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Understanding Glucose Transport by the Bacterial Phosphoenolpyruvate:Glycose Phosphotransferase System on the Basis of Kinetic Measurements in Vitro*

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The kinetic parameters in vitro of the components of the phosphoenolpyruvate:glycose phosphotransferase system (PTS) in enteric bacteria were collected. To address the issue of whether the behavior in vivo of the PTS can be understood in terms of these enzyme kinetics, a detailed kinetic model was constructed. Each overall phosphotransfer reaction was separated into two elementary reactions, the first entailing association of the phosphoryl donor and acceptor into a complex and the second entailing dissociation of the complex into dephosphorylated donor and phosphorylated acceptor. Literature data on the Km values and association constants of PTS proteins for their substrates, as well as equilibrium and rate constants for the overall phosphotransfer reactions, were related to the rate constants of the elementary steps in a set of equations; the rate constants could be calculated by solving these equations simultaneously. No kinetic parameters were fitted. As calculated by the model, the kinetic parameter values in vitro could describe experimental results in vivo when varying each of the PTS protein concentrations individually while keeping the other protein concentrations constant. Using the same kinetic constants, but adjusting the protein concentrations in the model to those present in cell-free extracts, the model could reproduce experiments in vitro analyzing the dependence of the flux on the total PTS protein concentration. For modeling conditions in vivo it was crucial that the PTS protein concentrations be implemented at their high in vivo values. The model suggests a new interpretation of results hitherto not understood; in vivo, the major fraction of the PTS proteins may exist as complexes with other PTS proteins or boundary metabolites, whereas in vitro, the fraction of complexed proteins is much smaller.

In many bacteria, the phosphoenolpyruvate:glycose phosphotransferase system (PTS)1 is involved in the uptake and concomitant phosphorylation of a variety of carbohydrates (for reviews, see Refs. 1 and 2). The PTS is a group transfer pathway; a phosphoryl group derived from phosphoenolpyruvate (PEP) is transferred sequentially along a series of proteins to the carbohydrate molecule. The sequence of phosphotransfer is from PEP to the general cytoplasmic PTS proteins enzyme I (EI) and HPr and, in the case of glucose, further to the carbohydrate-specific cytoplasmic IIA Glc, membrane-bound IIB Glc (the glucose permease), and glycose. For other carbohydrates, specific enzymes II exist (with the A, B, and C domains present either as a single polypeptide or as multiple proteins, depending on the carbohydrate that is transported), which accept the phosphoryl group from HPr (1–4).

Apart from its direct role in the above phosphotransfer and its indirect role in transport, IIA Glc is an important signaling molecule, mediating catabolite repression (reviewed in Refs. 1, 2, and 5). The presence or absence of a PTS substrate affects the IIA Glc phosphorylation state; in the absence of PTS substrate, phosphorylated IIA Glc predominates, which activates adenylate cyclase and hence increases the intracellular cyclic AMP level, thereby affecting the expression of a large number of genes. The presence of a PTS substrate, on the other hand, will lead to dephosphorylation of IIA Glc. Unphosphorylated IIA Glc can bind stochiometrically to the uptake systems for some non-PTS substrates for growth, inhibiting these uptake systems allosterically by so-called “inducer exclusion” and preventing the entry of the alternative carbon substrates into the cell to induce their own catabolic genes.

The individual components of the PTS have been characterized extensively, using kinetic and structural approaches (for a review, see Refs. 1, 2, 3, and 4). In the kinetic analysis of the PTS proteins, many Kms values for their substrates and products, as well as the equilibrium constants of the phosphotransfer reactions have been determined. A recent development is the direct determination, using a rapid quench method, of the forward and reverse rate constants of phosphotransfer between HPr and IIA Glc of Escherichia coli (6).

Metabolic control analysis is a quantitative framework developed by Kacser and Burns (7) and Heinrich and Rapoport (8) for describing the steady-state behavior of metabolic systems and the dependence of cellular variables (e.g. fluxes or intermediate concentrations) on parameters (e.g. enzyme concentrations). The intracellular concentrations of all four glucose PTS phosphotransferase system; PEP, phosphoenolpyruvate; EI, enzyme I; MeGlc, methyl α-D-glucopyranoside; PEG, polyethylene glycol.

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‡ The abbreviations used are: PTS, phosphoenolpyruvate:glycose
proteins in *Salmonella typhimurium* (9) and of IICB\textsuperscript{Glc} in *E. coli* (10) have been modulated in turn to determine the extent to which each of these proteins controls the PTS-mediated uptake rate *in vivo*. These dependences were quantified with so-called flux response coefficients, defined as the percentage change in the uptake rate upon a 1% increase in the enzyme concentration (see “Appendix” for mathematical definitions). Furthermore, titrations with different amounts of *E. coli* cell-free extracts (11) have enabled the quantification of the extent to which the glucose PTS proteins together control PTS-mediated phosphorylation activity *in vitro* at different protein concentrations. There was a remarkable difference between the results obtained *in vitro* and *in vivo*; *in vitro*, the four flux response coefficients added up to 0.8 (9), whereas *in vivo*, this sum varied between 1.5 and 1.8 depending on the total protein concentration (11). A value of greater than unity for this sum is in itself remarkable, since it reflects a higher order than linear dependence of flux on total protein concentration and contrasts strongly with the linear relationship between reaction rate and enzyme concentration usually found in enzyme kinetics. However, following these experiments, two additional questions are strongly affected by macromolecular crowding (11); it is involved in signal transduction through catabolite repression and inducer exclusion (see above); and a similar sequence of phosphoryl transfer is ubiquitous in the “two-component regulatory systems” (14, 15), which have been proposed to form an intracellular “phosphoneural network” (16). Any attempt at a deeper understanding of such cellular processes at a level beyond map making, component identification, and characterization will have to put the pieces of the puzzle together in a quantitative “systems framework.” This paper is such an attempt.

**Materials and Methods**

*Calculation of Rate Constants for Elementary Steps*—A phosphotransfer reaction from a phosphoryl donor AP to an acceptor E can be symbolized as follows.

\[
E + AP \xrightleftharpoons{k_{-1}} k_1 \rightarrow EP + A
\]

Scheme 1

We shall use Roman numerals as subscripts to designate the rate constants for such an overall phosphotransfer reaction; the forward rate constant is \(k_1\), and the reverse rate constant is \(k_{-1}\), yielding an equilibrium constant \(K_{eq}\) of \(k_1/k_{-1}\). This overall reaction can be divided further into two elementary reactions, the first involving association of E and AP into the complex EPA, and the second involving dissociation of the complex into EP and A as follows.

\[
E + AP \xrightarrow{k_1} EPA \xrightarrow{k_{-1}} EP + A
\]

Scheme 2

Scheme 2 has four elementary rate constants, i.e. the forward and reverse rate constants of both the association and dissociation reactions, which will be denoted by subscripts arabic numerals. Scheme 2 is an extended description of Scheme 1, and in general the two will not be valid simultaneously. However, if the concentration of the intermediate complex EPA is assumed to be constant because its rate of production equals its rate of consumption (i.e. assuming a steady state for EPA), both reaction Schemes 1 and 2 are valid descriptions of the same process, and their rate constants can be related (see below).

We calculated the values of the rate constants for Scheme 2 from experimental data. Four types of data were available: (a) equilibrium constants for phosphotransfer reactions, (b) \(K_m\) values of some enzymes for their substrates or products, (c) association or dissociation equilibrium constants for some enzymes and substrates or products, and (d) rate constants of the type \(k_1\) and \(k_{-1}\) for the overall phosphotransfer reactions (Scheme 1).

