Activation of Glyceraldehyde-Phosphate Dehydrogenase (NADP) and Phosphoribulokinase in Phaseolus vulgaris Leaf Extracts Involves the Dissociation of Oligomers

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ABSTRACT
Phosphoribulokinase (EC 2.7.1.19, ATP: d-ribulose-5-phosphate-1-phosphotransferase) resembles the NADPH-dependent glyceraldehyde-3-phosphate dehydrogenase (EC 1.2.1.13, d-glyceraldehyde-3-phosphate: NADPH oxidoreductase [phosphorylating]) of chloroplasts in that the activation of both of these enzymes involves the dissociation of oligomers (apparently tetrameric forms) with low catalytic activity to give protomers which possess higher catalytic activity. Gel filtration on Sepharose 6B has shown that the molecular weights of the oligomer and active protomer of phosphoribulokinase are, respectively, about 6.8 x 10^4 and 1.7 x 10^4, whereas the corresponding values for glyceraldehyde-3-phosphate dehydrogenase are 8.2 x 10^4 and 2.2 x 10^4. Activation of both enzymes occurs in response to either ATP, dithiothreitol, or cholate while the glyceraldehyde-3-phosphate dehydrogenase is also activated by NADPH. Activation/dissociation of these enzymes may involve conformational changes resulting from nucleotide binding, the reduction of sulfur bridges, and the cholate induced loosening of hydrophobic interactions.

It has been clearly established that light regulates the activities of a number of photosynthetic carbon cycle enzymes, namely the NADPH-dependent glyceraldehyde-P dehydrogenase (32), ribulose-bisP carboxylase (EC 4.1.1.39) (28), P-ribulokinase (16), d-fructose-1,6-bisP 1-P-hydrolase (EC 3.1.3.11) (9) and d-sedoheptulose-1,7-bisP 1-P-hydrolase (EC 3.1.37) (3). With the exception of ribulose-bisP carboxylase the main mechanism for the in vivo activation of these enzymes has been reported to depend on the generation of SH groups by the photosynthetic machinery, either directly as membrane-bound vicinal-dithiol groups (4) or indirectly through the mediation of ferredoxin-thioredoxin reductase and thioredoxin (10).

Most of the research effort in this field has concerned the NADP-dependent glyceraldehyde-P dehydrogenase whose activation has been shown to be correlated with the dissociation of a tetramer of about 600,000 mol wt to give protomers of about 145,000 mol wt (21, 22). The present knowledge of the activation and dissociation of this enzyme is summarized in Table I. Although not all of the substances listed in Table I can be expected to function in vivo these studies are valuable in contributing towards the understanding of the mechanism of regulation of this enzyme.

In this laboratory we have obtained results for bean leaf NADPH-dependent glyceraldehyde-P dehydrogenase similar to those reported for the spinach enzyme by Pupillo and Piccar (21, 22) and have found that a similar activation and dissociation mechanism occurs with P-ribulokinase (7, 8, 15, 27). A comparative account of the two enzymes is presented and certain discrepancies in the literature are noted.

MATERIALS AND METHODS

Chemicals and Enzymes. Except where otherwise indicated DTT and laboratory reagents were obtained from BDH Chemicals Ltd., Poole, Dorset, U. K., and biochemicals and enzymes were obtained from The Boehringer Corporation (London) Ltd., Lewes, East Sussex, U. K.

Plant Material. Plants of Phaseolus vulgaris L. c.v. Alabaster and c.v. Canadian Wonder were grown in Vermiculite as described previously (6). Plants for etioplast preparation were grown for 14 days at 23°C in total darkness and plants for chloroplast preparation were grown in the glasshouse under prevailing daylight and temperature conditions for 14–21 days. The activation experiments described in this paper were carried out with etioplast extracts because this work was part of an investigation of the process of greening. Similar results were obtained for the activation of chloroplast extracts when the leaves had been darkened for at least 1 h and the manipulations prior to extraction were performed in darkness.

