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INHIBITION OF 3-PHOSPHOGLYCERATE DEHYDROGENASE BY COMPOUNDS OTHER THAN L-SERINE

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Key Word Index—*Pisum sativum*; Leguminosae; pea; enzymology; 3-phosphoglycerate dehydrogenase; biosynthesis; control; serine; purine nucleotides.

Abstract—3-Phosphoglycerate dehydrogenase from etiolated pea epicotyls was not affected during *in vitro* assay by a range of hexose phosphates, amino group precursors and nucleotides at 1 mM but was significantly inhibited by 1 mM ATP and GTP. ADP and GDP gave slight inhibition at this concentration. NADH caused almost total inhibition at 0·45 mM.

A GREAT deal of evidence has accumulated to suggest that the control of amino acid synthesis is effected by the amino acids themselves through negative feed-back loops.[1] The first enzyme of the biosynthetic sequence unique to an amino acid has usually emerged as the most likely control point.[1] In agreement with this general rule in both bacteria[2] and plants[3–5] the enzyme sensitive to feed-back inhibition by serine appears to be 3-phosphoglycerate dehydrogenase. The plant enzyme seems specific for L-serine as inhibitor[3] but, as some recent reports have indicated that in certain organisms sugar phosphates and nucleotides can also control the activity of amino acid biosynthetic enzymes,[6–9] the effect of a wide range of compounds associated with the overall biosynthesis of serine has been tested on the *in vitro* activity of 3-phosphoglycerate dehydrogenase extracted from etiolated pea epicotyls.

When included at 1 mM in the assay, 2-oxoglutarate, glutamic acid, ammonium nitrate, glucose-1-phosphate, glucose-6-phosphate, fructose-6-phosphate, fructose-1-6-diphosphate, CTP, UTP, AMP and GMP were all without noticeable effect on the initial rate of reaction. ATP, GTP, ADP and GDP at 1 mM produced respectively 41% (10), 30% (5), 14% (5) and 10% (3) inhibition. The percentages are average inhibitions based on the number of determinations shown in parentheses.

Mixtures of inhibitors were tested for their effect on initial velocity relative to the effect of each compound individually. The results are shown in Table 1. If straightforward addition of inhibiting effect occurs then a mixture of 1 mM ATP and 1 mM GTP should cause 56% inhibition whereas a mixture of 1 mM ATP and 1 mM L-serine would be expected to give 73% inhibition. Comparison of these figures with those actually obtained (Table 1) indicate

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that the inhibitory effects of ATP and GTP are less than additive. Because of the structural similarity between ATP and GTP it seems likely that both these compounds act at the same site on the enzyme. The inhibitory effects of ATP and serine, however, are additive and so, presumably, mechanistically separate. This conclusion is supported by the marked variation in the persistence of the inhibitory effect of the two compounds when the enzyme extract was aged at 4–5° overnight. When assayed 1 hr from extraction, 0·5 mM L-serine produced 52% inhibition and 1mM ATP produced 41% inhibition. 24 hr after extraction these figures had changed to 1 and 40% respectively. During the storage period the enzyme lost 66% of its initial activity.

TABLE 1. EFFECTS OF MIXTURES OF INHIBITORS ON INITIAL VELOCITY OF 3-PHOSPHOGLYCERATE DEHYDROGENASE

Inhibitor	Inhibition (%)	Inhibitor	Inhibition (%)
ATP	37	ATP	44
GTP	29	L-Serine	57
ATP + GTP	45	ATP + L-serine	74

All inhibitors were incorporated in the assay at 1 mM. The data are based on averages of duplicate assays. A separate enzyme solution was used for each experiment.

In the standard assay for 3-phosphoglycerate dehydrogenase, the physiological products of the reaction, NADH and phosphohydroxypyruvate, were used as the substrates. The effect of varying the concentration of one of these compounds over a wide range while maintaining the other at the standard concentration was investigated and the results are shown in Fig. 1. Both compounds cause substrate inhibition but the effect of high levels of NADH is much more marked than that of high levels of phosphohydroxypyruvate.

