Isolation of the complex by complementation-coupled gel filtration offers an alternate method which is unique, is able to be carried out with facility, and renders a very pure product.

A study of the kinetic, allosteric, and subunit properties of the purified ASase–PRTase complex is now under way.

Acknowledgment

The author thanks Dr. Hermann Bujard for performing the analytical ultracentrifugation analysis, Drs. J. McCorquodale and K. Mizobuchi for stimulating discussions, and Dr. H. Zalkin of Purdue University for making available to us his unpublished manuscript on the purification and properties of the ASase subunit.

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Kinetic Mechanism of Maltodextrin Phosphorylase*

Julie Chao, George F. Johnson, and Donald J. Graves†

ABSTRACT: The kinetic mechanism of *Escherichia coli* maltodextrin phosphorylase has been investigated by (1) initial velocity experiments, (2) studies with inhibitors, and (3) isotopic exchange measurements at equilibrium. Rate equations have been derived for various mechanisms. These equations are different from the common two substrate systems because one of the substrates, polysaccharide, serves as a reactant in both the forward and reverse reactions. Double-reciprocal plots of initial velocity measurements were linear and showed converging line patterns. Inhibition by maltotetraose was competitive with respect to maltoheptaose and mixed with respect to P_i; uridine diphosphate glucose was competitive with respect to P_i and mixed with respect to maltoheptaose. The equilibrium exchange rates for $^{32}P_i \rightleftarrows$ glucose 1-phosphate and [^{14}C]glucose 1-phosphate $\rightleftarrows$ maltoheptaose increased when the concentrations of P_i and glucose 1-phosphate were increased (in constant ratio) at a fixed concentration of maltoheptaose. The exchanges also increased as a function of maltoheptaose concentration at a constant glucose 1-phosphate and P_i. The data are consistent with a rapid equilibrium random Bi-Bi kinetic mechanism for *E. coli* phosphorylase.

Maltodextrin phosphorylase of *Escherichia coli* has been purified and found to possess enzymatic properties similar to animal and plant polysaccharide phosphorylases (Schwartz and Hofnung, 1967). This enzyme was shown to contain 1 mole of pyridoxal phosphate/mole of protein indicating that maltodextrin phosphorylase is a simple monomeric system. Detailed kinetic studies of the complex multisite system of liver and muscle glycogen phosphorylases have been done (Lowry *et al.*, 1967; Maddaiah and Madsen, 1966), but their actual kinetic mechanisms have not yet been completely established. Mechanistic studies have been initiated on the simpler bacterial phosphorylase to firmly establish (1) its kinetic mechanism and (2) provide a framework for further studies, *e.g.*, pH dependence of kinetic parameters, to elucidate the role of specific functional groups and/or pyridoxal phosphate in phosphorylase catalysis.

The mechanism of action of maltodextrin phosphorylase has been derived from initial velocity experiments, inhibition studies, and isotopic exchange measurements and is consistent with a rapid equilibrium random Bi-Bi kinetic mechanism. A preliminary account of this work has been given (Chao and Graves, 1968).

Experimental Section

Materials. Glucose 6-phosphate dehydrogenase, phosphoglucomutase, potassium glucose 1-phosphate, uridine diphosphoglucose, and carrier-free [^{32}P]phosphoric acid in 1 N HCl were obtained from Calbiochem. TPN was purchased from Sigma Chemical Co. The substrate, maltoheptaose, and the inhibitor, maltotetraose, were gifts of Dr. Walter Verhue and Dr.

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Mukthar Abdullah. Phosphorylase was prepared from *E. coli* by following the procedure of Schwartz and Hofnung (1967). Other chemicals were of reagent grade.

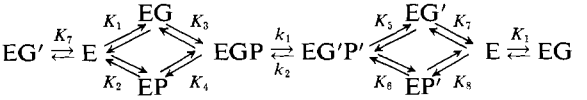
Methods. Initial velocity measurements were made in a Cary 15 spectrophotometer using the 0.1 absorbance slide wire. The reaction mixture samples were incubated at 28° by circulating water from a temperature-controlled bath through thermospacers. Reactions were initiated by addition of enzyme, and initial rates were determined with a coupled assay system described by Helmreich and Cori (1964). Phosphorylase activity was measured by following the production of TPNH at 340 mμ. Reactions were all carried out at pH 7.0. The phosphorylase concentration was 1.2 μg/ml. In the inhibition experiments, maltotetraose and uridine diphosphate glucose were used as inhibitors for the two substrates, P_i and dextrin, respectively. Velocity is expressed as molarity of product formed per minute.

