Rates of Phosphorylation and Dephosphorylation of Phosphoglycerate Mutase and Bisphosphoglycerate Synthase*

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Phosphoglycerate mutase and bisphosphoglycerate synthase (mutase) can both be phosphorylated by either glycerate-1,3-P, or glycerate-2,3-P, to form phosphohistidine enzymes. The present study uses a rapid quench procedure to determine if, for each enzyme, the formation of the phosphorylated enzyme and phosphate transfer from the enzyme can occur at rates consistent with the overall reactions. With bisphosphoglycerate synthase from horse red blood cells (glycerate-1,3-P* - glycerate-2,3-P,) at pH 7.5, 25°C, phosphorylation of the enzyme appears rate-limiting, k = 13.5 s⁻¹, compared with kcat = 12.5 s⁻¹ for the overall synthase rate. Phosphoryl transfer from the enzyme to phosphoglycerate occurs at 38 s⁻¹ at 4°C and was too fast to measure at 25°C. With chicken muscle phosphoglycerate mutase the half-times were too short to measure under optimal conditions. The rate of enzyme phosphorylation by glycerate-2,3-P, at pH 5.5, 4°C, could account for the overall reaction rate of 170 s⁻¹. The rate of phosphoryl transfer from the enzyme to glycerate-3-P was too rapid to measure under the same conditions. It is concluded that the phosphorylated enzymes have kinetic properties consistent with their participation as intermediates in the reactions catalyzed by these enzymes.

The central question about the mechanisms of phosphoglycerate mutase (3-PGA = 2-PGA) and bisphosphoglycerate synthase (mutase) (1,3-DPG → 2,3-DPG) is whether a phosphoryl enzyme functions in the direct path of catalysis. Early reports of the isolation of a phosphorylated form of phosphoglycerate mutase (1, 2) containing phosphoserine (1) were later concluded to be due to contamination with phosphoglucomutase (3). An intermediate containing bound glycerate-2,3-P, was subsequently proposed (3, 4). With the demonstration that both phosphoglycerate mutase (5-7) and synthase (8) can be phosphorylated on histidine residues, it becomes critical to show that these forms have kinetic properties consistent with their participation as intermediates in the catalytic reactions. Both phosphoglycerate mutase and bisphosphoglycerate synthase can catalyze the same three overall reactions which can be written with a phosphoenzyme intermediate:

- 2-PGA + E-P → E-P + 3-PGA
- 3-PGA + E-P → E-P + 3-PGA
- 2,3-DPG + E → E-P + PGA → 1,3-DPG + E → E + P,

Overall reaction | Designation | Steps involved
--- | --- | ---
2-PGA → 1,3-DPG | Phosphoglycerate mutase | 1, 4, and 5
1,3-DPG → 2,3-DPG | Bisphosphoglycerate synthase | 2, 3
2,3-DPG → PGA + Pi | Bisphosphoglycerate phosphatase | 1, 3

Abbreviations: 2-PGA, glycerate 2-P; 3-PGA, glycerate 3-P; 2,3-DPG, glycerate-2,3-P; 1,3-DPG, glycerate 1,3-P; E, free enzyme.

The phosphatase activity of phosphoglycerate mutase was studied by Grisolia and co-workers (e.g., Refs. 9 and 10) and by Sasaki et al. (11); its activation by glycolate-2-P was first shown by Rose and Liebowitz (12). The activation of phosphoglycerate mutase by glycerate-1,3-P, as well as by glycerate-2,3-P, was shown by Laforet et al. (13). Purified bisphosphoglycerate synthase was shown to have phosphatase activity activatable by glycolate-2-P; some phosphoglycerate mutase activity appeared inherent to it as well (8). Rosa et al. (14) observed that synthase and phosphatase activities of red cell hemolysates were not separated by electrophoresis. Subsequently, they showed that the same bands contained phosphoglycerate mutase activity (15). Sasaki et al. (16) and Kappel et al. (17) showed that the three activities are not separated during purification procedures.

