Biochem. J. (1980) **191**, 845–849
Printed in Great Britain

Light-mediated activation of stromal sedoheptulose bisphosphatase

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(*Received 26 August 1980/Accepted 9 September 1980*)

When isolated wheat (*Triticum aestivum*) chloroplasts were illuminated, the activity of sedoheptulose bisphosphatase increased severalfold. The rate of activation was limited by the size of the carbon pool, and experiments with a partially purified enzyme preparation showed that the degree of reductive activation of the enzyme is governed by the concentration of its substrate.

In common with several enzymes of the reductive pentose phosphate pathway, stromal sedoheptulose bisphosphatase (sedoheptulose 1,7-bisphosphate 1-phosphohydrolase, EC 3.1.3.37) is activated by photosynthetically generated reductants (Schürmann & Buchanan, 1975; Champigny & Bismuth, 1976; Breazeale *et al.*, 1978). Its activation is also favoured by the high Mg^{2+} (Anderson, 1974; Portis *et al.*, 1977) and low H^+ concentrations (Anderson, 1979) in the illuminated stroma. There are no previous reports, however, of activities sufficiently high to support observed rates of photosynthesis. The present paper summarizes procedures that allow the demonstration of such high activities and provides evidence that the reductive activation of sedoheptulose bisphosphatase also depends on the presence of its substrate.

Experimental

Materials

Biochemicals were purchased from Sigma, Poole, Dorset, U.K., auxiliary enzymes from Boehringer, Lewes, East Sussex, U.K., and Sephadex products from Pharmacia, Hounslow, Middx., U.K. Wheat (*Triticum aestivum* L., cv. Sappo) was grown in vermiculite under sunlight and supplementary incandescent lamps.

Isolation of chloroplasts

Protoplasts were isolated from wheat as previously described (Leegood & Walker, 1980*a*), and stored in the dark on ice for 3–4h before use. Protoplasts were collected by centrifugation (100*g* for 2min), resuspended in a medium containing 400mM-sorbitol, 10mM-EDTA (disodium salt) and 25mM-Tricine {*N*-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]glycine} (adjusted to pH8.4 with NaOH), and ruptured by passing them three times through a 20μm nylon mesh attached to a 1ml plastic syringe. Chloroplasts were collected by centrifugation (250*g* for 45s), resuspended in the same medium and used within 30min. Chlorophyll was determined by the method of Arnon (1949). The chloroplasts were more than 90% intact as determined by the ferricyanide technique (Lilley *et al.*, 1975).

Chloroplast illumination and O_2 evolution

O_2 evolution was monitored polarographically at 20°C (Delieu & Walker, 1972). Chloroplasts (50–100mg of chlorophyll·ml^{-1}) were illuminated (Delieu & Walker, 1972) with red light [I.C.I. Perspex (Lucite) red 400 filter and a Calflex C heat filter] at an intensity of 330W·m^{-2} in a medium containing 330mM-sorbitol, 10mM-EDTA, 25mM-Tricine, pH8.4, 0.2mM-P_i, and, as indicated, 10mM-$NaHCO_3$ and 0.1mM-dihydroxyacetone phosphate.

Partial purification of stromal sedoheptulose bisphosphatase

All operations were performed at 4°C. Wheat leaves (50g) were homogenized for 3min in 100ml of buffer A (50mM-Tris/HCl, pH7.8, containing 0.4mM-EDTA and 10mM-2-mercaptoethanol). The homogenate was filtered through four layers of muslin and centrifuged at 30000*g* for 15min. The supernatant was fractionated by addition of solid $(NH_4)_2SO_4$. Protein precipitating between 50 and 90% saturation was collected by centrifugation (27000*g* for 15min), dissolved in 4ml of buffer A supplemented with 200mM-NaCl and applied to a column (2cm^2 × 90cm) of Sephadex G-100 (Superfine grade) equilibrated with the same buffer. Sedoheptulose bisphosphatase activity was eluted (6ml·h^{-1}) in a single peak, and fractions free of both

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fructose bisphosphatase (EC 3.1.3.11) and fructose bisphosphate aldolase (EC 4.1.2.13) activity were combined. Before assay, portions of the preparation were desalted by passing through a column of Sephadex G-25M PD-10 equilibrated with 20mM-Tris/HCl, pH 8.2. The enzyme was purified approx. 25-fold.