Preparation of Etioplasts and Chloroplasts. Approximately 5 g of primary leaves were homogenized in an MSE "Atomix" top-drive homogenizer for two 5-s intervals at top speed with 40 ml of a semifrozen modified Walker (25) extraction medium (0.33 M sorbitol, 100 mM Na2HPO4, 100 mM KH2PO4, 20 mM NaCl, 5 mM MgCl2, and 0.4% BSA, adjusted to pH 7.5). The homogenate was squeezed through two layers of muslin and filtered through a further eight layers into a 50-ml centrifuge tube. The filtrate was centrifuged in an MSE "Highspeed 18" refrigerated centrifuge (8–X 50-ml angle head) with rapid acceleration to 4,000 g being followed by braking by hand, allowing a total elapsed time from rest to top speed and back to rest of 90 s. For etioplasts the centrifuge was held at top speed for 30 s before braking commenced. The supernatant was discarded and the surface layer of the plastid pellet was washed away with 2–3 ml of extraction medium. The plastid pellet was resuspended in 1 ml of extraction medium by the use of a pipette (1 ml) and a small spatula. All operations were carried out at 2–4°C. For larger scale preparations...
the leaves were ground for 3 s in Polytron PT 120 with a PT 25 head (Northern Media Supply Co., Hull, England).

**Enzyme Extraction.** Etioplasts and chloroplasts were osmotically shocked by suspension, for 5 min at 4 C, in sufficient buffer to give eventually 5-10 mg protein/ml of supernatant. For activation experiments the buffer comprised 100 mm Tris-Cl (pH 7.8), with 1 mm DTT, and for gel filtration it was 100 mm Tris-Cl (pH 8.0), with 10 mm EDTA and 0.01% 2-mercaptoethanol. The suspension was centrifuged at 38,000 g for 20 min and the supernatant was used.

**Gel Filtration.** Gel filtration was carried out on columns of Sepharose 6B (2.5- x 37-cm and 2.5- x 84-cm columns), Bio-Gel A-1.5m 100-200 mesh (1.5- x 86-cm column), and Sephadex G-100 (2.5- x 36-cm column) at 4 C in the cold room. Filtration was carried out in a downward direction with the rate of flow being regulated by a polystatic pump to 15 ml/h. The fractions were collected in test tubes with a BTL fraction collector and a Chromatimer set at 15 min per fraction. The buffer comprised 100 mm Tris-Cl (pH 8.0) and pH-adjusted 10 mm EDTA and 0.01% 2-mercaptoethanol. For some experiments other components were included in the incubation and elution buffer. Each fraction was assayed for enzyme activity (0.02-0.10 ml/assay).

The method of Andrews (5) was used to relate protein mol wt to the elution volume. The following standard proteins were obtained from Boehringer: Cyt c (assumed mol wt 12,500), chymotrypsinogen A (25,000), hen egg albumin (45,000), BSA (67,000), rabbit muscle glycerol-1-P dehydrogenase (78,000), rabbit muscle glyceraldehyde-P dehydrogenase (144,000), rabbit muscle aldolase (160,000), beef liver catalase (240,000), and beef liver glutamate dehydrogenase (640,000). The void volume was measured by Blue Dextran 2000.

**Enzyme Assays.** The assays were carried out at 25 C in a Unicam SP800 Spectrophotometer which recorded the consumption of NAD(P)H from the fall in A at 340 nm. The reaction mixture for the NADPH-dependent glyceraldehyde-3-P dehydrogenase assay comprised 50 μmol Tris-Cl (pH 7.8), 10 μmol MgCl₂, 4.5 μmol EDTA, 3 μmol ATP, 5 μmol 3-P-glycerate, 1 μg of P-glycerate kinase, 100 nmol of NADPH, and enzyme in a total volume of 1 ml. The reaction mixture for the P-ribulokinase assay comprised 50 μmol Tris-Cl (pH 7.8), 2 μmol DTT, 10 μmol MgCl₂, 2 μmol ATP, 40 μmol KC1, 2.4 μmol 2'-AMP, 5 μmol of a preparation of ribulose-5-P, and ribose-5-P (obtained by incubating ribose-5-P with Sigma ribose-P isomerase), 5 μg pyruvate kinase, 6 μg lactate dehydrogenase, 100 μmol NADH, and enzyme in a total volume of 1 ml. The assays were commenced by the addition of enzyme. ADP formed in the P-ribulokinase reaction was consumed by pyruvate kinase to give an equimolar amount of pyruvate. The rate of pyruvate production and hence of ADP production was determined from the consumption of NADH in the lactate dehydrogenase reaction. ADP, present as an impurity in the ATP, caused an initially high rate of NADH consumption, but within 2 min a lower steady rate was achieved. The rate was taken from between 1 and 3 min after the onset of the steady rate. As this lower rate was accounted for by both P-ribulokinase and ATPase activities it was necessary to carry out a control reaction without pentose-P so that a correction for any ATPase activity could be applied. Where preincubation treatments were carried out 0.5 ml of enzyme was mixed with 0.5 ml of the relevant effector and at the end of the appropriate incubation time at 25 C, 0.1 ml of the mixture was added to each assay.

