Rate and Equilibrium Constants for Phosphoryltransfer between Active Site Histidines of *Escherichia coli* HPr and the Signal Transducing Protein III*Glc*  

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The bacterial phosphoenolpyruvate:glycose phosphotransferase system (PTS) plays a central role in catabolizing many sugars; regulation is effected by phosphorylation of PTS proteins.

In *Escherichia coli*, the phosphoryltransfer sequence for glucose uptake is: PEP → Enzyme I(His51) → HPr(His15) → III*Glc*(His89) → II*Glc*(Cys421) → glycose. A rapid quench method has now been developed for determining the rate and equilibrium constants of these reactions.

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$ k_1 = 6.1 \times 10^7 \text{m}^{-1} \text{s}^{-1}, \ k_{-1} = 4.7 \times 10^5$ for the mutant II*Glc*/III*Glc*, $k_1 = 2.8 \times 10^5 \text{m}^{-1} \text{s}^{-1}, \ k_{-1} = 2.3 \times 10^5$. The derived $K_{eq}$ values agreed with the $K_{eq}$ obtained without use of the rapid quench apparatus. $K_{eq}$ for both reactions is 1–1.5.

The rate of phosphoryltransfer between HPr and wild type III*Glc* is close to a diffusion-controlled process, while the reactions involving the mutant II*Glc*/III*Glc* are 200-fold slower. These rate differences are explained by an hypothesis for the mechanism of phosphoryltransfer between HPr and III*Glc* based on the structures of mutant and wild type proteins (see Pelton et al. (1996) J. Biol. Chem. 271, 33446–33456).

The bacterial phosphoenolpyruvate:glycose phosphotransferase system (PTS) comprises at least two dozen cytoplasmic and membrane proteins. Among the diverse functions governed by the PTS is the translocation of PTS sugars across the cell membrane concomitant with their phosphorylation, and regulation of the expression of several non-PTS sugar operons (for reviews see Refs. 1–6).

In *Escherichia coli*, the phosphoryltransfer sequence for transport via the glucose-specific system is as follows: PEP → Enzyme I(His51) → HPr(His15) → III*Glc*(His89) → II*Glc*(Cys421) → glycose.

While this system has been extensively studied since discovery of the PTS (7), many important questions remain to be answered. For example, the complete, balanced equations are written as second order reactions, assuming binary complexes as the transition state intermediates, i.e. phospho-A + B = A-phospho-B = A + phospho-B. It is possible, however, that transient ternary, or possibly even quaternary complexes are formed during the reactions. Furthermore, the mechanisms of phosphoryltransfer are unknown, as are the rate-limiting steps during the transfer from PEP to glucose, the rates in the reverse direction and the equilibrium constants for each step in the pathway.

As a first approach to answering these questions, this paper describes a rapid quench method for determining the apparent rate and equilibrium constants of the phosphoryltransfer reactions. The protein pair HPr and III*Glc* was selected for initial studies for several reasons. First, phospho-HPr is a central branch point in phosphoryltransfer by the PTS, but it is very labile (8), and the technical problems encountered in working with this phosphoprotein had to be solved to collect meaningful data. Second, III*Glc* is a critical signal transducing protein in bacterial metabolism. It interacts with at least 10 other proteins, some by phosphoryltransfer reactions, and others by non-covalent binding (6); the latter are involved in regulating gene expression, which depends on the ratio of III*Glc* to phospho-III*Glc*. Third, important active site mutants of III*Glc* are available for comparative studies. Finally, in the only study on the phosphoryltransfer between HPr and III*Glc* (9), the technology available at the time gave only an estimate of the apparent $K_{eq}$.

