BBA 43202

Inhibition of spinach chloroplast fructose-1,6-diphosphatase by $MgATP^{2-}$, $MgADP^{-}$, and magnesium pyrophosphate ($MgP_2O_7^{2-}$)

Fructose-1,6-diphosphatase catalyzes the hydrolysis of fructose 1,6-diphosphate to fructose 6-phosphate and P_i. In photosynthetic cells it appears to be an important control point in the fixation of carbon dioxide by the reductive pentose phosphate cycle[1]. The mechanism of its regulation is not clear, and the present study is part of an attempt to clarify the problem.

Chloroplasts were extracted from store-bought spinach leaves by the method of JENSEN AND BASSHAM[2]. The chloroplast pellet was resuspended in distilled water and broken by sonication. After centrifugation at 2° for 30 min at 40000 × *g* the clear green supernatant was used as the crude extract of soluble protein. The crude extract was partially purified by ammonium sulphate fractionation and the fraction precipitating between 58 and 90% saturation was used as the enzyme preparation in these studies. The assay mixture contained in 1.0 ml: 50 μmoles Tris–HCl (pH 8.7), 5.0 μmoles $MgCl_2$, 2.0 μmoles EDTA (dipotassium salt), 1.0 μmole fructose 1,6-diphosphate, 0.2 μmole $NADP^+$, 5.0 μg phosphoglucose isomerase (D-glucose-6-phosphate ketol-isomerase, EC 5.3.1.9) and 1.5 μg glucose-6-phosphate dehydrogenase (D-glucose-6-phosphate:NADP oxidoreductase, EC 1.1.1.49). The reaction was started by adding 0.05 ml of an enzyme preparation containing 1.0 mg protein per ml. Reduction of the pyridine nucleotide was measured by following absorption at 340 mμ with a Cary (Model 14) spectrophotometer. After a delay of about 30 sec (possibly due to thermal equilibration) the reaction was linear at maximal rate for 2–3 min.

The activity of fructose-1,6-diphosphatase from animal and microbial systems is regulated by the allosteric inhibitor 5'-adenylate (AMP)[3–6]. However, AMP at a concentration of 20 mM has no effect on the activity of the enzyme from spinach chloroplasts. Other compounds which have no effect (at concentrations of 20 mM) include glucose 6-phosphate, fructose 6-phosphate, dihydroxyacetone phosphate, 3-phosphoglyceraldehyde, 3-phosphoglyceric acid, ribose 5-phosphate, ribulose 1,5-diphosphate, 6-phosphogluconate, phosphoenolpyruvate, UTP, CTP, NADPH.

Inhibition of activity was observed with $MgATP^{2-}$, $MgADP^{-}$ and magnesium pyrophosphate ($MgP_2O_7^{2-}$). (The stability constants for $MgATP^{2-}$ (ref. 7) and $MgP_2O_7^{2-}$ (ref. 8) are sufficiently high that one can assume that in the presence of excess Mg^{2+} the concentration of the free deficient ion is negligible; the stability constant for $MgADP^{-}$ (ref. 7) is lower, and at the excess Mg^{2+} concentrations used in this investigation 93–95% of the ADP is present as the complex and the remainder as the free ion.)

$MgATP^{2-}$ is a more effective inhibitor than either of the other two ions (Fig. 1). Thus, 50% inhibition is caused by about 2.5 mM $MgADP^{-}$ and $MgP_2O_7^{2-}$ whereas comparable inhibition is caused by only 0.74 mM $MgATP^{2-}$. The degree of inhibition caused by all three inhibitors varies with substrate and Mg^{2+} concentration. The results in Fig. 1 were obtained with a substrate concentration of 1.0 mM. If the concentration of fructose 1,6-diphosphate is decreased to 0.5 mM the concentrations of $MgP_2O_7^{2-}$, $MgADP^{-}$, and $MgATP^{2-}$ causing 50% inhibition are 0.9 mM, 1.6 mM and 0.53 mM, respectively. At a substrate concentration of 2.5 mM the respective

concentrations are 4.4 mM, 5.8 mM and 1.2 mM. Increasing or decreasing the free Mg^{2+} concentration affects the degree of inhibition in a similar way.

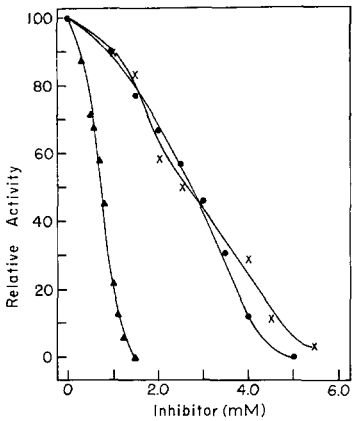

Fig. 1. The effect of $MgATP^{2-}$ (▲—▲), $MgADP^{-}$ (×—×) and $MgP_2O_7^{2-}$ (●—●) on fructose-1,6-diphosphatase activity. The substrate concentration is 1.0 mM and free Mg^{2+} concentration is 3.0 mM. The inhibitor concentrations are 1.5 mM. Activities are expressed as relative values with the activity in the absence of inhibitor taken to be 100.

PRIESS, WYMAN BIGGS AND GREENBERG[9] have observed a sigmoidal relationship between fructose-1,6-diphosphatase activity and substrate concentration, particularly at low Mg^{2+} concentration. This observation has been confirmed, and it has also been observed that the sigmoidal nature becomes more pronounced in the presence of the three inhibitors $MgATP^{2-}$, $MgADP^{-}$ and $MgP_2O_7^{2-}$. The relationship between activity and Mg^{2+} concentration is also sigmoidal and the three inhibitors enhance this feature. Hill plots of the data show that the interaction coefficients (n^*) with respect to substrate and with respect to Mg^{2+} concentration increase with increasing concentration of inhibitors (up to 2.5 mM).

