Control of Glucose Metabolism by Enzyme II$^{\text{Glc}}$ of the Phosphoenolpyruvate-Dependent Phosphotransferase System in Escherichia coli

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The quantitative effects of variations in the amount of enzyme II$^{\text{Glc}}$ of the phosphoenolpyruvate:glucose phosphotransferase system (PTS) on glucose metabolism in Escherichia coli were studied. The level of enzyme II$^{\text{Glc}}$ could be adjusted in vivo to between 20 and 600% of the wild-type chromosomal level by using the expression vector pTSG11. On this plasmid, expression of the structural gene for enzyme II$^{\text{Glc}}$, ptsG, is controlled by the tac promoter. As expected, the control coefficient (i.e., the relative increase in pathway flux, divided by the relative increase in amount of enzyme) of enzyme II$^{\text{Glc}}$ decreased in magnitude if a more extensive pathway was considered. Thus, at the wild-type level of enzyme II$^{\text{Glc}}$ activity, the control coefficient of this enzyme on the growth rate on glucose and on the rate of glucose oxidation was low, while the control coefficient on uptake and phosphorylation of methyl $\alpha$-glucopyranoside (an enzyme II$^{\text{Glc}}$-specific, nonmetabolizable glucose analog) was relatively high (0.55 to 0.65). The implications of our findings for PTS-mediated regulation, i.e., inhibition of growth on non-PTS compounds by glucose, are discussed.

The bacterial phosphoenolpyruvate:carbohydrate phosphotransferase system (PTS) is responsible for the translocation of carbohydrates across the cell membrane and their concomitant phosphorylation (22, 30). Transfer of the phosphoryl group from phosphoenolpyruvate (PEP) to the carbohydrates is catalyzed by two general, cytoplasmic PTS proteins, enzyme I and HPr, and one of a number of carbohydrate-specific permeases, which are localized in the cytoplasmic membrane. The permeases generally consist of one (enzyme II) or two (enzymes II and III) subunits.

Escherichia coli contains two glucose-specific PTS permeases (4, 7, 38). One of these permeases has a broad substrate specificity (mannose, glucose, fructose, and glucosamine) and is designated mannose permease, because it is required for growth on mannose as the sole carbon source (Fig. 1). The second system, enzyme II$^{\text{Glc}}$, is specific for glucose and the nonmetabolizable glucose analog $\alpha$-glucopyranoside (aMG). It consists of two subunits, the phosphomannomutase-carrying protein III$^{\text{Glc}}$ and the membrane-bound enzyme II$^{\text{Glc}}$, which catalyzes the actual glucose transport and phosphorylation (Fig. 1).

In addition to sugar uptake and phosphorylation, the PTS is involved in metabolic regulation (for reviews, see references 22, 30, and 33). Both the rate of synthesis of 3',5'-cyclic AMP by adenylate cyclase and the transport of several compounds which are not substrates of the PTS are influenced by the PTS. Presently, a model is favored in which the phosphorylation state of III$^{\text{Glc}}$ is of prime importance for the regulatory functions of the PTS (Fig. 1). In the absence of a PTS substrate, the PTS proteins are thought to be phosphorylated, while during transport of a carbohydrate, such as glucose, the PTS proteins (including III$^{\text{Glc}}$) could, at least partly, be dephosphorylated. Dephosphorylation of III$^{\text{Glc}}$, by the addition of aMG to intact Salmonella typhimurium cells, has been reported by Nelson et al. (24).

Glucose transport catalyzed by the PTS and PTS-mediated regulation are mostly described in a qualitative way; the quantitative aspects of the PTS functions have been much less investigated. For instance, for a proper understanding of PTS-mediated regulation, it is important to know which enzyme (or enzymes) controls the fluxes of phosphoryl groups through the PTS pathways. The control of an enzyme on the flux through a metabolic pathway can be determined by measuring the pathway flux as a function of varying amounts of the enzyme of interest. A useful model for a quantitative description of metabolic pathways has been put forward by Kacser and Burns (19) and Heinrich and Rapoport (13). They have developed a theoretical analysis of pathway fluxes and defined a control coefficient that expresses the degree to which individual steps influence the overall rate through a pathway.

