Phytochemistry, 1975, Vol. 14, pp. 1711–1713. Pergamon Press. Printed in England.

AMINO ACID ACTIVATION OF 3-PHOSPHOGLYCERATE DEHYDROGENASE FROM PEAS

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(*Received* 16 *January* 1975)

Key Word Index—*Pisum sativum*; Leguminosae; pea; 3-phosphoglycerate dehydrogenase; allosteric activation; methionine.

Abstract—When L-methionine was added to an extract of etiolated pea epicotyls activation of 3-phosphoglycerate dehydrogenase occurred and was complete in 20–30 min. Storage of extracts at 5° led to a loss in the ability of L-methionine to activate the enzyme and after 24 hr storage almost no activation was observed. On the basis of tests with 16 compounds the ability to activate 3-phosphoglycerate dehydrogenase was restricted to L-amino acids with intermediate-length side chains. There appears to be no requirement for a reactive group in the side chain. Gel-filtration showed that the higher levels of 3-phosphoglycerate dehydrogenase activity obtained after treatment with L-methionine are relatively stable.

INTRODUCTION

The enzyme 3-phosphoglycerate dehydrogenase catalyses the first step unique to the biosynthesis of L-serine from glucose and the enzyme from both bacterial [1] and plant [2,3] sources has been shown to be specifically inhibited by this amino acid. In addition, the enzyme from peas appears to be activated by L-methionine [4]. The possible physiological significance of this observation is not as obvious as the negative feedback effect of L-serine but it has been postulated that it might be an aspect of positive control between metabolic pathways; an example of what Jensen [5] has called metabolic interlock.

Previous work on activation by L-methionine [4] indicated that the extent of activation observed depended not only on the concentration of L-methionine in the assay but also on the length of the incubation period before addition of the substrate to start the reaction. This dependence on length of incubation suggests that activation by L-methionine is likely to be a different type of event from inhibition by L-serine for which no time dependence has been noted using standard spectrophotometric techniques. It is possible that the difference could be one simply of speed of conformational change [6] or it may be that activation is due to a more permanent structural change in the enzyme.

RESULTS AND DISCUSSION

Effect on 3-phosphoglycerate dehydrogenase activity of addition of L-*methionine to pea extracts*

A standard extract of etiolated pea epicotyls was prepared at room temperature and then divided into two portions, one of which was made 20 mM with regard to L-methionine. Both portions were maintained at 25° and the 3-phosphoglycerate dehydrogenase activity measured at intervals over a period of 3 hr (Fig. 1). The results indicate that initially there was a time-dependent increase in enzyme activity in the sample containing the added L-methionine, but that later a decline in activity occurred at a very similar rate to that observed in the untreated sample. Assay of the untreated extract with the addition of 0·2 mM L-methionine to the assay solution did not result in activation at any point.

In a second experiment an extract was made and stored at 5°. Duplicate portions (0·5 ml) were removed initially, and 4 and 24 hr later, and one

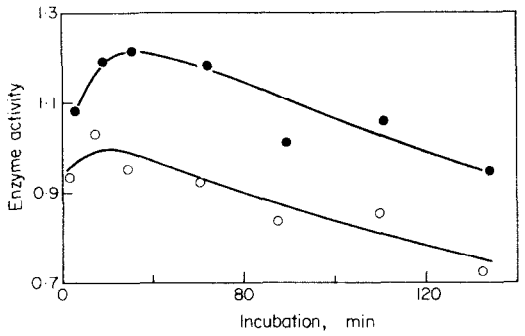

Fig. 1. Effect of L-methionine on 3-phosphoglycerate dehydrogenase activity of pea extract. Two 0·5 ml portions of pea extract were taken and one was made 20 mM with regard to L-methionine. Both samples were incubated at 25° and their 3-phosphoglycerate dehydrogenase activity assayed at intervals. No treatment (○); 20 mM L-methionine (●).

made 20 mM to L-methionine. The maximum 3-phosphoglycerate dehydrogenase activity of both samples incubated at 25° was 1·28, 1·02 and 0·45 μmol/min/min for the methionine treated sample and 0·80, 0·70 and 0·42 μmol/min/min for the untreated sample at the respective time intervals. The results indicate that the response of 3-phosphoglycerate dehydrogenase in extracts of pea epicotyls to L-methionine falls markedly over a period of 24 hr cold storage.

