Vol. 93, No. 1, 1980 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
March 13, 1980 Pages 166-171

DIFFUSION OF SOLUTES THROUGH CHANNELS PRODUCED BY PHAGE LAMBDA RECEPTOR PROTEIN OF ESCHERICHIA COLI: INHIBITION BY HIGHER OLIGOSACCHARIDES OF MALTOSE SERIES

Mary Luckey and Hiroshi Nikaido

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

Received January 28,1980

SUMMARY: Phage lambda receptor protein produces transmembrane diffusion channel, with some specificity toward oligosaccharides of the maltose series (Luckey and Nikaido, Proc. Nat. Acad. Sci. U. S. A., in press). When the purified protein was inserted into the phospholipid bilayers of liposomes, and the rates of influx of glucose and maltose through these channels were followed in the presence of maltopentaose, -hexaose, and -heptaose, low concentrations of the large oligosaccharides were found to inhibit strongly the flux of smaller sugars. These results are consistent with the notion that a configurationally specific binding site(s) exists within the channel.

The outer membrane of Escherichia coli contains several proteins which are involved in the facilitated diffusion of specific substrates and also function as receptors for bacteriophages and colicins (reviewed in ref. 1). One of these, the lambda receptor protein, is induced by growth on maltose and coded for by the lamB gene of the maltose regulon (2). In intact cells this protein allows the rapid diffusion of maltodextrins and maltose across the outer membrane (3), but other solutes penetrate through this channel extremely slowly or not at all (4). In contrast, in reconstituted membranes lamB protein seemed to produce a non-specific pore that allowed the diffusion of various small ions and molecules (5,6).

Recently we have shown that the lamB protein can discriminate among sugars of a similar size in an in vitro flux assay in liposomes (7). For example, the rate of maltose influx was forty times faster than that for sucrose in lamB protein-containing liposomes, and the discrimination became even more pronounced for larger oligosaccharides. Somewhat similar results were obtained more recently by T. Nakae and J. Ishii (personal communication). These results suggest that the lamB protein forms a transmembrane channel that allows the almost unhindered diffusion of very small solutes, but at the same time specif-

0006-291X/80/050166-06$01.00/0

ically recognizes the configuration of larger solutes perhaps via binding sites of low affinity. In the present work, we report that indeed low concentrations (1-4 mM) of higher oligosaccharides of the maltose series inhibit, up to 90%, the flux of glucose (45-90 mM) through this channel.

MATERIALS AND METHODS

Phospholipids were extracted as described (7) from E. coli K12, strain JC6724, grown in L broth at 42°C. The lamB protein was purified from outer membranes of porin-deficient E. coli mutant T19 (7). Maltoheptaose was prepared as described previously (7). Maltohexaose and maltopentaose were gifts of Dr. Taiji Nakae. Maltotriose and stachyose were obtained from Sigma, Dextran T-20 and Ficoll 400 from Pharmacia.

Liposomes were prepared as described for the rate permeation assay (7) with the following modifications. One to four micromoles of phospholipid were dried at the bottom of a large test tube (diameter: 20 mm), and resuspended in 0.2 ml of an aqueous solution of the purified lamB protein (0.1 to 1.0 μg) by sonication. The suspension was dried at 50°C with rapid shaking under evacuation with an oil pump, and kept in an evacuated dessicator overnight. Dextran T-20 (17%, w/v) in 5 mM Tris-Cl, pH 7.5 (0.3-0.9 ml) was added, and the tube was slowly rotated to wet the lipid film. Then it was incubated for 1 hr at 30°C without agitation, hand-shaken to produce a homogeneous suspension, and incubated at least for another hour at 30°C.

Rates of solute influx into liposomes containing the lamB protein were measured by the swelling assay as described (7). Depending on the batch of liposomes, there were some variations in the concentration of the sugar solutions isotonic to these liposomes; the isotonic concentration (usually 45-90 mM) was therefore determined by using an impermeable sugar, stachyose. For inhibition experiments, liposomes were diluted into appropriate isotonic solutions containing both substrate and inhibitor. In experiments where inhibitor concentration was varied by only a few mM, the isotonicity was maintained by small adjustment in substrate concentration. The diluent always contained 5 mM Tris-Cl, pH 7.5. Each batch of liposomes was tested to verify that solute permeation into control liposomes (without lamB protein) resulted in optical density change of less than 0.005 OD unit per min.

