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Contract No. W-7405-Eng-48

# THE PATH OF CARBON IN PHOTOSYNTHESIS. II. AMINO ACIDS

BY

W. Stepka, A. A. Benson and M. Calvin

25 May 1948

Berkeley, Calfiornia

| STANDARD DISTRIBUTION: SERIES A | COPY NUMBERS |
|---|---|
| Argonne National Laboratory | 1-8 |
| Armed Forces Special Weapons Project | 9 |
| Atomic Energy Commission, Washington | 10-11 |
| Battelle Memorial Institute | 12 |
| Brookhaven National Laboratory | 13-22 |
| Carbide & Carbon Chemicals Corporation (K-25 Area) | 23-26 |
| Carbide & Carbon Chemicals Corporation (Y-12 Area) | 27-30 |
| Columbia University (Failla) | 31 |
| General Electric Company | 32-35 |
| Hanford Directed Operations | 36-42 |
| Iowa State College | 43 |
| Kellex Corporation | 44-45 |
| Los Alamos | 46-48 |
| Massachusetts Institute of Technology | 49 |
| Monsanto Chemical Company, Dayton | 50-51 |
| National Bureau of Standards | 52-53 |
| Naval Radiological Defense Laboratory | 54 |
| NEPA | 55 |
| New York Directed Operations | 56-57 |
| Oak Ridge National Laboratory | 58-69 |
| Patent Advisor | 70 |
| Technical Information Division, ORDO | 71-85 |
| UCLA Medical Research Laboratory (Warren) | 86 |
| University of California Radiation Laboratory | |
| Information Division | 87-89 |
| Chemistry, Bldg 4 | 90 |
| Patent Dept. | 91 |
| University of Rochester | 92-93 |
| Western Reserve University (Friedell) | 94 |
| Office of Chicago Directed Operations | 95 |
| TOTAL | 95 |

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# The Path of Carbon in Photosynthesis. II. Amino Acids

by

W. Stepka, A. A. Benson and M. Calvin

From the Radiation Laboratory, Department of Chemistry and Division of Plant Nutrition, University of California, Berkeley, California*

25 May 1948

Abstract

The radioactive amino acids synthesized from $C^{14}O_2$ by green algae both in the light and in the dark after $CO_2$-free preillumination have been separated and identified using paper chromatography and radioautography. The radioactive amino acids identified were aspartic acid, alanine and smaller amounts of 3- and 4-carbon amino acids. This finding as well as the total absence of radioactive glutamic acid substantiates the mechanism for reduction of $CO_2$ previously postulated by members of this laboratory.

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* This paper is based on work performed under contract No. W-7405-Eng-48 with the Atomic Energy Commission in connection with the Radiation Laboratory, University of California, Berkeley, California.

For publication in Science.

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# THE PATH OF CARBON IN PHOTOSYNTHESIS. II. AMINO ACIDS

by

W. Stepka, A. A. Benson and M. Calvin

From the Radiation Laboratory, Department of Chemistry and Division of Plant Nutrition, University of California, Berkeley, California*

The amino acid constitutent 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 have been previously described (1,2) and involve the preparation of filter paper chromatograms of whole cell extracts (80% ethanol) or of amino acid mixtures obtained by adsorption on cation exchange resins from the plant extracts. (3). The paper chromatograms of the radioactive amino acids were either scanned with a Geiger counter or

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1. Fink, R. M. and Fink, K., Science, 107, 253 (1948)

2. Dent, C. E., Stepka, W. and Steward, F. C., Nature 160, 682 (1947)

3. Calvin, M. and Benson, A. A., Science, 107, 476 (1948)

* This paper is based on work performed under contract No. W-7405-Eng-48 with the Atomic Energy Commission in connection with the Radiation Laboratory, University of California, Berkeley, California.

radioautographed.

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

When the radioactive amino acids synthesized in the dark (1-minute) by preilluminated (10-minute) 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 for Scenedesmus. The following amino acids have been found in Chlorella extracts: glutamic acid, leucines, alanine, valine, glycine, and $\beta$-alanine. Chlorella which have been allowed to photosynthesize with $C^{14}O_2$ for 30 seconds form a predominant amount of radioactive aspartic acid with almost as much alanine. Minor radioactive products include $\beta$-alanine and serine. Dark (1-minute) $C^{14}O_2$ fixation by preilluminated (60-minute) Chlorella yields largely radioactive alanine.

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

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

** Aspartic acid may be as high as 75% according to co-crystallization assay.

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 (3) which inferred that the 3-carbon amino acids, alanine, serine and $\beta$-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.