Results

When sedoheptulose 1,7-bisphosphatase is added to a ruptured chloroplast extract, two P_i-yielding reaction sequences are initiated: the first is the sedoheptulose bisphosphatase reaction itself (eqn. 1), and the second is described by eqns. (2)–(5).

$$\text{Sedoheptulose 1,7-bisphosphate} + H_2O \rightarrow \text{sedoheptulose 7-phosphate} + P_i \quad (1)$$

$$\text{Sedoheptulose-1,7-bisphosphate} \rightarrow \text{dihydroxyacetone phosphate} + \text{erythrose 4-phosphate} \quad (2)$$

$$\text{Dihydroxyacetone phosphate} \rightarrow \text{glyceraldehyde 3-phosphate} \quad (3)$$

$$\text{Glyceraldehyde 3-phosphate} + \text{dihydroxyacetone phosphate} \rightarrow \text{fructose 1,6-bisphosphate} \quad (4)$$

$$\text{Fructose 1,6-bisphosphate} + H_2O \rightarrow \text{fructose 6-phosphate} + P_i \quad (5)$$

$$\text{Sedoheptulose 7-phosphate} + \text{glyceraldehyde 3-phosphate} \rightarrow \text{erythrose 4-phosphate} + \text{fructose 6-phosphate} \quad (6)$$

Determination of sedoheptulose bisphosphatase activity

Activity was measured at 20°C. Sedoheptulose bisphosphatase in chloroplasts was assayed by removing 80μl portions from the oxygen-electrode vessel and injecting them (within 2s) into an assay medium containing 50mM-Tris/HCl, pH 8.2, 1mM-EDTA, 0.8mM-sedoheptulose 1,7-bisphosphate, 10mM-$MgCl_2$ and 0.025% (v/v) Triton X-100. After 10min, 200μl of this mixture was removed for enzymic assay of fructose 6-phosphate (Kelly *et al.*, 1976). Simultaneously, 0.5ml of 30% (w/v) trichloroacetic acid was added to the remainder and its P_i content determined colorimetrically (Taussky *et al.*, 1953). Sedoheptulose bisphosphatase activity was determined by subtracting the total amount of fructose 6-phosphate from that of P_i (see the Results section) and was linear for at least 10min.

In order to measure sedoheptulose bisphosphatase activity in the partially purified preparation, 10μl (36μg of protein) of enzyme solution was incubated in a mixture (50μl) containing 50mM-Tris/HCl, pH 8.2, and, as specified, 5mM-$MgCl_2$, 5mM-dithiothreitol and up to 2mM-sedoheptulose 1,7-bisphosphate. After 7min, 0.45ml of an assay medium was added such that after addition the mixture contained 50mM-Tris/HCl, pH 8.2, 5mM-$MgCl_2$, 0.5mM-dithiothreitol, and 0.8mM-sedoheptulose 1,7-bisphosphate. Enzyme activity was determined by monitoring P_i release as described above without a concomitant correction for fructose bisphosphatase activity (which was absent). In a parallel experiment the amount of sedoheptulose 1,7-bisphosphate hydrolysed during preincubation was determined. Protein was determined by the procedure of Lowry *et al.* (1951).

To gauge sedoheptulose bisphosphatase activity, the amount of fructose 6-phosphate (which was used as a measure of the second source of P_i) was subtracted from the total P_i. Similar results were obtained when fructose 6-phosphate production was monitored continuously (Kelly *et al.*, 1976) or discontinuously. Transaldolase (EC 2.2.1.2) activity was too low to alter the size of the fructose 6-phosphate pool significantly (eqn. 6).

