Purification and Characterization of Phosphoglycerate Mutase from Methanol-grown *Hyphomicrobium* x and *Pseudomonas* AM1

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SUMMARY

Phosphoglycerate mutase has been purified from methanol-grown *Hyphomicrobium* x and *Pseudomonas* AM1 by acid precipitation, heat treatment, ammonium sulphate fractionation, Sephadex G-50 gel filtration and DEAE-cellulose column chromatography. The purification attained using the *Hyphomicrobium* x extract was 72-fold, and using the *Pseudomonas* AM1 extract, 140-fold. The enzyme purity, as shown by analytical polyacrylamide gel electrophoresis, was 50% from *Hyphomicrobium* x and 40% from *Pseudomonas* AM1. The enzyme activity was associated with one band. The purified preparations did not contain detectable amounts of phosphoglycerate kinase, phosphopyruvate hydratase, phosphoglycerate dehydrogenase or glycerate kinase activity. The molecular weight of the enzymic preparation was 32,000 ± 3000. The enzyme from both organisms was stable at low temperatures and, in the presence of 2,3-diphosphoglyceric acid, could withstand exposure to high temperatures. The enzyme from *Pseudomonas* AM1 has a broad pH optimum at 7.0 to 7.6 whilst the enzyme from *Hyphomicrobium* x has an optimal activity at pH 7.3. The cofactor 2,3-diphosphoglyceric acid was required for maximum enzyme activity and high concentrations of 2-phosphoglyceric acid were inhibitory. The $K_m$ values for the *Hyphomicrobium* x enzyme were: 3-phosphoglyceric acid, $6.0 \times 10^{-3}$ M; 2-phosphoglyceric acid, $6.9 \times 10^{-4}$ M; 2,3-diphosphoglyceric acid, $8.0 \times 10^{-6}$ M; and for the *Pseudomonas* AM1 enzyme: $3.4 \times 10^{-3}$ M, $3.7 \times 10^{-4}$ M and $10 \times 10^{-6}$ M respectively. The equilibrium constant for the reaction was $11.3 \pm 2.5$ in the direction of 2-phosphoglyceric acid to 3-phosphoglyceric acid and $0.09 \pm 0.02$ in the reverse direction. The standard free energy for the reaction proceeding from 2-phosphoglyceric acid to 3-phosphoglyceric acid was $-5.84$ kJ mol$^{-1}$ and in the reverse direction $+5.81$ kJ mol$^{-1}$.

INTRODUCTION

Previous studies on the carbon assimilation pathways for growth on methanol have shown that *Hyphomicrobium* x and *Pseudomonas* AM1 assimilate the carbon via the serine pathway (Harder, Attwood & Quayle, 1973; Heptinstall & Quayle, 1970). Further investigations showed that the product of the phosphoglycerate kinase activity in the pathway was 2-phosphoglyceric acid (Hill & Attwood, 1974) rather than the more usual product, 3-phosphoglyceric acid. This substantiated a previous suggestion that the conversion of 2-phosphoglyceric acid into 3-phosphoglyceric acid might be the initial step of gluconeogenesis rather than a direct step in the regeneration of the $C_1$ receptor molecule (Harder *et al.*, 1973). If this is so, phosphoglycerate mutase must be situated at a branch point in the carbon assimilation pathway. We therefore decided to purify the enzyme from these two organisms, investigate the properties of the purified enzymes and compare these with those of other purified phosphoglycerate mutase enzymes. The results are reported in this paper.
METHODS

Organisms and growth conditions. *Pseudomonas AMI* (NCIB9133) and *Hyphomicrobium* were maintained on slope cultures on an inorganic salts medium containing methanol (0.5%, v/v) as carbon and energy source and agar (1.5%, w/v). The organisms were subcultured weekly. Liquid cultures of *Pseudomonas AMI* were prepared as described by Heptinstall & Quayle (1970) with methanol (0.5%, v/v) as carbon and energy source. Liquid cultures of *Hyphomicrobium* were grown on methanol (0.5%, v/v) in the salts medium of Attwood & Harder (1972). Cultures were harvested towards the end of the exponential-growth phase by centrifuging at 6500 g for 20 min. The organisms were washed twice with 50 mM-sodium phosphate buffer pH 7.0 containing 5 mM-MgCl₂, and either resuspended in the same buffer supplemented with 2 mM-2-mercaptoethanol or frozen at −20 °C and stored.

