The Equilibrium Constant of the Phosphoglyceromutase Reaction

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The equilibrium constant of the phosphoglyceromutase reaction was determined over a range of pH (5.4–7.9), in solutions of different ionic strength (0.06–0.3) and in the presence of Mg^{2+} , at 30°C and at 20°C. The values obtained (8.65–11.65) differ substantially from previously published values. The third acid dissociation constants were redetermined for 2- and 3-phosphoglycerate, and in contrast with previous reports the pK values (7.03 and 6.97 respectively at zero ionic strength) were closely similar. The Mg^{2+} -binding constants were measured spectrophotometrically and the values, 286 mm^{-1} and 255 mm^{-1} for 2- and 3-phosphoglycerate at pH 7 and ionic strength 0.02, were also very similar. From the relative lack of effect of temperature, pH and ionic strength it is concluded that the equilibrium constant differs from unity largely because of entropic factors. At low ionic strength, in the neutral region, the pH-dependence can be attributed to the small difference in the acid dissociation constants, but the difference in dissociation constants does not explain the pH-dependence in the acid region or at high ionic strength. Within physiological ranges of pH, Mg^{2+} concentration and ionic strength there will be little variation in equilibrium constant.

Although there have been many measurements of the equilibrium constant for the phosphoglycerate mutase (EC 2.7.5.3) reaction the agreement between values is poor (Table 3). This constant is important in the Emden–Meyerhof pathway and in kinetic investigations (Britton & Clarke, 1972; Britton *et al.*, 1971). In the present paper we have therefore redetermined its value under a variety of conditions over a range of pH values and ionic strengths and in the presence of Mg^{2+} . As the change in equilibrium constant with pH was much smaller than that expected from the published values for the third acid dissociation constants for 2-phosphoglycerate and 3-phosphoglycerate (Johnson, 1960) these constants have also been determined; that of 2-phosphoglycerate was substantially different from the published values. In addition the Mg^{2+} -binding constants for 2- and 3-phosphoglycerate have been measured.

then the equilibrium can be represented as shown in Scheme 1. For this scheme the measured equilibrium constant K_{eq} ([3-phosphoglycerate]/[2-phosphoglycerate]) can be related to the equilibrium constant for the unliganded triply charged species, $K_{\text{eq},0}$, by the expression:

$$K_{\text{eq}} = \frac{\{1 + K_{\text{Mg}}^{3\text{-P-Gri}}[\text{Mg}_{\text{free}}^{2+}] + ([\text{H}^+]/K_i^{3\text{-P-Gri}})\}}{\{1 + K_{\text{Mg}}^{2\text{-P-Gri}}[\text{Mg}_{\text{free}}^{2+}] + ([\text{H}^+]/K_i^{2\text{-P-Gri}})\}} \quad (1)$$

$K_i^{3\text{-P-Gri}}$ and $K_i^{2\text{-P-Gri}}$ are the third acid dissociation constants for 3- and 2-phosphoglycerate, and $K_{\text{Mg}}^{3\text{-P-Gri}}$ and $K_{\text{Mg}}^{2\text{-P-Gri}}$ are the association constants for triply charged forms of 3- and 2-phosphoglycerate.

Materials and Methods

Materials

2-Phosphoglycerate and 3-phosphoglycerate as the trisodium salts, 2,3-diphosphoglycerate as the

Theory

If it is assumed that only the triply charged forms of 2- and 3-phosphoglycerate bind Mg^{2+} appreciably

Scheme 1. Ionic species of 2- and 3-phosphoglycerate that determine the equilibrium constant of the phosphoglycerate mutase reaction in the presence of Mg^{2+}

cyclohexylamine salt, ADP as the free acid, ATP and NADH as the sodium salts, and the enzymes (from rabbit muscle) phosphoglyceromutase [specific activity 30 units/mg, 2 mg/ml in 2.6M-(NH₄)₂SO₄], pyruvate kinase [specific activity 27 units/mg, 2 mg/ml in 2.8M-(NH₄)₂SO₄] and lactate dehydrogenase [specific activity 360 units/mg, 5 mg/ml in 2.2M-(NH₄)₂SO₄] were obtained from C. F. Boehringer G.m.b.H., Mannheim, Germany. Enzymic assay of both 2- and 3-phosphoglycerate (see below) indicated a purity of at least 95% as claimed by the manufacturers. Both gave single spots on paper chromatography on Whatman no. 1 paper with di-isopropyl ether-90% formic acid (3:2, v/v) (Eggleston & Hems, 1952) and development with molybdate (Burrows *et al.*, 1952). A.R. MgCl₂·6H₂O, KCl, triethanolamine and 8-hydroxyquinoline were obtained from Hopkin and Williams, Chadwell Heath, Essex, U.K. MgCl₂ solutions were standardized by titration with AgNO₃, with dichlorofluorescein as an indicator. Solutions of 8-hydroxyquinoline were freshly made up before use. All glassware was cleaned with Decon 75 (A. Gallenkamp and Co. Ltd., London E.C.2, U.K.).

