REGULATION OF SUGAR TRANSPORT IN ISOLATED BACTERIAL MEMBRANE PREPARATIONS FROM ESCHERICHIA COLI

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Abstract.—The experiments presented in this paper demonstrate that glucose transport via the phosphoenolpyruvate-phosphotransferase system as studied in isolated *E. coli* membrane preparations is subject to regulation by sugar-P's. Glucose transport in these preparations is inhibited by glucose-6-P, glucose-1-P, and, to a lesser extent, by other P-esters. There appear to be two inhibitory sites, one specific for glucose-1-P and related sugar-1-P's and the other specific for glucose-6-P and related sugar-6-P's. The inhibition by these sugar-P's is noncompetitive, and the inhibitory sites are separate, subject to independent control, and apparently are accessible from outside the membrane. Furthermore, glucose-6-P is able to reverse partially inhibition of glucose transport by glucose-1-P and vice versa. Preliminary evidence is presented indicating that glucose-1-P may play a central role in the regulation of sugar transport in general, by virtue of its ability to inhibit selectively the transport of sugars other than glucose.

Previous work from this laboratory demonstrated that isolated bacterial membrane preparations, in the absence of soluble proteins, catalyzed the concentrative uptake of proline,[1, 2] the facilitated diffusion of glycine and its ultimate conversion to phosphatidylethanolamine,[3, 4] and most recently, the translocation and accumulation of sugars as their phosphorylated derivatives.[5] The last study also demonstrated that the P-enolpyruvate-P-transferase system[6] was specifically responsible for the uptake of glucose and related sugars by isolated membrane preparations. Recent experimental results carried out in collaboration with Drs. Saul Roseman and Werner Kundig at Johns Hopkins University suggest that Enzyme II, the membrane-bound component of the P-transferase system, may be capable of undergoing conformational changes within the membrane matrix.[7] In addition, it has been demonstrated that the transport (vectorial phosphorylation) properties of the membrane can be functionally separated from the barrier properties of the membrane by a variety of techniques which apparently alter the phospholipids of the membrane without affecting the activity of the phosphotransferase system.[7, 8]

During these experiments, it became apparent that membrane preparations from *E. coli*, *Salmonella typhimurium*, or *B. subtilis* concentrated sugar-P to different levels depending on the growth and/or lysis conditions of the parent cells. To some extent, the differences resulted from a temperature-induced leakage of sugar-P from the intramembranal pool which also varied with growth conditions. However, even under conditions where no leakage was demonstrable, there were still considerable differences in the absolute level of sugar-P that was accumulated by these membrane preparations despite many morphologic and biochemical similarities. In addition, it was found with these and other membrane prepara-

tions that the ability of the membranes to carry out P-enolpyruvate-dependent phosphorylation of glucose or α-methylglucoside was inversely related to the ability of the membrane preparation to retain the phosphorylated sugar (i.e., transport).[8] Finally, the intramembranal concentration of α-methylglucoside-P in membrane preparations incubated under "leaky" conditions (i.e., 46°) and in the presence of high concentrations of α-methylglucoside was found to oscillate with respect to time. Both the period and the amplitude of the oscillations varied with the rate of leakage of α-methylglucoside-P from the intramembranal pool and the intramembranal concentration of α-methylglucoside-P.

These experiments led to the hypothesis that the P-enolpyruvate-P-transferase system—the glucose uptake mechanism—is subject to regulation by at least the immediate product of the transport reaction, glucose-6-P (or α-methylglucoside-P). The experiments to be presented in this paper demonstrate that the glucose uptake mechanism in isolated membrane preparations from *E. coli* is subject to rigorous control. Glucose transport is inhibited by glucose-6-P, glucose-1-P, and by a variety of related hexose-P's. The inhibitory sites for the 6-P and 1-P-esters are separate, distinct, and accessible from the outside of the membrane, and the inhibitory effects of glucose-6-P and glucose-1-P on glucose uptake by the membranes vary independently. Moreover, inhibition of glucose transport by 1-P-esters is antagonized by glucose-6-P, and vice versa. Finally, glucose-1-P specifically inhibits the uptake of fructose and galactose, indicating that it may be involved in the regulation of uptake and metabolism of sugars in general.

