Some Comparative Studies of Phosphoglycerate Kinase from Spinach, Wheat and Yeast *

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Phosphoglycerate kinase (ATP: 3-phospho-D-glycerate 1-phosphotransferase, EC 2.7.2.3) catalyzes the energy transforming reaction of glycolysis, gluconeogenesis and photosynthesis

In vitro experiments ¹ for the yeast enzyme show that Me^{2+} stands for Mg^{2+} , Ca^{2+} , Mn^{2+} , Co^{2+} , Zn^{2+} or Cd^{2+} and that the function of Me^{2+} in the catalytic reaction is to form complex with the nucleotide substrate. The two substrates bind independently to the enzyme.² Anions³ act as activators of the reaction at low concentrations and inhibitors at higher concentrations. The effects are most pronounced with SO_4^{2-} .^{3,4} Phosphoglycerate kinase has been isolated from a variety of sources. Until now most studies have been performed on the yeast enzyme, however. Very little is known about the enzyme from plants, disregarding some miscellaneous molecular and kinetic properties.^{5,6} For the yeast enzyme it is evident^{2,7} that multiple binding sites exist for both the substrates. ADP^8 and $1,3\text{-P}_2\text{-glycerate}^9$ appear preferentially to bind to sites outside the binding sites for the corresponding substrates MgATP^{2-} and 3-P-glycerate (cf. also ref. 10).

Experimental. Spinach phosphoglycerate kinase was purified from fresh leaves (cultivar Dominant) by affinity chromatography on ATP-ribosyl-adipoyl-dihydrazo-Sepharose according to Kuntz *et al.*¹¹ The same method was also used

for the enzyme from fresh wheat leaves (cultivar Swedish Amy). The yeast enzyme was prepared as described earlier.¹² The kinetic experiments were performed as described before^{2,8} in 50 mM Tris-HCl (pH 7.8, 25 °C) containing 0.10 M NaCl. The total Mg^{2+} and ATP concentrations were 1 mM if not stated otherwise.

Results. The spinach enzyme was eluted from the ATP-ribosyl-dihydrazo-Sepharose column with MgATP after unspecifically bound proteins had been removed with 0.7 M NaCl as described by Kuntz *et al.*¹¹ The wheat enzyme, and the earlier purified (cf. ref. 12) yeast enzyme (form 2), were eluted already with 0.3–0.7 M NaCl. For wheat a second peak appeared after elution with MgATP .

Kinetic studies of MgATP^{2-} and 3-P-glycerate at various fixed concentrations of 3-P-glycerate and MgATP^{2-} , respectively, show for the spinach enzyme that K_m for one of the substrates is independent of the concentration of the second substrate. The K_m value for MgATP^{2-} and 3-P-glycerate was 0.24 mM and 0.63 mM, respectively (mean of five experiments in each case). Similar results were earlier² obtained for the yeast enzyme. After the spinach enzyme had been kept frozen for some months K_m for 3-P-glycerate had increased to 1.1 mM (mean of four experiments). Attempts to make the transformation of this K_m value reversible were not successful. Kuntz and Krietsch reported⁵ that K_m for 3-P-glycerate is 1.1 mM, a value close to that obtained for the yeast enzyme under certain conditions (cf. ref. 2).

Inhibition of the spinach enzyme by ADP was linearly noncompetitive with either substrate, MgATP^{2-} or 3-P-glycerate . A competitive contribution was found in both cases. The results with both the spinach and the yeast⁸ enzyme give a strong indication for an ADP binding site outside the binding site for the nucleotide substrate. The kinetics appeared to fit to eqn. (1). The symbols used represent: A, MgATP^{2-} ; B, 3-P-glycerate ; I, ADP; K_A and K_B , the respective Michaelis constants; K_i , K'_i and K''_i the dissociation constant for the complex between I and the enzyme, the enzyme- MgATP and the enzyme-(3-P-glycerate) complex, respectively; v , the initial velocity; V , v at saturating conditions of both the substrates. K_m (3-P-glycerate) was 0.63 mM (cf. above) K_i was estimated to 0.1 mM, K'_i to 0.2 mM and K''_i to 0.3 mM.

Inhibition of the spinach enzyme by AMP was

$$v = \frac{V}{1 + \left(1 + \frac{[I]}{K'_i}\right) \frac{K_A}{[A]} + \left(1 + \frac{[I]}{K'_i}\right) \frac{K_B}{[B]} + \left(1 + \frac{[I]}{K_i}\right) \frac{K_A \cdot K_B}{[A][B]}} \quad (1)$$

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Fig. 1. The activity of spinach phosphoglycerate kinase with variable ATP-metal ion concentrations. MeATP stands for MgATP^{2-} ($\diamond$), MnATP^{2-} (∇), CoATP^{2-} ($\triangle$), CaATP^{2-} ($\circ$) and ZnATP^{2-} ($\square$). For the complex constants used see ref. 1. The total metal ion and ATP concentrations were 1:1; the 3-P-glycerate was 2 mM. The deviations from hyperbolic kinetics are probably due to the increasing free metal ion concentrations (see ref. 1).

