The Thermodynamic and Structural Differences among the Catalytically Active Complexes of Phosphoglucomutase: Metal Ion Effects†

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ABSTRACT: When the identity of the metal ion activator, M, is changed within the series, Zn^{2+}, Co^{2+}, Mg^{2+}, Ni^{2+}, Mn^{2+}, and Cd^{2+}, the equilibrium distribution among the central complexes in the phosphoglucomutase system is markedly altered. (The central complexes are E_P·M·Glc-6-P, E_D·M·Glc-1,6-P_2, and E_P·M·Glc-1-P, where E_P and E_D are the phospho and dephospho forms of the enzyme.) This altered distribution is caused by a metal-specific change in the equilibrium constant for transfer of the enzymic PO_3 group to bound glucose monophosphates: 65-fold as M is varied from Zn^{2+} to Cd^{2+}. This change in equilibrium is related to *metal-specific differences* in chemical potential of the phosphate group in the E_P·M complex; these *differences* in chemical potential *remain* in the E_P·M·Glc-1-P and E_P·M·Glc-6-P complexes, but essentially *disappear* in the E_D·M·Glc-1,6-P_2 complex. If glucose monophosphates are considered as substrates, and glucose bisphosphate as the product, there is a direct relationship between the equilibrium concentration of enzyme·substrate and enzyme·product complexes (when these are varied by changing the identity of the bound metal ion) and the ultraviolet spectrum of the equilibrium mixture of complexes, as assessed by difference spectroscopy (Peck, E. J., Jr., and Ray, W. J., Jr. (1969), *J. Biol. Chem. 244*, 3754). These spectral changes apparently are caused by an alteration in the conformation of the enzyme *during* transfer of a PO_3 group between the enzyme and the glucose phosphate moiety, or as the result of it. The extent to which conformational changes accompany group-transfer processes in other enzymic systems is not clear, but it is possible that analogous changes may help to account for the "half-of-the-sites reactivity" observed with a number of multimeric enzymes.

In the "absence" of a bivalent metal ion, the activity of phosphoglucomutase is reported as less than 10^{-5} of that observed in the presence of the most efficient activator, Mg^{2+} (Ray and Peck, 1972), and an even smaller value, 10^{-7} that of the Mg^{2+} enzyme, recently has been obtained (W. J. Ray, Jr., unpublished results). In addition to Mg^{2+}, a variety of bivalent metal ions elicit an extensive activation relative to the apparent rate in the absence of metals. Although the extent of activation varies markedly with the identity of the bound metal ion (Ray, 1969), an earlier study (Peck and Ray, 1969a) shows that the same tryptophan ultraviolet difference spectrum is produced by binding of each metal ion activator[1] and that there are no significant spectral differences among the various metal-ion complexes of the enzyme. By contrast, the difference spectra obtained on comparing the various metal-ion complexes of the enzyme in the presence of glucose phosphates (i.e., by comparing equilibrium mixtures of central complexes) are primarily tyrosine difference spectra, and the *intensities* of the difference spectra, relative to the Mg^{2+} complex, are characteristic of the *bound metal ion* (Peck and Ray, 1969b). Because a rough correlation is observed between the extent of the spectral shift produced by a given metal ion in the presence of bound glucose phosphates and the activation elicited by that ion, it has been suggested (see above reference) that metal binding in the presence of bound glucose phosphates produces a metal-specific structural change in the enzyme that is critical to catalysis.

In the present paper, the effect of the metal ion on the distribution among the central complexes, viz., the E_P·M·Glc-1-P, E_D·M·Glc-P_2, and E_P·M·Glc-6-P^2 complexes, is examined. The results indicate that, instead of eliciting a metal specific structural change, per se, metal binding alters the distribution among the central complexes, and that the central complexes themselves are sufficiently disparate, conformationally, to produce a significant difference in the environments of four or more tyrosine residues. Conformational changes that occur *during* (or subsequent to) the PO_3-transfer steps that interconvert these complexes must produce these spectral alterations.

Experimental Section

Materials. Phosphoglucomutase was prepared as previously described (Long and Ray, 1973). A molecular weight of 62 500 and an optical density of 0.77 for a solution of 1 mg per ml were used in calculating enzyme concentrations (*cf.* Ray and Peck,

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[1] Peck and Ray (1969a) suggest that the difference spectrum produced by binding of M^{2+} to E_P, which is not typical of a solvent-induced tryptophan spectrum, probably involved tyrosine residues as well. However, subsequent studies show that this difference spectrum is caused primarily by electrostatic effects and that little if any tyrosine perturbation is involved (W. J. Ray, Jr., E. S. Szymanski, and L. Ng, submitted for publication).

[2] The following abbreviations and symbols are used: E_P and E_D, the phospho and dephospho forms of phosphoglucomutase; M, a bivalent metal-ion activator; Glc-P_2, α-D-glucose 1,6-bisphosphate; Glc-1-P, α-D-glucose 1-phosphate; Glc-6-P, D-glucose-6-P; glucose-P or Glc-P, an equilibrium mixture of Glc-6-P and Glc-1-P; CC, the central complexes, E_P·M·Glc-1-P, E_D·M·Glc-P_2, and E_P·M·Glc-6-P; NTA, nitrilotriacetate; $K_{X(S)}$ the isotopic exchange constant for substrate S, at saturating concentrations of all other assay components; $K_{d(S)}$, the equilibrium dissociation constant for substrate, S; Tris, tris(hydroxymethyl)aminomethane; SD, standard deviation.

1972). For kinetics studies, solutions of about 1 mg per ml of the "metal free" enzyme (Ray and Mildvan, 1970) were converted to the various metal complexes by treating for an hour at room temperature with NTA-metal buffers in 20 mM Tris-Cl, pH 7.5: 2 mM NTA and 1 mM zinc acetate, or 1.8 mM cobalt acetate, or 1.8 mM manganese chloride; 1.2 mM NTA and 0.4 mM cadmium chloride. Further dilutions of the enzyme were made in the same solutions, except that 0.15 mg/ml of serum albumin (crystalline) was included. Glucose-1-P and glucose-6-P were chromatographically purified on 1.8 × 10 cm columns of Dowex-1-HCO_3, 8% crosslink, by using an elution gradient containing triethylammonium bicarbonate. The buffer subsequently was removed under vacuum. Synthetic α-D-glucose-1,6-P_2 (Hanna and Mendicino, 1970) was chromatographically purified in the manner described previously (Ray and Roscelli, 1964). Glucose-6-P dehydrogenase (Boehringer) was treated as described in an accompanying paper (Ray and Long, 1976b); $NADP^+$ was obtained from Sigma. All other materials were reagent grade or better.