RESULTS AND DISCUSSION

Higher oligosaccharides of maltose series diffuse through the lamB protein channel rather slowly. For example, maltoheptaose diffuses at a rate less than 1% of the rate of diffusion of glucose (7). Thus when both glucose and larger oligosaccharides were simultaneously added to liposomes whose bilayers contained the purified lamB protein, the rates of influx of larger oligosaccharides were negligible, and we were essentially observing the rate of influx of glucose. We added low (less than 4 mM) concentrations of higher oligosaccharides in comparison with high concentrations (45-90 mM) of glucose, and this further minimized the possibility that the influx of higher oligosaccharides contributed significantly to the overall flux of solutes into liposomes.

When the rates of influx of glucose through these channels were measured by the swelling rates of liposomes (see Methods), we found that the normally rapid influx of glucose through the lamB protein channel was greatly reduced in the presence of low concentrations of higher oligosaccharides of maltose series. Thus with one preparation of liposomes, maltotriose, -pentaose, -hexaose, and -heptaose, each at 4 mM, inhibited the glucose flux by 42, 69, 74, and 89%, respectively. In contrast, stachyose, raffinose, and sucrose at 4 mM did not show any significant inhibition (less than 8%). The observation that low concentrations of higher oligosaccharides of maltose series specifically blocked the influx of glucose supports our hypothesis that the lamB protein channel has site(s) with an affinity toward maltose and its higher homologs.

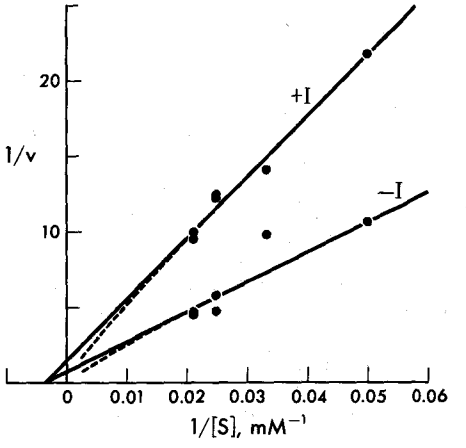

Fig. 1. Effect of maltoheptaose on glucose permeation into lamB protein-containing liposomes.

Liposomes were prepared with 4 μmol of phospholipids and 0.95 μg of purified lamB protein as described in Methods. They were diluted into glucose solutions containing 0 or 1 mM maltoheptaose. Stachyose was added to glucose solutions so that the total sugar concentration was 47 mM. Refractive index of all solutions was made identical by adding appropriate amounts of Ficoll 400, and Tris-Cl buffer was also added (see Methods). The swelling rates of liposomes, v, are expressed as d(1/optical density)/dt, where the unit of t is min.

Broken lines represent "theoretical curves" calculated by assuming that the observed flux is the sum of a "specific" component (K_M = 74 mM) and a non-specific component (with an infinitely high "K_M"). In the absence of the inhibitor, these components were assumed to contribute 36 and 64% of the total flux, respectively, at the glucose concentration of 20 mM. We further assumed that, in the presence of the inhibitor, the specific pathway was inhibited in a purely competitive manner (Ki = 0.8 mM), and that the binding of the inhibitor according to this value of Ki blocked 1/(1 + Ki) or 56% of the non-specific component. When the fraction assumed to go through the non-specific component at 20 mM glucose and 0 mM maltoheptaose was varied between 11 and 85%, the K_M expected for glucose changed over a wide range, but the model could still be made to fit the data with only small (< ±0.6) errors in 1/v between 1/(S) values of 0.02 and 0.05, and the Ki values obtained were always between 0.8 and 0.83 mM.

Fig. 1 shows the kinetics of inhibition of glucose flux by 1 mM maltoheptaose, at various concentrations of glucose. This is apparently an inhibition of "non-competitive" type, and "Ki" of 1.1 mM can be calculated by standard methods (8). When the concentrations of the inhibitors were varied in the presence of a fixed concentration of glucose ("Dixon plots"), straight lines were obtained as shown in Fig. 2. Here the "Ki" values for maltoheptaose, -hexaose, and -triose, calculated by assuming non-competitive kinetics (8), were 0.8, 1.0, and 3.2 mM, respectively. The reasonable agreement between the Ki values for maltoheptaose, obtained by two different methods, further confirms the formally "non-competitive" nature of the inhibition. Finally, we could show that maltose flux was similarly inhibited by maltoheptaose, with a "Ki" of 1.7 mM (Fig. 3).