Methods.—Whole cells: *E. coli* ML 308-225 was grown as described previously,[5] using 0.5% glucose, glycerol, or succinate, as indicated. Cells were harvested at approximately the end of the log phase of growth.

Preparation of membranes: Membranes were prepared by methods described previously,[5] with the exception that pancreatic RNase (Worthington, crystallized twice) was used in addition to DNase and at the same concentration.

Uptake studies: All the uptake studies to be described were carried out as described previously,[5] with the exception that 0.3 *M* LiCl (rather than 0.25 *M* potassium phosphate, pH 6.6) was used in the reaction mixtures, and 0.5 *M* LiCl during dilution, filtration, and washing.[9]

Results and Discussion.—Effect of glucose-6-P on sugar transport: The results shown in Figure 1 demonstrate that the initial rate of α-methylglucoside uptake by three different membrane preparations from *E. coli* ML 308-225 was inhibited by increasing concentrations of glucose-6-P. Furthermore, it can be seen that the $I_{0.5}$ for glucose-6-P (i.e., the concentration of glucose-6-P required for half-maximal inhibition) differed for each membrane preparation. Thus, for "glucose" membranes, the $I_{0.5}$ was approximately 5×10^{-4} *M;* for "glycerol" membranes, approximately 1.5×10^{-3} *M;* and for "succinate" membranes, approximately 3.0×10^{-3} *M*. It is *not* meant to be implied that the carbon source used for the growth of the parent cells was the critical variable responsible for the differences in uptake or sensitivity to glucose-6-P inhibition found for "glucose," "glycerol," or "succinate" membranes, but rather that the carbon source is the only known variable for these particular membrane preparations. Indeed, recent experiments have demonstrated that membranes prepared from

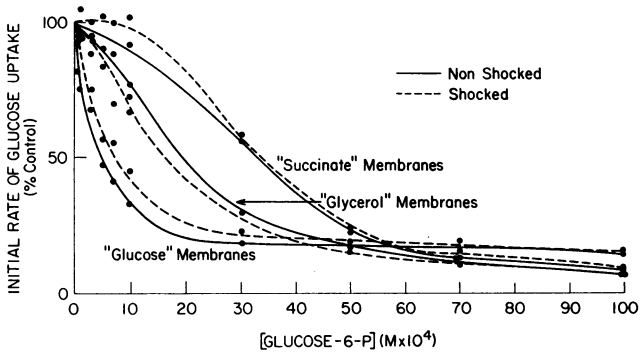

FIG. 1.—Inhibition of α-methylglucoside uptake by glucose-6-P. Membrane samples were prepared from *E. coli* ML 308-225 grown as described,[5] using glucose, glycerol or succinate as sole carbon sources. They were assayed for α-methylglucoside uptake as described in *Methods*, with the exception that the 15-min incubation prior to the addition of α-methylglucoside-^{14}C was omitted. Each reaction mixture contained (in a total of 0.1 ml) 50 mM potassium phosphate buffer, pH 6.6, 10 mM $MgSO_4$, 300 mM LiCl, 100 mM P-enolpyruvate, glucose-6-P in the concentrations given, 3.64×10^{-5} *M* α-methylglucoside-^{14}C (73.4 mCi/mmole), and 0.48, 0.61, or 0.41 mg of membrane protein for "glucose," "glycerol," or "succinate" membranes, respectively. The samples were incubated at 46° for 5 min, the incubations terminated, and subsequently handled as described in *Methods*. The "shocked" samples were treated in an identical manner, except that the membranes (in 0.1 *M* potassium phosphate buffer, pH 6.6) were added directly to an equal volume of a solution of glucose-6-P, at twice the concentrations given, before the other components of the reaction mixtures were added. The data are plotted as percentages of control samples which contained no glucose-6-P: "glucose" membranes, 0.61 and 0.54 nmole α-methylglucoside taken up/mg membrane protein/5 min for "nonshocked" and "shocked" samples, respectively; "glycerol" membranes, 1.27 and 1.15, respectively; and "succinate" membranes, 1.96 and 1.63, respectively.