Exchange Rate Measurements. The equilibrium reaction mixtures were prepared to contain inorganic phosphate and glucose 1-phosphate at their respective equilibrium concentrations at pH 7.0 (Hanes and Maskell, 1942). To ascertain that these reaction mixtures were fully equilibrated, aliquots were removed at various intervals and tested for P_i concentration by measuring the absorbancy of the phosphomolybdate complex at 310 mμ (Berenblum and Chain, 1938). Little or no change in concentration of inorganic phosphate was detected after 15 min of incubation with enzyme. At this time, trace amounts of $^{32}P_i$ and [^{14}C]glucose 1-phosphate were added to the reactions, and the equilibrium exchange rates were measured. The amount of radioactivity added as $^{32}P_i$ incorporated into glucose 1-phosphate was determined by first separating P_i and glucose 1-phosphate (Berenblum and Chain, 1938) and measuring the radioactivity of their respective fractions on a Nuclear-Chicago gas-flow counter, Model C-110B. Exchange of [^{14}C]glucose 1-phosphate into dextrin was assessed first by separating the two components by high-voltage electrophoresis with a Gilson Model D high-voltage electrophorator. Radioactive glucose 1-phosphate and dextrin were identified by radioautography with Kodak X-Ray film. The radioactive spots were cut out and counted on Tri-Carb liquid scintillation spectrometer, Packard Model 3310.

Results

Kinetic equations have been derived for a variety of mechanisms of enzyme-catalyzed reactions utilizing two substrates (Alberty, 1953, 1958; Cleland, 1963). The reaction catalyzed by polysaccharide phosphorylase is similar in that two substrates are involved in the reaction but is different in that one of the substrates, polysaccharide, can serve equally well in the back reaction as a product. The fact that initial velocities, thus measured, are always determined in the presence of one of the products makes the rate equations somewhat different than the common two-substrate systems. The consequences of this dual role of polysaccharide for different enzymatic mechanisms are presented in the following equations.

Mechanism I. RAPID EQUILIBRIUM RANDOM BI-BI.

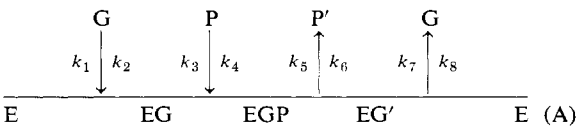


E, G, P, and P′, are, respectively, enzyme, glucosyl polymer, P_i, and glucose 1-phosphate. The complex EG represents binding of the polysaccharide for phosphorolysis; the complex EG′ indicates binding of G for elongation of the polysaccharide chain. Since both of these complexes would be present in either the forward or reverse reaction, these must be considered in the derivation of the initial velocity equations. The eight dissociation constants and two rate constants are given. The rate equation in relation to total enzyme, E_T, initial rate, V, and substrate concentrations for phosphorolysis is

$$\frac{E_T}{V} = \frac{1}{k_1} + \frac{K_4}{k_1(G)} + \frac{K_3\left(1 + \frac{K_1}{K_7}\right)}{k_1(P)} + \frac{K_1K_3}{k_1(G)(P)} \quad (1)$$

This equation is identical in form with the general equation for the rapid equilibrium random Bi-Bi mechanism, but differs in the 1/phosphate coefficient by the factor $(1 + (K_1/K_7))$. If we assume that the affinity of the enzyme for glucosyl polymer for synthesis and degradation is identical (*i.e.*, $K_1 = K_7$), then the coefficient of 1/phosphate is $2K_3$ instead of K_3.

Mechanism II. ORDERED BI-BI. The rate equation for

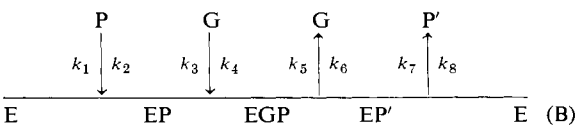


(A)

the mechanism where the glucosyl polymer is the first substrate to bind and the last product to dissociate is

$$\frac{E_T}{V} = \left(\frac{1}{k_5} + \frac{1}{k_7} + \frac{k_8}{k_1k_7}\right) + \frac{1}{k_1(G)} + \frac{(k_4 + k_5)}{k_3k_5(P)}\left[1 + \frac{k_2k_8}{k_1k_7}\right] + \frac{k_2(k_4 + k_5)}{k_1k_3k_5(G)(P)} \quad (2)$$

As was the case for mechanism I, this ordered mechanism does not give a rate equation which differs in form from the general equation for two substrate systems. The constant term and the coefficient for 1/phosphate do, however, differ significantly.



(B)

Where P_i is the first substrate to bind and glucose 1-phosphate is the last product to dissociate, the rate equation is

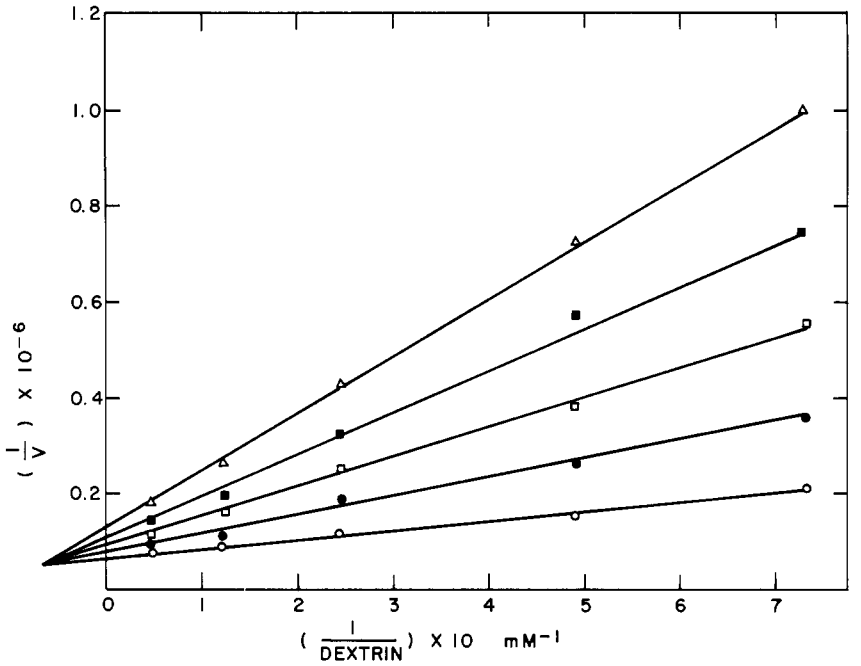
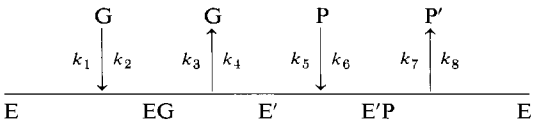


