Effect of Solute Size on Diffusion Rates through the Transmembrane Pores of the Outer Membrane of *Escherichia coli*

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ABSTRACT Nutrients usually cross the outer membrane of *Escherichia coli* by diffusion through water-filled channels surrounded by a specific class of protein, porins. In this study, the rates of diffusion of hydrophilic nonelectrolytes, mostly sugars and sugar alcohols, through the porin channels were determined in two systems, (*a*) vesicles reconstituted from phospholipids and purified porin and (*b*) intact cells of mutant strains that produce many fewer porin molecules than wild-type strains. The diffusion rates were strongly affected by the size of the solute, even when the size was well within the "exclusion limit" of the channel. In both systems, hexoses and hexose disaccharides diffused through the channel at rates 50–80% and 2–4%, respectively, of that of a pentose, arabinose. Application of the Renkin equation to these data led to the estimate that the pore radius is ~0.6 nm, if the pore is assumed to be a hollow cylinder. The results of the study also show that the permeability of the outer membrane of the wild-type *E. coli* cell to glucose and lactose can be explained by the presence of porin channels, that a significant fraction of these channels must be functional or "open" under our conditions of growth, and that even 10^5 channels per cell could become limiting when *E. coli* tries to grow at a maximal rate on low concentrations of slowly penetrating solutes, such as disaccharides.

INTRODUCTION

Cells of Gram-negative bacteria, for example, *Escherichia coli* and *Salmonella typhimurium*, are enclosed by a double layer of unit membrane (Glauert and Thornley, 1969). The inner membrane corresponds to the cytoplasmic membrane. The outer membrane is located outside the rigid peptidoglycan layer and corresponds to the outermost layer of the "cell wall" (Nikaido and Nakae, 1979). We have shown that the outer membrane of *E. coli* and *S. typhimurium* is permeable to monosaccharides, disaccharides, and trisaccharides by measuring the equilibrium distribution of labeled saccharides between the external medium and the "periplasmic space," i.e., the space between the outer and inner membranes (Decad and Nikaido, 1976). The component responsible for this permeability was identified as a special class of protein, porins, by the following approaches. (*a*) Porins were purified from the outer membranes,

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and membrane vesicles reconstituted from the purified porin and the lipid components of the outer membrane, i.e., lipopolysaccharides and glycerophospholipids, were shown to be permeable to sucrose and other small solutes (Nakae 1976 *a* and 1976 *b*). (*b*) Mutants with diminished levels of porin were shown to be impaired in their capacity to transport various nutrients into the cytoplasm, and consequently in their capacity to utilize them for growth (Bavoil et al., 1977; Lutkenhaus, 1977). The following points are noteworthy. (*a*) Each bacterial strain often produces a family of porins (Nakae, 1976 *a*; Nakae and Ishii, 1978). However, *E. coli* strain B and its derivatives (including B/r) produce only one kind of porin (Rosenbusch, 1974; Nakae, 1976 *b*; Bavoil et al., 1977). (*b*) Porins apparently produce nonspecific diffusion channels, as indicated by the following observations. The mutational loss of a single species of porin in *E. coli* B/r affects the transport of a wide variety of low molecular weight compounds, e.g., various sugars, sugar phosphates, amino acids, purines, pyrimidines, and even inorganic anions (von Meyenburg, 1971; Bavoil et al., 1977), and the insertion of porin into reconstituted membranes makes them permeable to any hydrophilic solute with a molecular weight < 600 (Nakae, 1976 *b*).

In this work, we examine the influence of the size of solutes on the rates of diffusion through the porin channels both in reconstituted membrane vesicles and in intact cells. The results allow us to estimate the effective size of the channel and lead us to a better understanding of the functions of the porin channel in a physiological context.

MATERIALS AND METHODS

Bacterial Strains

Derivatives of *E. coli* B were used, strain B(H) and two B/r strains, CM6 and CM7. CM6 is a *thyA drm tonA mal* strain, but can be considered a "wild-type" strain in terms of the production of porin. CM7 is a mutant of CM6 and contains an additional mutation, *kmt-7* (Bavoil et al., 1977), which, according to more recent genetic nomenclature (Bachmann and Low, 1980) should be called *ompB7.*

Chemicals

Generally, the best grades commercially available were used. Dextran T-20 was the product of Pharmacia (Uppsala, Sweden). Crude phospholipids were extracted from *E. coli* B according to the method of Folch et al. (1957). Porin trimers were purified to homogeneity by Dr. Y. Takeuchi of this laboratory from strain B(H) according to the procedure of Tokunaga et al. (1979).