*E. coli* III*Glc*, also designated IIAGlc*, is an 18.1-kDa protein containing two histidine residues, His75 and His76, in the active site. His76 accepts the phospho group from phospho-HPr, but His75 is conserved in many III or IIA type proteins (see Refs. 1–6 for reviews). The physiological significance of the His residues was tested by substituting glutamine for each His, giving the mutant proteins II*Glc*/III*Glc* and IIAGlc*/III*Glc*, respectively (10). The mutants exhibited some unexpected physiological and biochemical properties. For example, II*Glc*/III*Glc* accepted the phospho-glucose permease, the Enzyme II complex is designated II*Glc*/III*Glc* or III*Glc*/II*Glc* in the first system, and IIAGlc*/III*Glc* in the second.

**References**

1. The bacterial phosphoenolpyruvate:glycose phosphotransferase system, or PTS, comprises a group of cytoplasmic and membrane proteins with diverse functions in the bacterial cell (see Refs. 1–6 for reviews). The two proposed systems of nomenclature of the PTS have been reviewed (6). In brief, the general proteins of the system (not sugar-specific) are Enzyme I and HPr. The sugar-specific Enzyme II complexes comprise from one to as many as four (five?) separately encoded polypeptides, some or all of which are integral and peripheral membrane proteins. The proposed systems of nomenclature for the Enzyme II complexes are based on either the number of separate proteins in the complex, or, alternatively, on the function of the protein or protein domain in the phosphoryltransfer sequence. In the case of the *E. coli* glucose permease, the Enzyme II complex is designated III*Glc*/II*Glc* or III*Glc*/II*Glc* in the first system, and IIAGlc*/III*Glc* in the second.

2. The abbreviations used are: PTS, phosphoenolpyruvate:glycose phosphotransferase system; PEP, phosphoenolpyruvate; HPLC, high performance liquid chromatography.
structures are presented in the accompanying paper (11). It appeared possible that clarification of this apparent anomaly might be obtained by kinetic analysis of the phosphoryltransfer reactions and a detailed structural study of the proteins; the structures are presented in the accompanying paper (11).

We report here the apparent equilibrium constants ($K_{eq}$), the forward ($k_+$), and the reverse ($k_-$) rate constants for the transfer of the phosphoryl group between phospho-HPr and H75QIIIGlc, and between phospho-HPr and the mutant H75QIIIGlc.

These results, along with the structural information, suggest a mechanism for the phosphoryltransfer reaction (see accompanying paper; Ref. 11).

MATERIALS AND METHODS

Bacterial Strains—The mutant crr genes that encode H75QIIIGlc and H75QIIIGlc were constructed by site-directed mutagenesis as described (10), and transferred to the overproducing plasmid, pVEX-11 (12). The plasmids were used to transform E. coli BL21 (DE3) in which a kanamycin resistance cartridge was substituted for the coding region of the crr gene by Dr. Cing-Yuen Wong, to whom we are most grateful. In brief, the procedure was as follows. The plasmid pDS45 (13) was used as the source of the crr gene, which was precisely excised using the method of Kunkel et al. (14); after enlarging the size of the flanking region by ligating in a fragment of the gene for Enzyme I from plasmid pDS20 (13), the kanamycin resistance gene from pUC4-KIXX (Pharmacia Biotech Inc.) was ligated into the plasmid. The Km$^r$ gene was integrated by homologous recombination into the chromosome of E. coli V355 (recD1 1014) (15) by transformation, and, finally, E. coli strain BL21 (DE3) was transduced to kanamycin resistance using phage P1, creating strain CYW 14. III$^{Glc}$, the product of the crr gene, was not detected in extracts of CTY 14, using a sensitive, immunological rocket assay (9).