Protein determinations. Protein was measured by the Folin–Ciocalteu method of Lowry et al. (1951), using bovine serum albumin as the standard.

Enzyme assays. Phosphoglycerate mutase (EC. 2.7.5.3) activity was measured by: (i) continuous spectrophotometric assays in the direction of 3-phosphoglyceric acid to 2-phosphoglyceric acid and in the reverse direction; and (ii) a discontinuous assay in the direction of 3-phosphoglyceric acid to 2-phosphoglyceric acid. All continuous assay procedures were carried out using a Pye Unicam SP1800 spectrophotometer incorporating a constant-temperature cuvette housing. The continuous assay in the direction of 3-phosphoglycerate to 2-phosphoglycerate was a modification of the method described by Harder et al. (1973): 50 mM-imidazole buffer pH 7.3 was substituted for the Tris/HCl buffer in the original assay; the final magnesium chloride concentration was decreased to 0.5 mM; and the final 2,3-diphosphoglyceric acid concentration was increased to 0.2 mM. The continuous assay method in the reverse direction was a modified form of that given in *Biochemica Catalogue* (1968): 50 mM-imidazole buffer pH 7.3 was substituted for the 0.1 M-Tris/ethanolamine buffer pH 7.6 and the reaction was started by the addition of 2-phosphoglycerate (1 μmol ml⁻¹).

In the discontinuous assay, the reaction was allowed to run to completion in the direction of 3-phosphoglycerate to 2-phosphoglycerate. The incubation mixture consisted of 500 μmol imidazole/HCl buffer pH 7.3, 50 μmol MgCl₂, 20 μmol 2,3-diphosphoglyceric acid, extract or purified enzyme, and water to a final volume of 10 ml. The mixture was pre-incubated at 30 °C and the reaction was started by the addition of 50 μmol 3-phosphoglyceric acid. Samples (1.0 ml) were taken immediately and after known periods of time, added to 1 M-perchloric acid (1.0 ml) and allowed to stand for 5 min to terminate the reaction. 2 M-Potassium carbonate (0.3 ml) was added to neutralize the samples, and then water was added to give a final volume of 4.0 ml. The 2-phosphoglyceric acid present in the samples was measured spectrophotometrically and plotted against the time of incubation: the slope of the graph gave a measure of the rate of reaction.

Measurement of 2-phosphoglycerate. A reaction mixture (0.6 ml) containing 50 μmol Tris/HCl buffer pH 7.5, 0.5 μmol adenosine diphosphate, 0.15 μmol NADH, 0.5 μmol MgCl₂, 10 μg pyruvate kinase, 20 μg phosphopyruvate hydratase, and 10 μg lactate dehydrogenase, was incubated at 30 °C. The extinction of the reaction mixture at 340 nm was recorded and the reaction started by the addition of neutralized samples (0.4 ml) from the incubation mixture. The final extinction at 340 nm was recorded and the amount of 2-phosphoglycerate present in the sample calculated from the change in extinction.

Enzyme units. Enzyme activity was recorded as μmol NADH oxidized min⁻¹ (mg protein)⁻¹.
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for the continuous assays and as \( \mu \text{mol product formed min}^{-1} (\text{mg protein})^{-1} \) for the discontinuous assay.

**Incubation procedure for the direct measurement of the equilibrium constant.** Direct measurements of the equilibrium constant were made by adding purified enzyme to the reaction mixture, allowing the reaction to go to completion, and measuring the amounts of 2-phosphoglycerate and 3-phosphoglycerate present at the end of the reaction. The reaction mixture (2.0 ml) contained 100 \( \mu \text{mol imidazole/HCl buffer pH 7.3} \), 3.5 \( \mu \text{mol MgCl}_2 \), 9.4 \( \mu \text{mol 2,3-diphosphoglyceric acid} \), 10 \( \mu \text{mol 3-phosphoglyceric acid} \), and purified enzyme and water. Control reaction mixtures contained all the reactants except the substrates. The total enzyme added was 10 to 16 \( \mu \text{g protein} \) with an activity of 2 to 3 \( \mu \text{mol min}^{-1} \). The reaction mixture was incubated at 30°C, and samples were taken after 0, 1 and 2 h. The 2-phosphoglycerate and 3-phosphoglycerate present in the samples was measured by the discontinuous assay procedure. For 3-phosphoglycerate determinations, 25 \( \mu \text{g} \) of commercially purified rabbit muscle phosphoglycerate mutase was added to the reaction mixture.