Isotopic Exchange Studies. Assays to measure $K_{x(Glc\text{-}P)}$[2] were conducted at 24 °C in polypropylene centrifuge tubes by using variable concentrations of an equilibrium mixture of glucose-6-P and glucose-1-P (a 17.3/1 ratio; Atkinson et al., 1961) at 1 μM (saturating) glucose-1,6-P_2, and 20 mM Tris-Cl, pH 7.5. A metal ion buffer was used to provide saturating but not inhibiting concentrations of metal ion, and the enzyme was converted to the appropriate metal form prior to use; see above. In assays to measure $K_{x(Glc\text{-}P_2)}$, the glucose monophosphates were held constant at 0.1 mM and the glucose-1,6-P_2 concentration was varied. Both types of assays were initiated by adding a trace of [^{32}P]glucose-1-P to the equilibrium mixture containing enzyme. The former assays involved time intervals of 10 min; time intervals for the latter assays were 10 min for the Mg^{2+} enzyme, 4 h for the Co^{2+}, Mn^{2+}, and Cd^{2+} enzymes and 24 h for the Zn^{2+} enzyme. After adding carrier, the [^{32}P]glucose-6-P formed was measured as the soluble organic phosphate remaining after boiling in acid (to hydrolyze the 1-phosphate), treating with a triethylamine molybdate reagent to precipitate inorganic phosphate (Sugino and Miyoshi, 1964), cooling for 15 min at 0 °C, and centrifuging twice in an Eppendorff Microcentrifuge (see also Peck et al., 1968). Initial exchange rates were calculated from the fractional conversion of labeled substrate to labeled product in the manner described by Boyer (1959).

The Composition of the Central Complexes as a Function of the Bound Metal Ion. Solutions were prepared by using metal-free phospho-enzyme and the following materials (the final concentrations are indicated): enzyme, 20 mg per ml or 0.32 mM; metal ion, 0.32 mM (except for Ni^{2+} which was present at 0.58 mM, and one series of experiments in which 3.2 or 1.6 μM Mg^{2+} was used); glucose-6-P, 0.03 mM, and Tris-Cl buffer, pH 7.5, 20 mM. The mixtures were allowed to equilibrate at 24 °C for between 15 min and 1 h. Aliquots were quenched and the glucose phosphates assayed in the manner described in an accompanying paper (Ray and Long, 1976b).

Results

Isotopic Exchange Constants at Equilibrium. Values of the maximum isotopic exchange rate, R_{max}, and the isotopic exchange constants for glucose-P and glucose-1,6-P_2, $K_{x(Glc\text{-}P)}$ and $K_{x(Glc\text{-}P_2)}$, were obtained from double-reciprocal plots (not shown) of isotopic exchange rates (at equilibrium) and variable substrate concentration. When glucose-P was the variable substrate, glucose-1,6-P_2 concentration was maintained at a value of more than 100 $K_{x(Glc\text{-}P_2)}$, and $K_{x(Glc\text{-}P)}$ thus was measured directly. However, when glucose-1,6-P_2 was the variable substrate, it was sometimes necessary to use glucose-P concentrations that were greater than $K_{x(Glc\text{-}P)}$ only by several fold.[3] Hence, observed values of $K_{x(Glc\text{-}P_2)}$ were corrected to saturating glucose-P in the manner described in an accompanying paper (Ray and Long, 1976b). In addition, the dephosphoenzyme, E_D·M, binds glucose-1-P to form a dead-end complex (Ray and Peck, 1972), which is characterized by the dissociation constant $K_{I(Glc\text{-}P)}$, and $K_{x(Glc\text{-}P_2)}$ also was corrected for such binding by using the factor, $(1 + [Glc\text{-}P]/K_{I(Glc\text{-}P)})^{-1}$. $K_{I(Glc\text{-}P)}$ for the Mg^{2+} enzyme is 0.5 mM (Ray and Long, 1976b) and the value of this constant was taken to be the same for all of the metal forms of the enzyme for the following reasons: (a) the binding of glucose-1-P to E_D apparently does not depend significantly on the presence of a bound metal ion since glucose-1-P binds approximately equally to E_D and E_D·Mg (Ray et al., 1966); (b) inorganic phosphate and methyl phosphonate, which bind to the dephospho-enzyme competitively with glucose-1,6-P_2 (and competitively with glucose-P), bind approximately equally to complexes of the dephospho-enzyme with two different metal ions: Mg^{2+} and Mn^{2+} (Ray et al., 1973); (c) the strong phosphate binding site in E_D·Mn, where the phosphate group of glucose-P binds, is about 10 Å away from the bound metal ion (Ray and Mildvan, 1973); (d) the binding of both glucose-1-P and glucose-6-P to E_P·M also is essentially independent of the identity of M (see below). Table I, column 4, shows the corrected values of $K_{x(Glc\text{-}P_2)}$ as a function of the bound metal ion; in all cases the overall corrections were relatively small.[4] Columns 2 and 3, Table I, show the measured values of R_{max} and $K_{x(Glc\text{-}P)}$. The variation of R_{max} with metal ion is similar to the analogous variation of V_{max} in initial velocity studies (Ray, 1969), except in the case of Co^{2+}, where the R_{max} value is substantially less than might be expected from the corresponding V_{max} value. Values of

[3] In such experiments the concentration of enzyme must be kept much less than $K_{x(Glc\text{-}P_2)}$ in order to minimize the importance of glucose-1,6-P_2 generated via the reaction, E_P·M + Glc-1-P → E_D·M + Glc-P_2 (cf. Ray and Roscelli, 1964). With the maximum enzyme concentration thus fixed, as low a concentration of substrate as seemed reasonable was used in order to minimize assay intervals, which even so were quite lengthy (see Experimental Section). (In spite of the length of the assay intervals used, the R_{max}/E_0 values obtained were essentially identical with those observed directly in 10-min assays conducted under similar conditions but at higher enzyme concentrations and at saturating glucose-1,6-P_2.)

[4] Values of $K_{x(Glc\text{-}P_2)}$ for the Zn^{2+} and Mn^{2+} forms of the enzyme are less reliable than other exchange constants. In spite of the low concentrations of enzyme used in these studies (see footnote 3), blank values (no added glucose-1,6-P_2) that were about 10% of the maximum isotope exchange rates were obtained with these forms of the enzyme, whereas blank values were about 1% of the maximum rates with the Co^{2+} and Cd^{2+} enzymes. The former values, especially, were much too large to rationalize in terms of glucose-1,6-P_2, either generated in the manner noted in footnote 3 or present as a contaminant of the enzyme. Blank values were linear with time, directly proportional to enzyme concentration and independent of all other assay components over a 4-fold range. Hence, we conclude that the major portion of these blanks represents a slow reaction of E_D·M with glucose-1-P to give E_P·M + glucose, followed by the catalytic interconversion of glucose-1-P and glucose-6-P by the E_P·M thus produced. This conclusion is in line with the residual "glucose bisphosphate-independent activity" of the Mg^{2+} form of phosphoglucomutase observed earlier in initial velocity studies (Ray and Roscelli, 1964), and is supported by analogous initial velocity studies with the Zn^{2+} and Mn^{2+} enzyme forms (data not shown). In the case of the Co^{2+} and Cd^{2+} forms of the enzyme, the blank value was ignored; for the Mn^{2+} and Cd^{2+} enzymes, a correction was subtracted from each isotope exchange rate that was measured. This correction was equal to the blank value multiplied by the fraction of the enzyme calculated to be present as E_D·M.