One unit of enzyme consumed 1 μmol of ATP or NAD(P)H/ min at 25 C.

**RESULTS**

As for chloroplast extracts and purified enzyme preparations from spinach (19, 22, 29), bean etioplast extracts showed activation of NADPH-dependent glyceraldehyde-P dehydrogenase in response to preincubation with ATP and with NADPH. This enzyme activity increased by 6- to 8-fold in response to 30 min preincubation with 6 mm ATP and by 5-fold in response to a similar preincubation with 3 mm NADPH (7, 8, 27). These concentrations of activators were saturating with respect to activation and the half-times for activation at 25 C were 4 and 2 min, respectively, for 6 mm ATP and 2 mm NADPH. Like spinach chloroplast extracts (29) and a homogenate of pea apices (1) the bean etioplast extracts showed activation by DTT, a saturating concentration of 10 mm DTT giving a half-time of 7 min at 25 C for activation.

The P-ribulokinase activity of bean etioplast extracts was promoted by preincubation with either ATP or DTT (Fig. 1). Saturation was reached by 4 mm ATP (6- to 11-fold activation) and by 7 mm DTT (12- to 17-fold activation) the respective half times for 4 mm ATP and 10 mm DTT being 7 and 27 min, respectively. Quantitative variation in the activation observed with different etioplast extracts accounts for the differences between the time of preincubation curves and the effector concentration curves. The activation by DTT has been reported previously (1, 31) but

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**Table 1. Summary of the Present State of Knowledge of the Effectors Responsible for the Activation and Dissoication of NADPH-dependent Glyceraldehyde-P Dehydrogenase**

<table>
<thead>
<tr>
<th>Activators</th>
<th>References</th>
<th>Dissociators</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP*</td>
<td>18, 22, 29</td>
<td>ATP*</td>
<td>22</td>
</tr>
<tr>
<td>XTP</td>
<td>22</td>
<td>XTP</td>
<td>22</td>
</tr>
<tr>
<td>ITP</td>
<td>22</td>
<td>ITP</td>
<td>22</td>
</tr>
<tr>
<td>GTP</td>
<td>22</td>
<td>GTP</td>
<td>22</td>
</tr>
<tr>
<td>2'-AMP</td>
<td>22</td>
<td>2'-AMP</td>
<td>22</td>
</tr>
<tr>
<td>NADPH*</td>
<td>19, 22, 29</td>
<td>NADPH</td>
<td>11, 20, 22, 24</td>
</tr>
<tr>
<td>NADP*</td>
<td>18, 21, 22</td>
<td>NADP*</td>
<td>11, 21, 22, 24</td>
</tr>
<tr>
<td>DTT*</td>
<td>1, 19, 22, 31</td>
<td>DTT*</td>
<td>20, 22</td>
</tr>
<tr>
<td>DTT plus thioredoxin</td>
<td>31</td>
<td>Pi</td>
<td>24</td>
</tr>
<tr>
<td>Pi</td>
<td>22</td>
<td>Cholate*</td>
<td>23</td>
</tr>
</tbody>
</table>

*Confirmed in this investigation.
Wolosiuk and Buchanan (31) reported that ATP did not activate spinach P-ribulokinase, this being in direct contrast with our findings for the bean enzyme (Fig. 1). ATP is one of the substrates of P-ribulokinase and there is some disagreement as to whether the activity of the enzyme should be interpreted in terms of the energy charge of the reaction mixture (2, 17). These reports were of experiments in which preincubation of the enzyme with ATP prior to the completion of the reaction mixture was not carried out. When a sufficient preincubation treatment was used, as in this work, the activated rate of the P-ribulokinase reaction commences without any lag.