Kinetic studies of the phosphoglycerate mutase reaction suggested the likelihood of a ping-pong mechanism (18), although the evidence was considered inconclusive by others (19). Results of studies of rates of isotopic fluxes were consistent with the participation of a phosphoenzyme intermediate.
The kinetics of the bisphosphoglycerate synthase of human red cells (21) indicated the formation of ternary complexes and were therefore not suggestive of a mechanism involving phosphoenzyme. However, the ability of each enzyme to catalyze a phosphatase reaction under certain conditions led us to investigate further and demonstrate that both enzymes can be phosphorylated at single histidine residues by either glycerate-1,3-P₂ or glycerate-3,3-P₂ (5, 6, 8).

This study considers whether the kinetics of formation and utilization of these phosphoenzymes have the properties expected of intermediates in their respective enzymatic reactions. The rates of phosphorylation of the enzyme and phosphoryl transfer from the enzyme have been determined with a rapid mixing device that allows the measurement of rates as fast as about 20 ms. These rates have been compared with the maximal velocities of the reactions catalyzed by the two enzymes and found to be reasonable for steps in the overall processes.

**EXPERIMENTAL PROCEDURE**

The rapid quench apparatus was designed by D. P. Ballou (22). The reagents are placed in separate syringes which feed into a mixing chamber and then into a second mixing chamber where the quenching solution is injected. The length of time of reaction depends on the size of the second reaction cell and the length of tubing connecting it to the quenching cell. The apparatus was standardized according to Parma and Guthreid (23) using the alkaline hydrolysis of p-nitrophenylacetate for which the rate constant of 11.2 M⁻¹ s⁻¹ at 25°C was obtained by Drs. L.-J. Wong and C. F. Melford in this laboratory.

Two gas-tight syringes (Hamilton) held 0.5 ml each of the two reagents. Enzyme solutions routinely contained bovine serum albumin at 0.1 mg/ml for stability. Sodium dodecyl sulfate was satisfactory for stopping the reactions rapidly enough; the final concentrations used were 1.3% for phosphoglycerate mutase and 0.17% for bisphosphoglycerate synthase. Phenol was added to the quenched samples and the small molecules were extracted into aqueous medium as described earlier (5, 24).

Phosphoglycerate mutase was prepared from chicken breast muscle according to James et al. (25). Phosphoglycerate synthase was prepared from horse red blood cells using a modified procedure involving fractionation on a blue dextran column. Both proteins appeared homogeneous upon polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate and both have mobility indistinguishable from that of rabbit muscle phosphoglycerate mutase, that is, about 29,000 daltons (5).

Glycerate-1,3-[1-³P]P₂ (21) and glycerate-2,3-[U-³P]P₂ (12) were prepared as noted previously and used at specific activities of 10⁷ to 10⁸ cpm/μmol of P.

**Enzyme Assay**

In all cases maximal velocities were measured as shown by the failure of any substrate to increase the rate in spectrophotometric assays or by using extrapolated values from Lineweaver-Burk plots for assays involving radioactive substrates. Both enzymes have been characterized as having molecular weights of about 60,000 and consisting of two apparently identical subunits (5, 8).

The maximal extent of phosphorylation obtained with either glycerate-2,3-P₂ or glycerate-1,3-P₂ indicated that there is one reactive site per subunit. Values for k₅⁰ were obtained by dividing Vₐ₅₀ in terms of micromoles of product formed per second by the micromoles of active sites as indicated by the maximal extent of phosphorylation attainable with the same amount of enzyme. Therefore, the k is in terms of activity of subunits of 30,000 rather than molecules of 60,000.