Effect on 3-phosphoglycerate dehydrogenase activity of addition of a number of amino acids and related compounds to pea extracts

Several compounds related to L-methionine were tested to see if they could activate 3-phosphoglycerate dehydrogenase when added to pea extracts. This was done by carrying out a number of experiments similar to that described in Fig. 1. In a typical experiment four 0·5 ml samples of pea extract were taken. One was left untreated and a second made 20 mM with regard to L-methionine. The remaining samples were normally made 20 mM to test compounds and the enzyme activity of each sample assayed at intervals over a 2–3 hr period. Of the 16 compounds tested in this way, six gave rise to activation. In order of apparent effectiveness these were: L-homocysteine; L-methionine; L-homoserine; DL-norvaline together with DL-norleucine; and to a minor extent DL-ethionine (40 mM). The increase in activity produced by both L-homocysteine and L-homoserine was not stable and the activity of the samples had normally declined to the control level between 60 and 90 min after addition of the amino acids. The compounds tested which did not cause activation were: L-cysteine, propan-1-ol, D-methionine, 4-aminobutyric acid, L-glutamine, L-glutamic acid, 3-aminopropan-1-ol, *S*-adenosylmethionine, L-leucine and L-isoleucine. The latter two compounds were tested at 10 mM.

These results suggest that the ability to activate 3-phosphoglycerate dehydrogenase is restricted to L-amino acids with straight side chains of intermediate length, and there appears to be no need for a reactive group in the side chain. These results must be regarded with some caution, however, as the use of a crude pea extract could have led to interconversions of compounds during the experiment so that inert additives could have been transformed to activating compounds and vice versa added compounds with activating potential could have been rendered inoperative. The results could also have been affected by the presence of significant concentrations of activators in the pea extract and confirmation of the present list of activating compounds must await studies with purified enzyme systems.

Stability of 3-phosphoglycerate dehydrogenase activity after activation by L-methionine

The effect of passing untreated extracts of pea epicotyls and extracts treated with L-methionine through a small column of Sephadex G-200 was examined. Two samples of a freshly prepared pea extract were incubated at 25° for 1 hr. One of the samples was then made 20 mM with respect to L-methionine and 0·4 ml of the untreated extract were subjected to gel filtration through a column of Sephadex G-200 (total volume 9·8 ml; void column 1·6 ml; height 127 mm) using a K Pi buffer (0·1 M, pH 6·5) as eluant at a flow rate of 18 ml/hr. Fractions of 1·44 ml were collected so that all the 3-phosphoglycerate dehydrogenase activity was eluted in four fractions. The fractions were assayed for enzyme activity immediately on collection using 0·05 ml per assay. One hour after beginning the gel filtration of the untreated extract a 0·4 ml sample of the L-methionine treated extract was fractionated and assayed in the same way. Three experiments of this type were carried out and the average activity of the L-methionine treated samples was 131% of that of the untreated samples. After gel-filtration,

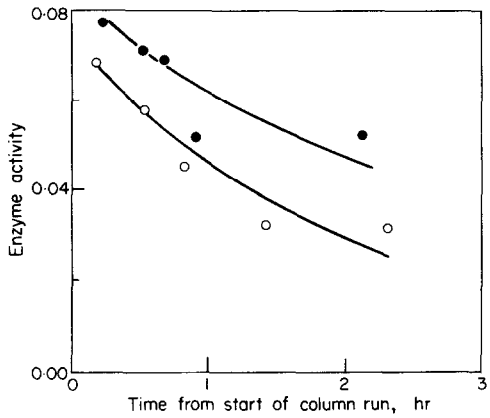

Fig. 2. Stability of 3-phosphoglycerate dehydrogenase after gel-filtration. The combined active fractions obtained after gel-filtration of both L-methionine treated (●) and untreated (○) pea extracts were incubated at 25° and the 3-phosphoglycerate dehydrogenase activity assayed at intervals using 0·05 ml of enzyme.

the average activity of the combined fractions obtained from the L-methionine treated sample was 127% of that of the fractions obtained from the untreated samples. It appears that the activated extract maintains its higher level of 3-phosphoglycerate dehydrogenase activity after gel-filtration. When the experiment was repeated with omission of the L-methionine treatment the two elution profiles obtained were indistinguishable.

After one experiment the active fractions obtained after gel filtration were assayed at intervals during a 2 hr period of storage at 25° (Fig. 2).

These results show that even 2 hr after removal of the activator by gel-filtration, the enzyme had no tendency to relax back to its former activity level. It seems, therefore, that activation of 3-phosphoglycerate dehydrogenase by L-methionine is not due to a rapid equilibrium binding between the amino acid and the enzyme as is normally envisaged to occur in allosteric modifications but that the relatively slow interaction with methionine in some way results in a more stable conformational change.

EXPERIMENTAL

The preparation of an extract of etiolated epicotyls of *Pisum sativum* (var. Meteor) and the assay of 3-phosphoglycerate dehydrogenase activity have been described previously [7]. Enzyme activity is reported as μmol NADH oxidized/min/ml of enzyme soln.

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