Proc. Natl. Acad. Sci. USA
Vol. 77, No. 1, pp. 167–171, January 1980
Biochemistry

Specificity of diffusion channels produced by λ phage receptor protein of *Escherichia coli*

(liposomes/reconstitution/outer membrane/facilitated diffusion/maltose)

MARY LUCKEY AND HIROSHI NIKAIDO

Department of Microbiology and Immunology, University of California, Berkeley, California 94720

Communicated by Clinton Ballou, October 1, 1979

ABSTRACT **The *lamB* protein, the receptor for phage λ, was purified from the outer membrane of *Escherichia coli* K-12 by extraction with Triton X-100 and EDTA, chromatography on DEAE-Sephacel in Triton X-100, exchange of Triton for cholate by gel filtration, and chromatography on Sephacryl S-200 in cholate, NaCl, and EDTA. The purified protein appeared to exist as several oligomeric species. In an equilibrium retention assay with reconstituted vesicles containing phospholipids and lipopolysaccharide, the *lamB* protein conferred permeability for disaccharides. In a liposome swelling assay designed to measure rates of diffusion, the *lamB* protein conferred permeability to phospholipid liposomes for a variety of substrates. The rates obtained indicate the permeation facilitated by the *lamB* protein is specific, discriminating among substrates by both size and configuration. For example, maltose diffused into liposomes 40 times faster than sucrose, about 8 times faster than cellobiose, and about 12 times faster than maltoheptaose. The results suggest that the *lamB* protein forms a transmembrane channel containing a site (or sites) that loosely interacts with the solutes.**

The outer membrane of Gram-negative bacteria has several pathways for the translocation of small molecules (reviewed in ref. 1). The passage of most small, hydrophilic molecules is accomplished by porins, which form nonspecific water-filled pores. Other outer membrane proteins are involved in the transport of specific nutrients, but the mechanism of the specific transport is not understood.

The λ phage receptor protein, product of the *lamB* gene (2), is required in *Escherichia coli* for the transport of maltose at micromolar concentrations and of maltodextrins of higher molecular weight (3). Because direct binding studies have failed to detect an affinity of *lamB* protein for maltose (4), the hypothesis that it also forms a fairly nonspecific pore has been attractive. Conductivity studies on black lipid membranes containing the *lamB* protein give evidence for a pore of fairly large diameter (5). Furthermore, while the current work was in progress, a paper by Nakae (6) appeared in which he claimed that membranes reconstituted from phospholipids, lipopolysaccharides, and *lamB* protein are nonspecifically permeable to all sugars tested except stachyose. In order to reconcile this apparent lack of specificity in reconstituted systems with the specificity for maltose and maltodextrins observed in intact cells, it has been proposed that the *lamB* protein produces a totally nonspecific channel, and that the specificity is derived entirely from the interaction of periplasmic maltose-binding protein with the *lamB* protein (7).

To examine the function of the *lamB* protein *in vitro*, we developed a purification scheme that utilized neither sodium dodecyl sulfate ($NaDodSO_4$) nor the organic solvents used in earlier works (5–8). These agents could alter the activities of the protein by partially denaturing it, so we wanted to employ milder treatments to extract and purify the protein. Furthermore, in this study we examined the rates of diffusion, in contrast to the equilibrium distribution studied by Nakae (6), of various saccharides across reconstituted membranes. We find that the *lamB* protein produces transmembrane channels of distinct specificity.

MATERIALS AND METHODS

Bacteria. All strains used were derivatives of *E. coli* K-12. Strain JC6724 (*endA*$^-$ *thi*$^-$ *gal*$^-$) was used as "wild type" with respect to the membranes. Strain T19 (*ompB*$^-$ *tsx-354 malB*$^+$) is a P1 transductant of *E. coli* AB2847 with *E. coli* B/r *ompB*$^-$ (strain CM7) as a donor (9).

Materials. [*U*-^{14}C]Maltose and [*glucose*-1-^{14}C]lactose were purchased from Amersham, [*U*-^{14}C]sucrose from New England Nuclear. [^{3}H]Raffinose and [^{3}H]stachyose were radiolabeled in our laboratory (10). [*methoxy*-^{3}H]Methoxydextran of about 40,000 daltons has been described (11). [*carboxyl*-^{14}C]Carboxyldextran was obtained from New England Nuclear and after a similar fractionation by gel filtration a 20,000-dalton fraction was used.