FIGURE 1: Double-reciprocal plot of initial reaction velocity, v, *vs.* dextrin. P_i concentrations were held constant at 0.42 (△), 0.56 (■), 0.83 (□), 1.67 (●), and 6.67 mM (○). Velocities are expressed as the molar concentration of TPNH formed in the reaction mixture over a period of 1 min after addition of enzyme. Other experimental details are given under Experimental Section.

$$\frac{E_T}{V} = \left(\frac{1}{k_5} + \frac{1}{k_7} + \frac{k_4k_6}{k_3k_5k_7}\right) + \frac{(k_4 + k_5)}{k_3k_5(G)} + \frac{k_6(G)}{k_5k_7} + \frac{1}{k_1(P)}\left[1 + \frac{k_2k_4k_6}{k_3k_5k_7}\right] + \frac{k_2(k_4 + k_5)}{k_1k_3k_5(G)(P)} \quad (3)$$

This equation is strikingly different not only in the significance of the coefficients but also in form from the general case. It predicts that a plot of the reciprocal of initial velocity will be a nonlinear function of the reciprocal of the concentration of the glucosyl polymer. The Lineweaver–Burk plot with respect to P_i will, however, be linear.

Mechanism III. BI-BI PING-PONG. E′ and E′P are,



respectively, glucosyl–enzyme and a glucosyl–enzyme complex with P_i. E, G, P, and P′ are as defined previously. The rate equation for this mechanism is

$$\frac{E_T}{V} = \left(\frac{1}{k_7} + \frac{1}{k_3}\right) + \frac{(k_2 + k_3)}{k_1k_3(G)} + \frac{(k_6 + k_7)}{k_5k_7(P)} \times \left[1 + \frac{k_2k_4}{k_1k_3}\right] + \frac{k_4(k_6 + k_7)(G)}{k_3k_5k_7(P)} \quad (4)$$

This equation predicts that a Lineweaver–Burk plot with respect to P_i or polysaccharide will be distinctly different from the usual Bi-Bi Ping-Pong mechanism. With respect to P_i the plots will show converging line patterns at different concentrations of the fixed substrate, polysaccharide. The slope of these lines, for this mechanism, actually increases with increasing concentration of polysaccharide. With respect to polysaccharide, Lineweaver–Burk plots should be nonlinear.

Initial Velocity Experiments. Results of initial velocity measurements with maltodextrin phosphorylase are shown in Figures 1 and 2. The kinetic data were obtained from studies in the direction of phosphorolysis utilizing maltoheptaose as a substrate. All the double-reciprocal plots are linear and show converging line patterns. Secondary plots of slopes and intercepts as a function of the reciprocal of the second substrate also give linear plots (Figures 3 and 4). These experimental findings tend to support mechanism I or II_A for *E. coli* phosphorylase and appear to exclude mechanisms II_B and III.

Inhibition Studies. As the measurement of initial velocities as described in the first section did not allow an unequivocal choice of a kinetic mechanism for *E. coli* phosphorylase, kinetic studies were undertaken in the presence of competitive inhibitors. It has been shown by Fromm (1964) that studies in the presence of competitive inhibitors will permit a choice between ordered and random mechanisms for two-substrate systems. This approach has been used in the study of the mechanism of yeast hexokinase (Zewe *et al.*, 1964). For mechanism I the rate equation in the presence of a competitive inhibitor for polysaccharide is

$$\frac{E_T}{V} = \frac{1}{k_1} + \frac{K_4}{k_1(G)}(1 + I/K_{i'}) + \frac{K_3}{k_1(P)} \times \left(1 + \frac{K_1}{K_7}\right) + \frac{K_1K_3}{k_1(G)(P)}(1 + I/K_i) \quad (5)$$

