INTRACELLULAR DISTRIBUTION OF THE EARLY PRODUCTS OF PHOTOSYNTHESIS

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One of the most important but least known aspects of the relationship between the chloroplast and rest of plant cell concerns the exchange of material between the plastid and its cytoplasmic environment. The intracellular fate of the early products of carbon fixation is unknown. Similarly the use by extraplastid reactions of photosynthetically produced ATP can only be inferred (Arnon, 1961) since direct measurements in vivo of exchange of ATP or TPNH between the chloroplast and hyaloplasm have not been made.

Except for the early observation by Frenkel (1941) that chloroplasts isolated in 0.5 M sucrose from Nitella that had been fed $C^{11}O_2$ for 25 minutes retained 80% of the fixed carbon, no attempts to determine the intracellular distribution of early photosynthetic products have been reported.

Kearney and Tolbert (1962) suggest, on the basis of experiments with spinach chloroplasts isolated in 0.35 M NaCl and subsequently fed $C^{14}O_2$, that a cyclic process for the transport of carbon products and assimilatory power of photosynthesis between the plastids and cytoplasm occurs. They concluded that phosphoglycolate was actively excreted from the chloroplasts, hydrolyzed to glycolate in the cytoplasm, and subsequently oxidized to glyoxalate. They believe that glyoxalate can re-enter the plastid.

Chloroplasts isolated in sucrose or salt solutions are subjected to severe leaching. When these chloroplasts are thrown into an artificial environment, unavoidable changes in permeability occur.

Compounds which are synthesized by isolated chloroplasts and which move out of these chloroplasts are not subjected to further metabolism in the normal sense. Plastid fragments contained within the plastid preparation are capable

of synthesizing carbon compounds that would diffuse into the surrounding medium. Because of these reasons, the nonaqueous method of isolating chloroplasts (Heber, 1957; Stocking, 1959) was studied for its usefulness in determining the intracellular movement of the early products of photosynthesis.

Method

Fully expanded leaves from greenhouse-grown tobacco plants (Nicotiana rustica L.) that had been kept in the dark for 36-48 hours were used. Plants were placed in the light for about one hour immediately before the experiment. Excised leaves were fed C^{14}-labeled CO_2 for times ranging from 5 seconds to 10 minutes. The exact light and dark sequence to which the leaves were subjected was varied in different experiments. Immediately after the feeding, the leaf was plunged into liquid nitrogen and then lyophilized at -18°C until dry. The time lapse between the end of the treatment and the complete freezing of the leaf was four or five seconds.

Plastids were separated from the more dense cellular materials by the nonaqueous method with hexane/carbon tetrachloride as the suspending medium (Stocking, 1959). Chlorophyll concentration and the C^{14} level in each fraction was determined. The magnitude of chloroplast contamination in the "nonchloroplast" fraction was determined by chlorophyll analyses and appropriate corrections were made. Less than one per cent of the total carbon fixed appeared in the supernatant after complete sedimentation of particles. This indicated that the early fixation products were essentially insoluble in the suspending medium and appreciable migration of these compounds during isolation of the plastids would not have occurred.

After isolation of the chloroplasts, the ethanol and water-soluble compounds in each fraction were separated by paper chromatography (Bassham and Calvin, 1957).

Results and Discussion

The severe leaching effect of isolating chloroplasts in 0.35 M NaCl was demonstrated when chloroplasts were isolated from sugar beet leaves fed $C^{14}O_2$ for 10 minutes prior to isolating in the aqueous solution (Table 1).

Table 1

Distribution of C^{14} in Fractions Isolated from Leaves Treated with $C^{14}O_2$

Fraction	% of recovered activity
0.35 M NaCl isolation (Sugar beet leaves, 10 minute feeding)	
200xg 1 minute precipitate	
Cellular debris, nuclei, cell walls	0.6
1000xg 7 minute precipitate	
Unwashed chloroplasts	2.6
1000xg 7 minute supernatant	
Nonplastid cytoplasmic and vacuolar material	96.8
Once washed chloroplasts	1.2
Wash solution	1.4
Nonaqueous isolation (Tobacco leaves, 10 minute feeding)	
Chloroplast fraction	54.3
Nonchloroplast fraction	45.3
Solvent	0.4

Sugar beet leaves normally do not form appreciable starch, and in this case less than three per cent of the total C^{14} fixed was retained in unwashed chloroplasts. About half of this activity was removed by one washing. These results are in contrast to those reported by Frenkel for Nitella. In Frenkel's experiment, radioactivity was probably retained in the chloroplasts in the form of insoluble starch grains formed during the relatively long feeding period in Nitella.