In this report, we describe the effect of variations in the amount of II$^{\text{Glc}}$ on the whole or on parts of the cellular metabolism of glucose in E. coli. We have been able to modulate the level of II$^{\text{Glc}}$ to between 20 and 600% of the wild-type level by using an adjustable expression vector. The application of an expression vector in control analysis has been described previously by Walsh and Kosland (39).

From our results, we conclude that at the wild-type level of II$^{\text{Glc}}$ activity, a variation in the amount of this enzyme has no effect on the growth rate in glucose-containing medium or on oxidation of glucose. However, under the same circumstances, i.e., near wild-type activity, the control coefficient of II$^{\text{Glc}}$ on uptake and phosphorylation of aMG is relatively high (0.55 to 0.65). Some consequences of these findings with respect to the model of the regulatory functions of the PTS are discussed.

**MATERIALS AND METHODS**

**Bacterial strains and plasmids.** The strains used are listed in Table 1. Plasmids pJF118HE (9) and pTSG11 were kindly provided by B. Erni (University of Bern, Bern, Switzerland).

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land). Plasmid pTSG11 (Fig. 2) contains the E. coli structural gene for II\textsuperscript{Glc}, ptsG, under the control of the tac promoter (8).

**Media and growth conditions.** For all experiments, cells were grown overnight in 25 ml of liquid medium A [1 g of (NH\textsubscript{4})\textsubscript{2}SO\textsubscript{4}, 10.5 g of K\textsubscript{2}HPO\textsubscript{4}, 4.5 g of KH\textsubscript{2}PO\textsubscript{4}, and 1 g of MgSO\textsubscript{4} per liter of demineralized water] with 0.5% glycerol, harvested by centrifugation, resuspended in 250 ml of medium A with 0.2% glucose, and grown to exponential phase (optical density at 540 nm of 0.5 to 1.0). All cultures were grown at 37°C on a rotary shaker and supplemented with thiamine (20 μg/ml), ampicillin (50 μg/ml), and the indicated amount of isopropyl-β-D-thiogalactopyranoside (IPTG).

**Chemicals.** [U-\textsuperscript{14}C]aMG (10.8 GBq/mmol) was obtained from the Radiochemical Centre, Amersham, England; [U-\textsuperscript{14}C]glycerol (0.3 GBq/mmol) was purchased from Du Pont, NEN Research Products, Boston, Mass.; α-phenylendiamine and PEP (as cyclohexylammonium salt) were obtained from Sigma Chemical Co., St. Louis, Mo.; IPTG, lactate dehydrogenase (from rabbit muscle), and NADH were obtained from Boehringer GmbH, Mannheim, Germany.

**Genetic methods.** Plasmid DNA was isolated according to Holmes and Quigley (16), and transformation of plasmid DNA was performed as described by Sambrook et al. (34). Preparation of P1 transducing lysates and transduction with bacteriophage P1 were performed as described by Arber (1). Excision of transposon Tn10 from the chromosome was performed according to Bochner et al. (2).

**Oxygen consumption and transport studies.** Oxygen consumption was measured with a Clark-type electrode in medium A and expressed as nanoatoms of O consumed per minute per milligram (dry weight [DW]) at 25°C. Transport of labelled compounds was performed as described previously (28).

**Preparation of cell extracts and enzyme assays.** Cells were ruptured by passage through an Amino French pressure cell at 1,100 kg/cm\textsuperscript{2}, and cell extracts were prepared as described earlier (28). The cell extracts were centrifuged at 230,000 × g for 2 h at 4°C. The activity of II\textsuperscript{Glc}, measured as PEP-dependent phosphorylation of aMG, was determined in the 230,000 × g pellet essentially as described by Kundig and Roseman (20). As a source of the soluble components of the glucose PTS, a high-speed supernatant of strain PPA211 was used. The activities of enzyme I and HPr in strain PPA211/ pTSG11 were determined in the high-speed supernatant of cell extracts according to Waygood et al. (40). In this assay, phosphorylation of aMG is catalyzed by the mannose perimase. For determination of enzyme I activity, a cell extract of S. typhimurium SB1690 was added as a source of the other components of the mannose PTS (HPr, I\textsuperscript{Man}, and II\textsuperscript{Man}). HPr activity in PPA211/pTSG11 was determined by addition of a cell extract of S. typhimurium ptsH strain SB2226 (containing enzyme I, I\textsuperscript{Man}, and II\textsuperscript{Man}).

