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NUCLEAR MAGNETIC RESONANCE STUDIES ON SUBSTRATE BINDING TO PHOSPHOGLUCOMUTASE

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SUMMARY

1. Phosphoglucomutase (α-D-glucose-1,6-bisphosphate: α-D-glucose-1-phosphate phosphotransferase, EC 2.7.5.1) has been assayed by monitoring the proton and phosphorus nuclear magnetic resonances of glucose 1-phosphate and glucose 6-phosphate as a function of time.

2. The activity of phosphoglucomutase in ^2H_2O is 70% of that in H_2O.

3. Both anomers of glucose 6-phosphate bind to phosphoglucomutase, although the enzyme is specific to the α-anomer.

4. The Mn^{2+}-binding site on phosphoglucomutase is 5.8 Å from the phosphorus of bound glucose 6-phosphate.

5. The protons of bound glucose 6-phosphate are about 7–10 Å from the Mn^{2+}-binding site.

INTRODUCTION

Phosphoglucomutase catalyses the interconversion between glucose 1-phosphate and glucose 6-phosphate. It requires a bivalent metal ion for activity, and is activated most efficiently by Mg^{2+}. The observations that the enzyme is still active in the presence of the paramagnetic ion Mn^{2+} (although its activity is only 5% of that obtained in the presence of Mg^{2+} [1]), and that Mn^{2+} binds much more tightly to the enzyme than to the substrate provide the basis of the magnetic resonance studies described in this paper. We have studied the relaxation which Mn^{2+} confers upon the nuclear magnetic resonance signals of glucose 6-phosphate in an attempt to define the relative positions of the metal and substrate binding sites on the enzyme, and to determine whether both anomers of this substrate bind to the enzyme. In the course of this study we have also developed a new assay for the enzyme using magnetic resonance as a means for measuring the concentration of substrate and product simultaneously.

MATERIALS AND METHODS

Phosphoglucomutase was prepared essentially by the method of Najjar [2], except that his second heat treatment was omitted, and after the first $(NH_4)_2SO_4$

crystallisation, the enzyme was passed through two columns, first a phosphocellulose column, P11, (5 mM phosphate, pH 6.5), then Sephadex G-100 (20 mM Tris, 150 μM KCl, pH 7.5). The enzyme gave a single band by gel electrophoresis (7% sodium dodecylsulfate acrylamide, 7% barbitone), and had a specific activity of about 650 μmoles $\cdot$ min^{-1} $\cdot$ mg^{-1} at 25 °C (for details of assays see below).

"Aristar" high grade Tris was obtained from BDH Chemicals Ltd., and its chloride was used throughout the experiments. Glucose 1-phosphate (disodium salt) was obtained from Boehringer. Metal ion impurities were removed from this substrate by extracting about 4 ml of aqueous solution several times with about 20 ml of a 1% solution of 8-hydroxyquinoline in chloroform. 8-Hydroxyquinoline was subsequently removed by extracting several times with about 20 ml of chloroform. The substrate was then lyophilised, redissolved in water, and its concentration measured colorimetrically, by acid hydrolysis followed by determination of inorganic phosphate [3].

An estimate of the quantity of metal ion impurity in the substrate was made by measuring the spin–spin relaxation time, T_2, of the glucose 1-phosphate phosphorus nuclear magnetic resonance. Trace amounts of paramagnetic metal ions considerably reduce the T_2 of this resonance; for example 1 part of 10^6 of Mn^{2+} reduces the T_2 from several seconds to about 1 s. EDTA was added to samples of glucose 1-phosphate until the T_2 of the phosphorus resonance reached its maximum limiting value, at which point the EDTA must have chelated all paramagnetic ions, and the concentration of EDTA at which this occurred gave therefore a rough estimate of the concentration of metal ion impurity. The metal ion impurity of glucose 1-phosphate was found to be reduced from about 1 part in 10^3 to 1 part in 10^4 (on a molar basis) by the purification procedure, a sufficient reduction for our experiments.

