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edited by

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PREFACE

The Sixth International Congress on Photosynthesis took place from 1 to 6 August 1983, on the Campus of the "Vrije Universiteit Brussel", in Brussels, Belgium. These Proceedings contain most of the scientific contributions offered during the Congress.

The Brussels Congress was the largest thus far held in the series of International Congresses on Photosynthesis. It counted over 1100 active participants. The organizers tried to minimize the disadvantages of such a large size by making maximum use of the facilities available on a university campus. Most contributions were offered in the form of posters which were displayed in a substantial number of classrooms. The discussion sessions, twice a day, four or five in parallel, took place in lecture rooms in the very vicinity of these classrooms. In this way it was attempted to generate the atmosphere of a small meeting. The unity of the subject Photosynthesis was preserved in the ten plenary lectures, organised in such a way that a general overview of two diverse topics was given every day. In addition, there were the five times four parallel symposia dealing with some sixteen general topics.

Every editor of proceedings of a congress is faced with the problem of editing and arranging the contributions, a problem compounded by the wide diversity and the large number of the 753 manuscripts. This editor did very little in the way of editing the papers: all papers were prepared, camera-ready, by the authors themselves and there was no proof-reading. The main reason for this was the need to ensure speedy publication. The contributions are arranged in four volumes but the Proceedings form one set. Although some attempts were made to bring related topics together in one volume, the volumes I to IV should be seen as a succession of chapters, rather than as volumes in their own right. Thus, artificial and arbitrary subdivisions were avoided. A page limit was imposed in order to prevent oversized volumes.

The contributions are arranged in chapters which have no direct relation to the sessions or symposia in which they were presented. The sole criterium for putting a contribution into a certain chapter was its contents. The contributions offered during the Round Table Discussion on Light-Controlled Development of the Photosynthetic Apparatus, July 29 to 30, 1983 in Antwerp, are also included in these Proceedings. They comprise most of the contents of Chapter 7 of Volume IV.

The early publication date of these Proceedings could not have been realised without the efforts of, and the pleasant cooperation with, Mr. Ad Plaizier of Martinus Nijhoff Publishing House. Thanks are due to all Congress members, whose active participation made the Congress a success and these volumes an important document on the state of photosynthesis research. The very much needed assistance of the Local Organizing Committee is gratefully acknowledged. The Photosynthetic Community is indebted to the "Vrije Universiteit Brussel" for making available its premises, facilities and staff. Thanks are also due to the administrative staff of the Congress: secretaries, hostesses, technicians and the two diligent computer programmers, Mr. W. Dierickx and Mr. B. Philips. Special appreciation goes to Ms Blanche van den Haute for her dedicated work in the preparation and the management of the Congress and her help in editing these volumes.

Brussels, March 1984

C. Sybesma, Editor

FRUCTOSE-2,6-BISPHOSPHATE AND C_4 PLANTS

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1. INTRODUCTION

Fructose-2,6-bisphosphate (Fru-2,6-P_2) is a regulatory metabolite that functions in determining the route of cytosolic carbon processing in plant cells--i.e., whether sucrose, the most important plant sugar, is synthesized or broken down (Cséke et al., 1982; Stitt et al., 1982). Our laboratory has recently described an enzyme preparation that catalyzes both the synthesis (via fructose-6-phosphate,2-kinase or Fru-6-P,2K) (Eq. 1) and the breakdown (via fructose-2,6-bisphosphatase or Fru-2,6-P_2ase) (Eq. 2) of Fru-2,6-P_2 in leaves of spinach, a C_3 plant (Cseke,Buchanan, 1983; Cseke et al., 1983).

(1) Fructose-6-phosphate + ATP $\xrightarrow{\text{Fru-6-P,2K}}$ Fructose-2,6-bisphosphate + ADP

(2) Fructose-2,6-bisphosphate + H_2O $\xrightarrow{\text{Fru-2,6-P}_2\text{ase}}$ Fructose-6-phosphate + Pi

Both the synthetic and degradatory activities in the spinach preparation are regulated allosterically by key leaf metabolites in a manner consistent with a role for chloroplasts in determining the fate of cytosolic carbohydrate flux in leaf cells.

Because of their differences from C_3 plants, the question arises about the status of Fru-2,6-P_2 in C_4 species. We have, therefore, investigated this problem and now report results on Fru-2,6-P_2 synthesis and function in Zea mays (corn), a classical C_4 species.

