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ROLE OF FERREDOXIN IN THE ACTIVATION OF SEDOHEPTULOSE DIPHOSPHATASE IN ISOLATED CHLOROPLASTS

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Summary

Sedoheptulose 1,7-diphosphatase activity of isolated spinach chloroplasts shows a requirement for (i) reduced ferredoxin and (ii) a protein factor. Activation by ferredoxin, reduced photochemically by chloroplast fragments, was optimal at pH 7.8 and at a Mg^{2+} concentration of 5 mM. The protein factor needed for activation appears to be the same as that required by the chloroplast fructose-1,6-diphosphatase that is activated by reduced ferredoxin. The results indicate that sedoheptulose-1,7-diphosphatase, like fructose-1,6-diphosphatase, is a regulatory enzyme whose activity in chloroplasts is controlled via ferredoxin by light.

We have previously reported that fructose-1,6-diphosphatase (FDPase), a key enzyme of carbon dioxide assimilation in chloroplasts, is activated photochemically by reduced ferredoxin in the presence of a protein factor in accordance with Eqns 1—3 [1,2].

$$2\ \text{Ferredoxin}_{\text{oxidized}} + H_2O \xrightarrow[\text{chloroplasts}]{\text{light}} 2\ \text{Ferredoxin}_{\text{reduced}} + 1/2\ O_2 + 2\ H^+ \quad (1)$$

$$\text{Ferredoxin}_{\text{reduced}} + \text{FDPase}_{\text{inactive}} \xrightarrow[\text{factor}]{\text{protein}} \text{FDPase}_{\text{active}} + \text{Ferredoxin}_{\text{oxidized}} \quad (2)$$

$$\text{Fru-1,6-}P_2 + H_2O \xrightarrow[Mg^{2+}]{\text{active FDPase}} \text{Fru-6-}P + P_i \quad (3)$$

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This discovery led to the finding that Fru-6-*P* formed in the fructose-1,6-diphosphatase reaction activates another enzyme of chloroplasts, ribulose-1,5-diphosphate carboxylase, the enzyme that catalyzes the fixation of carbon dioxide into ribulose-1,5-diphosphate to yield 3-phosphoglycerate [3]. Evidence was also presented that the carboxylase is deactivated by the substrate of the fructose-1,6-diphosphatase reaction, Fru-1,6-P_2. These findings led to the proposal of a regulatory mechanism in chloroplasts by which light, via ferredoxin, controls the activity of the regulatory enzymes of photosynthetic carbon dioxide assimilation [3].

We now report that another enzyme of carbon dioxide assimilation in chloroplasts, sedoheptulose-1,7-diphosphatase (SDPase) is activated by reduced ferredoxin and that, like the fructose-1,6-diphosphatase, its activity is dependent on a protein factor (Eqns 4,5). The results

$$\text{Ferredoxin}_{\text{reduced}} + \text{SDPase}_{\text{inactive}} \xrightarrow[\text{factor}]{\text{protein}} \text{SDPase}_{\text{active}} + \text{Ferredoxin}_{\text{oxidized}} \quad (4)$$

$$\text{Sed-1,7-}P_2 + H_2O \xrightarrow[Mg^{2+}]{\text{active SDPase}} \text{Sed-7-}P + P_i \quad (5)$$

further indicate that the protein factor required to activate the sedoheptulose-1,7-diphosphatase enzyme is identical to the one that is needed in the ferredoxin-fructose-1,6-diphosphatase reaction.

Table I shows the requirements for the release of inorganic phosphate from Sed-1,7-P_2 in a system derived from chloroplasts that contained, in addition to ferredoxin, chloroplast extract (the aqueous chloroplast fraction containing soluble enzymes) and chloroplast fragments (the chlorophyllous fraction containing components needed to photochemically reduce ferredoxin). The release of P_i in this system was strictly dependent on reduced ferredoxin as well as on Sed-1,7-P_2 and Mg^{2+}. The reaction was proportional to the ferredoxin concentration up to 50 μg of ferredoxin and showed an optimal pH of 7.8. In confirmation of the results of Anderson [4], we observed that the nonphysiological sulfhydryl reagent, dithiothreitol, could activate the sedoheptulose-1,7-diphosphatase in the absence of reduced ferredoxin. The products formed by the sedoheptulose-1,7-diphosphatase activated by either reduced ferredoxin or dithiothreitol were shown by thin-layer electrophoresis-chromatography to consist of Sed-1,7-P_2 and P_i [5].

The effect of Mg^{2+} concentration on the ferredoxin-activated sedoheptulose-1,7-diphosphatase of chloroplasts is shown in Fig. 1. The Mg^{2+} concentration required for maximum enzyme activity was about 5 mM. Higher concentrations of Mg^{2+} were inhibitory. It is noteworthy that the sedoheptulose-1,7-diphosphatase was not significantly active at any Mg^{2+} concentration tested in the absence of reduced ferredoxin or dithiothreitol. Such a requirement distinguishes the sedoheptulose-1,7-diphosphatase from the fructose-1,6-diphosphatase enzyme which could be activated at a Mg^{2+} concentration of 2 mM or greater in the absence of other activating agents [2].