Metal-free phosphoglucomutase was obtained by the method of Ray [1], dialysing the enzyme several times against 5 mM EDTA, then 0.5 mM EDTA in 100 mM Tris, pH 7.5, then against 100 mM Tris, pH 7.5, except that a solid-state chelating agent was not used in the EDTA-free solutions as the metal ion content of the Tris solutions was sufficiently small that a negligible amount of metal would bind to the enzyme. The experiments described below show that the enzyme was essentially free of both EDTA and metal ions.

Phosphorus NMR spectra were recorded on a Fourier Transform spectrometer, operating at 84.5 MHz, built in this laboratory. The magnet has been described in ref. 4 but the spectrometer has since then been considerably modified to accommodate Fourier Transform techniques [5]. Proton spectra were recorded on a Bruker spectrometer operating at 270 MHz. Spin–lattice relaxation times, T_1, were determined by the 180°–τ–90° pulse sequence [6]. Spin–spin relaxation times T_2 of the phosphorus resonances were determined by the Carr–Purcell pulse sequence [6], with the Meiboom–Gill modification [7]. Most of the results were obtained by phosphorus NMR.

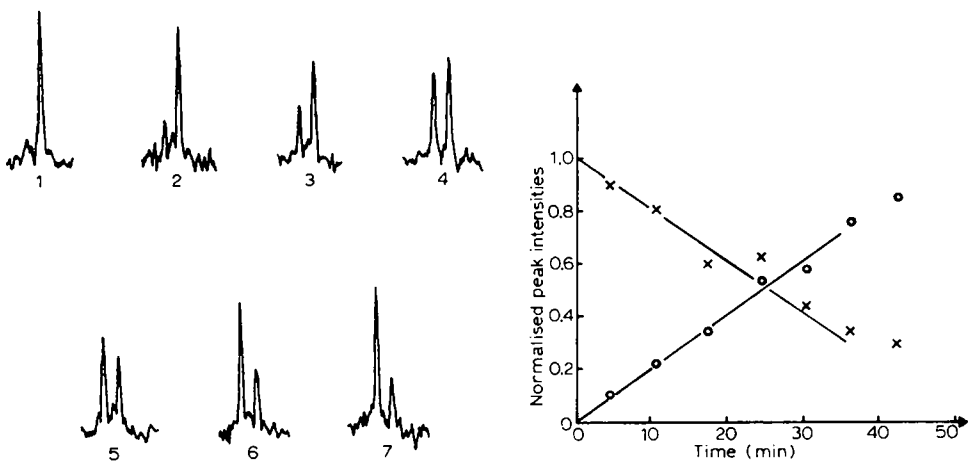
Assays of phosphoglucomutase

Phosphoglucomutase is normally assayed either by measuring colorimetrically the rate of formation of glucose 6-phosphate from glucose 1-phosphate, or in a coupled reaction [8]. We have assayed phosphoglucomutase by monitoring the glucose 1-phosphate and glucose 6-phosphate nuclear magnetic resonances as a function of time.

The spectra of glucose 1-phosphate and glucose 6-phosphate are easily distinguishable. At 25 °C, the phosphorus resonances are chemically shifted from each other by 2 ppm, and the proton spectra differ markedly in the position of the anomeric proton resonance. In glucose 1-phosphate, this proton resonance is a quartet 0.75 ppm downfield from the water peak, whereas in glucose 6-phosphate, which has two anomers, the resonance of the α-anomer is a doublet 0.5 ppm downfield, and the β-anomer a doublet 0.2 ppm upfield from the water peak. The intensities of the resonances give a direct measurement of the quantities of substrate and product present.

Since variable temperature facilities are available on both spectrometers, it was possible to assay the enzyme over a range of temperatures. Most of the assays were done at about 20 °C, but comparisons between the NMR and coupled assays were made at 25 °C.

Figs 1 and 2 show an assay done by phosphorus NMR of the Mg^{2+} enzyme, using 100 mM substrate. Owing to the low inherent sensitivity of phosphorus, it was



Figs. 1 and 2. Assay of phosphoglucomutase by phosphorus NMR. The left-hand or low-field peak in each spectrum is the glucose 6-phosphate resonance, the upfield peak the glucose 1-phosphate resonance. The enzyme was preincubated in 2 mM $MgCl_2$, 1 mM EDTA, 100 mM imidazole, 100 mM Tris, pH 7.5, and added, at a final concentration of 6.2 μg/ml to a solution containing 100 mM glucose 1-phosphate, 100 μM glucose 1,6-bisphosphate, 5 mM $MgCl_2$, 1 mM EDTA, 50 mM imidazole in 100 mM Tris, pH 7.5. Temperature 19.5 °C. ×, glucose 1-phosphate; ○, glucose 6-phosphate.

necessary to accumulate each spectrum for about 5 min to obtain sufficient signal to noise, but as long as the turnover rate is linear, the time of accumulation is unimportant.