where K_i and $K_{i'}$ are dissociation constants for the

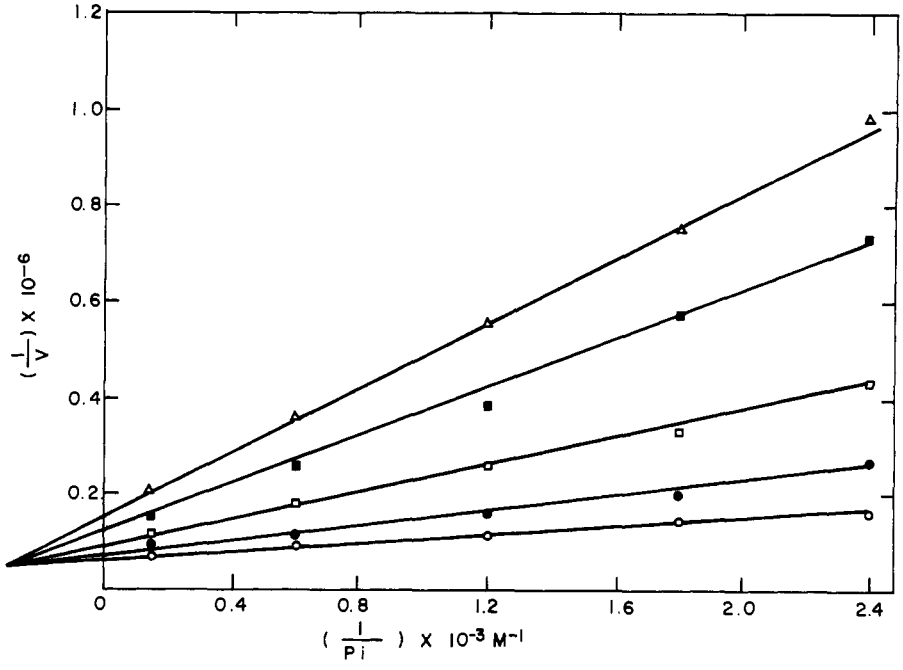


FIGURE 2: Double-reciprocal plot of initial reaction v *vs.* P_i. Dextrin concentrations were held constant at 1.36 (△), 2.04 (■), 4.12 (□), 8.24 (●), and 20.58 mM (○). Velocities are expressed as in Figure 1.

interaction of the inhibitor with the free enzyme and the enzyme–phosphate complex, respectively. In the presence of a competitive inhibitor for phosphate, the rate equation becomes

$$\frac{E_T}{V} = \frac{1}{k_1} + \frac{K_3}{k_1(P)}\left(1 + \frac{K_1}{K_7}\right)\left(1 + \frac{I}{K_i'}\right) + \frac{K_4}{k_1(G)} + \frac{K_1K_3}{k_1(G)(P)}(1 + I/K_i) \quad (6)$$

K_i and K_i' are dissociation constants for binding of inhibitor with free enzyme and enzyme–glucosyl polymer, respectively.

Equation 5 predicts that an inhibitor that is competitive with respect to polysaccharide will be mixed with respect to P_i; in the presence of a competitive inhibitor for phosphate eq 6 shows that corresponding symmetrical plots will be obtained.

In Figures 5–8 results of inhibition studies are illustrated for *E. coli* phosphorylase. Maltotetraose was found by us to be a competitive inhibitor for maltoheptaose and was used in experiments of Figures 5 and 6. It was shown earlier (Schwartz and Hofnung, 1967) and confirmed in this laboratory that maltotetraose is not further degraded by *E. coli* phosphorylase. Since Maddaiah and Madsen (1966) found that UDP-glucose was a competitive inhibitor for P_i with liver phosphorylase, UDP-glucose was used in the inhibition experiments of Figures 7 and 8. The data illustrated in the presence of two concentrations of maltotetraose (Figures 5 and 6) give Lineweaver–Burk plots which are predicted by eq 5. Similarly, the plots for inhibition by UDP-glucose (Figures 7 and 8) conform to the predictions of eq 6.

Fromm (1964) and Zewe *et al.* (1964) showed that for ordered mechanisms a competitive inhibitor for the second substrate will act as an uncompetitive inhibitor with respect to the first substrate. The rate equations for maltodextrin phosphorylase for the two ordered mechanisms in the presence of inhibitors, again, are different from the general case. For the ordered mechanism where the glycosyl polymer is the first substrate to bind and last product to dissociate (II_A), the rate equation predicts that a competitive inhibitor for phosphate will be an uncompetitive inhibitor with respect to the glucosyl polymer. A competitive inhibitor for the glucosyl polymer will act as a mixed inhibitor for phosphate. For the other ordered case (II_B), an inhibitor that binds at the same site as the glucosyl

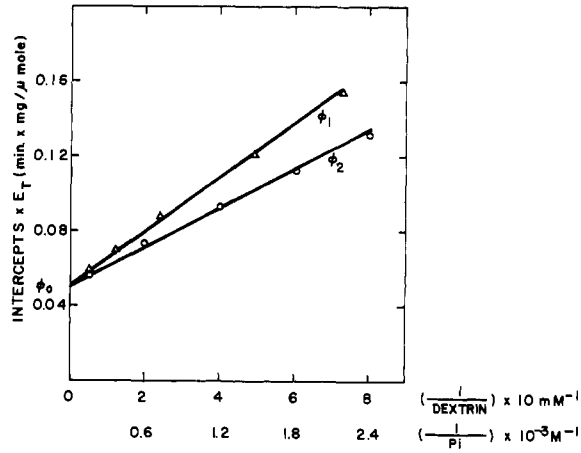


FIGURE 3: Secondary plots of the intercepts from the two primary Lineweaver–Burk plots. (○) Figure 1 and (△) Figure 2.

