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Short Communication

Studies on the Kinetics of Photosynthetic Products Synthesized by Different Preparations of Intact Spinach Chloroplasts[1]

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The earliest attempts to demonstrate CO_2 fixation by isolated chloroplasts resulted in rates considerably lower than those supported by the intact leaf. While leaves may have a fixation capacity in the order of 180 μmoles/mg of chlorophyll·hr, the rate obtained by chloroplasts isolated in 0.35 M NaCl was usually less than 5% of this value (1-3, 6). Then, Walker (11) observed rates as high as 25% of those of the intact leaf by substituting 0.33 M sorbitol for 0.35 M NaCl in the isolation medium. Also with sorbitol, Jensen and Bassham (7) obtained a chloroplast preparation capable of fixing CO_2 at a rate equal to that of the intact leaf. Both laboratories concluded, on the basis of microscopical observations, that chloroplasts isolated in sorbitol, in contrast to those prepared in NaCl, retained an intact membrane and possibly did not lose soluble proteins. However, the specific activities of the enzymes catalyzing the reductive pentose phosphate cycle were found by Gibbs *et al.* (5) to be identical in both kinds of chloroplasts.

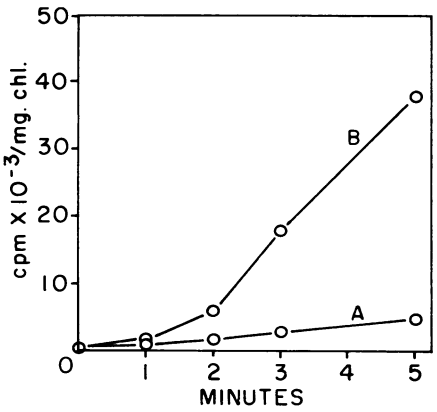

FIG. 1. Progress curves of CO_2 fixation by two spinach chloroplast Preparations. A: Prepared in NaCl; B: prepared in sorbitol.

The reason for the difference in the rates of photosynthesis between the two kinds of preparations remains unclear. It is possible that chloroplast preparations fixing CO_2 at rates approaching those of the intact leaf were merely a larger population of chloroplasts which retained their structural integrity, or that the new preparation method enabled additional fixation abilities or opened up new pathways. The present work was, therefore, concerned with a comparison of the CO_2 fixation processes of the two chloroplast preparations, namely a NaCl preparation suggested by Allen *et al.* (1) and modified by Gibbs and Calo (3) and a sorbitol preparation isolated according to the procedure of Jensen and Bassham (7). The kinetics of CO_2 fixation and photosynthetic products synthesized were studied during the early stages of $^{14}CO_2$ fixation. In addition, the distribution of the tracer within several compounds was examined.