Solute Penetration Rates in Reconstituted Vesicles

Multilamellar vesicles were made from *E. coli* phospholipids and *E. coli* B porin, essentially as described by Luckey and Nikaido (1980). The rate of penetration of nonelectrolytes was determined by the initial rate of swelling of these vesicles upon dilution into isotonic solutions of these compounds (Luckey and Nikaido, 1980).

Outer Membrane Permeability Intact Cells

The principles of this approach are described in Results. For the calculation of permeability coefficients, it was necessary to determine three growth parameters. All

growth experiments were carried out in AB medium (Clark and Maaløe, 1967) containing various sugars. The culture in Erlenmeyer flasks was aerated by shaking in a shaking water bath (New Brunswick Scientific Co., Inc., Edison, N. J.) at 37°C, and the growth was followed by reading the "optical density" of the culture in a spectrophotometer (Hitachi Ltd., Tokyo, Japan; model 124) For the determination of growth yield, *E. coli* strains were inoculated into AB medium containing limiting concentrations (usually up to 0.05%) of the carbon source, and growth was followed until it stopped as a result of the exhaustion of the carbon source. The yield in dry weight was calculated with the optical-density-to-dry-weight conversion curve prepared using strain CM6. For the determination of maximal growth rate and growth K_m, the cells were first grown overnight in AB medium usually containing 1% carbohydrate. A small portion of this culture was then diluted into fresh AB medium, and growth was followed by determining optical density at 450 nm for several hours. Maximal growth rates were calculated from the semilogarithmic plots of the growth rates measured during this stage. The culture was then diluted (usually when OD_{450} was ~2.0) at various ratios into several flasks containing prewarmed AB medium without the carbon source. These flasks were shaken at 37°C, and the growth was followed by determining optical density at 310 nm, rather than at 450 nm, to increase the sensitivity. The reciprocals of initial rates of growth in these flasks were plotted against the reciprocals of the initial carbohydrate concentrations, and the "growth K_m", i.e., the external carbohydrate concentration that allowed growth at one-half of the maximal rate, was read from these Lineweaver-Burk plots. These procedures are essentially those of von Meyenburg (1971). We found, however, that the addition of 0.01 volume of 10% $NaHCO_3$ to the AB medium just before use abolished the lag in growth frequently seen after extensive dilutions; therefore, $NaHCO_3$ was added to the medium in all the dilution experiments.

The growth K_m of CM6 for glucose and that of CM7 for glycerol were also determined by following growth by radioisotope incorporation. In these experiments the cultures were grown in AB medium containing either 0.02% [^{14}C] glucose (specific activity, 100 μCi/mg) or 0.1% [^{14}C]glycerol (specific activity, 20 μCi/mg) diluted into several flasks containing carbohydrate-free AB medium, and incorportion of ^{14}C into cellular material was followed by adding 1-ml aliquots of cultures to 10 ml of 5% trichloroacetic acid, filtering the suspension through a filter (Millipore Corp., Bedford, Mass.; pore diameter, 0.45 μm) and determining the radioactivity of the material on the filter with a liquid scintillation counter (Beckman Instruments, Inc., Fullerton, Calif.).

The rate of uptake or transport of the carbohydrate, V, was calculated by dividing the growth rate by the growth yield. For example, for strain CM7 growing on D-glucose, the doubling time at half-maximal growth rate was 4,800 s, and the growth rate was $\ln 2/4{,}800 = 1.44 \times 10^{-4}$ s^{-1}. Since 1 g of glucose (molecular weight, 180) gave rise to 0.52 g (dry weight) of CM7 cells, the rate of transport (or the rate of utilization), V, under these conditions is $1.44 \times 10^{-4} \div 0.52 \div 180 = 1.54 \times 10^{-6}$ mol · s^{-1} · g^{-1} or 1.54 nmol · s^{-1} · mg^{-1}.

RESULTS

Permeability of the Reconstituted Vesicles

In previous reconstitution studies from our laboratory (Nakae, 1976 *a* and 1976 *b*), the only information obtained was qualitative, that is, whether substantial amounts of labeled solute diffused out of the vesicles after an arbitrarily determined, rather long, period of incubation. Thus, the data

essentially gave near-equilibrium distribution of solutes rather than rates of diffusion through the channels. Since the rates of penetration give a better indication of the permeability properties of the outer membrane, they were measured in the present study by following the rates of swelling of porin-phospholipid vesicles in various solutions (see Materials and Methods and Fig. 1). Since phospholipid vesicles without porin did not show significant swelling in most of the sugar solutions used, the swelling rates in porin-containing liposomes indicate the rates of diffusion through porin channels (Fig. 1). The results (Fig. 2) demonstrate a strong dependence of diffusion rates on the size