cells in different phases of growth also show differences in uptake and sensitivity to glucose-6-P, indicating that there are other factors which could have been responsible for the differences in the membrane preparations. Thus, it is for convenience only that here and throughout the remainder of this paper the membrane preparations are referred to as "glucose," "glycerol," or "succinate" membranes. In any case, it is noteworthy that these half-maximal inhibitory concentrations of glucose-6-P are of the same order of magnitude as the concentrations of glucose-6-P or α-methylglucoside-P that are ultimately achieved by each of these membrane preparations during glucose or α-methyglucoside transport. Under optimal conditions, "glucose" membranes transported glucose or α-methylglucoside to an intramembranal glucose-6-P or α-methylglucoside-P concentration of approximately 1.1×10^{-3} *M*; "glycerol" membranes to approximately 3×10^{-3} *M*; and "succinate" membranes to approximately 6×10^{-3} *M*. This suggests that the different $I_{0.5}$ values for glucose-6-P in the three membrane preparations may be, therefore, of physiological significance, since the $I_{0.5}$'s appear to reflect the maximum concentrating ability of each membrane preparation with regard to sugar-P.

Figure 1 also shows that the inhibitory site for glucose-6-P is accessible from the outside of the membrane, as has already been suggested for the catalytic site.[7, 8] As shown by the broken lines in Figure 1, the $I_{0.5}$ for glucose-6-P in each

membrane preparation was the same whether the sugar-P was simply added to the reaction mixture (*solid lines*) or shocked into the membranes under conditions in which it can be shown that P-enolpyruvate is made accessible to the interior of the vesicles.[10]

Effect of glucose-1-P on sugar transport: The data presented in Figure 2 show that the relative order of sensitivity of glucose uptake to inhibition by glucose-1-P when studied in the same three membrane preparations is almost the reverse of that found for glucose-6-P. The initial rate of glucose uptake by "glucose" membranes was inhibited by only about 15 per cent over the range of glucose-1-P concentrations shown, whereas the rate of glucose uptake by "glycerol" or "succinate" membranes was markedly inhibited by glucose-1-P at concentrations considerably lower than those found for glucose-6-P. As shown, the $I_{0.5}$ for glucose-1-P with "glycerol" membranes was approximately

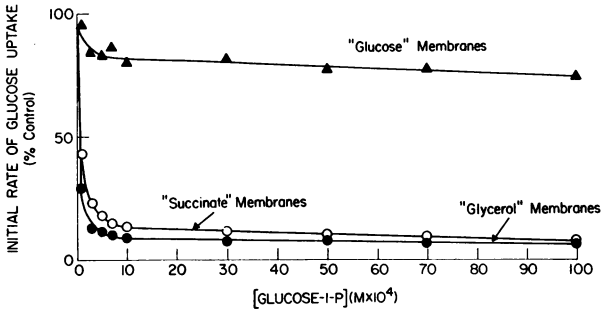

FIG. 2.—Inhibition of glucose uptake by glucose-1-P. Membrane samples were prepared from *E. coli* ML 308-225 grown as described with glucose, glycerol, or succinate as carbon sources. They were assayed for glucose uptake by incubation for 5 min at 46° in the presence of glucose-1-P in the concentrations shown and in the presence of 3.64×10^{-5} *M* ^{14}C-glucose (161 mCi/mmole). The data are plotted as percentages of control samples which contained no glucose-1-P· "glucose," "glycerol," and "succinate" membranes, 0.95, 2.1, and 5.3 nmoles glucose taken up/mg membrane protein/5 min, respectively.

5×10^{-5} *M;* and for "succinate" membranes, approximately 8×10^{-5} *M*. It is noteworthy that these $I_{0.5}$ values are within the same range as the intracellular glucose-1-P concentration in *E. coli*, which Lowry, Ward, and Glaser estimate to be approximately 2×10^{-5} *M*.[11] The inhibition of glucose transport in "glycerol" or "succinate" membranes appears to be a simple hyperbolic function of the glucose-1-P concentration, as shown here, but is a more complex, sigmoidal function of glucose-6-P concentration, as shown in Figure 1. As mentioned previously for glucose-6-P inhibition in these same three membrane preparations, it should be stressed that the differences in sensitivity to glucose-1-P are not necessarily related to the carbon source used for the growth of the parent cells.

