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A PORIN ACTIVITY OF PURIFIED λ-RECEPTOR PROTEIN FROM *ESCHERICHIA COLI* IN RECONSTITUTED VESICLE MEMBRANES

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SUMMARY. The receptor protein for bacteriophage λ was purified to homogeneity from a mutant strain of *Escherichia coli* K-12 producing reduced amounts of porin. In the reconstituted vesicle membranes the λ-receptor formed permeability channels that allowed the diffusion of maltose, lactose, sucrose, raffinose, amino acids, and nucleosides, but essentially not of stachyose. The permeability channels made of λ-receptor thus had a relatively low specificity for solute molecules. The active form of the protein seemed to be an oligomer of λ-receptor proteins.

The diffusion of small molecules through the outer membrane of Gram-negative bacteria has been studied extensively in recent years (see ref. 1 for review). We have reported that the outer membrane of *E. coli* and *S. typhimurium* contain permeability "channels" or "pores", which allow free diffusion of small uncharged molecules of less than 600 daltons (2,3). The major outer membrane proteins of around 35,000 daltons were identified as the pore-forming proteins, porin, in *E. coli* and *S. typhimurium* (4,5). Permeation of the solute through the channels was found to be rather non-specific in regards to the chemical structure of the solute (4,5).

In addition, the outer membrane harbors several specific transport proteins facillitating the diffusion of maltose, nucleoside, Fe^{3+}-chelator complexes, or vitamin B_{12} across the outer membrane (see ref. 6 for review). The receptor protein for phage λ was shown to increase the outer membrane permeability for maltose and maltotriose (7), to a certain degree, for other sugars such as lactose and glucose (8). In order to characterize this interesting transport mechanism, it is essential to purifiy λ-receptor protein and to

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Abbreviations: SDS, sodium dodecylsulfate, LPS, lipopolysaccharide

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incorporate them into an *in vitro* assay system. This paper reports purification and transport properties *in vitro* of the λ-receptors, and discuss the transport specificity of the outer membrane.

MATERIALS and METHODS

BACTERIAL STRAINS AND GROWTH CONDITIONS. *E. coli* K-12 strain T19 (*tsx, supE, kmt*, a gift from P. Bavoil) produces the λ-receptor abundantly but only trace amounts of porin, in maltose-containing medium (see ref. 9 for detail). Therefore the cells were grown in either L-broth or M9 medium containing 0.4% of maltose under vigorous aeration. For purification of porin, *E. coli* B was grown in L-broth (omitting maltose).

PURIFICATION OF λ-RECEPTOR. This is given at legend to Fig. 1.

PURIFICATION OF PORIN. Porin was purified from *E. coli* B by the procedure described elsewhere(10).

OTHER METHODS. Reconstitution of vesicle membranes and assay for permeability were described (5). Procedure for acrylamide gel electrophoresis in SDS was described previously (5). Protein was determined by the method of Lowry *et al.* as described (2). Purified porin was quantitated on the basis of its absorption coefficient at 278 nm (11). Phospholipids and LPS were prepared and quantitated as reported (4).

RESULTS

PURIFICATION AND PROPERTIES OF λ-RECEPTOR. The λ-receptor was purified to homogeneity by the procedure similar to that used for the purification of porin (10), from a mutant strain of *E. coli* K-12 producing reduced amounts of porin (9). The purified λ-receptor appeared to be homogeneous on SDS-acrylamide slab gel electrophoresis, whether or not the sample was heated at 100 C in SDS (Fig. 1). The unheated proteins migrated to the position close to the protein of 83,000 daltons or *feuB* protein (6), and the protein heated at 100 C for 5 min in SDS traveled to the position expected for a protein of about 48,000 daltons (Fig. 1). Since the behaviors of unheated and heated λ-receptors on SDS-gel electropherogram resembled that of the oligomer and the monomer of porin (10,11), it is suggested that the unheated λ-receptor exists in an oligomeric form. The oligomeric form of porin (similar preparation to Fig. 1 lane E) from *E. coli* and *S. typhimurium* was shown to contain three indentical subunits (10,11). The purified λ-receptor-oligomers retained their activity to inactivate λ-phage as assayed by the procedure described by Randall-Hazelbauer and Schwartz (12), but the heat dissociated monomers (similar to Fig. 1, lane D) fail to show the receptor activity (not shown). The results