When isolated chloroplasts were illuminated, a lag or induction phase occurred before the maximum rate of photosynthesis was attained (Fig. 1). After dihydroxyacetone phosphate raised the concentration of chloroplast intermediates (Lilley *et al.*, 1977; Leegood & Walker, 1980*a*) and, in accordance with previous results (Walker, 1976; Leegood & Walker, 1980*a*), decreased the length of the lag. Unlike several other light-activated reductive-pentose-phosphate-pathway enzymes (Leegood & Walker, 1980*a*), the rate of sedoheptulose bisphosphatase activation increased on addition of dihydroxyacetone phosphate, with maximum activity occurring after 3min compared with 7min in the absence of exogenous dihydroxyacetone phosphate (Fig. 1). In both cases, sedoheptulose bisphosphatase activity increased from a value in the dark that was insufficient to support the subsequent maximum rate of O_2 evolution to one that was ample (an sedoheptulose bisphosphatase activity of 33μmol·h^{-1}·mg of chlorophyll^{-1} theoretically maintains a rate of O_2 evolution of 100μmol·h^{-1}·mg of chlorophyll^{-1} if the product is triose phosphate). Maximum O_2 evolution and enzyme activity coincided.

When bicarbonate was excluded from the suspending medium, activation was relatively slow and, even after 15min, enzyme activity was only 60% of

that achieved with bicarbonate (Fig. 2). Conversely, chloroplast fructose bisphosphatase was highly active in similar bicarbonate-free experiments, and it has been suggested that complete reduction of the enzyme is not achieved in the presence of this competing electron acceptor (Leegood & Walker, 1980*b*).

If, as these results suggest, activation is regulated by the size of the chloroplast carbon pool, then supplementing that pool with a transportable intermediate should effect a more rapid activation. When a catalytic quantity of dihydroxyacetone phosphate was added to the bicarbonate-free medium, a rapid 'burst' of activation occurred, followed by a slower increase to a value well in excess of that required to support the highest rates of photosynthesis measured with intact chloroplasts (Fig. 2).

The mechanism by which the chloroplast carbon pool might regulate sedoheptulose bisphosphatase activation was investigated by using a partially purified enzyme preparation. Despite preincubation of the enzyme with substrate, Mg^{2+} or the reductant dithiothreitol, activation did not occur until all the components needed for enzyme activity were present (Table 1). When sedoheptulose bisphosphatase was

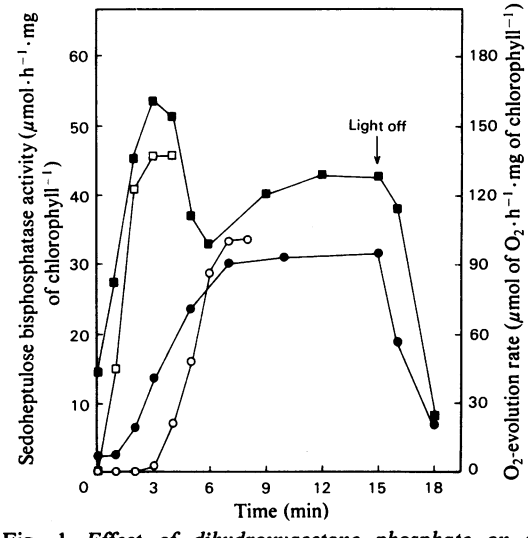

Fig. 1. *Effect of dihydroxyacetone phosphate on induction in isolated wheat chloroplasts in relation to the light-mediated activation and dark inactivation of sedoheptulose bisphosphatase*

Open symbols depict O_2 evolution and closed symbols enzyme activity. Measurements were made in the presence (■, □) and absence (●, ○) of 0.1 mM-dihydroxyacetone phosphate. Each assay mixture contained 10 mM-$NaHCO_3$.

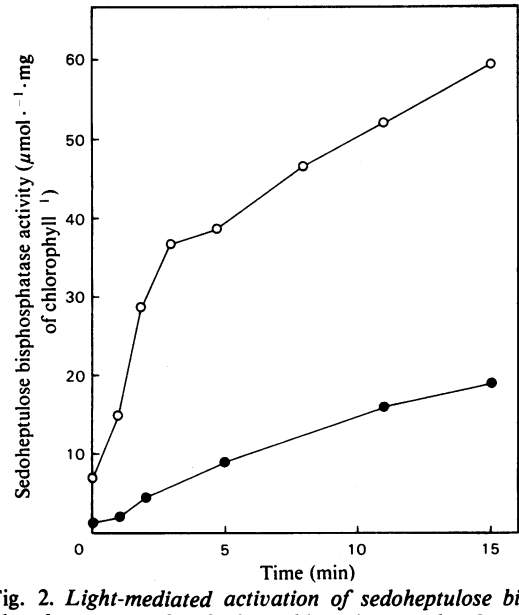

Fig. 2. *Light-mediated activation of sedoheptulose bisphosphatase in isolated wheat chloroplasts in the absence of $NaHCO_3$*