**Purification.** All procedures were carried out at 0 to 4°C unless otherwise stated.

Step 1. Preparation of crude sonic extract. The methanol-grown cells (7.0 g) were resuspended in 28 ml ice-cold 50 mM-sodium phosphate buffer pH 7.0 containing 5 mM-MgCl\(_2\) and 2 mM-2-mercaptoethanol. The cells were disrupted using an MSE ultrasonic disintegrator (model 60W) at 8 mV for 12 x 1 min exposures. The sonicator vessel was surrounded by a cooling mixture of powdered carbon dioxide and ice, and the sonicator was interrupted for 1 min after each minute of exposure to prevent local warming of the suspension. The cell debris was removed by centrifuging: 15000 \( g \) for 10 min for the *Hyphomicrobium* x extract; and 35000 \( g \) for 60 min for the crude *Pseudomonas AMI* preparation. The pellet was resuspended in buffer (28 ml) and the sonication and centrifugation procedures were repeated. The first and second supernatant fluids were combined to give the final crude sonic extract.

Step 2. Acid precipitation. Acetic acid (0.1 M) was added dropwise with careful and continuous stirring until the pH reached 5.0 with the *Hyphomicrobium* x extract, and pH 5.5 with that from *Pseudomonas AMI*. The extracts were then allowed to stand for 15 min and the precipitate formed was removed by centrifuging: 15000 \( g \) for 10 min for *Hyphomicrobium* x; and 35000 \( g \) for 60 min for *Pseudomonas AMI*. The precipitate was discarded and further acetic acid was added to both supernatant fluids until the pH reached 4.0. After standing for 15 min, the precipitate was collected by centrifuging as described previously, and dissolved in 14 to 15 ml 50 mM-sodium phosphate buffer pH 7.0 containing 5 mM-MgCl\(_2\) and 2 mM-2-mercaptoethanol.

Step 3. Heat treatment. 2,3-Diphosphoglyceric acid (5 mg ml\(^{-1}\)) was added to each acid precipitate in buffer, and then the mixture was brought to 60°C and maintained at this temperature for 15 min, before cooling to 4°C. The precipitate was removed by centrifuging as described in step 2.

Step 4. Ammonium sulphate fractionation. The supernatant fluid from step 3 was brought to 30% saturation by the addition, with continuous stirring, of solid ammonium sulphate. The preparation was then allowed to stand for 30 min, centrifuged at 15000 \( g \) for 10 min, and the precipitate was discarded. The supernatant fluid was taken to 50% ammonium sulphate saturation, and the precipitate was collected by centrifuging, as above, and dissolved in 4.0 to 5.5 ml 50 mM-sodium phosphate buffer pH 7.0 containing 5 mM-MgCl\(_2\) and 2 mM-2-mercaptoethanol.

Step 5. Sephadex G-50 gel filtration. The ammonium sulphate precipitate in buffer was
added to the top of a Sephadex G-50 medium grade column (15 x 1.5 cm), previously
equilibrated to the same buffer. The column was eluted with the same buffer, and fractions
(1.7 ml) were collected at a flow rate of 0.8 ml min⁻¹. The fractions were monitored for
(i) protein, using an LKB Uvicord I at 257 nm, and (ii) enzyme activity. Fractions showing
enzyme activity were combined.

Step 6. DEAE-cellulose column chromatography. The ammonium-free active fractions
(11 to 13 ml) were eluted from a column of DEAE-cellulose DE-52 (15 x 1.5 cm) with a
linear sodium chloride gradient (0 to 1 M) in 500 ml 10 mm-sodium phosphate buffer
pH 7.0 containing 5 mm-MgCl₂ and 2 mm-2-mercaptoethanol. Fractions (4.0 ml) were
collected at a flow rate of 0.4 ml min⁻¹ and monitored for protein. The protein peaks were
assayed for phosphoglycerate mutase activity, and the active fractions were combined and
stored at −20 °C.