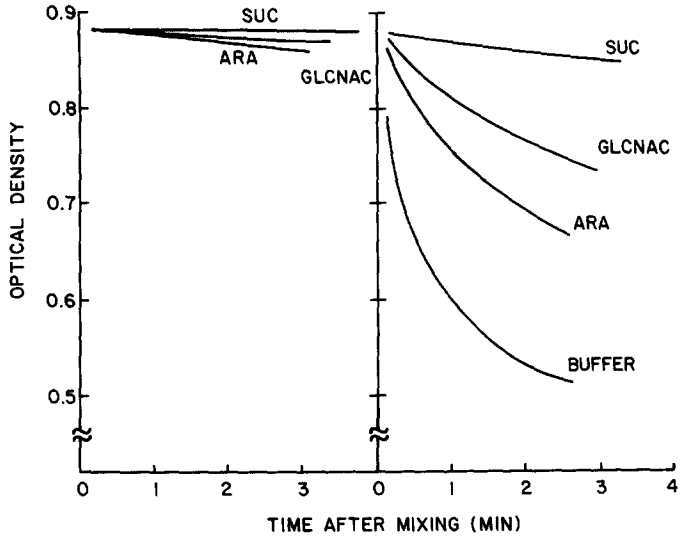

FIGURE 1. Optical density tracings in a liposome swelling experiment. Multilayer vesicles were made by resuspending 2.4 μmol of *E. coli* phospholipids in 0.6 ml of 17% (wt/vol) Dextran T-20 in 5 mM Tris-HCl buffer, pH 7.4, either without (*left*) or with (*right*) 0.5 μg of *E. coli* B porin as described in Materials and Methods. Portions (15 μl) of these suspensions were diluted into 0.63 ml of 0.04 M sugar solutions in 5 mM Tris-HCl buffer, pH 7.4, and the optical density was continuously recorded at 450 nm with a spectrophotometer at 25°C. The figure shows only the results obtained after dilution into L-arabinose (*ARA*), *N*-acetyl-D-glucosamine (*GLCNAC*), sucrose (*SUC*), and in the case of the porin-containing vesicles, 5 mM Tris-HCl buffer, pH 7.4.

of the solute. Thus, hexoses diffused, on an average, at ~70% of the rate of a pentose, arabinose, and the rates for disaccharides of hexose were almost two orders of magnitude lower than the rate of arabinose, although the size of all solutes tested was well within the exclusion limit of the pores as determined by the equilibrium diffusion assay (Nakae, 1976 *a* and 1976 *b*).

If the solutes indeed diffuse through the water-filled channels surrounded by the porin protein, the rates of solute diffusion should not be affected too much by the temperature. As shown in Fig. 3, the Q_{10} for the rate of diffusion of *N*-acetyl-D-glucosamine was ~1.25. This is very similar to the Q_{10} of the

free diffusion process in bulk water, which is between 1.2 and 1.3 in this temperature range according to the Stokes-Einstein equation. In contrast, the rate of diffusion of pentaerythritol into phospholipids-only vesicles was much more dependent on temperature (Fig. 3); this is typical of processes involving permeation through the hydrocarbon interior of the membrane (Galey et al., 1973).

Permeability of the Outer Membrane in Intact Cells

Although much information was obtained with the reconstituted vesicles,

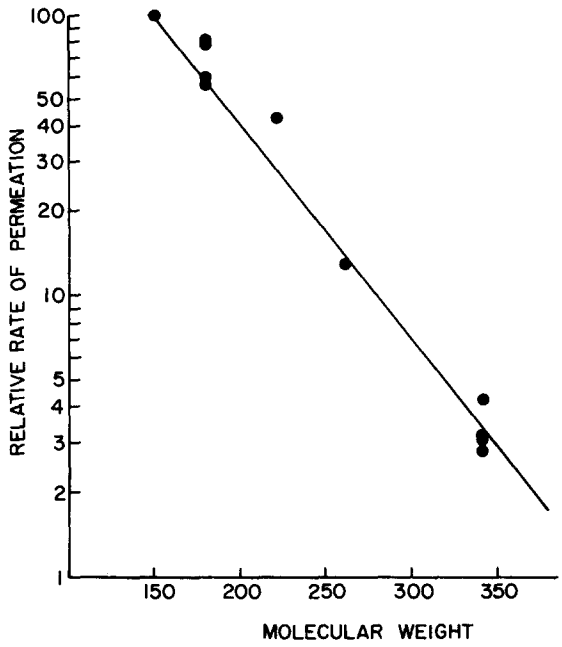

FIGURE 2. Rates of permeation of various sugars into phospholipid-porin vesicles. Rates were calculated from the results of swelling assays, some of which are shown in Fig. 1, as described by Luckey and Nikaido (1980). The sugars used are: mol wt 150, L-arabinose; mol wt 180 (from top to bottom), D-galactose, D-fructose, D-mannose, and D-glucose; mol wt 221, *N*-acetyl-D-glucosamine; mol wt 262, 2,3-diacetamido-2,3-dideoxy-D-glucose; mol wt 342 (from top to bottom), sucrose, melibiose, maltose, and lactose. Control experiments showed that permeation into phospholipid-only vesicles occurred at negligible rates (see Fig. 1).