First, the equilibrium constant was expressed as a function of the rate constants of the elementary steps as follows.

\[
K_{eq} = \frac{k_1}{k_{-1}} = \frac{k_1k_2}{k_1k_2}
\]

(Eq. 1)

Second, \(K_m\) values were related to the individual rate constants. Reaction Scheme 2 differs from that of a traditional enzyme in that the catalyst does not return unaltered. Only after EP has transferred its phosphoryl group to another molecule in additional reactions, free E is returned; also, the regeneration of AP requires additional reactions. Hence, the meaning of the Michaelis constant is also different. We applied the method analogous to that of Briggs-Haldane (17) by writing the differential equations and equating the time derivative of [EPA] to zero. This then allowed us to write rate equations for the zero product and the zero substrate case, which had the same form as the Michaelis-Menten equation. Hence, the \(K_m\) values of the “enzyme” E for AP and A (denoted with an asterisk to indicate this difference, i.e. \(K_{m,AP}\) and \(K_{m,A}\)) are defined as follows.

\[
K_{m,AP} = \frac{k_{-1} + k_2}{k_1}
\]

(Eq. 2)

and

\[
K_{m,A} = \frac{k_{-1} + k_2}{k_2}
\]

(Eq. 3)

The operational meaning of \(K_{m,AP}\) is the concentration of AP giving half-maximal rates when both [EP] and [A] are negligible; that of \(K_{m,A}\) is the concentration of A giving half-maximal reverse rates at zero [AP] and [E].

Third, association or dissociation equilibrium constants of enzymes and substrates or products were expressed in terms of rate constants of the elementary steps. Dissociation/association equilibrium constants of AP and A in Scheme 2 are given by the following.

\[
K_{d,AP} = \frac{1}{K_{m,AP}} = \frac{k_{-1}}{K_m}
\]

(Eq. 4)

and

\[
K_{d,A} = \frac{1}{K_{m,A}} = \frac{k_2}{K_m}
\]

(Eq. 5)

It may be noted that the former \(K_{d}\) refers to the association to E, whereas the latter \(K_{d}\) refers to the association to EP.
Kinetic Model of PTS

Parameters of the kinetic model

| Parameters                      | Units | In vivo | In vitro |
|---------------------------------|-------|---------|----------|
| [EII]_total                     | μM    | 5       | 0.04     |
| [HP]_total                      | μM    | 40      | 0.32     |
| [IIAGlc]_total                  | μM    | 15      | 0.12     |
| [IICBGlc]_total                 | μM    | 0.4     | 1.2      |
| Boundary metabolite concentrations |      |         |          |
| PEP                            | μM    | 2800    | 4000     |
| Pyruvate                       | μM    | 900     | 0        |
| MeGlc                          | μM    | 500     | 10,000   |
| MeGlc 6-P                      | μM    | 50      | 0        |

Rate constants (PTS step in parentheses)

|                   | μM⁻¹ min⁻¹ |         |
|--------------------|------------|---------|
| k₁ (PEP to EI)     | 294        | 1960*   |
| k⁻¹ (PEP to EI)    | 480,000    |        |
| k₂ (PEP to EI)     | 108,000    |        |
| k₃ (EI to HPr)     | 14,000     |        |
| k₄ (EI to HPr)     | 14,000     |        |
| k₅ (EI to HPr)     | 84,000     |        |
| k₆ (HP to IIAGlc)  | 3950       |        |
| k₇ (HP to IIAGlc)  | 21,960     |        |
| k₈ (HP to IICBGlc) | 21,960     |        |
| k₉ (HP to IICBGlc) | 4392       |        |
| k₁₀ (IIAGlc to IICBGlc) | 3334 | |
| k₁₁ (IIAGlc to IICBGlc) | 880 | |
| k₁₂ (IIAGlc to IICBGlc) | 2640 | |
| k₁₃ (IIAGlc to IICBGlc) | 960 | |
| k₁₄ (IICBGlc to Glycer) | 260 | |
| k₁₅ (IICBGlc to Glycer) | 380 | |
| k₁₆ (IICBGlc to Glycer) | 4800 | |

*Identical values were used for the rate constants under all conditions.

Reaction Scheme 2 may be divided further by including the step $E \cdot PA \leftarrow EP \cdot A$ explicitly, yielding three elementary steps in total. However, we did not consider this case, since insufficient data were available to assign values to all the rate constants in such a mechanism.

Model Parameters—To simulate the reactions of the PTS numerically, a few parameters other than the rate constants of the elementary reactions are required: the total concentration of each PTS protein and the concentrations of the boundary metabolites PEP, pyruvate, glucose (or methyl-D-glucopyranoside (MeGlc), its nonmetabolizable analogue), and glucose 6-phosphate (or MeGlc 6-phosphate). The subunit molecular masses of the cytoplasmic PTS proteins EI (63,489), HPr (9109), and IIAGlc (18,099) (19) were used to calculate intracellular concentrations of 5 μM (EI monomers), 20–100 μM (HPr), and 20–60 μM (IIAGlc) from the intracellular amounts of these proteins reported in the literature (20–23). An intracellular volume of 2.5 μl/mg dry mass (24–26) was assumed in the calculations. Because IIAGlc is a membrane protein, intracellular amounts were reported on the activity level. Intracellular IICBGlc amounted to 10 μmol/liter cytoplasmic volume, as calculated from the specific activity of the protein and the glucose phosphorylation activity of *E. coli* given in Ref. 27.

For *E. coli* growing exponentially on glucose, intracellular PEP levels of 60–300 μM (28, 29) and pyruvate levels of 0.36–8 mM (28, 30) have been reported. These PEP values may underestimate the intracellular concentration, due to the long filtration time (up to 60 s) employed by the authors for sampling (see discussion in Ref. 31). Furthermore, transport assays in our laboratory are routinely performed with washed, concentrated, and starved cell suspensions (32). Under these conditions, intracellular PEP and pyruvate levels have recently been determined for glucose-grown *E. coli*; the PEP concentration was 2.8 mM, and that of pyruvate was 0.9 mM (33). We used these values for our simulations of PTS uptake assays. The MeGlc concentration was set to 500 μM, a concentration used routinely for uptake assays. Intracellular MeGlc 6-phosphate was fixed at 50 μM. During PTS-mediated carbohydrate uptake, intracellular carbohydrate phosphates will accumulate, their concentrations increasing from initial values close to zero when the substrate is a nonmetabolizable analogue. Numerical simulation of the PTS requires, however, that the boundary metabolite concentrations be fixed; otherwise, calculation of a steady state will be impossible. The chosen low MeGlc 6-phosphate concentration reflects the situation during the initial stages of the uptake process; in addition, we verified that increasing this value to 6 mM had negligible effect (less than 0.1%) on the observed flux (at 6 mM Glc–P, the dissociation of IICBGlc · P · Glc into IICBGlc and Glc · P should be >99% irreversible; cf. “Appendix”).

When simulating PTS-mediated phosphorylation in *vitro*, the rate constants of the elementary steps were left unchanged. The dilution of the cytoplasmic proteins in our cell-free extracts in comparison to the intracellular environment was accounted for by calculating a dilution factor from the protein concentration of our extract and the reported intracellular total protein concentration of 0.25 g/ml (34). The intracellular concentrations of the PTS proteins were multiplied by this dilution factor to calculate their concentrations in the cell extract. Concentrations of the boundary substrates PEP, pyruvate, MeGlc, and MeGlc 6-phosphate were entered as employed under the experimental conditions.

All parameter values of the kinetic model are summarized in Table I. Concentrations of the PTS proteins and of the boundary metabolites are shown for the simulation of PTS activity in *vitro* and at two protein concentrations in *vitro*.

Numerical Methods—Simulations and steady-state calculations of the kinetic models were performed on an IBM-compatible personal computer.
Kinetic Model of PTS

Table II

Steady-state properties of the kinetic model and comparison with experimental values

The flux $J$, the flux response coefficients of the four glucose PTS proteins, and their sum $R_{\text{PTS}}$ were calculated with the parameter set of Table I for conditions in vivo and in vitro at two protein concentrations. $R_{\text{IIA}}$ and $R_{\text{IICB}}$ refer to the flux response coefficients with respect to the total concentrations of IIA$^{\text{Glc}}$ and IICB$^{\text{Glc}}$, respectively.

| Steady-state variable | In vivo  | Experiment$^a$ | In vitro 6 mg of protein/ml | In vitro 2 mg of protein/ml |
|-----------------------|---------|----------------|-----------------------------|-----------------------------|
| $J$ ($\mu$M min$^{-1}$) | $1.4 \times 10^4$ | $2.2 \times 10^4$ | 13                         | 6                         |
| $R_{\text{IIA}}$    | 0.05    | 0              | 0.07                        | ND                         |
| $R_{\text{IICB}}$   | 0.02    | $\leq 0.01$    | ND                          | ND                         |
| $R_{\text{IHP}}$    | 0.2     | $\leq 0.02$    | 0.05                        | 0.05                       |
| $R_{\text{P}}$      | 0.9     | 0.7            | 0.9                         | 0.9                        |
| $R_{\text{IIA}}$    | 1.1     | 0.7–0.8        | 1.7                         | 1.7                        |

$^a$ Experimental data from Ref. 9.

