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β-CARBOXYLATION PHOTOSYNTHESIS AND PHOTORESPIRATION IN HIGHER PLANTS

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SUMMARY

1. Compared with other species, plants which evolve little CO_2 in the light and fix CO_2 *via* the β-carboxylation pathway of photosynthesis have low glycolate oxidase and high phosphoenolpyruvate carboxylase activity.

2. Glycolate oxidase activity in extracts of plants from both groups was not activated by light or by FMN but was stimulated by incubation with glycolate. Increased O_2 uptake in the presence of FMN and light was found in all extracts, but this was due to a photoreduction of the pigment with the production of peroxide which stimulated the non-enzymic release of $^{14}CO_2$ from labeled glycolate or glyoxylate.

3. Glycolate-oxidase activity reflects an important difference in the pathways of carbon metabolism in the light in these two groups of plants. The data are consistent with the involvement of glycolate as a substrate for CO_2 production during photorespiration. 2-Carbon precursors of glycolate may be important intermediates in CO_2 fixation *via* the β-carboxylation pathway.

INTRODUCTION

Recent surveys have shown that a number of tropical grasses and other species evolve little CO_2 in air under normal conditions and show little enhancement of CO_2 assimilation in zero O_2[1–5]. Much evidence suggests that O_2 consumption and CO_2 evolution in the light (termed photorespiration) in plants such as tobacco involves the metabolism of photosynthetically produced glycolate[6]. Differences between species with respect to photorespiration may thus reflect differences in the rate of synthesis or metabolism of glycolate. The non-photorespiring species have been shown to incorporate CO_2 into oxaloacetate in the light by the β-caboxylation of phosphoenolpyruvate[7,8]. Although the primary carboxylation reaction is now well understood, the transfer of label from oxaloacetate to sugar phosphates is not.

The work reported here was designed to give a better understanding of glycolate metabolism in the light and its possible involvement in photorespiration and in the β-carboxylation pathway of photosynthesis. This communication deals primarily with the metabolism of glycolate in species which do or do not evolve significant quantities of CO_2 in the light under normal conditions. It shows that glycolate-oxidase activity is low in plants which evolve little CO_2 in the light and that this is always associated

with high phosphoenolpyruvate-carboxylase activity, characteristic of the β-carboxylation type of photosynthetic carbon fixation[7]. The reverse correlation is found in plants which produce CO_2 in the light and fix CO_2 *via* reactions of the Calvin cycle. These data support the hypothesis[10] that there is an intimate relationship between the deficient metabolism (and production) of glycolate in the light and the pathway of CO_2 fixation during the β-carboxylation type of photosynthesis.

MATERIALS AND METHODS

Leaves were collected from plants grown in the glasshouse in soil or in water culture (*Atriplex* spp. and spinach). Enzyme activities were assayed in cell free extracts of leaves. 5 g of leaves were taken from glasshouse-grown plants and chilled before blending for 90 sec in a medium consisting of 20 ml 0.1 M phosphate or 0.05 M Tris–HCl buffer at pH 8.0; 5 ml 0.05 M mercaptoethanol; 0.2 ml M $MgCl_2$; and 2 g of polyclar-AT[11] at 2°. The 10000 × *g* supernatant was used as an enzyme source and rates of activity were related to the trichloroacetic acid-precipitable protein content. Phosphoenolpyruvate carboxylase (EC 4.1.1.31) was assayed at 340 nm in the coupled system using endogenous malate-dehydrogenase activity[12]. Malate dehydrogenase (EC 1.1.1.37) was always assayed first and was regularly two or three orders of magnitude more active than phosphoenolpyruvate carboxylase. Internal standards, in the form of a known activity of purified, commercially available enzyme, were used in the malate dehydrogenase assay, and in the assay of other enzymes not reported here. These showed that crude extracts prepared as described contained no interfering substances. Likewise, glycolate-oxidase (EC 1.1.3.1) activity, which was assayed by O_2 consumption using a Rank O_2 electrode, was not significantly altered by partial purification on a Sephadex G-10 column.

