THE ACTIVATION OF 3-PHOSPHOGLYCERATE DEHYDROGENASE BY L-METHIONINE

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1. Introduction

Feedback inhibition within the metabolic pathways leading to amino acid biosynthesis is well known in a variety of organisms including higher plants [1, 2]. Most of the information refers to the effects of amino acids on their own biosynthesis but Jensen [3] has recently pointed out that a regulatory superstructure connecting various metabolic pathways may well exist. Interactions at this level seem more likely to be stimulatory rather than inhibitory.

This paper describes experiments which show that 3-phosphoglycerate dehydrogenase, the first enzyme of serine biosynthesis in peas [4, 5] is specifically and markedly activated by L-methionine.

2. Methods and materials

Seeds of *Pisum sativum* (var. Meteor) were obtained locally and grown as described previously [6]. Epicotyls were cropped after 8–10 days of growth and extracted in a pestle and mortar with potassium phosphate buffer pH 6.5, 0.1 M. The homogenate was strained through fine nylon mesh and then centrifuged at 15,000 *g* for 30 min. The freshly prepared supernatant was used as the enzyme extract in all the experiments described below. Operations were all carried out at room temperature.

Enzyme activity was measured as described previously [6]. A fixed order of addition of reactants to the cuvette was followed: buffer, NADH, serine (as appropriate), enzyme, methionine (as appropriate) and phosphohydroxypyruvate potassium salt. The cell contents were mixed after addition of the enzyme, methionine and phosphohydroxypyruvate. Unless otherwise stated, phosphohydroxypyruvate was added 1 min after the last of the other reagents.

Phosphohydroxypyruvate was prepared as described previously [6]. NADH, L-methionine, L-homoserine and L-threonine were obtained from Sigma, London. Hydroxypyruvate lithium salt was obtained from K and K Laboratories, California and all other reagents were supplied by B.D.H., Poole, England.

3. Results

3.1. *Effects of L-methionine concentration*

Assays were carried out incorporating L-methionine up to 15 mM with a standard pre-incubation time of 1 min after addition of L-methionine and before starting the reaction by addition of phosphohydroxypyruvate. The appropriate blanks indicated that L-methionine did not lead to a decrease in E_{340} in the absence of phosphohydroxypyruvate and the presence of L-methionine in the assays did not cause a deviation from initial zero order kinetics. The relationship obtained between L-methionine concentration and enzyme activity is shown in fig. 1.

3.2. *Effect of variation of pre-incubation time*

A series of assays containing 5.0 mM L-methionine was carried out with variation of the pre-incubation time from 0.5 to 8 min. The results are shown in fig. 2.

3.3. *Effect of related amino acids*

Several amino acids chemically and biochemically

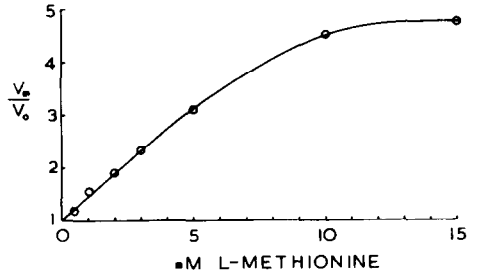

Fig. 1. The effect of L-methionine on 3-phosphoglycerate dehydrogenase activity measured as ΔE_{340}/min/assay. For conditions see text. V_m is the activity in the presence of L-methionine and V_0 is the activity in its absence.

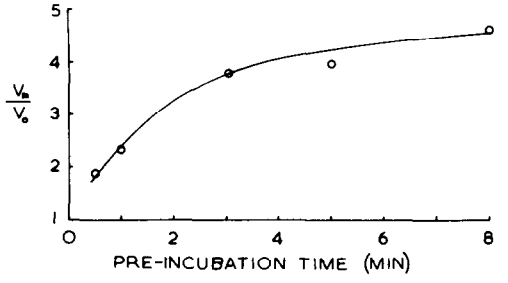

Fig. 2. The effect of length of pre-incubation time with 5.0 mM L-methionine on the activity of 3-phosphoglycerate dehydrogenase. See fig. 1 for an explanation of the symbols. For conditions see text.

related to L-methionine were tested for their effect on 3-phosphoglycerate dehydrogenase. The results are shown in table 1.

Activation of the enzyme appeared to be restricted to L-methionine as 10.0 mM DL-methionine exerted the same effect as 5.0 mM L-methionine and 10.0 mM DL-ethionine produced very little increase in activity. The members of the aspartate family other than L-methionine were without effect at 5.0 mM.