Maltitol was prepared by the reduction of maltose with sodium borohydride. Maltoheptaose resulted from limited hydrolysis of β-cyclodextrin (12). Maltotetraose was purified from amylose hydrolysates by charcoal/Celite column chromatography (13). Purity of the maltose oligosaccharides was determined by descending paper chromatography in ethyl acetate/pyridine/water (5:3:2, vol/vol).

Preparation and quantitation of lipopolysaccharide and phospholipids have been described (14), except that they were extracted from JC6724 grown to midlogarithmic phase in L broth. Outer membranes were prepared from T19 grown to midlogarithmic phase in L broth supplemented with 0.8% maltose as described (14).

Cholic acid was purchased from Sigma, recrystallized three times from boiling 70% ethanol to remove impurities, and neutralized with NaOH.

Protein was determined by the Lowry procedure (15), with 1% $NaDodSO_4$ present for samples containing Triton X-100 (16). Polyacrylamide slab gel electrophoresis was carried out by the method of Lugtenberg (17).

Permeation Assays. *Equilibrium method.* Vesicles were reconstituted by the method of Nakae (18). One micromole of phospholipid was dried under a stream of N_2 and then resuspended by sonication in lipopolysaccharide containing 0.5 μmol of heptose, with and without added protein. The suspension was dried under N_2 at 45°C and resuspended with brief sonication in 0.2 ml of 10 mM Hepes, pH 7.4, containing 0.1 M NaCl, 0.15 mM chloramphenicol, radiolabeled dextran, and radiolabeled

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

Abbreviation: $NaDodSO_4$, sodium dodecyl sulfate.

saccharide. The mixture was heated at 45°C for 30 min and allowed to cool for at least 2 hr. Then the suspensions were filtered on Sepharose 6B in 10 mM Hepes, pH 7.4/0.1 M NaCl/0.1 mM $MgCl_2$/3.3 mM NaN_3. Vesicles eluted at the void volume were collected and their radioactivities were measured in a liquid scintillation counter. Retention of substrates near equilibrium was indicated by the ratio of saccharide to dextran in the vesicle fractions; these ratios were normalized to the ratio obtained in vesicles not containing the protein.

Rate method. Rates of permeation were determined from the swelling rates of liposomes (19–21). *E. coli* phospholipids (5.3 μmol) were dried as a thin film at the bottom of a tube, and were first suspended in 0.2 ml of an aqueous solution of the purified *lamB* protein (3.8–15.2 μg) by sonication. The suspension was dried under a stream of N_2 at 50°C and, after 15 min in an evacuated desiccator, was finally resuspended, by Vortex mixing, in 1.5 ml of 17% (wt/vol) dextran T-20 (Pharmacia, lot 5351) containing 5 mM Tris·HCl, pH 7.5. Control vesicles were made similarly without the addition of *lamB* protein. Portions of these liposomes (usually 30 μl) were added to 0.63 ml of a 0.04 M solution of a sugar containing 5 mM Tris·HCl, pH 7.5, in a semimicro curvette, the contents were rapidly mixed, and the optical density was recorded at 500 nm with a Perkin-Elmer Hitachi model 128 spectrophotometer. It took 5–7 sec from the addition of liposomes to the beginning of the optical density trace, and the rate of swelling was determined as $d(1/OD)/dt$ from the optical density changes between 10 and 20 sec after the addition, by the procedure described in ref. 21. The assay was reproducible as long as the same batch of liposomes was used.

For experiments in which many different sugars were used, the sugar solutions were first adjusted to an apparent isotonicity with the liposomes by diluting slightly more concentrated solutions until the control liposomes, without the *lamB* protein, produced neither swelling nor shrinking. This procedure was necessary because of frequent uncertainties in the moisture content or the water of crystallization of various sugar preparations. Furthermore, the refractive index of all sugar solutions was adjusted to 1.3393 by adding appropriate amounts of Ficoll 400 (Pharmacia), in order to avoid the effect of refractive index differences of the diluting solutions on the extent of light scattering.

RESULTS

Purification of the *lamB* Protein. In order to minimize contamination by porins, a porin-deficient (*ompB*) strain, T19, was used as the source of *lamB* protein. Outer membranes from T19 cells induced with maltose were extracted with detergents under various conditions and examined by $NaDodSO_4$/polyacrylamide slab gel electrophoresis, in which the *lamB* protein can be recognized as a prominent 47,000-dalton band (22). We found maximal and preferential solubilization of the *lamB* protein to occur with 2% Triton X-100 in 10 mM Tris·HCl, pH 7.5/10 mM Na_2EDTA (Fig. 1, lanes a–d).