Fig. 3 shows various stages in the conversion of glucose 1-phosphate to glucose 6-phosphate as observed by proton NMR. The spectra were recorded in the continuous wave mode, using 100 mM substrate.

The activities obtained by phosphorus NMR agreed with those using the coupled assay, and the turnover rate of substrate increased linearly with enzyme concentration.

The assays by proton NMR must be done in ^2H_2O solution, otherwise the resonance from the water protons would mask the substrate resonances. We found,

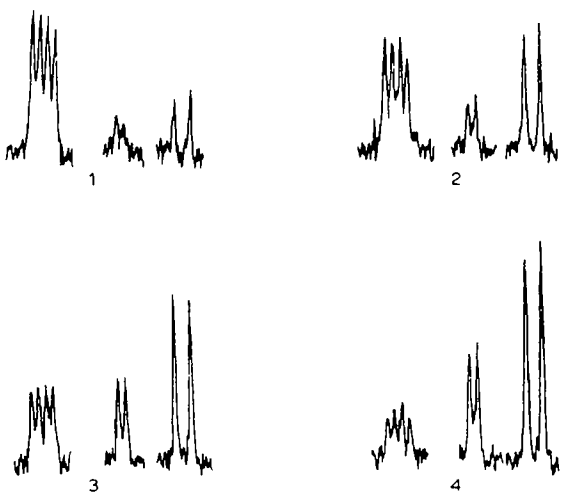


Fig. 3. Various stages in the conversion of glucose 1-phosphate to glucose 6-phosphate as observed by proton NMR. Each spectrum shows the quartet of glucose 1-phosphate, and the two doublets of glucose 6-phosphate.

in agreement with Ray (unpublished), that lyophilisation does not affect the activity of phosphoglucomutase. However, the activity of phosphoglucomutase in ^2H_2O was about 70% of that in H_2O. Thus lyophilised phosphoglucomutase gave the full activity if assayed in H_2O by phosphorus NMR, but when assayed in ^2H_2O by phosphorus or proton NMR, the enzyme gave only 70% activity.

The NMR assay requires about ten times the quantity of enzyme and substrate as the coupled and colorimetric assays, but it does have several advantages over the conventional assays. Firstly, no chemical modification is required, so the assay is totally non-destructive. Secondly, since the NMR assay does not perturb the reaction, the reaction may be followed as it proceeds. This can be done by the coupled assay, but not by the colorimetric method. Finally, whereas the colorimetric and coupled assays distinguish indirectly between glucose 1-phosphate and glucose 6-phosphate, and measure the concentration of only one substrate, the NMR method gives a direct, completely unambiguous way of observing both substrates simultaneously.

Since the NMR assay is capable of distinguishing between many substrates, it could be very useful in studying multienzyme systems, where it may be impossible by any other means to observe all the substrates.

Metal binding-site studies

In order to determine the relative positions of the metal and substrate binding sites, we added $MnCl_2$ to solutions containing metal-free phosphoglucomutase and glucose 1-phosphate and allowed the enzyme to convert glucose 1-phosphate to glucose 6-phosphate until equilibrium was reached. Typical solutions contained 100 μM enzyme, 10–50 μM Mn^{2+} and 100 mM substrate, so that at equilibrium there would be about 5 mM glucose 1-phosphate and 95 mM glucose 6-phosphate. For the time being, we shall neglect the presence of glucose 1-phosphate, but we shall consider the effects it produces later.