Measurements were made in the presence (○) and absence (●) of 0.1 mM-dihydroxyacetone phosphate.

Table 1. *Activation of stromal sedoheptulose bisphosphatase by dithiothreitol, Mg^{2+} and sedoheptulose 1,7-bisphosphate*

Enzyme (36 μg) was incubated for 7 min in 50 μl of a solution containing 50 mM-Tris/HCl, pH 8.2, and, as indicated, 5 mM-$MgCl_2$, 5 mM-dithiothreitol (DTT) and 0.8 mM-sedoheptulose 1,7-bisphosphate. Standard assay medium (0.45 ml) was then added and activity measured at zero time (see the Experimental section).

Preincubation conditions	Sedoheptulose bisphosphatase activity (μmol·min⁻¹·mg of protein⁻¹)
Control	0
DTT	0
DTT + sedoheptulose 1,7-bisphosphate	0
DTT + Mg^{2+}	0
Mg^{2+} + sedoheptulose 1,7-bisphosphate	0
DTT + Mg^{2+} + sedoheptulose 1,7-bisphosphate	0.56

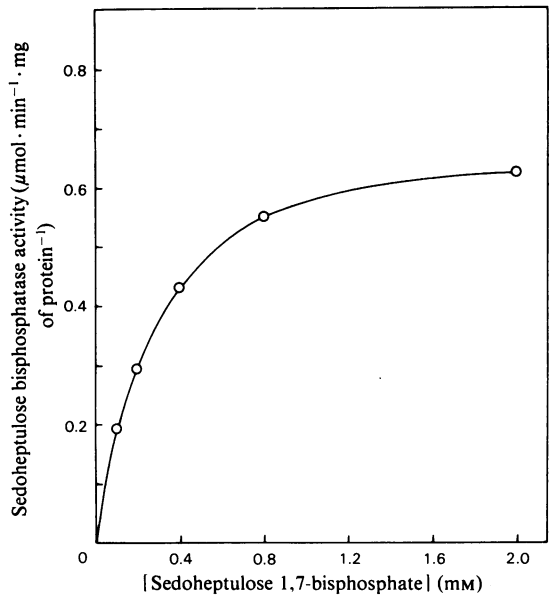

Fig. 3. *Effect of the sedoheptulose 1,7-bisphosphatase concentration during preincubation on stromal sedoheptulose bisphosphatase activity*

Enzyme (36 μg) was preincubated for 7 min in 50 μl of solution containing 50 mM-Tris/HCl, pH 8.2, 5 mM-dithiothreitol, 5 mM-$MgCl_2$ and various amounts of sedoheptulose 1,7-bisphosphate. Standard assay medium (0.45 ml) was then added and activity measured at zero time.

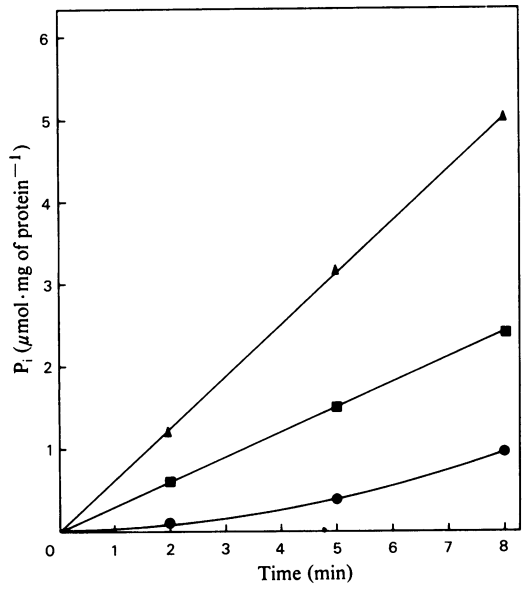

Fig. 4. *Stromal sedoheptulose bisphosphatase activity as measured in the standard assay medium (see the Experimental section)*

