CARBOXYLATION REACTIONS AND PHOTOSYNTHESIS OF CARBON COMPOUNDS IN ISOLATED MESOPHYLL AND BUNDLE SHEATH CELLS OF DIGITARIA SANGUINALIS (L.) SCOP.*

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SUMMARY: Bundle sheath and mesophyll cells were successfully separated from fully developed leaves of Digitaria sanguinalis (L.) Scop. to study the photosynthetic metabolism of these distinct cell types. Ribulose-1,5-diphosphate and ribose-5-phosphate enhanced CO_2 fixation by bundle sheath cells and had little effect on CO_2 fixation in mesophyll cells. In contrast, pyruvate induced CO_2 fixation in mesophyll cells and had no effect in bundle sheath cells. Phosphoenolpyruvate carboxylase is localized in mesophyll cells and ribulose-1,5-diphosphate carboxylase is localized in bundle sheath cells. The results establish that mesophyll cells fix CO_2 by a β-carboxylation while the bundle sheath cells fix CO_2 by carboxylation of ribulose-1,5-diphosphate.

Higher plants can be divided into at least two groups based on such factors as their photosynthetic capacity and photosynthetic carbon metabolism. Plants with a relatively low photosynthetic capacity assimilate CO_2 predominantly by RUDP** carboxylase through the pentose cycle. Plants with a relatively high photosynthetic capacity apparently photoassimilate CO_2 utilizing two carboxylases, PEP carboxylase and RUDP carboxylase. The details of the photoassimilation of CO_2 in these plants are unknown. It has been suggested that RUDP carboxylase and enzymes of the pentose cycle of photosynthesis are located in the chloroplasts of bundle sheath cells and that PEP carboxylase and enzymes of the C_4-cycle are located in the chloroplasts of mesophyll cells of plants with high photosynthetic capacity (1, 2). The relative importance and exact role of these two carboxylases

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**Abbreviations used are: RUDP, ribulose-1,5-diphosphate; R-5-P, ribose-5-phosphate; PEP, phosphoenolpyruvate; TCA, trichloroacetic acid; Chl, chlorophyll; pentose cycle, reductive pentose phosphate cycle; C_4-cycle, C_4-dicarboxylic acid cycle; and Tricine, N-tris-(hydroxymethyl)methylglycine.

in photosynthesis by these plants is not known.

In order to analyze the photosynthetic metabolism of chloroplasts in bundle sheath cells and chloroplasts in mesophyll cells, we have separated the bundle sheath and mesophyll cells of Digitaria sanguinalis (L.) Scop. D. sanguinalis has been identified as a plant with high photosynthetic capacity due to its low compensation point during photosynthesis (3, 4) and the anatomical characteristics of the chloroplasts in bundle sheath and mesophyll cells (5).

METHODS

Digitaria sanguinalis (L.) Scop. was grown in the greenhouse under conditions which provided 5-6000 ft-c of light on sunny days. After 6 to 8 weeks of growth, plants were harvested around midday. The grinding medium used for isolating the leaf cells contained 0.33 M sorbitol, 0.05 M Tricine (pH 8.0), 2 mM $NaNO_3$, 2 mM EDTA, 1 mM $MnCl_2$, 5 mM $MgCl_2$, 5 mM K_2HPO_4, and 2.5 mM dithiothreitol. Mesophyll cells and bundle sheath cells were separated by filtration after macerating leaves. Further detail and microscopic evidence for separation will be given in a manuscript in preparation.

The rate of CO_2 fixation by the two cell types was measured in 15 ml conical bottom tubes in a water bath at 30°. The reaction medium contained in addition to the cells, 4 mM $NaH^{14}CO_3$ having a specific radioactivity of 1 to 2 μc/μmole, and the same concentration of components used in the grinding medium. A small stirrer was placed in the bottom of each tube in order to keep the cells suspended in the medium during experimentation. The tubes were illuminated with 150 watt projector flood lamps producing 3000 ft-c of light at the surface of the bath.

For enzyme assays the leaves or isolated cells were ground in liquid nitrogen RUDP and PEP carboxylase were assayed by measuring the incorporation of $H^{14}CO_3^-$ in the presence of RUDP and PEP, respectively. Chlorophyll was determined in 96% ethanol using the absorption coefficients of Wintermans and De Mots (6).