**Immunological methods.** Determination of the amount of III\textsuperscript{Glc} was performed with rocket immunoelectrophoresis as described by Scholte et al. (36). To determine the amount of

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**TABLE 1. Strains used**

| Strain   | Genotype     | Construction | Reference or source |
|----------|--------------|--------------|---------------------|
| E. coli  |              |              |                     |
| CAG12078 | zce-726::Tnl0 |              | 37                  |
| PPA211   | ptsG2 ptsM1 glk-7 strA recA srl::Tnl0 |              | 26                  |
| PPA231   | ptsM1 glk-7 strA zce-726::Tnl0 | ZSC112 × P1 (CAG12078) | This study         |
| PPA233   | ptsM1 glk-7 strA Tnl0 excision | PPA231 | This study         |
| PPA234   | ptsM1 glk-7 strA recA srl::Tnl0 | PPA233 × P1 (PPA211) | This study         |
| ZSC112   | ptsG2 ptsM1 glk-7 strA |              | 4                   |
| S. typhimurium |              |              |                     |
| SB1690   | ptsI34 trpB223 |              | 3                   |
| SB2226   | ptsH38 trpB223 |              | 3                   |

FIG. 1. The PTS. In addition to the general proteins of the PTS, two permeases are shown, specific for mannose (III/II\textsuperscript{Man}) and glucose (III/II\textsuperscript{Glc}). Inhibition (−) of two different non-PTS uptake systems by II\textsuperscript{Glc} and activation (+) of adenylate cyclase by phosphorylated II\textsuperscript{Glc} (P−II\textsuperscript{Glc}) are indicated. S\textsubscript{1} and S\textsubscript{2} represent lactose, melibiose, maltose, and glycerol. CAMP, cyclic AMP.

FIG. 2. Map of plasmid pTSG11. Plasmid pTSG11 contains the structural gene for II\textsuperscript{Glc}, ptsG, under the control of the tac promoter (P\textsubscript{tac}). As a result of the presence of the lac repressor (lacI\textsuperscript{P}), the expression of ptsG can be modulated by the amount of IPTG added to the growth medium. pTSG11 contains the ampicillin resistance gene (bla). rnrB represents part of the E. coli rnrB operon containing the gene for 5S rRNA and its two transcriptional terminators.
II\textsuperscript{Glc} in membrane fractions of cell extracts, an enzyme-linked immunosorbent assay (ELISA) was developed. To the first, a poly microtiter plate (Maxisorp-F96; Nunc, Roskilde, Denmark), 50 \(\mu\)L of a membrane preparation (250 \(\mu\)g of protein per mL) was applied and serially diluted twofold 11 times in water. The plates were dried for 2 h at 65\(^\circ\)C and rinsed five times with PBST (140 mM NaCl, 3 mM KCl, 8 mM Na\textsubscript{2}HPO\textsubscript{4}, 1.5 mM KH\textsubscript{2}PO\textsubscript{4}, 0.1% [wt/vol] Tween 20, pH 7.5). Subsequently, 100 \(\mu\)L of an appropriate dilution of II\textsuperscript{Glc}-specific monoclonal antibody 5AS (gift from B. Ern) in PBST was added to each well and incubated for 1 h at 42\(^\circ\)C. Following five wash steps with PBST, the wells were incubated with 100 \(\mu\)L of 1:1,000 (in PBST) goat anti-mouse immunoglobulin G conjugated to horseradish peroxidase (Bio-Rad Laboratories, Richmond, Calif.) for 1 h at 42\(^\circ\)C. The plates were rinsed 10 times with PBST and incubated for 15 to 30 min at room temperature with color reagent (100 \(\mu\)L per well) containing 2 mg of o-phenylenediamine per mL, 100 mM sodium phosphate (pH 6.0), and 0.015% H\textsubscript{2}O\textsubscript{2}. The reaction was subsequently stopped with 50 \(\mu\)L of 2 N H\textsubscript{2}SO\textsubscript{4} per well, and absorption values were measured with a Titertec Multiscan Plus (ELFABoy, Helsinki, Finland) at 492 nm. The sigmoid-shaped dilution-absorption curve from each sample was compared with that of a wild-type sample; when the two curves were parallel, the ratio of dilutions at one absorption value gave the ratio of the amounts of II\textsuperscript{Glc}. The amount of II\textsuperscript{Glc} is given relative to the amount present in a wild-type strain.

