorentzian" function. Lorentzian spectra correspond to the noise expected for a random switch with different on and off probabilities, which are coupled by a chemical reaction (Verveen and De Felice, 1974; Conti and Wanke, 1975; De Felice, 1981):

$$S(f) = S_0/(1 + (f/f_c)^2) \quad \text{(A2)}$$

where S_0 is the plateau value of the power density spectrum at small frequencies. It is given by (Verveen and De Felice, 1974):

$$S_0 = 4 \cdot N \cdot i^2 \cdot p \cdot (1 - p) \cdot \tau \quad \text{(A3)}$$

where N is the total number of channels (blocked and unblocked) within the membrane, i is the current through one single open channel, and p is the probability that the channel is occupied by a carbohydrate (i.e., closed).

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