**Phosphoglycerate Mutase Activity**—The reaction was studied in the direction glycerate-2-P to glycerate-3-P. The incubations were for 5 min in 0.2-ml volumes at pH 7.5 and contained: 100 mM trithanolamine·Cl⁻ buffer, 0.5 mM glycerate-2,3-P₂, 1 mM glycerate-2-P, and enzyme sufficient to utilize about 10% of the substrate. At pH 5.5, incubations contained 100 mM ammonium acetate buffer, 2 mM glycerate-2,3-P₂, and 4 mM of glycerate-2-P. Reactions were stopped by the addition of 0.05 N HCl (0.10 ml), heated at 100°C for 3 min.

**RESULTS**

**Maximal Velocities of Enzymatic Reactions**—Individual steps in a reaction sequence must be at least as rapid as the overall reaction. Therefore, maximal velocities were determined for the three reactions catalyzed by each enzyme. The rates of phosphorylation of the enzymes with the 2 donor molecules and the rates of transfer of the phosphoryl groups to the acceptors glycerate-3-P or to water with the analog glycolate-2-P, are to be determined or lower limits set by the capability of the rapid quenching apparatus. If all of the rates of phosphorylation and transfer are compatible with the overall rates, then the isolated phosphoenzymes have the kinetic properties required of catalytic intermediates.

With the rapid quenching device used in these studies, a reaction could be stopped after 20 ms or up to 400 ms after initiation. This corresponds to a range of first order rate constants of 30 to 1.7 s⁻¹ from the expression k₅₀ = 0.69/t₅₀, where t₅₀ is the half-time of the reaction and can be taken directly from the semi-log plots of the data.

The maximal velocities of the three reactions catalyzed by phosphoglycerate mutase were measured under a variety of conditions (Table I). The phosphoglycerate mutase reaction is rapid and out of the range of measurement of the rapid quenching apparatus. The phosphatase reaction, activated by glycolate-2-P, is much slower with k₅₀ = 2.78 s⁻¹ at pH 7.5, 25°C, and the synthase activity is extremely slow, k₅₀ = 0.4 s⁻¹.

Table II shows the k₅₀ values for the same reactions catalyzed by bisphosphoglycerate synthase. In this case, the rates of the reactions are all in a favorable range for measurement. The phosphatase rates of the two enzymes are similar, whereas the other two reactions are catalyzed by very different rates. We have found that neither enzyme shows measurable phosphatase activity in the absence of an activator such as glycolate-2-P.

**Rate of Phosphorylation of Bisphosphoglycerate Synthase**—The rate of phosphorylation of bisphosphoglycerate synthase by glycerate-1,3-[1-³P]P₂ is shown in Fig. 1. At 25°C the observed half-time of 51 ms corresponds to a rate of phosphorylation of 13.5 s⁻¹, which is close to k₅₀ of 12.5 s⁻¹. At 4°C the phosphorylation rate of 3.9 s⁻¹ (t₅₀ = 176 ms) should be compared to k₅₀ of 3.57 s⁻¹. The glycerate-1,3-P₂ used in these studies contained no more than 10% glycerate-3-P. The presence of glycerate-3-P would tend to make the rate of enzyme phosphorylation appear to be slower than the true rate due to the transfer of the phosphoryl group from the enzyme to the

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1. L.-J. Wong and C. F. Melford, unpublished observations.
2. S. Dube and Z. B. Rose, manuscript in preparation.
Phosphorylation of Phosphoglycerate Mutase and Bisphosphoglycerate Synthase