**Protein and bacterial DW.** Protein was determined by the method of Peterson (27), using bovine serum albumin as a standard. Bacterial DW was determined as described by Herbert et al. (15).

**Phosphorylation via the PTS in toluene-treated cells.** Phosphorylation was measured essentially as described previously (35), with the following modifications. After harvesting and washing, cells were resuspended in 1/50 growth volume of buffer B (5 mM MgCl\textsubscript{2}, 10 mM KF, 0.5 mM dithiothreitol, 3 mM KCl, 50 mM potassium phosphate, pH 7.5), resulting in approximately 5 mg of bacteria (DW) per mL. For each culture, the amount of tolune needed to obtain maximal glucose PTS activity was determined; generally, 2 to 2.5 \(\mu\)L of toluene per mL of cell suspension gave the best results. Phosphorylation was measured with a SLM Aminco DW2000 double-beam spectrophotometer (\(A_1 = 350\) nm; \(A_2 = 375\) nm).

**RESULTS**

**Inducible expression of II\textsuperscript{Glc}.** *E. coli* PPA211 and PPA234 and plasmid pTSG11 (Fig. 2) were used to quantify the control of the II\textsuperscript{Glc} of the PTS on the bacterial glucose metabolism. On plasmid pTSG11, expression of the *E. coli* structural gene for II\textsuperscript{Glc}, *ptsG*, is dependent on the activity of the tac promoter. In the presence of the lacP repressor (also encoded by pTSG11), expression from the tac promoter can be adjusted by varying the amount of inducer, IPTG.

Strain PPA211 does not contain any chromosomally encoded II\textsuperscript{Glc}, II\textsuperscript{Man}, or glucokinase. As a consequence, this strain is unable to take up or metabolize glucose. By transforming PPA211 with plasmid pTSG11, we were able to vary in vivo the amount of II\textsuperscript{Glc}, which then represents the sole glucose uptake system in this strain. To compare the amount of II\textsuperscript{Glc} synthesized from the plasmid with the amount of this enzyme in a wild-type strain, we constructed a *ptsG*\textsuperscript{+} derivative of strain PPA211, designated PPA234.

Strain PPA211 containing plasmid pTSG11 was grown on glucose in the presence of different concentrations of IPTG. In the membrane fraction of these cells, the II\textsuperscript{Glc} activity was determined and appeared to increase linearly with the IPTG concentration (Fig. 3A). The maximum expression of II\textsuperscript{Glc} activity was reached at about 50 \(\mu\)M IPTG and corresponded to six to seven times the wild-type, chromosomal level (the values of the various experiments as determined for *ptsG*\textsuperscript{+} strain PPA234 are shown in Table 2). When the IPTG concentration was increased above 75 \(\mu\)M, the II\textsuperscript{Glc} activity decreased (Fig. 3A). In the absence of IPTG, the strain exhibited II\textsuperscript{Glc} activities that were about 20% of the wild-type level. This resulted possibly from read-through from other promoters present on the plasmid or from a low-level expression from the tac promoter despite the presence of the lacP repressor, because strain PPA231 without the plasmid showed no II\textsuperscript{Glc} activity (<1.0 nmol/mg/min).

On plasmid pTSG11, the natural promoter of *ptsG* was exchanged for the synthetic tac promoter. Because normally glucose induces the expression of *ptsG* (31, 38), we have investigated the effect of glucose on the expression of *ptsG* from pTSG11. When cells were grown on lactate, the relationship between IPTG concentration and II\textsuperscript{Glc} activity was similar to that of glucose-grown cells. For example, II\textsuperscript{Glc} activity in membranes isolated from strain PPA211/pTSG11 grown on minimal medium A containing 0.5% lactate in the presence of 20 \(\mu\)M IPTG was 48 nmol/mg/min; in the case of glucose-grown cells, the activity was 46 nmol/mg/min (Fig. 3A). Therefore, it was concluded that the presence of glucose during growth did not influence the expression of *ptsG* from pTSG11.