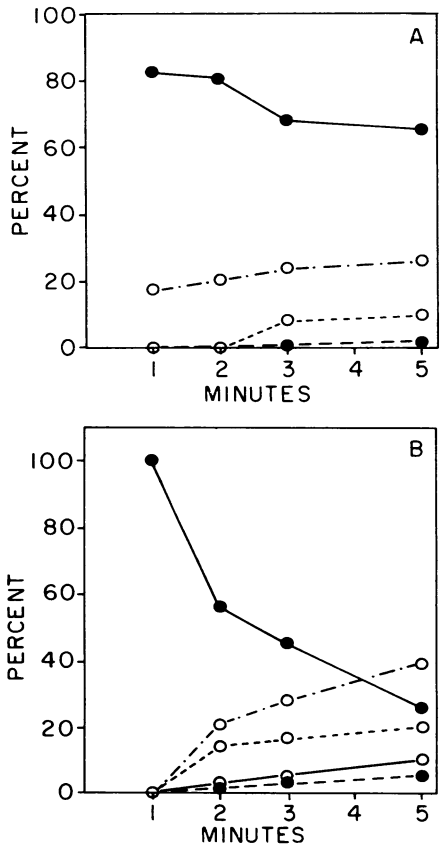

FIG. 2. Kinetics of photosynthetic products formed by two spinach chloroplast preparations. CO_2 fixation was conducted with chloroplasts prepared either according to Gibbs and Calo (3) (A) or to Jensen and Bassham (7) (B). The chloroplasts were incubated in 2-ml reaction mixtures at 20 C under N_2 and were illuminated at 4000 ft-c. At the designated time intervals, an aliquot of the reaction mixture was subjected to paper chromatography. The data represent the radioactivity detected on the paper chromatograms. ●——●: Glycerate-3-P; ○—·—○: dihydroxyacetone-P; ○——○: sugar-P; ○----○: glycolate; ●----●: starch.

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Chloroplasts were isolated from spinach leaves within 1 hr after the plants were harvested from a local commercial field. Two batches of chloroplasts were prepared simultaneously, one according to Gibbs and Calo (3) and the other, according to Jensen and Bassham (7). The 2-ml reaction mixtures were gassed by slowly bubbling N_2 before and after the addition of the chloroplasts, which contained 60 to 120 μg of chlorophyll. The experiments were performed in glass tubes (1.5 × 20 cm) at 20 C, which were illuminated from opposite sides with an intensity of 4000 ft-c.

CO_2 fixation rates and products were determined from samples which were taken from the reaction mixture and pipetted into concentrated formic acid to yield a final concentration of 4% formic acid. Products were separated by means of paper chromatography as described earlier (9).

The distribution of ^{14}C was determined in glycerate-3-P, dihydroxyacetone-P, and starch by the following procedure. Glycerate-3-P and dihydroxyacetone-P were eluted with water from the chromatograms; however, the starch spots were cut out, hydrolyzed while on paper with boiling 1 N HCl, and then extracted. HCl was then removed from the extract by placing the samples in a vacuum dessicator with NaOH pellets and P_2O_5. D-Glucose was added as carrier to the hydrolyzed starch samples which were then degraded by fermentation with *Leuconostoc mesenteroides* (4). Glycerate-3-P was incubated with potato phosphatase, and the glyceric acid formed was purified by paper chromatography with 1-butanol-propionic acid-water (6:3:4) as solvent, was located on the paper by autoradiography, and was then eluted by H_2O. Dihydroxyacetone-P was first converted into glycerate-3-P by incubation with triose-P isomerase and glyceraldehyde 3-P dehydrogenase. Carrier glyceric acid was added to the samples, and the degradation was conducted according to the method of Sakami (10) for serine.

A striking result (Fig. 1) of the present study is the 12-fold increase in CO_2 assimilation by chloroplasts prepared in sorbitol, compared to that by chloroplasts prepared in NaCl. The chloroplasts that were photosynthesizing at the lower rate attained a steady state of product formation almost immediately (Fig. 2A). In contrast, chloroplasts that fixed CO_2 at the higher rate did not reach a steady state distribution of radioactive CO_2 among the photosynthetic products during the experimental period (Fig. 2B). The definite lag in the CO_2 fixation rate in the sorbitol-prepared chloroplasts and the lesser one in the other preparation may be associated with the high concentration of newly formed glycerate-3-P. When the lag period in photosynthetic rate was ended, a fast conversion of glycerate-3-P into triose-P and sugar phosphates took place. This observation is in agreement with the report of Latzko and Gibbs (8), who noted that the lag in chloroplast photosynthesis was terminated when a certain level of pentose monophosphate was reached.

