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Selectivity for maltose and maltodextrins of maltoporin, a pore-forming protein of *E. coli* outer membrane

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Homogeneous maltoporin (lamB protein), an *Escherichia coli* outer membrane spanning protein, was incorporated in phospholipid planar bilayers. It generates aqueous channels distinct from those formed by the non-specific porin (OmpF) or by phosphoporin (phoE protein). The single conductance, 150 pS in 1 M NaCl, is much smaller than that of the porins. The channels, which are poorly selective for cations and voltage independent, are specifically inhibited by maltose and maltodextrins. This inhibition, observed in the absence of maltose binding protein, demonstrates that the selectivity of maltoporin for maltose and maltodextrins is an intrinsic property of the protein.

Maltoporin; Maltose-binding protein; Pore-forming protein; Single-channel conductance

1. INTRODUCTION

The product of the gene Lam B, maltoporin, is an integral membrane protein in the outer membrane of *Escherichia coli*. It behaves as a porin, inasmuch as it allows passive diffusion of molecules with a mass less than 600 Da. It also plays a specific role in the transport of maltose and maltodextrins [1]. It is essential for growth on maltodextrins if these contain three or more glucose units [2]. It was discovered at the cell surface as the receptor for several bacteriophages including bacteriophage lambda [3]. Like the other porins (OmpF, OmpC and PhoE proteins), the active form is a trimer (142 kDa) whose secondary structure consists predominantly of β-pleated sheets [4,5].

Conflicting evidence has been reported concerning the physical basis of the selectivity of maltoporin involving an interaction with maltose binding protein, a soluble periplasmic protein, and the size of the pore itself has been implied. It has been suggested by Heuzenroeder and Reeves [6] that the maltose binding protein confers specificity on the maltoporin pore by physically impeding the passage of molecules other than maltose through the channel. In contrast, Brass et al. [7] presented evidence that in vivo, the maltose binding protein does not modulate the activity of maltoporin as a general pore. The permeability of maltose through maltoporin reincorporated in liposomes in vitro has been reported to be 25-fold higher than that through the general (OmpF) porin while glucose diffuses at the same rate through the two protein channels [8,9]. Also, maltose flux appears to be inhibited by maltodextrins [10]. The existence of binding sites for maltodextrins has been suggested [11]. Neuhaus et al. [12] reported that incorporation of maltoporin into planar bilayers led to noisy levels of conductance. In those experiments, the addition of maltose binding protein resulted in the stabilization of discrete states, with a shift of the equilibrium of open and closed channels towards the closed state. Benz et al. [13] described two

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types of pore formed by a complex maltoporin preparation, a large pore of 2.7 nS and a smaller pore of 0.16 nS in 1 M KCl: only the smaller pore conductance was found to be inhibited by maltose and maltotriose.

In this report, we present results on the incorporation of highly purified maltoporin and demonstrate its functional reconstitution in planar bilayers. Its conductance properties are significantly distinct from those of porin and phosphoporin and are strongly affected by maltose and maltodextrins.

2. MATERIALS AND METHODS

2.1. *Protein preparation and chemicals*

Maltoporin was extracted and purified to homogeneity from *E. coli* W POP 1021 using the non-ionic detergent octyl-POE [14] according to the procedure described by Neuhaus [15]. As judged by SDS-PAGE using either a Coomassie blue or silver staining procedure, the maltoporin preparation contained neither lipoprotein, nor protein contaminant nor lipopolysaccharides (LPS). Maltose and glucose were purchased from Merck, maltotriose and maltoheptaose from Sigma.

2.2. *Vesicle formation*

Phospholipid vesicles were made using soybean lecithin (Sigma type IV) as described by Schindler and Quast [16]. Proteoliposomes were reconstituted from phospholipids and solubilized homogeneous maltoporin by detergent dialysis [17]. The molar protein-lipid trimer ratio was either 10^{-6}, 10^{-5} or 10^{-4}. Where indicated, proteoliposomes were further diluted with phospholipid vesicles to give final molar ratios of 10^{-8} and 10^{-9} corresponding to 300 and 30 trimers per bilayer area.

