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ENZYMATIC CARBOXYLATION OF RIBULOSE DIPHOSPHATE[1]

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Previous work[2] has indicated that ribulose-1,5-diphosphate acts as the primary carbon dioxide acceptor in photosynthesis. This conclusion was based upon observations with intact photosynthetic organisms. We now wish to report the demonstration of this carboxylation in a cell-free system.

A cell-free preparation was obtained from freshly harvested Chlorella by five-minutes treatment at 2-5° in a 9 Kc. Raytheon oscillator and subsequent removal by centrifugation of cell wall material and remaining whole cells. It has proved necessary to perform all operations rapidly (20-25 minutes from harvest) and in the cold ($< 5°$ C.) to obtain good fixation. Ribulose diphosphate was isolated from Scenedesmus which were killed rapidly in ethanol after thirty seconds nitrogen flushing following steady state photosynthesis in 4% carbon dioxide in air. Such conditions lead to maximum concentrations of ribulose diphosphate.[2] It was isolated from the extract by phenol chromatography of stripes of extract on oxalic acid-washed Whatman No. 4 filter paper, carrying spots of labeled ribulose diphosphate as markers.

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(1) The work described in this paper was sponsored by the U. S. Atomic Energy Commission.

(2) J. A. Bassham, A. A. Benson, L. D. Kay, A. Z. Harris, A. T. Wilson and M. Calvin, J. Am. Chem. Soc., 76, 1760 (1954).

Ribulose diphosphate or other substrates were added to the extract and $C^{14}O_2$ was introduced immediately. The results of one-minute exposure to $C^{14}O_2$ are embodied in Table I.

Complementary experiments with labeled ribulose diphosphate and this preparation demonstrated its rapid conversion to free sedoheptulose and a variety of normal metabolic intermediates which suggests a short lifetime for this substrate, limiting its availability for carboxylation. Similarly, labeled phosphoglycerate was largely converted to alanine in times longer than five minutes at room temperature.

Phosphoglycerate was identified by its chromatographic coordinates and cochromatography of the phosphatased compounds with authentic glyceric acid in four solvent systems. The degradation of the labeled glyceric acid showed 100% radioactivity in the carboxyl carbon,[3] and no (<2%) detectable activity for the β carbon.

It is clear that the extracts contain an enzyme (or enzymes) capable of catalyzing the carboxylation of ribulose diphosphate, specifically, to form phosphoglyceric acid. No intermediates between these compounds have been detected by this method which would have detected as little as an amount corresponding to 5% of the phosphoglyceric acid formed.

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(3) We wish to thank Dr. J. A. Bassham of this laboratory for doing the degradations of glyceric acid samples.

Table I

PRODUCTS FROM ONE MINUTE $C^{14}O_2$[a] FIXATION BY CELL-FREE PREPARATION FROM CHLORELLA

	Substrate added 0.1 μmoles				
Products	None	Ribulose-di-P[c,d]	Ribulose-5-P[f]	Ribose-5-P	Fructose-di-P
Phospho-glyceric acid	0[b]	320	0	0	0
Phospho-enol pyruvic acid	0	60	0	0	0
Alanine	0	60	0	0	0
Malic acid	750	80	600	720	900
Aspartic acid	100	10	50	50	40
Citric acid[e]	200	60	250	200	210

(a) Specific activity, 4.8×19^5 cpm/μmole.

(b) Counts per minute measured on paper chromatogram (self-absorption ~0.6) fixed during the first minute in 0.2 ml. solution containing contents of 10 mg. (wet weight) Chlorella cells.

(c) Isolated by water elution from ether-washed paper chromatograms of known amounts of Scenedesmus extracts. The amount, 0.1 μmole, was calculated assuming a cellular concentration of 10^{-3} Moles.

(d) It will be noted that fixation with this substrate is markedly lower than with the others. Eluates of a similarly treated blank chromatogram also inhibited fixation due to toxic constituents remaining on the paper (e.g. phenol, quinones, oxalic acid).

(e) In longer fixation periods radioactivity became incorporated in other tricarboxylic acid cycle intermediates (succinic, fumaric and glutamic acids). No sugar phosphate labeling was observed.

(f) We are indebted to Dr. B. L. Horecker for a sample of ribulose-5-P.