THEORY

General

In the presence of paramagnetic ions, the relaxation times T_1 and T_2 of dipolar nuclei are decreased according to the distance of the nucleus in question from the ion. The relaxation is given by the Solomon–Bloembergen equations [9, 10].

$$\frac{1}{T_{1M}} = \frac{2}{15}\frac{\gamma_I^2 g^2 S(S+1)\beta^2}{r^6}\left[\frac{3\tau_c}{1+\omega_I^2\tau_c^2} + \frac{7\tau_c}{1+\omega_S^2\tau_c^2}\right] + \frac{2}{3}S(S+1)\left(\frac{A}{\hbar}\right)^2\left(\frac{\tau_e}{1+\omega_S^2\tau_e^2}\right) \quad (1)$$

$$\frac{1}{T_{2M}} = \frac{1}{15}\frac{\gamma_I^2 g^2 S(S+1)\beta^2}{r^6}\left[4\tau_c + \frac{3\tau_c}{1+\omega_I^2\tau_c^2} + \frac{13\tau_c}{1+\omega_S^2\tau_c^2}\right] + \frac{1}{3}S(S+1)\left(\frac{A}{\hbar}\right)^2\left(\tau_e + \frac{\tau_e}{1+\omega_S^2\tau_e^2}\right) \quad (2)$$

The first terms arise from dipole–dipole interactions between the electron spin S and the nuclear spin I, characterised by a correlation time τ_C. This interaction is strongly distance dependent. The second terms arise from modulation of the scalar interaction which is only important for Mn^{2+} systems when the Mn^{2+} and nucleus are in contact, and then only in the case of T_2. We shall neglect the scalar interaction, and show that this is justified later on. γ_I is the magnetogynic ratio of the nucleus, β the Bohr magneton, and g the electron 'g' value, ω_S and ω_I are the electronic and nuclear Larmor precession frequencies, r is the distance between the nucleus and paramagnetic ion, and $A/\hbar$ is the electron-nuclear hyperfine coupling constant.

Presence of various metal complexes

In the phosphoglucomutase system, there are four metal species which can produce relaxation of the substrate resonances, as shown in Fig. 4. (The binding constant of Mn^{2+} to the enzyme is about 10^{-7} M [1], and is so tight that the free

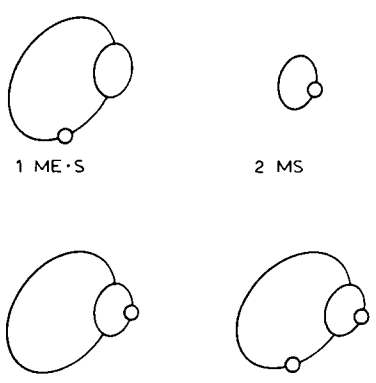


Fig. 4. Metal complexes which may affect the relaxation of the substrate resonances. Large ellipse represents enzyme, E; small ellipse represents substrate, S; circle represents metal, M. The complex we are interested in is Complex 1.

Mn^{2+} in solution produces negligible relaxation in the solutions we have used.) We wish to obtain information about the complex in which substrate binds to the Mn^{2+}–enzyme, and to do this, we must be able to separate the effects of the metal in this complex from its effects in any of the other metal complexes that may be present.

(In the following discussion, E will refer to metal-free enzyme, S to metal-free substrate, and M to metal ion. Thus ME is the metal–enzyme complex, MS the metal–substrate complex, and we are interested in the ME·S complex. E·SM and ME·SM are complexes in which metal–substrate is bound to enzyme.)

(i) Firstly, we shall show that, due to the low concentrations of E·SM and ME·SM, their effect on the observed substrate relaxation is negligible compared with that of ME·S and MS.

The binding constants of Mn^{2+} to glucose 6-phosphate and enzyme are about 40 mM* and 10^{-7} M respectively. From the data of Ray [1], the binding constant of Mn^{2+} to the ES complex is likely to be 10^{-7} M or tighter. Using these values, and assuming that MS binds to the enzyme with roughly the same binding constant as S, we find, in the solutions we have used, that the concentration of ME·SM is about 10^{-6} of that of ME·S, and the concentrations of E·SM and ME·SM are about 10^{-3} of that of MS. Because of the low concentration of ME·SM relative to ME·S, the contribution to the observed relaxation from the metal bound directly to the enzyme in the ME·SM complex is negligible.