RESULTS

Here, we shall describe the kinetic behavior of the model and compare this to experimental results obtained in vivo and in vitro. Furthermore, a parameter sensitivity analysis will be presented to determine to what extent the results depended on assumptions that were made during the derivation of the rate constants of the elementary steps from the phenomenological kinetic constants measured experimentally (see “Appendix”).

Steady-state Behavior—The steady-state flux predicted by the enzyme kinetic parameters of the PTS is shown in Table II for simulation of a PTS uptake assay in vivo and a phosphorylation experiment in vitro at two different protein concentrations. The corresponding experimental values are for comparison. For the modeled flux, agreement between model and experiment was good; although the modeled value was always lower than the experimental value, the discrepancy was only approximately 30% (i.e. less than a factor of 2) for conditions in vivo and in vitro. This discrepancy is small considering the fact that the calculation was based exclusively on experimentally determined parameters; no parameter value was fitted.

Next, we investigated whether the model would match experimental data obtained by varying the concentrations of the PTS proteins. The effect of changing in turn the concentrations of EI, HPr, IIA$^{\text{Glc}}$, and IICB$^{\text{Glc}}$ on PTS uptake activity (while keeping enzyme concentrations other than the modulated one constant) has been determined experimentally (9); these experiments were mimicked by numerical simulation. First we considered small variations, expressing the results in terms of response coefficients (i.e. the percentage change in flux for a 1% increase in enzyme concentration). Table II shows that the calculated response coefficients matched their experimental counterparts remarkably well.

A reservation concerning the use of response coefficients is that they refer to small parameter changes, whereas in biologically relevant cases, changes may well exceed 50%. We therefore also considered large variations in enzyme concentrations. Again the simulation results agreed remarkably well with experimental data: the modeled flux versus PTS protein concentration profiles matched the experimental ones (Fig. 1). An exception was the decrease in PTS uptake activity for high EI levels (9), which was not observed in the simulations. Fig. 1 also shows that changing the concentrations of EI, HPr, and IIA$^{\text{Glc}}$ around their wild-type levels had little effect on the flux; this was quantified by calculating the flux response coefficients of these proteins, as indicated on the graphs. The flux response coefficients of IIA$^{\text{Glc}}$ and IICB$^{\text{Glc}}$ were slightly higher than the experimentally reported values (cf. Table II).

A kinetic model will gain credibility if it can describe experimental data in vivo as well as in vitro without the need for adjustment of the kinetic parameters. The effect of concomitant changes in the concentrations of all proteins of the glucose PTS on its phosphorylation activity in vitro has been investigated experimentally by performing a PTS activity assay with different amounts of cell extract (11). We mimicked these experiments by numerical simulation (Fig. 2). As was observed experimentally (Figs. 1 and 2 in Ref. 11), the dependence of the flux on total protein concentration was more than linear but less than quadratic (Fig. 2a). Accordingly, the combined PTS flux response coefficient ($R_{\text{PTS}}$) decreased from almost 2 to around 1.7 as the protein concentration was increased from low values to 6 mg/ml (Fig. 2b).

In previous experiments, the macromolecular crowding agent PEG 6000 (37, 38) has been added to a PTS activity assay to mimic high intracellular macromolecule concentrations, and its effect was simulated with a simple kinetic model (11). We now proceeded to model macromolecular crowding with the complete model of the PTS by following a similar approach as in Ref. 11; the addition of PEG 6000 to the assay mixture was assumed to increase the on-rate constants for complex formation between the proteins and decrease the off-rate constants for complex dissociation by the same factor, $\alpha$. Comparing Fig. 2 with Fig. 1, $a$ and $b$, in Ref. 11, we see that the model agreed well with the experimental results; the addition of 9% PEG 6000 (simulated by $\alpha = 7$) stimulated the flux slightly at low protein concentrations and inhibited the flux at higher protein concentrations. A lower concentration (4.5%) of PEG 6000 (simulated by $\alpha = 4$) stimulated the flux over the whole range of protein concentrations. As was the case with PEG 6000 addition experimentally, the combined flux response coefficient ($R_{\text{PTS}}$) decreased more sharply in the model when the parameter $\alpha$ was increased (Fig. 2b); the decrease was sharper for 9% PEG 6000 and $\alpha = 7$ than for 4.5% PEG 6000 and $\alpha = 4$.

Metabolic control analysis of group transfer pathways has provided us with an analytical proof that the sum of the flux response coefficients of the proteins in a group transfer pathway can range from below 1 to 2 (12) and that values closer to 1 can result from increased complex formation between the proteins or a protein and a boundary metabolite. The absence of these complexes, on the other hand, leads to a value of 2 for this sum (39). As has been pointed out under “Discussion” of Ref. 11, decreasing $R_{\text{PTS}}$ values with increasing protein concentration...
suggest increased complex formation between the proteins of the PTS. We investigated this point further by calculating with the kinetic model, using parameter values simulating in vivo and values simulating in vitro at protein concentrations of 2 and 6 mg/ml (Table I), the fraction of the different PTS proteins that was free (uncomplexed) and the fraction that was complexed with other proteins or boundary metabolites (Table III). For each condition, the concentrations of all the species and their relative proportions are indicated. The kinetic parameters implied that the major fraction of the PTS proteins should exist in the complexed state under conditions in vitro (Table III). This was in accordance with the $R^2_{PTS}$ value close to 1 (Table II). Simulating conditions in vitro showed that the fraction of the proteins that existed in the complexed state was much lower but increased significantly with an increase in the protein concentration from 2 to 6 mg/ml. We also verified that increasing the parameter significantly with an increase in the protein concentration from existed in the complexed state was much lower but increased conditions in vitro showed that the fraction of the proteins that were free (uncomplexed) and the fraction that was complexed with other proteins or boundary metabolites (Table III).

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Parameter Sensitivity Analysis—Of course, the kinetic parameters used in the derivation of the rate constants (see “Appendix”) were subject to experimental variability. In addition, insufficient data were available in some cases to calculate the rate constants from the kinetic parameters, and assumptions had to be made (e.g. Equation 21). To establish how strongly the behavior of the model depended on the choices for the kinetic parameters, the sensitivity of the steady-state flux under conditions in vivo to changes in these parameters was calculated as outlined under “Appendix.” The sensitivity of the calculated flux to an uncertainty in a parameter value is quantified by the corresponding response coefficient.

The flux response coefficients of all model parameters (when simulating conditions in vivo) are listed in Table IV. In general, the flux response coefficients were small, indicating that the calculated flux did not depend crucially on the absolute magnitude of the chosen parameters, also when the reported literature values varied over a considerable range (e.g. $K_{\text{m}}^{\text{IIAGlc}}$ or $K_{\text{m}}^{\text{IIIV}}$). Notably, the parameters, the values of which had to be assumed altogether to solve for the rate constants of phosphotransfer reactions II–IV ($K_{\text{m}}^{\text{II}}, K_{\text{m}}^{\text{III}},$ and $K_{\text{m}}^{\text{IV}}$), had low flux response coefficients, which precludes any unusually large uncertainty in the calculated flux. The only parameters with large flux response coefficients were $K_{\text{m}}^{\text{IIAGlc}}$, $K_{\text{m}}^{\text{IICBGlc}}$, $k_{\text{V}}$, and $[\text{IICBGlc}]_{\text{total}}$ (0.5, 0.7, 0.3, 0.3, and 0.9, respectively). This was not entirely unexpected for the following reasons. First, modulations in both $K_{\text{m}}^{\text{IIAGlc}}$ and $K_{\text{m}}^{\text{IICBGlc}}$ resulted in an equal relative change in the rate constant $k_{\text{I}}$ (Equation 40), and the flux control coefficient of reaction 8 (i.e. from IIAGlc $\cdot$ P to IIAGlc and IICBGlc $\cdot$ P) in the model was high ($C_{7}^{\text{I}} = 0.6$); second, modulations in both $K_{\text{m}}^{\text{IICBGlc}}$ and $k_{\text{V}}$ resulted in an equal relative change in the rate constant $k_{10}$ (Equation 48), and the flux control coefficient of reaction 10 (i.e. from IICBGlc $\cdot$ P to IICBGlc and Glc $\cdot$ P) in the model was relatively high ($C_{10}^{\text{V}} = 0.3$); third, as shown by theory (12), both a high $C_{7}^{\text{I}}$ and a high $C_{10}^{\text{V}}$ are consistent with a high $R^2_{\text{EGP}}$, and finally, IICBGlc was the only PTS protein in the model with a high flux response coefficient, in agreement with experimental results (9, 10).
null
Kinetic Model of PTS