Purification of Proteins—Previously published methods were used to purify HPr (16) and wild type III$^{Glc}$ (12). H75QIIIGlc and H75QIIIGlc were purified by the same procedure used for wild type III$^{Glc}$. Enzyme I from E. coli was purified from an overproducing strain (17) by a modification of the method used for III$^{Glc}$. The cells were grown as described (17) in 3 liters of LB broth. The treatment of the cell suspension after harvesting was as described, except that, after pretreating all containers and the entire quench apparatus with albumin (1 mg/ml, Sigma, crystalline) to the reaction mixtures, and by adding 40 mM MgCl$_2$, 1 mM EDTA, 0.2 mM dithiothreitol, and 40 mM L-aminocaproic acid. This procedure yielded 200 mg of homogeneous Enzyme I as determined by densitometric scanning of SDS-polyacrylamide gels; the specific activity of the enzyme was the same as preparations made by the original method (19).

The experiments described below were conducted with a minimum of two independent preparations of each of the proteins. One sample of HPr was a generous gift from Dr. E. B. Waygood (University of Sask.

**FIG. 1.** Gel filtration chromatography of quenched reaction mixtures. An aliquot (200 µl) of a quenched reaction was chromatographed as described under “Experimental Procedures”; the column was first calibrated with the indicated standards.

**FIG. 2.** Time course of the phosphoryltransfer reaction between HPr and wild type III$^{Glc}$. A, $[^{32}P]$HPr + III$^{Glc}$ were mixed as described under “Experimental Procedures.” The data for this experiment appear in row 2 of Table I. B, $[^{32}P]$HII$^{Glc}$ + HPr. The data are shown in row 4 of Table I. ñ, time points from the rapid quench apparatus; •, hand-mixed time point.

katchewan, Saskatoon, Saskatchewan, Canada).

Protein Concentration—Three methods were used for determining the concentrations of the reactant proteins in the rapid quench experiments. (a) The colorimetric protein assay of Markwell et al. (20) was calibrated with protein standards whose concentrations were determined by Trudy Carr of this department using the Co fringe counting method in a Beckman model E analytical ultracentrifuge. (b) The lactate dehydrogenase-coupled assay (21) measures the quantity of pyruvate produced when a PTS protein is phosphorylated by PEP; since the reaction stops when the protein is fully phosphorylated, this method measures the amount of phosphorylatable protein in the assay. (c) In the very dilute solutions used for the rapid quench experiments, the concentration of the phosphoryl donor protein was determined from the specific activity of the $[^{32}P]$PEP used for its preparation, as was the quantity of $[^{32}P]$phosphoprotein product formed in the reaction.

Because the phosphoryltransfer reaction is so rapid, very dilute solutions of the proteins were used, and a serious possible source of error was the loss of protein during the quench experiment and on the HPLC column. A series of experiments was therefore undertaken to measure the recovery of $[^{32}P]$-labeled proteins through the entire procedure. Adsorption was, in fact, a problem but was resolved by adding albumin (1 mg/ml, Sigma, crystalline) to the reaction mixtures, and by pretreating all containers and the entire quench apparatus with albumin-containing solutions.

Under these conditions, protein concentrations were determined within ±10%.

Synthesis of $[^{32}P]$PEP.—The method of Roossien et al. (22) which utilizes $[^{32}P]$ATP as the starting material, was modified by reducing the quantity of all components to 15% of their original values except for the pyruvate kinase; the mixture was incubated for 15 min at 30 °C. All subsequent steps were conducted as previously reported. The resulting


TABLE I

| Initial concentration | [32P]HPr | [32P]IIIGlc | [32P]HPr | [32P]IIIGlc |
|-----------------------|---------|------------|---------|------------|
| HPr                   | 18      | 32         | 42      | 0          |
| 4.2                   | 9.4     | 86         | 0       | 0          |
| 12                    | 0       | 6.8        | 5.1     | 0          |
| 45                    | 0       | 31.9       | 7.3     | 0          |
| 20                    | 0       | 15         | 8.2     | 0          |

Constants for Phosphoryltransfer between HPr and III\textsubscript{Glc}

| $k_1$ | $k_{-1}$ | $K_{eq}$ |
|-------|----------|----------|
| 7.1   | 3.4      | 2.1      |
| 7.0   | 6.7      | 1.0      |
| 6.6   | 3.4      | 1.9      |
| 5.0   | 5.8      | 0.9      |
| 5.0   | 4.0      | 1.3      |