Chloroplast extracts were then subjected to gel filtration on a Sepharose 6B column in a way similar to that described by Pupillo and Piccari (21). The NADPH and NADH activities of the glyceraldehyde-P dehydrogenase and the P-ribulokinase activities in each fraction were then determined without any preincubation treatment. Peak elution of both NADPH and NADH activity of glyceraldehyde-P dehydrogenase occurred in fraction 28, at an elution volume of 104 ml, the NADH activity being 3.6 times greater than the NADPH activity (Fig. 2). A minor amount of NADPH activity was eluted in fraction 43, at a volume of 160 ml, there being no corresponding NADH activity detected. The peak of P-ribulokinase activity eluted in fraction 34, at a volume of 127 ml, while a prominent shoulder to this peak coincided with the glyceraldehyde-P dehydrogenase peak at fraction 28. The peaks close to fraction 28 will be described as high mol wt peaks as they eluted at a mol wt in excess of 600,000 whereas the peaks close to fraction 34 will be described as low mol wt peaks at values near 200,000. In addition to the enzyme activities plotted the effects of 30 min preincubation with 6 mM ATP were determined for some of the most active enzyme fractions eluted in the experiment (Fig. 2). For the high mol wt fraction 26 P-ribulokinase activity increased by 2.6-fold and glyceraldehyde-P dehydrogenase activity with NADPH increased by 14-fold and with NADH decreased by 50%, a result similar to that shown by the etioplast extracts (Fig. 1). In contrast, preincubation of the other main components (fractions 34 and 43) with ATP failed to bring about any activation.

Those fractions containing the high mol wt components (fractions 25-30) were combined and incubated for 24 h at 4 °C in either: (a) buffer (100 mM Tris-HCl [pH 8.0], 10 mM EDTA, and 0.01% 2-mercaptoethanol); (b) buffer plus 6 mM ATP; or (c) buffer plus 7 mM DTT. They were then subjected to refiltration on the Sepharose 6B columns with dimensions identical to the first one but each had been equilibrated with the corresponding incubation solution which was also used for the elution. The enzyme activities of each fraction were determined without further preincubation in the assays and the results are shown in Figure 3 and summarized in Table II. Refiltration in the same buffer (Fig. 3A) showed that the glyceraldehyde-P dehydrogenase activities reappeared in the high mol wt form but that some of the P-ribulokinase activity which had been present in the high mol wt form was present in the low mol wt form.

Preincubation and refiltration in either 6 mM ATP (Fig. 3B) or 7 mM DTT (Fig. 3C) resulted in the appearance of all of the P-ribulokinase activity in the low mol wt form. Comparison of the areas under peaks for the three treatments in Figure 3 showed that ATP and DTT had yielded a 6-fold increase in enzyme activity. Replicate filtrations and column calibrations provided estimates of 6.8 × 105 and 1.7 × 105 for mol wt of the high and low mol wt forms. It seems likely that activation of P-ribulokinase involves the dissociation of an oligomer to a protomer in a way analogous to that reported for spinach chloroplast glyceraldehyde-P dehydrogenase by Pupillo and Piccari (21).

The results for glyceraldehyde-P dehydrogenase activity on preincubation with 6 mM ATP are also in accord with the conclusions of Pupillo and Piccari (loc. cit.), our data indicating that an oligomer of about 8.2 × 105 mol wt dissociates to give a protomer of 2.2 × 105, with an increase of NADPH activity of 19-fold and a decrease of NADH activity of 60%. However incubation in 7 mM DTT resulted in a retarded elution of all of the glyceraldehyde-P dehydrogenase activity from Sepharose 6B column, there being a 3.5-fold increase in the NADPH activity and a 90% inhibition of the NADH activity. This retarded peak of activity corresponded to the peak eluting close to fraction 43 in Figure 2. The best estimate of the mol wt of this peak was obtained by gel filtration on a Sepadex G-100 column (2.5 × 36 cm) which gave a value of 3.1 × 105 and indicated that this fraction corresponded to the enzyme subunits whose mol wt have been reported to lie in the range of 3.6 × 104 to 4.3 × 104 (12, 13, 20).