RESULTS AND DISCUSSION

The mesophyll cell preparation of D. sanguinalis lacked the capacity to

fix substantial quantities of CO_2 in the absence of other substrates. However, 5 mM pyruvate induced a substantial rate of CO_2 fixation when the cells were illuminated. R-5-P has little effect on the CO_2 fixation by mesophyll cells (Fig. 1). Bundle sheath cells fix some CO_2 during illumination without addition of other substrates (Fig. 2) although this endogenous capacity is variable (Table II). R-5-P enhanced the CO_2 fixation by bundle sheath cells during illumination while pyruvate had no effect on the CO_2 fixation (Fig. 2). The enhancement of CO_2 fixation by pyruvate in the mesophyll cells indicates that β-carboxylation may be occurring in the mesophyll cells, and the R-5-P enhancement in the bundle sheath cells indicates that carboxylation of RUDP occurs in the bundle sheath cells.

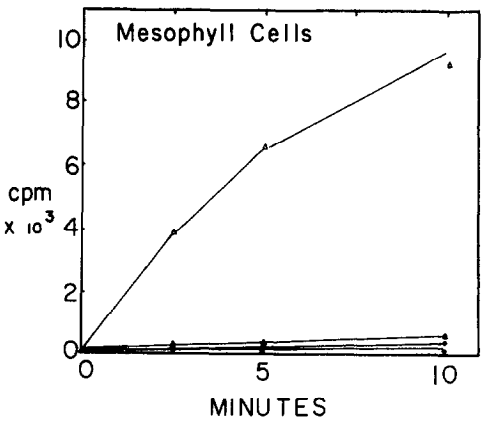

Figure 1. Effect of pyruvate and R-5-P on CO_2 fixation by mesophyll cells of Digitaria sanguinalis.

o——o, light; ▲——▲, light, 5 mM R-5-P; Δ——Δ, light, 5 mM pyruvate; ●——●, dark, 5 mM pyruvate. Rate of CO_2 fixation with light, 5 mM pyruvate was 11.9 μmoles of CO_2/mg Chl/hr. Each treatment had a volume of 225 μl and contained 22 μg of Chl. 50 μl samples were taken at the given time intervals and added to 50 μl 20% TCA in scintillation vials for radioactive counting. Other conditions were as described in METHODS.

Table I shows the levels of PEP and RUDP carboxylase in the whole leaf extracts and in extracts of the two types of cells from D. sanguinalis. RUDP carboxylase is located in the bundle sheath cells while the PEP carboxylase is predominantly in the mesophyll cells. Such differences in enzyme distribution

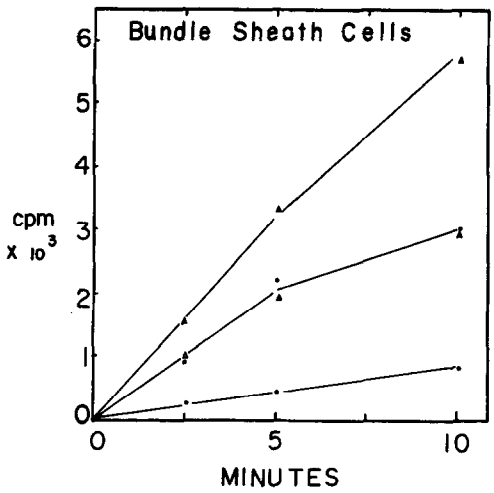

Figure 2. Effect of pyruvate and R-5-P on CO_2 fixation by bundle sheath cells of Digitaria sanguinalis.

o——o, light; ●——●, dark, 5 mM R-5-P; ▲——▲, light, 5 mM R-5-P; △——△, light, 5 mM pyruvate. Rate of CO_2 fixation with light and 5 mM R-5-P was 13.2 µmoles of CO_2/mg Chl/hr. Each treatment had a volume of 225 µl and contained 10 µg Chl. Other conditions as in Figure 1.

TABLE I

LEVELS OF RUDP CARBOXYLASE AND PEP CARBOXYLASE IN MESOPHYLL AND BUNDLE SHEATH CELLS OF DIGITARIA SANGUINALIS

Preparation	Expt. No.	RUDP Carboxylase	PEP Carboxylase
		µmoles of CO_2/mg Chl/hr	
Total Leaf	1	150	250
	2	115	223
Mesophyll Cells	1	12	635
	2	14	740
Bundle Sheath Cells	1	290	55
	2	225	54

suggest that the carbon assimilation during photosynthesis is different between the two types of cells.

