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RECONSTITUTION OF AMINO ACID SYNTHESIS BY COMBINING SPINACH CHLOROPLASTS WITH OTHER LEAF ORGANELLES

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Abstract—By adding leaf peroxisomes to purified intact chloroplasts, glycine synthesis was reconstituted. On adding leaf mitochondria, serine synthesis was also reconstituted. However, aromatic amino acid synthesis which was effected by purified chloroplasts was not enhanced on adding peroxisomes or mitochondria although the rate in whole leaves was considerably higher.

INTRODUCTION

Unpurified intact chloroplasts isolated according to Jensen and Bassham [1], as well as whole plants and microalgae, rapidly incorporate photosynthetically fixed CO_2 into amino acids. In contrast, purified intact chloroplasts isolated according to Larsson [2] synthesize only small amounts of amino acids [3], although photosynthetic activity is fully maintained.

The aim of the studies presented here is to prove the reconstitution of amino acid synthesis by combining CO_2-fixing chloroplasts with either leaf peroxisomes or leaf mitochondria and to compare this with the synthesis of unpurified chloroplasts [1] and whole plants. Furthermore, this question was extended to aromatic amino acids in view of the compartmentation of the shikimate pathway [4, 5].

RESULTS

Time course of amino acid synthesis in chloroplast suspensions of different purity

To favour optimal conditions for photo-oxidation and formation of glycolate (p_{CO_2}, p_{O_2}, light intensity; for Reviews see [6, 7]), firstly, HCO_3^- was added in amounts sufficient only for 5–10 min of photosynthesis; secondly, the width of cuvettes for illuminating chloroplast suspensions was reduced to 0.1 cm to achieve a high light intensity in suspensions of high density (*ca* 1 mg chlorophyll/ml).

As can be seen in Table 1, fully photosynthetically active purified chloroplasts [2] incorporated fixed CO_2 only in minimal amounts into amino acids, preferentially into Ala. On the other hand, unpurified chloroplasts [1] were able to incorporate considerable amounts of fixed CO_2 (10% on average) into amino acids. In the first minutes of illumination, a relatively high portion of Ala was formed. However, after 10 min the synthesis of Gly predominated. The amino acids in Tables 1 and 2 were fractionated by system I (see Experimental) yielding only the main amino acids (Asn + Glu + Asp), Ser, Gly and Ala. These fractions also included other amino acids (e.g. the aromatic amino acids in

Table 1. ^{14}C-Incorporation from $^{14}CO_2$ (via photosynthesis) into main amino acids of purified [2] and unpurified [1] chloroplasts after light exposure for 6 and 30 min

	Purified chloroplasts		Unpurified chloroplasts	
	Light			
	6 min	30 min	6 min	30 min
Photosynthetic carbon fixation* (μmol CO_2/mg chlorophyll/hr)	189		313	
% Photosynthetically fixed carbon in amino acids	0.013	0.019	0.686	10.92
Carbon fixation Asn + Gln + Asp	12.7	13.5	11.8	4.6
in amino acids Ser†	17.0	16.9	9.4	17.6
(sum = 100) Gly‡	9.8	12.7	33.1	63.4
Ala§	60.5	56.9	45.6	14.6

* Calculated from the 6 min value. Contamination with †Glu; ‡Trp; §Tyr + Phe.

Experimental conditions: vol. 0.575 ml; 0.375 and 0.33 mg chlorophyll in 0.5 ml medium C according to ref. [1]; 10.5 μmol $NaH^{14}CO_3$ (=30 μCi); temp. = 20 ± 2°; light intensity 2 × 10^6 erg/cm^2 sec.

Table 2. ^{14}C-Incorporation from $^{14}CO_2$ (via photosynthesis) into main amino acids by combinations of leaf organelles after light exposure for 30 min

	Purified chloroplasts			Unpurified chloroplasts
	No addition	Plus peroxisomes	Plus mitochondria‖	
Photosynthetic carbon fixation* (μmol CO_2/mg chlorophyll/hr)	146	101	176	161
% Photosynthetically fixed carbon in amino acids	0.20 ±0.10	3.26 ±1.28	2.22 ±0.89	3.99 ±3.49
Carbon fixation in amino acids (sum = 100) Asn + Gln + Asp	9.5	3.8	7.7	2.4
Ser†	13.7	8.3	27.6	13.0
Gly‡	17.5	64.8	30.6	72.0
Ala§	59.4	23.1	34.1	12.6
NADH–hydroxypyruvate reductase (μmol NADH/mg chlorophyll/hr)	12	101	108	58
Cytochrome *c* oxidase (μmol O_2/mg chlorophyll/hr)	0	0.39	2.05	trace
Experimental conditions: all values given in this table were from 4, 3, 4 and 3 experiments, respectively.				
ml vol.	0.575	1.075	1.075	0.575
mg chlorophyll in 0.5 ml medium C [1]	0.42	0.84	0.48	0.60
ml peroxisomes from sucrose gradient	—	0.5	—	—
ml mitochondria from sucrose gradient	—	—	0.5	—

10.5 μmol $NaH^{14}CO_3$ per experiment in 75 μl (=30 μCi); for further details see Table 1.
*, †, ‡, §—See Table 1.
‖ Contaminated with leaf peroxisomes.

minor amounts [4] which were classed in both tables with the main amino acids.