It could have been possible that because of synthesis of large amounts of II\textsuperscript{Glc}, the enzyme was not or not properly inserted in the cytoplasmic membrane and was therefore inactive. For this reason, we determined the amount of II\textsuperscript{Glc} immunologically. In an ELISA, the amount of II\textsuperscript{Glc} in cytoplasmic membranes was determined by comparing it with the amount of enzyme present in a *ptsG*\textsuperscript{+} strain. The relationship between the IPTG concentration present during growth and the amount of II\textsuperscript{Glc} in membrane preparations (Fig. 3B) appeared to be comparable to the relationship between IPTG concentration and II\textsuperscript{Glc} activity. Thus, at least up to 50 \(\mu\)M IPTG, the ratio of the amount of II\textsuperscript{Glc} and its activity was constant, and it was therefore concluded that the II\textsuperscript{Glc} present in the cytoplasmic membranes was in its active form.

Since the expression of II\textsuperscript{Glc} from pTSG11 was, at low IPTG concentrations, in the physiological range, this plasmid could be used to determine the control by II\textsuperscript{Glc} of glucose metabolism in *E. coli*.

**Control coefficient of II\textsuperscript{Glc} on growth rate and on oxidation of glucose.** As mentioned previously, strain PPA211 containing pTSG11 is able to take up glucose only via II\textsuperscript{Glc} expressed from the plasmid. When this strain was grown in batch cultures with glucose as the sole carbon and energy source, an increase of the II\textsuperscript{Glc} activity with respect to the wild-type level did not have an effect on the maximal growth rate (Fig. 4). Thus, under these excess-glucose conditions, II\textsuperscript{Glc} had a low control coefficient on the growth rate in the physiological range of the enzyme’s activity.

When the effect of the amount of II\textsuperscript{Glc} on the oxidation rate of glucose was determined, similar results were obtained (Fig. 5). Thus, a slight variation of II\textsuperscript{Glc} activity compared with the wild-type level had no effect on the oxidation rate of glucose. In other words, at the wild-type level, II\textsuperscript{Glc} had a low control coefficient on the oxidation rate of glucose.
In the case of both growth and glucose oxidation, small amounts of II^{Glc}, i.e., about 25% of the wild-type level, were able to support more than half the maximal growth rate and glucose oxidation rate that could be reached. This result suggests that under conditions in which glucose is amply available, II^{Glc} is present in great excess over the amount required for maximal growth or glucose oxidation.

**Control coefficient of II^{Glc} on the flux through the glucose PTS.** Growth on and oxidation of glucose involve rather complex metabolic pathways. In contrast, the glucose PTS is a much shorter pathway, comprising only four enzymes (Fig. 1). We have determined the effect of the amount of II^{Glc} on the flux through the glucose PTS. This flux is defined as transport of sugar by II^{Glc}, coupled to the flow of phosphoryl groups from PEP via enzyme I, HPr, III^{Glc}, and II^{Glc} to the sugar. As depicted in Fig. 6, the flux was measured in two

**TABLE 2. Characteristics of ptsG^+ strain PPA234**

| Determination                          | Value                  |
|----------------------------------------|------------------------|
| II^{Glc} activity                     | 22.0 ± 1.0 nmol/min/mg of protein (8) |
| Growth rate                            | 0.427 ± 0.010 h^{-1} (4) |
| Glucose oxidation                      | 1.37 ± 12 nmol/min/mg (DW) (6) |
| aMG uptake                             | 20.4 ± 1.3 nmol/min/mg (DW) (6) |
| aMG phosphorylation in                 | 4.3 ± 3.6 nmol/min/mg (DW) (4) |
| toluene-treated cells                  | 34.2 ± 18 nmol/min/mg (protein (4) |
| Enzyme I activity                      | 140 ± 9 nmol/min/mg of protein (4) |
| Amt of III^{Glc}                       | 4.2 ± 0.4 µg/mg of protein (4) |

* Cells of strain PPA234 containing pJF18EH were grown to exponential phase on glucose minimal medium. After harvesting, the cells were prepared for the different experiments as described in Materials and Methods. II^{Glc} activity in membranes and uptake of aMG by intact cells were determined with 0.5 mM [U-14C]aMG (specific activity, 140 cpmp/nmol). Oxidation by intact cells was determined with 10 mM aMG. Phosphorylation in toluene-treated cells was determined with 10 mM aMG. Activities of enzyme I and HPr and the amount of II^{Glc} were determined in high-speed supernatants of cell extracts. Determination of enzyme I and HPr activities, [U-14C]aMG (specific activity, 88 cpmp/nmol) was present at a concentration of 10 mM. Values ± standard error are from the number of determinations shown in parentheses.