Determination of 2-phosphoglycerate

Samples from the reaction 'mixtures' (0.5 ml) were added to 0.2 ml of 0.25M-HClO₄ in 10 mm light-path quartz cuvettes. After thorough mixing, 0.8 ml of 1M-NaOH was added to neutralize the solution and 2-phosphoglycerate was determined by enzymic conversion into lactate (Britton & Clarke, 1972).

Determination of total 2- and 3-monophosphoglycerate

Samples from the reaction 'mixtures' (50 μ l) were taken and the procedure for the determination of 2-phosphoglycerate was followed, with omission of HClO₄ and NaOH. Since phosphoglyceromutase was present in high concentration in the original solution (see below) both 2- and 3-phosphoglycerate were converted into lactate and thus total monophosphoglycerate was determined.

The volume of solution taken to determine 2-phosphoglycerate (0.5 ml) was ten times that taken to measure total monophosphoglycerate (50 μ l), so that when the equilibrium constant was measured (see below), the absorbance change was approximately the same in both determinations.

Determinations of the equilibrium constant of the phosphoglyceromutase reaction

2-Phosphoglycerate (3 mM) and 2,3-diphosphoglycerate (10 μ M) were incubated at 30°C with rabbit muscle phosphoglyceromutase (3 units, 10 μ l of enzyme solution; 2 mg/ml) in 3 ml of buffer. At 2½ h (3 h at pH 6.5) samples were taken for determination

of 2-phosphoglycerate and total monophosphoglycerate concentrations, and a further set of samples was taken 30 min later. The whole procedure was then repeated under identical conditions with 3-phosphoglycerate (3 mM) as the substrate. This experimental design was adopted to provide evidence for attainment of equilibrium. In practice no significant difference was found between any of the values. Mean values are therefore given in the Results section.

The buffer concentration was 33.3 mM in all cases and the buffers were sodium maleate (pH 5.5), imidazole-HNO₃ (pH 6.10 and 6.45), and Tris-HCl (pH 6.66, 7.10, 7.50 and 7.89). All pH values were determined with a glass electrode at the temperature at which the equilibrium constant was measured.

In some experiments tetraethylammonium nitrate or KCl was added to raise the ionic strength and MgCl₂ was added in a number of determinations.

Determination of the third acid dissociation constants of 2- and 3-phosphoglycerate

HCl (0.25M) was added with an Agla micro-syringe to 7.0 ml of a 10 mM solution of the trisodium salts of 2- or 3-phosphoglycerate at 30°C and the changes of pH were measured with a glass electrode. Solutions with ionic strength greater than 0.06 were obtained by adding KCl.

Determination of Mg²⁺-binding constants of 2- and 3-phosphoglycerate

The method adopted was essentially that of Burton (1959). Solutions (6 ml) containing 16.7 mM-Tris-HCl, pH 7.4, 0.833 mM-8-hydroxyquinoline, and 2- or 3-phosphoglycerate (0.95 mM) or sufficient KCl to give the same ionic strength were prepared. Each solution (3 ml) was placed in a 10 mm cuvette thermostatically maintained at 30°C and the remainder of the solution was used as a blank. MgCl₂ (60 mM) was added in 20 μ l batches from an Agla micro-syringe. The increase in E_{360} was corrected for the dilution caused by the MgCl₂ solution and the rate of change of absorbance with magnesium was extrapolated to zero magnesium concentration by fitting the data to the empirical polynomial:

$$I = p_1(1 - e^{-p_3[Mg^{2+}]}) + (a - p_1)(1 - e^{-p_2[Mg^{2+}]}) \quad (2)$$

where I is the optical increase in E_{360} , $[Mg^{2+}]$ is the total concentration of Mg^{2+} , p_1 , p_2 and p_3 are arbitrary constants, and a represents the final E_{360} when all of the 8-hydroxyquinoline is bound by Mg^{2+} . Fitting was done with an Atlas 360 computer by using program BMDX 85 (University of London Computer Centre).

