MEDIATION OF THE ALLOSTERIC RESPONSE OF 3-PHOSPHOGLYCERATE DEHYDROGENASE FROM PEAS

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1. Introduction

Earlier studies with plant extracts have shown that the first enzyme in the biosynthetic route to serine from 3-phosphoglycerate, 3-phosphoglycerate dehydrogenase, can be inhibited by low concentrations of its eventual end-product, L-serine [1,2]. In addition, the enzyme from peas can be activated by a range of amino acids including L-methionine [3,4]. However, desensitization of the pea enzyme to serine and methionine occurs on storage or on attempts at purification and this has greatly hindered the elucidation of the detailed mechanisms by which amino acids can alter the catalytic activity of 3-phosphoglycerate dehydrogenase. The results of experiments described in this paper suggest that these mechanisms are more complicated than was originally thought in that the enzyme from etiolated pea epicotyls appears to be normally associated with a reversibly bound compound, probably a nucleotide related to AMP, which sensitises the enzyme to allosteric effectors without itself having any effect on the catalytic activity of the enzyme.

2. Materials and methods

The method of preparation of extracts from etiolated epicotyls of *Pisum sativum* var. *Meteor,* assay of 3-phosphoglycerate dehydrogenase and determination of the effect of L-serine on this enzyme has been described previously [5,6]. The effect of methionine was determined by setting up a standard assay containing NADH, enzyme and 20 mM L-methionine in which the Na succinate buffer was replaced by K phosphate buffer, pH 6.5. The normal 1 min incubation period at 25°C was extended to 5 min before addition of K phosphohydroxy-pyruvate to start the reaction. A control assay containing no methionine was performed at the same time.

Dry ashing was carried out by carefully drying 1.0 ml of crude pea extract in an open crucible over a bunsen burner and then heating until all organic matter had been burnt off. The ash was then dissolved in 0.6 ml of K phosphate buffer (0.1 M, pH 6.5), the pH adjusted to 6.5 with HCl and the volume made up to 1.0 ml with the K phosphate buffer.

An ultrafiltrate was prepared by placing 1.0 ml of crude extract in an Amicon Ultrafiltration Model 12 fitted with a Diaflo ultrafiltration membrane UM 2 and operating at 25 lb/in^2 at room temperature until approx. 0.8 ml of ultrafiltrate had collected.

L-serine, L-methionine and all the compounds listed in table 1 were obtained from the Sigma Chemical Co., London.

3. Results and discussion

3.1. *Accelerated loss of allosteric sensitivity*

A crude extract of etiolated pea epicotyls was prepared and 0.5 ml dialysed in an open sac against 50 ml of K phosphate buffer (0.1 M pH 6.5) for 30 min at room temperature. The response of the 3-phosphoglycerate dehydrogenase activity in the extract to serine and methionine was followed and the results are shown in fig.1. The loss in allosteric sensitivity during the experiment was not accom-

Table 1
Restoration of allosteric sensitivity by defined compounds

Compound	Conc. in enzyme solution (mM)	% Activation by L-methionine	% Inhibition by L-serine
AMP	10	77	30
AMP	0.5	45	13
ADP	10	24	12
GMP	10	13	0
Adenosine	7	32	8
c-AMP	10	28	12
Kinetin	0.5	17	10
Kinetin-riboside	0.5	9	11

Solutions of the compounds given in the table were prepared at twice the stated concentration and then mixed with an equal volume of dialysed pea extract prepared as described in the text. After 60 min incubation at 25°C the effect of 20 mM L-methionine and 1.0 mM L-serine on the activity of 3-phosphoglycerate dehydrogenase in the diluted extracts was determined. Under these conditions the response of dialysed extract diluted 1:1 with K phosphate buffer (0.10 M, pH 6.5) was on average 20% activation by methionine and 6% inhibition by serine.

panied by any change in catalytic activity and the results suggest that a compound with relatively low molecular weight is required before 3-phosphoglycerate dehydrogenase from peas can show an allosteric response to amino acids.

