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ABSTRACT

A search for additional C^{14}-labeled sugar phosphates in extracts of Scenedesmus which had been exposed to $C^{14}O_2$ during photosynthesis has revealed the presence of radioactive xylulose 5-phosphate, and has led to its inclusion as an intermediate in the photosynthetic carbon-reduction cycle. No C^{14}-labeled tetrose could be detected in these experiments.

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In the proposed path of carbon in photosynthesis[1] it was suggested that the ribulose 5-phosphate detected among the photosynthetic products was formed by two reactions in which transketolase caused the transfer of a glycolyl group to 3-phosphoglyceraldehyde from sedoheptulose 7-phosphate in one case and from fructose-6-phosphate in the other. Recent evidence,[2,3,4] however, has indicated that these reactions proceed in two stages, (a) a transketolase reaction in which xylulose 5-phosphate is produced, and (b) conversion of the xylulose 5-phosphate to ribulose 5-phosphate by phosphoketopento-epimerase. A search was therefore made among the products of photosynthesis for xylulose 5-phosphate and also for erythrose 4-phosphate, which would be the other product produced by the action of transketolase on fructose 6-phosphate and 3-phosphoglyceraldehyde.

A suspension (total volume, 30.5 cc) containing washed Scendesmus cells (packed volume, 0.3 cc) and KH_2PO_4 solution (0.5 cc; 3.2 $\times 10^{-6}$M) in a thin vessel illuminated from approximately one foot on each side by two 150-w reflector flood lamps was left for 1 hour with a stream of 1% CO_2 in air. The suspension was then allowed to photosynthesize for 5 minutes with $NaHC^{14}O_3$ solution (0.9 cc; 360 μC) and flushed with air (1 minute). (Previous experience[5] indicated that these conditions would produce the highest level of triose phosphate labeling.) The combined 80% and 20% ethanol extracts were concentrated and applied to washed Whatman No. 4 papers, and the components analyzed in the usual way[6] by paper chromatography and radioautography.

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1 Bassham, Benson, Kay, Harris, Wilson, and Calvin, J. Amer. Chem. Soc. 76, 1760 (1954).

2 Srere, Cooper, Klybas, and Racker, Arch. Biochem. Biophys. 59, 535 (1955).

3 P. K. Stumpf and D. L. Horecker, J. Biol. Chem. 218, 753 (1956).

4 Horecker, Hurwitz, and Smyrniotis, J. Amer. Chem. Soc. 78, 692, (1956).

5 A. T. Wilson and M. Calvin, J. Amer. Chem. Soc. 77, 5948 (1955).

6 Benson, Bassham, Calvin, Goodale, Haas, and Stepka, J. Amer. Chem. Soc. 72, 1710 (1950).

Since dihydroxyacetone phosphate and erythrose 4-phosphate show little separation on the normal two-dimensional paper chromatogram, an enlarged area containing mainly triose phosphate was eluted and treated with purified phosphatase (prepared from Polidase "S") for 16 hours at 35°. The pentulose monophosphate and pentulose diphosphate areas were examined in the same way. After paper chromatographic analysis, no erythrose or erythulose could be detected among the phosphatased products from any of these areas. However, rechromatographing the trailing end of the ribulose-xylulose areas obtained from phosphatased pentulose monophosphate or diphosphate areas produced radioactive spots which coincided exactly with carrier xylulose spots, as revealed by spraying with orcinol-trichloractic acid. In addition, a component coinciding with glyceraldehyde was detected from the phosphatased triose phosphate area. Checks on the purity of the phosphatase used showed that it produced erythrose from authentic erythrose 4-phosphate (with no detectable erythulose) and ribose from ribose 5-phosphate (with no detectable ribulose or xylulose).