Calculation from the data of Näseänen (1952) showed that at pH 7.4 only about 75% of the 8-hydroxyquinoline should be complexed at a Mg^{2+} concentration of 20 mM, whereas 98% should be complexed at pH 8.8. The parameter a was therefore determined at pH 8.8 in 20 mM- MgCl_2 . At this pH the E_{360} was slightly dependent on Tris concentration, values of 1.068, 1.108 and 1.128 being recorded at Tris concentrations of 16.7, 8.33 and 4.17 mM respectively. The E_{360} was therefore extrapolated to zero Tris concentration to give a value for a of 1.142.

In the absence of ligand, the initial slope (A) as Mg^{2+} is added is given by the expression (Burton, 1959):

$$A = \frac{aK_1}{[\text{H}^+]\left(1 + \frac{K_1[\text{HOx}]}{[\text{H}^+]}\right)} \quad (3)$$

where K_1 is the association constant for the reaction

and HOx is the protonated form of hydroxyquinoline. Substitution into eqn. (3) of the initial slope determined from eqn. (2) gave a value for K of $4.425 \times 10^6 \pm 0.099 \times 10^6$, in good agreement with the value 4.61×10^6 reported by Näseänen (1952).

The initial slope (B) in the presence of the ligand is given by the equation:

$$B = \frac{aK_1}{[\text{H}^+]\left\{1 + \frac{K_1[\text{HOx}]}{[\text{H}^+]} + \frac{K_{\text{Mg}}^{\text{P-Gri}}K_1^{\text{P-Gri}}[\text{P-Gri}]}{(K_1^{\text{P-Gri}} + [\text{H}^+])}\right\}} \quad (4)$$

$K_{\text{Mg}}^{\text{P-Gri}}$ is the association constant of the triply charged form of the ligand for Mg^{2+} , P-Gri is the total concentration of the ligand and $K_1^{\text{P-Gri}}$ is its third acid dissociation constant. Eqn. (4) was derived by following the procedure of Burton (1959) with the assumption that only the triply charged ligand binds Mg^{2+} to an appreciable extent. $K_{\text{Mg}}^{\text{P-Gri}}$ was determined from the difference $(1/A) - (1/B)$.

Results

Equilibrium constant in the absence of Mg^{2+}

Data for the equilibrium constants are shown in Table 1 and Fig. 1. At 30°C and at low ionic strength ($I = 0.06$ – 0.07) the equilibrium constant varied between 8.56 and 11.65 over the pH range 5.42–7.89. The equilibrium constant $K_{\text{eq},0}$ for the triply charged species (eqn. 1, Theory section) may be expected to be independent of pH. Assuming $K_{\text{eq},0} = 11.77$ (eqn. 1) and taking the values for the third acid dissociation constants for 2-phosphoglycerate and 3-phosphoglycerate determined in the present paper ($I = 0.065$; $\text{p}K_1^{3-\text{P-Gri}} = 6.81$; $\text{p}K_1^{2-\text{P-Gri}} = 6.86$), a theoretical line ----, Fig. 1) is obtained which is a reasonable fit

to the experimental data in the neutral range, although there is an appreciable deviation at low pH values. In contrast with the same value of $K_{\text{eq},0}$ and with previously reported values for the dissociation constants ($I = 0.065$; $\text{p}K_1^{3-\text{P-Gri}} = 6.42$; $\text{p}K_1^{2-\text{P-Gri}} = 7.16$; Johnson, 1960) a theoretical line (----, Fig. 1) is obtained which deviates markedly from the experimental points, and the fit would not be materially improved by adopting a different value for $K_{\text{eq},0}$.

A limited series of measurements at 20°C (Table 1, Fig. 1) indicated that the equilibrium constant may be marginally smaller at this temperature. Observations were also made at 30°C in the presence of added KCl or tetraethylammonium nitrate to give an ionic strength similar to that of mammalian plasma (Table 1, Fig. 1). The equilibrium constant was decreased by 5–10% although the pattern of pH-dependence showed little change. The pH-dependence of the constant in this case cannot be explained by the difference in $\text{p}K_1$ values, since both the $\text{p}K_1$ values are decreased by the high ionic strength (see below) and the pH curve should be displaced to a more acid pH range.