Schwarz (23) reported that the addition of 1% sodium cholate to the homogenizing buffer during and after the homogenization of primary leaves of Phaseolus vulgaris gave an increase in the activity of NADPH-dependent glyceraldehyde-P dehydrogenase in the extract. She attributed this to the release of the enzyme from cell membranes. The high mol wt form of bean P-ribulokinase was obtained by gel filtration as in Figure 2 and after 24 h incubation in 1% buffered sodium cholate (the buffer was 100 mM Tris-HCl [pH 8.0], 10 mM EDTA, and 0.01% 2-mercaptoethanol) it was run through a Sepharose 6B gel column (2.5 × 84 cm) which had been equilibrated with the cholate buffer. All of the P-ribulokinase eluted as the low mol wt form and the activity recovered was twice that of the high mol wt form used in this experiment (Table II). High and low mol wt fractions of the enzyme obtained by gel filtration of a bean chloroplast extract were then incubated in buffered 1% cholate at 4 °C, aliquots were removed at intervals and assayed for P-ribulokinase activity without further preincubation. There was a 3.5-fold rise in the activity of the high mol wt fraction in 24 h while activity of the low mol wt fraction showed an 18% fall in activity (Fig. 4). It seems likely that this fall of activity represented enzyme degradation and that the initial rise in the activity of the low mol wt fraction resulted from some contamination by the high mol wt fraction. Figure 5 shows the effects of 30-min incubation at 4 °C of the two P-ribulokinase forms in various concentrations of cholate in buffer. 5% cholate was the most effective concentration tested, yielding a 3.6-fold increase in the activity of the high mol wt form and a 31%
ACTIVATION OF TWO CHLOROPLAST ENZYMES

Table II. Gel filtration of P-ribulokinase

Fractions from a Sepharose 6B filtration of chloroplast extract were incubated in buffer (100 mM Tris-HCl [pH 8.0], 10 mM EDTA, and 0.01% 2-mercaptoethanol) or buffer plus an additional component for 24 h at 4°C prior to refiltration through an identical Sepharose 6B column, containing the incubation medium, and determination of the distribution of P-ribulokinase activity eluted.

<table>
<thead>
<tr>
<th>P-ribulokinase Form Investigated</th>
<th>Additions to Incubation Medium</th>
<th>Recovered as High Mol Wt Form</th>
<th>Recovered as Low Mol Wt Form</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Units Added</td>
<td>Initial Activity</td>
<td>Units Initial Activity</td>
</tr>
<tr>
<td>High mol wt</td>
<td>None</td>
<td>2.8</td>
<td>2.5</td>
</tr>
<tr>
<td>High mol wt</td>
<td>6 mM ATP</td>
<td>2.8</td>
<td>0.0</td>
</tr>
<tr>
<td>High mol wt</td>
<td>7 mM DTT</td>
<td>2.8</td>
<td>0.0</td>
</tr>
<tr>
<td>High mol wt</td>
<td>1% cholate</td>
<td>1.3</td>
<td>0.0</td>
</tr>
<tr>
<td>Low mol wt</td>
<td>None</td>
<td>10.6</td>
<td>0.0</td>
</tr>
</tbody>
</table>

Fig. 3. Elution profiles of the combined high mol wt fractions (fractions 25-30) of Figure 2 after 24 h further incubation and passage through Sepharose 6B columns of identical dimensions to that used in Figure 2. The standard buffer was used for preincubation and elution with the following additions: A, none; B, 6 mM ATP; C, 7 mM DTT. Other details as for Figure 2 except that 5 ml of material (2.4 mg protein) was used in each case and 2.5-ml fractions were collected.