Mean ± S.D. 6.1 ± 0.6 4.7 ± 1.3 1.4 ± 0.5

FIG. 3. Time course of the phosphoryltransfer reaction between HPr and H75QIIIGlc. A. $^{[32P]}$HPr + H75QIII\textsubscript{Glc}. The data are shown in row 2 of Table II. B. $^{[32P]}$H75QIII\textsubscript{Glc} + HPr. The data are given in row 4 of Table II. c, time points from the rapid quench apparatus; ▲, hand-mixed time points.

[32P]PEP contained approximately 0.8 mCi of radioactivity in 10 nmol of PEP.

The specific activity of the $^{[γ-32P]}$ATP used in the synthesis of [32P]PEP was 1.1 × 10\textsuperscript{8} GBq/mol, and after rigorous purification, the product was diluted with unlabeled PEP to about 1.0 × 10\textsuperscript{8} GBq/mol for phosphorylating the proteins. PEP concentrations were determined by the pyruvate kinase assay (23), which were reproducible to 4%. Hydrolysis of the [32P]PEP during storage at 4 °C and pH 7.6 was measured by thin-layer chromatography on polyethyleneimine-cellulose plates (Baker-flex cellulose PEI-F) developed with 2 \text{m} sodium formate, pH 3.4, and autoradiographed. The rate of hydrolysis was 0.16%/day.

The errors of the methods therefore yield an estimated error of <5% for the specific activities of the [32P]PEP.

Preparation of $^{[32P]}$HPr and $^{[32P]}$III\textsubscript{Glc}—Reaction mixtures contained (final volumes, 0.3 ml): 5 nmol of the protein to be phosphoryl-

ated, a 4–7-fold excess of [32P]PEP (adjusted to the desired specific activity), 5 mM MgCl\textsubscript{2}, 50 units of Enzyme I, 1 mg/ml albumin, 0.35 M triethylamine bicarbonate, pH 7.6. For phosphorylating III\textsubscript{Glc}, 0.2 nmol of HPr was added to serve as a catalyst, and the mixtures were incubated for 15 min at room temperature. An HPLC Pharmacia Superose 12HR 10/30 column was used to separate the proteins, giving about the same resolution as the Superdex column described below. The Superose column was equilibrated and eluted with a mixture of 17 mM KHCO\textsubscript{3}, 3 mM K\textsubscript{2}CO\textsubscript{3}, pH 9.5, and 1 mg/ml bovine serum albumin, at a flow rate of 0.5 ml/min. Peak fractions containing the phosphorylprotein were pooled and stored at −70 °C.

Rapid Quench Apparatus—An Update Instrument Co. (Madison, WI) syringe drive apparatus and two Wiskind mixers were used (24–26). It was essential to maintain constant ram speed and displacement, and to know their precise values. In recent models of the instrument, the ram is computer-controlled. In our instrument, the original model 1501/1502 motor control circuitry was replaced with an Intel 486-based computer control designed and written in C source code by Dr. Charles Long (Department of Chemistry, The Johns Hopkins University, Baltimore, MD). The original model 1500 controllable power supply was retained. This modification provided a continuous profile of the ram velocity and a computation of average ram velocity immediately following each experiment.

A set of reaction hoses was prepared from PEEK plastic tube (Upchurch Scientific) and calibrated (27) by measuring the rate of p-nitrophenyl acetate hydrolysis (data not shown).