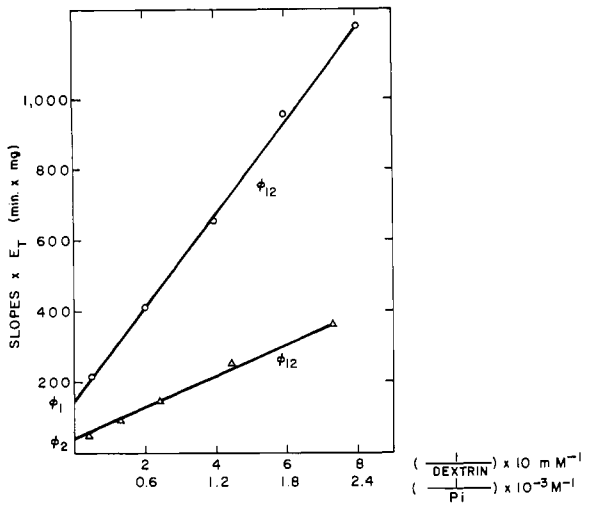


FIGURE 4: Secondary plots of the slopes from two primary Lineweaver–Burk plots. (○) Figure 1 and (△) Figure 2.

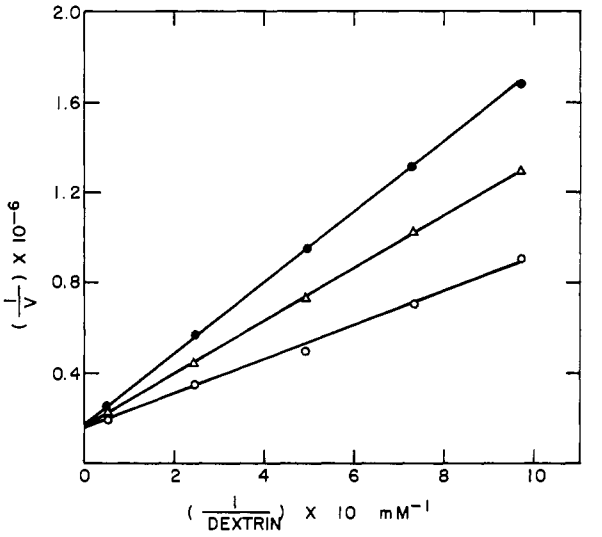


FIGURE 5: Double-reciprocal plot of initial reaction velocity *vs.* dextrin in the presence and in the absence of maltotetraose. P_i concentration was held constant at 1.67 mM and dextrin varied in the concentration range 20.58–1.03 mM. Maltotetraose concentrations are none (○), 4.12 mM (●), and 8.24 mM (△). Velocities are expressed as in Figure 1.

polymer will not give kinetics typical of competitive inhibition, since both nonlinearity and different $1/V$ intercepts will be obtained at various levels of inhibitor. Inhibition with respect to phosphate will be uncompetitive and linear. Since parallel lines were not observed in any of the inhibition plots and the inhibitors for the glucosyl polymer and for phosphate give linear competitive kinetics, these results support strongly the rapid equilibrium random Bi-Bi mechanism for *E. coli* phosphorylase.

Isotopic Exchange Measurements. Boyer (1959) and Boyer and Silverstein (1963) have developed the theory for the relationship of substrate and product interchanges at equilibrium to enzyme mechanisms. The measurement of isotopic exchange rates at equilibrium has been used extensively by many investigators and is a helpful tool in elucidating the kinetic mechanism for two substrate systems. For mechanism I, the exchange rate between $^{32}P_i \rightleftarrows$ glucose 1-phosphate is given by eq 7,

$$V = \frac{k_1 E_T}{1 + \frac{K_1 K_3 K_{equil}}{K_5 K_7} + \frac{1}{(G)}\left[K_4 + \frac{K_1 K_3 K_{equil}}{K_8}\right] + \frac{1}{(P)}\left[K_3 + \frac{K_1 K_3}{K_7}\right] + \frac{K_1 K_3}{(G)(P)}} \quad (7)$$

