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Short Communication

Photosynthetic Assimilation of Carbon Dioxide and Acetate by Isolated Chloroplasts[1]

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Chloroplasts isolated by methods similar to that described by Arnon et al. (1) are capable of incorporating acetate carbon into both lipid and non-lipid materials when fortified with appropriate cofactors (2, 3, 4, 5). The actual rate of incorporation of acetate carbon in these preparations was, however, very small compared with the normal rate of photosynthesis (1–2 μmoles/mg chlorophyll·hr) by such chloroplasts (1). In the few cases where the rate of incorporation of acetate carbon can be computed (2, 5) an upper limit of approximately 6 mμmoles/mg chlorophyll·hour was found. Concurrent rates of CO_2 fixation were not published. Jensen and Bassham (6, 7) have recently prepared chloroplasts capable of fixing CO_2 at rates in excess of 100 μmoles/mg chlorophyll·hour. We have looked for acetate utilization in chloroplasts prepared by this method and have fractionated the products into water soluble and water insoluble and lipid fractions. The products of photosynthesis using ^{14}C-labeled bicarbonate have been fractionated in a similar way in order to compare acetate and CO_2 as substrates for lipid synthesis, in the same chloroplasts. No attempt in either case was made to fractionate further the lipid fraction. Where this has been done in previous work with broken chloroplasts (5, 6) more than half of this isotope incorporated from labeled acetate was shown to be in fatty acids, with a small fraction in glycerides. In our experiments, chloroplasts were capable of CO_2 fixation at a maximal rate of 96 μmoles/mg chlorophyll·hour under N_2 and were able to continue photosynthesis for up to 1 hour at slightly reduced rates (table I). Air inhibited the rate of photosynthesis by about 25 % in the presence and absence of acetate but had no effect on the distribution of ^{14}C assimilated. Furthermore, we found that the presence of 1 mM acetate did not affect either the uptake of $^{14}CO_2$ or the distribution of isotope arising from the fixation of $^{14}CO_2$. On the other hand, 10 mM acetate inhibited the rate of photosynthesis by about 50 % in the presence and absence of CO_2 but had no effect on the distribution of ^{14}C assimilated.

Table I. *Distribution of ^{14}C Incorporated into Illuminated Spinach Chloroplasts from Sodium Bicarbonate and Acetate*

Chloroplasts were prepared from freshly harvested mature leaves of *Spinacia oleracea* by the method of Jensen and Bassham (7). Photosynthesis was measured at 20° in small tubes in which the reaction mixture of 2.5 ml volume was aerated with a stream of N_2 or air. The light intensity was 2000 ft-c. The reaction mixture contained in mM amounts: N-2 hydroxyethylpiperazine-N′-2 ethanesulfonic acid buffer adjusted with NaOH to pH 7.6, 50; sorbitol, 330; $NaNO_3$, 2; potassium EDTA, 2; $MnCl_2$, 1; $MgCl_2$, 1; K_2HPO_4, 0.5; $Na_4P_2O_7$, 2; NaCl, 20; $NaH^{14}CO_3$, 10 mM, was included in all except $^{14}CH_3COONa$ treatments where $NaHCO_3$, 10 mM, was substituted. Radioactivity in both $NaH^{14}CO_3$ and $^{14}CH_3COONa$ was 3×10^7 cpm. Chlorophyll content was 195 μg. At intervals samples were pipetted in ice-cold formic acid (4 % final concentration) and aliquots plated as described by Walker (8). When $^{14}CH_3COONa$ was included, 2 drops of 5 N acetic acid were added to the planchets during drying to remove residual ^{14}C-acetate. For fractionation of products three replicate aliquots of each sample were placed on Whatman No. 1 chromatography paper and the radioactivity was determined using a Geiger tube with a Mylar end window. Distilled water was passed as an ascending solvent 20 cm beyond the spot in order to separate the water-soluble products from an insoluble residue which after drying at room temperature was counted. A mixture of CH_3OH – $CHCl_3$ (1:3, v/v) was passed over the origin and the radioactivity extracted was designated as lipid.

Time	$NaH\ ^{14}CO_3$, 10 mM		$^{14}CH_3COONa$, 1mM	
	Water – Soluble			
	N_2	Air	N_2	Air
min	cpm	cpm	cpm	cpm
5	8500	5470	70	104
15	15,030	24,870	90	148
30	61,210	45,350	152	134
60	79 290	60,000	258	116
	Water – Insoluble, Non-lipid			
5	300	175	43	83
15	1820	815	58	86
30	4220	2,370	82	100
60	5750	3,500	47	78
	Lipid			
5	0	18	39	15
15	150	125	15	16
30	270	280	20	14
60	365	300	45	38

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ited $^{14}CO_2$ uptake roughly 20 %. We observed that CO_2 is by 10-fold a better substrate for the synthesis of material soluble in methanol:chloroform than acetate yet this fraction comprises at best 0.6 % of the total uptake of isotope. In addition, little incorporation of acetate into any fraction occurs after the first 5 minutes, while incorporation of CO_2 into all fractions continues for at least 60 minutes. These results suggest that unlike the broken chloroplast preparations of previous workers (4, 5) intact chloroplasts carrying on high rates of CO_2 fixation utilize acetate to only a limited extent. This may be due to competition in these preparations for ATP or TPNH between enzymes of the reductive pentose phosphate cycle and acetate activation. Acetate thiokinase activity although shown in preparations of broken chloroplasts, appears to contribute barely any product to the photosynthetic yield of intact chloroplasts from fully expanded leaves. The inability of these chloroplasts to utilize either glycolate (P. W. Ellyard, personal communication) or formate has also been noted. The rate of lipid synthesis from $^{14}CO_2$ may be limited at any step beyond glycerate 3-P formation as only traces of intermediates related to pyruvate are found (7, 8). Seasonal appearance of alanine has, however, been observed in spinach chloroplasts (9) and it is therefore possible that lipid synthesis also functions seasonally.

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