Triton/EDTA extracts were adsorbed to DEAE-Sephacel, then eluted with 0.1% Triton X-100/10 mM Tris·HCl, pH 7.5, with a linear NaCl gradient up to 0.6 M, at 4°C (Fig. 1, lane e and Fig. 2). A large peak containing the *lamB* protein eluted at 0.25 M NaCl.

The DEAE fractions 82–96 (5.1 mg of protein) were pooled and concentrated to 1 ml by dialysis against Ficoll 400 and mixed with 1 ml of 10% sodium cholate by sonication. This sample was applied to a 1.27 × 56 cm column of Sephacryl S-200 equilibrated with 1% sodium cholate/10 mM Tris·HCl, pH 7.5, in order to replace the protein-bound Triton X-100 with cholate. Analysis of the eluate fractions showed that most proteins were eluted at 0.33 column volume (Fig. 1, lane f), whereas most of the Triton (measured by absorbance at 280 nm) came out at 0.60 column volume.

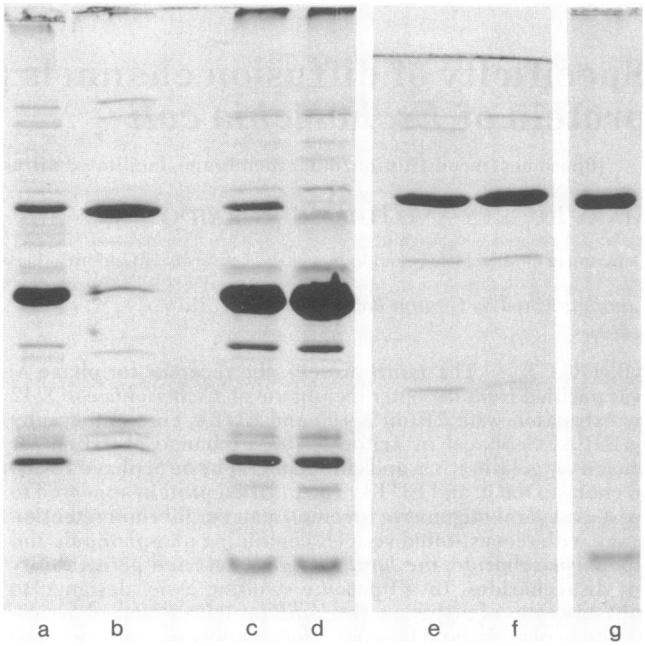

FIG. 1. Purification of the *lamB* protein: protein composition on $NaDodSO_4$/polyacrylamide slab gels. T19 outer membrane (lane a) (containing 30 mg of protein) was extracted once with 30 ml of 2% Triton X-100/5 mM $MgCl_2$/10 mM Tris·HCl, pH 7.5, and then twice with 30 ml each of 2% Triton X-100/10 mM Na_2EDTA/10 mM Tris·HCl, pH 7.5 (extract, lane b, and pellets, lanes c and d) with 2-min sonication at 25°C, 20-min shaking at 37°C, and 60-min centrifugation at 20,000 × *g*. The extracts were chromatographed on DEAE-Sephacel, Sephacryl S-200, and Sephacryl S-200 in high salt as described in *Results*. Lanes e, f, and g are the peak fractions containing *lamB* protein from DEAE-Sephacel (fraction 90), Sephacryl S-200 (fraction 20), and Sephacryl S-200 in high salt (fraction 96), respectively. Samples applied were boiled 2 min in sample buffer and contained from 10 to 35 μg of protein.