studies with intact cells were necessary for the following reasons. (*a*) The vesicle assay only gives relative values of permeability, not the absolute values of permeability coefficient. (*b*) The properties of the porin in intact cells could be different from those in reconstituted vesicles. The conformation of the porin might be affected by its association with neighboring molecules, and the possibility of partial denaturation during purification can never be completely ruled out. (*c*) From the vesicle studies, it is not clear whether the porin

channels alone can explain most of the permeability properties of the outer membrane.

The permeability of outer membrane in intact cells was measured by studying the growth behavior of *E. coli* cells. The principles of the approach are as follows (see Fig. 4).

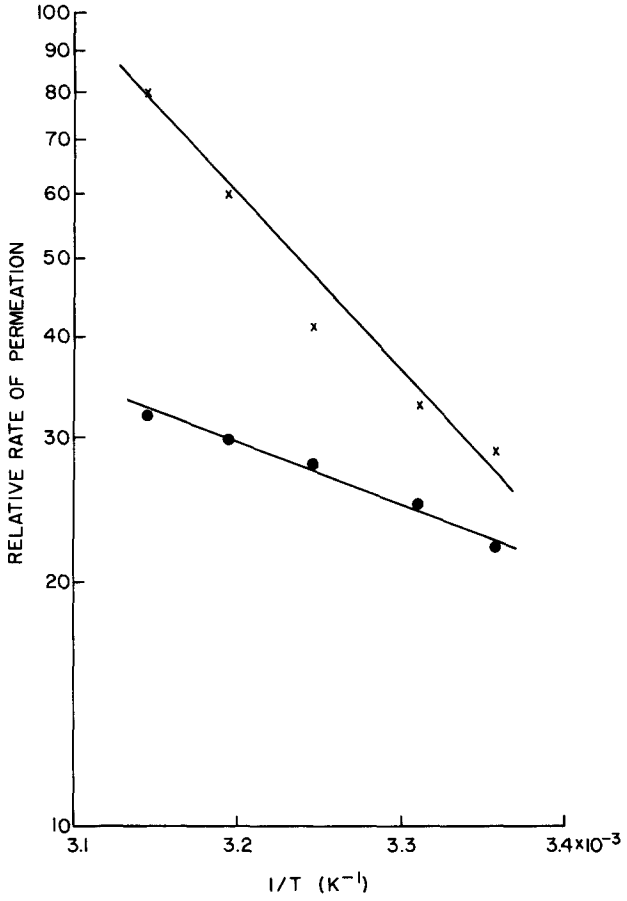

FIGURE 3. Temperature dependence of diffusion processes through porin channels and through phospholipid bilayer. Rate of diffusion into vesicles was determined by the initial rate of the swelling of vesicles at various temperatures. (●) Rates of swelling of vesicles reconstituted from 2.4 μmol *E. coli* phospholipids and 0.5 μg porin upon dilution into 0.04 M *N*-acetyl-D-glucosamine. (×) Rates of swelling of vesicles made from *E. coli* phospholipids upon dilution into 0.04 M pentaerythritol.

From Fick's first law, the rate of diffusion of solutes across the outer membrane, V, is given by

$$V = P \cdot A \cdot (C_o - C_p), \quad (1)$$

where P, A, C_o, and C_p are the permeability coefficients of the outer membrane,

area of the outer membrane, concentration of the solute in the external medium, and its concentration in the periplasmic space, respectively. At steady state, V is also equal to the rate at which the solute is further transported from the periplasm into the cytoplasm via the active transport system of the cytoplasmic membrane. In the assay used in this work, we grew *E. coli* under conditions under which the rate of transport of the carbon (and energy) source limited the growth rate. Thus, we were able to calculate V from the growth rate and growth yield (see Materials and Methods). A was found to be 131 cm^2/mg cells (dry weight) in a closely related organism, *Salmonella typhimurium*