Polyacrylamide gel electrophoresis of protein. Discontinuous polyacrylamide gel electro-
phoresis was carried out at 0 to 4 °C with 7.5 % (w/v) polyacrylamide gels at pH 5·5, 7·5
and 8·5, as described by Davis (1964). Enzyme samples were dialysed against the appropriate
half-strength running buffer for 20 h at 0 to 4 °C. Dialysed samples (120 μg protein) together
with 0.05 % bromophenol blue marker dye (final concentration 0.001 %) were added to the
gels in 15 % (w/v) sucrose. Proteins on the gels were stained with 0.25 % Coomasie Blue in
methanol/acetic acid/water (5:7:88, by vol.) and destained by soaking in methanol/acetic
acid/water (5:7:88, by vol.) in the presence of Dowex 1X-8-50 resin for 20 h at 37 °C. The
bands on the stained gels were located by scanning the gels at 540 nm in a Pye Unicam
SP1800 spectrophotometer fitted with a scanning densitometer attachment (SPI809). Gels
with no added enzyme were prepared to provide the base line for the peak profiles, and
the areas under the band peaks were calculated. Unstained gels were cut into 2 mm slices,
which were eluted with buffer and assayed for enzyme activity.

Molecular-weight determination by gel filtration. The molecular weight of the purified
enzyme was determined by chromatography of the enzymes on a calibrated column of
Sephadex G-200 by the procedure of Andrews (1965).

Chemicals. Diethyl acetal barium salt of D-glyceraldehyde 3-phosphate and all coenzymes,
enzymes and substrates were obtained from Boehringer (London), Lewes, East Sussex
BN7 1LG. Bovine serum albumin was from Armour Pharmaceutical, Eastbourne, Sussex.
β-Glycerol phosphate, β-DL-chloroacetic acid and DL-serine were from Sigma. DL-O-
Phosphoserine and hydroxyproprionic acid phosphate dimethyl ketal were from Calbiochem.
DL-Glyceric acid was from Koch-Light.

RESULTS

Purification of the enzymes

Data from typical purifications are presented in Table 1. The enzyme from *Hyphomicro-
bium* X was purified 72-fold with an activity yield of 16 %; and that from *Pseudomonas* AM1
was purified 140-fold with an activity yield of 33 %. An identical purification procedure
carried out in the absence of mercaptoethanol resulted in an enzyme preparation from
*Hyphomicrobium* X with a 20-fold purification and an activity yield of 8 %, and a 120-fold
enzyme purification and 22 % activity yield from *Pseudomonas* AM1. The location of the
purified enzyme from both organisms in the eluate fractions from a calibrated column of
Sephadex G-200 indicated a molecular weight of 32000 ±3000. Polyacrylamide gel electro-
phoresis showed that the purified enzyme activity was associated with only one protein band,
which constituted 50 % of the final protein concentration in the *Hyphomicrobium* X pre-
paration and 40 % in the *Pseudomonas* AM1 preparation. Although the enzyme preparations
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Table 1. Purification of phosphoglycerate mutase from crude sonic extracts of methanol-grown *Hyphomicrobium X* and *Pseudomonas AMI*

