

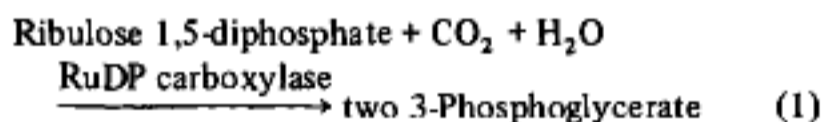
A REGULATORY MECHANISM FOR CO₂ ASSIMILATION IN PLANT PHOTOSYNTHESIS: ACTIVATION OF RIBULOSE-1,5-DIPHOSPHATE CARBOXYLASE BY FRUCTOSE 6-PHOSPHATE AND DEACTIVATION BY FRUCTOSE 1,6-DIPHOSPHATE

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One of the puzzling features of carbon assimilation in photosynthesis is the low affinity for CO₂ of

ribulose 1,5-diphosphate (RuDP) carboxylase in the initial CO₂ incorporation reaction (eq. 1).



Purified RuDP carboxylase shows a K_m for bicarbonate of about 2×10^{-2} M [1-4] and isolated intact chloroplasts carrying out complete photosynthesis show an apparent K_m for bicarbonate of about 0.05×10^{-2} M [5, 6]. We now report evidence for a new mechanism which increases the affinity of RuDP carboxylase for CO₂ (supplied as bicarbonate) *in vitro*

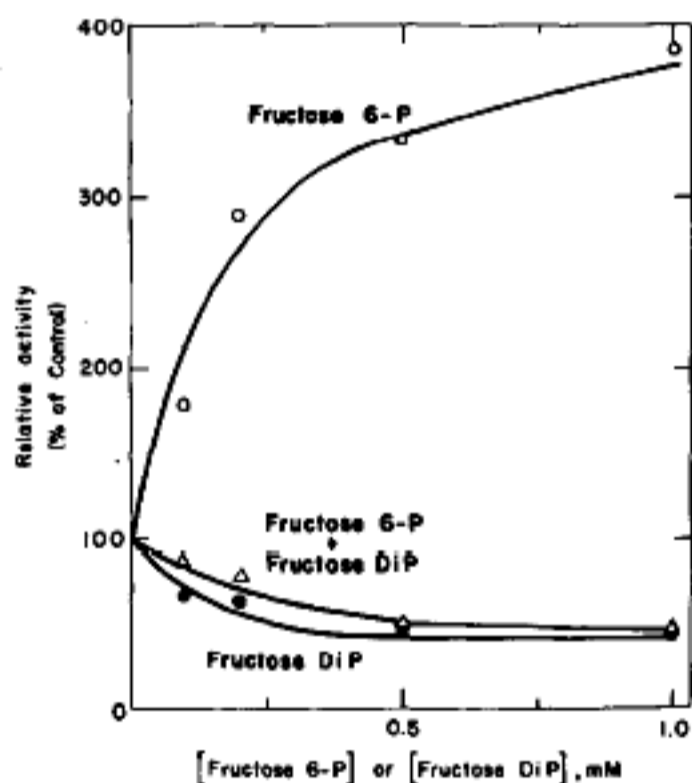


Fig. 1. Effect of fructose 6-phosphate and fructose 1,6-diphosphate on the activity of RuDP carboxylase. The reaction mixture contained the following (added in the order indicated): Tricine buffer, pH 7.5, 200 mM; EDTA, 0.06 mM; MgCl₂, 1 mM; reduced glutathione, 5 mM; RuDP carboxylase (purified to homogeneity by a procedure based on that of Paulsen and Lane [3]), 20 μg; fructose 6-phosphate and fructose 1,6-diphosphate, concentrations as shown; NaHC¹⁴CO₂ (5×10^{-5} cpm μmole), 1 mM; and RuDP, 0.3 mM. Volume, 0.5 ml; temperature, 25°; reaction time, 10 min. The reaction, carried out in scintillation vials, was stopped with 0.05 ml 6 N HCl; samples were evaporated to dryness and the ¹⁴C-phosphoglycerate (PGA) formed was determined in a scintillation counter. The control treatment gave 690 cpm PGA formed. PGA was identified as the reaction product in each of the treatments by thin-layer electrophoresis-chromatography [11].

