OUTER MEMBRANE OF GRAM-NEGATIVE BACTERIA. XVII. SPECIFICITY OF TRANSPORT PROCESS CATALYZED BY THE λ-RECEPTOR PROTEIN IN ESCHERICHIA COLI

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SUMMARY: The outer membrane of Gram-negative bacteria contains (a) "porin" proteins that form transmembrane channels and allow diffusion of various hydrophilic, small molecules (Nakae, J. Biol. Chem., 251, 2176-2178, 1976), and (b) proteins which catalyze the specific transport of unique classes of compounds, e.g. the λ-receptor protein facilitates the diffusion of maltose and maltotriose (Szmelcman et al., Eur. J. Biochem., 65, 13-19, 1976). When strains of Escherichia coli B/r and K-12 containing the λ-receptor but not porin were constructed and compared with those containing neither of them, it was found that in the former strains the transmembrane diffusion of glucose and lactose, but not of histidine and 6-aminopenicillanic acid, was significantly accelerated. These results suggest that λ-receptor may facilitate the diffusion of sugars other than maltose.

Gram-negative bacteria are covered with the outer membrane, which serves as a permeability barrier against macromolecules and many hydrophobic substances and yet allows the rapid diffusion of small, hydrophilic molecules (1-3). Diffusion across the outer membrane takes place normally by one of the following mechanisms. (a) One or more outer membrane proteins with molecular weights around 35,000, called porins (4), produce transmembrane pores as shown by reconstitution studies (4,5). Porin-produced channels allow the diffusion of a wide variety of small molecules (5). (b) In addition to the non-specific pathway, the outer membrane contains several other proteins whose mutational loss leads to reduced transport rates for specific substances. These proteins are presumably involved in facilitated diffusion processes. Examples of such systems include: the tonA protein (=T1, T5, φ80, colicin M receptor; ferrichrome transport)(6,7); feuB protein (=colicin B receptor; Fe^{3+}-enterochelin transport)(8,9); btu protein (=colicin E receptor; vitamin B_{12} transport)(10); tsx protein

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(=T6 receptor; nucleoside transport)(11); and lamB protein (=λ receptor; maltose and maltotriose transport)(12).

Diffusion rate across the outer membrane can be evaluated by two methods. First, when the rate becomes reduced, for example by the deficiency of porin in kmt mutants (13; Bavoil, Nikaido, and von Meyenburg, submitted for publication) or of λ-receptor in lamB mutants (14), the overall rate of transport becomes limited by the diffusion across the outer membrane, especially at lower concentrations of substrate. In other words, reduction of outer membrane permeability increases the "K_m" of the overall transport process (12,13), even though a substrate, after diffusion through the outer membrane, is transported across the cytoplasmic membrane via a specific system usually with a very low K_m. Second, passage of β-lactams across the outer membrane can be followed by measuring the hydrolysis of β-lactams by intact cells containing excess β-lactamase in the periplasmic space (15-17).

During our recent study of kmt (porin-deficient) mutants of E. coli, we found that the transport phenotypes of these strains were influenced by the presence or absence of λ-receptor. A more detailed study, reported here, suggests that λ-receptor can catalyze the diffusion of glucose and lactose, in addition to that of maltose and maltotriose.

MATERIALS AND METHODS

Bacterial strains. E. coli B/r strains were derived from CM6 (=CP366, ref. 13), a thy drm tonA strain. This strain contained also mutation(s) in the "malB" cluster, which contains the lamB gene (14), and was consequently Mal^- as well as deficient in the λ-receptor. Its porin-deficient derivatives were CM7 (=CP367)(kmt-7), CM31 (kmt-31), and CM32 (kmt-32). Strains that had regained the λ-receptor, but not porin, were obtained from these porin-deficient derivatives either amongst the "revertants" that grew rapidly on 0.02% lactose minimal plates, or by introducing the functional "malB" region by transduction with P1kc grown on a "$malB^+$" K-12 strain, and selecting for Mal^+ transductants.

E. coli K-12 strains were derivatives of AB2847 (F^- aroB351 malT354 tsx-354), obtained from E. coli Genetic Stock Center. This mutant failed to produce λ-receptor owing to the mutation in the regulatory gene malT. When this strain was infected with P1bc grown on CM6 or CM7, the Aro^+ transductants contained various combinations of porin and λ-receptor, as both kmt and malT were co-transducible with aroB (Bavoil et al., submitted for publication).

Table 1. Adsorption of λ phage particles by various strains.