Simulated distribution of PTS protein species under different conditions

Steady-state calculations were performed with the parameters of Table I for conditions in vivo and two protein concentrations in vitro.

| Species          | Concentration | Percentage of total | In vivo | Concentration | Percentage of total | In vitro | Concentration | Percentage of total |
|------------------|---------------|---------------------|---------|---------------|---------------------|---------|---------------|---------------------|
| **EI species**   |               |                     |         |               |                     |         |               |                     |
| E1 · P · Pyr     | 3.1           | 61.0                | 0.12    | 0.3           | 0.9                 | 0.7     |
| E1               | 0.27          | 5.4                 | 0.01    | 0.02          | 0.02                |         |
| E1 · P           | 1.2           | 23.8                | 39.7    | 99.3          | 118                 | 98.3    |
| E1 · P · HPr     | 0.49          | 9.8                 | 0.16    | 0.4           | 1.2                 | 1.0     |
| Total E1         | 5.0           | 100                 | 40      | 100           | 120                 | 100     |
| **HPr species**  |               |                     |         |               |                     |         |               |                     |
| E1 · P · HPr     | 0.5           | 1.0                 | 0.2     | 0.04          | 1                   | 0.1     |
| HPr              | 1.3           | 2.6                 | 27      | 6.8           | 67                  | 5.5     |
| HPr · P           | 30            | 59.6                | 364     | 90.9          | 1069                | 89.1    |
| HPr · P · IIA^Glc| 18            | 36.9                | 9       | 2.2           | 63                  | 5.2     |
| Total HPr        | 50            | 100                 | 400     | 100           | 1200                | 100     |
| **IIA^Glc species** |           |                     |         |               |                     |         |               |                     |
| HPr · P · IIA^Glc| 18            | 46.2                | 9       | 2.8           | 63                  | 6.6     |
| IIA^Glc          | 0.6           | 1.6                 | 28      | 8.2           | 63                  | 6.6     |
| IIA^Glc · P      | 15            | 38.5                | 280     | 87.4          | 798                 | 53.1    |
| IIA^Glc · P · IICB^Glc| 5.5    | 13.7               | 5       | 1.6           | 37                  | 3.8     |
| Total IIA^Glc    | 40            | 100                 | 320     | 100           | 960                 | 100     |
| **IIICB^Glc species** |         |                     |         |               |                     |         |               |                     |
| IIA^Glc · P · IIICB^Glc | 5.5  | 54.7           | 5.1     | 6.3           | 37                  | 15.2    |
| IIICB^Glc        | 1.4           | 14.1                | 72      | 90.2          | 183                 | 78.4    |
| IIICB^Glc · P    | 0.1           | 1.2                 | 0.01    | 0.01          | 0.04                | 0.02    |
| IIICB^Glc · P · MeGlc| 3.0 | 29.9          | 2.8     | 3.5           | 20                  | 8.4     |
| Total IIICB^Glc  | 10            | 100                 | 80      | 100           | 240                 | 100     |
| Proteins in complexes |        | 63                | 4       | 10            |                     |         |
| Proteins uncomplexed |         | 37                | 96      | 90            |                     |         |

directly on kinetic data. Experimentally determined parameters taken from the literature were entered in the model; they were not subject to a fitting procedure. The flux calculated with the model was generally insensitive to changes in the kinetic parameters that were used in the derivation of the kinetic rate constants (Table IV), indicating that our selection of a parameter from a range of literature values, or the assumption of a value where no data were available, did not bias the result significantly. The high parameter sensitivities toward \( K^s_{\text{IIAGlc}} \) and \( k_{\text{IV}} \) (and hence to \( K^s_{\text{IIAGlc}} \) and \( k_{\text{V}} \)) were in agreement with the suggestion (9), based on the determination of \( R^s_{\text{IIICB}} \) in vivo, that the flux control coefficients of the fourth and fifth phosphotransfer reactions (from phosphorylated IIA^Glc to glucose or MeGlc) in vivo are high.

In the construction of the model, we have made some simplifications. First, we did not take into account that EI can dimerize. The dimer is the active form (43–45); the monomer–dimer transition (46, 47). Detailed studies of the monomer–dimer transition (46, 48, 49) have also shown that the monomer cannot be phosphorylated by PEP, leading to a model proposing that an active EI dimer is phosphorylated by PEP and then dissociates into two phosphorylated monomers, which subsequently pass the phosphoryl group to HPr and redimerize (1, 50, 51). This cycle was not included in the present kinetic model, since insufficient data were available on the additional rate constants involved. Moreover, at EI concentrations present in vivo, it should be virtually only dimer. Since there is no suggestion of cooperativity of the two subunits during phosphorylation, we conclude that it is valid to treat EI as a monomer for the kinetic equations presented in this paper.

Second, IIICB^Glc, the membrane-bound glucose permease, has also been shown to exist in dimeric form (52, 53), and immunoprecipitation experiments have suggested that four IIA^Glc molecules are bound to the IIICB^Glc dimer (53). We have not included this phenomenon in the kinetic model either for lack of kinetic details.

Third, the kinetic model ignores the vectorial nature and compartmentation of the uptake process. The reactions are simulated as if they occurred in a well stirred reactor; however, PTS-mediated uptake entails that extracellular glucose or MeGlc be taken up and phosphorylated to yield intracellular glucose 6-phosphate or MeGlc 6-phosphate. For modeling purposes, this should have no consequences, since there are no extracellular pools of variable metabolites. The extracellular glucose (or MeGlc) concentration is a fixed parameter, as is the intracellular glucose 6-phosphate (MeGlc 6-phosphate) concentration; it was not required, therefore, to enter the different volumes of the extracellular and intracellular compartments in the model.

A final simplification concerns the organism described. Most of the kinetic data used in the model are from E. coli, while the physiological uptake experiments with varying PTS protein levels were performed on S. typhimurium. Because of the identity of HPr and near identity of EI and IIA^Glc in the two organisms (2), this should pose no problem. Even the IIICB^Glc proteins from both organisms have very similar kinetic properties despite differences in their isoelectric points and specific activities (27). In addition, variation of intracellular IIICB^Glc levels in E. coli and S. typhimurium leads to very similar physiological responses (9, 10). We have therefore combined as much as possible of the available data on both organisms into a general model of the glucose PTS in enteric bacteria.

Calculations with the kinetic model show that under conditions in vivo the largest proportion of the PTS exists as long living transition state complexes, either between two PTS proteins and the bound phosphoryl group or between a protein, a boundary substrate, and the bound phosphoryl group (Table III). In contrast, under conditions in vitro a much larger frac-
tion is uncomplexed. This is in agreement with the control theory (12), which relates \( R_{\text{PTS}} \) values approaching 2 to the absence of protein-protein complexes and \( R_{\text{PTS}} \) values near 1 to the prevalence of these complexes in significant proportions. In fact, complexes between HPr and IIAGlc (40), as well as IIAGlc and IICBGlc (53) have been demonstrated biochemically, although both proteins were unphosphorylated. It is likely that their interaction will be much stronger if one of the two proteins is phosphorylated, since this situation obtains during the normal sequence of phosphotransfer along the PTS. Therefore, complexes between the PTS proteins may well exist for significant lifetimes in the cell.

