SELECTIVITY OF THE *ESCHERICHIA COLI* OUTER MEMBRANE PORINS *ompC* AND *ompF*

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1. Introduction

The cell envelope of *Escherichia coli* consists of two distinct membranes separated by a peptidoglycan layer. The cytoplasmic membrane contains phospholipids and proteins which are responsible for active transport and the respiratory chain. The outer membrane represents a protective coat which contains lipopolysaccharides in addition to proteins and phospholipids. A distinctive feature of the outer membrane is the presence of a few protein species present in very large numbers (review [1]). In *E. coli* K-12 two of these proteins (coded for by the *ompC* and the *ompF* genes; the porin nomenclature used here is that proposed in [2]) form non-specific pores or channels which allow the diffusion of small hydrophilic molecules across the outer membrane [3–5]. Strains without any of the *ompC* and *ompF* porins exhibit either reduced viability and transport functions or express a new pore-forming major protein [6].

The expression of the *ompC* and *ompF* porins is regulated by the genetic locus *ompB* [6]. The total amount of these two porins remains about constant, although the relative proportion of the two proteins varies depending upon the composition of the growth medium. The protein coded for by the *ompC* gene is favored at high osmotic strength [7,8] or in the presence of a fermentable carbon source [7,9,10]. Other factors such as growth temperature and the composition of the lipopolysaccharide have also been shown to affect the relative proportions of *ompC* and *ompF* porins [9]. The peptide composition of the *ompC* porin is very similar (but not identical) to that of the *ompF* porin [11,12].

It is of interest whether the physiological regulation of the relative amounts of the *ompC* and *ompF* porins results in an altered permeability of the outer membrane channel. At low substrate concentrations adenylic acid (AMP) penetrates more readily into *E. coli* cells containing *ompF* porin than into those producing only the *ompC* protein [3]. Here, non-metabolizable compounds were used and their entry into an enlarged periplasmic space was measured directly.

2. Materials and methods

2.1. *Bacterial strains and growth conditions*

Escherichia coli K-12 MC 4100 (Δ *lac*, *bgl*$^+$, *ompC*$^+$, *ompF*$^+$, *nal*^{S}, *str*^{R}), MH 450.1 (Δ *lac*, *bgl*$^+$, *ompC*$^+$, *ompF*:Tn 5, *nal*^{S}, *str*^{R}), and MH 150 (Δ *lac*, *bgl*$^+$, *ompC*:Tn 5, *ompF*$^+$, *nal*^{S}, *str*^{R}) were kindly provided by Michael Hall.

The cells were grown at 37°C in medium 63 [14] + 1% tryptone (Difco) to mid-log phase. The cells were harvested and washed twice with equal volumes of 50 mM morpholinopropanesulfonic acid (MOPS), pH 7. The cells were resuspended in MOPS to give an A_{600} of 3.0.

2.2. *Microfuge procedure*

Reaction mixture (1 ml) was placed on top of 0.5 ml silicone oil [15] in a 1.5 ml microfuge tube and centrifuged at 13 000 × *g* for 1 min. The cell pellet in the test tube tip was cut off with a razor blade after carefully removing the aqueous supernatant and some of the silicone oil. The cell pellet or samples of

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the supernatant were placed in scintillation fluid and counted.

The total sample volume, the extracellular (contaminating) fluid and the periplasmic space were measured in parallel experiments using ^3H_2O, [^{14}C]inulin (which does not penetrate through the outer membrane) and [^{3}H]taurine (which only enters the periplasmic space) as probes. All radiochemicals were from New England Nuclear (Boston MA) and purified chromatographically before use. In a typical experiment the total sample volume was 6.7 μl; 1.2 μl were extracellular fluid, 3.4 μl were the expanded periplasmic space and 2.1 μl were the cytoplasmic space. The periplasmic space in plasmolyzed cells accounted for ~60% of the cell volume as compared to ~25–30% in normal cells [15]. Higher concentrations of sucrose resulted in a somewhat higher degree of plasmolysis but the cells could not be separated from the incubation medium by the silicone oil method.

2.3. *Preparation of [^{3}H]stachyose*

[^{3}H]Stachyose was prepared using the galactose oxidase technique [16]. Galactose oxidase was from Sigma (St Louis MO); NaB^3H_4 was from New England Nuclear (Boston MA). Tritiated stachyose was purified by descending paper chromatography in 1-propanol:H_2O (3:1).