There was little, if any, uptake or hydrolysis of glucose-6-P or glucose-1-P that could be detected when the appropriately labeled sugar-P was incubated with the membrane preparations under the same conditions as used in these experiments.

Studies on the initial rates of α-methylglucoside uptake as a function of increasing sugar concentration in the presence and absence of glucose-6-P or glucose-1-P revealed noncompetitive modes of inhibition for both sugar-P's in the three membrane preparations. It is also interesting that the K_m for α-methylglucoside in the three membrane preparations in the absence of glucose-6-P or glucose-1-P was essentially the same (approximately 2×10^{-6} M), whereas the V_{max} values differed considerably (approximately 0.65 nmole of α-methylglucoside taken up/mg membrane protein/5 minutes with "glucose" membranes, approximately 1.88 with "glycerol" membranes; and approximately 5.5 with "succinate" membranes). Detailed results of these experiments will be published elsewhere.[8]

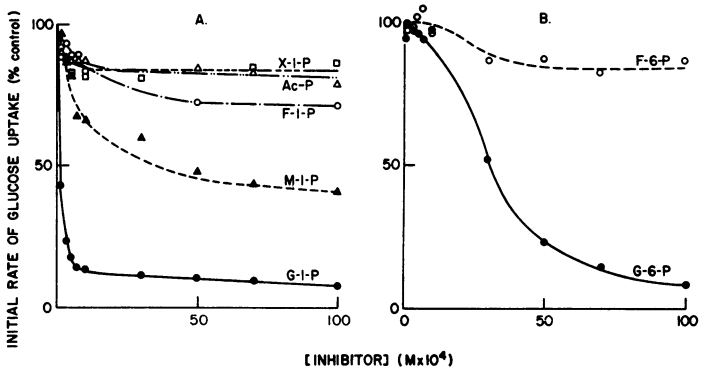

FIG. 3.—The effect of various compounds on glucose uptake by isolated membrane preparations. Membrane samples were prepared from *E. coli* ML 308-225 grown with succinate as sole carbon source. Assays for glucose uptake were for 5 min at 46° in the presence of one of the compounds shown. The concentrations of the inhibitors used are also shown in the figure. Assays were carried out as described, and the glucose-^{14}C (161 mCi/mmole) used was at a concentration of 3.64×10^{-5} M. The data are plotted as percentages of control samples which contained none of the compounds tested. The control value, average of 20 determinations, was 4.8 nmoles glucose taken up/mg membrane protein/5 min (range, 3.94 to 5.85). Inhibition by 1-P-esters is shown in (*A*), 6-P-esters in (*B*).

Specificity of inhibition: The data presented in Figure 3*A* and *B* show the effects of a variety of compounds on the initial rate of glucose uptake by membranes prepared from succinate-grown cells. It can be seen that the inhibitors fall into two classes—those which mimicked the inhibition by glucose-1-P (*A*) and those which mimicked the inhibition by glucose-6-P (*B*). In the former category (see Fig. 3*A*), in addition to glucose-1-P, mannose-1-P, fructose-1-P, acetyl-P, and xylose-1-P, each inhibited glucose uptake with decreasing effectiveness. In the latter category (see Fig. 3*B*), only fructose-6-P was an effective inhibitor of glucose uptake, although it was a much less potent inhibitor than glucose-6-P. A number of other compounds were completely ineffective as inhibitors of glucose transport. Included in this final group were α-glycerol-P, dihydroxyacetone-P, 3-P-glycerate, 1,2-P_2-glycerate, 2-P-glycerate, glucose-1,6-P_2, 6-P-gluconate, galactose-1-P, galactose-6-P, fructose-1,6-P_2, mannose-6-P,

carbamyl-P, ATP, 3',5'-AMP, and acetylcoenzyme A. Studies to be published elsewhere on the ability of each of these compounds to compete with either glucose-6-P or glucose-1-P also indicate that there are only two classes of inhibitor sites, one for 1-P and for one 6-P-esters.