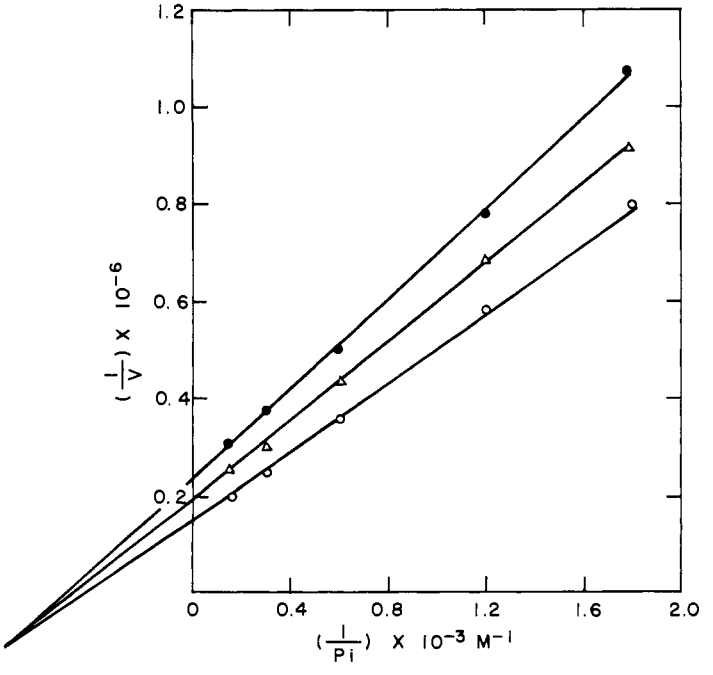


FIGURE 6: Double-reciprocal plot of velocity *vs.* P_i in the presence and in the absence of maltotetraose. Dextrin concentration was held constant at 4.12 mM and P_i varied in the concentration range 6.67–0.56 mM. Maltotetraose concentrations are none (○), 4.12 mM (△), and 8.24 mM (●). Velocities are expressed as in Figure 1.

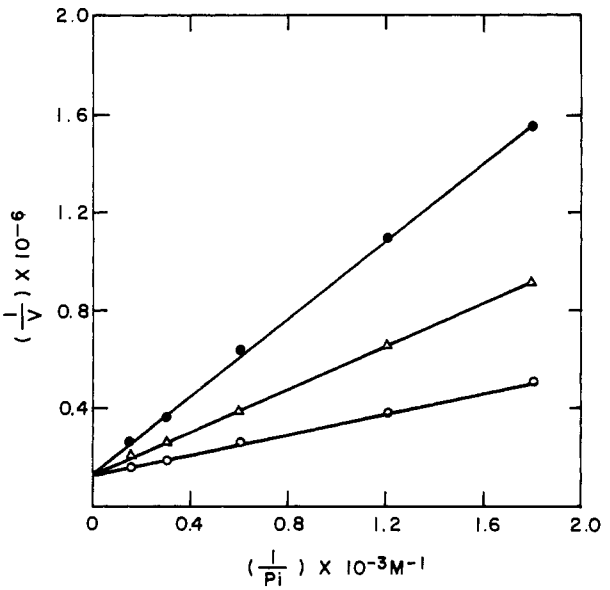


FIGURE 7: Double-reciprocal plot of initial reaction v *vs.* P_i in the presence and in the absence of UDP-glucose. Dextrin concentration was held constant at 8.24 mM and P_i varied in the concentration range 6.67–0.56 mM. UDP-glucose concentrations are none (○), 0.5 mM (△), and 1 mM (●). Velocities are expressed as in Figure 1.

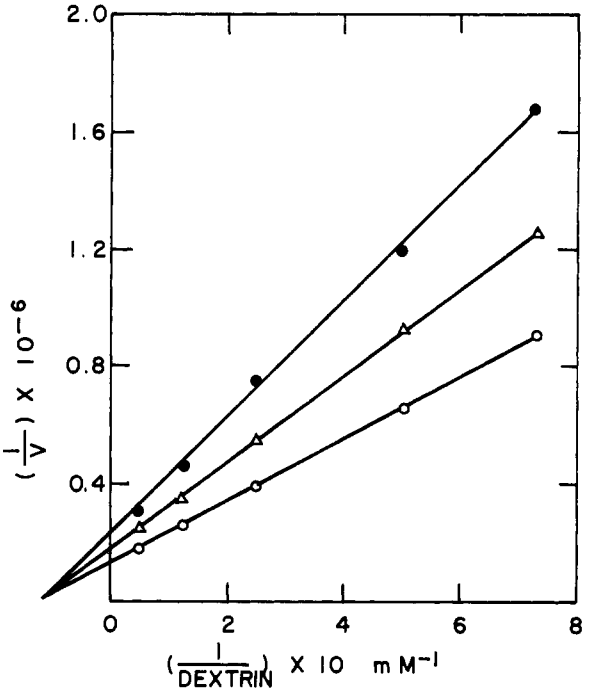


FIGURE 8: Double-reciprocal plot of initial reaction v *vs.* dextrin in the presence and in the absence of UDP-glucose. P_i concentration was held constant at 1.67 mM and dextrin varied in the concentration range 20.58–1.36 mM. UDP-glucose concentrations are none (○), 0.5 mM (△), and 1 mM (●). Velocities are expressed as in Figure 1.

where K_{equil} = (G')(P')/(G)(P) = (P')/(P). The equation for the exchange rate between [^{14}C]glucose 1-phosphate ⇄ dextrin is identical with the equation for the $^{32}P_i$ ⇄ glucose 1-phosphate exchange.