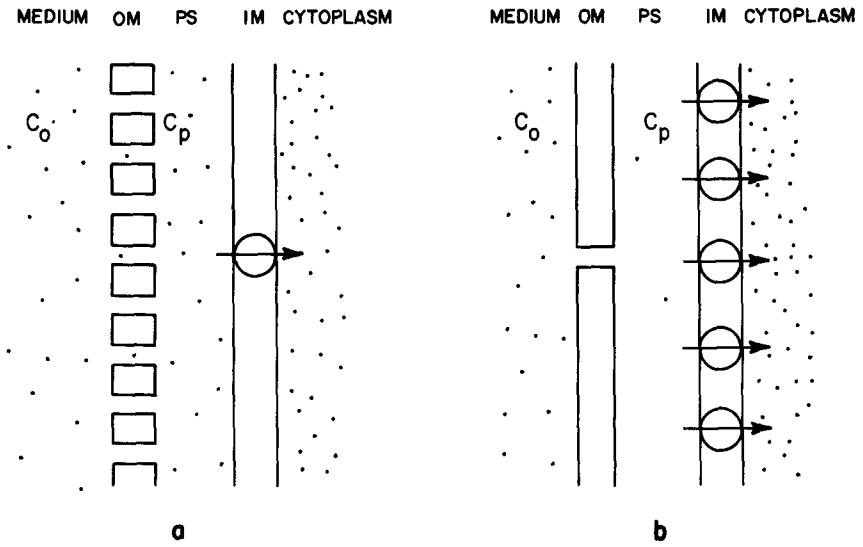

FIGURE 4. Schematic representation of the nutrient transport by *E. coli* cells. In *a*, the outer membrane is highly permeable as a result of the presence of a large number of pores (as in the wild-type strain). The overall transport process tends to become limited by the active-transport process across the cytoplasmic membrane, especially when the latter has a low V_{max}. Thus, C_p approaches C_o. In *b*, the permeability of the outer membrane is limited due to the reduced number of porin channels, as in the porin-deficient mutants used in this study. In this situation, the overall process tends to become limited by the diffusion step across the outer membrane, especially when the active transport system has a high capacity, i.e., high V_{max}. Thus, C_p becomes much lower than C_o. OM, outer membrane; PS, periplasmic space; IM, inner (cytoplasmic) membrane.

(Smit et al., 1975), and C_o is known. Unfortunately there is no convenient way to measure C_p for the calculation of P. Our approach to solving this problem was to create conditions under which $C_p \ll C_o$, so that C_p can be neglected without affecting the accuracy of results. Thus,

$$P \simeq V/(A \cdot C_o). \tag{2}$$

Two experimental maneuvers were used to ensure that C_p was much lower than C_o. Firstly, we used solutes that are efficiently removed from the

periplasmic space by active transport systems located in the cytoplasmic membrane (Fig. 4). Sugars are usually transported by systems with a high V_{max} and low K_m (Koch, 1971), and we therefore used them as solutes. Secondly, it was easier to lower C_p if the permeability of the outer membrane was lowered (Fig. 4). We therefore used mutants CM7, CM30, and CM31 of *E. coli* B/r, which produce < 1% of the number of porins found in the parent, wild-type strain CM6 (Bavoil et al., 1977). The *ompB* mutation responsible for this change is most probably a regulatory mutation that does not affect the structure of the porin protein (Ichihara and Mizushima, 1978; Sato and Yura, 1979).

For convenience and reproducibility, and to make certain that C_p was negligible, we determined V at a solute concentration C_o that corresponded to the "growth K_m," which is the value of C_o that gave half-maximal growth rates (Table I). Because the growth rate is limited by the transport rate, which is determined by the Michaelis-Menten relationship, C_p under these conditions must be equal to, or less than, the K_m of the active transport system. The K_m

TABLE I

GROWTH K_m VALUES FOR VARIOUS CARBON SOURCES

	Growth (transport) K_m			
	Parent	Porin-deficient mutants		
Substrate	CM6	CM7	CM30	CM31
		μM		
Glycerol	N.D.	60,60*	55	160
L-Arabinose	N.D.	300	280	370
D-Glucose	6*	500	480	760
Lactose	70	7,000	4,700	9,000

Each value is an average of the results of at least two experiments.

* Values determined with radioactive substrates (see Materials and Methods).

values of the overall transport processes (in the wild-type *E. coli*) were 0.9 μM for glycerol (Hayashi and Lin, 1965), 6 μM for glucose, and 70 μM for lactose (see Table I). Since the true K_m of the transport system of the inner membrane is always lower than the "K_m" of the overall system, the former and the C_p would be expected to be lower than the values listed above. The arabinose-binding protein has a K_d of 0.2 μM (Parsons and Hogg, 1974), and the arabinose transport system would be expected to have a K_m not too much higher than this value. When these estimated K_m values of the active transport systems, i.e, the approximate values of C_p, are compared with the C_o, or "growth K_m" values for the mutants in Table I, we find that the former usually are 1–2% or less of the latter. Thus, with the mutants under our conditions of assay, C_p is indeed negligible in comparison with C_o, and we can use Eq. 2 without introducing much error.