The protein-containing fractions were again concentrated to 1 ml and mixed with 1 ml of 10% sodium cholate plus NaCl and Na_2EDTA to final concentrations of 1.0 M and 10 mM, respectively. Chromatography on Sephacryl S-200 (1.27 × 92 cm) in 1% sodium cholate/10 mM Tris·HCl, pH 7.5/1 M

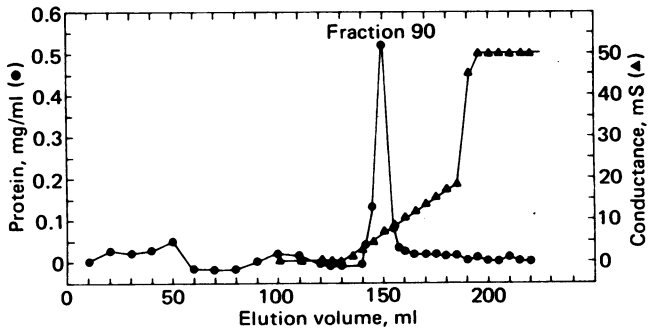

FIG. 2. Ion-exchange chromatography of Triton X-100/EDTA extracts. Extracts containing 17.4 mg of protein were adsorbed at 4°C to a column of DEAE-Sephacel (1.5 × 11 cm) that had been equilibrated with 10 mM Tris·HCl, pH 7.5/0.1% Triton X-100. When no more protein was eluted by the equilibrium buffer, a 60-ml linear gradient from 0 to 0.6 M NaCl in 10 mM Tris·HCl, pH 7.5/0.1% Triton X-100 was applied, followed by a wash of 2 M NaCl in 10 mM Tris·HCl, pH 7.5/1.0% Triton X-100. Conductance was measured on a Radiometer type CDM2b conductivity meter.

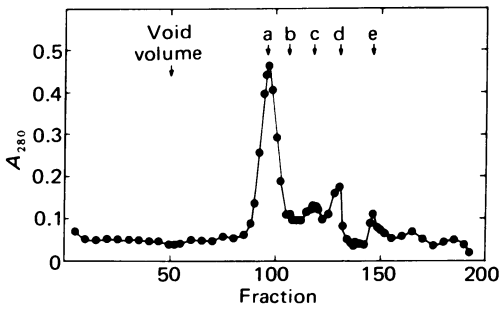

FIG. 3. Gel filtration of *lamB* extract in cholate/NaCl. The sample, containing 5.1 mg of protein in 10 mM Tris·HCl, pH 7.5/4% sodium cholate/1 M NaCl/10 mM Na_2EDTA, was applied to a column of Sephacryl S-200 (92 × 1.27 cm) that had been washed with 10 mM Tris·HCl, pH 7.5/1% sodium cholate/1 M NaCl/10 mM Na_2EDTA and was eluted with the same high-salt buffer. Fraction size was 0.7 ml. Protein was determined by absorbance at 280 nm. Arrows indicate the fractions examined by $NaDodSO_4$/polyacrylamide gel electrophoresis (see Fig. 4).

NaCl/10 mM EDTA resulted in the separation of the major protein species into three peaks (Fig. 3). The major peak contained the *lamB* protein and only a low molecular weight contaminant (Fig. 4 and Fig. 1, lane g). When this *lamB* protein was examined on $NaDodSO_4$ gels without boiling it appeared to exist as several oligomeric species (Fig. 4). The purified protein was concentrated and then dialyzed for 20 hr against 10 mM Tris·HCl, pH 7.5, with two changes of buffer, and stored at 4°C. The yield was 1.9 mg of protein.

In the reconstitution assay described in the next section, it took 35.6 μg protein of crude outer membrane to achieve the efflux of 80% of intravesicular [^{14}C]maltose, but only 7.6 μg of the purified *lamB* protein was required for this purpose. This shows a considerable enrichment in specific activity, although these assays are at best semiquantitative.

Activity of the Purified *lamB* Protein in Equilibrium Distribution Assay. The permeability-conferring activity of the purified protein was examined by using the method devised by Nakae (18). This involves the incorporation of 7.5–20 μg of the purified protein into vesicle membranes containing phospholipids and lipopolysaccharide, and, after the formation of vesicles in the presence of radioactively labeled saccharides, examination of the efflux of intravesicular saccharides during the gel filtration of vesicles. Because the gel filtration takes 10–15 min, even a slowly penetrating solute tends to diffuse out of the vesicles more or less completely.

