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The role of phospho*enol*pyruvate in the simultaneous uptake of fructose and 2-deoxyglucose by *Escherichia coli*

(glycose transport system/kinetics of phosphorylative transport)

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ABSTRACT **Nonmetabolizable glucose analogs inhibit the growth of *Escherichia coli* on a wide variety of carbon sources. This phenomenon was investigated with particular reference to the effect of 2-deoxyglucose (2DG) on growth on fructose as sole carbon source. When the inhibitor is supplied in sufficiently low concentrations, the initial arrest of growth is overcome; this relief of inhibition is aided by means that increase the availability of phospho*enol*pyruvate (PEP) to the growing cells, such as the use of L-aspartate instead of ammonium chloride as sole nitrogen source for growth, and the introduction of the *pps*$^+$ allele into a *pps*$^-$ strain. Studies with [^{14}C]2DG showed that the analog or its 6-phosphate as such did not inhibit growth but that 2DG exerted its effect by competing for intracellular PEP and lowering its concentration below that needed to sustain growth. Direct measurements of the PEP-dependent phosphorylation of 2DG and of fructose by permeabilized *E. coli* showed that the apparent K_m for PEP was nearly 7 times higher for 2DG than it was for fructose, although the apparent V_{max} for 2DG was nearly 3 times that for fructose; this explains the ability of cells to overcome the inhibition by low, but not by high, concentrations of 2DG.**

Hexoses such as fructose, mannose, and glucose are taken up by enteric bacteria via a phospho*enol*pyruvate (PEP)-dependent phosphotransferase system (PTS). In this system, phosphate groups from PEP are sequentially transferred to the hexoses as they cross the plasma membrane, via sugar-specific membrane-spanning proteins, so that the hexoses taken up appear inside the cells as either the 1- or 6-phosphate esters (1, 2). At concentrations of fructose below 5 mM, fructose enters *Escherichia coli* predominantly via a membrane-spanning protein, II^{Fru}, and appears in the cytoplasm as fructose 1-phosphate (3). Glucose and its nonmetabolizable analogs methyl α-glucoside and 5-thioglucose are translocated via a protein designated II^{Glc} or PtsG, whereas mannose and 2-deoxyglucose (2DG) enter the cells via II^{Man}; all these hexoses appear in the cytoplasm as the 6-phosphate esters.

Although each of the membrane-spanning proteins of the PTS is sugar-specific and will effect the transport and concomitant phosphorylation of only a fairly limited range of hexoses, the cytoplasmic proteins that catalyze the transfer of the phosphate from PEP to the hexoses exhibit no such specificity. In each case, an enzyme (enzyme I) catalyzes the transfer of phosphate to a histidine residue in a carrier protein, from which that phosphate is subsequently transferred, directly or via additional carrier proteins, to the incoming hexose. The simultaneous provision of two hexoses to *E. coli* thus imposes a need upon the cells to choose whether to direct the flux of phosphate equally to the uptake of each hexose, as happens when fructose and glucitol are utilized simultaneously (4), or to channel that flux so that one substrate is taken up in preference to another, as happens when glucose is used in preference to other substrates of the PTS (5–8). The competition for uptake between hexoses transported respectively via II^{Glc} and II^{Man} has been elegantly studied by Scholte and Postma (9), who concluded that uptake of the preferred sugar resulted in a partial dephosphorylation of one of the carrier proteins so that the less favored hexose received an inadequate supply of phosphate. In their experiments, insufficiency of PEP was ruled out as imposing a rate-limiting step.

Not only glucose, which is transported via II^{Glc}, but also its noncatabolizable analog 2DG, which is taken up via II^{Man}, are utilized in preference to fructose; in consequence, 2DG powerfully inhibits the growth of *E. coli* on fructose (6–8). By using 2DG in concentrations below 0.5 mM, we have obtained evidence that, contrary to the earlier view (9), the extent to which each of two competing sugars is taken up by the cells can be determined by the availability of PEP.