Synthesis of amino acids by combining purified chloroplasts with other leaf organelles

In contrast to purified chloroplasts [2], the reconstituted system consisting of these chloroplasts plus added leaf peroxisomes formed amino acids in comparable amounts to unpurified chloroplasts [2]; this was demonstrated by another series of experiments (Table 2). In this combination, Gly predominated over Ala and Ser. A similar rate of synthesis was obtained by combining purified chloroplasts [2] plus added leaf mitochondria. The mitochondrial fraction contained a relatively large amount of leaf peroxisomes. This might explain the high rate of amino acid synthesis, especially of Ser. Glu, α-KGA, pyridoxal-5-P, each 0.5–1 mM, and 1–5 mM $NaNO_2$ added to the suspensions inhibited rather than stimulated the synthesis. Only a negligible synthesis of amino acids was found in experiments with CO_2 in

Table 3. ^{14}C-Incorporation from $^{14}CO_2$ (via photosynthesis) into amino acids by chloroplasts, combinations with other organelles (both 30 min light) and whole plants (70 min light)

	Purified chloroplasts			Unpurified chloroplasts	Plants
	No addition	Plus peroxisomes	Plus mitochondria†		
Photosynthetic carbon fixation* (μmol CO_2/mg chlorophyll/hr)	57	87	90	10	
% Photosynthetically fixed carbon in amino acids	0.34	1.14	3.72	0.44	1.76
Carbon fixation in amino acids (sum = 100)					
Asn + Gln + Asp	9.3	9.0	11.8	1.8	17.0
Glu	1.3	2.0	1.0	not det.	4.5
Ser	7.6	15.3	14.1	8.4	26.7
Gly	31.3	47.8	16.0	72.5	6.8
Ala	40.3	20.7	49.7	12.9	28.4
Phe	6.0	1.9	5.4	3.0	5.7
Tyr	1.8 } 10.2	1.0 } 5.1	0.7 } 7.3	1.2 } 5.4	5.7 } 16.5
Trp	2.4	2.2	1.2	1.2	5.1

* See Table 1.
† Contaminated with leaf peroxisomes
Exposure of whole plants to $^{14}CO_2$ (100 μCi per plant) was performed as described in ref. [9] except that the plants were enclosed in plastic bags. To prevent $^{14}CO_2$-contamination of roots in the earth, the culture vessels were covered with a layer of plaster coated with cold glue. Experimental conditions for combinations of leaf organelles, see Table 2.

the dark by adding peroxisomes or mitochondria. Osmotically shocked (broken) chloroplasts plus leaf organelles were also inactive. Non-reversible, non-phosphorylating NADP-linked D-glyceraldehyde-3-phosphate dehydrogenase [8] was absent from purified chloroplasts as marker enzyme for cytosol [2].

Synthesis of amino acids with special reference to Phe, Tyr and Trp

In further experiments (Table 3), aromatic amino acids were separated from the other amino acids by rechromatography with system II (see Experimental). Leaves of whole plants incorporated photosynthetically fixed CO_2 into aromatic amino acids 2 to 3 times faster than that of chloroplasts and their combinations with other organelles (see sum of aromatic amino acids in Table 3).

DISCUSSION

The reconstitution of amino acid synthesis suggests metabolic interactions between chloroplasts, peroxisomes and mitochondria working not only in the cell but also after combining the isolated organelles. This shows that unpurified chloroplasts [1] contaminated with peroxisomes and mitochondria are able to work as well as 'multi-organelle complexes' (peak III-chloroplasts) [2] present to some extent in these suspensions. The transport of substrates of glycolate metabolism [6, 7] from organelle to organelle seems to be no problem even in suspensions. For this reason, concentration of each amino acid depends largely on the quota of participating organelles.

It can be inferred from the results of the above experiments that at least a part of the shikimate pathway is localized in plastids. This agrees with results of recent studies [4, 5] using the method of preserving the substrates within the chloroplasts by lowering the translocation rate across the envelope (for reviews on this topic see [10, 11]). A plastidic isoenzyme of shikimate dehydrogenase has also been identified [12]. On the other hand, it is assumed from the considerable synthesis of aromatic amino acids in leaves of whole plants that this also takes place in compartments other than plastids.

EXPERIMENTAL

Isolation of intact spinach chloroplasts was according to refs. [1] and [2].

Isolation of peroxisomes and mitochondria from spinach leaves. The supernatant of isolation procedure for chloroplasts (in medium A [1]) was centrifuged at 10000 *g* (10 min, 4°). The pellet obtained was suspended in 25% sucrose and layered on a 5 ml tube containing a continuous gradient from 60 to 25% sucrose. (Preparation of gradient: a tube containing 2.5 ml 60% sucrose with 2 mM EDTA (at the bottom) plus 2.5 ml 25% sucrose with 2 mM EDTA (at the top) was sealed with Parafilm and deposited horizontally for 17 hr at 4°.) The layered gradient was centrifuged at 80000 *g* (at the surface of soln; 4.5 hr; 4°). After this, 0.5 ml fractions were drained from the bottom of the gradient and were assayed for NADH–hydroxypyruvate reductase and cytochrome *c* oxidase as marker enzymes for leaf peroxisomes and mitochondria, respectively. Particle separation by this procedure was obtained as in ref. [13].

Enzyme assays were performed as described in the following refs.: NADH–hydroxypyruvate reductase (EC 1.1.1.29) in [14]; cytochrome *c* oxidase (EC 1.11.1.5) in [13] but conc of reduced cytochrome at time $t = 0$ was only 25 μM; and non-reversible, non-phosphorylating NADP-linked D-glyceraldehyde-3-phosphate dehydrogenase (EC 1.2.1.9) in [8].

Isolation and determination of amino acids as dansyl derivatives see ref. [4, 15]; system I was Si gel with C_6H_6–Py–HOAc (20:5:1); system II was micropolyamide with H_2O–HCO_2H (100:3).

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