When the vesicles were reconstituted in the presence of radiolabeled oligosaccharides and dextran, the following results were obtained (Table 1). (*i*) The *lamB* protein-containing membrane was impermeable to dextran: a similar percentage of added radioactive dextran was retained in the filtered vesicles

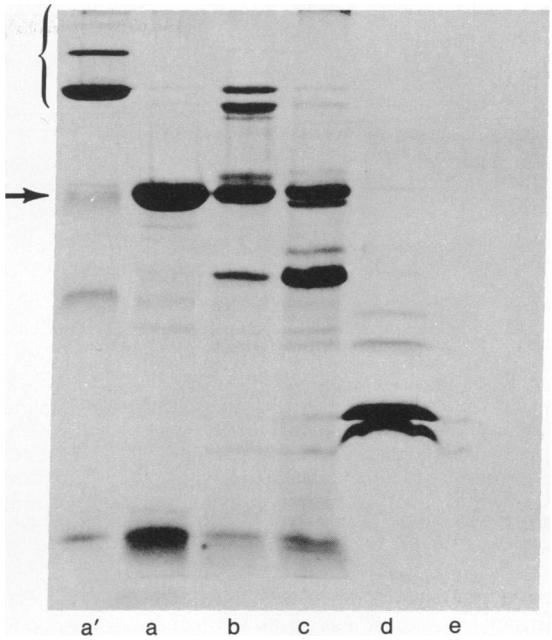

FIG. 4. Protein composition of peaks separated by gel filtration. Fractions from gel filtration on Sephacryl S-200 in cholate and high salt (shown in Fig. 3) were concentrated by overnight precipitation in ethanol and then dissolved in equal volumes of distilled water and sample buffer. The arrow marks the position of the 47,000-dalton band, monomers of the *lamB* protein. The first sample (lane a') was not heated and shows the protein of the major peak (peak a) in several high molecular weight bands (marked by brace). The remaining samples, a through e, were boiled 2 min and contain the peak fractions marked with corresponding labels on Fig. 3. Because the first peak (peak a) contained largely high molecular weight oligomers, the presence of the *lamB* protein in peaks b and c may be due to its migration on Sephacryl also as monomer. Sample e contained no protein; its absorbance at 280 nm was due to residual Triton X-100. Samples a' through d contained 10–50 μg of protein.

in the presence or absence of the added *lamB* protein (not shown). (*ii*) Similarly, the *lamB* protein did not confer permeability to stachyose and raffinose: the retention of these sugars by vesicles was similar to that in the absence of the protein. (*iii*) In contrast, the *lamB* protein made the vesicle membrane permeable to disaccharides: the vesicles containing protein retained less of these sugars in comparison with vesicles not containing protein. Furthermore, there was a hint of some discrimination among disaccharides because the efflux of maltose often appeared to be more extensive than that of sucrose, especially when smaller amounts of the *lamB* protein were used.

Rates of Diffusion of Various Sugars Across the *lamB*-Containing Vesicle Membranes. Because the assay with ra-

Table 1. Equilibrium permeation assay for vesicles reconstituted with the *lamB* protein*

lamB protein		Solutes				
		Normalized $^{14}C/^{3}H$ ratio with [^{3}H]dextran and			Normalized $^{3}H/^{14}C$ ratio with [^{14}C]dextran and	
Preparation	Amount, μg	[^{14}C]Maltose	[^{14}C]Lactose	[^{14}C]Sucrose	[^{3}H]Raffinose	[^{3}H]Stachyose
I†	10.0	0.45	0.40	0.45	1.0	1.0
II‡	19.0	0.17	0.17	0.26	ND	ND
	7.5	0.22	0.35	0.50	ND	ND

ND, not determined.

* Reconstitution employed the method of Nakae (18). The ratios given represent the degree of retention of oligo- and disaccharides relative to the degree of retention of dextran; thus the ratio of 1.0 indicates lack of permeability and the ratios less than 1.0 the efflux of oligo- and disaccharides from vesicles during the gel filtration step.

† This preparation was frozen and thawed several times.

‡ This preparation was stored at 4°C.

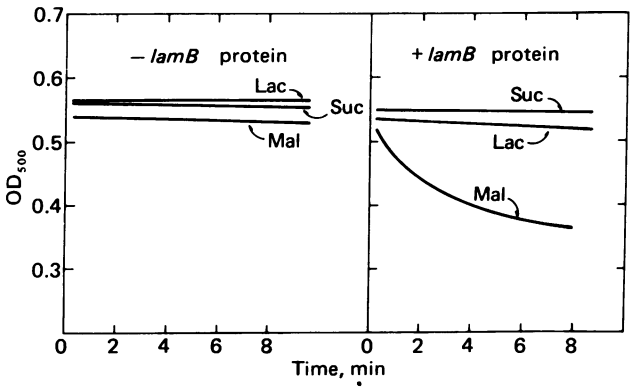

FIG. 5. Optical density tracings in liposome swelling experiment. *E. coli* phospholipids were made into liposomes with and without 3 μg of purified *lamB* protein per mg of phospholipids. Portions (30 μl) of this suspension (3.3 μmol of phospholipid per ml) were diluted into 0.04 M solutions of lactose, sucrose, or maltose, and OD was recorded at 500 nm. The curves were redrawn from the actual recorder tracings.