The continuing high level of radioisotope in glycerate-3-P in saline-prepared chloroplasts could have been the result of a slowly functioning reductive pentose phosphate cycle. This possibility was tested by determining the location of ^{14}C in the carbon atoms of glycerate-3-P, dihydroxyacetone-P, and the water-insoluble polyglucan (most likely starch). The movement of isotope through the carbon atoms of glycerate-3-P and dihydroxyacetone-P was actually faster in chloroplasts photosynthesizing at the lower rate, particularly in the early samples (Table I). Clearly, a higher fixation rate should have resulted in an increased turnover rate of the intermediates of the cycle, and concomitantly, a faster spread of isotope in the intermediates. Inasmuch as this was not found, we conclude that chloroplasts prepared in sorbitol contain a higher level of unlabeled carbon compounds which eventually are converted to ribulose 1,5-diphosphate. This ribulose 1,5-diphosphate acts as unlabeled carrier material and

Table I. *Distribution of ^{14}C in Glycerate-3-P, Dihydroxyacetone-P, and Starch Glucose Formed during Photosynthetic CO_2 Fixation by Two Spinach Chloroplast Preparations*

These compounds were isolated from the incubation mixtures, the progress curves of which are given in Figure 1. The carbon content is based on COOH, CH_2OH, or C-4 = 100. The actual value of COOH, CH_2OH, or C-4 in millimicrocuries per milligram of carbon is given in parentheses.

Compound Degraded	Chloroplasts Prepared in Sorbitol[1]				Chloroplasts Prepared in NaCl[2]			
	^{14}C content after reaction time of:							
	1 min	2 min	3 min	5 min	1 min	2 min	3 min	5 min
Carbons of glycerate-3-P								
COOH	100(1.04)	100(1.12)	100(2.77)	100(5.80)	100(0.23)	100(0.48)	100(0.53)	100(0.98)
CHOH	29	49	62	77	86	95	85	98
CH_2OP	25	53	64	76	49	67	71	91
Carbons of dihydroxyacetone-P								
CH_2OH	...[3]	100(0.71)	100(1.20)	100(2.90)	...	100(0.25)	100(0.34)	100(0.48)
C = O	...	40	75	87	...	82	85	92
CH_2OP	...	37	77	84	...	67	78	90
Carbons of starch glucose								
C-1	...	...	34	52	...	...	50	71
C-2	...	...	21	86	...	...	44	43
C-3	...	...	83	95	...	...	81	85
C-4	...	...	100(0.12)	100(1.90)	...	...	100(0.07)	100(0.16)
C-5	...	...	34	77	...	...	25	54
C-6	...	...	38	72	...	...	37	59

[1] According to Jensen and Bassham (7), rate of photosynthesis was 67 μmoles/mg of chlorophyll·hr.

[2] According to Gibbs and Calo (3), rate of photosynthesis was 5.8 μmoles/mg of chlorophyll·hr.

[3] Not degraded; radioactivity was too low.

dilutes out the labeled products formed from $^{14}CO_2$ in the early time periods. A similar decrease in the rate of tracer distribution among the carbon atoms of glycerate-3-P was demonstrated earlier when unlabeled fructose 1,6-diphosphate was added to a medium that contained photosynthesizing NaCl-prepared chloroplasts (2).

The distribution of ^{14}C in polyglucan glucose was less affected by the chloroplast preparation. This is most likely the result of a slower incorporation of isotope into the polysaccharide. As in a previous study (6), the distribution of label was asymmetric.

In summary, there is little doubt that chloroplasts prepared in sorbitol assimilate CO_2 at a much higher rate than those isolated in NaCl. However, the chloroplasts inferior in rate may be better suited for kinetic studies because of an apparent lack of high, and possibly variable, endogenous levels of intermediates of the reductive pentose phosphate cycle. We conclude that it is this higher endogenous level of intermediates that is a contributing factor to the observed differences in photosynthetic rates. However, these levels cannot be the sole factor since addition of ribose 5-phosphate or fructose 1,6-diphosphate to chloroplasts prepared in NaCl did not increase their photosynthetic rate to the rate obtained with sorbitol-prepared chloroplasts isolated from the same leaves (data not shown). Finally, it seems safe to conclude from the chromatograms and from the isotopic distribution data that both of the chloroplast preparations are assimilating CO_2 by the same pathway.

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