TABLE I: Rate and Equilibrium Constants for the Interaction of Glucose Phosphates with Phosphoglucomutase as a Function of the Activating Metal Ion.[a]

Metal Ion	$\frac{R_{max}}{E_0}$[b] (s^{-1})	$K_{x(Glc\text{-}P)}$[c] (μM)	$K_{x(Glc\text{-}P_2)}$[d] (nM)	$\frac{10^4 \times (E_D\cdot M)(Glc\text{-}P_2)}{(E_P\cdot M)(Glc\text{-}6\text{-}P)}$[e] ($K_{Tr\text{-}1}$[f])	$\frac{E_D\cdot M\cdot Glc\text{-}P_2}{E_P\cdot M\cdot Glc\text{-}6\text{-}P}$[g] ($K_{Tr\text{-}1}$[b])	$\frac{E_P\cdot M\cdot Glc\text{-}1\text{-}d}{E_P\cdot M\cdot Glc\text{-}6\text{-}P}$[j]	$K_{d(Glc\text{-}P_2)}$[g] (nM)	$K_{d(Glc\text{-}6\text{-}P)}$[h] ($\mu M$)
Zn^{2+}	0.17	28	0.7	0.3	0.23	0.31	5	43
Co^{2+}	3.8	17	8.	4	0.7	0.24	23	33
Mg^{2+}	93	20[i]	10[i,j]	5	1.6	0.39	19	58
Ni^{2+}					1.7	0.30		
Mn^{2+}	7	3.6	0.6	2	6	0.42	0.7	28
"None"[k]				34[l]	4.8	0.48	75[m]	70[m]
Cd^{2+}	1.1	2.5	8	40	15	(0.5)[n]	9	42

[a] At 24 °C and pH 7.5, in the presence of metal-ion buffers (see Experimental Section). [b] The maximum isotopic exchange rate at saturating glucose-P and glucose-1,6-P_2 for [^{32}P]Glc-1-P → [^{32}P]Glc-6-P. [c] From equilibrium isotope exchange studies conducted at saturating glucose-1,6-P_2; $K_{x(Glc\text{-}6\text{-}P)} = 0.95K_{x(Glc\text{-}P)}$; $K_{x(Glc\text{-}1\text{-}P)} = 0.055K_{x(Glc\text{-}P)}$. [d] From equilibrium isotope exchange studies conducted at 0.1 mM glucose-P. Minor corrections were applied to observed values for the use of glucose-P concentrations that were not quite saturating as well as for nonproductive binding of glucose-P (see Results). [e] Calculated from the ratio, $K_{x(Glc\text{-}P_2)}/K_{x(Glc\text{-}6\text{-}P)}$; identified in eq 3. [f] Calculated from data in Table II. [g] Values of $K_{x(Glc\text{-}P_2)}$ were multiplied by the inverse of the fraction of the central complexes in the E_D·Glc-P_2 form to give $K_{d(Glc\text{-}P_2)}$ values; see Results. [h] Values of $K_{x(Glc\text{-}6\text{-}P)}$ were multiplied by the inverse of the fraction of the central complexes in the E_P·Glc-6-P form to give $K_{d(Glc\text{-}6\text{-}P)}$ values; see Results. [i] See accompanying paper (Ray and Long, 1976b). [j] Value also corrected for formation of the inactive complexes of Mg^{2+} and glucose-1,6-P_2 (see Results). [k] Equilibrium established with 1 mol % Mg^{2+}; see Results. [l] Calculated from the corresponding value for the Mg^{2+} enzyme and the ratio of the dissociation constants for E_P·Mg and E_D·Mg. [m] Calculations described in a supplemental section; see paragraph at the end of this paper concerning supplementary material. [n] Unreliable value due to relatively large fractional error in values appearing in Table II.

$K_{x(Glc\text{-}6\text{-}P)}$ and $K_{x(Glc\text{-}1\text{-}P)}$ (not shown) can be calculated from $K_{x(Glc\text{-}P)}$ in the manner described in footnote *c*, Table I. By using metal-ion buffers, the free metal ion was maintained at a concentration sufficiently high to saturate the binding site in all enzyme forms present, but, except in the case of Mg^{2+} (see Ray and Long, 1976b), at a concentration sufficiently low to prevent the formation of significant amounts of the metal complexes of glucose phosphates. Under these conditions, isotope exchange constants for glucose-6-P, glucose-1-P, and glucose-1,6-P_2, respectively, are equal to $(E_P\cdot M)(Glc\text{-}6\text{-}P)/(CC)$, $(E_P\cdot M)(Glc\text{-}1\text{-}P)/(CC)$, and $(E_D\cdot M)(Glc\text{-}P_2)/(CC)$; here (CC) is the sum of the concentrations of the central complexes *at equilibrium*. The data in Table I thus indicate that each of these ratios depends on the identity of the bound metal ion.

From the above definitions, $K_{x(Glc\text{-}P_2)}/K_{x(Glc\text{-}6\text{-}P)}$ is equal to the equilibrium constant for phosphate transfer from the phospho-enzyme to the 1-position of free glucose-6-P, $K_{Tr\text{-}1}$[f]: $E_P\cdot M + Glc\text{-}6\text{-}P \rightleftharpoons E_D\cdot M + Glc\text{-}P_2$ (see also Peck et al., 1968). Values of $K_{Tr\text{-}1}$[f] (column 5, Table I) also are sensitive to the identity of the bound metal ion.

The Equilibria among the Central Complexes as a Function of the Bound Metal Ion. The values of $K_{x(Glc\text{-}P)}$ and $K_{x(Glc\text{-}P_2)}$ in Table I also can be used to determine the amount of phospho-enzyme that must be added to a solution of glucose-6-P so that at equilibrium essentially all of the glucose phosphates will be bound to the enzyme (Ray and Long, 1976b). Based on this and other considerations, concentrations of 0.32 and 0.03 mM for the phospho-enzyme and glucose-6-P were chosen. Under these conditions, *free* glucose monophosphates were never significantly larger than 10% of the total (see columns 4 and 7, Table II), while free glucose-1,6-P_2 was entirely negligible. (The expected concentration of free glucose-1,6-P_2 can be calculated from $K_{Tr\text{-}1}$[f] values and the concentrations of free glucose-6-P and free E_P·M.) Hence, the distribution among glucose-6-P, glucose-1-P, and glucose-1,6-P_2 in quenched aliquots of such equilibrium mixtures should provide an accurate assessment of the original distribution among the central complexes, E_P·M·Glc-6-P, E_P·M·Glc-1-P, and E_D·M·Glc-P_2, after a small correction for free glucose-6-P and free glucose-1-P,[5] provided quenching artifacts can be avoided (see below).