Table III shows that only 1.6% of the total IIAGlc exists in the free unphosphorylated state when simulating MeGlc uptake in vivo. The question arises how the PTS can still regulate other systems by inducer exclusion under these conditions, because unphosphorylated IIAGlc binds stoichiometrically to its target proteins. Additional simulations (54) have shown that introduction of a step for binding of free unphosphorylated IIAGlc to a target protein leads to a redistribution of the IIAGlc forms and that a significant fraction of the total IIAGlc can bind to the target protein if the dissociation equilibrium constant is in the range of 0.2–1 \( \mu \text{M} \), as reported for glycerol kinase and IIAGlc in the presence of Zn(II) (55). However, the addition of glucose by itself to a suspension of E. coli cells (i.e. under non-inducer exclusion conditions) has been shown to lead to >95% dephosphorylation of IIAGlc after 15 s (33). How does this relate to the 39% IIAGlc·P (i.e. >5%) calculated by the model when simulating glucose (or MeGlc) uptake in vivo (Table III)? One explanation can certainly be based on the intracellular concentrations of PEP and pyruvate, and specifically their ratio, which drops from 3.0 to less than 0.1 after the cells have been challenged with glucose for 15 s and recovers to 0.2 after 30 s (33). Using these PEP and pyruvate concentrations, the model predicts IIAGlc·P to be 7% of the total IIAGlc 15 s after glucose addition (14% after 30 s), which agrees much better with the experimental results. Why did we not use the lower PEP/pyruvate ratios in our simulations? It is a well known fact that the PTS-mediated MeGlc uptake rate decreases with time (the slope of the curve decreases), and to ensure that we simulate initial uptake rates, we entered the concentrations of PEP and pyruvate in our model as they were determined just prior to glucose addition.

The second-order rate constant for phosphotransfer between HPr and IIAGlc reported by Meadow and Roseman (6) (6.1 \( \times 10^3 \text{ M}^{-1} \text{s}^{-1} \)) is much larger than the value of 6 \( \times 10^{-1} \text{ M}^{-1} \text{s}^{-1} \) reported by Misset et al. (56). The larger value is essential for obtaining the results presented here. Using the lower value of Misset et al., the simulated flux was significantly lower, and the flux response coefficients of EI and HPr were significantly higher, which did not match the experimental results (data not shown). We conclude, therefore, that the use of the second order rate constant of IIAGlc phosphorylation reported in Ref. 56 in our kinetic model yielded results that did not correspond with the strain and conditions used in the experiments determining the flux response coefficients in vivo and that the experimental improvement of measuring the phosphotransfer directly between the two proteins (6) resulted in values that improved the fit of the model to MeGlc uptake data in vivo. In contrast, the second order rate constant of EI phosphorylation by PEP (2 \( \times 10^4 \text{ M}^{-1} \text{s}^{-1} \)) reported in Ref. 47 agrees very well with the 360 \( \mu \text{M}^{-1} \text{min}^{-1} \) found by Meadow and Roseman.2

Remarkably, the effects of the macromolecular crowding agent PEG 6000 on PTS activity in vitro could be simulated with the kinetic model by assuming increased on-rates for protein-protein complex formation and decreased off-rates for its dissociation. An increased relative fraction of complexes between the proteins could in principle be achieved by increasing the on-rates for complex formation or by decreasing the off-rates for complex

### Table IV

| Parameter | PTS step | Equation | Units | Reference value | \( R^b \) |
|-----------|---------|----------|-------|-----------------|--------|
| \( K_{\text{mPEP}} \) | PEP to EI | \( p \) | \( \mu \text{M} \) | 300 | \( 3 \times 10^{-3} \) |
| \( K_{\text{mphyr}} \) | PEP to EI | \( p \) | \( \mu \text{M} \) | 2000 | \( 6 \times 10^{-3} \) |
| \( K_{\text{mIII}} \) | PEP to EI | \( p \) | Dimensionless | 1.5 | \( 4 \times 10^{-2} \) |
| \( k_I \) | PEP to EI | \( p \) | \( \mu \text{M}^{-1} \text{min}^{-1} \) | 360 | \( 2 \times 10^{-3} \) |
| \( K_{\text{mHPr}} \) | EI to HPr | \( p \) | \( \mu \text{M} \) | 7 | \( 2 \times 10^{-2} \) |
| \( k_{\text{III}} \) | EI to HPr | \( p \) | \( \mu \text{M}^{-1} \text{min}^{-1} \) | 12,000 | \( 7 \times 10^{-2} \) |
| \( K_{\text{mHPr} \cdot P} \) | EI to HPr | \( p \) | \( \mu \text{M} \) | 480 | \( 2 \times 10^{-2} \) |
| \( k_{\text{IV}} \) | EI to HPr | \( p \) | \( \mu \text{M}^{-1} \text{min}^{-1} \) | 1 | \( 3 \times 10^{-3} \) |
| \( K_{\text{III}} \) | HPr to IIA | \( p \) | \( \mu \text{M} \) | 1 | \( 3 \times 10^{-1} \) |
| \( K_{\text{IV}} \) | HPr to IIA | \( p \) | \( \mu \text{M} \) | 660 | \( 7 \times 10^{-1} \) |
| \( k_{\text{IV}} \) | HPr to IIA | \( p \) | \( \mu \text{M}^{-1} \text{min}^{-1} \) | 240 | \( 3 \times 10^{-1} \) |
| \( K_{\text{III}} \) | IICBG | \( p \) | \( \mu \text{M} \) | 1 | \( 3 \times 10^{-1} \) |
| \( k_{\text{III}} \) | IICBG | \( p \) | \( \mu \text{M}^{-1} \text{min}^{-1} \) | 20 | \( 3 \times 10^{-1} \) |
| \( K_{\text{mIICBG}} \) | IICBG to Glc | \( p \) | \( \mu \text{M} \) | 1.5 | \( 4 \times 10^{-6} \) |
| \( k_{\text{IV}} \) | IICBG to Glc | \( p \) | Dimensionless | 5.9 \( \times 10^{5} \) | \( 7 \times 10^{-6} \) |
| \( [\text{EI}]_{\text{total}} \) | | | \( \mu \text{M}^{-1} \text{min}^{-1} \) | 240 | \( 3 \times 10^{-1} \) |
| \( [\text{HPr}]_{\text{total}} \) | | | \( \mu \text{M} \) | 5 | \( 5 \times 10^{-2} \) |
| \( [\text{IIA}]_{\text{total}} \) | | | Dimensionless | 50 | \( 2 \times 10^{-2} \) |
| \( [\text{IICBG}]_{\text{total}} \) | | | \( \mu \text{M} \) | 40 | \( 2 \times 10^{-1} \) |
| \( [\text{PEP}] \) | | | \( \mu \text{M} \) | 10 | \( 9 \times 10^{-1} \) |
| \( [\text{Fumarate}] \) | | | \( \mu \text{M} \) | 2800 | \( 2 \times 10^{-2} \) |
| \( [\text{pyruvate}] \) | | | \( \mu \text{M} \) | 900 | \( 5 \times 10^{-2} \) |
| \( [\text{MeGlc}] \) | | | \( \mu \text{M} \) | 500 | \( 1 \times 10^{-2} \) |
| \( [\text{MeGlc 6-P}] \) | | | \( \mu \text{M} \) | 50 | \( 7 \times 10^{-6} \) |

2 N. D. Meadow and S. Roseman, unpublished results.
dissociation or both. Minton (57) has argued on thermodynamic grounds that the association of monomers to homopolymers should be stimulated by the addition of crowding agents mainly via an enhancement of the on-rate. As reasoned in Ref. 11, however, PEG 6000 resulted in both a stimulation and an inhibition of the flux, depending on the protein concentration, and this can only be achieved by a simultaneous effect on both the on-rate and the off-rate. Indeed, even for the simplistic assumption that the on-rate and off-rate constants are affected to the same extent (but in opposite directions) by PEG 6000 addition and furthermore that complex formation between the different PTS proteins is enhanced to the same extent, the agreement between model and experiment was remarkable when comparing the effect of two PEG 6000 concentrations with two values for the parameter \( \alpha \), *i.e.* the factor by which the rate constants are affected (cf. Fig. 1 in Ref. 11 and Fig. 2* in this paper). It may be noted that when calculating the PTS transport rate in *vivo*, we set the crowding parameter \( \alpha \) to 1, *i.e.* implying that we used the concentrations of PTS proteins on the basis of number of molecules per intracellular cytoplasmic volume without taking into account the volume exclusion effects accompanying macromolecular crowding. In parallel calculations (not shown here), we set \( \alpha \) to values different from 1. The results were closer to experimental observations on some counts but farther off on others. More *in vivo* experimental work is needed; our model may be helpful here.