The findings of isotopic exchange experiments at equilibrium are illustrated in Figures 9 and 10. In Figure 9 maltoheptaose was held constant at 61.7 mM. P_i and glucose 1-phosphate were varied at a constant ratio (3:1:1) up to a concentration of P_i approximately five times its K_M value. From Figure 9 it can be seen that the isotopic exchange rates between glucose 1-phosphate and P_i and glucose 1-phosphate and maltoheptaose increase and begin to level off as saturation of the enzyme with P_i and glucose 1-phosphate is approached. In Figure 10 P_i and glucose 1-phosphate were held in a constant ratio at a fixed concentration, while maltoheptaose was increased in concentration to over 20 times its K_M value. Again both exchanges increased and leveled off as predicted by the theoretical equation for mechanism I. Theoretical equations for ordered mechanism II_A predict that the exchange between [^{14}C]glucose 1-phosphate and maltoheptaose should be considerably inhibited by increasing amounts of glucose 1-phosphate and P_i. For mechanism II_B the exchange between glucose 1-phosphate and P_i should be drastically reduced as the enzyme becomes saturated with maltoheptaose. For mechanism II_A the extent to which the [^{14}C]glucose 1-phosphate and maltoheptaose exchange may be inhibited depends upon the rate of dissociation of the EG' complex relative to the rate of reaction of glucose 1-phosphate with the EG' complex. If the latter is much less than the former, no inhibition of exchange would occur at all reasonable concentrations of glucose 1-phosphate. Similarly the inhibition of the exchange between glucose 1-phosphate and maltoheptaose for ordered mechanism II_B depends upon the relative rate of the dissociation of the EP' complex and its reaction with maltoheptaose. The results of Figures 9 and 10 show that the exchange rate between glucose 1-phosphate and maltoheptaose is considerably faster than the exchange between glucose 1-phosphate and P_i. Since the glucose 1-phosphate and P_i exchange should be equal to or greater than the glucose 1-phosphate and maltoheptaose exchange for mechanism II_A, these data alone eliminate this mechanism. The exchange data do not eliminate mechanism II_B. However, the inhibition experiments described earlier completely eliminated mechanism II_B. Therefore, it would appear that the isotopic exchange data must be rationalized in terms of a random mechanism. This is possible, if the interconversion steps between the ternary complexes are not solely rate limiting. Similar results were obtained by Fromm *et al.* (1964) in their study of yeast hexokinase.

Kinetic Constants. By using the data from Figures 1–4, we calculated the kinetic coefficients (Dalziel, 1957) and the corresponding kinetic constants for the rapid equilibrium random Bi-Bi mechanism (Table I). Only the kinetic constants k_1, K_2, and K_4 could be evaluated since it is not known whether the dissociation constants K_1 and K_7 are identical.

Discussion

The reactions catalyzed by *E. coli* phosphorylase and other polysaccharide phosphorylases are distinctly

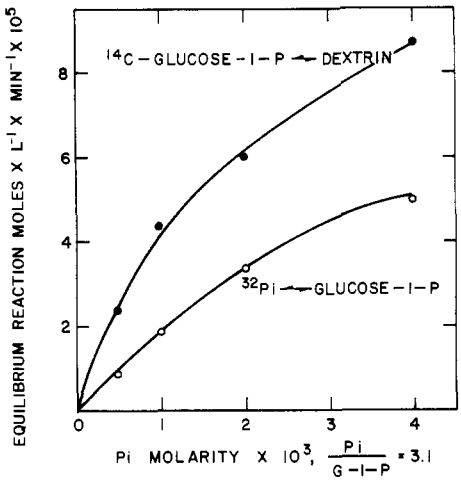


FIGURE 9: The effect of P_i and glucose 1-phosphate concentrations on equilibrium reaction rates catalyzed by phosphorylase. The reaction mixtures contained at 30°: 20 mM glycerophosphate and 0.5 mM dithiothreitol (pH 7.0), 61.74 mM dextrin, 10 μg of *E. coli* phosphorylase/ml, P_i, and glucose 1-phosphate (pH has been adjusted to 7.0) as shown in the figure. After 15-min incubation, trace amounts of $^{32}P_i$ and [^{14}C]glucose 1-phosphate were added to separate reaction mixtures. Aliquots were removed and analyzed to extent of interchange as discussed in the Experimental Section.

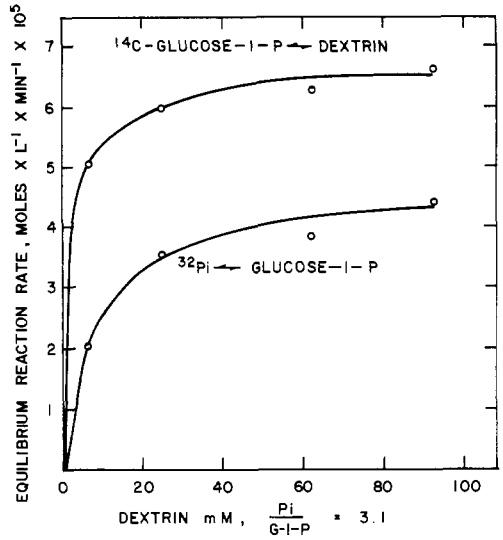


FIGURE 10: The effect of dextrin concentration on equilibrium reaction rates catalyzed by phosphorylase. The reaction mixtures contained at 30°: 20 mM glycerophosphate and 0.5 mM dithiothreitol (pH 7.0), 1 mM P_i and 0.32 mM glucose 1-phosphate, 10 μg of *E. coli* phosphorylase/ml, and dextrin as shown in the figure. Other experimental details are given in Figure 9.