The fixation of ^{14}C added as $NaH^{14}CO_3$ was measured by counting acidified aliquots of the reaction mixture on glass filter discs. $^{14}CO_2$ produced from [1-^{14}C]-glycolate or [1-^{14}C]glyoxylate was absorbed on KOH-soaked glass-filter discs and dried at 70°. The discs were counted in toluene–2,5-diphenyloxazole scintillant. The light source used in some experiments was a 250-W Philips Photolita lamp mounted behind a water filter 12 inch from the reaction vessels. Enzymes, substrates and cofactors were obtained from Sigma and Boehringer. Labeled compounds were obtained from the Radiochemical Centre, Amersham, Great Britain.

RESULTS AND DISCUSSION

Plants which fix CO_2 *via* the β-carboxylation pathway of photosynthesis have unusually high phosphoenolpyruvate-carboxylase activity and low ribulose-1,5-diphosphate-carboxylase activity[9]. This distinction is clearly shown in Fig. 1 by the time course of $^{14}CO_2$ fixation in extracts of two representative species of Atriplex with phosphoenolpyruvate and ribulose 1,5-diphosphate as substrates. A further distinction between these plants relates to the production of CO_2 in the light[1–5]. Table I shows the inverse correlation between glycolate-oxidase activity, which may be responsible for the initial reactions leading to CO_2 production in the light[6], and phosphoenolpyruvate-carboxylase activity. Activities of these enzymes in duplicate samples from seven species are given and the relevant data on $^{14}CO_2$ fixation and evolution in the light is

summarised. In these, and thirteen other species examined, high glycolate-oxidase activity was associated with low phosphoenolpyruvate-carboxylase activity and *vice versa*.

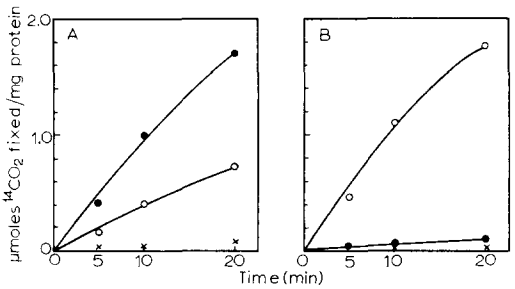

Fig. 1. Time course of $^{14}CO_2$ fixation in extracts of *A. spongiosa* (A) and *A. hastata* (B). The incubation media contained, in 1.2 ml, 1.0 ml extract, 1 μmole of phosphoenolpyruvate, 2.5 μmoles of glutamate and 5 units of aspartate amino transferase (EC 2.6.1.1) (●—●); 1.0 ml extract and 1μmole of ribulose 1,5-diphosphate (○—○); 1.0 ml extract and no substrate (×—×). All contained 2.25 μmoles of $NaH^{14}CO_3$ with approx. 0.5 μC ^{14}C.

TABLE I

ENZYME ACTIVITY IN CELL-FREE EXTRACTS OF LEAVES AND ITS RELATION TO CO_2 FIXATION AND EVOLUTION IN THE LIGHT

Phosphoenolpyruvate carboxylation (nmoles phosphoenolpyruvate/min/mg protein) measured by coupling with endogenous malate dehydrogenase. $NADH^+$ oxidation followed at 340 nm in vessel containing 5 μmoles sodium phosphoenolpyruvate, 100 μmoles Tris–HCl (pH 7.4), 20 μmoles $MgCl_2$, 0.3 μmole $NADH^+$ and 0.1 ml enzyme extract in a total volume of 3 ml.