Equilibrium constant in the presence of Mg^{2+}

Measurements were made at 30°C in the presence of MgCl_2 at the various substrate concentrations (Table 2). Despite the evidence for substantial binding of Mg^{2+} (see below), the apparent equilibrium constant was very similar to that in the absence of Mg^{2+} .

Dissociation constants of 2- and 3-phosphoglycerate

The apparent third acid dissociation constants are plotted against ionic strength in Fig. 2. Extrapolation to zero ionic strength gives values of $\text{p}K_1 = 7.03$ and $\text{p}K_1 = 6.97$ for 2- and 3-phosphoglycerate respectively.

Measurement of Mg^{2+} -binding constants of 2- and 3-phosphoglycerate

The Mg^{2+} -binding constants were determined at pH 7.4 and at an ionic strength of 0.02 from the initial slopes of the Mg^{2+} titration curves as described in the Materials and Methods section. Values of 394 ± 26 (s.e.m. of five experiments) M^{-1} and 337 ± 22 (s.e.m. of five experiments) M^{-1} were obtained for 2- and 3-phosphoglycerate respectively. These values were calculated for the triply charged forms of the monophosphoglycerate by assuming that the doubly ionized forms do not bind Mg^{2+} appreciably and by using the acid dissociation constants reported above. Any binding by the doubly charged forms would be expected to be very much less than that of the triply charged form because of the lower charge,

Table 1. *Equilibrium constant (K_{eq}) of the glyceromutase reaction at different pH values in solutions of various ionic strengths*
 The buffers (33.3 mM) were Tris-HCl (pH 7.89–6.66), imidazole-HNO₃ (pH 6.45–6.10) and sodium maleate (pH 5.5).

Ionic strength $I = 0.06-0.07$ and at 30°C											
pH	5.42		6.20	6.45	6.66	7.10	7.50		7.89		
$K_{eq} \pm \text{s.e.m.}$	8.65 ± 0.01	10.02 ± 0.05	10.50 ± 0.07	10.72 ± 0.03	11.40 ± 0.07	11.59 ± 0.06	11.65 ± 0.03				
No. of observations	16	24	8	18	8	12	14				
Ionic strength $I = 0.3$ and at 30°C											
pH	6.97	7.45	7.89	7.01	7.55	7.80	8.12				
$K_{eq} \pm \text{s.e.m.}$	$10.06 \pm 0.03^*$	$10.45 \pm 0.04^*$	$11.10 \pm 0.04^*$	$10.43 \pm 0.03^\dagger$	$10.84 \pm 0.09^\dagger$	$11.14 \pm 0.11^\dagger$	$11.47 \pm 0.05^\dagger$				
No. of observations	16	16	16	16	8	8	16				
* Ionic strength adjusted to 0.3 by addition of KCl.											
† Ionic strength adjusted to 0.3 by addition of tetraethylammonium nitrate.											
Ionic strength $I = 0.06-0.07$ and at 20°C											
pH	6.22	7.24	8.06								
$K_{eq} \pm \text{s.e.m.}$	9.81 ± 0.07	11.28 ± 0.05	11.74 ± 0.04								
No. of observations	7	4	6								

Table 2. *Equilibrium constant for the phosphoglyceromutase reaction in 16.7 mM-Tris-HCl buffer, pH 7.4, containing MgCl₂ at 30°C*

All values are the means of duplicated or triplicated observations. [Mg²⁺ (free)] was calculated from the binding constants reported in the present paper. [Mg²⁺ (total)] was measured as described in the Materials and Methods section. *K_{eq}* is the apparent equilibrium constant; *K_{eq, 0}*, the equilibrium constant for the triply charged unliganded species, was calculated from eqn. (1) with the constants derived in this paper.