**FIG. 4. Dependence of the maximal growth rate on the activity of II^{Glc}.** Cells of strain PPA211/pTSG11 were grown exponentially on minimal medium containing 0.2% glucose and other supplements as described in Materials and Methods. From the same cells, membranes were isolated and II^{Glc} activity was determined as described in the legend to Fig. 3 (the arrow indicates wild-type [WT] activity).
were mined for mM (0) glucose activity. IIGIc strain 6188RUYTER from the...

ways, first as the uptake of αMG (a II\textsubscript{Glc}-specific glucose analog) by intact cells and second as the phosphorylation of αMG by toluene-treated cells. It is clear that the flux through the glucose PTS depended to a much greater extent on the II\textsubscript{Glc} activity than did the growth rate and the rate of glucose oxidation. The slight difference in the dependence of uptake and phosphorylation on the activity of II\textsubscript{Glc} reflected the difference in the process studied. From these graphs, it was possible to calculate control coefficients as defined by Heinrich and Rapoport (13) and Kacser and Burns (19). The control coefficients (relative increase in pathway flux, divided by the relative increase in amount of enzyme) of II\textsubscript{Glc} on αMG uptake by intact cells and on αMG phosphorylation by toluene-treated cells were 0.65 and 0.55, respectively. It is known that the presence of II\textsubscript{Glc} is required for complete induction of the general PTS proteins, enzyme I and HPr, during growth (5, 21, 31) on glucose, while the amount of III\textsubscript{Glc} in the cell is more or less constant (5, 36). During our experiments, the amount of II\textsubscript{Glc} was varied, and it was therefore possible that the amounts of enzyme I and HPr also varied. In that case, the effects ascribed to II\textsubscript{Glc} could, at least partially, be caused by variations in the amounts of enzyme I, HPr, or III\textsubscript{Glc}. Therefore we measured the activity of enzyme I and HPr and the amount of III\textsubscript{Glc} in the same cells used for the studies with II\textsubscript{Glc}. The HPr activity and the amount of III\textsubscript{Glc} did not vary much with the II\textsubscript{Glc} activity but fluctuated around their respective wild-type values (Fig. 7). The enzyme I activity was also more or less constant except at very low II\textsubscript{Glc} activities (Fig. 7). Therefore, at wild-type II\textsubscript{Glc} activities, only an increase in the activity of II\textsubscript{Glc} could account for an increase in flux through the glucose PTS.

Effect of II\textsubscript{Glc} activity on inducer exclusion. We have investigated the effect of variations in the activity of II\textsubscript{Glc} on the phosphorylation state of III\textsubscript{Glc} indirectly by measuring...
TABLE 3. Correlation between II^{Glc} activity and inhibition of glycerol uptake

| Strain                  | [IPTG] (µM) | II^{Glc} activity (nmol/min/mg of protein) | Rate of glycerol transport (nmol/min/mg (DW)) | Inhibition (%) |
|-------------------------|-------------|------------------------------------------|-----------------------------------------------|---------------|
| PPA234/pJF118EH         | 0           | 22.5                                     | 15.5                                          | 0.7           | 95            |
| PPA211/pJF118EH         | 0           | 0.5                                      | 15.7                                          | 1             |
| PPA211/pTSG11           | 0           | 5.3                                      | 11.5                                          | 1.9           | 83            |
| PPA211/pTSG11           | 10          | 20.9                                     | 12.0                                          | 1.0           | 92            |
| PPA211/pTSG11           | 50          | 128                                      | 11.8                                          | 1.0           | 92            |

* Cells were grown overnight on glycerol minimal medium and subsequently grown for 1 h on glucose minimal medium as described in Materials and Methods. Cells were then prepared for transport of [U-14C]glycerol (specific activity, 165 cpm/nmol) as described. αMG was added to a concentration of 10 mM 3 min before the uptake experiment was started.

glucose-limited were glycerol functional under these circumstances. Our results have shown that glucose-limited cells grown in the absence of PTS substrates (33) expressed glucose-limited cells grown in the absence of PTS substrates (33). Differentially, III^{Glc} inhibits, for instance, the enzyme glycerol kinase and in this way, indirectly, glycerol uptake by cells.