2. MATERIALS AND METHODS

Previously described methods were used for the growth of corn plants and for preparation of extracts for enzyme fractionation (Nishizawa,Buchanan, 1981) as well as for the isolation of mesophyll and bundle sheath cells by differential grinding (Ghirardi,Melis, 1983). The Fru-6-P,2K/Fru-2,6-P_2ase preparation was obtained from extracts by polyethylene glycol (0 to 15%) precipitation followed by DE 52 and hydroxyapatite column chromatography. Pyrophosphate-D-fructose-6-phosphate-1-phosphotransferase (PFP) was isolated from similar extracts by polyethylene glycol (5 to 15%) fractionation followed by DE 52 chromatography. Previously described methods were used for the enzymic assay of Fru-6-P,2K (Cséke,Buchanan, 1983) and for Fru-2,6-P_2ase (Cséke et al., 1983) with spinach leaf PFP as target enzyme.

3. RESULTS AND DISCUSSION

3.1. Regulatory properties of Fru-6-P,2K and Fru-2,6-P_2ase.--As found for spinach, the Fru-6-P,2K preparation used in these studies contained Fru-2,6-P_2ase activity (Cséke,Buchanan, 1983). Also as for spinach, Pi increased the activity of corn leaf Fru-6-P,2K by lowering the $S_{0.5}$ for its fructose-6-phosphate and ATP substrates; the activation by Pi was reversed by 3-phosphoglycerate (PGA), a metabolite transported counter to Pi by

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chloroplasts (Cséke et al., 1983). Fru-6-P,2K was also inhibited by another metabolite transported counter to Pi, viz., dihydroxyacetone phosphate (DHAP). Certain typical intermediates of C_4 photosynthesis (phosphoenolpyruvate, oxalacetate) also inhibited Fru-6-P,2K but others did not (pyruvate, malate). UDP-glucose and sucrose also did not influence the enzyme (data not shown).

The Fru-2,6-P_2ase present in the preparation had a high affinity for Fru-2,6-P_2. Activity with this substrate was higher than related sugar phosphates in the physiological (μM) range. In contrast to Fru-6-P,2K, Fru-2,6-P_2ase showed no requirement for a divalent cation and was inhibited (rather than stimulated) by fructose-6-phosphate. Fru-2,6-P_2ase was also inhibited by metabolites found to inhibit corn leaf Fru-6-P,2K--i.e., phosphoenolpyruvate, DHAP and PGA. Substances which decreased Fru-2,6-P_2ase activity but were without effect on Fru-6-P,2K were UDP-glucose, pyruvate and malate (data not shown).

3.2. Cellular localization of Fru-6-P,2K and Fru-2,6-P_2ase.--The finding of enzymes catalyzing the synthesis and hydrolytic breakdown of Fru-2,6-P_2 raises the question of their cellular location--i.e., whether they are present in the mesophyll or bundle sheath. As shown in Table I, Fru-6-P,2K and Fru-2,6-P_2ase activities were found to occur mainly if not exclusively in the bundle sheath, suggesting that both the synthesis and degradation of Fru-2,6-P_2 takes place in mesophyll cells.

TABLE 1. Cellular localization of Fru-6-P,2K and Fru-2,6-P_2ase in corn leaves

Enzyme	Mesophyll	Bundle sheath
Fru-6-P,2K	11.3	1
Fru-2,6-P_2ase	20	1
Phosphoenolpyruvate carboxylase (mesophyll marker)	300	1
NADP-malate dehydrogenase (mesophyll marker)	8	1
Ribulose-1,5-bisphosphate carboxylase/ oxygenase (bundle sheath marker)	1	4.3

3.3 PFP in corn leaves.--In view of the finding of Fru-6-P,2K and Fru-2,6-P_2ase in corn leaves, the question arises as to its function. We, therefore, undertook an investigation to determine the cellular distribution and properties of PFP previously reported to be present in corn leaves (Carnal, Black, 1983). We found that corn leaves contain two different PFP isozymes, one in the mesophyll and the other in the bundle sheath (Fig. 1). The enzymes were found to differ in charge, in the ratio of their forward to reverse reactions, and in their regulatory properties, but were similar in other respects examined. Both forms of PFP were activated by Fru-2,6-P_2 ($A_{0.5}$ = 0.08 μM), but only the mesophyll enzyme was activated by UDP-glucose and, less effectively, by glucose-1,6-diphosphate (respective $A_{0.5}$ of 0.05 μM and 0.05 mM).