Due to the large rotational tumbling time of the enzyme, relaxation of the MS complex when bound to the enzyme may be more efficient than that of the free MS complex, and therefore its effect on the observed relaxation cannot be neglected merely because of its low concentration. However, the contribution to the observed phosphorus resonance T_2 from the MS complex when bound to the enzyme is limited by the time it takes for the substrate in this complex to exchange with free substrate, which requires dissociation both of metal from substrate and of substrate from enzyme. The contribution from the MS complex is limited by the lifetime of the metal in the complex which is almost certainly shorter than the exchange time of the E·SM complex. Therefore, due to the low concentrations of E·SM and ME·SM, their effect in the observed phosphorus T_2 will be negligible.

The dependence of T_1 on correlation time is such that T_1 relaxation of either phosphorus or protons in the MS complex bound to the enzyme cannot be more than about ten times as efficient as in the free MS complex, so again due to their low concentrations, the contributions of E·SM and ME·SM to the observed T_1 relaxation will be negligible.

If the active enzyme complex involves a metal bridge, and therefore ME·S and E·MS are equivalent and may be represented as EMS, then the complex ME·MS will not exist. There are therefore only two complexes we need consider, MS and EMS, and the above discussion is very much simplified.

(ii) Thus we have shown that the only metal complexes we need consider are ME·S and MS, and we shall now consider the relaxation they produce.

* The binding constant of Mn^{2+} to glucose 6-phosphate was obtained from measurements of the T_2 of the phosphorus resonance at varying metal and glucose 6-phosphate concentrations, and taking into account the temperature at which they were done (20 °C), was in rough agreement with the value obtained by Cohn and Townsend [11].

The contribution to the relaxation of the substrate resonances from the paramagnetic ion in the ME·S and MS complexes is given by [12, 13]:

$$\frac{1}{T_{iP}} = \frac{[ME\cdot S]}{[S]}\frac{1}{(T_{iM}+\tau_M)_E} + \frac{[MS]}{[S]}\frac{1}{(T_{iM}+\tau_M)_S} \quad i = 1,2 \tag{3}$$

The equation for T_2 involves the assumption that the chemical shift of the metal complexes from the free substrate is much less than $1/T_{2M}$. This is true for the Mn^{2+}–proton interaction [14], and is therefore also true for the Mn^{2+}–phosphorus interaction, as the larger hyperfine coupling constant produces a greater effect on the relaxation rate than on the shift. Subscript E refers to the ME·S complex, S to the MS complex, τ_M is the lifetime of the relevant metal complex. In the case of the ME·S complex, since Mn^{2+} dissociates so slowly from the enzyme (with a half-life of about 15 min [1]), $(\tau_m)_E$ is the lifetime of substrate on the enzyme. $(\tau_m)_S$ is the lifetime of Mn^{2+} on free substrate.

The metal dissociation constants are given by

$$K_E = \frac{[M][ES]}{[ME\cdot S]} \qquad K_S = \frac{[M][S]}{[MS]}$$

Substituting for MS and ME·S in Eqns 3, we obtain the equations:

$$\frac{1}{fT_{iP}} = \frac{\dfrac{[ES]}{K_E}\dfrac{1}{(T_{iM}+\tau_M)_E} + \dfrac{[S]}{K_S}\dfrac{1}{(T_{iM}+\tau_M)_S}}{1 - \dfrac{[ES]}{K_E} + \dfrac{[S]}{K_S}} \tag{4}$$

where f is the ratio of total metal ion, $[M]_T$, to substrate. Now since K_E is about 10^{-7} M, and K_S is 40 mM, then as long as there is an excess of enzyme over metal,

$$\frac{[ES]}{K_E} \gg \frac{[S]}{K_S}, 1$$

Therefore the denominator in Eqn 4 is simply ES/K_E and the equation may be written

$$\frac{1}{fT_{iP}} = \frac{1}{(T_{iM}+\tau_M)_E} + \frac{[S]}{[ES]}\frac{K_E}{K_S}\frac{1}{(T_{iM}+\tau_M)_S} \tag{5}$$

where the first term still gives the relaxation from the ME·S complex, and the second term gives the relaxation from the MS complex. Using the functional form of this equation, it is possible to distinguish the contribution to the substrate relaxation from Mn^{2+} bound directly to substrate in solution.

