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# Specificity of the glucose channel formed by protein D1 of *Pseudomonas aeruginosa*

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**Protein D1 from the outer membrane of *Pseudomonas aeruginosa* was purified and reconstituted into proteoliposomes. Many small molecules were shown to diffuse through the D1 channel in the proteoliposomes, and the permeation rates of D-glucose, L-glucose, D-xylose, and L-xylose were much higher than expected for their size. This finding and the permeation rates of various glucose analogs suggest that, although the channel has a specific recognition site for glucose, it functions in a manner very different from that of the glucose carrier of erythrocytes.**

The glucose carrier of animal cells, especially that of human red blood cells, is one of the best studied specific systems involved in facilitated diffusion processes [1–3]. Its binding site appears to interact with the solute molecule via the hydrogen bond formation to the oxygen atoms at C1, C3, and C6 [1,3].

Bacteria, in contrast to animal cells, more frequently use active transport systems to take up nutrients from the external environment. However, Gram-negative bacteria are covered by an additional membrane layer, the outer membrane [4], and the nutrient molecules first traverse this membrane usually via simple diffusion through non-specific, water-filled channels in porins [4], before they are taken up by the active transport system located in the inner, cytoplasmic membrane. In addition to porins, the outer membrane often contains proteins involved in the uptake, via facilitated diffusion, of nutrients that cannot be taken up with sufficient rates through the porin pathway. An example is the phage lambda receptor protein, or LamB protein, of *Escherichia coli*, which produces a specific channel for the diffusion of maltose and maltodextrins [5,6]. In *Pseudomonas aeruginosa*, it has been hypothesized [7] that an outer membrane protein D1 [8,9] may produce a glucose-specific channel, because its synthesis is induced by growth in the presence of glucose [8] and because liposomes reconstituted from purified protein D1 allowed an efflux of radioactive glucose initially trapped in the intravesicular space [7]. However, the specificity of the channel is not yet well-defined, and the radioisotope efflux assay suggested that also sucrose penetrated through this channel [7]. Furthermore, our curiosity on the specificity of this channel was stimulated by recent reports [10,11] that *P. aeruginosa* mutants resistant to a novel β-lactam antibiotic, imipenem (*N*-formimidoylthienamycin [12]), are lacking an outer membrane protein, which had an apparent molecular weight similar to D1. We have confirmed by two-dimensional gel electrophoresis that the protein missing in imipenem-resistant mutant was indeed D1 (Trias and Nikaido, to be published). Since this suggests

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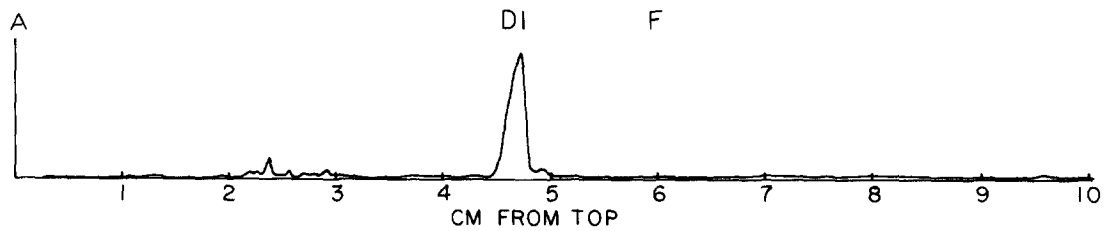


Fig. 1. Densitometer scanning of the slab SDS-polyacrylamide electrophoretogram of purified protein D1. Protein D1 preparation used in the proteoliposome swelling experiments was analyzed by slab SDS-polyacrylamide gel electrophoresis [13], and the Coomassie blue-stained gel was scanned with a Helena Lab scanner. The abscissa shows distance from the top of the separating gel, and the position where the protein F is expected to migrate is marked by the letter F.

that the protein D1 allows also the diffusion of imipenem, whose structure does not resemble that of glucose in any obvious manner, we studied the specificity of the D1 channel by quantitatively measuring the rates of diffusion of various solutes into reconstituted liposomes. The results show that the D1 protein indeed produces a glucose-specific diffusion pathway, but the details of solute recognition mechanism seem to be very different from that of the glucose carrier of the erythrocytes.