Replicate aliquots of equilibrium mixtures established with each metal ion were quenched alternatively in acid and base. In the case of the Co^{2+} enzyme, which is about 25% as active as the Mg^{2+} form in initial velocity studies (Ray et al., 1973), the same results, within experimental error, were obtained with 0.5 or 1 N acid as well as 1 or 2 N base (see Table II). The same results also were obtained with two other less active metal forms of the enzyme, the Cd^{2+} and Zn^{2+} forms, on quenching with 0.5 N acid or 2 N base. However, small but significant differences between acid and base quenching, that were independent of the concentration of acid or base in the quenching solution, were observed with the Mn^{2+} enzyme (Table II) which is less active than the Co^{2+} enzyme in the initial velocity studies (Ray, 1969) but more active in isotope exchange experiments (see Table I). Larger differences between the results obtained by acid and base quenching were observed for the Ni^{2+} enzyme, which is 70% as active as the Mg^{2+} enzyme, but the differences were smaller than those observed with the Mg^{2+} enzyme (see Table II, as well as Table I of a previous paper (Ray and Long, 1976b)). These results show that the glucose phosphate complexes of the more reactive metal forms of the enzyme undergo some PO_3 transfer during either acid or base quenching, or both, but that complexes with the less active forms do not.

[5] The $K_{x(Glc\text{-}P)}$ value for the Ni^{2+} enzyme was not measured because of the instability of this metal form of the enzyme in *dilute* solution (Ray, 1969), and corrections for free glucose monophosphates in equilibrium mixtures involving this form of the enzyme were arbitrarily taken as intermediate between those for the Mg^{2+} and Mn^{2+} forms of the enzyme, because the spectral properties of the central complexes involving Ni^{2+} are between those of the complexes involving the indicated metal ions (see Discussion).

TABLE II: The Equilibrium Distribution of the Central Complexes of Phosphoglucomutase as a Function of the Bound Metal Ion.[a]

Metal Ion[b]	Glc-6-P Obsd[c] (μM) Acid	Glc-6-P Obsd[c] (μM) Base	Corr[d] (μM)	E_P·M·Glc-6-P % (Acid Only)[e]	Glc-1-P Obsd[c] (μM) Acid	Glc-1-P Obsd[c] (μM) Base	Corr[d] (μM)	E_P·M·Glc-1-P % (Acid Only)[e]	Glc-P_2 Obsd[c] (μM) Acid	Glc-P_2 Obsd[c] (μM) Base	E_D·M·Glc-P_2 % (Acid Only)[e]
Zn^{2+} (4)	20.3 ± 0.2	20.0 ± 0.4	2.5	65 ± 0.5	5.6 ± 0.1	5.6 ± 0.4	0.14	20 ± 0.5	4.1 ± 0.3	4.4 ± 0.1	15 ± 1
Co^{2+} (4)	16.3 ± 0.8[f]	15.2 ± 0.6[f]	1.6	52 ± 3	3.6 ± 0.3[f]	4.5 ± 0.3[f]	0.09	12.5 ± 1	9.9 ± 0.6[f]	10.3 ± 0.5[f]	35 ± 2
Mg^{2+} (7)	11.1 ± 0.6[g]	6.4 ± 0.3[g]	1.8	33 ± 2	3.8 ± 0.4[g]	2.2 ± 0.1[g]	0.10	13 ± 1.5	15.2 ± 0.3[g]	21.2 ± 0.7[g]	54 ± 2.5
Ni^{2+} (5)	10.6 ± 0.2	7.7 ± 0.2	(0.8)[h]	33.5 ± 0.5	3.0 ± 0.3	2.4 ± 0.2	(0.05)[h]	10 ± 1	16.4 ± 0.3	19.8 ± 0.0	56.5 ± 1
"None" (5)	5.0 ± 0.3[i]		(0.7)[j]	14.5 ± 1	2.1 ± 0.2		(0.04)[j]	7 ± 1	22.9 ± 0.3		78.5 ± 1
Mn^{2+} (5)	4.2 ± 0.2[f]	3.6 ± 0.6[f]	0.3	13 ± 0.5	1.6 ± 0.2[f]	3.6 ± 0.3[f]	0.02	5.5 ± 0.5	24.1 ± 0.4[f]	22.9 ± 0.6[f]	81.5 ± 1
Cd^{2+} (5)	2.0 ± 0.2	1.8 ± 0.6	0.2	6 ± 0.5	0.9 ± 0.2	0.6 ± 0.3	0.01	3 ± 0.5	27.1 ± 0.3	27.6 ± 0.6	91 ± 1

[a] At pH 7.5 and 24 °C, in the presence of metal ion buffers (see Experimental Section). [b] The numbers in parentheses refer to the number of separate quenchings that were conducted with acid; an equal number of quenchings were conducted with base. [c] The concentration of sugar phosphate in an equilibrium mixture initially containing 0.32 mM phosphoglucomutase and 0.03 mM glucose-6-P; values obtained by quenching aliquots of the mixture with 0.5 N $HClO_4$ or 2 N KOH are shown together with the standard deviation. [d] The concentration of free sugar phosphate calculated from the $K_{X(Glc\text{-}P)}$ values in Table I and the initial conditions in footnote *c* (cf. Ray and Long, 1976b). [e] The estimated percent of the central complexes in the indicated form plus the standard deviation (see Results). Only the values obtained with acid quenching are shown and each value, together with its standard deviation is rounded to the nearest half percent. [f] Average of results obtained by use of 0.5 N or 1 N acid, or 1 N or 2 N base in the quenching solution; see Results. [g] Average of results obtained with 0.5, 1, or 2 N acid or 0.5, 1, or 2 N base in the quenching solution (Ray and Long, 1976b). [h] Arbitrary correction; see footnote 5. [i] Average of six time aliquots removed between 3 and 24 h after mixing. [j] Correction calculated in the manner described in a supplemental section; see paragraph at the end of this paper concerning supplementary material.

As was noted earlier (Ray and Long, 1976a), attempting to maximize the rate of inactivation of enzyme–substrate and enzyme–product complexes in the present system by mixing with acid seems more reasonable than by mixing with base, since in the formation of these complexes the basic form of the glucose phosphates is the form that binds to the enzyme. Thus, protonating the phosphate group of a substrate or product not only would greatly reduce its chances of recombining with the enzyme, but also might well speed its dissociation, whereas an analogous effect is not expected with base. Moreover, if inactivation by base requires direct unfolding of the enzyme·glucose phosphate complexes, the equilibria among the central complexes could be altered during the quenching process (e.g., if one complex unfolded more readily than another). Hence, in cases where a significant difference was observed between the results obtained by quenching with acid or base, the results obtained with acid were used in subsequent calculations; however, both sets of results are shown in Table II for each metal ion in terms of the percent of the central complexes that would be present in each form if no quenching artifacts had occurred. An additional reason for using the results obtained with acid is related to the plot in Figure 2, which is described in the Discussion section, and is indicated in footnote 6. However, differences observed in mixtures quenched in acid and base never were large, and only small quantitative changes would be produced by either using the average of the two results or using only those obtained by quenching in base.