3.4. *Action of L-methionine on glycerate dehydrogenase*

Glycerate dehydrogenase was assayed in the same plant extract under the same conditions as was 3-phosphoglycerate dehydrogenase with the substitution of 5 μmoles of hydroxypyruvate lithium salt for phosphohydroxypyruvate in each assay. Under these standard conditions 5.0 mM L-methionine was without effect on the rate of reduction of hydroxypyruvic acid.

3.5. *Interaction of L-methionine and L-serine*

3-Phosphoglycerate dehydrogenase is known to be inhibited by low concentrations of L-serine [7] and a series of experiments was carried out to determine the relationship between the effect of L-serine and that of L-methionine. Assays were carried out over a range of L-serine concentrations but employing a fixed L-methionine level of 5.0 mM.

In no experiment was any evidence obtained to suggest that the percentage inhibition produced by L-serine

Table 1

The effect of selected amino acids on the activity of 3-phosphoglycerate dehydrogenase. Assay conditions were as described in the text and activity was measured as ΔE_{340}/min/assay.

Amino acid	Concn (mM)	Enzyme activity	Activity ratio
None	–	0.050	1.00
L-Threonine	5.0	0.047	0.94
L-Isoleucine	5.0	0.051	1.02
L-Lysine	5.0	0.050	1.00
L-Homoserine	5.0	0.051	1.02
L-Methionine	5.0	0.130	2.60
DL-Methionine	5.0	0.062	1.24
DL-Methionine	10.0	0.127	2.54
DL-Methionine	10.0	0.060	1.20

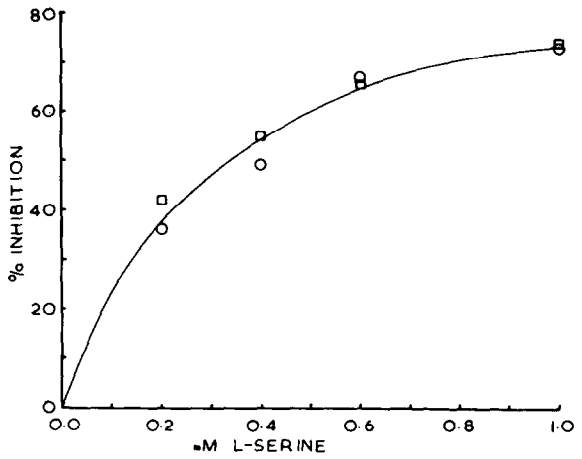

Fig. 3. The inhibitory effect of L-serine on 3-phosphoglycerate dehydrogenase alone (○) and on the enzyme after pre-incubation for 1 min with 5.0 mM L-methionine (□). For conditions see text.

was different in the case of the activated enzyme as compared to that of the untreated enzyme. The average values of data obtained from experiments on four separate extracts are shown in fig. 3 and this graph supports the conclusion that the effects of L-serine and L-methionine on 3-phosphoglycerate dehydrogenase are independent. Jensen [3] observed this same independence of action with prephenate dehydratase, an enzyme which is activated by L-methionine and inhibited by L-phenylalanine.

4. Discussion

The evidence summarised in figs. 1 and 2 and in table 1 indicates that L-methionine acts as a specific activator of 3-phosphoglycerate dehydrogenase from peas. The amino acid failed, however, to stimulate the closely related enzyme, glycerate dehydrogenase, so demonstrating that a general effect of L-methionine on dehydrogenases was not being observed.

Some similarities exist between the present case and that of prephenate dehydratase [3]. Both enzymes are relatively more sensitive to the inhibitor than to the activator (L-methionine in both cases) although in higher concentration ranges L-methionine produces marked activation resulting in up to four to five times the activity observed in its absence. Again in both cases, the percentage inhibition produced by the inhibitor appears to be independent of the presence of L-methionine. Thus, as stated by Jensen [3] for prephenate dehydratase, the quantitative significance of L-methionine activation of 3-phosphoglycerate dehydrogenase will be greater at low levels of L-serine.

The significance of this activation, *in vivo*, is unknown and at first sight appears to run against the normal principles of feed-back control. Serine is probably involved in the biosynthesis of methionine [8] so the most likely effect of methionine would be to act as an inhibitor of the enzymes of serine biosynthesis. However, it may be that the activation effect is part of a secondary control system, as suggested by Jensen [3] whose main function is to correct any inbalances in the relative concentrations of the amino acids.

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