In another experiment, a freshly picked spinach leaf, whose stem was immersed in a culture medium, was left to photosynthesize for 5 minutes in a stream of 4% CO_2 in air. The CO_2 supply was removed and the surface of the leaf swept with air for 1 minute. After a partial vacuum was created in the cell, $C^{14}O_2$ (generated previously from 5 mg $BaC^{14}O_3$--82 μc/mg) was sucked in and the leaf allowed to photosynthesize for 5 minutes. The spinach leaf was then plunged into boiling 80% ethanol (200 cc). This extract and a succeeding 20% ethanol (200 cc) extract were combined, concentrated, and then analyzed in the usual way[6] by paper chromatography and radioautography. After treatment of all the sugar monophosphate areas with phosphatase, followed by rechromatography, the ribulose-xylulose areas were examined as above and the presence of appreciable quantities of xylulose demonstrated. Again the main triose spot coincided exactly with glyceraldehyde. No erythrose could be detected.

It seems highly probable, therefore, that the xylulose detected arose originally from xylulose 5-phosphate and that the photosynthetic cycle should be amended to include this compound (see Fig. 1). Similarly the glyceraldehyde probably arose from glyceraldehyde 3-phosphate, whose presence had already been inferred from the detection of dihydroxyacetone phosphate. The failure to detect erythrose indicates that free erythrose 4-phosphate, if present, must be in a concentration $<$ 7% of that of the triose phosphate. This may indicate that the rate constants of the reactions that utilize erythrose 4-phosphate[7] are sufficiently high to keep its steady-state concentration low.

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[7] H. L. Kornberg and E. Racker, Biochem. J. 61, iii, (1955).

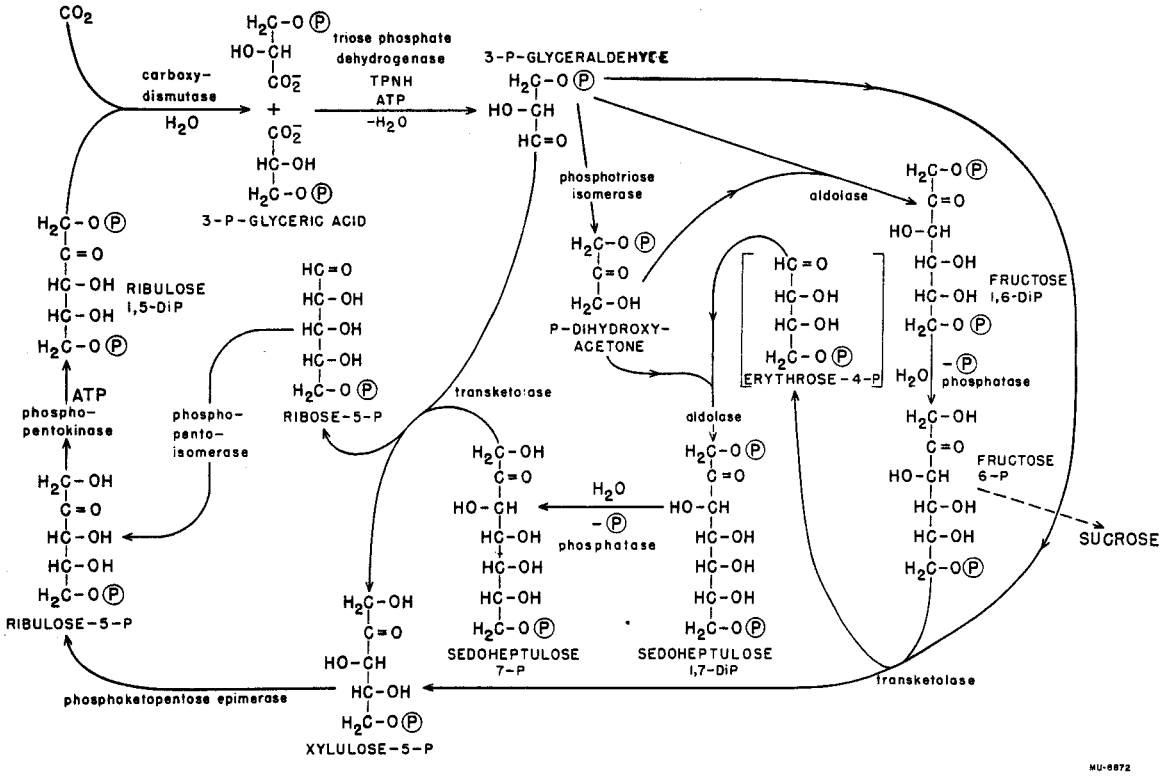

Fig. 1. The photosynthetic cycle.