The Effect of Adenosine Triphosphate on Phosphoglycerate Mutase Activity from *Hyphomicrobium* x and *Pseudomonas* AM1 Grown on Reduced One-carbon Compounds

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INTRODUCTION

When *Hyphomicrobium* sp. and *Pseudomonas* AM1 are grown on reduced one-carbon compounds, they assimilate carbon via the serine pathway (Harder, Attwood & Quayle, 1973; Heptinstall & Quayle, 1970). Detailed studies, using cell-free extracts of *Hyphomicrobium* sp., on the conversion of glycerate into phosphoenolpyruvate suggested that 2-phosphoglycerate was the product of the glycerate kinase reaction. This implies that phosphoglycerate mutase activity is not part of the serine pathway but may act as the first enzyme in the biosynthesis of cell material.

Furthermore, ATP appeared to stimulate the activity of phosphoglycerate mutase in crude cell-free extracts and this stimulation was antagonized by AMP. It was suggested that the phosphoglycerate mutase activity might be regulated by an adenylate charge effect (Harder *et al.*, 1973). In partially purified preparations of glycerate kinase from *Hyphomicrobium* x and *Pseudomonas* AM1 (Hill & Attwood, 1974) and in crude cell-free extracts of *Pseudomonas* MA (Newaz & Hersh, 1975), 2-phosphoglycerate is the product of the glycerate kinase reaction. However, the apparent stimulation of phosphoglycerate mutase activity by ATP has not been detected in crude cell-free extracts of *Pseudomonas* MA (Newaz & Hersh, 1975). We have now studied in more detail the effects of AMP, ADP and ATP on the activity of phosphoglycerate mutase in crude cell-free extracts, partially purified enzyme preparations and purified enzyme preparations from *Hyphomicrobium* x and *Pseudomonas* AM1.

METHODS

Growth of organism and preparation of cell-free extracts. *Hyphomicrobium* x was grown and harvested and crude cell-free extracts were prepared as previously described (Harder *et al.*, 1973). *Pseudomonas* AM1 (NCIB9133) was grown as described by Salem, Hacking & Quayle (1973) using methanol 0.5% (v/v) as carbon source; cells were harvested and crude cell-free extracts were prepared as above.

Protein determinations. Protein was measured by the Folin-Ciocalteu method (Lowry *et*

Table 1. *Phosphoglycerate mutase activity of protein peaks eluted from a Sephadex G-200 column: the effect of adenosine triphosphate (5 mM)*

Phosphoglycerate mutase activity was measured using the continuous spectrophotometric assay in the direction 3-phosphoglycerate to 2-phosphoglycerate as described in Methods. Peaks 1, 2 and 4 alone were inactive in the presence or absence of ATP.

Protein peak	Phosphoglycerate mutase activity [$\mu\text{mol min}^{-1} (\text{mg protein})^{-1}$]			
	<i>Hyphomicrobium</i> x		<i>Pseudomonas</i> AM1	
	Plus ATP	No ATP	Plus ATP	No ATP
Peak 3	0.37	0.40	0.21	0.21
Peak 1 + peak 2 + peak 3 + peak 4	0.53	0.33	0.11	0.08
Peak 1 + peak 3	0.33	0.33	0.13	0.13
Peak 2 + peak 3	0.55	0.33	0.26	0.16
Peak 4 + peak 3	0.30	0.30	0.12	0.13

al., 1951), using bovine serum albumin (Armour Pharmaceutical Co., Eastbourne, Sussex) as the standard.

Partially purified enzyme preparations. Cell-free extract (4.0 ml) was added to the top of a column of Sephadex G-200 (70 $\times$ 2.5 cm) equilibrated with 10 mM-sodium phosphate buffer pH 7.0 containing 5 mM-magnesium chloride, 2 mM-2-mercaptoethanol and 0.1 M-sodium chloride. The protein was eluted from the column with the same buffer. Fractions (4.0 ml) were collected and monitored for protein and phosphoglycerate mutase activity. Those fractions showing enzyme activity were pooled and used as the partially purified enzyme preparation.

Purified enzyme preparations. Phosphoglycerate mutase was purified by acid precipitation, heat treatment, ammonium sulphate fractionation, Sephadex G-50 gel filtration and DEAE-cellulose DE-52 column chromatography, as described by Hill & Attwood (1976).

Enzyme assays. Spectrophotometric assays were carried out using a Pye Unicam SP1800 spectrophotometer at 30°C. The amounts of enzyme to be assayed were such that the rates were linear with respect to time for at least 3 min and were proportional to the amounts of extract added.

Phosphoglycerate mutase (EC. 2.7.5.3) activity was measured by continuous and discontinuous assays in either direction as described by Hill & Attwood (1976), except that the magnesium ion concentration was increased to 5 mM to overcome any possible loss in available magnesium ion due to complex formation with nucleotides.

3-Phosphoglycerate kinase (EC. 2.7.2.3) activity was measured as described by Roustan *et al.* (1973).

D-Glyceraldehyde-3-phosphate dehydrogenase (EC. 1.2.1.12) was assayed as described in the *Biochemica Catalogue* (1968).

Chemicals, purified enzymes, nucleotides and coenzymes were obtained from Boehringer.