TABLE I
Rates of reactions catalyzed by phosphoglycerate mutase

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Conditions</th>
<th>Maximal velocity $k_{max}$</th>
<th>Donor 1,3-DPG</th>
<th>Donor 2,3-DPG</th>
<th>Acceptor 3-PGA</th>
<th>Acceptor analog glycerate 2-P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mutase</td>
<td>pH 7.5, 4°C</td>
<td>510 s$^{-1}$</td>
<td>&gt;100$^{a}$</td>
<td>&gt;100$^{a}$</td>
<td>&gt;100$^{a}$</td>
<td>&gt;100$^{a}$</td>
</tr>
<tr>
<td>2-PGA-3-PGA</td>
<td>pH 7.5, 25°C</td>
<td>1197 s$^{-1}$</td>
<td>165 s$^{-1}$</td>
<td>325 s$^{-1}$</td>
<td>1333 s$^{-1}$</td>
<td>2.78 s$^{-1}$</td>
</tr>
<tr>
<td>Phosphatase</td>
<td>pH 7.5, 25°C</td>
<td>2.78 s$^{-1}$</td>
<td>&gt;100$^{a}$</td>
<td></td>
<td>3.59 s$^{-1}$</td>
<td>3.59 s$^{-1}$</td>
</tr>
<tr>
<td>Synthase</td>
<td>pH 7.5, 25°C</td>
<td>0.40 s$^{-1}$</td>
<td>1.57 s$^{-1}$</td>
<td>&gt;100$^{a}$</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$^{a}$ Lower limit estimated from observation that reaction was already complete in 22 ms.

**FIG. 1.** Rate of phosphorylation of bisphosphoglycerate synthase with glycerate-1,3-[1-14C]P$_2$. Upon mixing in the rapid quench apparatus all reactions contained: N-tris(hydroxymethyl)methyl-2-aminoethane sulphonate-Na buffer, pH 7.5, 10 mM; bovine serum albumin, 0.05 mg/ml; and enzyme, 0.13 FM. At 4°C with glycerate-1,3-[1-14C]P$_2$, 0.5 (x) or 1 μM (A); 25°C, glycerate-1,3-[1-14C]P$_2$, 0.5 μM (C). Free enzyme is designated E.

**TABLE II**
Rates of reactions catalyzed by bisphosphoglycerate synthase

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Conditions</th>
<th>Maximal velocity $k_{max}$</th>
<th>Donor 1,3-DPG</th>
<th>Donor 2,3-DPG</th>
<th>Acceptor 3-PGA</th>
<th>Acceptor analog glycerate 2-P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Synthase</td>
<td>pH 7.5, 4°C</td>
<td>3.57 s$^{-1}$</td>
<td>3.9 s$^{-1}$</td>
<td>12.5 s$^{-1}$</td>
<td>13.5 s$^{-1}$</td>
<td>13.5 s$^{-1}$</td>
</tr>
<tr>
<td></td>
<td>pH 7.5, 25°C</td>
<td>12.5 s$^{-1}$</td>
<td>13.5 s$^{-1}$</td>
<td>3.9 s$^{-1}$</td>
<td>13.5 s$^{-1}$</td>
<td>13.5 s$^{-1}$</td>
</tr>
<tr>
<td>Phosphatase</td>
<td>pH 7.5, 25°C</td>
<td>2.57 s$^{-1}$</td>
<td>2.3 s$^{-1}$</td>
<td>16 s$^{-1}$</td>
<td>&gt;100 s$^{-1}$</td>
<td>&gt;100 s$^{-1}$</td>
</tr>
<tr>
<td>Mutase</td>
<td>pH 7.5, 25°C</td>
<td>1.7 s$^{-1}$</td>
<td>2.3 s$^{-1}$</td>
<td>&gt;100 s$^{-1}$</td>
<td>&gt;100 s$^{-1}$</td>
<td>&gt;100 s$^{-1}$</td>
</tr>
</tbody>
</table>

* Lower limit estimated from observation that reaction was already complete in 22 ms.