dioactive sugars was too insensitive to detect small differences in diffusion rates, a rate assay for solute penetration was devised. This method is based on the swelling assay used by Bangham, De Gier, and others (19–21), with some modifications. (*i*) A purified channel-forming protein (in this case the *lamB* protein) was incorporated into the phospholipid bilayer. (*ii*) The refractive index of the intravesicular solution was increased by making liposomes in a dextran solution, so that the sensitivity of the assay was improved and the light scattering behavior of the liposomes was less affected by the number of lamellae, interlamellar distance, etc.

We thus made *lamB* protein-containing liposomes in a solution containing 17% (wt/vol) Dextran T-20, an impermeant solute, and followed the turbidity of the liposome suspension after a rapid dilution into solutions of various sugars. Penetration of the sugar into liposomes caused the liposomes to swell, due to the osmotic pressure exerted by the dextran in the intravesicular space, and the swelling was detected as a decrease in turbidity. As shown in Fig. 5, such a decrease was observed upon the dilution of *lamB* protein-containing liposomes in maltose, but little or no decrease was seen upon the dilution of such liposomes in slowly penetrating sugars such as sucrose, or upon the dilution of control liposomes, made without the addition of *lamB* protein, in solutions of any sugars.

As was shown by Bangham *et al.* (19), the permeability of the liposome membrane to any solute is proportional to the initial rate of change of the reciprocal of optical density. The initial rates for a number of substrates are shown in Table 2. The *lamB* protein channel appears to be rather unspecific when the solute is small (cf. serine in Table 2) but to exert an increasing degree of discrimination as the solute becomes larger. The details of these results will be analyzed in *Discussion*.

It may be argued that the turbidity of the liposomes and their light scattering are dependent on the size, multi-layered structure, and interlamellar distance, and the swelling of one liposome preparation cannot be compared with that of another. We, however, believe that our comparison between *lamB* protein-containing liposomes and control liposomes is quite valid for the following reasons. (*i*) Reconstitution in the presence of [^{3}H]dextran showed that the internal volume of vesicles containing *lamB* protein was similar, within 10–20%, to that of the protein-free liposomes when exceedingly low protein-to-lipid ratios such as in Table 2 were employed. It is possible to conceive of two populations of vesicles—for example, one small and unilamellar and the other large and multilamellar—

Table 2. Rates of swelling of *lamB* protein-containing liposomes in solutions of various sugars

	$d(1/OD_{500})/dt$, min^{-1}		Relative rate of permeation	
	Liposome I	Liposome II	mol basis	wt basis
Maltoheptaose	0.02		2.5	8
Maltotetraose	0.15		19	37
Stachyose	<0.005			
Maltotriose	0.52		66	97
Isomaltotriose	0.10		13	19
Raffinose	<0.005			
Melezitose	<0.005			
Trehalose	0.60		76	76
Maltose	0.79	0.20	100	100
Isomaltose	0.51		65	65
Cellobiose	0.10		13	13
Gentiobiose	0.33		42	42
Sucrose	0.02		2.5	2.5
Turanose	0.34		43	43
Palatinose	0.37		47	47
Melibiose	0.26		33	33
Lactose	0.07		9	9
Di-*N*-acetylchitobiose	<0.005			
Maltitol	0.57		72	72
D-Glucose		0.58	290	153
D-Mannose		0.32	160	84
D-Galactose		0.45	225	118
D-Fructose		0.27	135	71
D-Glucosamine·HCl*		0.37	185	116
N-Acetyl-D-glucosamine		0.10	50	32
Sorbitol		0.42	210	110
Mannitol		0.30	150	79
DL-Serine*†		0.39	195	60

For details see text. The rates shown are approximate because the first 7–10 sec of the swelling reaction could not be recorded. Liposomes I and II contained 4 and 1 μg of purified *lamB* protein per mg of phospholipids, respectively.

* These solutes penetrated slowly into control liposomes. Their rates of permeation are therefore only approximate values.