Having obtained the contribution from the ME·S complex by using the Solomon–Bloembergen equation we can obtain information about the distance of the Mn^{2+} binding site from the glucose 6-phosphate binding site.

RESULTS AND DISCUSSION

To determine which of the terms in Eqn 5 was dominant, the T_2 values of the glucose 6-phosphate phosphorus resonance were measured at fixed enzyme and sub-

strate concentrations (apart from dilution effects), but varying Mn^{2+} concentration, so that the amount of metal-free enzyme varied, but substrate remained constant. Addition of metal produced dilution of the solutions, and it makes the calculations simpler if we work in terms of quantities of substrate and enzyme, rather than their concentrations. To take into account the small dilution effects, it is necessary merely to replace[S] and [ES] in Eqn 5 by the quantitites of metal-free substrate and enzyme, which we have expressed in μmoles. The results are shown in Table I.

TABLE I

T_2 VALUES OF THE GLUCOSE 6-PHOSPHATE PHOSPHORUS RESONANCE IN THE PRESENCE OF PHOSPHOGLUCOMUTASE AND VARYING AMOUNTS OF $MnCl_2$

Solutions contained 100 μl substrate (0.485 M) and glucose 1,6-bisphosphate (0.48 M), 300 μl enzyme (6.5 mg/ml), in 100 mM Tris, pH 7.5, and 10-μl aliquots of 0.5 mM $MnCl_2$ were added. Thus the solutions contained 46 μmoles glucose 6-phosphate, 31.5 nmoles enzyme and $MnCl_2$ as indicated in the table.

Amount of $MnCl_2$ added (nmoles)	T_{2P} (ms)	$\frac{1}{fT_{2P}}$ ($10^{-4} \cdot s^{-1}$)	Amount of metal-free enzyme (nmoles)
10	225	2.04	215
15	162	1.89	165
20	111	2.07	115
25	91	2.02	65
30	64.5	2.38	15
35	37	2.55	

The Carr–Purcell pulse sequence produces for each line at or near resonance an exponential decay of time constant T_2 characteristic of that line. The glucose 6-phosphate phosphorus resonance appears as a single line, but consists in fact of a superposition of two resonances, one from the α-anomer and one from the β-anomer. The decay obtained by the Carr–Purcell sequence is similarly a superposition of the two decays of different time constants, and a logarithmic plot of the decay will be non-linear. (The rate of anomerisation is so slow [15] that any averaging of the signals by this process can be ignored.) All the decays observed in these experiments gave linear logarithmic plots, as shown in Fig. 5, indicating the presence of only one T_2, or two similar ones (a logarithmic plot is noticeably non-linear if the two time constants differ by more than a factor of 3). Thus the phosphorus resonances of the α- and β-anomers of glucose 6-phosphate have the same or similar T_2 values, and must therefore be equally affected by Mn^{2+}. Since we show that relaxation is due to the metal in the ME·S complex, this means that the β-anomer of glucose 6-phosphate, which is assumed not to be a substrate of phosphoglucomutase [8], nevertheless binds to the enzyme. If we assume the phosphorus to Mn^{2+} distance to be the same for both anomers, then the β-anomer of glucose 6-phosphate must bind to phosphoglucomutase with more or less the same binding constant as the α-anomer. Therefore, in the phosphorus experiments, we consider the glucose 6-phosphate to be a single species, regarding the two anomers as identical as far as relaxation by Mn^{2+} is concerned.

From Table I, we see that $1/fT_{2P}$ for the phosphorus resonance of glucose 6-

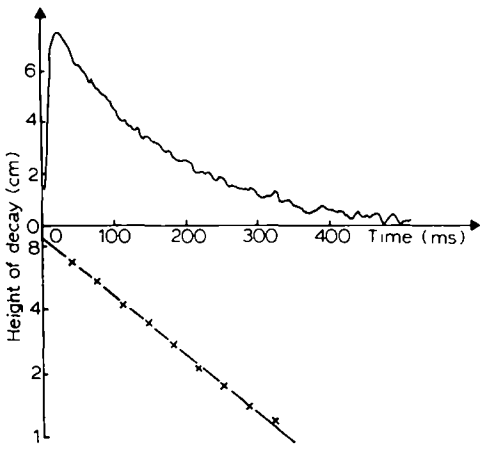


Fig. 5. A typical phosphorus decay as obtained by the Carr–Purcell sequence, with a linear logarithmic plot of the decay below.

phosphate is independent of the quantity of metal-free enzyme, over the range 65–215 μmoles, so that, using Eqn 5, it is clear that $1/fT_{2P} = 1/(T_{2M} + \tau_M)_E$, which means that the observed relaxation must be almost entirely due to the metal in the ME·S complex.