Activity was assayed using the continuous spectrophotometric method in the direction 3-phosphoglyceric acid to 2-phosphoglyceric acid.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Vol. (ml)</th>
<th>Total activity (µmol min⁻¹)</th>
<th>Total protein (mg)</th>
<th>Specific activity (µmol min⁻¹ (mg protein)⁻¹)</th>
<th>Recovery (%)</th>
<th>Purification factor</th>
</tr>
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<tbody>
<tr>
<td><em>Hyphomicrobium X</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crude sonic extract</td>
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<td>19.0</td>
<td>426</td>
<td>0.04</td>
<td>100</td>
<td>1</td>
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<td>Acid precipitation pH 4.0</td>
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<td>12.9</td>
<td>45</td>
<td>0.29</td>
<td>68</td>
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<td>6.0</td>
<td>10</td>
<td>0.62</td>
<td>32</td>
<td>14.0</td>
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<td>Sephadex G-50 filtration</td>
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<td>4.4</td>
<td>1.29</td>
<td>32</td>
<td>29.0</td>
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<tr>
<td>DEAE-cellulose eluate</td>
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<td>3.0</td>
<td>1</td>
<td>3.22</td>
<td>16</td>
<td>72.0</td>
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<tr>
<td><em>Pseudomonas AMI</em></td>
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<tr>
<td>Crude sonic extract</td>
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<td>638</td>
<td>0.14</td>
<td>100</td>
<td>1</td>
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<tr>
<td>Acid precipitation pH 4.0</td>
<td>18</td>
<td>73.8</td>
<td>237</td>
<td>0.31</td>
<td>81</td>
<td>2.2</td>
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<tr>
<td>Heat supernatant</td>
<td>16</td>
<td>74.6</td>
<td>64</td>
<td>1.17</td>
<td>82</td>
<td>8.2</td>
</tr>
<tr>
<td>(NH₄)₂SO₄ fractionation</td>
<td>5.4</td>
<td>78.0</td>
<td>33.5</td>
<td>2.33</td>
<td>86</td>
<td>16.3</td>
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<tr>
<td>Sephadex G-50 filtration</td>
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<td>70.4</td>
<td>29</td>
<td>2.45</td>
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<tr>
<td>DEAE-cellulose eluate</td>
<td>13</td>
<td>29.7</td>
<td>1.5</td>
<td>20.03</td>
<td>33</td>
<td>140.0</td>
</tr>
</tbody>
</table>

were not homogeneous, they did not have detectable activities of phosphoglycerate kinase (EC. 2.7.2.3), phosphopyruvate hydratase (EC. 4.2.1.11), phosphoglycerate dehydrogenase (EC. 1.1.1.95), or glycerate kinase (EC. 2.7.1.31).

**Stability of the purified enzyme**

When solutions of the purified enzyme stored at 100 µg ml⁻¹ in 10 mM-sodium phosphate buffer pH 7.0 containing 5 mM-MgCl₂ and 2 mM-mercaptoethanol at −20 °C were subjected to a weekly freezing and thawing cycle, they lost 50% of their initial specific activity after nine weeks, with the preparation from *Hyphomicrobium X*, and after 10 weeks, with the preparation from *Pseudomonas AMI*. With uninterrupted storage at −20 °C, only 13% of the initial specific activity of the *Hyphomicrobium X* preparation was lost after 10 weeks, whilst with the *Pseudomonas AMI* preparation, 10% was lost over this period.

Samples of *Hyphomicrobium X* purified enzyme (50 to 60 µg protein ml⁻¹) incubated at 30 °C in the absence of the cofactor 2,3-diphosphoglyceric acid lost 57% of their initial specific activity in 30 min, whereas incubation in the presence of 2,3-diphosphoglyceric acid (5 mg ml⁻¹) at 60 °C resulted in the loss of only 17% of the initial specific activity in 30 min. Similarly, samples of *Pseudomonas AMI* purified enzyme (10 to 20 µg protein ml⁻¹) incubated at 30 °C for 30 min without protective 2,3-diphosphoglyceric acid lost 38% of the initial specific activity, but in the presence of the cofactor at 60 °C lost only 13% of the initial activity.

**Characterization of the enzyme**

Both enzyme preparations required the addition of the cofactor 2,3-diphosphoglyceric acid for maximum activity. Without it, no activity could be recorded with the enzyme preparation from *Hyphomicrobium X*; whilst with the *Pseudomonas AMI* enzyme preparation, an 81% stimulation in activity was observed on the addition of the cofactor. Both enzyme preparations required the addition of an –SH protective group to the reaction mixture for
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Fig. 1. pH dependence of activity of phosphoglycerate mutase from (a) *Hyphomicrobium x*, (b) *Pseudomonas AMI*. Phosphoglycerate mutase activity was assayed as described in Methods. Imidazole/HCl buffers were used in the range pH 5.8 to 7.6 and Tris/HCl buffers from pH 7.2 to 8.6. The activities in the presence of Tris/HCl buffers have been corrected for the inhibitory effect of the Tris buffer (see Results). ○, △: Activity measured in the direction of 3-phosphoglyceric acid to 2-phosphoglyceric acid. ●, ▲: Activity measured in the direction of 2-phosphoglyceric acid to 3-phosphoglyceric acid.

total enzyme activity. This could be provided by mercaptoethanol, dithioerythritol, cysteine or reduced glutathione.