2.3. *Formation of the bilayer and electrical measurements*

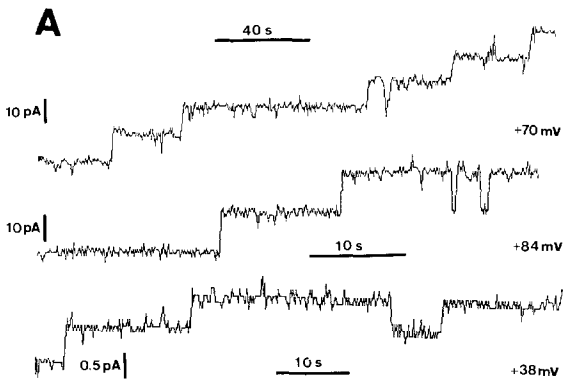
Two different experimental approaches have been used: (i) phospholipid vesicles and vesicles containing maltoporin were transformed into planar membranes via monolayer assembly as described by Schindler [18]; or (ii) highly purified maltoporin in detergent was injected in front of a preformed bilayer obtained as above as described by Pattus and co-workers [19]. By using this last experimental approach, the number of induced channels varies 2- or 3-fold. Final protein concentration was 5 to 30 $\mu g \cdot ml^{-1}$. The bilayers were formed over a circular aperture (2×10^{-4} cm^2). Electrical measurements and the criteria for bilayer formation and characteristics were as described by Schindler [18]. The sign of electrical potential always refers to the *cis* compartment to which either protein-containing vesicles were added or purified protein injected. Ag/AgCl electrodes, inserted in the aqueous compartments on both sides of the bilayer, were used in experiments performed with chloride salts. V_m current membrane potentials were measured using agar salt bridges and determined as described by Raymond et al. [20]. All experiments were carried out at room temperature with 10 mM Tris-acetate (pH 7.4) containing 10 mM $CaCl_2$ or 10 mM $MgCl_2$ and 3 mM NaN_3 in addition to various salt concentrations.

Single-channel recordings were filtered at 1 kHz by means of low pass filter EG & G model 113 before being recorded on an FM tape recorder (Racal FM) and digitized at 20 ms sampling rate for computer analysis (Z-80, CP/M3 system). Analysis of single-channel recordings was carried out mainly through computerised data processing. Current amplitude histograms were used to evaluate single-channel amplitude.

3. RESULTS

3.1. *Maltoporin channel conductance*

Fig.1A shows successive induction of well defined conductance steps following injection of maltoporin in a dilute detergent solution (see section 2). Analogous to other systems [19,21], these increments can be attributed to incorporation and activation of pores formed by maltoporin within the bilayer. After induction, the current reaches a steady state where fluctuations occur frequently (fig.1B). These fluctuations, corresponding to opening and closing events of channels, have the same size as the induction steps shown in A. Their frequency is independent of the membrane potential applied. In contrast to the OmpF and PhoE proteins, the pores formed by maltoporin do not exhibit a negative resistance property and are opened at high potential. This is illustrated by the steady state *I-V* curve (fig.2). The steady-state cur-



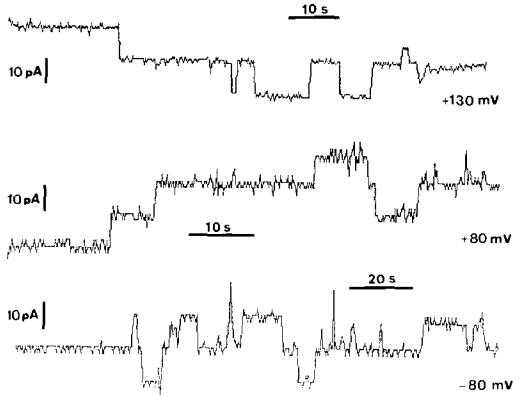


Fig.1. (A) Stepwise increase in current after injection of maltoporin in a detergent solution (1% octyl-POE) in front of a preformed asolectin bilayer at membrane potentials of 70, 84 and 38 mV. The voltage was applied before injecting maltoporin. The planar bilayers are formed by the apposition and fusion of two monolayers according to Schindler [18]. (B) The current trace at steady state after the induction of maltoporin pores at an applied membrane potential of −80, 80 and 130 mV. Stepwise current fluctuations corresponding to the inductions steps are clearly resolved. Note that the frequency of the fluctuations is independent of the membrane potential. The small fluctuations resolved at high time resolution correspond to noise due to the acquisition system. The buffer was Hepes-NaOH (10 mM), and contained 1 M NaCl, 10 mM $CaCl_2$ and 3 mM NaN_3 at pH 7.4.