Now $f = [M]_T/[S]$, and since almost all the metal is bound to the enzyme,

$$= \frac{[ME \cdot S]}{[S]} \tag{6}$$

However, because of the presence of glucose 1-phosphate, which binds competitively with glucose 6-phosphate, the glucose 6-phosphate binds to only 77% of the enzyme (Ray, W. J., personal communication), and a correcting factor of 0.77 must be introduced into Eqn 6 to take this into account.

Thus:

$$\frac{1}{T_{2M} + \tau_M} = \frac{2.00 \cdot 10^4}{0.7} = 2.9 \cdot 10^4 \text{ s}^{-1}$$

The presence of the dephosphoenzyme–glucose 1,6-bisphosphate complex was not considered. If it is significant (Ray, W. J., personal communication) then the appropriate correction should be made.

The fact that the first addition of Mn^{2+} produced as much relaxation per mole of Mn^{2+} added as subsequent additions indicates that the enzyme was free of EDTA, and because $1/fT_{2P}$ remained constant until the metal concentration was roughly that of the enzyme, the enzyme must have been essentially metal-free before addition of Mn^{2+}, so that the Mn^{2+} could bind stoichiometrically to the enzyme.

To determine the relative importance of T_{2M} and τ_M, it was necessary to study the temperature dependence of T_2:

$$fT_{2P} = T_{2M} + \tau_M$$

The temperature dependence of T_{2P} is shown in Fig. 6, together with the values of T_{2M} and τ_M which fit the curve. The curve was fitted by assuming slow exchange at low

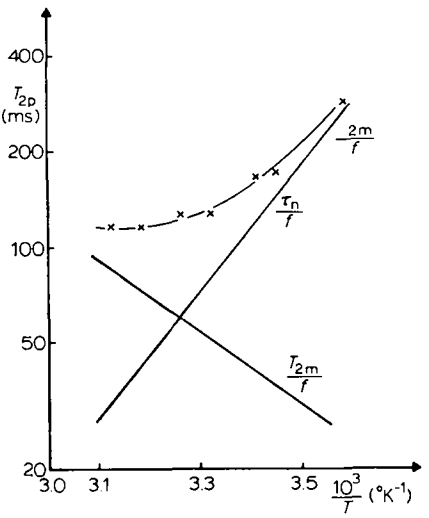


Fig. 6. The temperature dependence of the T_2 of the glucose 6-phosphate phosphorus resonance plotted logarithmically, and the values of τ_M and T_{2M} which fit this curve.

temperature, so that the slope of the curve at low temperature gave the activation energy for τ_M, and the difference between the curve and the deduced τ_M value at an temperature gave T_{2M}. In this way, the activation energy for τ_M was found to be about 8 kcal/mole and for T_{2M} about −5 kcal/mole. Since $1/T_{2M} \approx \tau_C$, the activation energy for τ_C is about 5 kcal/mole.

In this system, $1/\tau_C = 1/\tau_R + 1/\tau_S$ where τ_R is the rotational correlation time of substrate on the enzyme, and τ_S is the Mn^{2+} electron spin relaxation time, which is assumed to be given by the Bloembergen–Morgan equation [16]. From the frequency dependence of τ_S, it is very likely that, at the high magnetic field of 50 kG, τ_S is very much greater than τ_R, so that $1/\tau_C = 1/\tau_R$.

Thus it is likely that the activation energy we have obtained is that for τ_R.

This was checked by studying the temperature dependence of the T_2 of the glucose 6-phosphate phosphorus resonance in the presence of the Mg^{2+}–enzyme, looking at the diamagnetic contribution of the metal–enzyme to T_2. Since the diamagnetic contribution is a small effect, T_2 is much greater than τ_M, and so the substrate must be in fast exchange. The activation energy obtained must therefore be that for T_2, and since now $1/\tau_C = 1/\tau_R$, we obtain the activation energy for τ_R. This was found to be 5 kcal/mole, in good agreement with the value obtained for the Mn^{2+}–enzyme complex. So even if the Bloembergen–Morgan equation is not totally valid in our system, it is very likely that in the ME·S complex, $\tau_C = \tau_R$.