Table I, column 6, shows values of the equilibrium constant for the PO_3-transfer step, E_P·M·Glc-6-P $\rightleftharpoons$ E_D·M·Glc-P_2 (i.e., for $K_{Tr\text{-}1}{}^b$) as a function of the bound metal ion. These values, which were calculated from the data in columns 4 and 11, Table II, change by about 65-fold in going from the Zn^{2+} to the Cd^{2+} form of the enzyme. Thus, noncovalent interactions involving the enzymic phosphate group in E_P·M·Glc-6-P and the same group in E_D·M·Glc-P_2 must change in a manner that depends on the identity of the bound metal ion. By contrast the equilibrium constant for the overall conversion of E_P·M·Glc-6-P to E_P·M·Glc-1-P is essentially independent of the metal ion (column 7, Table I): average value, 0.36 ± 0.09 (SD).

Equilibria among the Enzyme-Glucose Phosphate Complexes in the "Absence" of a Metal Ion. In order to aid in interpreting the effects of the various metal ions on the equilibria among the central complexes, an analogous equilibrium was established with metal-free enzyme, after addition of 1 mol % Mg^{2+}. Although the enzyme–substrate complexes are essentially inactive in the absence of a metal ion activator (Ray and Peck, 1972), equilibria among E_P·Glc-1-P, E_D·Glc-P_2, and E_P·Glc-6-P can be established via dissociation and reassociation of a small amount of a metal ion such as Mg^{2+}. Since the compositions of equilibrium mixtures established with 1 mol % Mg^{2+} were different from those obtained when all of the enzyme was present as its Mg^{2+} complex, and since no differences were observed in time aliquots taken from the former mixtures at intervals between 3 and 24 h after mixing (see Table II), equilibrium undoubtedly was achieved in this study. Moreover, the 1 mol % Mg^{2+} present probably did not significantly distort the equilibrium involving the metal-free enzyme forms, because Mg^{2+} binds approximately equally to E_P and the equilibrium mixture of E_P·Glc-1-P, E_D·Glc-P_2, and E_P·Glc-6-P (Ray et al., 1966) and thus should be bound predominately to E_P, which was present in a tenfold excess over the enzyme–glucose phosphate complexes. In addition, the same results were obtained with a mixture which was identical except that only 0.5 mol % Mg^{2+} was used (data not shown). The procedure for calculating dissociation constants for E_P·

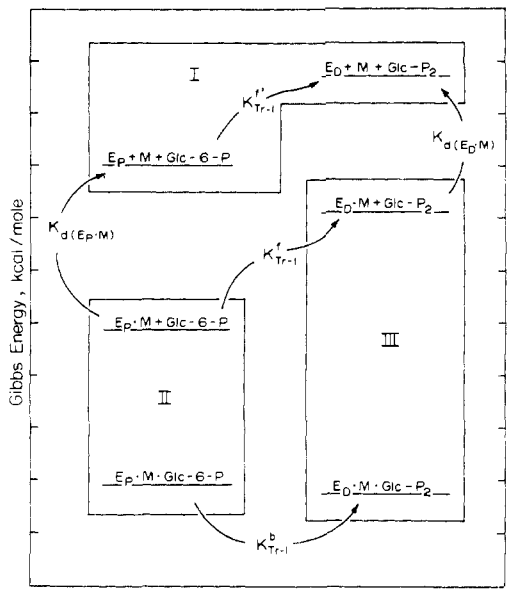

FIGURE 1: A Gibbs energy diagram for interconversion of various species in the phosphoglucomutase–metal ion–substrate system. $\Delta G^{\circ\prime}$ values were calculated for the Mg^{2+} enzyme from equilibrium constants either given in Table I or in Ray and Peck (1972). All values refer to pH 7.5; however, data were obtained both at 24 °C and other data at 30 °C (see Results). Species whose relative positions either are unchanged or essentially unchanged when Mg^{2+} is replaced by other activating metal ions are enclosed in solid lines (see Results). The arrows identify equilibrium constants that are considered in the Discussion section.

Glc-6-P and E_D·Glc-P_2, columns 8 and 9, Table I, from data obtained in these experiments (Table II) is described in a supplemental section; however, the accuracy of the constants obtained in this manner undoubtedly is less than that of other dissociation constants in Table I (see below).

Dissociation Constants for the Central Complexes. The equilibrium dissociation constants of the various sugar phosphates from their complexes with the enzyme, E_D·M·Glc-P_2, E_P·M·Glc-6-P, and E_P·M·Glc-1-P, can be calculated as the product of the appropriate equilibrium isotope exchange constant and the reciprocal of the fraction of the central complexes comprising the complex in question at equilibrium, e.g., $K_{d(Glc\text{-}6\text{-}P)} = (E_P{\cdot}M)(Glc\text{-}6\text{-}P)/(E_P{\cdot}M{\cdot}Glc\text{-}6\text{-}P) = K_{x(Glc\text{-}6\text{-}P)}[CC]/[E_P{\cdot}M{\cdot}Glc\text{-}6\text{-}P]$ (see accompanying paper, Ray and Long, 1976b). Estimates of the true dissociation constants for glucose-6-P and glucose-1,6-P_2 are shown in the last two columns of Table I. The similarity of the K_d values for glucose-6-P—the average value is 41 ± 11 μM (SD), excluding the value in the absence of a metal ion—as well as the similarity of the corresponding values for glucose-1-P (not shown) plus the relatively constant values of $[E_P{\cdot}M{\cdot}Glc\text{-}1\text{-}P]/[E_P{\cdot}M{\cdot}Glc\text{-}6\text{-}P]$ (column 7) shows the lack of dependence of the noncovalent interactions between the enzyme and substrate in E_P·M·Glc-P on the identity of M. In fact the dissociation constant for E_P·Glc-6-P only is about twice the average value for that of E_P·M·Glc-6-P. In the case of E_P·M·Glc-P_2, the variation in $K_{d(Glc\text{-}P_2)}$ with metal ion is about twice that for $K_{d(Glc\text{-}P)}$, on a fractional basis (excluding the value obtained with Mn^{2+} enzyme and that measured in the absence of a metal ion; see Discussion), but still does not depend to a large extent on the identity of M, and the dissociation constant for E_D·Glc-P_2 only is about fivefold larger than the average value for E_D·M·Glc-P_2. Thus, most of the variation in K_x values in columns 3 and 4, Table I, is simply the result of differences in the distribution of species among the central complexes.