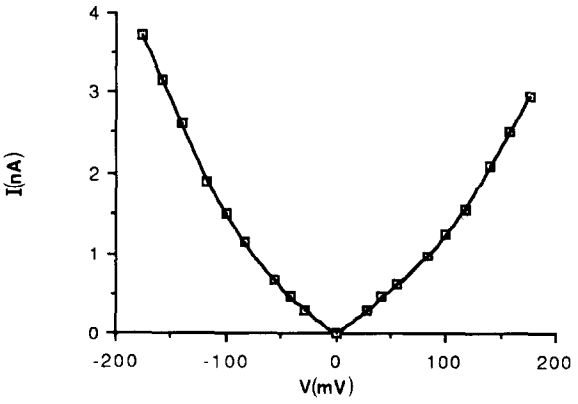


Fig.2. Current-voltage curve of maltoporin pores contained in a phospholipid bilayer. The steady-state current (in absolute terms) was determined after voltage steps from 0 mV up to a given potential. Between each voltage step, the steady-state current was measured at 50 mV for reference. Note the slight asymmetry of the curve. The experimental conditions are identical to those in fig.1.

rent resulting from the ionic flux through many channels is strictly proportional to the current flowing through a single channel at any voltage. The single-channel conductance is constant between −80 mV and +80 mV (150 ± 20 pS, in 1 M NaCl) while it increases at higher potentials with a slight asymmetry depending on polarity. In order to measure the ionic selectivity of maltoporin, a 4.4-fold NaCl (0.88 M:0.2 M) gradient was established across the bilayer. The zero current potential of −7 ± 2 mV obtained indicates a slight preference (1.6-fold) for cation over anion.

Transformation of maltoporin-containing vesicles into planar bilayers via monolayer self-assembly [18] allows the number of trimers incorporated in the membrane to be monitored. Planar bilayers obtained from vesicles with a high lipid-to-protein ratio (10^8) do not display well-defined conductance properties. By decreasing the lipid-to-protein ratio by several orders of magnitude, stepwise conductance inductions are observed. Most of the induction steps are multiples of the single-channel conductance observed upon injecting the protein in the aqueous phase, indicating that activation of aggregate occurs. The smallest induction steps and most of the fluctuations observed correspond to single-channel events found by injection. At steady state the yield of activated pores