The data presented in Figure 4 demonstrate that inhibition of glucose transport by glucose-1-P is antagonized by glucose-6-P and vice versa. The experiment presented in Figure 4 shows the initial rate of glucose uptake in "succinate" membranes as a function of increasing concentrations of glucose-6-P in the presence of two concentrations (half-maximal and maximal inhibitory concentrations) of glucose-1-P. It can be seen that the inhibition by glucose-1-P at both concentrations was partially overcome by low concentrations of glucose-6-P in the reaction mixtures. Furthermore, glucose-1-P, especially at 10^{-4} M, partially abolished the inhibitory effects of high concentrations of glucose-6-P.

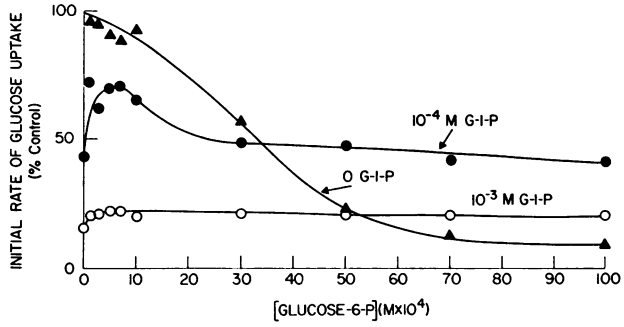

FIG. 4.—Effect of increasing concentrations of glucose-6-P on glucose transport in the presence of glucose-1-P. Membrane samples prepared from *E. coli* ML 308-225 grown with succinate as a carbon source were assayed for glucose uptake for 5 min at 46° in the presence or absence of glucose-6-P or glucose-1-P as shown. The assays were carried out with ^{14}C-glucose (161 mCi/mmole) at 3.64×10^{-5} M. The data are plotted as a percentage of a control sample which contained no glucose-6-P or glucose-1-P (5.3 nmoles glucose taken up/mg membrane protein/5 min).

Thus, the inhibition exerted by glucose-6-P and glucose-1-P together was always less than would have been expected from the additive effects of the two when used independently. The mechanism by which these two inhibitors antagonize each other is obviously complex but may have resulted from the close physical proximity of the inhibitory sites, resulting in distortion of one site when the other was occupied by its ligand. Such a mechanism has been postulated by Stadtman *et al.*[12] for the cumulative inhibition of glutamine synthetase. Glucose-6-P inhibition in the presence of inhibitory concentrations of mannose-1-P produced the same effect.[8]

Thus far, the data are consistent with the hypothesis that the glucose transport mechanism in isolated membrane preparations is subject to product inhibition by glucose-6-P and to feedback inhibition by glucose-1-P. Furthermore, inhibition by these sugar-P's is noncompetitive, and the inhibitory sites are distinct, possibly close physically, and under independent control.

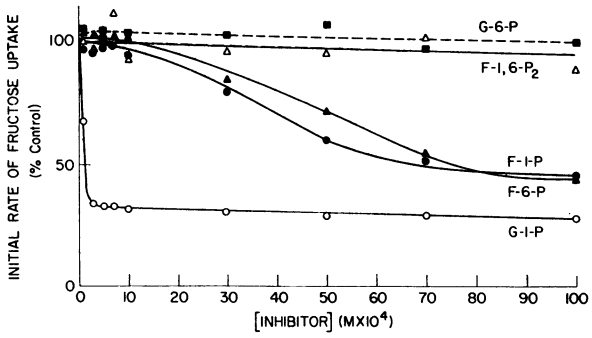

FIG. 5.—Inhibition of fructose uptake by various sugar-P's. Membrane samples prepared from *E. coli* ML 308-225 grown with succinate as a carbon source were assayed for fructose uptake for 5 min at 46° in the presence of a given sugar-P in the concentrations shown. The assays were carried out with ^{14}C-fructose (177 mCi/mmole) at 3.64×10^{-5} *M*. The data are plotted as percentages of control samples containing no sugar-P; the average value of the controls (five determinations) was 1.74 nmoles fructose taken up/mg membrane protein/5 min.