**Release of glyc erate-2,3-P$_4$ should be more rapid than the initial phosphorylation by glyc erate-1,3-P$_2$.**

$$E + 1,3-DPG \fast E \cdot 1,3-DPG \rightarrow E-P\cdot 3-PGA$$

The rate of phosphorylation of the synthase by glycerate-2,3-[U-14C]P$_2$ at pH 7.5, 25°C, is 2.3 s$^{-1}$ from the data in Fig. 2. In this experiment the enzyme concentration was 0.13 FM, and with 0.5 or 1 μM glycerate-2,3-P$_2$ the same rate was obtained, indicating a first order reaction. Therefore, the association of glycerate-2,3-P$_2$ with the enzyme is relatively rapid and far in the direction of complex formation, while the transfer of the phosphoryl group to the enzyme is slow. This transfer appears to limit the rate of the phosphatase reaction for which $k_{max}$ is 2.6 s$^{-1}$ and indicates that the properties of the acceptor glycerate-2-P do not limit the overall rate and therefore no other acceptor should give a faster rate. The 5-fold slower rate of phosphorylation of the enzyme by glycerate-2,3-P$_2$ correlates with the difference in $k_{max}$ between the synthase reaction which uses glycerate-1,3-P$_2$ and the phosphatase and mutase reactions which use glycerate-2,3-P$_2$. In all cases the subsequent steps for the reactions must be more rapid. For example, the dissociation of E-P-PGA cannot be rate-limiting.

$$E + 2,3-DPG \fast E \cdot 2,3-DPG$$

**Rate of Phosphoryl Transfer from Phosphorylated Bisphosphoglycerate Synthase**—For these experiments phosphoryl enzyme was formed by mixing excess enzyme with the phosphoryl donor so that no rephosphorylation by the donor was possible. If rephosphorylation should occur, the apparent transfer rate would be slower than the true rate. At 25°C with glycerate-3-P as the acceptor, as in the synthase reaction, the transfer was complete in the shortest time measurable, 22 ms, indicating $k$ is at least 100 s$^{-1}$. At 4°C, the rate of transfer at pH 7.5 is 38 s$^{-1}$ and at pH 5.5 it is 13.8 s$^{-1}$ (Fig. 3). Therefore at pH 7.5, 25°C, the reaction is:

$$E + 2,3-DPG \fast E + P + PGA$$

which is the reverse of Equation 2. Comparison of the rates of phosphorylation, 2.3 s$^{-1}$, and phosphoryl transfer, >100 s$^{-1}$, indicates this step is far in the direction of transfer.

$$K = \frac{E \cdot P \cdot PGA}{E \cdot 2,3-DPG} = \frac{k_r}{k_t} \approx 2.3 > 100$$

phosphoglycerate. In the experiments described in Fig. 1, the rate of phosphorylation was not affected by increasing the glycerate-1,3-P$_2$ from 0.5 to 1 μM. Since the enzyme concentration was 0.13 FM, it appears that the 4-fold excess of substrate over enzyme was sufficient to convert all of the enzyme to enzyme-substrate complexes, $K_{diss} \leq 3 \times 10^{-6}$ M compared with a $K_a$ of 4 μM. The rate of phosphoryl transfer is then the observed first order rate ($E$·glycerate-1,3-P$_2$ → E-P·glycerate-3-P) (Fig. 1). The agreement between the overall rate, $k_{max}$, and rate of phosphorylation by glycerate-1,3-P$_2$ (Equation 1) indicates that the additional steps leading to the formation and release of glycerate-2,3-P$_4$ should be more rapid than the initial phosphorylation by glycerate-1,3-P$_2$. 

$$E + 1,3-DPG \fast E \cdot 1,3-DPG \rightarrow E + 2,3-DPG$$

$$E + P + PGA \rightarrow E + 2,3-DPG$$

$$E \cdot 2,3-DPG \rightarrow E + 2,3-DPG$$

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that there had been rapid phosphorylation of 70% of the
concentration of glycerate-2,3-P, in order to eliminate the
possibility that the substrate had protected the enzyme against
glycerate-2,3-P,, the extrapolation to the ordinate indicates
denaturation. In this experiment, at saturating levels of
phosphorylation was complete in the shortest measurable time, about 22 ms. At
saturating levels of pH 5.5, 4°, the results shown in Fig. 5 were obtained. There was
still more rapid than enzyme phosphorylation, and not rate-
limiting. The rates are summarized in Table II.