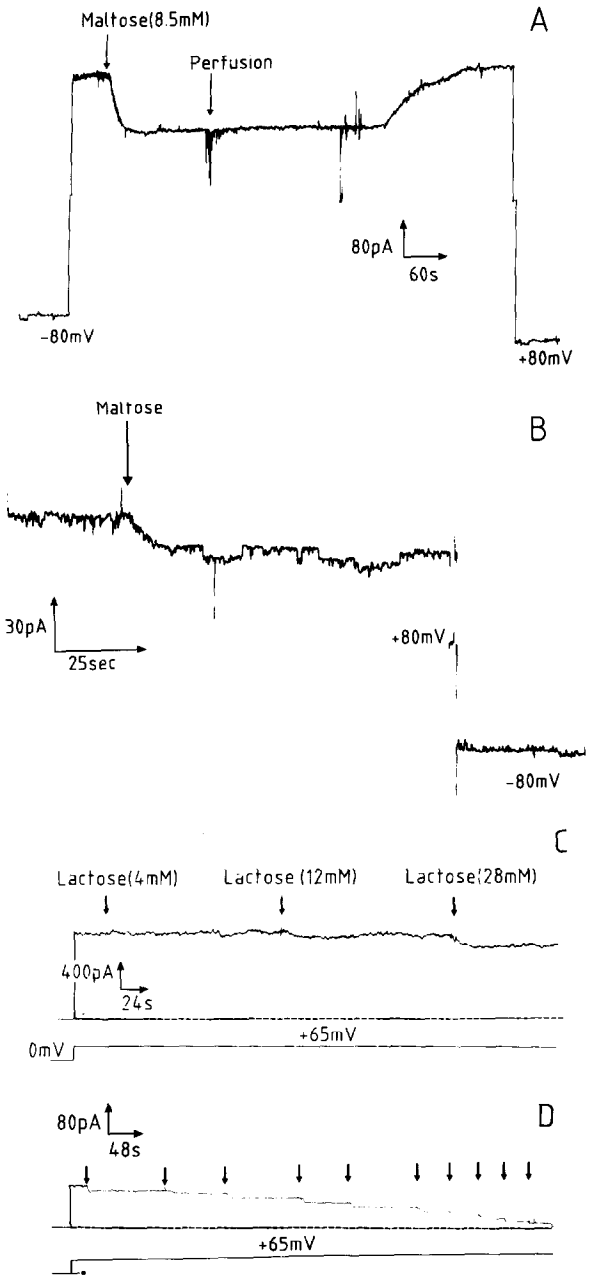


Fig.3. (A) Inhibition of maltoporin conductance by maltose. At the time indicated by the arrow, 8.5 mM maltose (final concentration) was added to the *cis* compartment at 80 mV applied voltage. The inhibition is reversible. By perfusing (arrow) the *cis* compartment with buffer without maltose (10 times the volume), the initial level of current is reestablished. The slight increase in the current is due to new incorporation of maltoporin pores. Maltoporin trimers were injected into the front of a preformed asolectin bilayer in the *cis* compartment. The conditions are those described in fig.1. (B and C) Discrimination between two disaccharides of identical molecular mass: the effect of maltose and lactose on maltoporin pores. (B) Inhibition of maltoporin conductance following the injection of maltose (2.5 mM). Note that the pores still fluctuate at a slightly decreased size of the maltoporin pore in the presence of maltose. (C) The slight inhibition of maltoporin pore conductance following the injection of lactose in the cis compartment at 4, 12 and 28 mM (final concentration) respectively. (D) Inhibition of maltoporin pore conductance by increasing the amount of maltotriose. Conditions are those given for fig.1. At the time indicated (arrows), increasing amounts of maltotriose (40, 122, 200, 350, 580 and 860 μM, 1.3, 2, 3.7, 6.6 and 9.4 mM final concentration) were injected into the *cis* compartment. Note that complete inhibition was reached at 9.4 mM maltotriose.

is significant (7–20%). It is not clear whether the lack of channel activation at high lipid-to-protein ratios arises from defective reconstitution at the vesicle level or whether it is due to denaturation within the monolayer, or malfunctioning of the protein within the planar bilayer. With both reconstitution methods, no effect of, and no requirement for, lipopolysaccharides or maltose binding proteins was observed. Unlike the previously observed stabilisation of channel conductance [12], the maltoporin-induced conductance did not change detectably if purified maltose binding protein was present on either side of the bilayer or if it was preincubated with maltoporin before reconstitution.

3.2. *The influence of maltose and maltodextrins*

The significant biological role of maltoporin in vivo appears to be the facilitation of the diffusion of maltose and maltodextrins through the outer membrane of *E. coli*. We have investigated the effect of maltose and maltodextrins on maltoporin pore conductance in our system. As shown in fig.3A, injection of maltose (8.5 mM) into the aqueous compartment leads to a drastic drop in the ionic current crossing the bilayer. At this maltose concentration the current was decreased by nearly 50%. The effect is reversible; the initial level of conductance is reestablished upon removal of the disaccharide. The single-channel conductance is decreased in the presence of maltose although the

Table 1

Inhibition constants of maltose and maltodextrins

Maltose and maltodextrin compounds	NaCl (M)	Inhibition constants (M)	
		Injection into *cis* compartment	Injection into *trans* compartment
Maltose	1	$10 \pm 3 \times 10^{-3}$	$10 \pm 3 \times 10^{-3}$
	0.1	$13 \pm 2 \times 10^{-3}$	ND
Maltotriose	1	$7.6 \pm 2 \times 10^{-4}$	$5.8 \pm 2 \times 10^{-4}$
Maltoheptaose	1	$2.4 \pm 0.4 \times 10^{-4}$	ND