The comprehensive kinetic model of the PTS presented in this paper may be used to predict the flux through the PTS for different induction levels of the glucose PTS proteins. Furthermore, the phosphorylation states of all the proteins (*i.e.* the ratio between the phosphorylated and unphosphorylated forms) can be computed, which has important implications for signaling (*e.g.* by IIA\(_{\text{Glc}}\) in inducer exclusion and the activation of adenylate cyclase). There is a distinct possibility for improvement and further validation of the model by measuring the phosphorylation state of IIA\(_{\text{Glc}}\) under different conditions, as described in Ref. 33, and comparing the results with the model predictions. We have used only flux data to assess the performance of the model in describing experimental results; inclusion of the concentrations of the phosphorylated and unphosphorylated protein forms should be an interesting and valuable addition. We have presented preliminary reports (54) on the use of the kinetic model to simulate the interaction of the signal transducer IIA\(_{\text{Glc}}\) with its target proteins under conditions that lead to the phenomena of inducer exclusion (2) and reverse inducer exclusion (58). An ambitious goal is the incorporation of the present model in a much larger model describing the glycolysis of enteric bacteria. Realization of such a project should provide interesting new insights into metabolic regulation, especially since glycolysis following PTS-mediated uptake differs from that in other organisms in that the phosphoryl donor for the initial carbohydrate phosphorylation during PTS-mediated uptake is PEP and not ATP.

The kinetic equations presented here are a first step in devising a testable model for quantifying sugar uptake by the PTS, and we have used a particular set of conditions (*i.e.* glucose-grown cells) to test the model. However, the model is adaptable to other experimental conditions, for instance with cells grown on other carbon sources or with leaky mutant PTS proteins, provided that the necessary parameters are defined. Under such conditions, it may well be that PTS proteins other than IICB\(_{\text{Glc}}\) become the major rate determinants for sugar uptake. For instance, in the extreme case, if EI were all monomer or was somehow converted to all monomer, sugar uptake would halt. The importance of the present model is that it can readily be adjusted to account for unknown factors that affect functioning of the PTS as they are characterized and their kinetic effects are determined *in vitro*. These factors can include regulators on the genetic level; *e.g.* Mcr, a negative regulator of the *ptsHI* operon and *ptsG*, has recently been proposed to bind to unphosphorylated IICB\(_{\text{Glc}}\), so that conditions leading to IICB\(_{\text{Glc}}\) dephosphorylation (glucose transport, *ptsHerr* deletion) may result in IICB\(_{\text{Glc}}\) sequestering Mcr, leading to *ptsHI* and *ptsG* activation (59).

Since signal transduction along two-component regulatory systems (14, 15) involves phosphoryl transfer similar to the PTS, our modeling results suggest that complex formation between the different signaling proteins may well be expected to occur as well. This could strongly influence their control properties in that the combined flux response, and thus the speed of signal transmission, depends on macromolecular crowding and differs between intracellular conditions and dilute solutions *in vitro*. Furthermore, this pattern of signal transfer is not limited to prokaryotes. For example, phosphoryl transfer through the components of a two-component system was demonstrated to be part of the osmosensing response in yeast (60). In addition, a chimeric protein consisting of the sensing domain of the *E. coli* aspartate receptor and the cytosolic portion of the human insulin receptor was able to activate the insulin pathway in response to aspartate (61), demonstrating the generality of the signal transfer mechanism. Therefore, the novel aspects of metabolic behavior described in this paper may also apply to eukaryotic signal transduction. Most importantly, we show here how it is possible to analyze these systems quantitatively, in order to evaluate their properties and predict their dynamic behavior in the living cell.

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**APPENDIX**

**Metabolic Control Analysis**

Metabolic control analysis is a quantitative framework originally developed by Kacser and Burns (7) and Heinrich and Rapoport (8) to quantify the control of the steady-state behavior of metabolic systems. An important entity in this analysis is the so-called flux response coefficient, which quantifies the extent to which a change in a parameter of a metabolic pathway affects the flux through that pathway and is defined mathematically as follows,

\[
R'_{pj} = \left( \frac{\partial J}{\partial p_j} \right) \frac{p_j}{J} - \left( \frac{\partial \ln J}{\partial \ln p_j} \right) \frac{p_j}{J}
\]

(Eq. 8)

where \( J \) is the steady-state flux through the pathway and \( p_j \) is the modulated parameter. Operationally, \( R'_{pj} \) can be envisaged as the percentage change in \( J \) upon a 1% increase in \( p_j \). The parameter \( p_j \) can, for example, be the concentration of an external metabolite that affects the pathway flux or the concentration of an enzyme in the pathway. When measuring a flux response coefficient, the system is allowed to relax to a new steady state after perturbation in the parameter \( p_j \) while keeping all of the other parameters \( p_k \) constant, as indicated by subscript \( p_k \).

The extent to which any catalytic component (*e.g.* an elementary step of a reaction mechanism) controls the flux is quantified by a control coefficient, which, for a step \( i \) of the system, is defined (62) as follows,

\[
C_{pi}^i = \frac{\langle \partial \ln J/\partial p_i \rangle_{p_k}}{\langle \partial \ln J/\partial p_i \rangle_{p_k}}
\]

(Eq. 9)

where \( p_i \) is any parameter that affects step \( i \) specifically. Subscript \( p_k \) indicates, as above, that the other parameters \( p_k \) remain constant and that the entire system relaxes to a new
steady state after a change in $p_i$; subscript $s_i, p_i$ indicates that
the change in the rate $v_i$ of the independent step $i$ is considered
locally at constant reactant and product concentrations (63).
For step $i$, referring to an elementary reaction in an enzyme
mechanism (as the individual phosphotransfer reactions of the
PTS), the control coefficient has also been termed the friction
coefficient (64).

**Derivation of Rate Constants for PTS Reactions**

Here we derive elementary rate constants, as shown in
Scheme 2 under “Materials and Methods,” for the five phospho-
transfer reactions of the glucose PTS from available kinetic
data for the PTS components, using the relationships in Equations
1–7. In some cases, insufficient data were available, and
additional assumptions had to be made, as indicated clearly. To
ensure that all quantities were expressed in the same units, we
consistently converted all concentrations to micromolar and all
time units to minutes.

**Phosphotransfer from Phosphoenolpyruvate to EI**—The first
phosphotransfer reaction from PEP to EI can be written sche-
nematically as follows,

\[
EI + PEP \rightleftharpoons EI \cdot P + Pyr
\]

**SCHEME 3**

\[
EI + PEP \rightleftharpoons EI \cdot P \cdot Pyr \rightleftharpoons EI \cdot P + Pyr
\]

**SCHEME 4**

where Scheme 3 shows the direct phosphotransfer and Scheme
4 includes the complex EI-·P-·Pyr explicitly.

Reported $K_m$ values of Scheme 4 (i.e. of EI for PEP) range
from 0.2 to 0.4 mM (43, 65), and values of EI·P for Pyr range
from 1.5 to 3 mM (66). Furthermore, the equilibrium constant
for the above reaction is 1.5 (44). Using a rapid quench method
as described in Ref. 6, the forward rate constant of the overall
phosphorylation reaction (i.e. $k_1$ in Scheme 3) has been deter-
mined as $6 \times 10^6$ M$^{-1}$ s$^{-1}$. Selecting values of 0.3 and 2 mM
for $K_mPEP$ and $K_mPyr$, respectively, the following set of equations
can be derived using the approaches outlined under “Materials
and Methods.”

\[
K_{mPEP} = \frac{k_1 + k_2}{k_2} = 300 \, \mu M
\]

**Eq. (10)**

\[
K_{mPyr} = \frac{k_1 + k_2}{k_2} = 2000 \, \mu M
\]

**Eq. (11)**

\[
K_{eq} = \frac{k_1}{k_{-1}k_2} = \frac{k_1k_2}{k_{-1}k_{-2}} = 1.5
\]

**Eq. (12)**

\[
k_1 = \frac{k_1k_2}{k_{-1}k_2} = 6 \times 10^6 \, \text{M}^{-1}\text{s}^{-1} = 360 \, \mu \text{M}^{-1}\text{min}^{-1}
\]

**Eq. (13)**

Equations 10–13 can be solved simultaneously for the ele-
mentary rate constants $k_1$, $k_{-1}$, $k_{-2}$, and $k_2$ of Scheme 4 as follows.

\[
k_1 = \frac{k_1[K_mK_{mPEP} + K_{mPyr}]}{K_{eq}K_{mPEP}} = 1960 \, \mu \text{M}^{-1}\text{min}^{-1}
\]

**Eq. (14)**

\[
k_{-1} = \frac{k_{-1}K_{mPyr}}{K_{m}} = 480,000 \, \text{min}^{-1}
\]

**Eq. (15)**

\[
k_2 = k_1K_{mPEP} = 108,000 \, \text{min}^{-1}
\]

**Eq. (16)**

$^3$ N. D. Meadow and S. Roseman, unpublished results.