These results indicate that higher oligosaccharides of maltose series do indeed bind to site(s) within the lamB protein channel with a reasonable affinity. However, the affinity would still be too low for detection by some

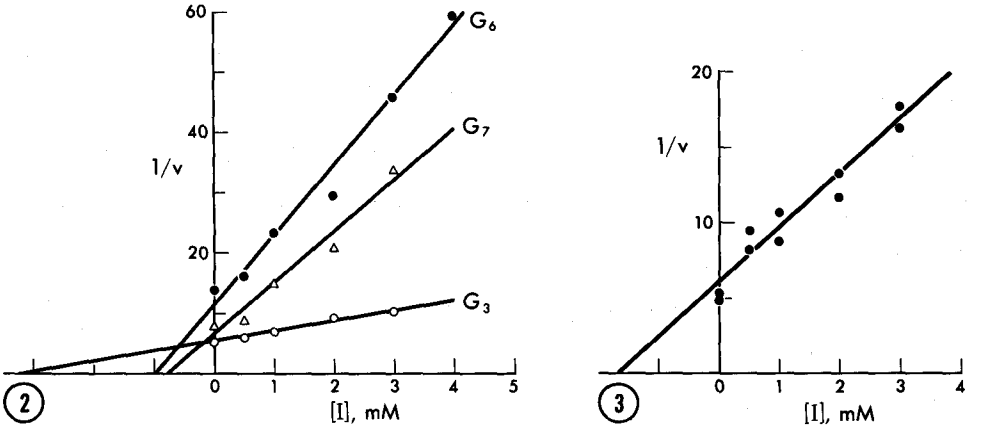

Fig. 2. Effect of various oligosaccharides of maltose series on the rate of glucose influx into lamB protein-containing liposomes.
Liposomes were prepared from 2 μmol of phospholipids and 0.19 μg lamB protein. Each inhibitor shown was tested with a different batch of liposomes made on different days, and the isotonic concentrations for the liposomes were 60, 90, and 65 mM, respectively, for experiments with maltotriose (G_3), maltohexaose (G_6), and maltoheptaose (G_7). For other details see Methods.

Fig. 3. Inhibition of maltose influx into lamB protein-containing liposomes by maltoheptaose. Liposomes were prepared from 2 μmol phospholipids and 0.57 μg lamB protein, and were diluted into 60 mM maltose containing various concentrations of maltoheptaose. For details see Methods.

of the conventional binding assays, and this explains the previous failure to detect the binding of maltose by this protein (9).

How do we interpret the "non-competitive" kinetics of inhibition? We suspect that this is an artefact, because of the following. First, a true non-competitive inhibitor should exhibit identical Ki regardless of the nature of the substrate, but in our case Ki of maltoheptaose was different depending on whether glucose or maltose was used as the substrate. Furthermore, if glucose (or maltose) and higher oligosaccharides bind to the same recognition site, competitive rather than non-competitive kinetics is expected. One explanation of these observations is based on the previous observation that the lamB channel allows the non-specific penetration of small solutes such as monovalent cations (5), Tris, or serine (7). Since glucose is rather small, we can imagine that some of the glucose molecules diffuse through the channel non-specifically, i.e. without ever binding to the specific site. In fact broken lines in Fig. 1 illustrate that it is perfectly possible to get an apparently "non-competitive" kinetics in the presence of a competitive inhibition of the "specific" component of the flux, if a non-binding pathway of penetration is assumed to coexist with the specific pathway. (Unfortunately we cannot test the regions where the broken lines deviate significantly from the solid lines, because the viscosity of the isotonic dextran solutions would become too high). These considerations suggest that the "Ki" values obtained from these experiments probably correspond to upper limit estimates.

After this work has been completed, we leared that T. Ferenci and coworkers (personal communication) found that a fluorescent derivative of amylopectin bound to the surface of E. coli cells containing lamB protein, and that the binding could be inhibited by oligosaccharides of the maltose series. The Kd value for maltotetraose, deduced from these experiments, was 0.3 mM, and thus has the same order of magnitude as our Ki values. Our work thus is not only consistent with these results but extend them in showing that the binding occurs inside the channel, not at functionally inert sites on the external surface of the protein.

ACKNOWLEDGEMENT

We thank Drs. T. Ferenci and T. Nakae for making their results available to us before publication. This work was supported by a U.S. Public Health Service grant AI-09644 and an American Cancer Society grant BC-20. M. Luckey is an American Cancer Society Postdoctoral Fellow (PF-1540).

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