MATERIALS AND METHODS

All sugars were of the D configuration. Chemicals and enzymes were purchased from Boehringer Mannheim and from Sigma, ^{14}C-labeled fructose and 2DG were from Amersham. The strains of *E. coli* used were wild type for the uptake of most sugars; strain HK 1711 also carried a mutation in the gene (*pps*) specifying PEP synthase. The introduction of the normal (*pps*$^+$) allele into this strain by P1 phage-mediated transduction, to yield strain HK 1719, was performed as described (10), transductants being selected for their ability to grow on lactate as sole carbon source. Cultures of bacteria were grown with shaking in liquid medium containing the appropriate carbon source at 10 mM, the amino acids required by these strains (histidine, arginine, threonine, and leucine) at 40 μg/ml, and salts (11); 2DG was added to such cultures as specified in the text. Measurements of growth and of the uptake of ^{14}C-labeled substrates have been described (8). For determination of the incorporation and retention of [^{14}C]2DG by *E. coli* growing on fructose, the labeled material was added (0.05 mM) to cells, grown on 10 mM fructose and 0.2 mM 2DG, suspended in 5 ml of 10 mM fructose growth medium at an initial density of 0.1 mg (dry mass)/ml; samples (0.2 ml) of the growing cultures were filtered at intervals through a Gelman filter (0.45-μm pore size) and washed, and their radioactivity was assayed with a Beckman scintillation counter. Where aspartate was the sole nitrogen source, ammonium salts were omitted from the salts medium (11) and 2.5 mM L-aspartate was used instead. The PEP-dependent phosphorylation of fructose and of 2DG by cells rendered permeable with toluene in ethanol (16:84, vol/vol) was measured as described (12).

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Abbreviations: 2DG, 2-deoxyglucose; PEP, phospho*enol*pyruvate; PTS, phosphotransferase system.

RESULTS AND DISCUSSION

The growth on fructose of the *pps*$^-$ strain HK 1711 of *E. coli* is exquisitely sensitive to the presence of nonmetabolizable analogs of glucose: even at concentrations as low as 0.04 mM, 2DG inhibits growth (Fig. 1*a*). However, this growth stasis is but temporary: after a lag, the duration of which is dependent on the concentration of 2DG present in the medium, growth resumes and is maintained thereafter at a rate not significantly different from that observed with fructose as sole carbon source. This behavior raises three immediate questions: (*i*) why does 2DG inhibit growth, (*ii*) how is this inhibition overcome, and (*iii*) is the pattern of inhibition by 2DG, and its relief with time, peculiar to fructose (or other sugar substrates of the PTS) or is it observed also when the growth substrate is one taken up by facilitated diffusion or by active transport?

A clue to the first and second of these questions was provided by the paradox that, although there were obvious differences in the sensitivity to 2DG during growth of cells that had overcome the initial inhibition and of cells that had not previously encountered 2DG, no significant differences were observed in the rates at which washed suspensions of cells thus grown took up either 0.1 mM [^{14}C]fructose or 0.1 mM [^{14}C]2DG, or in the rates at which such cells, rendered permeable with toluene (12), effected the PEP-dependent phosphorylation of these hexoses. Moreover, sonic extracts of cells that had grown on fructose in the presence of 2DG contained no more glucose 6-phosphate phosphohydrolase than did extracts of cells that had grown in the absence of 2DG. It was conceivable that some component of the PTS, necessary for the transport both of fructose and of 2DG, became rate-limiting during growth on fructose and that that resulted in a reduction in the ability of 2DG to compete with fructose for phosphorylation.