† Other small molecules that showed rapid permeation included L-glutamic acid and Tris.

that are identical in enclosed volume per mg of phospholipid. This, however, can be excluded because it predicts a wide difference in turbidity between these vesicles when they are made in media of low refractive indices (23), whereas we found almost identical turbidity between protein-containing and non-containing vesicles. Thus, the addition of proteins apparently caused little change in the size or the number of layers per vesicle. (*ii*) In the original De Gier approach (19–21), most of the light scattering was caused by the phospholipid bilayers themselves (23). We, however, incorporated an intravesicular fluid of high refractive index (17% dextran) into liposomes, so that much of the scattering directly reflects changes in intravesicular volume, and is less affected by the number of layers or the interlamellar distance.

DISCUSSION

To study the activity of intrinsic membrane proteins in reconstitution assays it is obviously advantageous to solubilize them in cholate, which can be easily removed by dialysis (24). The *lamB* protein, however, could not be extracted efficiently from outer membranes by cholate. We thus took a two-step approach, first extracting the protein in Triton X-100 and then, after purification by ion-exchange chromatography, exchanging Triton X-100 with cholate. The small size of cholate micelles

enabled us to fractionate membrane proteins on the basis of size (Figs. 3 and 4). This general strategy may be useful for the purification of other intrinsic membrane proteins.

As was already shown in other laboratories (5, 6), the purified *lamB* protein conferred hydrophilic permeability to phospholipid bilayer membranes (Table 1). The earlier studies, however, suffered from the fact that only the penetration of ions could be studied in the "black lipid film" approach (5), and that even slightly permeant solutes almost completely exited out of the vesicles during the long time necessary for filtration (6). In this study we have circumvented these problems and measured the rates of transmembrane diffusion by using the liposome swelling assay.

The values of $d(1/OD)/dt$ in this assay reflect the degree of permeability of the liposome membrane toward a given solute (19–21). That the permeability toward sugars was conferred by the *lamB* protein was seen from the total absence of permeability in control liposomes (Fig. 5), as well as from the fact that $d(1/OD)/dt$ was proportional to the amount of *lamB* protein added in the range of 0.7–4.2 μg of protein per mg of phospholipid (data not shown). Cholate did not produce significant permeability even at 200 μg/mg of phospholipid.

Table 2 shows that the *lamB* protein makes the membrane permeable to a wide variety of compounds but discriminates among closely related compounds. The rapid penetration of serine confirms the conclusion that the protein allows the rather nonspecific diffusion of small molecules and ions (5). Yet some degree of discrimination is already seen at the level of monosaccharides. Thus glucose diffused most rapidly, and changes at C-1, C-2, C-4, or C-6 produced small but significant reductions in diffusion rates. Especially noteworthy is the situation with *N*-acetyl-D-glucosamine, in which presumably the existence of a bulky substituent group at C-2 reduces the permeation rate to less than 20% of that of glucose. At the disaccharide level, the discrimination becomes stronger, and sugars such as sucrose diffuse at a rate less than 5% of that of maltose. At the level of trisaccharides, the selectivity becomes even more pronounced. Thus the diffusion of raffinose and melezitose, which contain the sucrose structure, could not be detected at all. Furthermore, although isomaltose diffused at 65% of the rate of maltose, isomaltotriose penetrated at less than 20% of the rate of diffusion of maltotriose.

What is the explanation of these observations at the molecular level? The present results by themselves cannot distinguish between the formation of pores by the *lamB* protein and its functioning as a rotating carrier. However, the high rate of flux catalyzed by this protein in intact cells, as well as the relative lack of specificity toward very small solutes, tends to support the former possibility. As a working hypothesis we therefore propose that the *lamB* protein produces a transmembrane channel with a site (or sites) that interacts with the solutes in a configuration-specific manner. Because of the looseness of interaction, the binding of maltose cannot be detected by the conventional assay (4), and the channel allows the diffusion of a wide variety of solutes. However, the channel becomes highly discriminatory for higher oligosaccharides because they interact with the sites repeatedly or more tightly.