TABLE I

REQUIREMENTS FOR FERREDOXIN-ACTIVATED SEDOHEPTULOSE-1,7-DIPHOSPHATASE IN ISOLATED SPINACH CHLOROPLASTS

The complete system contained 0.5 ml of chlorplast extract (equivalent to 0.5 mg chlorophyll, see ref. 1), chloroplast fragments (0.05 mg chlorophyll) heated for 5 min at 55°C to destroy their oxygen-evolving capacity, spinach ferredoxin (0.1 mg), and the following (μmol): Tris · HCl buffer (pH 7.8), 100; neutralized reduced glutathione, 2.5; Sed-1,7-P_2 (sodium salt) (obtained from Sigma Chemical Co., St. Louis Mo.), 1.5; sodium ascorbate, 10; 2,6-dichlorophenol indophenol, 0.1. Final volume, 1.5 ml; gas phase, nitrogen; light intensity, 20 000 lux. The reactions were carried out at 20°C in Warburg vessels containing the Sed-1,7-P_2 in the sidearm. After 5-min equilibration with nitrogen, the vessels were preilluminated for 20 min. Sed-1,7-P_2 was added from the sidearm, and the reaction continued for 20 min. In the last treatment (dark, complete, ferredoxin not reduced), the vessel was kept in the dark throughout the preillumination and reaction periods. The reaction was stopped with 0.2 ml of 3 M $HClO_4$, the precipitated protein was centrifuged off, and an aliquot was used for the P_i determination [2]. Another aliquot was neutralized with 3 M K_2CO_3 and applied to thin-layer plates for separation and identification [5]. Results similar to the above were obtained with freshly prepared unheated chloroplasts in which water rather than ascorbate 2,6-dichlorophenol indophenol served as electron donor.

Treatment	P_i released (μmol)
Light, complete	0.29
Light, ferredoxin omitted	0.05
Light, Sed-1,7-P_2 omitted	0.0
Light, chloroplast extract omitted	0.0
Light, chloroplast fragments omitted	0.01
Light, $MgCl_2$ omitted	0.01
Dark, complete (ferredoxin not reduced)	0.01

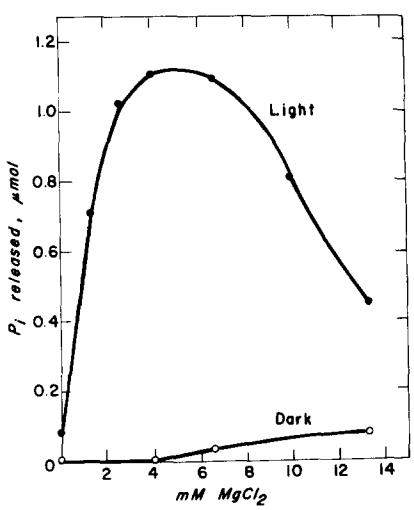

Fig.1. Effect of $MgCl_2$ concentration on ferredoxin-activated sedoheptulose-1,7-diphosphatase. Except for varying the $MgCl_2$ concentration, conditions were as described in Table I.

However, the sedoheptulose-1,7-diphosphatase resembles the fructose-1,6-diphosphatase enzyme in the requirement for a protein factor for activation by reduced ferredoxin. Addition of a partly purified preparation (that showed high protein-factor activity in the ferredoxin-fructose-1,6-diphos-

TABLE II

EFFECT OF PARTLY PURIFIED FERREDOXIN-FRUCTOSE-1,6-DIPHOSPHATASE PROTEIN FACTOR ON ACTIVATION OF SEDOHEPTULOSE-1,7-DIPHOSPHATASE BY PHOTOREDUCED FERREDOXIN

Experimental conditions were as given in Table I.

Treatment	P_i released (μmol)
Chloroplast extract, 0.25 ml	
(1) Control	0.06
(2) + fructose-1,6-diphosphatase protein factor	0.40
(3) + fructose-1,6-diphosphatase protein factor, - ferredoxin	0.04
Chloroplast extract, 0.50 ml	
(1) Control	0.95
(2) + fructose-1,6-diphosphatase protein factor	1.32
(3) + fructose-1,6-diphosphatase protein factor - ferredoxin	0.11

phatase system) increased the activity of the sedoheptulose-1,7-diphosphatase at low levels of chloroplast extract up to 6-fold (Table II). It seems likely, therefore, that the ferredoxin-linked activation of the fructose-1,6-diphosphatase and sedoheptulose-1,7-diphosphatase enzymes may involve similar mechanisms.

In sum, these results provide evidence that the sedoheptulose-1,7-diphosphatase enzyme of chloroplasts, like its fructose-1,6-diphosphatase counterpart, is a regulatory enzyme activated by reduced ferredoxin (cf. refs 4,6). Activation of the sedoheptulose-1,7-diphosphatase is dependent on a protein factor that appears to be the same as that required to activate the fructose-1,6-diphosphatase enzyme. The relationship of the sedoheptulose-1,7-diphosphatase enzyme of chloroplasts that is activated by reduced ferredoxin to the sedoheptulose-1,7-diphosphatase enzyme detected in spinach leaves by Racker and Schroeder [7] remains to be determined.

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References

1 Buchanan, B.B., Kalberer, P.P. and Arnon, D.I. (1967) Biochem. Biophys. Res. Commun. 29, 74—79
2 Buchanan, B.B., Schürmann, P. and Kalberer, P.P. (1971) J. Biol. Chem. 246, 5952—5959
3 Buchanan, B.B. and Schürmann, P. (1973) J. Biol. Chem. 248, 4956—4964
4 Anderson, L.E. (1974) Biochem. Biophys. Res. Commun. 59, 907—913
5 Schürmann, P., Buchanan, B.B. and Arnon, D.I. (1972) Biochim. Biophys. Acta 267, 111—124
6 Pedersen, T.A., Kirk, M. and Bassham, J.A. (1966) Physiol. Plant. 19, 219—231
7 Racker, E. and Schroeder, E.A.R. (1958) J. Biol. Chem. 74, 326—344