Fig. 4. Effect of incubation in 1% cholate on the activity of the high and low mol wt forms of P-ribulokinase. The two forms of the enzyme were obtained by similar gel filtration to that shown in Figure 2. Incubation was carried out in a solution of 100 mM Tris-HCl (pH 8.0), 10 mM EDTA, 0.01% 2-mercaptoethanol, and 1% cholate at 4°C. Enzyme activity was determined after various times. (▲): High mol wt form; (△): low mol wt form.

longer periods of incubation necessary for the performance of gel filtration experiments no activity of this enzyme was detected in the column eluates from cholate treatments. Thus the apparent lability of bean glyceraldehyde-P dehydrogenase in the presence of cholate has limited our follow-up of the work of Schwarz (23).

DISCUSSION

ATP, DTT, and cholate are all responsible for both the dissociation and activation of the high mol wt form of P-ribulokinase (Table II). Those compounds which have been shown, in this laboratory and elsewhere, to activate or dissociate NADPH-dependent glyceraldehyde-P dehydrogenase are listed in Table I. We have found that ATP, DTT, and cholate each function both as activators and dissociators of P-ribulokinase. The activation of P-ribulokinase by DTT has been reported by other workers (1, 31) as has activation by DTT plus thioredoxin (31). In each case the list of activators is longer than the list of dissociators because
Activation studies have predominated over dissociation studies. However, all of the compounds listed as dissociators are also known to be activators, and, as far as we know, no known activator has been conclusively shown to not bring about dissociation. We conclude, for both enzymes, that their activation involves the dissociation of a high mol wt form to yield a low mol wt form. As gel filtration yields some activity of each enzyme in the position of high mol wt form it seems likely that the high mol wt forms possess reduced activity than rather being inactive. Pupillo and Piccarì (22) considered that the high mol wt form of glyceraldehyde-P dehydrogenase did not possess activity with NADPH. Apart from this latter point our conclusions about glyceraldehyde-P dehydrogenase are similar to those of Pupillo and Piccarì (21, 22). The close similarities in activation/dissociation between glyceraldehyde-P dehydrogenase and P-ribulokinase seem to be of particular interest and importance.

Table III shows the estimated mol wt of the different forms of the two enzymes based on gel filtration. Although there are inevitable discrepancies between experiments carried out in different laboratories for the gel filtration of broad peaks of activity (Figs. 2 and 3 and ref. 21) and for velocity sedimentation in sucrose gradients (22), our conclusions are close to those of Pupillo and Piccarì and we consider that both enzymes are activated by the dissociation of an oligomer (high mol wt form) to a protomer (low mol wt form). Our best estimates of the mol wt of the oligomer and protomers and of the glyceraldehyde-P dehydrogenase subunit were obtained on Sepharose 6B and Sephadex G-100 columns and are given in Table IV. SDS-polyacrylamide gel electrophoresis of the NADPH-dependent glyceraldehyde-P dehydrogenase has been reported to give two distinct subunits with estimated mol wt of 3.6–3.9 \times 10^4 (12, 13, 20). This evidence suggests that our estimate of 3.1 \times 10^4 for the subunit mol wt is somewhat low.

There is a substantial discrepancy between our results and those of Pawlik and Latzko (20) who estimated the mol wt of the two forms of spinach glyceraldehyde-P dehydrogenase to be 2.4 \times 10^5 and 7.9 \times 10^4 on the basis of filtration on Bio-Gel A-1.5m. We consequently filtered bean chloroplast extracts on a similar Bio-Gel column and found some retardation of our fractions compared to the Sepharose 6B result. Table IV shows the estimated mol wt of the enzyme oligomers and protomers and suggests that the lower values obtained by Pawlik and Latzko (20) may have resulted from binding of glyceraldehyde-P dehydrogenase to the Bio-Gel. As we have previously observed nucleotide binding to Bio-Gel P-2 (26) it may be expected that a nucleotide binding enzyme like glyceraldehyde-P dehydrogenase would show retardation on a Bio-Gel column.

Our findings do permit a number of conclusions about these enzymes and the nature of the binding of their protomers. Although the two enzymes show many similar properties there were consistent differences between the two enzymes in the elution of the oligomers and of the protomers. Thus the two enzyme activities belong to distinct polypeptides and they do not reside either in the same oligomer or the same protomer. In neither case do we know whether the oligomer yields identical protomers on dissociation or whether the protomers all possess identical enzymic activity. If each oligomer yields protomers of identical size it is likely that each oligomer will be a tetramer.