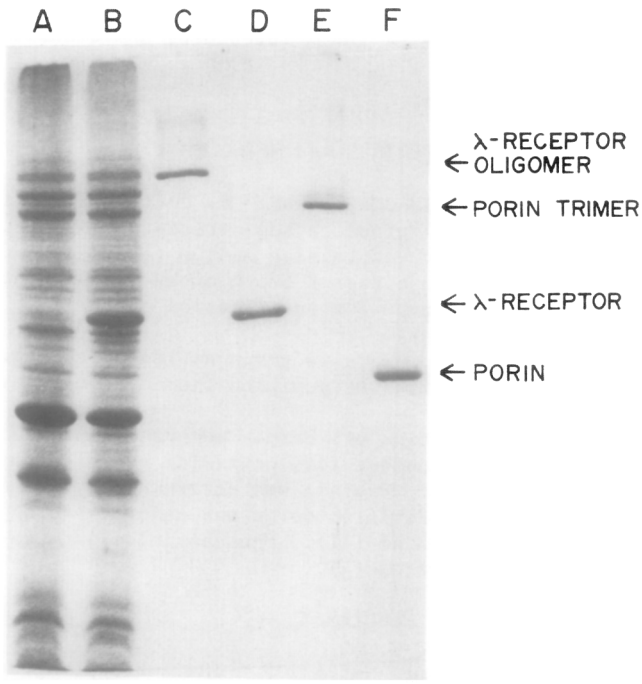

Fig. 1. Electrophoretic mobilities of the λ-receptor protein. SDS-gel containing 10% acrylamide was used as described earlier (5). A. The envelope fraction (20 μg protein) prepared from the strain T19 (grown in M9-glucose medium) was subjected to electropnoresis after heating at 100 C for 5 min in "sample buffer" (5). B. The envelope fraction (20 μg protein) of the strain T19 (grown in M9-maltose medium), heated at 100 C for 5 min. C. The purified λ-receptor (2 μg protein),unheated. The λ-receptor protein was purified by the following procedure. The total envelope fraction of the strain T19 was prepared as described (5). The envelope fraction containing 3,200 mg protein was sequentially treated with the following solutions and was centrifuged at 80,000xg for 30 min each time. (i) 320 ml of 3% SDS and 10 mM Tris-HCl buffer, pH7.5 at 35 C for 30 min. (ii) 320 ml of 0.25% SDS, 10 mM Tris-HCl buffer, pH7.5, at 35 C for 30 min. (iii) 160 ml of 0.25% SDS, 10 mM Tris-HCl buffer, pH7.5, 5 mM EDTA, 0.05% β-mercaptoethanol and 3 mM NaN_3 at room temperature for 30 min. The pellet fraction from the last wash (200 mg protein) was re-suspended in 50 ml of the solution containing 0.25% SDS, 0.3 M MaCl, 10 mM Tris-HCl buffer, pH7.5, 5 mM EDTA, 0.05% β-mercaptoethanol and 3 mM NaN_3, and was centrifuged at 80,000xg for 60 min, after left at room temperature for 30 min. The supernatant fraction containing 20 mg protein was applied onto a Sepharose 4B column (2.5 x 90 cm) equilibrated with the solution containing 0.25% SDS, 0.3 M NaCl, 10 mM Tris-HCl buffer, pH7.5, 5 mM EDTA, 0.05% β-mercaptoethanol and 3 mM NaN_3, after concentration to about 3 ml by dialysis against dry Ficoll 400. A symmetric single peak with a K_d of 0.68 was seen when protein was located by determination of absorption at 280 nm (result not shown). A portion of peak fraction was subjected to electrophoresis. D. The purified λ-receptor (2 μg protein). heated. The sample similar to the above C was heated at 100 C for 5 min in "sample buffer". E. The purified porin trimer (2 μg protein) from *E.coli* B. Porin was purified as described (10). F. The purified porin monomer. The sample similar to the above E. was heated at 100 C for 5 min in "sample buffer".

are consistent with the works from other laboratories (13,14). The oligomer of purified λ-receptor was found not to be dissociated into "monomers" by heating in SDS for 5 min at temperature up to 70 C. It became partially and completely dissociated by heating at 90 C and 100 C, respectively (result not shown).