Species	*Glycolate oxidase**	*CO_2 evolution in light*	*CO_2 assimilation in zero O_2*	*Phospho-enolpyruvate carboxylase*	*β-carboxylation photosynthesis*
Maize	11, 9	Little[8]	Unchanged[3,4]	238, 242	Yes[9]
Sorgum	24, 25	Little[8]	Unchanged[3,4]	250, 370	Yes[9]
A. spongiosa	15, 22	—	—	675, 542	Yes[10]
A. hastata	85	Much[5,7]	Stimulated[5]	30, 29	—
Spinach	99, 114	Much[7]	—	30, 25	No
Tobacco	70, 69	Much[8]	Stimulated[3]	7, 7	No[9]
Wheat	74, 73	Much[8]	Stimulated[3,4]	4, 4	No[9]

* O_2 consumption (nmoles O_2/min/mg protein)measured on addition of 10 μmoles sodium glycolate to 1 ml of leaf extract in 2.3 ml 0.1 M phosphate buffer (pH 8.0).

Plants fell into two groups consistent with this distribution of enzyme activity; those known to produce CO_2 in the light and to fix CO_2 *via* conventional Calvin-cycle reactions and those which are known to produce little CO_2 in the light and to fix CO_2 *via* the β-carboxylation of phosphoenolpyruvate. The different behaviour of the two Atriplex species is of particular interest and these plants will be used in further studies.

Particular attention was given to the glycolate oxidase assay. Endogenous O_2 uptake in all extracts was negligible and that due to the addition of glycolate was almost completely inhibited by 3.3 mM α-hydroxy-2-pyridinemethane sulphonate. Flavin mononucleotide, the prosthetic group of glycolate oxidase, is usually added during assay for this enzyme. FMN was not added to the assays reported in Table I for it was found that the stimulation of O_2 uptake by FMN (0.18 mM) was exactly equal

to the rate of O_2 uptake measured when FMN was added to boiled extracts and was not sensitive to α-hydroxy-2-pyridinemethane sulphonate.

If the activity of glycolate oxidase reflects an important difference in the pathways of carbon metabolism in the light in these two groups of plants, the control of glycolate-oxidase activity becomes of particular interest. This enzyme is reportedly activated by substrate[13] or by FMN, which stimulates CO_2 production when fed to maize plants in the light[14]. All efforts to show that the latter increase in CO_2 production was due to increased glycolate-oxidase activity (O_2 consumption) were unsuccessful. In all experiments FMN failed to raise the rate of O_2 consumption when the rate measured with boiled enzyme + FMN was taken into account.

The large stimulation of O_2 uptake with FMN in the light (Table II) was identical in control and boiled extracts. Preliminary experiments show that light-dependent O_2 uptake with FMN is supported only by blue light (< 500 nm) and is exactly halved in the presence of catalase. This strongly suggests that photoreduction of FMN and production of peroxide are responsible for the observed O_2 uptake. However, in tissues and extracts FMN stimulated $^{14}CO_2$ release from [1-^{14}C]glycolate, particularly in the light (Table II). $^{14}CO_2$ production was always several orders of magnitude slower than O_2 consumption but reflected the activity of glycolate oxidase in the extracts. In the dark $^{14}CO_2$ production was inhibited by α-hydroxy-2-pyridinemethane sulphonate, as was the increased $^{14}CO_2$ production in the light + FMN. The FMN-stimulated release of $^{14}CO_2$ was substantially reduced by the addition of catalase.

The data suggest that CO_2 production from glycolate is controlled, in the first place by relative glycolate-oxidase activity, and in the second by the non-enzymic

TABLE II

PARTIAL REACTIONS OF GLYCOLATE METABOLISM IN CELL-FREE EXTRACTS OF REPRESENTATIVE SPECIES

Reaction conditions	*O_2 uptake* (nmoles/min per mg protein)*	*$^{14}CO_2$ evolution (nmoles substrate/30 min per mg protein) from*	
		[1-^{14}C]Glycolate	*[1-^{14}C]Glyoxylate*
Maize			
Substrate, light	<5	0.4	4.6
Substrate + FMN**	400	9.2	75.5
Substrate + FMN + catalase***	—	5.7	42.7
Substrate + FMN + α-hydroxy-2-pyridinemethane sulphonate§	—	5.8	—
Tobacco			
Substrate, light	73	1.5	6.7
Substrate + FMN	183	39.0	60.5
Substrate + FMN + catalase	—	15.0	23.1
Substrate + FMN + α-hydroxy-2-pyridinemethane sulphonate	—	2.1	—

* Measured as in Table I.

