TECHNICAL PAPERS

The Path of Carbon in Photosynthesis: II. Amino Acids[1]

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The amino acid constituents of the green algae *Chlorella pyrenoidosa* and *Scenedesmus* D-3 have been examined after exposure to $C^{14}O_2$, using the method of paper chromatography. Not only have the free amino acids been identified, but the radioactive members of the group have been ascertained.

The methods used in these experiments, which have been previously described (*2, 3*), involve the preparation of filter paper chromatograms of whole-cell extracts (80% ethanol) or of amino acid mixtures obtained by absorption on cation exchange resins from the plant extracts (*1*). The paper chromatograms of the radioactive amino acids were either scanned with a Geiger counter or radioautographed.

In *Scenedesmus* we have found the following amino acids, listed in the order of decreasing relative intensity of ninhydrin color on the chromatogram: glutamic acid, "unknown,"[2] alanine, serine, arginine, valine, aspartic acid, leucines, phenylalanine, tyrosine, α-aminobutyric acid (?), lysine, β-alanine, threonine, glycine, and proline.[3] The radioactive amino acids photosynthesized by *Scenedesmus* from $C^{14}O_2$ in 30 sec (*1*) include predominantly aspartic acid[4] with somewhat less alanine. Other radioactive amino acids synthesized under these conditions and detected by radioautography included asparagine, serine, β-alanine, and phenylalanine.

When the radioactive amino acids synthesized in the dark (1 min) by preilluminated (10 min) *Scenedesmus* were separated, the predominant radioactive product was aspartic acid with somewhat less labeled alanine. Radioactive phenylalanine is synthesized in much smaller amount.

The analysis of *Chlorella* is not yet as complete as that for *Scenedesmus*. The following amino acids have been found in *Chlorella* extracts: glutamic acid, leucines, alanine, valine, glycine, and β-alanine. *Chlorella* which have been allowed to photosynthesize with $C^{14}O_2$ for 30 sec form a predominant amount of radioactive aspartic acid with almost as much alanine. Minor radioactive products include β-alanine and serine. Dark (1 min) $C^{14}O_2$ fixation by preilluminated (60 min) *Chlorella* yields largely radioactive alanine.

In all paper chromatograms the glutamic acid ninhydrin spot was strongly evident. In no case was any radioactivity found coincident with this spot. In cases where glutamine was present, no corresponding radioactivity was observed.

Thus, it appears that in both dark reduction of $C^{14}O_2$ and photosynthesis the same pattern of radioactivity in the amino acids occurs. In both cases the amino acids which have been identified correspond to the 3- and 4-carbon amino acids. This is in accord with the tentative scheme proposed earlier (*1*), which inferred that the 3-carbon amino acids, alanine, serine, and β-alanine, have their origin in pyruvic acid and the 4-carbon ones have the origin of their carbon skeletons in oxalacetic acid. The positive determination of the absence of radioactive glutamic acid is to be taken as evidence against the participation of the tricarboxylic acid cycle in the anabolic path of CO_2 in photosynthesis.

References

1. CALVIN, M., and BENSON, A. A. *Science*, 1948, **107**, 476.
2. DENT, C. E., STEPKA, W., and STEWARD, F. C. *Nature, Lond.*, 1947, **160**, 682.
3. FINK, R. M., and FINK, K. *Science*, 1948, **107**, 253.

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[2] Identical with Spot #23 of Dent, Stepka, and Steward and very probably the same compound reported as (b) with chromatograms of *E. coli* digest (A. Polson. *Nature, Lond.*, 1948, **161**, 351).

[3] Due to the yellow color of this ninhydrin spot, it is not possible to compare its intensity.

[4] Aspartic acid may be as high as 75% according to co-crystallization assay.

Significance of Distribution of Fluorescein in Sarcoma 180[1]

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Moore, in a recent report (*2*) on the use of previously injected fluorescein in the diagnosis of human tumors at operation, stated that under the conditions of his study (dose—5 cc of 20% solution intravenously; duration between injection and operation—3–8 hrs) fluores-

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