Total monophospho- glycerate (mM)	Ionic strength	[Mg ²⁺ (total)] (mM)	[Mg ²⁺ (free)] (mM)	<i>K_{eq}</i>	<i>K_{eq, 0}</i>
8.87	0.085	4.53	1.97	11.11	11.89
4.50	0.059	4.53	3.00	11.64	12.45
2.24	0.046	4.53	3.62	11.30	12.09
2.73	0.038	3.33	2.44	11.36	11.69
1.56	0.032	3.33	2.79	11.31	11.64

Fig. 1. *Variation of the equilibrium constant with pH, ionic strength and temperature*

Equilibrium constants: ●, at *I* = 0.06–0.07, and at 30°C; ▲, at *I* = 0.06–0.07, and at 20°C; ■, at *I* = 0.30 in the presence of KCl and at 30°C; ○, at *I* = 0.30 in the presence of tetraethylammonium nitrate and at 30°C. For details see the text. The line ---- shows the expected variation of the equilibrium constant with pH at *I* = 0.06–0.07 with published values (Johnson, 1960) of the acid dissociation constants. The line - - - shows the expected variation with the values for the acid dissociation constants determined in the present paper (see the text).

and Wold & Ballou (1957) could find no evidence for the doubly charged form of 3-phosphoglycerate binding Mg²⁺ in acid–base–titration studies. The apparent binding constants at pH 7.4 (i.e. binding to total monophosphoglycerate) for 2- and 3-phosphoglycerate are respectively 0.286 mM^{–1} and 0.255 mM^{–1} at pH 7.4 and 30°C (ionic strength 0.02).

The dependence of the parameter *a* (see the Materials and Methods section) on the concentration of Tris suggested some complexing of the Tris

Fig. 2. *Apparent third acid dissociation constants of 2- and 3-phosphoglycerate plotted against ionic strength at 30°C*

▲, *pK_i* of 2-phosphoglycerate; ●, *pK_i* of 3-phosphoglycerate. The ionic strength was adjusted by the addition of KCl. For other details see the text.

by Mg²⁺. To test further for Mg²⁺ binding the initial slopes of Mg²⁺–titration curves of 0.833 mM-hydroxyquinoline were measured at pH 7.91 in 16.67 mM-, 8.33 mM- and 4.17 mM-Tris–HCl buffers with KCl added to maintain the ionic strength at 0.02. The initial slopes (3.60 × 10^{–2} M^{–1}) were the same in each case. An association constant of 20 M^{–1} between Mg²⁺ and the un-ionized base would have led to a 10% difference in slope and it was concluded that the binding constant must be smaller than this value. It follows that at pH 7.4, where the proportion of base in the un-ionized form is much less, binding of Mg²⁺ by Tris will be negligible, as concluded by Milstein (1961).

Discussion

The equilibrium constants reported here for the phosphoglyceromutase reaction differ considerably from published values (Table 3). The previous values,

Table 3. *Previously published values for the equilibrium constant (K_{eq}) of the phosphoglyceromutase reaction*

Methods: a, optical rotation in neutral molybdate; b, optical rotation in 1 M-HCl; c, enolase assay (Rodwell *et al.*, 1957). Optical-rotation data have been calculated with the revised data of Ballou & Fischer (1954) on the optical rotation of 2-phosphoglycerate.

K_{eq}	pH	Temp. (°C)	Source of enzyme	Method	Authors
10.8	7.6	20	Not given	a	Meyerhof & Schulz (1938)
3.65	7.6	24	Muscle	b	Meyerhof & Oesper (1949)
4.1	—	—	Muscle	a }	Ballou & Fischer (1954)
4.0	0	100	Nil (1 M-HCl)	b }	
5.0	6.8	37	Rabbit muscle	a	Cowgill & Pizer (1956)
5.8	5.0–7.2	25	Yeast	a	Chiba & Sugimoto (1959)
6.3	4.6–6.5	30	Yeast	c	Rodwell <i>et al.</i> (1957)
6.0–10	? 7.6	30	Wheat germ	c	Ito & Grisolia (1959)

which do not agree well between themselves, were determined under a variety of conditions. However, the present observations have shown that the equilibrium constant is virtually independent of temperature (over the range 20–30°C) and Mg^{2+} concentration, and dependent to only a small extent on pH (pH 5.5–8.0), and ionic strength ($I = 0.03$ – 0.30). These factors therefore do not explain the scatter of values. However, the determination of the equilibrium constant by the enolase-coupled method are subject to considerable uncertainty, since the E_{240} of phosphoenolpyruvate is dependent on the free Mg^{2+} concentration, and not only the phosphoenolpyruvate but also 2- and 3-phosphoglycerate bind Mg^{2+} . Further, the calculation depends on the enolase equilibrium constant, which is also $[Mg^{2+}]$ -dependent (Wold & Ballou, 1957; Pizer, 1962).