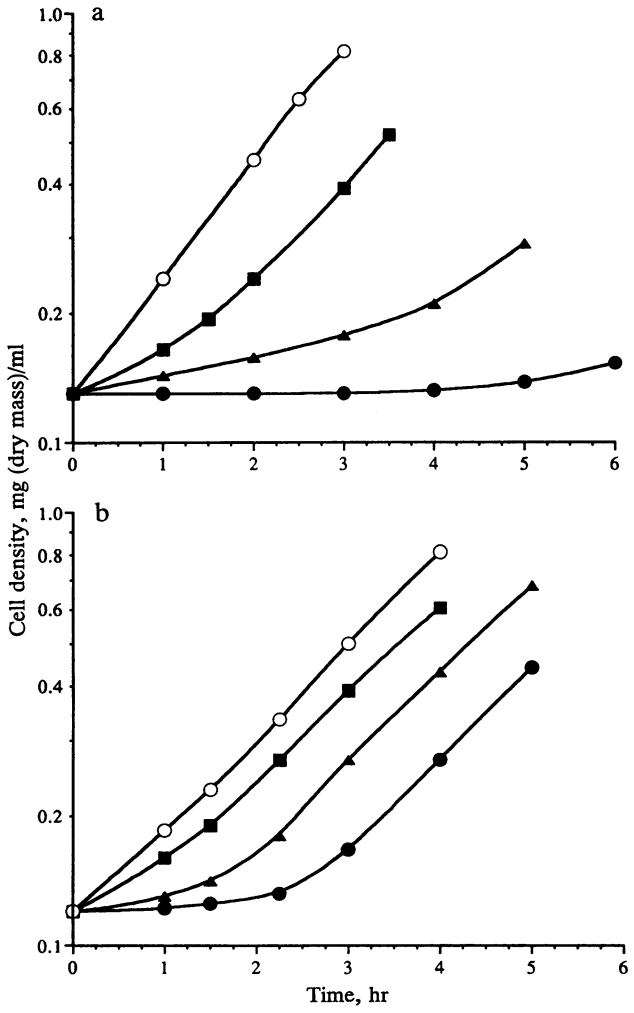

FIG. 1. Effect of 2DG on the growth of *E. coli* on fructose as sole carbon source. Strains HK 1711 (*pps*) (*a*) and HK 1719 (*pps*$^+$) (*b*) grew on 10 mM fructose alone (○) and on this hexose in the presence of 40 μM (■), 60 μM (▲), or 100 μM (●) 2DG.

PEP is essential for growth on fructose in at least three ways: it is the reactant of the PTS without which fructose cannot be taken up and metabolized; it is a precursor for the biosynthesis of many cell components; and, particularly, it is the only C_3 compound that can serve to maintain the tricarboxylic acid cycle by the anaplerotic fixation of carbon dioxide (13). PEP is required also for the uptake of 2DG but, whereas the catabolism of fructose via glycolysis results in a net gain of one mole of PEP for each mole of fructose taken up, the uptake of 2DG results in a net loss of PEP which, in the absence of PEP synthase (14), cannot be made good from the pyruvate formed. The overall reactions may be written as

$$(i)\ \text{fructose}_{(out)} + 2P_i + ADP + 2NAD^+ = PEP_{(in)} + \text{pyruvate}_{(in)} + ATP + 2NADH + 2H^+$$

$$(ii)\ 2DG_{(out)} + PEP_{(in)} = 2DG\ 6\text{-phosphate}_{(in)} + \text{pyruvate}_{(in)}.$$

It was thus conceivable that the inhibition of growth by 2DG was, at least in part, the consequence of an insufficiency of PEP. Such an insufficiency had previously been invoked to explain the poor growth on glucose of mutants impaired in 6-phosphofructokinase activity (15, 16).

Evidence in support of this explanation was provided by two types of experiment. When the gene specifying PEP synthase (*pps*$^+$) was introduced into strain HK 1711 by phage-mediated transduction, the resultant strain, HK 1719, was significantly less sensitive to inhibition by 2DG both during growth on fructose (Fig. 1*b*) and in washed cell suspension (Table 1). Second, strain HK 1711 growing on fructose with 2.5 mM aspartate as sole nitrogen source was also less sensitive to inhibition by 2DG than when ammonium salts provided the nitrogen for growth: the growth patterns observed were virtually identical with those illustrated in Fig. 1*b* and are therefore not shown separately. Similarly, the presence of aspartate reduced the sensitivity to 2DG of washed suspensions of cells taking up [^{14}C]fructose (Table 2) while also stimulating the uptake of fructose and permitting more 2DG to be retained by the cells. The use of aspartate as sole nitrogen source would necessarily introduce oxaloacetate into the cells, which would supply PEP through the action of PEP carboxykinase.