The conductance of the maltoporin pore, asymmetrically incorporated in planar bilayers, is inhibited by maltose and maltodextrins, irrespective of which side the compounds are injected

fluctuation frequency (fig.3B) does not change significantly. This indicates that diffusion of maltose within the lumen of the pore hinders the diffusion of cations and anions through the pore, yet it does not affect the equilibrium between open and closed pores. In contrast to maltose, injection of lactose into the aqueous compartment leads to a very slight inhibition of the current only (fig.3C). At a concentration of 28 mM, the residual current still represents 90% of the initial one while at the same concentration of maltose; it is decreased to 20% (not shown). Upon stepwise increase of maltotriose concentration in the aqueous compartment, an even more marked decrease in the current is observed (fig.3D). The apparent inhibition constants listed in table 1 were calculated [22] by plotting the residual conductance as a function of the concentration of either maltose or maltodextrins. Irrespective of the ionic strength (1 M NaCl or 0.1 M NaCl) and of the applied membrane potential, an inhibition constant of 10 ± 3 mM was found for maltose, independent of its concentration. Maltodextrins such as maltotriose and maltoheptaose led to inhibition at lower concentrations (table 1). The effect of maltodextrins and maltose was found independently of the reconstitution methods used (injection or vesicle transformation). With the latter method, an asymmetrical orientation of maltoporin in the planar bilayer is expected. However, we found identical inhibition constants whichever side maltose or maltodextrins were injected.

4. DISCUSSION

The single-channel data presented in this study show that in artificial membranes, maltoporin forms a unique type of channel of 0.15 nS single conductance steps (in 1 mM NaCl). This corresponds to the small channel described by Benz et al. [13]. The absence of larger channels is likely to reflect the homogeneity of the preparations used in this study. Ionic flow through the maltoporin channel is 4-times lower than that through the porin channels. Ion transfer, which is ohmic at low potentials, is enhanced at higher potentials with a slight but significant asymmetry depending on the polarity of the applied potential. Channel fluctuations, however, occurred independently of membrane potential. It was shown with the porins OmpF and the PhoE proteins that induction of channels in planar bilayers occurred through 2 nS conductance steps or multiples thereof [23,24]. Moreover, fluctuations occurred only through 0.6 nS conductance steps. When a single porin or phosphoporin trimer was activated, it closed at high voltage through three consecutive, and time-independent, closing events. In contrast, induction and channel fluctuation of maltoporin always occurred through single unit conducting steps.

The detection of the inhibitory effects of maltose and maltodextrins on maltoporin pore conductance affords new insights into the characteristics of the maltoporin channels. Thus the observed inhibition is unlikely to be due to a

real blocking effect. Since maltose is an uncharged molecule, its passage through the channel cannot be measured directly in our system. It can be concluded from our observations, though, that the maltoporin pore itself is able to discriminate two related disaccharides (maltose and lactose, cf. fig.3B and C) of identical mass, and thus to act as a selective filter for maltose and maltodextrins. This hypothesis is in agreement with the following observations in an assay of swelling liposomes. Firstly, a higher diffusion of maltose occurs through maltoporin than through OmpF pores, and secondly, the flux of maltose is inhibited by the presence of maltodextrins such as maltotriose and maltoheptaose in the same range of concentration as those we have found [8–10]. Finally, a discrimination among sugars is observed with maltoporin channels but not with OmpF porin [25].

In our study, maltose binding protein did not modulate the maltoporin pore properties. Conductance levels were stable in the absence of maltose binding protein, such that the latter had no significant effect on channel lifetime. Previously it was reported that this periplasmic protein shifted the equilibrium of the open and closed state in favour of the closed state [12]. The preferred explanation was that the maltose binding protein modifies the channel-forming characteristics of maltoporin. The possibility that with the binding protein, purified on an agar column, tightly bound oligosaccharides were introduced cannot be strictly discounted. It is also possible that strain difference or a requirement for a third component other than lipopolysaccharides was the cause of these observations.

Our results confirm in vivo studies which indicate that maltoporin contributes to the transport specificity for maltodextrins [7]. Although it appears that maltose binding protein is not required to confer the specificity of maltoporin, we cannot rule out that interaction between it and maltoporin occurs in vivo and may play a role in a different step of the maltose uptake pathway. Diffusion of maltodextrins through maltoporin pores is most likely due to the presence of multiple maltosyl binding sites as suggested previously [11,26]. Using the asymmetrical incorporation of maltoporin by the fusion of monolayers to bilayers [18], an easy access exists to both the outer and the periplasmic side of the maltoporin. Combined with the use of mutations affecting either the binding of maltodextrins or maltose transport and the structural analyses, an elucidation of the structure of the selectivity filter pore protein and the topology of the binding site should be possible at the molecular level.

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