Table 3 shows that addition of 10 mM αMG did not inhibit glycerol uptake in ptsG ptsM strain PPA211, which was expected because this mutant strain did not contain any functional II^{Glc} and was therefore unable to take up αMG. In contrast, glycerol uptake was inhibited 95% by addition of αMG to the ptsG mutant strain PPA234. Furthermore, when strain PPA211/pTSG11 was grown in the absence of IPTG (the amount of II^{Glc} then corresponded to about 20% of the wild-type level), glycerol uptake was inhibited more than 80% by addition of αMG. This indicated that 20 to 30% of the wild-type activity of II^{Glc} is sufficient for almost complete inhibition of glycerol uptake by addition of αMG.

**DISCUSSION**

In this report, we describe the control by II^{Glc} of glucose metabolism in E. coli. Using an inducible expression vector, the effects of varying the amount of II^{Glc} on the growth rate in a glucose-containing medium, on the oxidation rate of glucose, and on the uptake and phosphorylation of αMG were determined.

Our results show that at the wild-type level of II^{Glc} activity, this enzyme had a low control coefficient on the growth rate in batch cultures containing glucose. According to Jensen and Pedersen (18), the control of growth lies in the availability of building blocks for synthesis of cellular components. This implies that under most circumstances, the machinery polymerizing the building blocks to cellular structures is saturated. Consequently, II^{Glc} did not control growth, apparently other steps in metabolism control the growth rate under these circumstances. Dijkhuizen et al. (6) have determined that the lactose permease has a high control coefficient on the growth rate under lactose-limited conditions. Furthermore, Hunter and Kornberg (17) have shown that under glucose-limited conditions, the uptake capacity of glucose via the PTS limits growth. In these experiments, glucose-limited conditions were obtained by growing cells in chemostat cultures. During growth of the strains, which contain an intact PTS, in glucose-limited chemostat cultures, the glucose concentration in the culture fluid is usually lower than 10 µM (32).

Furthermore, because the apparent K_{m} of II^{Glc} for glucose is about 10 µM (38), the enzyme is operating at rates below its V_{max} under glucose-limited conditions. It is possible that II^{Glc} has a higher control coefficient on the growth rate under these conditions.

Apart from the effects on growth rate, we have determined the control coefficients of II^{Glc} on oxidation of glucose and on uptake and phosphorylation of the glucose analog αMG. Oxidation of glucose involves a large number of catabolic steps, while uptake and phosphorylation of αMG represent only the first two steps in the catabolism of glucose. These two reactions are catalyzed by the glucose PTS, which consists of four proteins (Fig. 1). Generally, when the length of a metabolic pathway (i.e., the number of enzymatic steps involved) is decreased, it is to be expected that the flux control of an enzyme in this pathway increases. This is observed in our experiments. II^{Glc} did not control the rate of oxidation of glucose (i.e., a large number of catabolic steps) but had a relatively high control coefficient (0.55 to 0.65) on uptake and phosphorylation of αMG (i.e., the first two steps of catabolism).

A few reports have appeared that deal with determination of the flux-controlling properties of PTS enzymes. However, in these studies, flux control is described in a more qualitative way. For instance, Ghosh et al. (10) used E. coli strains overproducing enzyme I and concluded that overproduction of enzyme I did not lead to increased uptake and phosphorylation of αMG. These results are in agreement with a high control coefficient of II^{Glc} on uptake and phosphorylation of αMG. The metabolic control theory states that the sum of the enzyme control coefficients on the flux through an investigated pathway equals 1. Thus, if the control coefficient of II^{Glc} on the flux through the glucose PTS is 0.55 to 0.65, there is still 0.35 to 0.45 of control left to distribute between enzyme I, HPr, and III^{Glc}. Because apparently enzyme I does not control the flux through the glucose PTS, this leaves only HPr and III^{Glc}. Experiments are in progress to determine the flux control exerted by these PTS enzymes.