Rapid Quench Experiments—In these experiments, the phosphoryl donor was injected into the first Wiskind mixer simultaneously with the acceptor protein. The mixture was then passed through a calibrated reaction hose and entered the second mixer at the same time as the quench solution; the quenched reaction mixture was collected for analysis. Equal volumes of each solution were injected into the apparatus using 1-ml Hamilton syringes (model 1001, C style; Hamilton Corp., Reno, NV). The solutions all contained 1 mg/ml albumin, and were as follows. (a) The donor $^{32P}$-labeled protein was diluted to the desired concentration with the bicarbonate, carbonate buffer used for its elution from the Superose 12 10/30 column; (b) the phosphoryl-acceptor protein was diluted with a solution containing potassium phosphate buffer (0.1 M, pH 6.5), 1 mM EDTA, and 5 mM MgCl\textsubscript{2}; (c) the quench solution was 5 \text{m} urea, 3 \text{m} KOH.

The efficiency of the quenching fluid was determined by mixing each of the proteins or phosphoproteins with quench fluid followed by the relevant second proteins. These experiments showed that the quench solution stopped the phosphoryltransfer reaction within 5 ms. These quench premix values, although small, were subtracted from the data before the simulations were performed.

Unless otherwise stated, the ram speed was 15 mm/s, so that equal volumes of the protein solutions were driven through the mixer at flow rates of 500 \text{µl/s} in the first mixing chamber; the reaction mixture and quench solution were mixed at 750 \text{µl/s} in the second chamber. To obtain reaction times longer than 0.6 s, a calibrated hose of 350 \text{µl/s} was added to the second chamber. To obtain reaction times longer than 0.6 s, a calibrated hose of 350 \text{µl/s} was quantitatively filled with reactants at a flow rate of 500 \text{µl/s}, the ram was then paused for the desired length of time, and the contents of the hose were then quantitatively expelled with a second push of the ram. Typically, the distance of the pushes ranged from 17 to 25 mm, and the syringes delivered 16.7 \text{µl/mm}, sufficient to obtain about 600 \text{µl} of quenched reaction mixture. Experiments were performed at ambient temperature, which ranged from 25 to 26.5 °C during the course of this work. Quenched reaction mixtures were immediately frozen on dry ice and stored at −70 °C.

Each frozen mixture was thawed immediately before injecting a 200-\text{µl} aliquot onto an HPLC Pharmacia Superdex 75HR 10/30 column, equilibrated with 3 \text{m} urea, 35 mM Na\textsubscript{2}PO\textsubscript{4}, pH 12.1. A Waters model 590 pump was used to operate the column at flow rates of 0.5–1.0 \text{ml/min} of the urea, phosphate buffer. Fractions were counted in a Packard 2200CA liquid scintillation counter using Packard UltimaGold XR scintillation mixture. The resolution obtained by the column is shown in Fig. 1, and the recovery of $^{[32P]}$ injected onto the column was at least 95%. It is important to note that the column resolves $^{[32P]}$PP, [32P]ATP, and [32P]PEP.
Results. However, when the mutant H75QIIIGlc is used, the rate is much higher than the hydrolysis of phospho-HPr does not significantly affect the quench experiments. The solutions were preincubated for 5 min at pH 7.5 (8). Phosphoryltransfer is so rapid between wild type IIIGlc and HPr that the transfer of the phosphoryl group from phospho-HPr to IIIGlc or from HPr to phospho-IIIGlc is shown in Fig. 2.

The transfer of the phosphoryl group from phospho-IIIGlc to HPr occurred while the solutions were in the syringes or after they were mixed, and the extent of hydrolysis at each time point.

Fig. 4. Effect of flow rate into the first mixing chamber on the rate of phosphoryltransfer. [32P]HPr + III\textsuperscript{H75Q}Glc. Flow rate: +, 270 \( \mu \text{l/s} \); \( \Theta \), 500 \( \mu \text{l/s} \); \( \Delta \), 700 \( \mu \text{l/s} \).

from the proteins, and we could therefore determine whether hydrolysis occurred while the solutions were in the syringes or after they were mixed, and the extent of hydrolysis at each time point.

