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EFFECT OF ABSCISIC ACID ON PHOTOSYNTHETIC PRODUCTS OF *LEMNA MINOR*

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Key Word Index—*Lemna minor;* Lemnaceae; duckweed; photosynthesis; ^{14}C distribution; abscisic acid.

Abstract—*Lemna minor* fronds were grown on nutrient only, or nutrient plus 10^{-6} M abscisic acid (ABA) for 2 or 8 days. After various $^{14}CO_2$ pulse-chase time periods, the fronds were harvested and the photosynthetic products separated into acidic, lipid, residue, sugar and amino acid fractions. Compared with the control fronds, total ^{14}C-fixation was 15% higher in the 2 day ABA-treatment and 6% lower in the 8 day ABA-treatment. This pattern was reflected in all the fractions examined, and it appeared that ABA did not alter the distribution of ^{14}C between the photosynthetic products during the $^{14}CO_2$ pulse. During the chase, less ^{14}C was lost from the carbohydrate fractions in the ABA-treated fronds than in the control fronds. The results indicate that the previously reported ABA-mediated increase in carbohydrate levels was a consequence of decreased degradation rather than an increase in synthesis from assimilated carbon.

INTRODUCTION

In recent years abscisic acid (ABA) has been reported to increase starch in guard cells [1], in roots of *Lemna minor* [2] and in whole *L. minor* fronds [3]. In the whole *L. minor* fronds net photosynthetic rate was higher after 2 days growth in 10^{-6} M ABA, however, after 8 days in 10^{-6} M ABA net photosynthetic rate was decreased while high starch and sugar levels were maintained [4]. Since frond multiplication rate and respiration were depressed by ABA treatments [4], the high carbohydrate levels may have occurred due to decreased assimilate utilisation. A second possibility is that ABA altered the flow of assimilated carbon into the carbohydrate pools. ABA has been reported to affect the distribution of carbon between the photosynthetic products of *Pennisetum typhoides* [5]. The present investigation was designed to assess the possibility that: (1) after 2 days growth in ABA, the increased net photosynthetic rate led to an increase in the synthesis of carbohydrates, (2) after 8 days, ABA maintained the high carbohydrate levels by altering the distribution and flow of photosynthetically assimilated carbon.

RESULTS AND DISCUSSION

Total ^{14}C fixation in the control and ABA-treated fronds is shown in Fig. 1. During the $^{14}CO_2$ pulse, fronds from the 2 day ABA treatment fixed 15% more ^{14}C than the control fronds, while the 8 day ABA treatment resulted in 6% lower ^{14}C fixation. This is in agreement with the results found when ^{14}C fixation was determined by total frond combustion, and with net photosynthetic rates measured by IR gas analysis [4]. In the control and 2 day ABA-treated fronds similar total radioactivity levels occurred during the chase, while the radioactivity in the 8 day ABA-treated fronds was consistently lower.

In the acidic fraction (organic acids and phosphate esters) the radioactivity expressed as a percentage of the total was initially high and decreased during the pulse (Table 1), indicating that this fraction contained some of the first labelled intermediates in the assimilatory system. The percentage of the total radioactivity, during the pulse-chase, was similar in the control and ABA-treated fronds, suggesting that ABA did not have a direct effect on the movement of carbon into or out of the intermediates in the Calvin cycle.

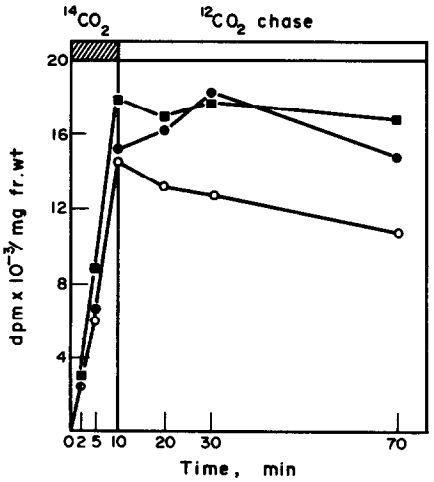

Fig. 1. Total radioactivity, by addition of all the fractions, during a 10 min pulse and subsequent chase. Fronds were grown in the presence of 10^{-6} M ABA for (●) 0 days, (■) 2 days and (○) 8 days. Each value is the mean of 2 replicates.

