ASYMMETRIC DISTRIBUTION OF C^{14} IN THE GLUCOSE PHOSPHATES FORMED DURING PHOTOSYNTHESIS[1,2]

OTTO KANDLER[3] AND MARTIN GIBBS[4]

DEPARTMENT OF BIOLOGY, BROOKHAVEN NATIONAL LABORATORY, UPTON, NEW YORK

While Bassham, Benson, Kay, Harris, Wilson and Calvin (1) have found a symmetrical distribution of C^{14} in fructose isolated from Scenedesmus following a brief photosynthesis in $C^{14}O_2$, Gibbs and Kandler (2) have recently reported an asymmetric labeling pattern in the glucose moiety of sucrose and starch both in Chlorella as well as the leaves of several higher plants. In our previous communication only the glucose of the end products of photosynthesis was investigated. In this note, the distribution of tracer in compounds more closely associated with the photosynthesis cycle will be presented, namely the several glucose phosphate esters. Labeled $C^{14}O_2$ was used in photosynthesis by dense Chlorella suspensions (1.5 ml packed cells per 100 ml water) under two conditions: (1) illumination of the algae for 5 minutes in a nitrogen atmosphere before the introduction of $C^{14}O_2$ for a period of 10 seconds and (2) incubation of the cells with the $C^{14}O_2$ in the dark for five minutes before photosynthesis occurred for one minute. The photosynthesis was terminated by pouring the Chlorella suspension into boiling 80 % ethanol. The 80 % ethanol extract and two 20 % ethanol washings were concentrated under vacuum to 1 ml. This residue was analyzed by two-dimensional paper chromatography (solvents: water saturated phenol and butanol–propionic acid). The areas of the chromatogram occupied by the sugar phosphates were eluted and the eluate was subjected to enzymatic hydrolysis by phosphatase. The resulting free sugars were then separated by two-dimensional paper chromatography using the same solvent system as for the original plant extract. Glucose was detected in four distinct spots on the chromatogram of the original plant extract (fig 1).

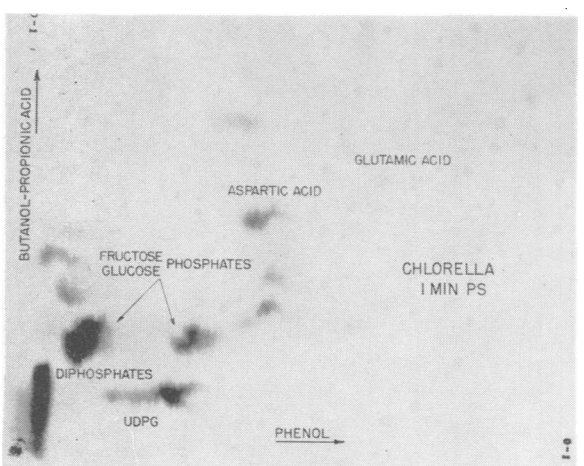

FIG. 1. Chromatogram of Chlorella extract (1-min photosynthesis) indicating the 4 glucose phosphate areas.

TABLE I

DISTRIBUTION OF C^{14} IN GLUCOSE PHOSPHATE ESTERS

EXPT NO.	AREA	TRACER CONTENT OF GLUCOSE CARBON ATOMS					
		1	2	3	4	5	6
		cpm/mg carbon					
1	Monophosphate	51	43	186	243	19	35
2	UDPG *	88	72	468	530	43	59
3	Unknown glucose phosphate	216	200	838	1074	117	141
4	Monophosphate	85	90	550	595	12	16
5	UDPG	83	80	730	811	12	20

In experiments 1, 2 and 3, the Chlorella was incubated with the $C^{14}O_2$ in the dark for 5 min before an illumination of 4000 ft-c for 1 min was begun.

In experiments 4 and 5, the Chlorella was illuminated 5 min in a nitrogen atmosphere before the introduction of $C^{14}O_2$. Photosynthesis time was 10 sec. Illumination intensity was 4000 ft-c

* UDPG = Uridinediphosphate glucose.

Analogous to the chromatograms published by Bassham, Shibata, Steenberg, Bourdon and Calvin (3), glucose was found in the diphosphate area (close to the origin and associated with fructose and ribulose), monophosphate area (again associated with fructose) and the uridine diphosphate glucose (UDPG) area. The fourth area finds no analogy on the chromatograms of the California group. It is located on all of our chromatograms between the diphosphate and the phosphoglyceric acid areas and contains tracer in a ratio of approximately 60 % glucose and 40 % fructose. The glucose of the various areas was degraded by the *Leuconostoc mesenteroides* method which permits a determination of the C^{14} content of the individual carbon atoms (4).

The data listed in table I shows that in each glucose phosphate ester, the specific activity of carbon atom 3 (C-3) was significantly less than that of C-4; however, C-1 (glucose aldehyde carbon) and C-2 were higher than that of C-5 and C-6. This asymmetric type of labeling in the glucose phosphate esters is

[1] Received August 3, 1956.

[2] Research carried out at the Brookhaven National Laboratory under the auspices of the U. S. Atomic Energy Commission.

[3] Aided by a grant from the Rockefeller Foundation. Permanent address: Botanical Institute, University of Munich, Munich, Germany.

[4] Present address: Department of Biochemistry, Cornell University, Ithaca, New York.

similar to that reported in our earlier communication for the glucose moiety of sucrose and starch.

If a lower activity in C-3 in relation to C-4 was caused by the presence of a pool of unlabeled dihydroxyacetone phosphate as suggested by the California group (1), then a corresponding dilution of C-1 and C-2 would be expected. The marked difference between the ratio of C-3 to C-1 and/or C-2 and C-4 to C-5 and/or C-6 indicates that it is unlikely that two equal types of triose phosphates are combined to yield a hexose phosphate.

Summary

Photosynthesis was carried out with Chlorella using labelled $C^{14}O_2$: 1) after illumination for 5 minutes in atmosphere of N_2 and for 10 seconds after introduction of $C^{14}O_2$ and 2) incubation of the cells with $C^{14}O_2$ in the dark for 5 minutes and then 1 minute of light. The glucose was isolated from the sugar phosphates, and the labelling in the carbon atoms followed. The labelling pattern is not that which is expected if two equal triosephosphates combined to yield an hexosephosphate.

Literature Cited

1. Bassham, J. A., Benson, A. A., Kay, L. D., Harris, A. Z., Wilson, A. T. and Calvin, M. The path of carbon in photosynthesis. XXI. The cyclic regeneration of carbon dioxide acceptor. Jour. Amer. Chem. Soc. 76: 1760–1770. 1954.
2. Gibbs, M. and Kandler, O. Asymmetric distribution of C^{14} in sugars formed during photosynthesis. Proc. Natl. Acad. Sci., U. S. (In press).
3. Bassham, J. A., Shibata, K., Steenberg, K., Bourdon, J. and Calvin, M. The photosynthetic cycle and respiration: Light-dark transients. University of California Radiation Laboratory Report 3331. 1956.
4. Gunsalus, I. C. and Gibbs, M. The heterolactic fermentation. II. Position of C^{14} in the products of glucose dissimilation by *Leuconostoc mesenteroides*. Jour. Biol. Chem. 194: 871–875. 1952.