From a series of reciprocal inhibition experiments, Scholte and Postma (35) concluded that in S. typhimurium, II^{Glc} and III^{Glc}, and III^{Glc} and II^{Man} competed for the pool of phosphorylated enzyme I and HPr and that consequently the flow of phosphorylation through enzyme I and HPr was the rate-limiting step in the PTS reactions. Although the authors did not quantify the control properties of enzyme I and HPr, it is possible that these proteins do control the flux through the glucose PTS to a certain extent. However, because the data presented in this report indicate that in E. coli the control coefficient of II^{Glc} on the flux through the glucose PTS is 0.55 to 0.65, it is not likely that the control coefficient of enzyme I or HPr approaches 1 (i.e., is rate limiting). We suggest that the control on the flux through the glucose PTS is distributed among enzyme I, HPr, III^{Glc}, and II^{Glc}, with the largest part on II^{Glc}. This distribution of control over several enzymes is generally found when flux-controlling properties of enzymes are determined (12).

According to Scholte and Postma (35), the rate-limiting properties of enzyme I and HPr in S. typhimurium were in agreement with the model of the PTS-mediated regulation, which predicts a net dephosphorylation of PTS components, in particular III^{Glc}, in the presence of a PTS substrate (Fig. 1). In this model, phosphorylated III^{Glc} accounts for the inhibition of uptake of several non-PTS substrates (glycerol, lactose, maltose, and melibiose). The relatively high control of II^{Glc} on the flux through the glucose PTS, presented in this report, seems to be in contradiction with this model. A high
control coefficient of II\text{Glc} on the flux through the glucose PTS could mean that the supply of phosphoryl groups to II\text{Glc} is faster than the rate at which II\text{Glc} donates its phosphoryl groups to its sugar substrate. In other words, III\text{Glc} would be phosphorylated even in the presence of a substrate of the glucose PTS. To investigate the effect of II\text{Glc} activity on the phosphorylation state of III\text{Glc}, we have measured the inhibition of glycerol uptake by addition of αMG as a function of the II\text{Glc} activity. It appeared that at a II\text{Glc} activity comparable to the induced wild-type level, glycerol uptake was almost completely inhibited (>90%) by addition of αMG. Thus, in that case III\text{Glc} must be dephosphorylated to such an extent that it is able to inhibit glycerol uptake.

To explain the data of αMG uptake and inhibition of glycerol uptake, the kinetic characteristics of the enzymes should be taken into account. During αMG uptake, II\text{Glc} had a high control coefficient on the flux through the glucose PTS. Therefore, if we assume Michaelis-Menten kinetics, II\text{Glc} was operating at rates near its V\text{max} during αMG uptake and phosphorylation. In other words, the concentration of phosphorylated III\text{Glc} must have been high relative to the K\text{m} of II\text{Glc} for phosphorylated III\text{Glc}. This K\text{m} value has been reported to be 3.4 μM in S. typhimurium (23) and 5 μM in E. coli (11), while the intracellular concentration of III\text{Glc} is estimated to be 30 to 50 μM (36). Thus, even if III\text{Glc} is only partly in the dephosphorylated state, the concentration of phosphorylated III\text{Glc} is higher than the K\text{m} value. The target molecule of III\text{Glc} for inhibition of glycerol uptake is glycerol kinase (29). According to Heller et al. (14), the intracellular concentration of glycerol kinase is about 15 μM. With respect to the K\text{d} of III\text{Glc} for glycerol kinase, several values have appeared in the literature. Postma et al. (29) have reported a value of 50 μM for S. typhimurium (pH 7.5), while Novotny et al. (25) claimed that this K\text{d} is pH dependent in E. coli: 10 μM at pH 7.0 and 4 μM at pH 6.0. If we assume a low K\text{d} (see also reference 33) and a stoichiometric interaction between dephosphorylated III\text{Glc} and glycerol kinase, then severe inhibition of glycerol kinase is possible when III\text{Glc} is only partly present in the dephosphorylated state.

Thus, although II\text{Glc} controls the flux through the glucose PTS to a large extent, this is not necessarily in contradiction with the presently used model of the regulatory functions of the PTS. To elucidate regulation involving the PTS, experiments are now in progress to determine the flux-controlling properties of enzyme I, HPr, and III\text{Glc}. Finally, to understand the regulatory properties of III\text{Glc}, the characteristics of the interactions between this enzyme and its target proteins should be quantified.

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