Protein D1 was extracted from the outer membrane of *P. aeruginosa* strains PAO1 [8] or 3C [10] with Triton X-100/EDTA, and was purified by ion exchange chromatography on DEAE-Sephacel (Pharmacia) as described by Hancock and Carey [7]. The results presented below were obtained with D1 of strain 3C, but very similar data were obtained also with that of strain PAO1. The final preparation contained only a very small 'shoulder' running just ahead of the major D1 band, possibly corresponding to D2 (Fig. 1). We emphasize especially the absence, in our preparation, of the protein F, which functions as the major nonspecific porin in this species [13,14]. The protein (10 or 20 $\mu$g) was then reconstituted with 6 $\mu$mol of egg phosphatidylcholine (Sigma Type IX-E, treated as specified earlier [15]) and 0.3 $\mu$mol of dicetyl phosphate [15], except that the dried film of the lipid-protein mixture was rehydrated in 0.6 ml of 20 mmolal stachyose/5 mM Tris-HCl (pH 7.5). Solutions of test solutes made in 5 mM Tris-HCl (pH 7.5) were adjusted by using a Wesco model 5500 osmometer so that they were isoosmotic with the 20 mmolal stachyose/5 mM Tris-HCl (pH 7.5). The proteoliposomes made with the D1 protein were then diluted into these solutions, and the rates of influx of various solutes were determined

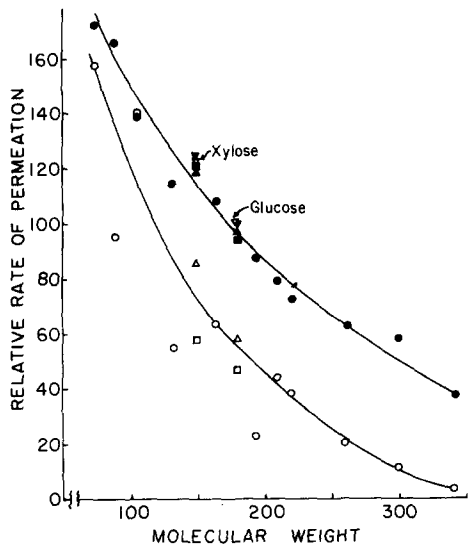


Fig. 2. Rates of diffusion of solutes of different sizes through the protein D1 and porin F channels. The rates were determined by measuring the swelling rates of proteoliposomes reconstituted as described in the text, and were normalized to that of D-glucose (equilibrium mixture of anomers), taken as 100. For D1 protein, liposomes containing 20 mmolal stachyose were used throughout, whereas for F porin we used liposomes, which contained 15% (w/v) Dextran T-40 (Pharmacia) and were isoosmotic with about 42 mmolal stachyose, except for the measurement of imipenem diffusion, which required the use of 20 mmolal stachyose-containing liposomes because of the low solubility of imipenem. Each data point represents an average of 2–4 experiments. Filled symbols represent data obtained for porin F, and empty symbols those for protein D1. The molecular weights and the compounds used are: 75, glycine; 89, L-alanine; 105, L-serine; 132, glycylglycine; 164, 2-deoxy-D-glucose; 194, $\beta$-methyl-D-glucoside; 210, D-glycero-D-glucoheptose; 221, *N*-acetyl-D-glucosamine; 262, 2,3-diacetamido-2,3-dideoxy-D-glucose; 299, imipenem; and 342, sucrose. Three pentoses with $M_r = 150$ were used: D-xylose (▼, ▽), L-arabinose (▲, △), and D-lyxose (■, □). Three hexoses with $M_r = 180$ were: D-glucose (▼, ▽), D-galactose (▲, △), and D-mannose (■, □).

from the initial rates of swelling of the liposomes obtained from the spectrophotometric trace of turbidity [15]. As a control, swelling rates of proteoliposomes containing the nonspecific porin F, prepared as described earlier [13], were also determined. Most chemicals were obtained from Sigma, with the exception of 2,3-diacetamido-2,3-dideoxy-D-glucose (U.S. Biochemicals Corp.) and imipenem (Merck Sharp and Dohme Research Laboratories).

As seen in Fig. 2, the rates of penetration of solutes through the porin F channel were a smooth function of the molecular size, here expressed as molecular weight. Diffusion through the D1 protein was also very roughly dependent on the size of the solute; this observation, as well as the high rates of flux through this protein, suggests that D1 functions as a channel rather than a carrier. In contrast to the diffusion through the porin channel, however, compounds with an identical size often diffused with strikingly different rates through the D1 channel. Especially the permeation rates of D-glucose and D-xylose were much higher than those of other compounds of comparable sizes, suggesting that the channel is indeed specific for these homomorphous [16] sugars.