PERMEABILITY PROPERTIES OF THE VESICLES RECONSTITUTED FROM λ-RECEPTOR. The oligomers of the λ-receptor were assayed for permeability-conferring activity, after lowering the concentration of SDS by dialysis. Vesicles were reconstituted from λ-receptor protein, phospholipids, and LPS. It was found that the vesicle membranes containing the λ-receptor were equally permeable to maltose, lactose, and sucrose, and to some extent, raffinose. These membranes thus showed a molecular-sieving property, but at the same time relatively low specificity for the solute molecules (Fig. 2A). The result was similar to the permeability properties of membranes containing porin (4,5). The membranes reconstituted from the heated λ-receptors, which were presumably monomeric, were not permeable to saccharides.

If λ-receptor acts as carriers for certain sugar molecules as was suggested (7), the rate of transport of such sugars is expected to be reduced at low temperature. This possibility was tested by assaying permeability of the λ-receptor containing vesicles to various sugars at 0 C and 25 C. Fig. 2B shows that the extent of maltose permeability was very close to that of sucrose and lactose, and also that the permeability of each sugar at 0 C and 25 C were comparable. I also assayed for permeability of the λ-receptor-containing membranes to amino acids and nucleosides, and found that glutamic acid and thymidine were highly permeable through the vesicle membranes, but arginine, leucine and uridine were relatively less permeable (Table 1). Theoretical ground for the different permeability of these compounds is not clear at present. Addition of various concentration of non-radioactive compounds to extravesicular space did not produce any significant effect on the outward diffusion of intravesicular solutes (Table 1).

If the λ-receptor acted as a carrier for both maltose and other sugars, it was assumed that the substrate competition for diffusion should occur in

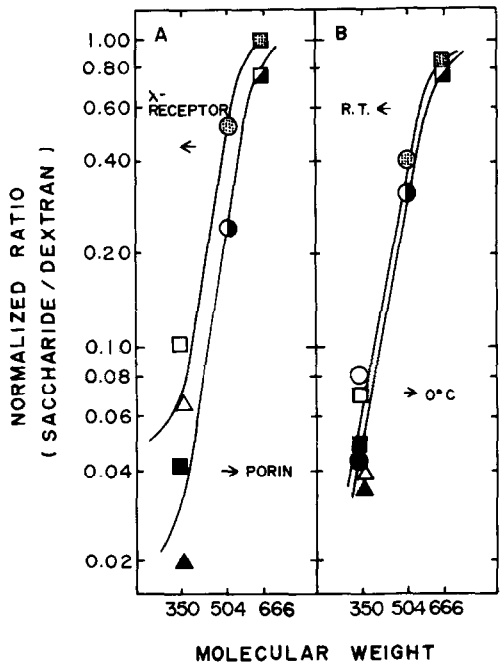

Fig. 2. The permeability of the reconstituted vesicle membranes to oligosaccharides. Vesicles were reconstituted from 0.5 μmol of phospholipids, 0.075 μmol of LPS and 100 μg of λ-receptor oligomers or 100 μg of porin trimers in the presence of [^{14}C]maltose / [^{3}H]dextran, [^{14}C]lactose / [^{3}H]dextran [^{14}C]sucrose / [^{3}H]dextran, [^{3}H]raffinose / [^{14}C]dextran, or [^{3}H]stachyose/ [^{14}C]dextran. The assay procedure for permeability was described (5). The figure shows the logarithms of normalized ratios of oligosaccharides and dextran (oligosaccharide/dextran) as previously described (5). A. Permeability of the vesicle membranes reconstituted from the λ-receptors or porins, at 25 C. Note, A direct comparision of permeability of λ-receptor-vesicle to porin-vesicle cannot be made, since we do not know the numbers of pore(s) made per polypeptide. B. Permeability of the vesicle membranes reconstituted from λ-receptors. Permeability assay was made at 0 C or 25 C. Symbols: △ or ▲ , □ or ■ , ○ or ● , ◎ or ◕ , and ▦ or ◪ for maltose, lactose, sucrose, raffinose and stachyose, respectively.

the presence of maltose and other sugars. I tested this possibility assaying the efflux of [^{14}C]maltose and [^{3}H]sucrose from vesicles containing both of these sugars. Results suggested little competition between two sugars (not shown).