In contrast to the very low amount of C^{14} retained in the aqueously isolated chloroplasts, approximately half of the C^{14}-labeled products of 10 minute photosynthesis was retained by chloroplasts isolated nonaqueously from tobacco leaves. About 10 per cent of the label in this chloroplast fraction was insoluble in hot ethanol and water and was presumably starch.

The amount of the total fixed C^{14} that was retained by chloroplasts isolated nonaqueously from tobacco leaves treated in the light with $C^{14}O_2$ from 5 seconds to ten minutes is shown in Table 2.

Table 2

Intracellular Distribution of Radioactivity in Tobacco Leaves Treated with $C^{14}O_2$ in the Light for the Times Indicated

Time of treatment	Per cent of total C^{14} fixed	
	In chloroplasts	In nonchloroplast material
5 secs.	81.0	19.0
	77.5	22.3
	75.4	24.6
	71.2	28.6
	57.5	43.4
10 secs.	62.0	37.4
	55.7	43.9
	42.0	57.5
20 secs.	70.2	28.8
	67.9	31.9
	62.6	36.5
	61.5	37.9
	59.4	40.7
	53.0	46.1
40 secs.	68.2	31.0
	63.2	36.1
	47.4	52.2
60 secs.	55.1	44.5
	49.9	49.7
90 secs.	48.7	50.8
	43.0	56.6
	33.6	65.6
120 secs.	53.4	46.2
	50.6	49.0
10 min.	54.3	45.3

Prior to the $C^{14}O_2$ feeding, the leaves were illuminated in a closed chamber without added CO_2 for 5 minutes. This treatment would have reduced the CO_2 level in the chamber and would have resulted in the photosynthetic buildup of ATP and TPNH in the cells just prior to the $C^{14}O_2$ feeding. After 60 seconds of photosynthesis, approximately 50% of the newly formed carbon compounds are found outside of the chloroplasts. As would be expected, the per cent of the

total fixed carbon that is present in the chloroplasts at the end of the treatments increased with decreasing time of treatment. At 5 seconds, on the average, at least 70% of the fixation products were present in the chloroplasts.

The variability of the results reported within one time period is indicative of the difficulty and possible limitation of the method. Although study of the isolated chloroplast fractions with the phase microscope, or light microscope, failed to reveal significant visible contamination (Stocking and Ongun, 1962), this does not rule out the presence of variable minor cytoplasmic contamination. Probably of more importance as far as the usefulness of the method is concerned is the fact that leaves are composed of a wide variety of cells some of which photosynthesize but many of which do not. Even within the population of photosynthesizing cells a wide difference in the rate and amount of $C^{14}O_2$ fixed within the shorter time series undoubtedly exists. Thus the results of analyses of a gross separation of chloroplasts may reflect the average distribution of radioactivity between the plastid and nonplastid leaf material but, especially under short feeding experiments, may not represent the true intracellular distribution. Use of more uniform photosynthesizing cells such as synchronized algal cultures should obviate some of the difficulty caused by this type of variability.

In spite of the variability encountered the results show that a very rapid migration of carbon compounds occurs from the chloroplast into the cytoplasm. Chromatography of the chloroplast and nonchloroplast fractions should yield information on the site of synthesis and rates of movement of specific compounds. After a 20 second treatment with $C^{14}O_2$, sucrose appears in the chloroplast fraction only (Fig. 1). The origin of the chromatograms is at the lower right with phenol/water in the horizontal and butanol/propionic acid/water development in the vertical directions. Appreciable sucrose did not appear in the nonchloroplast fraction until 1 to 2 minutes after $C^{14}O_2$ feeding started. Its appearance here could be explained as resulting from either a diffusion from the plastid or from a secondary synthesis occurring outside of the chloroplast. These possibilities are being investigated.

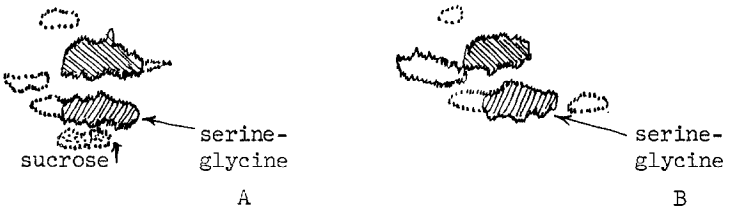

Fig. 1. Tracing of photograph of radioautographs from chloroplast and nonchloroplast fractions isolated nonaqueously from tobacco leaves after 20 seconds of feeding with $C^{14}O_2$. A. Chloroplasts. B. Nonchloroplast material. Phosphorylated compounds not shown.

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