*pH activation curves*

The curves (Fig. 1) for the purified enzymes showed definite maximal activity for *Hyphomicrobium x* mutase at pH 7.3, but with the preparation from *Pseudomonas AMI*, maximum activity was recorded over the pH range 7.0 to 7.6. The nature of the buffer did not affect the pH optimum pattern; but the initial velocities observed in the Tris/HCl, sodium phosphate, Tris/maleate and Tris/ethanolamine buffers showed 15, 25, 29 and 48% inhibition respectively over the values obtained with imidazole buffers. The initial velocity of the reaction increased linearly with both enzyme preparations up to at least 18 μg protein ml⁻¹. The specific activities of the enzymes at pH 7.3 and 30 °C were 3 to 5 μmol (mg protein)⁻¹ min⁻¹ and 20 to 25 μmol (mg protein)⁻¹ min⁻¹ from *Hyphomicrobium x* and *Pseudomonas AMI* respectively.

*Substrate concentrations*

The apparent $K_m$ values for the substrates 2-phosphoglycerate, 3-phosphoglycerate and the cofactor 2,3-diphosphoglycerate were calculated by standard procedures from enzyme rates measured over a range of substrate concentrations, using the assay procedures described, at pH 7.3 (0.05 M-imidazole/HCl buffer). Double reciprocal plots (Lineweaver & Burk, 1934; Eadie, 1942; Hofstee, 1952) were used to calculate the $K_m$ values (Table 2). The equilibrium constant for the system was calculated using the $K_m$ and $V_{max}$ values obtained, and also from direct measurements of the reactants and enzyme products at equilibrium (Table 2). High concentrations of 2-phosphoglycerate (10 mM) resulted in 14% enzyme inhibition with the *Hyphomicrobium x* preparation and 35% inhibition with the enzyme from *Pseudomonas AMI*. Using the equation $\Delta G^\circ = -RT\ln K_m$, the apparent standard free energy change for the reaction was calculated to be $-5.84$ kJ mol⁻¹ in the direction of 2-phosphoglycerate to 3-phosphoglycerate and $+5.81$ kJ mol⁻¹ in the reverse direction.
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Table 2. Kinetic characteristics of the purified phosphoglycerate mutases

<table>
<thead>
<tr>
<th></th>
<th>Hyphomicrobium x</th>
<th>Pseudomonas AM1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( K_m )</td>
<td></td>
</tr>
<tr>
<td>3PGA</td>
<td>6.0 ( \times ) 10^{-3} M</td>
<td>3.4 ( \times ) 10^{-3} M</td>
</tr>
<tr>
<td>2PGA</td>
<td>6.9 ( \times ) 10^{-4} M</td>
<td>3.7 ( \times ) 10^{-4} M</td>
</tr>
<tr>
<td>2,3-diPGA</td>
<td>8.0 ( \times ) 10^{-4} M</td>
<td>10 ( \times ) 10^{-4} M</td>
</tr>
<tr>
<td>( V_{max} ) (μmol min^{-1})</td>
<td>( K_m )</td>
<td>( K_m )</td>
</tr>
<tr>
<td>3PGA</td>
<td>0.20</td>
<td>0.25</td>
</tr>
<tr>
<td>2PGA</td>
<td>0.26</td>
<td>0.25</td>
</tr>
<tr>
<td>2,3-diPGA</td>
<td>0.08</td>
<td>0.16</td>
</tr>
</tbody>
</table>

Substrate specificity

Studies with the purified enzymes failed to detect any activity with the following substrates: \( \beta \)-glycerol phosphate (5 or 10 mM), DL-O-phosphoserine (5 or 10 mM), DL-glyceric acid (5 or 10 mM), \( \beta \)-DL-chloroacetic acid (5 or 10 mM), DL-serine (5 or 10 mM) and DL-glyceraldehyde 3-phosphate (2.5 or 5 mM). With hydroxypyruvic acid 3-phosphate, activity was detected at 3 to 7% of the activity with 3-phosphoglyceric acid.