From the graph in Fig. 6, we find at 20 °C that $T_{2M}/f = 40 \pm 10$ ms and $\tau_M/f = 120 \pm 10$ ms and therefore that the lifetime τ_M of the substrate on the enzyme is about $3 \cdot 10^{-5}$ s.

To determine the value of τ_R, we measured the T_1 of the glucose 6-phosphate phosphorus resonance, since at a given temperature, $T_{1M}/T_{2M} = 7/6 + 2/3\,\omega I^2 \tau_C^2$ from Eqns 1 and 2. The results are shown in Table II.

As in the case of the T_2 studies, the first term in Eqn 5 predominates for T_1. Since $1/T_{2P} \gg 1/T_{1P}$, the substrate must de in fast exchange for T_1.

TABLE II

T_1 VALUES OF THE GLUCOSE 6-PHOSPHATE PHOSPHORUS RESONANCE IN THE PRESENCE OF PHOSPHOGLUCOMUTASE AND VARYING AMOUNTS OF $MnCl_2$

Solutions identical to those described in Table I.

Amount of $MnCl_2$ added (nmoles)	T_{1P} (s)
10	6.31
30	4.48
50	3.82

Therefore, $1/fT_{1P} = 1/T_{1M} = 335 \pm 80\ s^{-1}$.

From the ratio of T_{1M} to T_{2M}, we find a correlation time $\tau_C = \tau_R = 4 \cdot 10^{-8} \pm 1 \cdot 10^{-8}$ s. This is very likely to be the rotational tumbling time of the whole MES complex.

From Eqns 1 and 2 we can then calculate the distance of the glucose 6-phosphate phosphorus nucleus from the Mn^{2+} bound to phosphoglucomutase to be 5.8 ± 0.4 Å.

There are several assumptions we have made in this calculation. Ignoring the scalar interaction, the Mn^{2+} to phosphorus distance obtained is 5.8 Å, which is consistent with the fact that the scalar interaction is only important when the nucleus is adjacent to the Mn^{2+} ion. If we assume scalar interaction to be present, then the distance we would obtain would be larger than 5.8 Å, which is inconsistent. Thus it is valid to ignore the scalar term in Eqn 2.

Secondly, we have ignored the possibility that the glucose 1-phosphate phosphorus is closer to the Mn^{2+} than the glucose 6-phosphate phosphorus, is therefore relaxed more efficiently, and may transfer this relaxation to the glucose 6-phosphate. However this transfer cannot occur at more than the catalytic turnover rate, which is about 25 μmoles/s at 20 °C, and this will produce a negligible effect.

Finally, it is known that Mn^{2+} binds to phosphoglucomutase at secondary sites with a binding constant of about 10^{-5} M [17]. Thus about 10^{-2} of the Mn^{2+} is bound at these sites. The question arises as to what happens if these sites are nearer the glucose 6-phosphate than the main binding site, and so produce much more efficient relaxation. If it were assumed that these sites produce the dominant relaxation mechanism, then we would obtain a value for $(\tau_M)_E$ of about 10^{-7} s, which is highly improbable. Thus we can assume that the relaxation observed is due to Mn^{2+} binding at the main binding site.

Preliminary studies of the proton relaxation rates of glucose 6-phosphate indicate that the Mn^{2+} ion on phosphoglucomutase is further away from the protons of glucose 6-phosphate than it is from the phosphorus nucleus.

Since the magnetic field was higher for the proton than for the phosphorus experiments, the correlation time for the proton–Mn^{2+} interaction should also be τ_R, and using the value we obtained for τ_R, the protons seem to be in the range 7–10 Å from the Mn^{2+} ion.

However, interpretation of the results is difficult, due to the complexity of the proton spectrum of glucose 6-phosphate. But it was found that the β-protons were

relaxed to roughly the same extent as the α-protons, showing again that the β-anomer also binds to phosphoglucomutase.

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