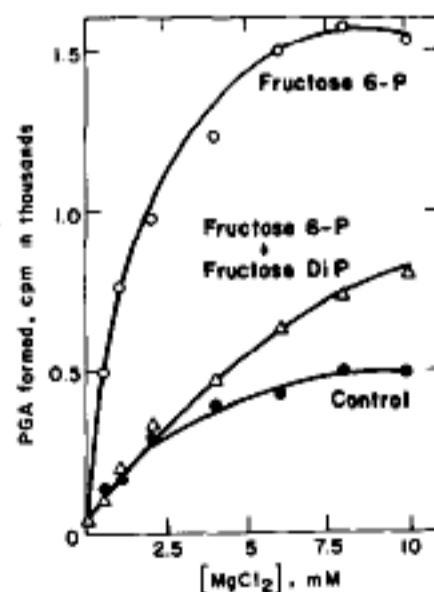


Fig. 2. Effect of [MgCl₂] on RuDP carboxylase activation by fructose 6-phosphate and deactivation by fructose 1,6-diphosphate. Except for varying the MgCl₂ concentration and adding the indicated fructose 6-phosphate and fructose diphosphate at 0.5 mM, conditions were as described for fig. 1.

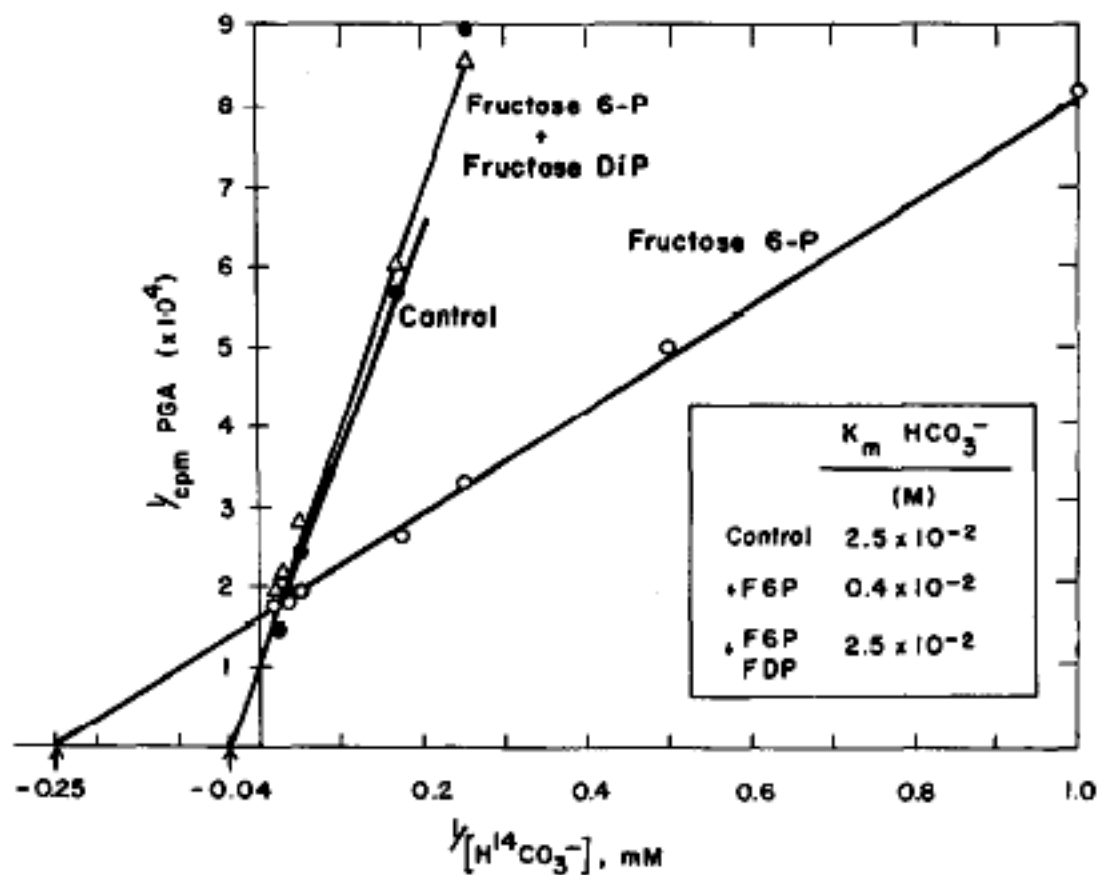


Fig. 3. Effect of fructose 6-phosphate and fructose 1,6-diphosphate on the K_m of RuDP carboxylase for HCO_3^- . Except for varying the HCO_3^- concentration and adding the indicated fructose 6-phosphate and fructose diphosphate at 0.5 mM, conditions were as described for fig. 1.

and which may govern the activity of the enzyme *in vivo*.