Rate of Phosphorylation of Phosphoglycerate Mutase—The
phosphoglycerate mutase reaction is too fast for the measurement of the rates of its component steps in the rapid quench apparatus (Table I). However, it would be possible to eliminate the phosphoenzyme as a plausible intermediate if its rates of formation and transfer were observed to be too slow. Positive evidence for participation of the phosphoenzyme could come from observation of rates of steps involved only in the slower secondary reactions catalyzed by the enzyme. Phosphorylation of the enzyme by glycerate-2,3-[U-32P]P, at pH 7.5 and 4° or 4° was complete in the shortest measurable time, about 22 ms. At pH 5.5, 4°, the results shown in Fig. 5 were obtained. There was a burst of protein phosphorylation followed by a slower linear rate of formation of additional phosphoenzyme. Particular care was taken to be sure that the quenching process was adequate at the shortest times and in the presence of the highest concentration of glycerate-2,3-P, in order to eliminate the possibility that the substrate had protected the enzyme against denaturation. In this experiment, at saturating levels of glycerate-2,3-P, the extrapolation to the ordinate indicates that there had been rapid phosphorylation of 70% of the enzyme in a shorter time than is required by the kcat of 165 s⁻¹.

In a scheme similar to that proposed for the synthase (Equation 2), it would appear that the first two steps are in a rapid equilibrium with the phosphorylation of the mutase occurring much more rapidly than the rate of 2.3 s⁻¹ observed for the synthase. The third step is the slow release of the first product and readjustment of the preceding equilibrium with the further formation of phosphoenzyme.

The phosphorylation by glycerate-1,3-P, is very slow with a rate of 1.57 s⁻¹ at pH 7.5 and 25° (Fig. 6). This is to be compared with kcat for the glycerate-1,3-P, → glycerate-2,3-P, reaction, which is 0.40 s⁻¹ (Table I). The apparent Kₐ of glycerate-1,3-P, in the synthase reaction was very high at 20 μM. It is probable in the usual kinetic experiment that once glycerate-2,3-P, is generated it competes effectively with glycerate-1,3-P,. The single turnover experiment (Fig. 6) indicates that there is a high affinity of glycerate-1,3-P, >3 x 10⁻⁸ M, for the enzyme, since 0.5 μM glycerate-1,3-P, was sufficient to give the maximal rate of phosphorylation with 0.25 μM enzyme. The rates are summarized in Table I.

Rate of Phosphoryl Transfer by Phosphoglycerate Mutase—When the acceptor was glycerate-3-P, the normal substrate of the phosphoglycerate mutase reaction, the transfer of the phosphoryl group from preformed phosphoenzyme was already completed in 22 ms, the shortest time that could be measured. This was true at pH 7.5, 4°, with 5 mM glycerate-3-P and at pH 5.5, 4°, with 2.5 or 5 mM glycerate-3-P as second substrate.

With the substrate analog glycolate-2-P to release the
phosphoryl transfer was readily measured. Fig. 7 shows that at
by glycerate-2,3-P,. However, the $K_m$ values of the two
phosphoryl group, as in the phosphatase reaction, the rate of
phosphoryl transfer was readily measured. Fig. 7 shows that at
by glycerate-2,3-P,. However, the $K_m$ values of the two
phosphoryl donors are similar, i.e., 0.5 $\mu M$ for glycerate-1,3-P$_2$ in
the synthase reaction (21) and 0.6 $\mu M$ for glycerate-2,3-P$_2$ in
the phosphatase reaction activated by glycolate-2-P (12). The $K_i$
of glycerate-2,3-P$_2$ in the synthase reaction is 0.8 $\mu M$ (21). It is
believed that this enzyme is also the functional bisphos-
phoglycerate phosphatase of the red cell. We have found that
phosphatase activity in the absence of added activator is
undetectable.$^*$ Glycolate-2-P, the activator used in this study,
is not present in red cells, but ions of the cell, particularly the
combination of $P_i$ and $Cl^-$, have been shown to activate the
phosphatase (12). There is no reason to believe that the phos-
phoglycerate mutase activity of this enzyme is physiologically
significant in view of the high level of phosphoglycerate mutase
in cells.