Calculation according to Eq. 2 produced the values of P shown in Table II. Clearly, the size of the solute has a profound influence on diffusion rates.

Indeed, the agreement between these results and those obtained with vesicles containing purified porin is striking: the ratio of the permeability coefficients for arabinose, glucose, and lactose in intact cells is 1:0.46:0.02 (Table II), and that in reconstituted vesicles is 1:0.56:0.03 (Fig. 2). These results not only indicate that porins in the vesicle produced channels of unaltered properties, but also confirm the idea that porin channels are the major routes of penetration for most small, hydrophilic molecules through the outer membrane.

We emphasize that very similar results were obtained with the three independent mutants used (Table II). This is important because it argues against the possibility that *ompB* mutations alter the properties of the porin channel, and that we are simply observing diffusion through the altered, inefficient channels. This result also is further evidence against the possibility that some of the solutes may be diffusing through an alternate pathway. If this were the case, the more permeable mutant (e.g., CM30) should have more residual porin channels open than the less permeable mutant (e.g., CM31). Since the alternate channel and the porin channel would be expected to have

TABLE II

PERMEABILITY OF OUTER MEMBRANE

Diffusing molecule	Hydrated radius*	Maximum growth rate	Growth yield	Permeability coefficient (P)		
				CM7	CM30	CM31
	nm	s^{-1}	*g cells/g substrate*		*μm/s*	
Glycerol	0.31	2.34×10^{-4}	0.58	2.84	3.10	1.07
L-Arabinose	0.38	2.50×10^{-4}	0.41	0.52	0.56	0.42
D-Glucose	0.42	2.88×10^{-4}	0.52	0.24	0.245	0.155
Lactose	0.54	2.88×10^{-4}	0.59	0.008	0.012	0.006

* From Schultz and Solomon (1961).

different pore sizes, the effect of the solute size should be different, depending on the different properties of these channels. Clearly, such a situation was not observed.

DISCUSSION

Experimental Systems

In the present study, the rates of diffusion of nonelectrolytes through the water-filled channels created by the porin protein were determined in two experimental systems, in vesicles reconstituted from purified porin and in intact cells. The vesicle system had several advantages. Results could be generated much more rapidly with this system, a wide range of solutes could be used, and one could be certain that the solutes were penetrating through the porin channels. On the other hand, the intact cell system permitted the examination of the functions of porin in its normal environment, and also allowed the determination of the absolute values of permeability coefficients. It is gratifying that varying the size of the solutes produced exactly the same

effect in both systems (Fig. 2 and Table II), a result indicating that the vesicle system serves as a faithful model of the outer membrane in terms of the permeability of the porin channel.

In the vesicle system, we measured swelling rates by dilution into isotonic solutions of nonelectrolytes. We are aware that this method only gives approximate values of the permeability coefficient (Kedem and Katchalsky, 1958). Yet we have chosen this method, for the following reasons. (*a*) With cells and single-layer liposomes, the minimum volume method (Sha'afi et al., 1970) theoretically gives more correct values. However, this method cannot be used for multilamellar liposomes such as those used in this work, because at the point of apparent minimal volume or maximal turbidity the inner parts of the liposomes are still shrinking, and the turbidity increase caused by this shrinking is compensated for by swelling of the outermost layers. Thus, with multilayered liposomes, the point of maximal turbidity does not necessarily correspond to the point of zero volume flux, and the minimum volume method and its variants become inapplicable, despite claims to the contrary (van Zoelen et al., 1978). (*b*) The isotonic swelling rates deviate from the solute permeability coefficients because the rapid influx of solvent drags in significant numbers of solute molecules. However, in our system most of the solvent (water) influx apparently occurs through the phospholipid bilayers of the vesicles, as indicated by the observation that the swelling rates in water of porin-containing vesicles are not much faster than those of vesicles that do not contain porins. Since the solutes used could not penetrate through the bilayer, the effect of solvent drag probably had minimal effect on the accuracy of the permeability coefficients obtained in our experiments.

Effective Radius of the Porin Channel

Although our previous work with reconstituted vesicles indicated that a trisaccharide, raffinose, diffused through the channel, whereas a tetrasaccharide, stachyose, did not (Nakae, 1976 *b*), we could not immediately determine the pore size from these data. This was because the test solutes could assume flexible conformations in water, and because in this assay we could obtain only qualitative data on the presence or absence of penetration after a fairly long equilibration. Thus, the pore radius could have been smaller than the Stokes radius of raffinose, and yet raffinose could have diffused by assuming a cylindrical conformation. On the other hand, the pore radius could have been larger than the Stokes radius of stachyose, and yet stachyose diffusion could have been scored as negative, simply because not enough time was allowed for the slow penetration of this sugar.