Table 1. Radioactivity, expressed as a percentage of the total fixed, during a 10 min $^{14}CO_2$-pulse and subsequent chase. Fronds were grown in the presence of 10^{-6} M ABA for 0 days (C), 2 days and 8 days. Each value is the mean of 2 replicates

		$^{14}CO_2$ pulse			$^{12}CO_2$ chase		
		2 min	5 min	10 min	20 min	30 min	70 min
Acidic fraction	C	39	34	29	15	11	11
	2d	49	35	29	15	11	9
	8d	33	34	29	15	12	12
Glucose + fructose	C	11	7	7	10	12	16
	2d	11	9	4	10	10	19
	8d	8	9	5	8	8	11
Amino acids	C	25	22	22	13	7	9
	2d	22	22	23	15	8	7
	8d	24	23	24	15	11	9

The lipid fraction initially contained 1% of the total radioactivity. This increased to 6%, during the 70 min pulse-chase, as would be expected for end products of assimilated carbon. No differences were found between control and ABA-treated fronds.

After treatment of the residue with amyloglucosidase all radioactivity in the residue became soluble and corresponded to glucose by PC. Consequently, all residue radioactivity was considered to be in starch. Figure 2 shows that there was a steady flow of ^{14}C into starch during the pulse and in the case of the control and 2 day ABA-treated fronds this continued during the first 20 min of the chase. In all three treatments there was a loss of radioactivity after a 60 min chase.

The sucrose content of the control was 290 mg/g, while after growth for 2 days and 8 days in 10^{-6} M ABA, the sucrose content was 2750 and 2940 mg/g resp. The starch content of the control was 13.2 mg/g while after growth for 2 days and 8 days in 10^{-6} M ABA the starch content was 19.4 and 35.0 mg/g resp. Due to the increase in size of the starch pool the sp. act. of starch was lower in the ABA-treated fronds. However, the relative changes in sp. act., after a 60 min chase, showed that 2 day and 8 day ABA-treated fronds retained 75 and 67%, respectively, of their sp. act. while the control fronds had lost 46% of their maximum sp. act. (Fig. 2). This indicated that the ABA treatments resulted in a decreased movement of recently fixed carbon out of starch.

The proportion, of the total radioactivity, found in glucose and fructose was similar for the control and ABA-treated fronds (Table 1). The sp. act. data for sucrose (Fig. 3) showed there was no loss of ^{14}C during the chase in the ABA-treated fronds, while the sp. act. of sucrose in the control fronds decreased during the chase. These results indicate that the movement of recently assimilated carbon out of sucrose was decreased, and that the ABA-mediated increase in sucrose levels was a result of decreased sucrose utilisation rather than increased synthesis.

The percentage radioactivity in the amino acid fraction (Table 1) showed that the amino acids contained a high proportion of the total label during the pulse, indicating that ^{14}C was rapidly incorporated into amino acids. Controls and ABA-treated fronds showed a similar pattern throughout the pulse-chase.

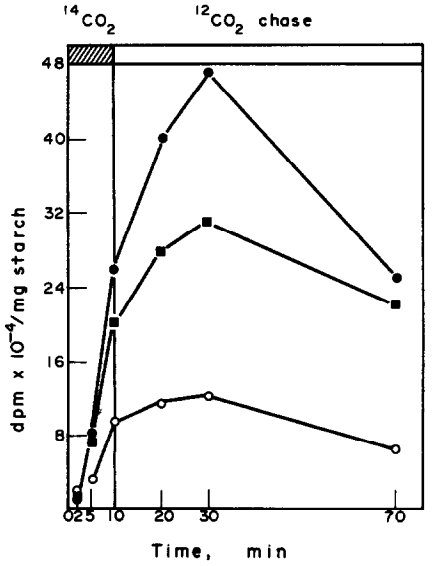

Fig. 2. Specific activity of starch during a 10 min pulse and subsequent chase. Key as in Fig. 1.

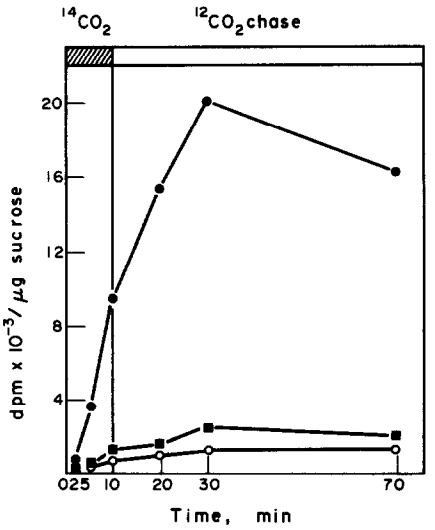

Fig. 3. Specific activity of sucrose during a 10 min pulse and subsequent chase. Key as in Fig. 1.