A Gibbs Energy Diagram for PO_3 Transfer. The above results can be summarized by the Gibbs energy diagram in Figure 1. This diagram was constructed by using the equilibrium constants for the Mg^{2+} enzyme, although the metal ion is indicated as M to facilitate comparisons of changes induced by other metal ions. Data summarized by Ray and Peck (1972) also were used. The latter data were obtained at 30 °C, while the data in Table I were collected at 24 °C; however, this difference is not expected to produce significant differences in the positions of the various species. Solid lines enclosed species whose *relative* positions are independent or essentially independent of the *identity* of the metal ion (a) by definition, e.g., enclosure I, or (b) as determined experimentally, e.g., enclosure II, or III.

TABLE III: Gibbs Energy Changes as a Function of the Bound Metal Ion.[a]

Metal Ion	$(\Delta G_{Tr-1}{}^{\circ\prime})^f$	$(\Delta G_{Tr-1}{}^{\circ\prime})^b$
Zn^{2+}	+6.2	+0.9
Co^{2+}	+4.7	+0.2
Mg^{2+}	+4.6	−0.3
Ni^{2+}		−0.3
Mn^{2+}	+5.1	−1.1
"None"	+3.4[b]	−1.0
Cd^{2+}	+3.3	−1.6

[a] Calculated at 24 °C and pH 7.5; unless otherwise indicated data in Tables I and II were used; for a definition of constants, see eq 2 and 3. [b] Calculated from the corresponding values for the Mg^{2+} enzyme plus the dissociation constants for E_P·Mg and E_D·Mg (Ray and Peck, 1972).

Discussion

Two PO_3-transfer steps are required to complete the phosphoglucomutase reaction (Ray and Peck, 1972):

$$E_P{\cdot}M{\cdot}Glc\text{-}1\text{-}P \rightleftharpoons E_D{\cdot}M{\cdot}Glc\text{-}P_2 \rightleftharpoons E_P{\cdot}M{\cdot}Glc\text{-}6\text{-}P \quad (1)$$

However, since noncovalent interactions between the enzyme and either glucose-1-P or glucose-6-P in the first and last of these complexes are essentially independent of M (see Table I, column 7, and Results section), in a study of metal ion effects such as the present one it seems reasonable in some places to omit a distinction between E_P·M·Glc-1-P and E_P·M·Glc-6-P and refer to the two, together, as E_P·M·Glc-P, so that E_P·M·Glc-P and E_D·M·Glc-P_2 become a (bound) substrate-product pair (see Ray and Long, 1976b).

Structural Differences between Enzyme–Substrate and Enzyme–Product Complexes. One of the striking aspects of this study is the systematic shift in equilibrium between E_P·M·Glc-P and E_D·M·Glc-P_2 as the identity of the metal ion is changed within the series, Zn^{2+}, Co^{2+}, Mg^{2+}, Ni^{2+}, Mn^{2+}, and Cd^{2+}. In terms of equilibrium constants, the extent of the shift is about 65-fold, as is indicated by the ratio, $[E_D{\cdot}M{\cdot}Glc\text{-}P_2]/[E_P{\cdot}M{\cdot}Glc\text{-}6\text{-}P]$, column 6, Table I. (The change in the analogous ratio for glucose-1-P (not shown) is similar, for the reason noted above.) Thus, the central complexes are predominately E_P·M·Glc-P when M = Zn^{2+} and predominately E_D·M·Glc-P_2 when M = Cd^{2+}, and physical differences in the observed properties of the central complexes involving Zn^{2+} and Cd^{2+} should be related primarily to differences between enzyme–substrate and enzyme–product complexes rather than to differences in the bound metal ion, per se, partly because there seems to be little conformational difference among the E_P·M complexes themselves (Peck and Ray, 1969a)

or among the E_D·M complexes (J. W. Long and W. J. Ray, Jr., unpublished results), and partly because the binding of glucose phosphates also is independent of the *identity* of M (see below).

Two properties of the central complexes have been studied as a function of the activating metal ion: the ultraviolet absorption spectrum (via difference spectroscopy) and the exposure of aromatic residues to the aqueous environment (via solvent perturbation techniques). Both suggest that conformational differences are produced by the above changes. Thus, the tyrosine peaks in the metal-specific difference spectrum that are generated in the presence of bound substrate (Peck and Ray, 1969b) are red-shifted in the (central) complexes involving Zn^{2+} and blue-shifted in those involving Cd^{2+} if the complexes involving Mg^{2+} are used as a reference. That there is a relationship between the magnitude of these metal-specific difference peaks and the composition of the central complexes is shown by the plots (●) in Figure 2.[6] Thus, as the composition of the central complexes changes from predominately E_P·M·Glc-P to predominantly E_D·M·Glc-P_2, the spectral shift changes from positive to negative in a manner that is roughly proportional to the fraction of the central complexes present as E_P·M·Glc-P or E_D·M·Glc-P_2. (A similar conclusion also holds if E_P·M·Glc-1-P and E_P·M·Glc-6-P are treated separately; see Figure 2.)

To the extent that the metal-dependent difference spectra whose $\Delta\epsilon_{286}$ values are plotted in Figure 2 reflect conformational differences between E_P·M·Glc-P and E_D·M·Glc-P_2, a conformational change must accompany the interconversion of this substrate–product pair. Moreover, the results of solvent perturbation studies reported previously (Peck and Ray, 1969b) suggest that these spectral differences are indeed the rosines: partially buried residues, accessible to water and small perturbants, and buried residues, essentially inaccessible to all solvent molecules. Thus, since small perturbants such as D_2O and dimethyl sulfoxide do not distinguish among the tyrosine residues of the central complexes involving Zn^{2+}, Mg^{2+}, and Cd^{2+} (Figure 4, top, Peck and Ray, 1969b), changes in residues inaccessible to such perturbants, i.e., buried residues, must be responsible for part (and possibly most) of the spectral change that was referred to above. However, under the same conditions, larger perturbants such as glycerol and sucrose do recognize significant differences in tyrosine exposure (Figure 4, bottom, Peck and Ray, 1969b); hence, partially buried residues, accessible to both water and small perturbants, but not large perturbants, also may contribute to the spectral differences among the central complexes.

Although there is no reliable procedure for accurately estimating the number of tyrosines whose environment is different in the E_P·M·Glc-P and E_D·M·Glc-P_2 complexes, differences in the solvent perturbation spectra produced by sucrose are large enough to implicate at least three such residues (Peck and Ray, 1969b), and at least one completely buried residue also must be involved (see above). In fact, it is possible that a sizable fraction of the 17 or so tyrosines in phosphoglucomutase (cf. Ray and Peck, 1972) is affected to a small extent rather than four residues being affected to a large extent. *If* so, conformational changes produced by the substrate–product interconversion may well be propagated to points substantially removed from the active site. That binding effects can be propagated to many distant points in a protein molecule is supported by the studies of Wickett et al. (1974), in which the effect of inhibitor binding on tritium exchange in the lysozyme system was examined. Hence, to the extent that such effects are propagated by means of conformational changes, a conformational change involving distant points in the protein must accompany interconversion of enzyme·substrate and enzyme·product complexes if the change produced by substrate binding differs from that produced by the binding of product.