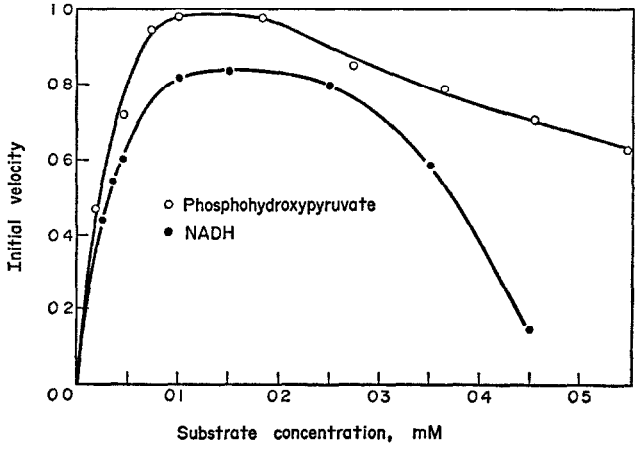

FIG 1. THE EFFECT OF VARIATION OF NADH OR PHOSPHOHYDROXYPYRUVATE CONCENTRATION ON THE INITIAL VELOCITY OF 3-PHOSPHOGLYCERATE DEHYDROGENASE.
In experiments where the NADH concentration was varied the phosphohydroxypyruvate concentration was 0·18 mM. When the concentration of phosphohydroxypyruvate was varied, NADH was used at 0·20 mM. Otherwise assay conditions were as stated in the text. Initial reaction velocity is given as μmol NADH oxidized/min/ml of enzyme.

Under the conditions of the experiments described 3-phosphoglycerate dehydrogenase from pea epicotyls is insensitive to a range of sugar phosphates which are precursors of serine during biosynthesis from glucose and also to a number of compounds involved in the transfer of the amino group into the amino acid. However, as well as showing substrate inhibition by NADH and phosphohydroxypyruvate the enzyme is inhibited by the purine triphospho- and diphospho-nucleotides. The present experiments do not give detailed information on the mechanism or the type of binding site involved in the nucleotide inhibition but do indicate that the nucleotides and serine act as separate and additive inhibitors. It is therefore possible that under *in vivo* conditions the activity of 3-phosphoglycerate dehydrogenase, and so the synthesis of serine, could be partially controlled by the concentration of ATP, ADP, GTP and GDP as well as by that of serine.

In the cells studied the biosynthetic route to serine seems likely to lead from hexose derived from the cotyledons via either Embden–Meyerhoff pathway or pentose phosphate pathway degradation to 3-phosphoglyceraldehyde and from this point via a common pathway involving 3-phosphoglycerate, phosphohydroxypyruvate and phosphoserine.[10] Whichever actual route is considered, the ATP consumed per mol of serine produced is balanced by the production of ATP by substrate level phosphorylation. However, in the case where triose is produced through the action of the Embden–Meyerhoff sequence then the production of 1 mol of serine gives rise to 2 mol of NADH if an amino group donor is available, or to 1 mol of NADH if ammonia is incorporated through the action of NADH-dependent glutamate dehydrogenase. When triose is produced via the pentose phosphate pathway then 6 mol NADPH and 1 or 2 mol NADH can be produced again depending on the source of the amino group. Through the operation of exchange reactions between NAD and NADP[11] and between cytoplasm and mitochondria,[12,13] regeneration of the reduced nucleotides would be expected to go along with a certain production of ATP under aerobic conditions. Thus from the point of view of biochemical energetics the biosynthesis of serine from glucose is a catabolic rather than an anabolic process and so some control over serine biosynthesis by such compounds as NADH, ATP and GTP does not appear unreasonable.

EXPERIMENTAL

Epicotyls of *Pisum sativum* (var. Meteor) were extracted after 8–10 days growth in the dark using a pestle and mortar with potassium phosphate buffer pH 6·5, 0·1 M. The extract was centrifuged at 1000 *g* for 10 min and the supernatant solution used as the enzyme preparation in all the experiments described. Operations and enzyme storage were all carried out at 0–4°. 3-Phosphoglycerate dehydrogenase was assayed as described previously[3] but using 0·01 ml enzyme solution and the total assay volume was 1·0 ml.

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