Strain[1]	"50,000 dalton band"	λ particles adsorbed[2] (% of input)
CM6	-	<15
CM7	-	<15
CM7-R32	+	<15
CM7-R39	+	42
CM7-R41	+	66
CM31-R5	+	62
CM31-R7	+	66
CM32-R3	+	36
CM6-T1	+	94
CM7-T1	+	88
HfrG6	+	>95

[1]The "revertants" (see Methods) were named by the strain number of the original mutant followed by the letter R plus the revertant number, e.g. CM7-R32. The transductants were named by the strain number of the recipient, followed by the letter T and the transductant number, e.g. CM6-T1. Both transductants in this Table were Mal^+ transductants receiving the functional "malB" region from a K-12 donor (Methods). HfrG6, a standard λ-sensitive K-12 strain, was a gift from Dr. G. L. Hazelbauer.

[2]Adsorption assay was carried out by incubating 2 x 10^8 exponential phase cells, grown up in L broth and washed in 10 mM $MgSO_4$, with 4 x 10^5 plaque-forming units of phage $\lambda c_I 190$, in 10 mM $MgSO_4$ (final volume: 1 ml) at 37° C for 10 min. The infectious centers were killed by dilution into $CHCl_3$-saturated L broth, and the remaining free phage particles were assayed by using HfrG6 as the indicator bacteria.

Cell envelope fractions of all strains used were examined by SDS polyacrylamide slab gel electrophoresis as previously described (18), in order to ascertain the presence or absence of porin and λ-receptor protein (apparent molecular weights, approx. 35,000 and 50,000, respectively).

Growth and transport K_m determinations. This was carried out as described previously (13). In some cases, the Lineweaver-Burk plots showed concave upward deviation in the region of small $[S]^{-1}$. Because of this reason, the "K_m" values here were determined as the substrate concentrations that gave half-maximal growth rate or rate of incorporation, on the basis of the maximal rates obtained with the wild type cells.

Rates of β-lactam diffusion across the outer membrane. This was determined according to the procedures described previously (15-17), after the introduction

of the R plasmid R1 by conjugation. 6-Aminopenicillanic acid (Sigma) was used as the substrate.

RESULTS AND DISCUSSION

"Revertants" of the porin-deficient mutants. The kmt mutants of E. coli B/r contain no or few porin molecules in the outer membrane, and consequently grow very slowly in minimal media containing low concentrations of carbon sources, e.g. lactose. Rapidly growing, spontaneous "revertants" were selected from these mutants (Methods). When cell envelope proteins of these "revertants" were analyzed by slab gel electrophoresis, some were found to have regained porin, but others were found to overproduce non-porin envelope proteins while still remaining porin-deficient (Bavoil et al., submitted for publication). The most frequently obtained of the latter class produced a "50,000 dalton" protein which, on comparison with gels of lamB$^+$ and lamB strains, appeared to be identical with the lamB gene product, i.e. the λ-receptor protein. Many of the "revertants" producing the 50,000 dalton protein indeed adsorbed phage λ, in contrast to the parent strains (Table 1). (The fact that the adsorption rates were slow or negligible in some revertants may be due to the mutationally altered structure of the λ-receptor protein).

Several of these λ-receptor-producing, porin-deficient "revertants" were indeed shown to have an increased permeability toward glucose and lactose, as judged by the decreased "K_m" values for the transport of these sugars, measured as described in Methods. Although these results suggest that the λ-receptor may facilitate the diffusion of glucose and lactose, we have not studied the revertants further, because of the possibility that some may contain altered λ-receptor.

Studies with transductants. Another piece of evidence suggesting a role for λ-receptor protein in the transport of substances other than maltose came from studies of the phenotypes of aroB$^+$ transductants of a K-12 strain, AB2847. This strain has both aroB and malT mutations. Therefore, by using P1bc grown in either CM6 or CM7, aroB$^+$ transductants containing various combinations of kmt and malT alleles could be constructed (see the AB2847 derivatives listed in

Table 2. "K_m" for growth on glucose or lactose, and for L-histidine transport, in strains containing various combinations of porin and λ-receptor.

Class	Porin[1]	λ-receptor	Strain[2]	"Transport K_m" (μM) K-12 strains Glc	Lac	His	B/r strains Glc	Lac	His
1	-	-	AB2847-T17[3]	500	3000	0.3			
			CM7				700	7000	3
2	-	+	AB2847-T19[4]	200	300	0.3[5]			
			CM7-T1				200	2500	3[5]
3	+(B)	+	AB2847-T5	3	60	ND[6]			
			CM6-T1				ND	70	0.2
4	+(B)	-	CM6				1[7]	70	0.2
5	+(K)	-	AB2847-T15[8]	3	60	0.1			

[1] Porin(s), when present, were classified into either the B/r-type (B), or K-12-type (K), by their behavior on SDS acrylamide slab gel electrophoresis.
[2] See Methods and the footnotes to Table 1. AB2847-T5 was isolated by using CM6 as the transductional donor, and other AB2847 transductants by using CM7.
[3] Another transductant of the same class, containing the kmt-32 allele, gave similar K_m values for glucose and lactose.
[4] Four other transductants of the same class, containing either kmt-7 or kmt-32 allele, gave similar K_m values for glucose and lactose.
[5] Very similar K_m values were obtained even when the λ-receptor was fully induced by growing the strains in 0.4% maltose-minimal medium.
[6] Not determined.
[7] From ref. 13.
[8] Two other transductants of the same class gave similar K_m values for glucose and lactose.