Is the specificity of the *lamB* protein channel sufficient to explain the selectivity of this permeation pathway in intact cells? Growth studies employing *ompB* mutants (lacking porins) have indicated that the *lamB* protein can facilitate the diffusion of glucose and lactose (25). Our present results are in qualitative agreement with this finding, but, quantitatively, we find that glucose and maltose diffuse with similar rates, on mass basis, in our reconstituted vesicles (Table 2), whereas in intact cells maltose penetrates through the outer membrane much more rapidly than glucose (25). Most likely other components of the maltose transport system do enhance the rate of maltose diffusion *in vivo*, and not that of nonmaltose sugars. The maltose binding protein can do this simply by enhancing the concentration gradient of free maltose (or maltodextrins) across the outer membrane. Thus the diffusion through the *lamB* protein channel may be inherently slow, probably because of the interaction of solutes with sites on the channel walls.

Finally, we point out that this study represents one of the few cases in which a protein involved in a facilitated diffusion process has been isolated and successfully reconstituted into liposome membranes (26). This system appears to have significant advantages over the animal cell carrier systems because a genetic approach can be utilized. Already a number of mutants of *lamB* gene are known (27).

This work was supported by grants from the National Institutes of Health (AI 9644) and The American Cancer Society (BC-20). M.L. is a Postdoctoral Fellow of the American Cancer Society (PF-1540).

1. Nikaido, H. (1979) *Agnew. Chem. (Int. Ed.)* **18,** 337–350.
2. Hofnung, M. (1974) *Genetics* **76,** 169–184.
3. Szmelcman, S., Schwartz, M., Silhavy, T. J. & Boos, W. (1976) *Eur. J. Biochem.* **65,** 13–19.
4. Randall-Hazelbauer, L. & Schwartz, M. (1973) *J. Bacteriol.* **116,** 1436–1446.
5. Boehler-Kohler, B. A., Boos, W., Dieterle, R. & Benz, R. (1979) *J. Bacteriol.* **138,** 33–39.
6. Nakae, T. (1979) *Biochem. Biophys. Res. Commun.* **88,** 774–781.
7. Endermann, R., Hindennach, I. & Henning, U. (1978) *FEBS Lett.* **88,** 71–74.
8. Sandermann, H., Bavoil, P. & Nikaido, H. (1978) *FEBS Lett.* **95,** 107–110.
9. Bavoil, P., Nikaido, H. & von Meyenburg, K. (1977) *Mol. Gen. Genet.* **158,** 23–33.
10. Decad, G. M. & Nikaido, H. (1976) *J. Bacteriol.* **128,** 325–336.
11. Hancock, R. E. W. & Nikaido, H. (1978) *J. Bacteriol.* **136,** 381–390.
12. French, D., Levine, M. & Pazur, J. H. (1949) *J. Am. Chem. Soc.* **71,** 356–358.
13. Whelan, W. J., Bailey, J. M. & Roberts, P. J. P. (1953) *J. Chem. Soc.* 1293–1298.
14. Smit, J., Kamio, Y. & Nikaido, H. (1975) *J. Bacteriol.* **124,** 942–958.
15. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193,** 265–275.
16. Sandermann, H. & Strominger, J. L. (1972) *J. Biol. Chem.* **247,** 5123–5131.
17. Lugtenberg, B., Meijers, J., Peters, R., van der Hoek, P. & van Alphen, L. (1975) *FEBS Lett.* **58,** 254–259.
18. Nakae, T. (1976) *J. Biol. Chem.* **251,** 2176–2178.
19. Bangham, A., De Gier, J. & Greville, G. D. (1967) *Chem. Phys. Lipids* **1,** 225–246.
20. De Gier, J., Mandersloot, J. G. & van Deenen, L. L. M. (1968) *Biochim. Biophys. Acta* **150,** 666–675.
21. Bangham, A. D., Hill, M. W. & Miller, N. G. A. (1974) in *Methods in Membrane Biology,* ed. Korn, E. D. (Plenum, New York), Vol. 1, pp. 1–68.
22. Braun, V. & Krieger-Brauer, H. J. (1977) *Biochim. Biophys. Acta* **469,** 89–98.
23. Chong, C. S. & Colbow, K. (1976) *Biochim. Biophys. Acta* **436,** 260–282.
24. Kagawa, Y. (1972) *Biochim. Biophys. Acta* **265,** 297–338.
25. von Meyenburg, K. & Nikaido, H. (1977) *Biochem. Biophys. Res. Commun.* **78,** 1100–1107.
26. Kagawa, Y. (1978) *Biochim. Biophys. Acta* **505,** 45–93.
27. Braun-Breton, C. & Hofnung, M. (1977) *FEMS Microbiol. Lett.* **1,** 371–374.