Enzyme (36 μg) was preincubated for 7 min in a solution containing 50 mM-Tris/HCl, pH 8.2, 5 mM-dithiothreitol, 5 mM-$MgCl_2$, and 0 mM- (●), 0.2 mM- (■), and 2 mM- (▲) sedoheptulose 1,7-bisphosphate. The standard assay medium contained 0.8 mM-sedoheptulose 1,7-bisphosphate.

incubated for several minutes in complete reaction mixtures containing various amounts of substrate, then injected into a standard assay medium (containing 0.8 mM-sedoheptulose 1,7-bisphosphate), the degree of activation varied. The concentration of sedoheptulose 1,7-bisphosphate required for half-maximum activation was 0.26 mM (Fig. 3). At least 7 min preincubation was required before activity increased to a constant value, and after injection into the standard assay medium activity was linear for at least 8 min (Fig. 4). In experiments in which one or more components of the reaction mixture were omitted during preincubation, some activation still occurred in the assay medium, even with 10-fold dilution of the dithiothreitol (Fig. 4). The degree of activation was therefore estimated at zero time. Neither dihydroxyacetone phosphate nor fructose 6-phosphate, both increasing during induction (Leegood & Walker, 1980*a*), increased the rate of sedoheptulose bisphosphatase activation.

Whereas the activation of sedoheptulose bisphosphatase was influenced by adding dihydroxyacetone phosphate to isolated chloroplasts, inactivation was not. The enzyme was almost completely inactive after 3 min of darkness in both experiments (Fig. 1).

Discussion

The sedoheptulose bisphosphatase activities measured in these experiments depend, to a certain degree, on the nature of the assay medium. Conditions were therefore chosen to be similar to those believed to exist in the stroma of illuminated chloroplasts (a high $[Mg^{2+}]$ and an alkaline pH). If the degree of sedoheptulose bisphosphatase activation is not significantly altered on rupture of the chloroplast, then the activation patterns should reflect those occurring *in vivo*. Obviously the absolute activities will depend on the extent to which the experimental conditions accurately reflect those in the illuminated stroma. Nevertheless, it seems clear that the activity of sedoheptulose bisphosphatase at the start of illumination is unlikely to be sufficient to support the subsequent rate of photosynthesis, and is apparently much lower than that of other reductive-pentose-phosphate-pathway enzymes (Champigny & Bismuth, 1976; Leegood & Walker, 1980*a*). Before a high rate of photosynthesis can be attained, sedoheptulose bisphosphatase must be activated severalfold.

Sedoheptulose bisphosphatase activation appears to be limited by the size of its substrate pool and requires Mg^{2+} and a light-generated reductant. The

addition of triose phosphate to wheat chloroplasts effects a rapid increase in the concentration of fructose 6-phosphate (Leegood & Walker, 1980*a*). A similar increase in the concentration of sedoheptulose 1,7-bisphosphate would almost certainly follow [as CO_2 fixation proceeds at a high rate under such conditions (Leegood & Walker, 1980*a*; Lilley *et al.*, 1977)], and this accords with the relatively rapid rate of enzyme activation observed. When appreciable carbon fixation was prevented by omitting bicarbonate, thereby restricting the increase in sedoheptulose 1,7-bisphosphate, activation was correspondingly slow. Previous reports of low sedoheptulose bisphosphatase activities may well be attributable to this factor (Champigny & Bismuth, 1976).

Chloroplast fructose bisphosphatase activation also depends on the substrate (Wolosiuk *et al.*, 1980); however, there is a fundamental difference between this dependency and that of sedoheptulose bisphosphatase. Thus although fructose bisphosphatase activation can be effected by incubation with fructose 1,6-bisphosphate and a reductant before initiating the reaction with Mg^{2+}, sedoheptulose bisphosphatase activation, with dithiothreitol as reductant, does not occur until all the components required for catalysis are present. This type of condition has been called 'concerted hysteresis' (Frieden, 1970).