Our previous detailed kinetic studies of bisphosphoglycerate
synthase, which showed sequential kinetics, were done with
enzyme isolated from human red blood cells (21). The demon-
stration of phosphorylation was also with that enzyme (8).
Stable enzyme intermediates are often thought not to play a
part in reactions that analyze as sequential. In the present
study we have prepared enzyme from horse red cells in order to
have an adequate source of fresh starting material. Many
properties of the enzymes from the two sources are similar but
the horse enzyme shows ping-pong kinetics. The kinetic results
suggest that with the human red cell enzyme the first product
does not leave the enzyme until the second substrate is bound,
implicating separate binding sites for the two substrates. The
horse synthase may have an essentially similar mechanism
with separate binding sites for phosphoryl donor and acceptor.

The phosphorylation rate of phosphoglycerate mutase is too
rapid to measure at pH 7.5. Since phosphorylation is complete
in 22 ms, the half-time is at least 3 to 4 times faster or has as a
lower limit a rate of 100 s$^{-1}$. At pH 5.5, 4$^\circ$, $k_{cat}$ is 165 s$^{-1}$. Fig. 5
shows that phosphorylation of the enzyme is rapid enough to be
a component of observed overall reaction. The additional slow
rate could be due to the slow dissociation of the phosphoglycer-
ate produced in the phosphorylation reaction as shown in Fig. 8.
The scheme shows the phosphorylation of the enzyme and the
slow dissociation of the first product, which comes from the
phosphoryl donor. The overall phosphorylation rate is faster
than the linear secondary phase of phosphoenzyme
formation shown in Fig. 5. Therefore it is postulated that the
slow reaction is not on the usual reaction path and that there is a
second site at which the substrate molecule can bind whether or
not the first product has left. The interconversion of the two
phosphoglycerates occurs in the reactions shown in the triangle
of Fig. 8. Phosphoenzyme is regenerated. It is known that
glycerate-2,3-P$_2$ is not released in most turnovers of the enzyme
(18). From this scheme one can suggest that glycerate-2,3-P$_2$ is
released instead of phosphoglycerate when a substrate mole-
ule reacts with the donor site rather than with the acceptor
site. From kinetic studies of the phosphoglycerate mutase
reaction, Grisolia and Cleland (18) suggested that the mecha-
nism is ping-pong with inhibition of the binding of each
substrate by the other substrate. Rate studies done in conjunc-
tion with the present work support this mechanism.

The conclusion that the phosphoenzyme participates in the

\footnote{Z. B. Rose, unpublished observations.}

**DISCUSSION**

These studies indicate that the phosphorylated forms of
phosphoglycerate mutase and bisphosphoglycerate synthase
have the kinetic properties required of intermediates in the
catalytic processes.

Bisphosphoglycerate synthase appears to function with
phosphorylation of the enzyme as the rate-limiting step.
Phosphorylation by glycerate-1,3-P$_2$ is much more rapid than
that by glycerate-2,3-P$_2$. However, the $K_m$ values of the two
phosphoglycerate mutase reaction substantiates the often postulated similarity of mechanism between that enzyme and phosphoglucomutase (27). Phosphoglucomutase is isolated mainly as the phosphoserine derivative in which the phosphate functions in phosphoryl transfer. The cofactor, glucose-1,6-P₂, serves to rephosphorylate the enzyme as needed. The phosphorylated form of phosphoglycerate mutase, a histidine derivative, is less stable and the enzyme is isolated in the free form which shows an absolute requirement for glycerate-2,3-P₂.

REFERENCES
Rates of phosphorylation and dephosphorylation of phosphoglycerate mutase and bisphosphoglycerate synthase.
Z B Rose and S Dube


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