Activity of homogeneous preparations of the carboxylase was increased up to 4-fold by 1 mM fructose 6-phosphate (F6P) (fig. 1). The F6P-activated carboxylase was fully deactivated by the addition of fructose 1,6-diphosphate (FDP). The activation by F6P (and deactivation by FDP) was independent of

the concentration of enzyme or RuDP and was observed at MgCl_2 concentrations ranging from 0.5 to 10 mM (fig. 2). The sharpest activation was obtained at 1 mM MgCl_2 .

Activation by F6P was markedly influenced by the concentration of bicarbonate: it was most pronounced at low concentrations and was maximal at 1 mM or less bicarbonate. 1 mM bicarbonate was also found to be optimal for complete photosynthesis by isolated intact chloroplasts.

Evidence that F6P acts by increasing the affinity of RuDP carboxylase for CO_2 (added as bicarbonate) is shown in fig. 3. F6P lowered the K_m of the carboxylase for bicarbonate from 2.5×10^{-2} M to 0.4×10^{-2} M. The addition of FDP to F6P gave the expected return to the original K_m for bicarbonate.

These results indicate that chloroplast RuDP carboxylase is indeed subject to regulatory control (cf. [7, 8]) but that at physiological levels of CO_2 the activity of the enzyme may be controlled by the relative concentrations of F6P and FDP. The levels of F6P and FDP may in turn be regulated by the ferredoxin-dependent FDPase system of chloroplasts that is actuated by light [9, 10]. A regulation

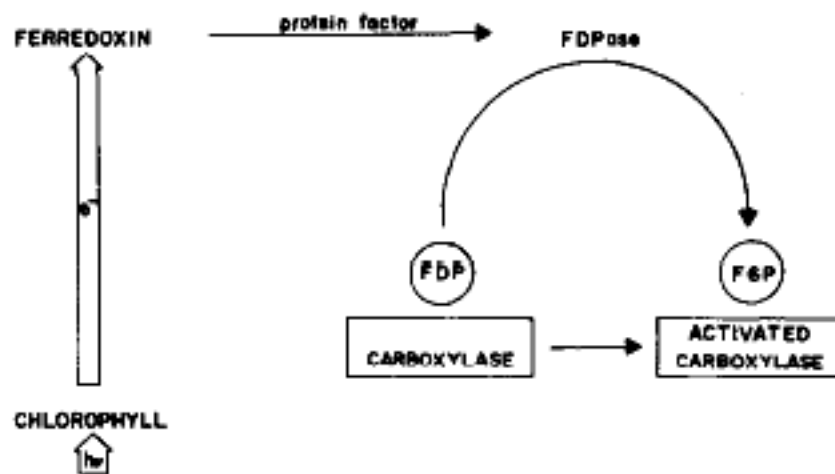


Fig. 4. Role of light in the regulation of fructose diphosphatase (FDPase) and RuDP carboxylase in spinach chloroplast.

mechanism for RuDP carboxylase for which there is now evidence in chloroplasts is summarized in fig. 4.

Acknowledgements

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References

- [1] A. Weissbach, B.L. Horecker and J. Hurwitz, *J. Biol. Chem.* 218 (1956) 795.
- [2] E. Racker, *Arch. Biochem. Biophys.* 69 (1957) 300.
- [3] J.M. Paulsen and M.D. Lane, *Biochemistry* 5 (1966) 2350.
- [4] N. Kawashima and S.G. Wildman, *Ann. Rev. Plant Physiol.* 21 (1970) 325.
- [5] R.G. Jensen and J.A. Bassham, *Proc. Natl. Acad. Sci. U.S.* 56 (1966) 1095.
- [6] M. Gibbs, E. Latzko, R.G. Everson and W. Cockburn, in: *Harvesting The Sun*, eds. A. San Pietro, F.A. Green and T.J. Army (Academic Press, New York and London, 1967) p. 111.
- [7] T.A. Pedersen, M. Kirk and J.A. Bassham, *Physiol. Plant* 19 (1966) 219.
- [8] T. Sugiyama, N. Nakayama and T. Akazawa, *Arch. Biochem. Biophys.* 126 (1968) 737.
- [9] B.B. Buchanan, P.P. Kalberer and D.I. Arnon, *Biochem. Biophys. Res. Commun.* 29 (1967) 74.
- [10] B.B. Buchanan, P. Schürmann and P.P. Kalberer, *J. Biol. Chem.* 246 (1971) 5952.
- [11] P. Schürmann, B.B. Buchanan and D.I. Arnon, *Biochim. Biophys. Acta* 267 (1972) 111.