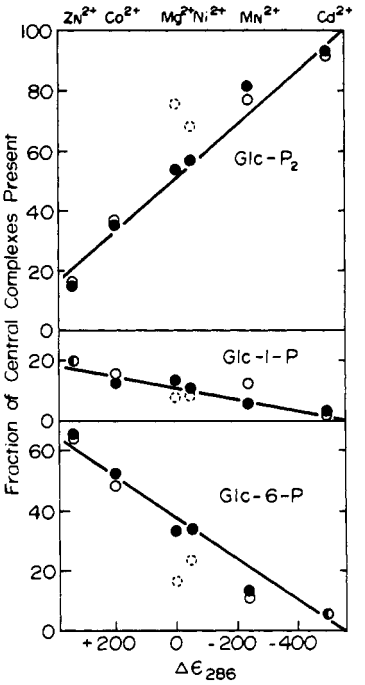

FIGURE 2: Variation in the percent of the central complexes present at equilibrium as E_D·M·Glc-P_2, E_P·M·Glc-6-P, or E_P·M·Glc-1-P, as a function of the extinction coefficient at 286 nm for the difference spectrum of (enzyme + glucose phosphate + metal ion) vs. (enzyme + glucose phosphate + Mg^{2+}). The percent of the complexes present was taken from Table II: (●) results from acid quenching; (○) results from base quenching; (◌) results from base quenching that were discarded for reasons noted in the Results. Values of $\Delta\epsilon_{286}$ were taken from experiments analogous to those of Peck and Ray (1969b) but in which the central complexes involving Mg^{2+} instead of Zn^{2+} were used as the reference.

The Possible Significance of Conformational Changes during Catalysis. There are three different types of reasons why a conformational change might occur during or as the result of the catalytic process and, although each ultimately is related to the inability of a *rigid* binding site to interact optimally with both the reactants and the products of a chemical process (cf. Jencks, 1969), it is difficult to decide among them. Thus, the conformational change in question might be (a) a peculiarity of the phosphoglucomutase system and similar systems that utilize a reaction sequence involving two group-transfer steps (which require alternative binding interactions) to complete the catalytic cycle (cf. Ray et al., 1973); (b) an enabling aspect of the catalytic process such as is required in a "continuous compensation" mechanism (Hammes, 1964); (c) a permissive feature of the enzymic reaction, that, if blocked, would oppose the catalytic process, but that does not

[6] A regular change in $\Delta\epsilon_{286}$ with a change in the apparent fraction of E_P·M·Glc-P or E_D·M·Glc-P_2 present is calculated from the results obtained by use of acid quenching (●, Figure 2) whereas an irregular change is calculated from the results obtained with base (○, Figure 2). The attractive explanation given below for a regular change in spectral properties with a change in composition of the central complexes contributed to the decision to use only results obtained by means of acid quenching (as opposed to the average of results obtained by both procedures) in the subsequent analysis (see also the Results section) although both types of results are included in Figure 2 and in Table II.

provide a significant *driving force* for the bond-breaking and bond-making steps, e.g., a change or series of changes by which the conformation of the binding site of the enzyme "follows" the course of the reaction.

It also seems reasonable to point out that the half-of-the-sites reactivity found in some multimeric enzymes (cf. Lazdunski, 1972) might be related to conformational changes that *accompany* the interconversion of *stoichiometrically identical* enzyme–substrate and enzyme–product complexes. In fact, since a permissive change (see above) in the conformation of a binding site during catalysis in a monomeric enzyme system can become an enabling change relative to catalysis in an analogous dimeric system if the originally permissive changes are tightly coupled across the subunit–subunit interface, a possible origin of such effects is suggested.

The Mechanism of the Metal-Specific Alteration in the Equilibrium Ratio of Enzyme–Substrate and Enzyme–Product Complexes. The following generalizations describe trends in the constants listed in columns 5, 6, 8, and 9, Table I, that indicate how the metal ion alters the equilibria among the central complexes. These trends are summarized in the energy level diagram of Figure 1.

(a) The Binding of Glucose Monophosphates to the Phospho-Enzyme. The dissociation constants for E_P·M·Glc-6-P complexes, column 9, Table I, do not depend substantially on the *identity* of M (nor do the corresponding constants for the combined glucose monophosphates; see Results). To show this independence, the positions of E_P·M + Glc-P and of E_P·M·Glc-P are enclosed within a box in Figure 1. Such a relationship makes a contact interaction between the metal ion and glucose monophosphates in E_P·M·Glc-P seem unlikely, but does not rule it out completely. In fact, it is difficult to decide how much of a change in dissociation constant with a change in M one might reasonably expect within the series studied if a contact interaction were involved. Taking $M^{2+}HPO_4^{2-}$ as a model, a variation of about tenfold would be expected (Sillen and Martell, 1964, 1971), but within the restricted environment of a ternary enzyme–metal–substrate complex, an even larger change would not be surprising. Moreover, although glucose-6-P binds to the *metal-free* phospho-enzyme only about half as well as to the "average" enzyme–metal complex (column 9, Table I), a larger difference than this should be observed if in E_P·M·Glc-P, glucose-P were bound within the coordination sphere of the metal ion. (Methyl phosphonate anion, which is an inhibitor competitive with glucose-1-P, binds some 10 Å distant from Mn^{2+} (Ray and Mildvan, 1972); this also suggests that the anionic phosphate group of bound glucose-P does not interact directly with the metal ion.) A direct interaction between the metal ion and an hydroxyl group of the sugar ring also seems unlikely, but cannot be ruled out.

(b) The Binding of Glucose Bisphosphate to the Dephospho-Enzyme. The variation in $K_{d(Glc\text{-}P_2)}$ (column 8, Table I) with metal ion is about twice as large as the corresponding variation in $K_{d(Glc\text{-}P)}$, even if the values obtained with Mn^{2+} and without a metal ion are excluded (see Results). However, as a first approximation, $K_{d(Glc\text{-}P_2)}$ is taken as independent of the *identity* of M, as is indicated by enclosure III, Figure 1 (except in the case of Mn^{2+}; see below), since metal-specific differences in $K_{d(Glc\text{-}P_2)}$ are much smaller than the corresponding differences in the equilibrium constant, $K_{Tr\text{-}1}{}^b$, for the PO_3-transfer process involving bound reactants and products:

$$E_P\text{·M·Glc-6-P} \rightleftharpoons E_D\text{·M·Glc-P}_2 \qquad (2)$$

(see column 6, Table I). A stronger interaction between M and bound glucose-1,6-P_2 than between M and bound glucose-P is indicated by the greater increase in $K_{d(Glc\text{-}P_2)}$ on removal of the metal ion (columns 8 and 9, Table I). However, this difference can be easily rationalized in terms of the increased charge on glucose-1,6-P_2 relative to glucose-P, without invoking a contact interaction.