[32P]Phospho-HPr spontaneously hydrolyzes at significant rates on the minute time scale at the pH used for these experiments, or even at pH 7.5 (8). Phosphoryltransfer is so rapid between wild type III\textsuperscript{H75Q} and HPr that the hydrolysis of phospho-HPr does not significantly affect the results. However, when the mutant H75QIIIGlc is used, the rate is much slower, and the simulated kinetics included a step for the hydrolysis of phospho-HPr. The rate constant that gave the best fit for this hydrolysis was \( 3 \times 10^{-4} \text{ M}^{-1} \text{s}^{-1} \), which agrees with the published value (8).

Rate constants for the forward and backward transfer reactions were estimated from the experimental data using the kinetic simulation program, KINSIM (28) as modified by Anderson et al. (29), which allows entry of experimental data collected at unequal intervals of time.

**Determination of Equilibrium Constants**—Apparent \( K_{\text{eq}} \) constants were obtained in two ways. The ratios of the rate constants, determined independently in each direction, gave one value. A second value was obtained without using the rapid quench apparatus, i.e., by hand mixing samples.

In the hand-mixed experiments, solutions containing [32P]HPr and either IIIGlc or H75QIIIGlc were prepared as described for the rapid quench experiments. The solutions were preincubated for 5 min at 25 °C, mixed, and incubated for 30 s (wild type) or for 5 min (H75QIIIGlc), at which time a one-third volume of quench solution was added and the samples frozen until analyzed. The distribution of radioactivity between HPr and IIIGlc was determined by the HPLC method. It is important to emphasize that the hand mixed experiments utilized much higher concentrations of proteins than employed in the rapid quench studies.

**RESULTS**

**Rate of Phosphoryltransfer from [32P]Phospho-HPr to IIIGlc and from [32P]Phospho-IIIGlc to HPr**—A typical progress curve for the transfer of the phosphoryl group from phospho-HPr to wild type III\textsuperscript{H75Q} is shown in Fig. 2A. The reverse reaction is shown in Fig. 2B. The data in each direction fit a model of second order kinetics over the entire time course.

A summary of several such experiments is presented in Table I. It should be emphasized that there was as much as a 10-fold difference in initial concentrations of some of the proteins used in these experiments. Nevertheless, excellent agreement was obtained in the different experiments, with a maximum variation of 2 for the derived rate constants. Most importantly, there is excellent agreement between the rate constants obtained by starting the reaction with phospho-HPr and III\textsuperscript{H75Q} or with HPr and phospho-IIIGlc.

**Rate of Phosphoryltransfer from [32P]Phospho-HPr to IIIGlc and from [32P]Phospho-H75QIIIGlc to HPr**—Progress curves for the phosphoryltransfer between HPr and the H75QIIIGlc mutant are shown in Fig. 3. As with the wild type proteins, the reactions fit second order reaction kinetic models over the entire time course, and reactions measured from either direction yielded the same values for the rate constants (Table II).

**Validity of Measured and Calculated Kinetic Constants**—Extensive preliminary experiments showed that the rapid quench experiments were subject to a number of errors, some of which, such as ram speed, protein concentrations, protein losses, etc., are discussed above. A number of additional parameters were investigated to determine the significance of the calculated rate constants.

(i) The constants should be independent of the initial concentrations of the reactants, and they are, as shown in Tables I and II.

(ii) An important source of error in rapid quench measurements is insufficient mixing of the reactants on a time scale relevant to the shortest time being used, i.e., mixing must be so efficient that the solution is homogeneous well before collecting the first time point. Fig. 4 shows that this goal was achieved. In this experiment, three flow rates were used, ranging from 270 to 670 \( \mu \text{l/s} \) into the first mixing chamber, and the results show that the data are free of artifacts caused by incomplete mixing. In other experiments, flow rates ranging from 150 to 500 \( \mu \text{l/s} \) into the first mixing chamber also had no effect on the estimated rate constants (data not shown). These experiments satisfy the conditions of the velocity probe (30). In addition, in the experiments using H75QIIIGlc, excellent agreement was obtained between time points that were collected at 500 \( \mu \text{l/s} \) from the rapid quench apparatus and data that were obtained by mixing the reactants by hand (Fig. 3).