The preliminary experiments to be presented in the remainder of this communication suggest that glucose-1-P may not only be involved in the control of glucose uptake and metabolism, but may also play an important role in the regulation of the transport, and thus the metabolism, of other sugars, as well. The experiment presented in Figure 5 shows the effects of various sugar-P's on fructose transport by "succinate" membranes. Fructose transport was selected, in this instance, because it had already been shown that the membranes had a

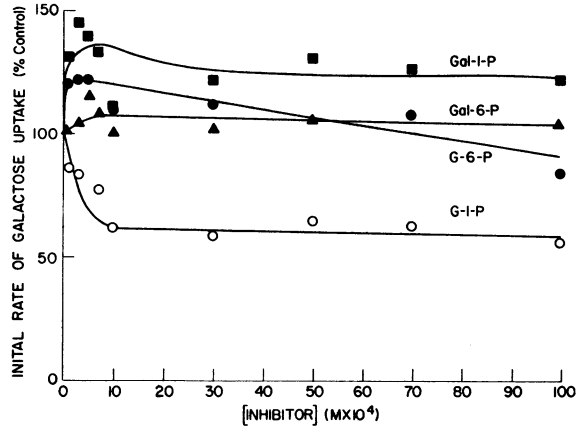

FIG. 6.—Inhibition of galactose uptake by various sugar-P's. Membrane samples prepared from *E. coli* $K_2$1t grown as described previously[5] were assayed for galactose uptake for 5 min at 46° in the presence of a given sugar-P in the concentrations shown. The assays were carried out as described in *Methods*, using galactose-^{14}C (35.4 mCi/mmole) at 3.64×10^{-5} *M*. The data are plotted as percentages of control samples containing no sugar-P (average value for four determinations, 0.42 nmole galactose taken up/mg membrane protein/5 min).

constitutive transport system for this sugar.[5] It can be seen that neither glucose-6-P nor fructose-1,6-P_2 inhibited fructose transport to any significant extent over the concentration range shown. Fructose-6-P and fructose-1-P both inhibited fructose uptake in a sigmoidal fashion with $I_{0.5}$'s of about 4.5×10^{-3} M. Finally, as shown, glucose-1-P exerted a marked inhibitory influence on fructose uptake with an $I_{0.5}$ of about 10^{-4} M.

Figure 6 shows the results of a study in which galactose uptake by membranes prepared from *E. coli* $K_2$1t[5] was studied in the presence of increasing concentrations of sugar-P's. *E. coli* $K_2$1t was selected because membranes prepared from *E. coli* ML 308-225 do not have any constitutive activity toward galactose. It can be seen that only glucose-1-P produced any significant inhibition of galactose transport—glucose-6-P, galactose-6-P, and galactose-1-P were all ineffective. It is noteworthy that despite the observation that glucose-1-P produced only about 40 per cent inhibition of galactose uptake in this experiment, in the same membrane preparation, glucose transport was *not* inhibited by glucose-1-P over this concentration range. These two experiments, especially when considered in conjunction with the independent experiments of Ward and Glaser,[13] provide a strong preliminary indication that sugar-P's, glucose-1-P in particular, may be central metabolites in the regulation of carbohydrate transport and utilization, in general. It is tempting, in this regard, to postulate that glucose-1-P may be involved in certain aspects of diauxie,[14] especially since there is convincing evidence available[15] that the entrance of sugar into the cell plays an important role in this phenomenon.

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[8] Kaback, H. R., in *Current Topics in Membranes and Trnsport*, ed. A. Kleinzeller and F. Bronner (New York: Academic Press), in preparation.
[9] Of a number of salts and other osmotic stabilizers, LiCl is most effective in preventing the leakage of sugar-P from the intramembranal pool (see ref. 8).
[10] As demonstrated previously,[5] very high P-enolpyruvate concentrations (0.1 M) were required for uptake of sugars by membrane preparations, and even at these concentrations, uptake was not maximal. However, it has recently been shown that when the membranes are subjected to mild osmotic shock in the presence of P-enolpyruvate (see legend for Fig. 1), the initial rate of uptake of α-methylglucoside as a function of increasing P-enolpyruvate concentration shows saturation kinetics with an apparent K_m of about 2.5×10^{-3} M. For further details, see ref. 8.
[11] Glaser, L., unpublished information.
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[13] Drs. Ward and Glaser of Washington University at St. Louis have arrived at the same conclusion regarding glucose-1-P from their studies on β-galactosidase synthesis in mutant cells defective in α-D-glucose-1-P phosphohydrolase. Their observations will be published in the near future.
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