Quantitative determinations were made on amino acids separated by high voltage electrophoresis. Comparison with standards indicated that 5 radioactive amino acids were aspartic acid, glutamic acid, serine, alanine and glycine. During the $^{14}CO_2$-pulse the proportion of radioactivity in each amino acid was similar in the control and ABA-treated fronds. During the chase, it was found that the proportion of radioactivity in serine dropped by 26% in the control fronds compared with 12% in the ABA-treated fronds.

Overall the results indicated that, in *Lemna*, ABA did not alter the distribution of photosynthetically assimilated carbon as was reported for the C_4 plant *Pennisetum typhoides* [5]. Subsequently, Bauer *et al* [6] have reported that ABA did not alter the distribution of ^{14}C in the photosynthetic products of *L. minor* during a 10 sec $^{14}CO_2$ pulse; they suggest that the ABA-mediated effect found with *Pennisetum* may have been a consequence of changes in stomatal aperture and hence CO_2 concentration at the fixation site.

The data reported here indicate that the higher ^{14}C fixation rate, after 2 days growth in ABA, was reflected in all the fractions examined and did not result in an increase in the flow of assimilated carbon into the carbohydrates. The ABA-mediated increase and maintenance of high carbohydrate levels appeared to be a consequence of decreased degradation rather than increased synthesis.

ABA has been reported to inhibit GA stimulated α-amylase synthesis in alleurone layers of barley [7], and to inhibit α-amylase activity *in vitro* [8]. However, preliminary experiments [3] indicated that, in *Lemna*, ABA did not affect α-amylase but appeared to decrease chloroplast membrane permeability to 3-phosphoglyceric acid. The decreased movement of ^{14}C out of the carbohydrates, the previously reported ABA-mediated decrease in respiration and elevated carbohydrate accumulation [4] may be a consequence of decreased carbon flow from the assimilatory to the respiratory sites, possibly due to ABA effects on the chloroplast membrane.

EXPERIMENTAL

Lemna minor fronds were grown as previously described [4] and 10^{-6} M ABA added to the nutrient 8 or 2 days before further treatment.

$^{14}CO_2$ pulse-chase. Using the apparatus previously described [4], duplicate flasks of ABA-treated and control fronds were exposed to air-containing $^{14}CO_2$ for 2, 5 or 10 min. In the pulse-chase treatments fronds were fed air-containing $^{14}CO_2$ for 10 min followed by 10, 20 or 60 min chasing with air.

Extraction and fractionation. Following harvesting and recording of fr. wt [4], (*ca* 1g per flask), fronds were ground and extracted in hot 80% EtOH. Homogenate was centrifuged and the pellet washed 2× with 80% EtOH. Homogenate was then filtered, residue washed and filtrate combined with the supernatants. Residue and filter paper were combusted in a sample oxidiser and counted by liquid scintillation. Combined supernatants and filtrate were evaporated and taken up in 2 × 4 ml $CHCl_3$–MeOH–H_2O (1:2:1). A sample of the $CHCl_3$ phase was counted by liquid scintillation and was considered to represent the lipid fraction. The total MeOH–H_2O phase was applied to a 10 ml cation exchange column (Dowex AG50 × 8, H^+ form) and the effluent applied to a 10 ml anion exchange column (Dowex AG1 × 8, HCO_3^- form). The sugar, amino acid and acid fractions were obtained from the ion exchange columns [9].

Sugar fraction. The sugar fraction was evaporated, taken up in 1 ml H_2O and a 0.5 ml sample counted by liquid scintillation. The sugars in a 50 μl sample, or standards, were separated by PC in EtOH–HOAc–H_2O (10:5:2) on Whatman no. 1 paper. The radioactive peaks were identified using a strip counter and the corresponding areas counted by liquid scintillation.

Amino acid fraction. The amino acid fraction was evaporated, taken up in 1 ml 0.1 N HCl and a 0.5 ml sample counted by liquid scintillation. Individually labelled amino acids were separated by high-voltage electrophoresis. A 50 μl sample, standards and visual dye marker were run on Whatman no. 3MM paper in HCO_2H–HOAc–H_2O (25:78:897), pH 1.85, for 45 min at 4 kV. Radioactive areas were detected by autoradiography and counted by liquid scintillation.

Residue. Control and ABA-treated fronds were labelled with ^{14}C and the residue extracted as before. To the residue was added 30 mg amyloglucosidase (300 units/g from *Aspergillus*) and 8 ml NaOAc buffer, pH 4.5, and incubated at 60° for 20 hr. The soluble radioactivity was counted by liquid scintillation and a sample run in the PC system previously described. For sp. act., the sugar and starch contents were determined in parallel samples as previously described [4]. Liquid scintillation counting was carried out in a scintillation spectrometer with an activity analyser for quench correction. The scintillant used consisted of toluene: methoxyethanol: PPO, 120:8:1.

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