**Phosphotransfer from EI to HPr**—The second phosphotrans-
fer reaction from phosphorylated EI to HPr can be written
schematically as follows.

\[
EI \cdot P + HPr \rightleftharpoons EI + HPr \cdot P
\]

**SCHEME 5**

\[
EI \cdot P + HPr \rightleftharpoons EI \cdot P \cdot HPr \rightleftharpoons EI + HPr \cdot P
\]

**SCHEME 6**

Again, the former reaction, Scheme 5, describes direct phos-
photransfer, whereas the latter (Scheme 6) includes the com-
plex EI·P·HPr explicitly.

Reported $K_m$ values of EI for HPr range from 2.7 to 9 μM (43,
65, 67–69); we shall assume a value of 7 μM here. The associ-
ation constant, $K_a$ for EI and HPr is $10^5$ M$^{-1}$ (abstract cited in
Ref. 1), although this most probably refers to the interaction
between unphosphorylated EI and HPr. The interaction be-
tween a phosphorylated and an unphosphorylated protein is
likely to be stronger than that between two unphosphorylated
proteins, since the former situation obtains during the normal
phosphotransfer reaction sequence. Recently, the rate con-
stants of the overall phosphorylation reaction (Scheme 5) were
determined directly using the rapid quench method (see above):

\[
k_{-2} = 2 \times 10^6 \, \text{M}^{-1}\text{s}^{-1} \quad \text{and} \quad k_{-1} = 8 \times 10^6 \, \text{M}^{-1}\text{s}^{-1}
\]

The equilibrium constant for phosphotransfer from EI to HPr,
as calculated from $k_{-2}$ and $k_{-1}$, is 25; this value is higher than the
2.3–8.9 reported previously (44). Because of their direct deter-
mination, we shall use the above values for $k_{-2}$ and $k_{-1}$ and 25 for
$K_{eq}$. The combined equilibrium constant for the first two
PTS reactions (i.e. phosphotransfer from PEP to HPr) is $1.5 \times
25 = 37.5$ when proceeding from the measured rate constants,
which likewise exceeds the experimental value of 10.8 ± 7.7
reported previously (44).

We can derive the following set of equations.

\[
K_{mHPr} = \frac{k_3 + k_4}{k_3} = 7 \, \mu M
\]

**Eq. (18)**

\[
k_{-2} = \frac{k_4k_4}{k_3k_3} = \frac{2 \times 10^6 \, \text{M}^{-1}\text{s}^{-1}}{12,000 \, \mu \text{M}^{-1}\text{min}^{-1}} = 1 \, \mu \text{M}^{-1}\text{min}^{-1}
\]

**Eq. (19)**

\[
k_{-1} = \frac{k_3k_4}{k_3 + k_4} = \frac{8 \times 10^6 \, \text{M}^{-1}\text{s}^{-1}}{480 \, \mu \text{M}^{-1}\text{min}^{-1}}
\]

**Eq. (20)**

Equations 18–20 contain insufficient information to solve for
the rate constants $k_3$, $k_{-2}$, $k_4$, and $k_{-4}$ unambiguously. Some
additional choice has to be made for $k_3$, $k_{-2}$, or $k_{-4}$. For ex-
ample, fixing the association equilibrium constant $K_a$ of EI-P
and HPr allows calculation of all of the rate constants. We assumed
the association of EI-P and HPr to be 10-fold stronger that of
unphosphorylated EI and HPr,

\[
k_{-1} = k_2k_3 = 10^6 \, \text{M}^{-1} = 1 \, \mu \text{M}^{-1}
\]

**Eq. (21)**

This yields the following rate constants for the elementary
steps in Scheme 6.

\[
k_3 = \frac{K_{mHPr}K_{mHPr}k_{-1}}{K_{mEI}K_{mHPr} - 1} = 14,000 \, \mu \text{M}^{-1}\text{min}^{-1}
\]

**Eq. (22)**

\[
k_{-3} = \frac{K_{mHPr}k_{-1}}{K_{mEI}K_{mHPr} - 1} = 14,000 \, \text{min}^{-1}
\]

**Eq. (23)**
Phosphotransfer from HPr to IIAGlc—The third phosphotransfer reaction from phosphorylated HPr to IIAGlc can be written schematically as follows.

\[
\text{HPr} \cdot P + \text{IIAGlc} \xrightarrow{k_{\text{III}}} \text{HPr} \cdot \text{IIAGlc} \cdot P
\]

\text{SCHEME 7}

\[
\text{HPr} \cdot P + \text{IIAGlc} \xrightarrow{k_{5}} \text{HPr} \cdot \text{IIAGlc} \cdot P
\]

\text{SCHEME 8}

As above, Scheme 7 shows the direct phosphotransfer, and Scheme 8 includes the direct HPr \cdot P \cdot \text{IIAGlc} explicitly. Values of 0.3 \mu M (69) and 2.7 \mu M (56) have been reported for the \(K_m^*\) of IIAGlc for HPr-P. The association constant \(K_a\) for HPr and IIAGlc was measured as 10^4 to 10^5 M^{-1} (40); however, this was measured for unphosphorylated HPr, not HPr-P. As for the interaction between EI and HPr, the \(K_a\) of HPr-P and IIAGlc will most probably be larger than that for unphosphorylated HPr and IIAGlc because HPr-P donates a phosphoryl group to IIAGlc during the normal phosphotransfer reaction sequence. The rate constants for the overall phosphorylation reaction (Scheme 7) have been published: \(k_{\text{III}} = 6.1 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}\) and \(k_{-\text{III}} = 4.7 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}\) (6). The equilibrium constant for phosphotransfer from HPr to IIAGlc, as calculated from \(k_{\text{III}}\) and \(k_{-\text{III}}\), is 1.3; this value is 10-fold higher than the 0.13 reported previously (70).

Assuming a value of 1.2 \mu M for the \(K_m^*\) of IIAGlc for HPr \cdot P and using the directly determined rate constants, we can derive the following set of equations.

\[
K_{\text{III}} = \frac{k_{5}k_{6}}{k_{5} + k_{6}} = 1.2 \mu M
\] (Eq. 26)

\[
k_{-\text{III}} = \frac{k_{5}k_{6}}{k_{5} + k_{6}} = 4.7 \times 10^7 \text{ M}^{-1} \text{ s}^{-1} = 2820 \mu M^{-1} \text{ s}^{-1}
\] (Eq. 27)

As was the case for phosphotransfer between EI and HPr, Equations 26–28 contain insufficient information to solve for the rate constants \(k_{5}, k_{-5}, k_{5},\) and \(k_{-6}\) unambiguously. We have assumed additionally a value of 1 \mu M^{-1} for the association constant of HPr \cdot P and IIAGlc. This is identical to the association constant of EI \cdot P and HPr, and 10–100-fold larger than the association constant of unphosphorylated HPr and IIAGlc (see above). With the additional assumption,

\[
K_{\text{II}} = k_{5}k_{-5} = 1 \mu M^{-1}
\] (Eq. 29)

and using Equations 26–28, the following rate constants for the elementary steps in Scheme 8 were calculated.