Table I shows the effect of substitutions at various positions of the glucose molecule. Interestingly, the 1-hydroxyl group does not seem essential because (i) α-D-glucose and β-D-glucose diffuse at similar rates, and (ii) 1-deoxy-D-glucose (1,5-anhydro-D-sorbitol) permeates through the D1 channel nearly as rapidly as D-glucose. However, bulky substituents at C-1 do produce decrease in permeation as seen with α- and β-methyl-D-glucosides. The 2-OH group seems very important, because the rate of diffusion was much slower for 2-deoxy-D-glucose, and for the 2-epimer of D-glu-

TABLE I

RELATIVE RATES OF PERMEATION OF GLUCOSE AND ITS ANALOGS THROUGH THE PORIN F CHANNEL AND PROTEIN D1 CHANNEL

| Compound [a] | $M_r$ | Comment | Relative permeation rate [b] | |
|---|---|---|---|---|
| | | | porin F | protein D1 |
| D-Glucose | 180 | | 100 | 100 |
| 1-Deoxy-D-glucose | 164 | no OH at C1 | 107 | 82 |
| α-D-Glucose | 180 | axial OH at C1 | n.d. [c] | 98 |
| β-D-Glucose | 180 | equatorial OH at C1 | n.d. | 103 |
| Methyl-α-D-glucoside | 194 | axial substitution at C1 | 95 | 19 |
| Methyl-β-D-glucoside | 194 | equatorial substitution at C1 | 92 | 23 |
| 2-Deoxy-D-glucose | 164 | no OH at C2 | 98 | 64 |
| D-Mannose | 180 | axial OH at C2 | 101 | 47 |
| *N*-Acetyl-D-glucosamine | 221 | equatorial substitution at C2 | 71 | 38 |
| D-Allose | 180 | axial OH at C3 | 98 | 88 |
| 2,3-Diacetamido-2,3-dideoxy-D-glucose | 262 | equatorial substitutions at C2, C3 | 62 | 20 |
| D-Galactose | 180 | axial OH at C4 | 100 | 58 |
| 6-Deoxy-D-glucose | 164 | no OH at C6 | n.d. [c] | 96 |
| D-Xylose | 150 | no C6 | 126 | 123 |
| D-Glycero-D-glucoheptose | 210 | C7 attached to C6 | 78 | 44 |
| L-Glucose | 180 | enantiomer of D-glucose | 100 | 89 |
| *myo*-Inositol | 196 | ring O replaced by CHOH | n.d. | 119 |
| D-Glucitol | 182 | open chain alcohol | 82 | 55 |

[a] Equilibrium mixtures of anomers were used for all reducing sugars, except for α- and β-D-glucose, which were used within 5 min after dissolution into buffer.

[b] The results were normalized to the penetration rate of D-glucose, which was taken as 100. Each figure represents an average of at least two experiments.

[c] Not determined.

cose, D-mannose. Significant decrease in permeability was also seen when the 4-OH group was moved to the axial position as in D-galactose. In contrast, the removal of the 6-OH group or even the $CH_2OH$ group at C-6 did not produce decreases in permeability. Similar observations could be made for pentoses: D-xylose, which is homomorphous with D-glucose showed the highest permeability, and its 2-epimer, D-lyxose, the lowest permeability among pentoses (Fig. 2 and data not shown).

These results are different from those on the binding specificity of the red blood cell glucose carrier, which shows a much lower affinity to D-xylose [3]. Furthermore, in contrast to the red blood cell glucose carrier which does not transport L-glucose at all [1–3], the D1 channel allows the diffusion of L-glucose at a rate nearly identical to that of D-glucose (Table I). Similarly, L-xylose is transported at almost the same rate as D-xylose (data not shown). Perhaps these surprising observations can be understood if the binding site within the D1 protein recognized only some of the equatorial oxygens at C-2 through C-4 of D-glucose in the C1 chair conformation, because corresponding oxygen atoms of L-glucose (in the most stable 1C chair conformation) may occupy nearly superimposable positions, as shown by the comparison of CPK space-filling models of these compounds.

The D1 protein also seems to allow the passage of smaller compounds with little obvious structural resemblance to glucose, such as amino acids. This could be due to the channel partially functioning as a small, non-specific pore; similar observations have been made also with proteoliposome swelling studies with the LamB protein [5]. However, even the passage of these compounds may involve their binding, albeit with low affinity, to the binding site in the D1 protein. The higher permeation rate of L-serine, with its hydroxyl group, than the smaller L-alanine (Fig. 2) seems to favor this hypothesis. Finally, imipenem penetrated the D1 channel with a rate approximately expected from its molecular weight, and at present we do not know whether the imipenem sensitivity of the wild type *P. aeruginosa* cells can be explained by its permeation through the D1 channel. We must emphasize here that our swelling assays were carried out with a fixed, rather high (20 mM) concentration of the test solutes, and detailed kinetic analysis at varying solute concentrations is needed for a better understanding of the molecular mechanism of permeation as well as the physiological role of this channel.

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