DISCUSSION

Studies on maltose and maltotriose transport in the outer membrane of *E. coli* showed that the λ-receptor facillitated the diffusion of these sugars preferentially (7,12). The determination of "transport K_m" using the strain

Table 1. Permeabilities of various substance through the vesicle membranes reconstituted from the purified λ-receptor.

Experiment I[a]			Experiment II[b]			
Test material	Intravesicular concentration	Normalized ratio	Test material	Intravesicular concentration	Extravesicular concentration	Normalized ratio
Maltose	0.13×10^{-3} (M)	0.016	Maltose	0.13×10^{-3} (M)	10^{-4} (M)	0.041
	1.13×10^{-3}	0.022			10^{-3}	0.035
Sucrose	0.5×10^{-3}	0.071			10^{-2}	0.040
	1.5×10^{-3}	0.060	Sucrose	0.5×10^{-3}	10^{-4}	0.111
Lactose	0.09×10^{-3}	0.038			10^{-3}	0.104
	1.09×10^{-3}	0.033			10^{-2}	0.103
Glutamic acid	1.25×10^{-6}	0.046	Lactose	0.09×10^{-3}	10^{-4}	0.067
					10^{-3}	0.068
Arginine	15.4×10^{-6}	0.283			10^{-2}	0.072
Leucine	0.4×10^{-6}	0.282	Glutamic acid	1.25×10^{-6}	10^{-6}	0.062
Uridine	0.27×10^{-6}	0.285			10^{-4}	0.078
Thymidine	1.08×10^{-6}	0.074			10^{-2}	0.053

Vesicles were prepared from 0.5 μmol of phospholipids, 0.075 μmol of LPS and 100 μg of the purified λ-receptors as reported previously (4,5). Permeability of membranes was assayed by exit of [^{14}C] or [^{3}H]test material from the vesicles that had been preloaded with [^{14}C]material and [^{3}H]dextran (20,000 daltons), or [^{3}H]material and [^{14}C] dextran (20,000 daltons) (4,5). Results were expressed as normalized ratio of [^{14}C] or [^{3}H]test material / [^{3}H] or [^{14}C] dextran.

(a) Permeability was assayed at 25 C, 10 min after dilution with the medium described previously (5), free of test material. (b) Vesicles were prepared under fixed concentration of test material and assayed at 25 C, 2 min after dilution using the medium containing various concentration of non-radioactive test material.

carrying *kmt, malT* (porin$^-$, λ-receptor$^-$) mutations and the strain carrying *kmt, malT*$^+$ (porin$^-$, λ-receptor$^+$) alleles suggested that the λ-receptor facilitated the diffusion of glucose and lactose in addition to its already known function in maltose and maltotriose transport (8). The present *in vitro* study revealed that the purified λ-receptors formed the permeability channels in vesicle membranes and allowed the transmembrane diffusion of maltose, sucrose, lactose, glutamic acid, thymidine, and to some extent, for leucine arginine, and raffinose. But only a little permeability for stachyose was observed, suggesting both molecular sieving property and some unknown discriminating mechanism of the membranes. The result did not strictly rule out the possibility that the present preparation of λ-receptor was "denatured" in such way that resulted in the reduced solute specificity. However the available lines of evidence [e.g. lack of binding of λ-receptor to maltose

or maltotriose (15); lack of conformational change of λ-receptor in the presence of maltose or maltotriose (Nakae, T. unpublished result); relatively low specificity of λ-receptors for sugar transport *in vivo*(8); and the formation of ion permeable pores in black lipid membranes (14)] do not favor the above interpretation. It is still possible that the current methods for reconstitution of vesicle membranes and for permeability assay could not distinguish the relatively low transport specificity present in λ-receptor channel. Other possibilities to be tested in future include (i) if a mutation at *kmt* gene results, pleiotropically, a dropping of transport specificity from the λ-receptor-channel; (ii) Whether some other membraneous or non-membraneous component(s) [most likely outer membrane protein(s) or the periplasmic protein (s)] associate with the λ-receptor-channels and somehow control the specificity of the transmembrane diffusion of the solute; and (iii) Whether the λ-receptor-channel facillitates a diffusion of higher maltose-oligosaccharides.

After this work was completed I learned that the pore-forming activity of λ-receptor protein was also demonstrated independently by M. Luckey and H. Nikaido using liposome vesicles (personal communication)

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