That it was competition for PEP, rather than the intracellular concentration of 2DG or 2DG 6-phosphate, that medi-

Table 1. Effect of PEP synthase activity on the inhibition by 2DG of the uptake of 0.1 mM [^{14}C]fructose

Concentration of 2DG, μM	Inhibition of fructose uptake, %	
	HK 1711 (*pps*)	HK 1719 (*pps*$^+$)
0	0	0
50	62	32
100	69	40
200	78	56

The rates at which 0.1 mM [^{14}C]fructose was taken up by washed suspensions of the cells in the absence of 2DG were 10 nmol/min per mg of dry mass (strain HK 1711) and 7.5 nmol/min per mg of dry mass (strain 1719). Inhibition is expressed as the percent decrease in the rates in the presence of 2DG.

Table 2. Effect of aspartate on the inhibition of fructose uptake by 2DG

Content of incubation mixture	Rate of fructose uptake, %
Fructose	100
Fructose + 2DG	17
Fructose + aspartate	122
Fructose + aspartate + 2DG	69

The rate at which 0.1 mM [^{14}C]fructose was taken up by washed suspensions of strain HK 1711, which had been grown on 10 mM fructose plus 2.5 mM aspartate as nitrogen source, was 7.8 nmol/min per mg of dry mass. Rates observed in the presence of 10 μM 2DG and/or 0.1 mM L-aspartate are expressed as percentages of this rate.

ated the inhibitory effects of the glucose analog, was demonstrated also by measurements of the amounts of the ^{14}C accumulated by cells growing on 10 mM fructose in the presence of 0.05 mM [^{14}C]2DG under various conditions. There was an immediate uptake of the glucose analog, to 10–18 nmol/mg (dry mass) of cells, which was followed by a steady loss of the labeled material from the growing bacteria, at rates linear with increase in cell density. But, whereas the Pps$^-$ strain HK 1711 lost the ^{14}C initially accumulated at ≈47 nmol/mg (dry mass) of increase when growing with ammonium salts as nitrogen source, the rate of loss decreased to 12–15 nmol/mg (dry mass) of increase when PEP was supplied additionally through the utilization of aspartate as nitrogen source (Fig. 2). As expected from this hypothesis, the Pps$^+$ strain HK 1719, during growth on fructose in the presence of [^{14}C]2DG, lost the isotope initially taken up at ≈30 nmol/mg (dry mass) of increase (data not shown), which is a rate lower than that observed with the Pps$^-$ strain growing under the same conditions but faster than when that strain grew with aspartate as nitrogen source. These results also show that the accumulation of 2DG 6-phosphate is not inhibitory as such, which is contrary to findings published previously by one of us (7) and supports the view of Scholte and Postma (9) on this matter.

Direct evidence on the role of the PEP concentration in determining whether 2DG is taken up via the PTS in preference to fructose or vice versa was provided by measurements of the rates at which these hexoses were phosphorylated by cells rendered permeable with toluene (12). At PEP concentrations of 1 mM or above, not only was 2DG phosphorylated much more rapidly than was fructose, but the rates observed when both substrates were present together were not significantly different from those observed with 2DG alone. However, at PEP concentrations below 0.1 mM, the addition of fructose to permeabilized cells phosphorylating 2DG decreased the rates observed; this apparent inhibition became progressively more evident as the PEP concentration used was reduced to the lowest level at which it was feasible to conduct such measurements (0.04 mM). Direct measurement of the kinetics of phosphorylation of 1 mM fructose and of 1 mM 2DG, with various quantities of PEP, are illustrated as a Hanes plot in Fig. 3. From a nonlinear least-squares fit directly to the hyperbola, it was calculated that the apparent K_m for PEP was 0.46 ± 0.10 mM when 2DG was the substrate, whereas it was 0.07 ± 0.01 mM when fructose was used; however, the apparent V_{max} values for PEP with these substrates were 51 ± 6 and 19 ± 1 nmol/min per mg (dry mass) of permeabilized cells, respectively. It has been shown (17) that the PEP content of *E. coli* growing on glucose is, on average, 0.21 μmol per g of dry mass—i.e., ≈0.05 mM. This suggests that whereas 2DG may have an advantage over fructose in competing for PEP in resting cells, it will cease to compete effectively with fructose for PEP when the concentration of that C_3 compound falls to the even lower levels likely to be present during growth on fructose, when PEP has to fulfill both biosynthetic and anaplerotic purposes. This will be exacerbated by lowering the concentration of 2DG in the medium: whereas at saturating concentrations of PEP (1 mM), the apparent K_m for 2DG was ≈0.07 mM, that for fructose was too low to be measured accurately by the method used but was probably 1 order of magnitude less.