In contrast to these difficulties generated by the equilibrium distribution assay, our determination of the *rates* of diffusion of various solutes should enable us to estimate the effective pore radius in a reliable manner, because the penetration rates should be affected predominantly by the time-averaged, or Stokes, radius of the solutes. The effect of solute size on the rate of penetration through pores is expressed by the well-known Renkin equation (Renkin, 1954),

$$a/a_o = [1 - (r/R)]^2 [1 - 2.104 (r/R) + 2.09 (r/R)^3 - 0.95 (r/R)^5], \quad (3)$$

in which a, a_o, r, and R are the effective area of the pore, the total cross-sectional area of the pore, radius of the solute, and radius of the pore, respectively. We have calculated the theoretically expected rates of diffusion of the solutes listed in Table II by multiplying their free diffusion coefficient

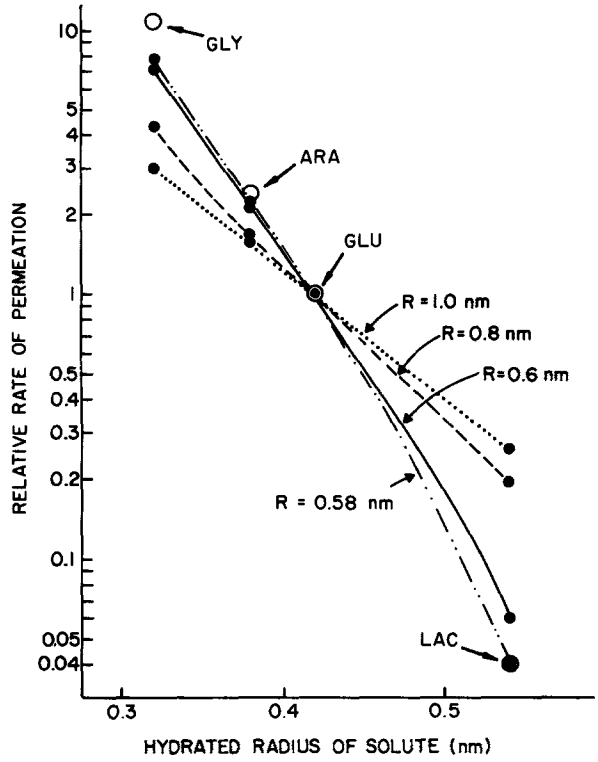

FIGURE 5. Comparison of the rates of permeation observed in intact cells with theoretically predicted rates for cylindrical pores of various radii (R). (○) Relative rates of diffusion of glycerol (*GLY*), L-arabinose (*ARA*), D-glucose (*GLU*), and lactose (*LAC*) through the outer membrane; the rates in Table II were first normalized to the rate of permeation of glucose, and then averages of the three strains were computed. (●) Theoretically predicted rates computed by multiplying the free diffusion coefficients of the solutes with the Renkin factor (see text) and then normalizing all values to the predicted rates for glucose. It is possible that glycerol diffuses faster than predicted because it also utilizes the non-porin pathway, i.e., diffusion through the lipid bilayer region of the membrane.

in water (calculated from the Stokes-Einstein radii data in Table III of Schultz and Solomon [1961]) with the Renkin factor a/a_o; it is seen in Fig. 5 that the experimentally observed data fit best with the values predicted by assuming $R = 0.58$ nm. Although the absolute value of this effective radius should not be taken too literally because of the uncertain validity of the Renkin equation

for these small pores and the possible deviation of the pore shape from a perfect cylinder, it is gratifying to see that the estimated radius, 0.58 nm, corresponds roughly to the Stokes radius of raffinose, the largest saccharide seen to go through these channels in our earlier, qualitative assays (Nakae, 1976 *a* and 1976 *b*).

The radius of *E. coli* porin channels has been estimated to be 0.465 nm from the conductivity of porin-containing planar lipid bilayers (Benz et al., 1978). This was based on two assumptions: (*a*) porins exist as trimers, and a trimer contains one central channel, and (*b*) conductivity within the channel is identical to the conductivity of bulk solutions. We believe that both of these assumptions are incorrect. Firstly, the careful measurement of single-step conductance increase in porin-containing planar bilayers (Schindler and Rosenbusch, 1978), as well as electron microscopy of negatively stained outer membrane preparations (Steven et al., 1977), indicate that a trimer of porin contains three channels. Secondly, since the size of the hydrated ions is of the same order of magnitude as the size of the pore, it is obviously difficult to believe that the mobility of these ions through the channel can be similar to that in the bulk solution. If we recalculate, assuming the presence of three channels per trimer, that both K^+ and Cl^- ions are associated with two molecules of water (Bockris, 1949), and that the hydrated ions go through the channel in a manner predicted by the Renkin equation, the conductivity data of Benz et al. (1978) actually lead to an estimation of pore radius of 0.55 nm. This is very close to our own estimate.