linearly competitive with MgATP^{2-} and linearly noncompetitive (a competitive contribution occurred) with 3-P-glycerate up to AMP concentrations of 3 mM. The kinetics appeared to fit into eqn. (2):

$$v = \frac{V}{1 + \left(1 + \frac{[I]}{K_i'}\right) \frac{K_A}{[A]} + \frac{K_B}{[B]} + \left(1 + \frac{[I]}{K_i}\right) \frac{K_A K_B}{[A][B]}} \quad (2)$$

the symbols as for Eqn. 1 but I stands for AMP and K_i and K_i' for the dissociation constant for the complex between I and the enzyme and the enzyme-(3-P-glycerate) complex, respectively.

K_M (3-P-glycerate) was 1.1 mM (cf. above) K_i was estimated to 0.2 mM and K_i' to 0.4 mM. Very similar results were earlier⁸ obtained for the yeast enzyme. From the K_i values it is evident that the spinach enzyme has a higher affinity for AMP than the yeast enzyme has.

The metal ion specificity of the spinach enzyme was tested. The ATP and metal ion concentrations (molar ratio 1/1) were varied in separate experiments with Mg^{2+} , Ca^{2+} , Mn^{2+} , Co^{2+} and Zn^{2+} , respectively. The rate profiles, Fig. 1, appeared very close to those of the yeast enzyme (Fig. 1 in ref. 1). The most significant differences were observed for CaATP^{2-} . Compared to the yeast enzyme and MgATP^{2-} , CaATP^{2-} is only

Fig. 2. The effect of SO_4^{2-} on the activity of phosphoglycerate kinase from yeast ($\triangle$), spinach ($\circ$) and wheat ($\square$), (the wheat enzyme eluted with 0.3–0.7 M NaCl). The total ATP concentration was 1 mM, Mg^{2+} and 3-P-glycerate 2 mM.

about half as active as a substrate for the spinach enzyme. The K_m values are within 0.1 and 0.4 mM, increasing in the following order

similar to the yeast enzyme.

The SO_4^{2-} effects on the enzymes were very different (Fig. 2): For the yeast enzyme activation occurred at low and inhibition at high SO_4^{2-} concentrations; for the spinach enzyme SO_4^{2-} inhibition was quite obvious, possible activation can be suspected only; and for the wheat enzyme, eluted from the affinity column with NaCl, only activation showed up. If the SO_4^{2-} activation is related to the fact that the enzyme was eluted from the column by fairly low NaCl concentrations is still an open question. The wheat enzyme fraction eluted with MgATP (see above) showed a SO_4^{2-} rate profile (not shown in Fig. 2) very similar to the yeast enzyme, even if the activation was only one fifth of the value obtained with the yeast enzyme.

Conclusions. Phosphoglycerate kinase from various sources appear to have many molecular and kinetic properties in common. Some differences between the spinach, wheat and yeast enzymes have been observed, however: 1. in behaviour on an affinity column labelled with a substrate homologue, note that two forms of the wheat enzyme were obtained (*cf.* Refs. 12 and 13); 2. in sensitivity to sulfate ion; 3. in the affinity for AMP and 4. in the relative efficiency of the Ca^{2+} activation.

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6. Cavell, S. and Scopes, R. K. *Eur. J. Biochem.* 63 (1976) 483.
7. Larsson-Raźnikiewicz, M. *Arch. Biochem. Biophys.* 158 (1973) 754.
8. Larsson-Raźnikiewicz, M. and Arvidsson, L. *Eur. J. Biochem.* 22 (1971) 506.
9. Schierbeck, B. and Larsson-Raźnikiewicz, M. *Biochim. Biophys. Acta* 568 (1979) 195.
10. Wrobel, J. A. and Stinson, R. A. *Eur. J. Biochem.* 85 (1978) 345.
11. Kuntz, G. W. K., Eber, S., Kessler, W., Krietsch, H. and Krietsch, W. K. G. *Eur. J. Biochem.* 85 (1978) 493.
12. Arvidsson, L., Schierbeck, B. and Larsson-Raźnikiewicz, M. *Acta Chem. Scand. B* 30 (1976) 228.
13. Pacold, I. and Anderson, L. E. *Plant Physiol.* 55 (1975) 168.

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1. Larsson-Raźnikiewicz, M. *Eur. J. Biochem.* 17 (1970) 183.
2. Larsson-Raźnikiewicz, M. *Biochim. Biophys. Acta* 132 (1967) 33.
3. Larsson-Raźnikiewicz, M. and Jansson, J. R. *FEBS Letters* 29 (1973) 345.
4. Khamis, M. M. and Larsson-Raźnikiewicz, M. *Biochim. Biophys. Acta* 657 (1981) 190.
5. Kuntz, G. W. K. and Krietsch, W. K. G. In Wood, W. A., Ed., *Methods in Enzymology*, Academic, New York, 1982, Vol 90, Chapter 19, p. 110.