3.2. *Restoration of allosteric sensitivity*

An aliquot of dialysed pea extract prepared as described above was incubated at 25°C with an equal volume of a crude pea extract which had been held at 100°C for 1 min and then clarified by centrifugation. The response of the enzyme mixture to both serine and methionine was determined at

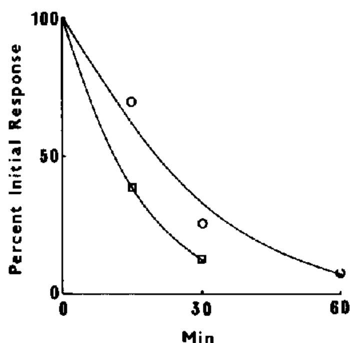

Fig.1. Loss of allosteric sensitivity on dialysis. A crude extract of pea epicotyls was dialysed as described in the text for 1 h and the effect of 1.0 mM L-serine (□) and 20 mM L-methionine (○) on the 3-phosphoglycerate dehydrogenase activity of the extract was determined at intervals.

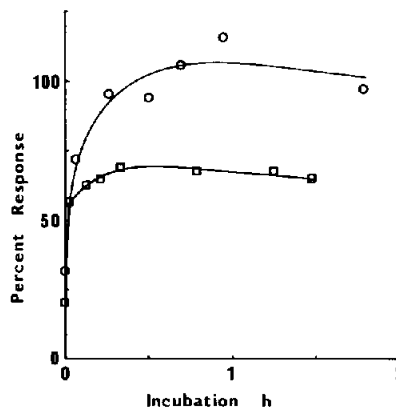

Fig.2. Restoration of allosteric sensitivity. A dialysed extract of pea epicotyls was mixed with an equal volume of heat treated extract as described in the text and the effect of 1.0 mM L-serine (□) and 20 mM L-methionine (○) on the 3-phosphoglycerate dehydrogenase activity of the mixture determined at intervals.

intervals and the restoration of allosteric sensitivity is shown in fig.2. Similar results were obtained if the heated extract was replaced by an ultrafiltrate of crude pea extract prepared using a 1000 molecular weight cut-off membrane. These results support the conclusion of the previous section that a low mol. wt. compound is necessary before 3-phosphoglycerate dehydrogenase can show an allosteric response. Furthermore, as the restoration of allosteric sensitivity takes a period of several minutes it appears that the mediating compound binds reversibly to the enzyme rather than forming a complex with the allosteric effectors.

3.3. *Nature of the allosteric mediator*

A similar response to that shown in fig.2 was obtained if the heated pea extract was replaced by a hot water extract of yeast so indicating that the mediating compound is not unique to peas. A variety of compounds were tested for their effectiveness in restoring allosteric sensitivity to a dialysed extract (table 1) and only AMP gave a positive response. A concentration of several mM was necessary to restore allosteric sensitivity to the same extent as that achieved by using an ultrafiltrate of pea extract whose total nucleotide content based on E_{260} calculated as AMP was less than 4 μM. This appears to rule out AMP as the natural mediator but the fact that it can restore allosteric sensitivity together with the findings that heated pea extract was rendered ineffective by dry ashing or by stirring with Dowex 50 H^+ resin at low pH but not by boiling for 10 min are consistent with the natural compound being a simple nucleotide closely related to AMP.

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References

[1] Slaughter, J. C. and Davies, D. D. (1968) Biochem. J. 109, 749–755.
[2] Rosenblum, I. Y. and Sallach, H. J. (1970) Arch. Biochem. Biophys. 137, 91–101.
[3] Slaughter, J. C. (1970) FEBS Lett. 7, 245–247.
[4] Slaughter, J. C. (1975) Phytochemistry 14, 1711–1713.
[5] Slaughter, J. C. and Davies, D. D. (1968) Biochem. J. 109, 743–748.
[6] Slaughter, J. C. (1973) Biochem. J. 135, 563–565.