Further evidence that it is competition for PEP that accounts for the escape from inhibition by low concentrations of 2DG of growth on PTS substrates was provided by the observation that, even at a concentration as low as 0.05 mM, 2DG virtually arrests growth on glycerol or gluconate, but there is no escape from this inhibition over at least 8 hr. In these cases and as proposed by Scholte and Postma (9), the

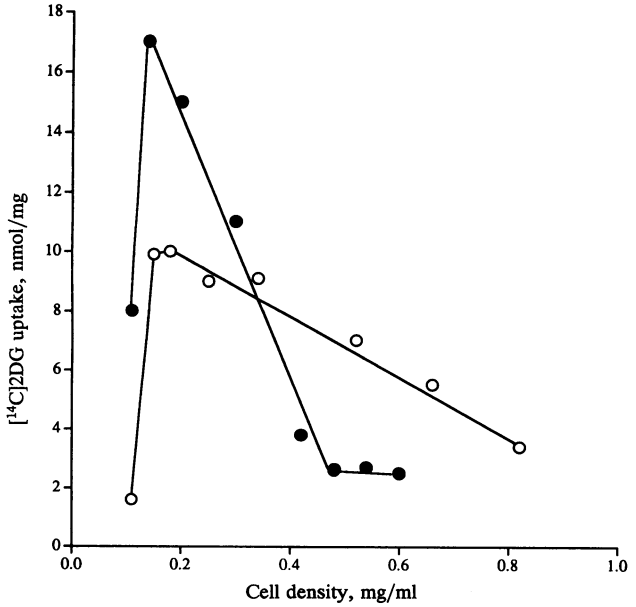

FIG. 2. Uptake and retention of ^{14}C-labeled 2DG by *E. coli* strain HK 1711 (*pps*) growing on 10 mM fructose to which [^{14}C]2DG was added (50 μM) at the first point shown, with ammonium salts (●) or L-aspartate (○) as sole nitrogen source.

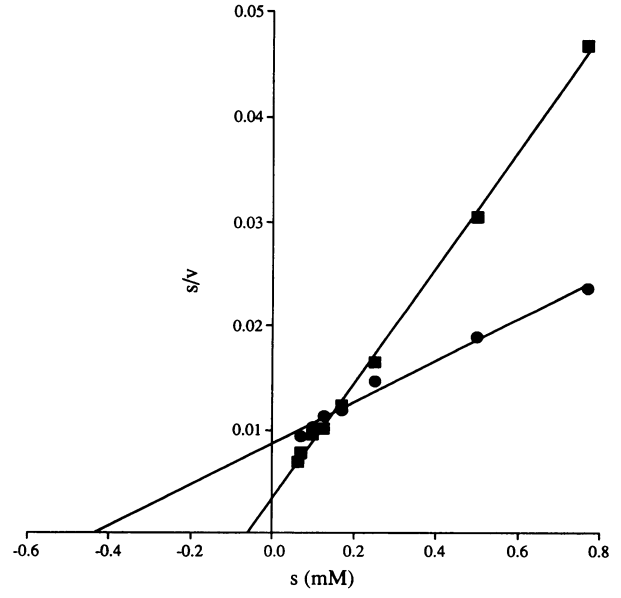

FIG. 3. Hanes plot of the kinetics of phosphorylation of 1 mM fructose (●) or 1 mM 2DG (■) by various amounts of PEP in permeabilized suspensions of *E. coli* strain HK 1711. s, Substrate (PEP) concentration; v, reaction velocity [nmol/min per mg (dry mass)].

inhibition by 2DG is likely to be mediated by dephosphorylation of one of the cytoplasmic components of the PTS, such as the factor III^{Glc}, which is known to inhibit the active transport of a variety of sugars (2).

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