(c) The Effect of the Metal Ion on the Chemical Potential of the Phospho-Enzyme. Since the relative positions of the species within enclosures II and III, Figure 1, are independent of the *identity* of M, metal specific effects on $K_{Tr\text{-}1}{}^b$ (eq 2) are caused primarily by alterations in the *relative* positions of these enclosures, which in turn are produced by *differences* in binding of M to E_P and E_D. Thus, if metal binding produced a change in distance between enclosures I and III (which is a measure of K_d for E_D·M) identical with that produced between I and II (which is a measure of K_d for E_P·M), no *metal specific* effects on the central complexes would be observed. (The actual distance between enclosures I and II, which is a measure of the dissociation constant for E_P·M, varies from about 6.2 kcal/mol for M = Mg^{2+} to about 15.6 kcal/mol for Zn^{2+} (cf., Ray, 1969).) In fact, changes in the binding of M to E_P and E_D are equivalent to changes in the chemical potential of the phosphate group in E_P·M as a function of M, and several experiments have been designed to show that the enzymic phosphate in the binary E_P·M complex is directly coordinated to M. Although all of these produced negative results (Ray and Mildvan, 1973, and unpublished results), none were conclusive, and the precise relationship between the enzymic PO_3 group and bound M remains undefined; however see the following section.

To the extent that the above generalizations about enclosures II and III hold, the metal-specific change in $(\Delta G_{Tr\text{-}1}{}^{\circ\prime})^f$ for the equilibrium involving free reactants and products

$$E_P\text{·M} + \text{Glc-6-P} \rightleftharpoons E_D\text{·M} + \text{Glc-P}_2 \qquad (3)$$

must parallel the metal-specific change in $(\Delta G_{Tr\text{-}1}{}^{\circ\prime})^b$ (eq 2), i.e., metal-specific changes in chemical potential of the enzymic phosphate group in E_P·M are not (appreciably) altered by the binding of glucose-P. (Alternative support for this conclusion can be obtained by comparing the independently determined values of $K_{Tr\text{-}1}{}^f$ and $K_{Tr\text{-}1}{}^b$ in columns 5 and 6, Table I.) Hence, metal-specific changes in the equilibrium among the central complexes arise from differences in metal ion binding to E_P·Glc-P and E_D·Glc-P_2.

Does the Metal Ion Participate Directly in PO_3 Transfer? In the process defining $(\Delta G_{Tr\text{-}1}{}^{\circ\prime})^f$ (eq 3), the enzymic PO_3 group must move entirely out of the sphere of influence of the bound metal ion (even though it may not be directly coordinated with the metal ion initially). By contrast, in the process defining $(\Delta G_{Tr\text{-}1}{}^{\circ\prime})^b$ (eq 2) the enzymic PO_3 group presumably moves only through a distance sufficiently great to free it from the serine oxygen of the enzyme and attach it to the oxygen of the bound glucose-P molecule. Since the phosphate groups in *free* glucose-1,6-P_2 (by definition) cannot be affected by the identity of the *bound* metal ion in E_D·M, the phosphate groups in E_D·M·Glc-P_2 apparently are not affected by the *identity* of the metal ion either; otherwise altering the metal ion would not produce a parallel change in $(\Delta G_{Tr\text{-}1}{}^{\circ\prime})^b$ and $(\Delta G_{Tr\text{-}1}{}^{\circ\prime})^f$. Hence, during the process, E_P·M·Glc-P → E_D·M·Glc-P_2, the enzymic PO_3 group moves out of the sphere of influence of the bound metal ion to an extent sufficient to essentially eliminate *metal specific effects* (although not the overall effect of the metal ion; see previous section). (In the case of Mn^{2+}, the PO_3 group apparently does not become nearly so free from the in-

fluence of the metal ion, and this effect shows up both in terms of an anomalously tight binding of glucose-1,6-P_2 (see above) and a nonparallel change in $(\Delta G_{Tr\text{-}1}°')^f$ and $(\Delta G_{Tr\text{-}1}°')^b$ when M changes from any other metal ion to Mn^{2+}.)

If, in the E_P·M·Glc-P complex, the enzymic phosphate group were directly coordinated with, and its chemical potential determined by, the bound metal ion, eliminating essentially all of the metal-specific influence on the enzymic phosphate group *during* the PO_3-transfer step would necessitate removal of the PO_3 group from the coordination sphere of the metal ion either *during* or subsequent to this step. Since such a dissociation step *during* a process involving the making and breaking of covalent bonds does not seem attractive from a mechanistic standpoint, either the enzymic PO_3 group in E_P·M·Glc-P is not directly coordinated to the metal ion in the first place (and the metal-specific change in its chemical potential is produced indirectly) or if it is in the coordination sphere of the bound metal it remains there during PO_3 transfer, but the initial (E_D·M·Glc-P_2) complex that is produced is subsequently converted into a more stable E_D·M·Glc-P_2 complex in which the coordination between the transferred PO_3 group and the metal ion has been ruptured.

The Coupling between the Chemical Potential of the Enzymic Phosphate and the Structural Differences among the Central Complexes. Although metal ions produce alterations in the spectral properties of tryptophan residues when they bind to the free enzyme,[1] no *metal-specific* alterations are observed, viz., all metal ions, including Mn^{2+}, produce the same spectral changes (Peck and Ray, 1969a). Hence, metal-specific differences in chemical potential of the enzymic phosphate group, per se, do not produce spectral differences. The subsequent binding of glucose phosphates produces additional spectral changes in tryptophan residues and alters the spectral properties of tyrosines as well (Peck and Ray, 1969b). However, part of the alteration in tyrosine residues is *metal specific,* and the metal-specific part appears *in response* to changes in the E_P·M·Glc-P $\rightleftharpoons$ E_D·M·Glc-P_2 equilibrium. Thus, changes in this equilibrium together with the spectral differences produced by these changes are the *consequence* of alterations in the chemical potential of the enzymic phosphate group—not the cause of it—and show up as conformational differences only in the presence of glucose phosphates. In fact, the ability of the metal ion to alter the chemical potential of the enzymic phosphate group may well be nearly incidental to its role in catalysis. Thus Cd^{2+} appears to bind without significantly altering the chemical potential of E_P (see previous section), although Cd^{2+} accelerates the PO_3-transfer process by at least 10^5-fold relative to whatever transfer occurs in the absence of a bound metal—in spite of the fact that Cd^{2+} is only 1% as efficient an activator as Mg^{2+} (Ray, 1969).

In any case, the variation of the above equilibrium with metal ion provides an unusual opportunity to study structural differences in enzyme-substrate and enzyme-product complexes; this system will be investigated more thoroughly in subsequent studies.

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Supplementary Material Available

Additional information concerning calculations as noted in the text (1 page). Ordering information is given on any current masthead page.

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