\[
h_{5} = \frac{K_{\text{III}}k_{5}k_{6}K_{\text{III}}}{K_{\text{III}}K_{\text{III}}K_{\text{III}} + 1} = 21,960 \mu M^{-1} \text{ s}^{-1}
\] (Eq. 30)

\[
h_{-5} = \frac{K_{\text{III}}k_{5}k_{6}K_{\text{III}}}{K_{\text{III}}K_{\text{III}}K_{\text{III}} + 1} = 21,960 \mu M^{-1} \text{ s}^{-1}
\] (Eq. 31)

\[
h_{6} = \frac{K_{\text{III}}k_{5}k_{6}K_{\text{III}}}{K_{\text{III}}K_{\text{III}}K_{\text{III}} + 1} = 4392 \mu M^{-1} \text{ s}^{-1}
\] (Eq. 32)

\[
h_{-6} = \frac{K_{\text{III}}k_{5}k_{6}K_{\text{III}}}{K_{\text{III}}K_{\text{III}}K_{\text{III}} + 1} = 3384 \mu M^{-1} \text{ s}^{-1}
\] (Eq. 33)

However, we have reservations about both published values for \(K_m^*\). In order for the assay conditions in the paper by Reizer et al. (69) to measure the \(K_m^*\) of the reaction that the authors claim, all of the HPr must have been phosphorylated, whereas all of the IIAGlc must have been dephosphorylated. Whereas the former condition may well have been true, one cannot assume that the latter condition always held, because of (a) the speed of the HPr-P-IIAGlc phosphotransfer reaction and (b) the low concentrations of IIAGlc used in comparison with the higher HPr concentrations. In the paper by Misset et al. (56), the fact that no IIAGlc was added to the assays calls into question just which PTS was being measured, especially in the light of the structural similarity and functional exchangeability of the IIa domains of various sugar-specific systems (see e.g. Refs. 71 and 72). For these reasons, we also modeled phosphoryl transfer between HPr-P and IIAGlc as a direct reaction (Scheme 7), using the published values of \(k_{\text{III}}\) and \(k_{-\text{III}}\), and omitting \(K_{\text{III}}\) (Eq. 26) as well as the assumption of \(K_m^*\) (Equation 29). The modeling results did not differ significantly from the ones presented in Figs. 1 and 2 (data not shown). For reasons of consistency, we therefore modeled all five phosphotransfer reactions as proceeding via an explicit transition state complex.

Phosphotransfer from IIAGlc to IICBGlc—The fourth phosphotransfer reaction from phosphorylated IIAGlc to IICBGlc can be written schematically as follows.

\[
\text{IIAGlc} \cdot P + \text{IICBGlc} \xrightarrow{k_{\text{IV}}} \text{IIAGlc} + \text{IICBGlc} \cdot P
\]

\text{SCHEME 9}

\[
\text{IIAGlc} \cdot P + \text{IICBGlc} \xrightarrow{k_{-\text{IV}}} \text{IIAGlc} \cdot P + \text{IICBGlc}
\]

\text{SCHEME 10}

Scheme 9 shows the direct phosphotransfer, and Scheme 10 includes the transition state complex IIAGlc-P-IICBGlc explicitly. The \(K_m^*\) of IICBGlc for IIAGlc-P ranges from 1.7 to 5 \mu M (22, 69, 70, 73). Recently, the rate constants of the overall phosphorylation reaction (Scheme 9) were determined directly using the rapid quench method (see above): \(k_{\text{IV}} = 1.1 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}\) and \(k_{-\text{IV}} = 4 \times 10^6 \text{ M}^{-1} \text{ s}^{-1}\); however, this is 10-fold higher than the 0.13 reported previously (70).

Assuming a value of 1.2 \mu M for the \(K_m^*\) of IIAGlc for HPr \cdot P and using the directly determined rate constants, we can derive the following set of equations.

\[
K_{\text{IIAGlc-P}} = \frac{k_{5} + k_{6}}{k_{5}} = 4 \mu M
\] (Eq. 34)

\[
k_{-\text{IV}} = \frac{k_{5}k_{6}}{k_{5} + k_{6}} = 1.1 \times 10^7 \text{ M}^{-1} \text{ s}^{-1} = 660 \mu M^{-1} \text{ s}^{-1}
\] (Eq. 35)

\[
k_{-\text{IV}} = \frac{k_{5}k_{6}}{k_{5} + k_{6}} = 4 \times 10^6 \text{ M}^{-1} \text{ s}^{-1} = 240 \mu M^{-1} \text{ s}^{-1}
\] (Eq. 36)

No additional literature data were found. In order to solve for the rate constants \(k_{5}, k_{-5}, k_{5},\) and \(k_{-6}\), one further independent equation (i.e., one assumption) is needed as above. We assumed an association constant of 1 \mu M^{-1} for IIAGlc-P and IICBGlc (identical to the association constants of EI \cdot P and HPr and of HPr \cdot P and IIAGlc). This yielded the following additional equation,

\[
k_{\text{IV}} = k_{5}k_{6}K_{\text{IIAGlc-P}} = 880 \mu M^{-1} \text{ s}^{-1}
\] (Eq. 38)
**Kinetic Model of PTS**

$$k_7 = \frac{k_{IV}K_{MEG}}{K_{MEG} + V_{IV}} = 880 \text{ min}^{-1} \quad (\text{Eq. 39})$$

$$k_9 = \frac{k_{IV}K_{MEG}}{K_{MEG} + V_{IV}} = 2640 \text{ min}^{-1} \quad (\text{Eq. 40})$$

$$k_{10} = \frac{k_{IV}K_{MEG}}{K_{MEG} + V_{IV}} = 960 \text{ } \mu\text{M}^{-1} \text{ min}^{-1} \quad (\text{Eq. 41})$$

**Phosphotransfer from IICBGlc to Glucose**—The fifth and final phosphotransfer reaction from phosphorylated IICBGlc to glucose (Glc) can be written schematically as follows.

$$\text{IICBGlc} \cdot \text{P} + \text{Glc} \rightleftharpoons \text{IICBGlc} + \text{Glc} \cdot \text{P}$$

**Scheme 11**

Using 20 \( \mu\text{M} \) for the \( K_a \) of IICBGlc for glucose and the \( K_{eqV} \) and \( k_V \) values above, the following set of equations,

$$K_{eqV} = \frac{k_{-3} + k_{10}}{k_9} = 20 \text{ } \mu\text{M} \quad (\text{Eq. 42})$$

$$K_{Glc} = \frac{k_{-7} + k_{10}}{k_9} = 1.5 \mu\text{M} \quad (\text{Eq. 43})$$

$$k_V = \frac{k_{10}}{k_{-7} + k_{10}} = 5.9 \times 10^4 \quad (\text{Eq. 44})$$

was solved simultaneously to calculate the rate constants of the elementary steps in Scheme 12,

$$k_9 = \frac{k_{10}K_{Glc}}{K_{eqV} - K_{Glc}} = 260 \text{ } \mu\text{M}^{-1} \text{ min}^{-1} \quad (\text{Eq. 46})$$

$$k_{-7} = \frac{k_{10}K_{Glc}K_{eqV}}{K_{eqV} - K_{Glc}} = 389 \text{ min}^{-1} \quad (\text{Eq. 47})$$

$$k_{10} = \frac{k_{10}K_{Glc}}{K_{eqV} - K_{Glc}} = 4800 \text{ min}^{-1} \quad (\text{Eq. 48})$$

**Parameter Sensitivity Analysis**

The flux response coefficients toward the model parameters in Table IV were calculated as follows. Consider, as an example, \( R_{K_{eqV}}^I \) (i.e. the parameter sensitivity toward \( K_{eqV} \)). From Equation 8 we have the following,

$$R_{K_{eqV}}^I = \left( \frac{\delta \ln \phi}{\delta \ln K_{eqV}} \right)_{\phi}$$

$$= R_k^I \left( \frac{\delta \ln k_1}{\ln K_{eqV}} \right)_{\phi} + R_k^I \left( \frac{\delta \ln k_2}{\ln K_{eqV}} \right)_{\phi} + R_k^I \left( \frac{\delta \ln k_3}{\ln K_{eqV}} \right)_{\phi}$$

$$\quad + R_k^I \left( \frac{\delta \ln k_4}{\ln K_{eqV}} \right)_{\phi}$$

$$= \frac{-2000}{1.5 \times 300 + 2000} = -0.82 \quad (\text{Eq. 52})$$

The other derivatives were calculated similarly, enabling determination of the flux response coefficient \( R_{K_{eqV}}^I \) using Equation 50. The other parameter sensitivities in Table IV were obtained by following a similar approach.

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