TABLE II

EFFECT OF VARIOUS SUBSTRATES ON CO_2 FIXATION BY MESOPHYLL AND BUNDLE SHEATH CELLS OF DIGITARIA SANGUINALIS*

Treatment and Substrate Added	Mesophyll Cells	Bundle Sheath Cells
	µmoles of CO_2/mg Chl/hr	
light, no addition	0.4	1.2
dark, no addition	0.1	1.0
light, PEP	1078	198
dark, PEP	968	183
light, R-5-P	0.4	32.2
dark, R-5-P	0.3	8.1
light, R-5-P, ADP	4.6	67.0
dark, R-5-P, ADP	3.3	28.7
light, RUDP	1.3	34.3
dark, RUDP	1.7	17.0
light, ADP	0.6	11.0
dark, ADP	0.3	1.8

*Concentrations of additives were 2 mM ADP, 4 mM PEP, 5 mM R-5-P, 2 mM RUDP. Each treatment had a volume of 112 µl and contained 5.3 µg Chl for mesophyll cells or 5.6 µg Chl for bundle sheath cells. 40 µl aliquots were taken at 2.5 and 5 min after adding $NaH^{14}CO_3$, and added to 40 µl 20% TCA in scintillation vials for counting.

Table II shows the effect of several substrates on CO_2 fixation by mesophyll and bundle sheath cells of D. sanguinalis. The mesophyll cells have a very high capacity to fix CO_2 when provided with PEP whereas R-5-P and RUDP enhance the CO_2 fixation in bundle sheath cells. Again, the effects of substrates on the two cell types provide some evidence for a β-carboxylation occurring in mesophyll cells and a pentose carboxylation in the bundle sheath cells during photosynthesis.

The enhancement of CO_2 fixation by PEP in the bundle sheath cell preparation (Table II) indicates that part of the PEP carboxylase may be located in bundle sheath cells. The bundle sheath cells which have high RUDP carboxylase activity and possibly the pentose cycle of photosynthesis may be analogous to low photosynthetic capacity plants. Spinach, a low photosynthetic capacity plant, photoassimilates CO_2 predominantly by the pentose cycle and contains PEP carboxylase activity comparable to bundle sheath cells (unpublished data). PEP has been found in other experiments in this laboratory to enhance CO_2 fixation by 60 to 70 μmoles of CO_2/mg Chl/hr in isolated spinach mesophyll cells, either in the dark or light. Comparable PEP carboxylase activity has been reported in a number of plants having low photosynthetic capacity (7).

Baldry, Bucke, and Coombs (8) reported a light-induced fixation of CO_2 by sugarcane (a C_4-cycle plant) chloroplasts with PEP of approximately 2 μmoles CO_2/mg Chl/hr. The PEP carboxylation was not active in the absence of light, and pyruvate and R-5-P did not enhance CO_2 fixation. Whole cell preparations would seem superior to isolated chloroplasts in their capacity to fix CO_2 since less damage to the chloroplasts would be expected. Whole cell preparations also would allow the chloroplasts to function in a more natural state in association with other organelles and cytoplasmic enzymes.

The end products of the substrate enhancement of CO_2 fixation in the two cell types of D. sanguinalis are being analyzed. We can tentatively conclude that the primary end products of the pyruvate and PEP stimulation of CO_2 fixation during illumination of mesophyll cells is oxalacetate (75%) and malate (15%).

Although the relative amount of carbon fixation which occurs in the bundle sheath cells and mesophyll cells in vivo is not known, isolated bundle sheath cells and mesophyll cells of D. sanguinalis have a high capacity to fix CO_2 when provided with certain substrates. It is not yet known if the chloroplasts of the bundle sheath cells and mesophyll cells of high photosynthetic capacity plants are capable of operating a photosynthetic carbon reduction cycle independently or if the cells are dependent upon one another as suggested by Slack and Hatch (1).

This question is under investigation in these leaf cell types. We currently favor independent photosynthetic cycles since in leaves both types of cells accumulate the storage product of photosynthesis, starch (5).

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