Inhibition of 3-Phosphoglycerate Dehydrogenase from *Pisum sativum* by Purine Nucleotides

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The inhibition of 3-phosphoglycerate dehydrogenase from etiolated pea epicotyls by purine nucleoside di- and tri-phosphates is linear, competitive with regard to NADH, and the nucleotides are mutually exclusive in their binding. Free ATP and ADP are more effective inhibitors than are the respective magnesium complexes.

3-Phosphoglycerate dehydrogenase (D-3-phosphoglycerate-NAD⁺ oxidoreductase, EC 1.1.1.–) catalyses the first divergence from the glycolytic sequence in the biosynthetic route to serine from glucose, and the enzyme from etiolated peas has been shown to be inhibited *in vitro* by low concentrations of L-serine (Slaughter & Davies, 1968b). A further investigation into other compounds associated with this route of serine biosynthesis indicated that adenosine and guanosine di- and tri-phosphates could also significantly inhibit the pea enzyme *in vitro* (Slaughter, 1973). As mixtures of serine and ATP gave additive inhibition and there was a marked difference in the retention of sensitivity towards the two inhibitors during storage, it was assumed that the mechanism involved was quite different for each inhibitor. On the basis of the percentage inhibition produced in standard assays by 1 mM concentrations the nucleotides were ranked in the following order of effectiveness: ATP, GTP, ADP and GDP. However, the mode of action of the nucleotides was not investigated.

The present paper presents the results of a kinetic investigation into the action of these purine nucleotides as inhibitors of 3-phosphoglycerate dehydrogenase obtained from etiolated pea epicotyls.

Materials and methods

Epicotyls of *Pisum sativum* (var. Meteor) were extracted after 8–10 days' growth in the dark by using a pestle and mortar with 0.1 M-potassium phosphate buffer, pH 6.5. The extract was centrifuged at 1000g for 10 min and the supernatant solution used as the enzyme preparation in all the experiments described. All operations and enzyme storage were carried out at room temperature.

3-Phosphoglycerate dehydrogenase was assayed as described previously (Slaughter & Davies, 1968a) but by using 0.01 ml of enzyme solution in a total assay volume of 1.0 ml.

ATP, ADP, AMP and GTP were obtained from Sigma (London) Chemical Co., London S.W.6, U.K., and NADH was obtained from the Boehringer Corp. (London) Ltd., London W.5, U.K. MgCl₂ was

A.R. grade from BDH Chemicals Ltd., Poole, Dorset, U.K. Potassium phosphohydroxypyruvate was prepared by the method of Ballou & Hesse (1956) from hydroxypyruvic acid phosphate dimethylketal tri-cyclohexylammonium salt obtained from Calbiochem Ltd., London W.1, U.K.

Results and discussion

The structural similarity between the inhibitory nucleotides and NADH suggested that the mode of action of the nucleotides might be competition for the NAD area of the catalytic site. The Lineweaver-Burk plot shown in Fig. 1(a) indicates that binding of ATP and ADP is mutually exclusive with binding of NADH. Fig. 1(a) also shows that no inhibition was found with 1 mM-ATP over a wide range of NADH concentrations, in confirmation of the earlier observation that it was without effect under standard assay conditions (Slaughter, 1973). No effect on reaction rate has been found when sodium pyrophosphate was included in standard assays (NADH concentration, 0.1 mM) at up to 10 mM. The effect of mixtures of ATP and GTP was investigated and the parallel lines obtained when $1/v$ was plotted against ATP concentration at various GTP concentrations (Fig. 1b) indicates that the binding of ATP and GTP is mutually exclusive (Cleland, 1970). Fig. 2 shows the effect of 1 mM-ATP and 1 mM-L-serine on 3-phosphoglycerate dehydrogenase activity in a fresh extract and in the same extract after 24 h storage at 5°C. During this period the total enzyme activity of the extract fell by 60%.

The results shown in Fig. 1 are in agreement with the hypothesis that the inhibitory nucleotides bind at the NADH area of the catalytic site and that complexes containing mixtures of NADH and nucleotides or more than one nucleotide molecule are not formed. The kinetic evidence does not rule out the possible existence of a specific site for nucleotides quite separate from the catalytic site, but the stability of the inhibitory effect of ATP during a period of overall enzyme instability (Fig. 2) suggests

Fig. 1. Effect of purine nucleotides on 3-phosphoglycerate dehydrogenase

(a) Effect of adenine nucleotides at 1 mM over a range of NADH concentrations. Other assay conditions were as described in the text. ATP, ■; ADP, □; AMP, △; no addition, ○. (b) Effect of mixtures of ATP and GTP. Assay conditions were as described in the text. No added GTP, ■; 0.25 mM-GTP, ○; 0.50 mM-GTP, △; 1.00 mM-GTP, □. Units of $1/v$ are reciprocals of enzyme activity measured as change in E_{340}/min per ml of enzyme.

Fig. 2. Effect of ATP and L-serine on fresh and aged epicotyl extracts

The inhibitory effect of ATP and L-serine was determined immediately after extraction and again after 24 h storage at 5°C . The total enzyme activity fell 60% during the storage period. ATP (fresh extract), ○; ATP (aged extract), △; L-serine (fresh extract), ■; L-serine (aged extract), □.

that action is through binding at the active site. The failure of AMP and pyrophosphate to cause inhibition indicates that the minimum structure necessary to interfere with NADH binding is a purine nucleoside

diphosphate which in the case of ADP is identical with part of the structure of the normal coenzyme, NAD.

The effect of MgCl_2 on inhibition by ATP and ADP was investigated by inclusion of MgCl_2 at 1 and 10 mM in standard assays (NADH concentration, 0.1 mM). In all cases the presence of the magnesium salt led to a loss in the inhibitory power of the adenine nucleotides. Inhibition by 1 mM-ATP fell from 62% in the absence of added MgCl_2 to 55% in the presence of 1 mM- MgCl_2 and to 29% in the presence of 10 mM- MgCl_2 . Comparable values for inhibition by 1 mM-ADP were 54, 52 and 39% respectively. MgCl_2 alone had no effect on the activity of the enzyme in standard assays. It seems, therefore, that the magnesium-nucleotide complexes are less effective inhibitors than the free nucleotides. This observation is in line with the reported effect of free and magnesium-complexed ATP and ADP with enzymes which use these nucleotides as substrates (Purich & Fromm, 1972).

It is not known whether the inhibitory effect of purine nucleotides on 3-phosphoglycerate dehydrogenase has any physiological significance in controlling the biosynthesis of serine, but, if this is the case, it appears that the concentration of magnesium *in vivo* would also be of great importance. It is therefore possible to postulate the existence of a control system which acts at the level of purine nucleotide-enzyme interaction but which is operated through

fluctuations in the cellular concentration of magnesium rather than through fluctuations in the overall nucleotide concentration. The likely importance of magnesium concentration in modifying any controlling influences of adenylate charge has been discussed at length by Purich & Fromm (1972) for a number of mammalian enzymes, but at the moment insufficient information seems to exist to allow a similar consideration of the situation in plants.

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