The lack of marked effect of pH on the equilibrium constant was unexpected in view of the difference in pK values of 0.74 unit previously reported (2-phosphoglycerate $pK = 7.48$, 3-phosphoglycerate $pK = 6.74$; Johnson, 1960). If there were such a difference in the pK values the relative absence of pH effect would imply that the equilibrium constants for the doubly and triply ionized forms were themselves dependent on pH. However, the redetermined values (2-phosphoglycerate $pK = 7.03$, 3-phosphoglycerate $pK = 6.97$) are in reasonable agreement with the assumption that at low ionic strength the equilibrium constants for the individual ionic species are relatively, although not completely (see below), independent of pH. The results also indicate that the equilibrium constants for the triply charged species ($K_{eq,0}$) and the doubly charged species ($K_{eq,1}^1$) are not very different ($K_{eq,0}/K_{eq,1}^1 = 1.15$). The phosphate and carboxyl groups are closer together in 2-phosphoglycerate than in 3-phosphoglycerate. Electrostatic repulsion between the phosphate and carboxyl groups appears therefore not to be very important in determining either the equilibrium constant or the acid dissociation constants. The lack of effect of

ionic strength on the difference in dissociation constants and the relatively small fall in equilibrium constant with ionic strength (indicating that the activity coefficient of 2-phosphoglycerate is only slightly more sensitive to ionic strength than the activity coefficient of 3-phosphoglycerate) are both consistent with this view. The apparent lack of importance of the electrostatic-repulsion factor might possibly be partly due to the compensatory effects of hydrogen bonding in 2-phosphoglycerate as discussed below.

Although with the redetermined acid dissociation constants the theoretical curve (Fig. 1) fits the experimental results quite closely at neutral pH values, there is a systematic deviation at low pH which is difficult to explain in terms of dissociation constants. Further, the variations in equilibrium constant with pH at high ionic strength obtained by the addition of KCl or tetraethylammonium nitrate (Fig. 1) cannot be explained by changes in the ionization of 2- and 3-phosphoglycerate, since the dissociation constants are displaced to the acid region under these conditions. These findings suggest that under some conditions $K_{eq,0}$ and $K_{eq,1}^1$ may be affected by pH. In the presence of KCl, complexing of the phosphoglycerates by K^+ might be a factor affecting the equilibrium constant, but almost identical values were obtained with tetraethylammonium nitrate, where complexing would not be expected. McQuate & Utter (1959) noted similar discrepancies in their determination of the equilibrium constant of the pyruvate kinase reaction.

If an equilibrium constant of 11.77 is assumed it follows that the standard free-energy change (ΔG_{298}^0) for the conversion of 2-phosphoglycerate into 3-phosphoglycerate is -6140 J. If this were solely due to a change in enthalpy (ΔH^0) then it follows from the Van't Hoff relationship that the equilibrium constant at 20°C should be 1.086 times the value at 30°C. Since it is clear that the change with temperature is very much less than this and indeed

may be in the opposite direction (Fig. 1, Table 1) the net enthalpy change must be considerably smaller. This is consistent with the other evidence just discussed that electrostatic repulsion between the phosphoryl and carboxyl groups is not very important in determining the equilibria. Model building suggests that internal hydrogen bonding may occur more extensively with 2-phosphoglycerate than with 3-phosphoglycerate, and it is possible that the enthalpy change from hydrogen bonding in 2-phosphoglycerate may partly compensate for enthalpy changes caused by electrostatic repulsion. Since the enthalpy change is small the equilibrium constant must be largely determined by a difference in entropy. A major factor would appear to be the limitation of rotational movement in 2-phosphoglycerate, owing to steric crowding. Internal hydrogen bonding in 2-phosphoglycerate might also contribute to the rigidity of the molecule.

The similarity of the Mg^{2+} -binding constants of 2- and 3-phosphoglycerate suggests that bidentate effects are not important with either ligand and that binding is related to the total charge on the molecule. The fact that the equilibrium constant is virtually independent of Mg^{2+} concentration indeed suggests that the differences between the constants is even less than indicated by the measurements with 8-hydroxyquinoline. A similar lack of bidentate effects is found with glucose 1- and 6-phosphate and glucose 1,6-diphosphate (Ray & Roscelli, 1966).

In the erythrocyte the free Mg^{2+} concentration varies with metabolism (Rose, 1968) and in cells in general there must be considerable differences in pH, ionic strength and temperature. The present experiments, however, indicate that the equilibrium constant of the phosphoglyceromutase reaction is substantially the same under all conditions.

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