Table 2). Initial growth studies showed that all of the ten kmt malT$^+$ (i.e. porin$^-$ λ-receptor$^+$) transductants tested grew more rapidly than the ten kmt malT (porin$^-$ λ-receptor$^-$) transductants, in minimal medium containing 0.03% lactose, and suggested that the λ-receptor can partially substitute for the channel-forming function of porins. A more detailed study of several representative

strains (Table 2) showed clearly that in λ-receptor-producing strains the apparent affinity for glucose and lactose transport was significantly increased, suggesting that these sugars diffused through the outer membrane more rapidly.[1]

Transport (Table 2) and β-lactam hydrolysis (Methods) experiments did not reveal any effect of λ-receptor on the permeability of the outer membrane to histidine and 6-aminopenicillanic acid. However, even in the strains lacking both porin and λ-receptor, the transport K_m for histidine remained quite low (0.3 μM), and 6-aminopenicillanic acid diffused at significant rates, about 15% of that seen in the wild type. Presumably E. coli K-12 contains alternative mechanisms that permit amino acids and β-lactams to cross the outer membrane. Thus if the λ-receptor produced only small increases in permeability, they would have been difficult to detect because of this large "residual" activity. Accordingly, we turned to strains with the B/r background, since they seemed to contain many fewer non-porin transport systems.

The λ-receptor protein was introduced into B/r strains CM6 and CM7 by transduction (Methods). Comparison of the λ-receptor-producing (lamB$^+$) kmt transductants with the non-producing (lamB) kmt mutant (Table 2) confirms the finding that the presence of λ-receptor accelerates the diffusion of lactose and glucose across the outer membrane. Although histidine transport in the porin-deficient, λ-receptor-deficient strain (CM7) was severely limited, the addition of the λ-receptor to this strain did not affect the transport "K_m" (cf. CM7-T1).

The efficiency of porin and λ-receptor in the transport of maltose is under study, but it is already known that the strains carrying λ-receptor but not porin (e.g. CM7-T1) can grow on 0.005 to 1% maltose as rapidly as strains producing both porin and λ-receptor (e.g. CM6-T1).

In conclusion, the presence of λ-receptor protein in porin-deficient strains of E. coli B/r and K-12 lowers the overall transport "K_m" for glucose and lactose Since overall transport in porin-deficient mutants is limited mainly by diffusion

[1]These results cannot be explained by contamination of these sugar preparations with maltose, as the latter sugar will be rapidly consumed away by malT$^+$ strains, and will not affect the subsequent course of growth.

rate across the outer membrane (Bavoil et al., submitted for publication), the λ-receptor appears to facilitate the transmembrane diffusion of glucose and lactose, in addition to its already established role in maltose and maltotriose transport. The minimal values for the permeability of the outer membrane to various sugars can be estimated from the growth rates and the "K_m" values (Bavoil et al., submitted for publication). These calculations show that, in the K-12 line, the λ-receptor-dependent permeability of the outer membrane to lactose and glucose is less than 20% and 2%, respectively, of the permeability allowed by the presence of porins in the parent strain. However, these low values are likely to be largely due to the use of lactose- and glucose-grown cells containing only low levels of λ-receptor (35% and 15% of the fully induced level, respectively, as judged by the scanning of the stained slab gel) in the K_m determination, and the λ-receptor may be rather efficient in transporting these non-maltose sugars.

Finally, we must mention that these results do not rigorously rule out the alternative possibility that the presence of λ-receptor or other components of the maltose transport system affects transport "K_m" through indirect mechanisms, e.g. by increasing the amounts of "residual" porin incorporated, or through effects on the active transport of glucose and lactose across the cytoplasmic membrane. On the other hand, the available lines of evidence (e.g. absence of porin bands in the SDS polyacrylamide gels of λ-receptor-containing strains; the improvement in the transport of sugars, but not of an amino acid or a β-lactam) do not favor this interpretation. In vitro reconstitution experiments with the purified protein should furnish the unequivocal proof that the λ-receptor can catalyze the diffusion of sugars other than maltose and maltotriose.

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