DISCUSSION

Phosphoglycerate mutase has been purified previously from many eukaryotic tissues including rabbit skeletal muscle (Cowgill & Pizer, 1956), chicken breast muscle (Torralba & Grisolia, 1966) and from one prokaryotic organism \( Escherichia coli \) (D'Alessio & Josse, 1971). In the above organisms, the phosphoglycerate mutase reaction was not considered to be the first step in carbohydrate biosynthesis. A comparison of the properties of the purified phosphoglycerate mutases from the \( C_1 \)-utilizing bacteria \( Hyphomicrobium \) x and \( Pseudomonas \) AM1 with the reported data for other purified phosphoglycerate mutases shows them to be very similar in many instances. For example, both the enzymes purified in the present study were stable on storage at \(-20\,^\circ\mathrm{C}\) and, in the presence of the protective coenzyme 2,3-diphosphoglyceric acid, at a temperature of \( 60\,^\circ\mathrm{C}\) for 30 min.

Data reported suggest that there are two classes of phosphoglycerate mutase enzyme: those which have no cofactor requirements and those which require 2,3-diphosphoglyceric acid for total activity (Grisolia, 1962). The requirement for the cofactor has been shown with enzymes purified from rabbit muscle (Pizer & Ballou, 1957) and yeast (Rodwell, Towne & Grisolia, 1957). Neither of the plant preparations, purified from wheat and rice germ, required the cofactor (Ito & Grisolia, 1959; Fernandez & Grisolia, 1960). The enzyme purified from both \( Hyphomicrobium \) x and \( Pseudomonas \) AM1 required the cofactor for total activity, and without it no activity was detected in the preparation from \( Hyphomicrobium \) x. Reported evidence also suggests that enzymes dependent on 2,3-diphosphoglyceric acid are inhibited by a high concentration of 2-phosphoglyceric acid, whereas mutases whose activity is independent of 2,3-diphosphoglyceric acid do not show this inhibition (Fernandez & Grisolia, 1960). This was confirmed in the present study: both the purified enzymes were inhibited by high concentrations of 2-phosphoglyceric acid. The
enzyme preparations were relatively substrate specific. No activity could be detected with a series of compounds and only a low level of activity was detected with hydroxypyruvic acid 3-phosphate. These results agree with those reported for crystalline phosphoglyceric acid mutase purified from rabbit muscle (Pizer & Ballou, 1959).

Muscle and yeast enzymes display maximal activity at pH 5-9 whilst both plant enzymes show activity over a wide pH range (pH 6 to 10) with an optimum activity around pH 9-0. The enzyme from Hyphomicrobium X gave maximum activity at pH 7-3 whereas the Pseudomonas AM1 enzyme displayed its maximum activity over the pH range 7-0 to 7-6. Our results are consistent with data reported for mutases dependent on 2,3-diphosphoglyceric acid (Fernandez & Grisolia, 1960).

The kinetic properties of the phosphoglycerate mutases from Hyphomicrobium X and Pseudomonas AM1 were measured and the $K_m$ values for the two substrates and cofactor were comparable with reported data (Grisolia, 1962). Using these $K_m$ values, and by direct measurement, the equilibrium constant for the reaction was shown to be $11.3 \pm 2.5$ for the reaction proceeding from 2-phosphoglycerate to 3-phosphoglycerate and the reciprocal value $0.09 \pm 0.02$ in the reverse direction. These values, although higher than those reported for yeast phosphoglycerate mutase (Rodwell et al., 1957), were comparable with those quoted for the wheat-germ enzyme (Ito & Grisolia, 1959) and for commercially available crystalline enzyme from rabbit muscle (Clarke, Birch & Britton, 1974). The standard free energy change for the reaction at 30 °C, calculated from $K_m$ values, was $-5.84$ kJ mol$^{-1}$ for the reaction proceeding in the direction of 2-phosphoglycerate to 3-phosphoglycerate, and $+5.81$ kJ mol$^{-1}$ in the reverse direction. These agree with the reported figure of $-4.35$ kJ mol$^{-1}$ at 25 °C (Pizer, 1962). Thus it appears that, despite the unique position of phosphoglycerate mutase in the $C_1$ assimilatory pathway of these organisms, the phosphoglycerate mutases purified from the two bacteria have properties very similar to those reported for the enzyme purified from other widely differing sources.

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REFERENCES


Phosphoglycerate mutase in $C_1$ utilizers