** Reaction mixture contained 1 ml leaf extract, 2.5 μmoles substrate and 0.5 μmole FMN in 1.035 ml.

*** Reaction mixture contained 1 ml leaf extract, 2.5 μmoles substrate, 0.5 μmole FMN, and 200 μg catalase in 1.045 ml.

§ Reaction mixture contained 1 ml leaf extract, 2.5 μmoles substrate, 0.5 μmole FMN and 4 μmoles of α-hydroxy-2-pyridinemethane suphonate.

decomposition of glyoxylate by peroxide[15]. This interpretation is supported by parallel experiments with [1-^{14}C]glyoxylate (Table II) which show marked stimulation of decarboxylation when FMN was added to extracts in the light. Reversal of this decarboxylation by catalase suggests that photoreduction of FMN stimulates the production of peroxide and the non-enzymic destruction of glyoxylate. FMN evidently does not stimulate $^{14}CO_2$ evolution *via* activation of glycolate oxidase.

These observations may explain the FMN effect in intact maize leaves[14], the apparent light stimulation of glycolate oxidase in chloroplast fragments[16], and underline the difficulties in relating CO_2 production from glycolate to glycolate-oxidase activity alone. Light-stimulated O_2 uptake and CO_2 production in the presence of FMN resembles the blue light stimulation of O_2 uptake in Chlorella in which a flavin is evidently involved[17].

The activation of glycolate oxidase in crude extracts by incubation or extraction with substrate is well established[13]. In the present work, extracts from both low and high glycolate oxidase plants have been activated in this way but results are somewhat variable. It is possible, however, that the activity of this enzyme reflects the level of glycolate produced in the tissue during photosynthesis. Thus it is very low in etiolated leaves[13] and is low in plants such as Atriplex and sugar cane which produce little labelled glycolate during photosynthesis in $^{14}CO_2$ (refs. 8, 18). Glycolate-oxidase activity is high in plants, such as tobacco, which are known to produce much glycolate[6].

The correlation between glycolate-oxidase activity and CO_2 production in the light in different species reported here supports the hypothesis that glycolate oxidation is a major component of CO_2 production in the light[6]. This reaction controls the production of glyoxylate, which may subsequently yield CO_2 by enzymic or non-enzymic decarboxylation, or may be metabolised to amino acids. These data also underline the importance of the photo-production of peroxides which stimulate the non-enzymic decomposition of glyoxylate[19]. High temperatures inhibit catalase[20] and the accumulation of peroxides may explain increased CO_2 production at high temperatures[3,6].

The significance of low glycolate-oxidase activity in plants which fix CO_2 *via* the β-carboxylation pathway requires much further study. It is consistent with low glycolate production in these species and suggests that glycolaldehyde, the probable 2-carbon precursor of glycolate, may be otherwise involved during CO_2 fixation *via* the β-carboxylation pathway. It is possible that glycolaldehyde or a related compound is the intermediate involved in the transfer of label from the C_4 position of oxaloacetate to sugar phosphate. Either a 'transcarboxylation' or a condensation reaction could yield pyruvate and hydroxypyruvate. Phosphopyruvate synthase would then regenerate the CO_2 acceptor phosphoenolpyruvate[21], and the action of glycerate dehydrogenase and glycerate kinase could convert hydroxypyruvate to 3-phosphoglycerate. This hypothesis and the metabolism of glycolate and its precursors in these two groups of plants are being investigated.

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OESER *et al.*[22] and other short contributions from N. E. TOLBERT's laboratory confirm the low glycolate oxidase activity in non-photorespiring species, reported here.

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