(iii) Another consideration is the quality of the simulation with the KINSIM curve fitting program. One test was to substitute arbitrary values of \( k_1 \) and \( k_{-1} \) for those derived from the simulations, and to determine how these substitutions affected the curve with respect to the data points. In general, a ±20% change in the rate constants gave a "poor fit."

(iv) The \( K_{eq} \) values determined directly, and from the ratios of the rate constants (see "Discussion") were in good agreement.

**Determination of Equilibrium Constants**—One important measure of the validity of the rate constants is whether they yield \( K_{eq} \) values \((k_1/k_{-1})\) that agree with values obtained independently by hand mixing. The \( K_{eq} \) values derived from the kinetic data are shown in Tables I and II. The results obtained by the second method, using higher concentrations of proteins

| Initial concentration | [32P]HPr | H75QIIIGlc | [32P]HPr | H75QIIIGlc |
|-----------------------|---------|-----------|---------|-----------|
| 8.5                   | 9.9     | 36        | 0       | 0         |
| 8.4                   | 16      | 74        | 0       | 0         |
| 7.6                   | 17      | 77        | 0       | 0         |
| 42                    | 0       | 20        | 11      |           |

Mean ± S.D. 2.8 ± 0.9 2.3 ± 0.3 1.2 ± 0.4
mixed by hand are given in Tables III and IV. The two sets of values are in good agreement.

**Table III**

| Reaction | Initial concentration | $K_{eq}$ | Mean ± S.D. |
|----------|----------------------|---------|-------------|
| I        | HPPr | [32P]HPPr | III$^{Glc}$ |
| 1        | 42   | 107      | 620        | 0.9        |
| 2        | 42   | 107      | 410        | 1.2        |
| 3        | 42   | 107      | 410        | 1.1        |
| 4        | 42   | 107      | 205        | 1.2        |
| 5        | 42   | 107      | 205        | 1.2        |
| 6        | 19   | 49       | 205        | 1.0        |
| 7        | 19   | 49       | 205        | 1.0        |

**Table IV**

| Reaction | Initial concentration | $K_{eq}$ | Mean ± S.D. |
|----------|----------------------|---------|-------------|
| I        | HPPr | [32P]HPPr | III$^{Glc}$ |
| 1        | 67   | 193      | 835        | 1.1        |
| 2        | 67   | 193      | 835        | 1.0        |
| 3        | 67   | 193      | 418        | 1.6        |
| 4        | 67   | 193      | 418        | 1.3        |
| 5        | 67   | 193      | 209        | 1.7        |
| 6        | 67   | 193      | 209        | 1.9        |
| 7        | 34   | 97       | 209        | 1.3        |
| 8        | 34   | 97       | 209        | 1.5        |
| 9        | 34   | 97       | 104        | 1.5        |
| 10       | 34   | 97       | 104        | 2.0        |

**DISCUSSION**

The following reactions were studied by the methods described above:

**Phospho-HPPr + III$^{Glc}$ ⇌ phospho-III$^{Glc}$ + HPPr**

**REACTION I**

**Phospho-HPPr + H75QIII$^{Glc}$ ⇌ phospho-H75QIII$^{Glc}$ + HPPr**

**REACTION II**

Apparent rate constants were found to be: wild type III$^{Glc}$, $k_1 = 6.1 \times 10^7 M^{-1} s^{-1}$, $k_1 = 4.7 \times 10^7 M^{-1} s^{-1}$; H75QIII$^{Glc}$, $k_1 = 2.8 \times 10^5 M^{-1} s^{-1}$, $k_1 = 2.3 \times 10^5 M^{-1} s^{-1}$.

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