Although certain characteristics of the inhibition described here therefore resemble those of several known 'regulatory enzymes'[4,10–13], the role of the three inhibitors in the control of fructose-1,6-diphosphatase activity *in vivo* is not clear. Thus, their combined concentrations decrease when chloroplasts or Chlorella cells are transferred from the light to darkness[1,14]. This change is the reverse of that which would be expected if the inhibitors were responsible for the decrease in activity of the diphosphatase thought to occur when photosynthetic cells are transferred from illuminated conditions to darkness.

The original observations of PEDERSEN, KIRK AND BASSHAM[1] also suggested that the activity of ribulose-1,5-diphosphate carboxylase (3-phospho-D-glycerate carboxy-lyase (dimerizing), EC 4.1.1.39) also decreased when Chlorella cells are transferred from light to darkness. Of possible interest is the fact that preliminary observations indicate that the same three inhibitors ($MgATP^{2-}$, $MgADP^{-}$, $MgP_2O_7^{2-}$) also inhibit the activity of the carboxylating enzyme extracted from spinach chloroplasts. (The inhibitors do not have a general inhibitory effect on chloroplast enzymes since they fail to inhibit the activity of aldolase (ketose-1-phosphate aldehyde-lyase, EC 4.1.2.7) and triose phosphate dehydrogenase (D-glyceraldehyde-3-phosphate: NADP oxidoreductase (phosphorylating), EC 1.2.1.13) from spinach chloroplasts.)

The work was done while on leave of absence in The Biodynamics Laboratory, Lawrence Radiation Laboratory, University of California, Berkeley, Calif. and was supported by the U.S. Atomic Energy Commission.

Botany Department,
University College London,
London (Great Britain)

IAN MORRIS

1 T. A. PEDERSEN, M. KIRK AND J. A. BASSHAM, *Physiol. Plantarum*, 19 (1966) 219.
2 R. G. JENSEN AND J. A. BASSHAM, *Proc. Natl. Acad. Sci. U.S.*, 56 (1966) 1095.
3 B. L. HORECKER, S. PONTREMOLY, O. ROSEN AND S. ROSEN, *Federation Proc.*, 25 (1965) 1521.
4 K. TAKETA AND B. M. POGELL, *J. Biol. Chem.*, 240 (1965) 651.
5 C. GANCEDO, M.C. SALAS, A.GINER AND A.SOLS, *Biochem. Biophys. Res. Commun.*, 20 (1965) 15.
6 A. M. UNDERWOOD AND E. A. NEWSHOLME, *Biochem. J.*, 95 (1965) 767.
7 W. J. O'SULLIVAN AND D. D. PERRIN, *Biochemistry*, 3 (1964) 18.
8 L. G. SILLEN AND A. E. MARTELL, *Chem. Soc. London Spec. Publ.*, 17 (1964).
9 J. PRIESS, M. L. WYMAN BIGGS AND E. GREENBERG, *J. Biol. Chem.*, 242 (1967) 2292.
10 D. E. ATKINSON, J. A. HATHAWAY AND E. C. SMITH, *J. Biol. Chem.*, 240 (1965) 2682.
11 M. P. GHOSH AND J. PRIESS, *J. Biol. Chem.*, 241 (1966) 4491.
12 N. GENTNER AND J. PRIESS, *Biochem. Biophys. Res. Commun.*, 27 (1967) 417.
13 B. KEECH AND G. J. BARRITT, *J. Biol. Chem.*, 242 (1967) 1983.
14 J. A. BASSHAM, M. KIRK AND R. G. JENSEN, *Biochim. Biophys. Acta*, 153 (1968) 211.

Received May 20th, 1968

Biochim. Biophys. Acta, 162 (1968) 462–464

BBA 43204

Phytochrome spectrum of Pisum leaves and stems

Reports in this journal[1] and elsewhere[2,3] indicate that the phytochrome difference spectrum obtained on etiolated Pisum (pea) leaves, or extracts of them, differs substantially from corresponding spectra obtained from other sources, including Pisum stems. These interesting observations seemed to accord with suggestions made by the writer (ref. 4, p. 318), and attempts were immediately made to confirm them in this laboratory. This communication summarizes the failure of these attempts.

At first, it seemed unnecessary to repeat the earlier procedures precisely, particularly in view of the likelihood (see later) that they might introduce errors. A difference of the magnitude reported[1] in phytochrome spectra should be easily detected by more straight-forward methods, so the following were adopted. Seedlings of *Pisum sativum* cv. Alaska were grown for 7 days in total darkness at about 26° (ref. 5). Samples of stem tissue consisted of 20–50 5-mm segments cut just below the apical hook, while leaf samples comprised 15–50 apical buds cut just above the hook and excluding as much stem tissue as possible. Immediately after cutting, the samples were packed in cylindrical aluminum cells 14 mm in diameter (*cf.* ref. 5), held on ice for about 10 min, then exposed on ice to 10–30 min of white incandescent light, about 45000 lux, to saturate protochlorophyll conversion. Spectra were then taken at ice temperature using the Biospect Model 61 (Agricultural Specialties Co., Beltsville, Md.), a single-beam recording spectrophotometer capable of measuring at high ab-