The effectiveness of certain nucleotides as activators/dissociators would be expected to be mediated through their binding to either catalytic sites (NADPH-dependent glyceraldehyde-P dehydrogenase has a catalytic site for NADP(H) and P-ribulokinase has one for ATP) or to regulatory sites (the effectiveness of ATP on NADPH-dependent glyceraldehyde-P dehydrogenase activity suggested that there should be a regulatory site for ATP). The binding of nucleotides might alter protomer conformation or impose steric effects on protomer : protomer bonds, thus bringing about dissociation by imposing strain on the bonding between the protomers.

DTT or reduced thioredoxin or reduced vicinal-SH groups would be expected to bring about activation and dissociation through the reduction of sulfur bridges linking protomers or indirectly from conformational changes arising from the cleavage of the bridges. Activation by reduced thioredoxin (30, 31) or reduced vicinal-SH (4) (30, 31) or reduced vicinal-SH (4) (30, 31) or reduced vicinal-SH (4) (30, 31) or reduced vicinal-SH (4) (30, 31) or reduced vicinal-SH (4) appears to be more rapid than with DTT alone (Fig. 1). Either or both of the first two of these activating systems are likely to function in vivo and of course DTT is not a naturally occurring substance. DTT is of interest in that it eventually brings about dissociation of glyceraldehyde-P dehydrogenase to subunit size.

It is likely that cholate is effective in bringing about activation and dissociation through its effect in decreasing interaction between hydrophobic domains on the protomers. Although cholate was effective on both activation and dissociation of P-ribulokinase its effects on glyceraldehyde-P dehydrogenase were complicated by the apparent lability of this enzyme in cholate, so that some activation was observed but attempts to detect dissociation were unsuccessful.

Our findings for both enzymes are consistent with their activation being dependent on oligomers (probably tetramers) dissociating to give protomers. The activation/dissociation process may be brought about by nucleotide binding, by sulfur bridge reduction or modification and by the depression of hydrophobic interactions. In vivo activation probably depends on vicinal-SH reduction (4)

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Table III. Comparison of the Estimated Mol Wt of the Glyceraldehyde-P Dehydrogenase and P-ribulokinase

Forms Obtained by Gel Filtration on Sepharose 6B, Bio-Gel A-1.5m, and Sephadex G-100 Columns

<table>
<thead>
<tr>
<th>Gel Filtration Column</th>
<th>Glyceraldehyde-P Dehydrogenase</th>
<th>P-ribulokinase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Oligomer</td>
<td>Protomer</td>
</tr>
<tr>
<td>Sepharose 6B</td>
<td>8.2 \times 10^4</td>
<td>2.2 \times 10^4</td>
</tr>
<tr>
<td>Bio-Gel A-1.5m</td>
<td>7.1 \times 10^4</td>
<td>1.2 \times 10^4</td>
</tr>
<tr>
<td>Sephadex G-100</td>
<td>3.1 \times 10^4</td>
<td></td>
</tr>
</tbody>
</table>
and/or thioredoxin reduction (10) although it is also possible that the binding of naturally occurring nucleotides has an activating role or a stabilizing effect.

The regulation of these two enzymes also involves a complementary deactivation process which is observed when intact leaves are transferred from light to darkness and which has also been reported for intact chloroplasts (4, 14). It may be presumed that deactivation involves protomer aggregation as Pupillo and Piccari (22) were able to bring about both processes with \( \text{NAD}^+ \). We have not been able to demonstrate protomer aggregation in this laboratory but have not used \( \text{NAD}^+ \) as an effector. Comparatively little is known about the mechanism of deactivation of these photosynthetic carbon pathway enzymes, for instance Buchanan and co-workers (10, 30) were unable to elucidate any mechanisms of NADPH-glyceraldehyde-P dehydrogenase deactivation but reported that oxidized glutathione and dehydroascorbate were both effective in deactivating P-ribulokinase (31).

**LITERATURE CITED**