TABLE I[a]

Kinetic Coefficients		Corresponding Kinetic Constants	
ϕ_0	= 0.05 mg of protein × min per μmole	k_1	= 20 μmoles/min per mg
ϕ_1	= 149 min × mg	K_4	= 3 mM maltoheptaose
ϕ_2	= 36 min × mg	K_2[b]	= 3.4 mM P_i
ϕ_{12}	= 0.45 mole × min × mg		

[a] ϕ_0, ϕ_1, ϕ_2, and ϕ_{12} are, respectively, $1/k_1$, K_4/k_1, $(K_3/k_1)(1 + K_1/K_7)$, and K_1K_3/k_1. [b] From the intersection in the upper left-hand quadrants of Figure 2.

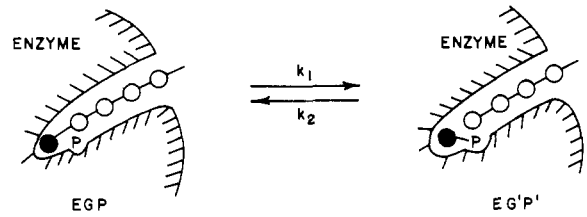


FIGURE 11: Schematic representation of the complexes for enzyme–glucosyl polymer–P_i and enzyme–glucosyl polymer–glucose 1-phosphate at equilibrium.

different from the usual two-substrate systems in that one of the substrates, polysaccharide, serves as a reactant in the forward and reverse reactions. The degree to which these glucosyl polymers can serve in both reactions depends upon the size of the polymer and the enzyme in question (Brown and Cori, 1961). Under limited attack, phosphorylases usually do not distinguish between substrate and product. The consequence of the dual function of polysaccharide has been considered in this work and presented in the various rate equations for maltodextrin phosphorylase. It was assumed that the complexes EG and EG′ indicate, respectively, binding of dextrin for degradation and chain elongation. Schematically, these interactions are represented in the complexes EGP and EG′P′ illustrated in Figure 11. It can be seen that P_i and the phosphoryl group of glucose 1-phosphate occupy common sites in the two complexes. The glucosyl moiety of glucose 1-phosphate (dark circle of the EG′P′ complex) is bound at the site where the nonreducing end of the glucosyl polymer binds when phosphorolysis takes place.

Our results of initial velocity measurements with and without inhibitors and of isotopic exchange reactions at equilibrium support strongly a rapid equilibrium random Bi-Bi mechanism for maltodextrin phosphorylase. Maddaiah and Madsen (1966) also suggested from their kinetic studies that liver phosphorylase possessed a rapid equilibrium random Bi-Bi kinetic mechanism. For their mechanism the complexes EG and EG′ also signified, respectively, binding of enzyme with the substrate, glycogen, and the product, a glycogen molecule with one less glucosyl residue. The formation of an EG′ complex from substrate was not included in their scheme. Consequently, the rate equation of Maddaiah and Madsen was simpler than ours and corresponded to that for the general case for a random

mechanism (Alberty, 1958). It is interesting to compare the significance of their constants with those expected for our random mechanism. The kinetic coefficients, ϕ_0 and ϕ_1, and the intersection point of the upper left-hand quadrant of the double-reciprocal plot with respect to phosphate yield the same identities for the two cases. The intersection point in the double-reciprocal plot with respect to the glucosyl polymer does not give the same constant. The equation used by Maddaiah and Madsen (1966) predicts that the intersection points in the direction of phosphorolysis and synthesis correspond to $-1/K_1$ and $-1/K_7$, respectively. They found that these constants agreed very well numerically. For our random mechanism, the intersection point is the same for both directions and is $-(1/K_1 + 1/K_7)$. Equal values for the intersection points, therefore, do not mean anything in regard to the relative affinities of enzyme for the polymer for synthesis and degradation, they just verify what is predictable for the random mechanism. Also, it is because the intersection point of Figure 1 is $-(1/K_1 + 1/K_7)$ that the dissociation constants K_1, and K_3 could not be evaluated. These constants can only be evaluated when K_1 equals K_7.

For maltodextrin phosphorylase, it might be expected that various dead-end inhibitor complexes could be formed. Such a complex might be EG'P where the glucosyl polymer is bound as illustrated in the EG'P' complex but in the presence of P_i. For the random mechanism, the inclusion of the dead-end inhibitor complex, EG'P, does not alter the form of the rate equation. The kinetic coefficient, ϕ_0, for this case becomes $(1/k_1)(1 + K_4/K_9)$, where K_9 is the dissociation constant for the reaction, EG'P $\rightleftarrows$ EP + G. If such a complex did occur, k_1 could not be evaluated, and therefore, no dissociation constants could be calculated from the kinetic coefficients. Since our experiments do not allow us to decide whether such complexes exist or not, dissociation constants and k_1 were calculated and tabulated in Table I for comparative purposes.

It was found by Schwartz and Hofnung (1967) that maltoheptaose, maltohexaose, and maltopentaose can be degraded by *E. coli* phosphorylase but maltotetraose cannot. Our studies show that maltotetraose is bound by the enzyme, as we observed that maltotetraose is a competitive inhibitor with respect to maltoheptaose (Figure 5). Inhibition can be explained by binding of maltotetraose as illustrated in the EG'P' complex of Figure 11 or by binding of its nonreducing end at the same position as the nonreducing end of maltopentaose in the EGP complex. For the latter case, orientation of the catalytic site with substrate would have to be wrong to explain the lack of enzymic degradation of maltotetraose.

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