RESULTS AND DISCUSSION

Cell-free extracts of *Hyphomicrobium* x and *Pseudomonas* AM1 were assayed for phosphoglycerate mutase activity with and without added nucleotides using the continuous spectrophotometric assay in the direction 3-phosphoglycerate to 2-phosphoglycerate. Added ATP apparently stimulated activity in both extracts. This stimulation was antagonized by AMP

and ADP. When the purified enzyme preparations (72-fold purification for *Hyphomicrobium* x and 140-fold purification for *Pseudomonas* AM1) were used instead of the crude extracts, the nucleotides did not affect the phosphoglycerate mutase activity. These observations were confirmed using the continuous assay in the direction 2-phosphoglycerate to 3-phosphoglycerate and the discontinuous assay procedure in both directions. They suggested that either the observations using crude cell-free extracts were an artefact or that the purification procedure had affected the enzyme structure so that it was no longer sensitive to the addition of nucleotides. Therefore partially purified enzyme preparations from *Hyphomicrobium* x (37.5-fold) and from *Pseudomonas* AM1 (9.7-fold), prepared by Sephadex G-200 filtration, were assayed for phosphoglycerate mutase activity. Added nucleotides had no effect on the activity of the enzyme. Since the purification step used was a mild treatment it appeared that the initial stimulatory effect was an artefact due to the use of crude extracts.

The elution pattern obtained from the Sephadex G-200 column had four protein peaks one of which showed phosphoglycerate mutase activity. When an equal volume of one of the other three peaks was added to that containing the phosphoglycerate mutase activity, the activity of the mixture measured in the direction 3-phosphoglycerate to 2-phosphoglycerate was stimulated by ATP (Table 1). This stimulation was antagonized by AMP or ADP. The peak causing the effect (peak 2) was named the modifier peak. Thus the partial purification had separated the stimulatory element from the phosphoglycerate mutase activity. When a sample from the modifier peak was added, in the presence of ATP, to the purified phosphoglycerate mutase preparation only a slight stimulation in activity was recorded.

The apparent stimulation in enzyme activity on the addition of ATP had been detected by continuous spectrophotometric assays in the direction 3-phosphoglyceric acid (3PGA) to 2-phosphoglyceric acid (2PGA). In this assay the 2-phosphoglyceric acid produced as a result of phosphoglycerate mutase activity is initially converted into phosphoenolpyruvate by phosphoenolhydratase and then to pyruvate and lactate through the action of pyruvate kinase and lactate dehydrogenase. Lactate dehydrogenase converts the pyruvate to lactate concomitantly with the oxidation of NADH. This oxidation is followed and used to calculate the rate of phosphoglycerate mutase activity.

Thus any enzyme(s) which utilizes 3-phosphoglyceric acid as the initial substrate and brings about the oxidation of NADH in the presence of ATP could be the cause of an apparent stimulation of phosphoglycerate mutase activity by ATP. The presence of 3-phosphoglycerate kinase and glyceraldehyde-3-phosphate dehydrogenase in the crude cell-free extracts and in the modifier peak and/or the partially purified mutase preparation would be compatible with the experimental observations and this suggestion.

Crude cell-free extracts of *Hyphomicrobium* x contained both 3-phosphoglycerate kinase [sp. act. $0.24 \mu\text{mol min}^{-1} (\text{mg protein})^{-1}$] and glyceraldehyde-3-phosphate dehydrogenase

[sp. act. $0.05 \mu\text{mol min}^{-1} (\text{mg protein})^{-1}$]; the partially purified enzyme preparation contained 3-phosphoglycerate kinase [sp. act. $46.30 \mu\text{mol min}^{-1} (\text{mg protein})^{-1}$] and glyceraldehyde-3-phosphate dehydrogenase [sp. act. $0.03 \mu\text{mol min}^{-1} (\text{mg protein})^{-1}$]. The modifier peak contained glyceraldehyde-3-phosphate dehydrogenase [sp. act. $0.14 \mu\text{mol min}^{-1} (\text{mg protein})^{-1}$] and 3-phosphoglycerate kinase [sp. act. $0.07 \mu\text{mol min}^{-1} (\text{mg protein})^{-1}$]. The more highly purified preparation of phosphoglycerate mutase contained neither enzyme activity. Similar results were obtained with *Pseudomonas* AM1. Therefore in crude extracts with added ATP, the oxidation of NADH measured in the spectrophotometric assay not only reflected the activity of the phosphoglycerate mutase but also the rate of conversion of 3-phosphoglycerate to 1,3-diphosphoglycerate and hence to glyceraldehyde 3-phosphate. Without added ATP the oxidation of NADH measured was a result of mutase activity only. Sephadex G-200 gel filtration separated the two enzymes required for the overall conversion of 3-phosphoglycerate to glyceraldehyde 3-phosphate into different protein peaks and the resulting partially purified mutase preparation showed no apparent stimulation by ATP until a sample of the modifier peak was added. The more highly purified mutase preparation showed only slight stimulation in the presence of ATP on addition of the modifier peak. This slight increase in activity reflects the fact that the modifier peak contained both glyceraldehyde-3-phosphate dehydrogenase and trace amounts of 3-phosphoglycerate kinase activity, the latter limiting the amount of stimulation detected. Thus the evidence which led to the suggestion that phosphoglycerate mutase in *Hyphomicrobium* x grown on reduced one-carbon compounds may be under adenylate charge regulation was due to interference in the assay procedure by other enzymes.

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