The hysteretic properties of sedoheptulose bisphosphatase may be important in the regulation of the reductive pentose phosphate pathway. If activation involves the conversion of an inactive enzyme into an active conformation, then the proportion of the active forms appears to be governed by the sedoheptulose 1,7-bisphosphate concentration. Given a rapid increase in the concentration of sedoheptulose 1,7-bisphosphate, the kinetic characteristics of sedoheptulose bisphosphatase would then slowly respond until a new steady-state concentration of substrate is established. Frieden (1970) describes the role of this type of response as a 'time-dependent buffer' or damping device, which may serve to prevent rapid changes in the concentrations of other intermediates in the pathway. Sedoheptulose bisphosphatase may then provide a fine control that helps to maintain the intermediate pools at concentrations optimal for photosynthesis. For example, two-thirds of the pentose phosphate regenerated in the reductive pentose phosphate pathway is formed in the second transketolase (EC 2.2.1.1) reaction, in which sedoheptulose 7-phosphate is a C_2 donor. This reaction must compete for glyceraldehyde 3-phosphate, which is also consumed in the first transketolase and first aldolase reactions. A mechanism that would ensure that sedoheptulose 7-phosphate was not produced more rapidly than absolutely necessary would help to contain this competition and allow those reactions to proceed in parallel in a balanced fashion.

Although the above suggestions add a new dimension to present concepts of regulation of the reductive pentose phosphate pathway, they do not detract from the view, for which there is already considerable evidence, that lags lasting longer than half a minute are more easily explained in terms of substrate deficiency than inadequacy of catalysis.

This work was supported by the U.K. Agricultural Research Council. I. E. W. is also supported by a Royal Commission for the Exhibition of 1851 Overseas Scholarship. We are grateful to Professor E. Latzko and Dr. G. J. Kelly for helpful discussions.

References

Anderson, L. E. (1974) *Biochem. Biophys. Res. Commun.* **59**, 907–913

Anderson, L. E. (1979) in *Encyclopedia of Plant Physiology* (Gibbs, M. & Latzko, E., eds.) (*Photosynthesis*), vol. 2, pp. 271–281, Springer-Verlag, Berlin

Arnon, D. I. (1949) *Plant Physiol.* **24**, 1–15

Breazeale, V. D., Buchanan, B. B. & Wolosiuk, R. A. (1978) *Z. Naturforsch.* **33**, 521–528

Champigny, M.-L. & Bismuth, E. (1976) *Physiol. Plant.* **36**, 95–100

Delieu, T. & Walker, D. A. (1972) *New Phytol.* **71**, 201–225

Frieden, C. J. (1970) *J. Biol. Chem.* **254**, 5788–5799

Kelly, G. J., Zimmermann, C. & Latzko, E. (1976) *Biochem. Biophys. Res. Commun* **70**, 193–199

Leegood, R. C. & Walker, D. A. (1980*a*) *Arch. Biochem. Biophys.* **200**, 575–582

Leegood, R. C. & Walker, D. A. (1980*b*) *FEBS Lett.* **116**, 21–24

Lilley, R. McC., Fitzgerald, M. P., Reinits, K. G. & Walker, D. A. (1975) *New Phytol.* **75**, 1–10

Lilley, R. McC., Chon, C. J., Mosbach, A. & Heldt, H. W. (1977) *Biochim. Biophys. Acta* **460**, 259–272

Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275

Portis, A. R., Chon, C. J., Mosbach, A. & Heldt, H. W. (1977) *Biochim. Biophys. Acta* **461**, 313–325

Schürmann, P. & Buchanan, B. B. (1975) *Biochim. Biophys. Acta* **376**, 189–192

Taussky, H. H., Shorr, E. & Kurzmann, G. (1953) *J. Biol. Chem.* **202**, 675–685

Walker, D. A. (1976) in *The Intact Chloroplast* (Barber, J., ed.), pp. 235–278, Elsevier, Amsterdam

Wolosiuk, R. A., Perelmuter, M. E. & Chehebar, C. (1980) *FEBS Lett.* **109**, 289–293