3. Results and discussion

The transfer of the carbohydrates raffinose (M_r 504) and stachyose (M_r 667) across the outer cell wall into the periplasmic space of the two strains of *E. coli* lacking either the *ompF* or the *ompC* porin was measured using the microfuge procedure [15]. The bacterial cells were plasmolyzed to provide a larger periplasmic volume accessible to the carbohydrates. Raffinose equilibrated with the enlarged periplasmic space of both strains within 15 s (not shown). This is the lower limit of time resolution with this experimental procedure. The entry of stachyose was much slower (fig.1). The measured time course of equilibration, however, showed no significant differences in uptake between the cells with the *ompC* or the *ompF* porin. In addition the parent cell *E. coli* K-12 MC 4100 grown on tryptic soy broth (Difco) which results in the almost exclusive synthesis of the *ompC* protein [6], and grown on nutrient broth (Difco) which results primarily in the synthesis

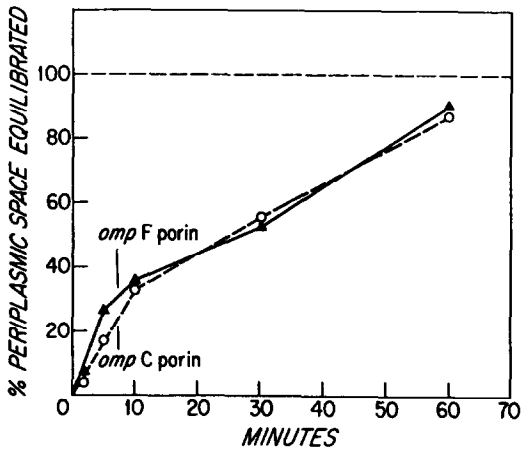

Fig.1. Stachyose entry in the periplasmic space of *E. coli* K-12. Strain MH 450.1 (○) and MH 150 (▲) were grown and prepared for the experiment as in section 2. At zero time the cells were mixed 1:1 to give a final concentration of 50 mM MOPS (pH 7), 150 mM sucrose (to enlarge the periplasmic space), 100 mg stachyose/ml and 0.02 μCi [^{3}H]stachyose/ml. At times indicated 1 ml samples were withdrawn in duplicate and centrifuged through silicone oil (microfuge technique [15]). Shown are the mean values of 5 independent experiments; there are no statistically significant differences between the 2 curves.

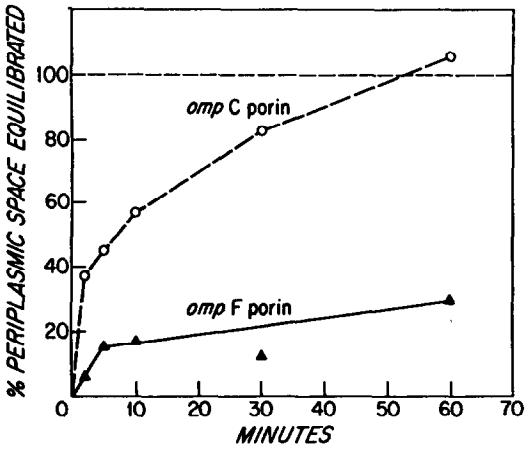

Fig.2. Permeation of formyl-methionyl–leucyl–phenylalanine into the periplasmic space of *E. coli* K-12. Strain MH 450.1 (○) and MH 150 (▲) were grown and prepared for the experiment as in section 2. The reaction mixture contained 50 mM MOPS (pH 7), 150 mM sucrose, 20 mg f-Met–Leu–Phe/ml (from Sigma) and 2 μCi [^{3}H]f-Met–Leu–Phe/ml (purchased from New England Nuclear). The cells were centrifuged, processed and the equilibration of the periplasmic space calculated as in section 2. The mean values of 3 independent experiments are shown.

of the *ompF* protein [6], gave essentially the same results (not shown). This indicates that the properties of the two pores toward these carbohydrates are identical within the sensitivity of our experimental methods.

The rate of entry of the chemotactic peptide f-Met–Leu–Phe (M_r 438) was faster in the presence of the *ompC* channel compared to the *ompF* channel (fig.2). Indeed, entry of the peptide was drastically reduced when only the *ompF* protein was present. From a comparison of the molecular masses of raffinose and f-Met–Leu–Phe one could expect a very rapid equilibration into both cells, neglecting other important factors as, e.g., hydrophobicity. The peptide is 15% smaller than raffinose, a sugar which equilibrated with the periplasmic space of both strains within 15 s. The time course of entry into the cell with the *ompC* porin, however, was similar to stachyose which is 50% larger than the peptide.

The two porins act as a molecular sieve towards uncharged compounds such as carbohydrates. However, adenylic acid (a strong acid), permeates predominantly through the *ompF* pore [3], whereas formyl-methionyl–leucyl–phenylalanine (a weak acid) preferred the *ompC* porin. This is the first occasion that a solute has been reported to permeate more efficiently through the *ompC* porin than through the *ompF* channel. The results suggest that there may be differences in charged groups inside the two porin channels. The finding that the *ompC* and *ompF* protein have different isoelectric points [12] lends further support to this idea.

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