Permeability of the Outer Membrane of Wild-Type E. coli

We may now ask whether the properties of the porin channel we have seen are capable of explaining the permeability characteristics of the wild-type *E. coli*. The permeability coefficient expected on theoretical grounds can be calculated as follows:

$$P_{theory} = (D/d)\ (a_o/A)\ (a/a_o), \tag{4}$$

where D, d, and a_o are the free diffusion coefficient, thickness of the membrane, and the total cross-sectional areas of all the pores present, respectively, and A and a/a_o are the total area of the membrane and the Renkin correction factor as defined in Eqs. 1 and 3, respectively. Since one cell of *E. coli* B is reported to have 10^5 porin molecules (Rosenbusch, 1974), each of which can be assumed to produce a pore of 0.6 nm in radius, the total cross-sectional area of the channels per cell is 1.13×10^5 nm^2. The average surface area per cell for *E. coli* B is ~ 3 μm^2 or 3×10^6 nm^2. By substituting appropriate values in Eq. 4, we get 0.4×10^{-2} cm · s^{-1} for P_{theory} for glucose.

This expected value can now be compared with the experimentally determined value of the outer membrane permeability. Unfortunately, in a wild-type strain, C_p in Eq. 1 is not negligibly small in comparison with C_o. Thus, the values of $P_{observed}$ obtained by neglecting C_p will be only minimal estimates. By using values of V at the "growth K_m" of 6 μM (Table I), we get $P_{observed} \geq 0.2 \times 10^{-2}$ cm · s^{-1}. It is seen that the P_{theory} fits very well with the $P_{observed}$.

Similar calculations for lactose diffusion in CM6 showed again that the P_{theory} (0.25×10^{-3} cm $\cdot$ s^{-1}) is in the same range as the $P_{observed}$ ($\geq 0.1 \times 10^{-3}$ cm $\cdot$ s^{-1}). Since many of the numbers used for calculation lack precision, one should be careful in the interpretation of these results. Yet two conclusions appear to be warranted. (*a*) These results are consistent with the idea that porin channels of ~0.6 nm radius are responsible for the transmembrane diffusion of saccharides in mutants as well as in the wild-type cells of *E. coli*. (*b*) The results rule out the model in which most of the channels are closed under physiological conditions; this is important in view of the observation of Schindler and Rosenbusch (1978) that porin channels can be closed by membrane potentials exceeding a certain limit.

Permeability of the Outer Membrane in a Physiological Context

The knowledge of the size of the pores and the magnitude of the permeability coefficients of the outer membrane is useful in considering the biological significance of the barrier properties of this membrane. For many nutrients, *E. coli* has high affinity transport systems with overall K_m values in the neighborhood of 1 μM (Koch, 1971). For such systems, and especially for systems with high V_{max}, the presence of a very large number (10^5 per cell) of porin molecules is absolutely essential; otherwise the diffusion through the outer membrane will quickly become limiting at low external substrate concentrations, and, thus, the "K_m" of the overall system will become much higher than the true K_m of the active transport system of the inner membrane, the *E. coli* cells thus losing all the potential advantages of having high-affinity transport systems.

Another important point is that there is a very large dependence of the penetration rates on solute size. Thus, the outer membrane is fully permeable to a trisaccharide, raffinose, when equilibrium distribution assays are used. Yet, even for lactose, which is much smaller, already the diffusion *rate* is about 60-fold lower than that of arabinose. Thus, in comparison with the rate of uptake necessary for the maximal growth rate of *E. coli*, the diffusion of lactose across the outer membrane becomes limiting, even with the presence of 10^5 porin molecules per cell, at an external lactose concentration of ~200 μM or less. This consideration then explains why the active transport system of lactose has such an exceptionally low affinity among sugar transport systems of *E. coli* (Koch, 1971; Kaback and Hong, 1973). Since the diffusion across the outer membrane becomes limiting at such high external concentrations, presumably there is no sense in producing a high-affinity transport system located in the inner membrane. Another disaccharide, maltose, is transported with a very low K_m by intact cells of *E. coli* (Szmelcman et al., 1976). However, in this case, maltose diffuses through the outer membrane via a specific channel, the λ-receptor protein (Luckey and Nikaido, 1980), and in mutants lacking this protein the diffusion through the porin channels becomes limiting at expected external concentrations, thus raising the "overall K_m" of transport from 1 μM to 100 μM (Szmelcman et al., 1976). We can thus see that porins alone cannot produce an "overall transport K_m" for disaccharides much lower than 10^{-4} M.

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