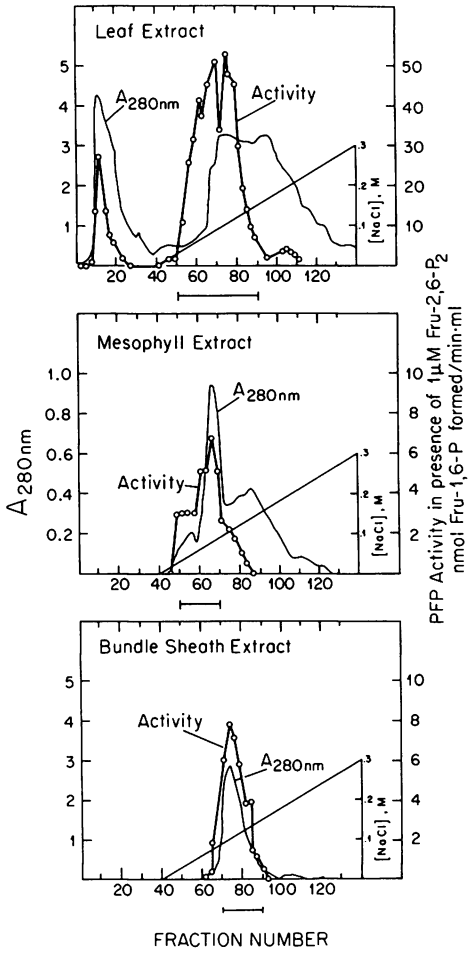

Fig. 1. Demonstration of mesophyll and bundle sheath forms of PFP in corn leaves.

Activation by UDP-glucose was unique in being time dependent (maximal activation was observed after 30 min). Also, it is noteworthy that the reverse reaction (fructose-1,6-bisphosphate hydrolysis) differed from C_3 PFP's in not showing a requirement for P_i or Fru-2,6-P_2 (C. Cséke, unpublished; Van Schaftingen et al., 1982). It is significant that the rate of the reverse reaction relative to the forward reaction was greater with the bundle sheath than with the mesophyll enzyme. Certain properties of the PFP's from C_3 and C_4 sources are summarized in Table II (next page).

4. CONCLUDING REMARKS

The present results provide evidence that corn leaves contain two different forms of PFP, one in the mesophyll cells that is activated by Fru-2,6-P_2, and a second in the bundle sheath that is activated by UDP-glucose, glucose-1,6-diphosphate as well as Fru-2,6-P_2. The PFP isozymes differed in charge and in the ratio of their forward and reverse reaction rates. Because of the confinement of the enzymes catalyzing the synthesis (Fru-6-P,2K) and degradation (Fru-2,6-P_2ase) of Fru-2,6-P_2 to the mesophyll, it would seem that PFP of the bundle sheath is regulated mainly by UDP-glucose. Thus, whereas the pattern of regulation of Fru-6-P,2K and Fru-2,6-P_2ase by metabolite effectors seems clear,

TABLE II. Comparison of PFP's of C_4 and C_3 plants

Property	C_4 (corn) Mesophyll	C_4 (corn) Bundle sheath	C_3 (spinach) Parenchyma
PFP Forward Reaction			
Stimulation by Fru-2,6-P_2	+	+	+
K_a for Fru-2,6-P_2	0.08 uM	0.08 uM	0.012 uM
Maximal Fru-2,6-P_2 activation	20-X	20-X	30-X
Stimulation by UDP-glucose	-	+	-
K_a for UDP-glucose		0.15 mM	
Maximal UDP-glucose activation		20-X	
PFP Reverse Reaction			
Stimulation by Fru-2,6-P_2	-	-	+
Pi requirement	-	-	+
Mg^{++} requirement	-	-	+
Hysteretic	+	+	-

the physiological basis for the differential regulation of the two C_4 PFP's remains to be determined. A related question concerns the basis for the differential rates of the forward and reverse reactions catalyzed by the mesophyll and bundle sheath preparations. Based on the current results, it would seem that the bundle sheath enzyme is ideally suited to function in the reverse direction (sucrose synthesis), whereas the mesophyll enzyme would function mainly in the Fru-2,6-P_2 dependent direction (sucrose breakdown).

5. ACKNOWLEDGEMENT

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