Title
PROTEIN PHOTOSYNTHESIS

Authors
Bassham, J.A.
Morawiecka, Bronislawa
Kirk, Martha.

Publication Date
1963-12-01

UCRL-11141

University of California

Ernest O. Lawrence Radiation Laboratory

PROTEIN PHOTOSYNTHESIS

Berkeley, California

DISCLAIMER

submitted to Biochim. and
Biophys. Acta

UCRL-11141

UNIVERSITY OF CALIFORNIA
Lawrence Radiation Laboratory
Berkeley, California

AEC Contract No. W-7405-eng-48

PROTEIN PHOTOSYNTHESIS

J. A. Bassham, Bronislawa Morawiecka and Martha Kirk

December 1963

UCRL 1141

PROTEIN PHOTOSYNTHESIS

J.A. Bassham, Bronislawa Morawiecka and Martha Kirk
and Department of Chemistry
Lawrence Radiation Laboratory, University of California, Berkeley, Calif. (U.S.A.)

SUMMARY

Kinetic studies have been performed on the appearance of ^{14}C in free amino acid pools and in the amino acid moieties of protein in Chlorella pyrenoidosa during steady-state photosynthesis with $^{14}CO_2$. At the same time, we have determined the total concentrations of free and bound amino acids, permitting us to calculate the specific radioactivity of the free and bound pools of certain primary amino acids. Comparison of the specific activities of the free amino acid pools with the rate of ^{14}C-labeling of the corresponding pools of bound amino acids shows that there is a direct kinetic precursor-product relationship between the actively turning over pools of free amino acids and the bound amino acids in the protein. In the case of certain amino acids, notably glycine, the actively turning over pool of free amino acid is very small, leading to "saturation" when the average specific activity of the entire free amino acid pool of glycine is only 0.015. The total rate of labeling with ^{14}C of the bound amino acids in protein appears to account for only a fraction of the steady state rate of labeling of the free amino acid pools. Explanations are offered for the apparent discrepancy.

INTRODUCTION

Much evidence has been presented to support the view that the formation of reduced carbon compounds from carbon dioxide during photosynthesis is accomplished by a series of dark reactions utilizing reduced and chemically energetic cofactors formed by light reactions[1-3].

According to this concept, photochemically produced reducing agents, such as reduced chloroplast ferredoxin and nicotinamide adenine dinucleotide, and chemically energetic compounds such as adenosine triphosphate, provide the reducing equivalents and chemical energy required to reduce carbon dioxide via the basic carbon reduction cycle to 3-phosphoglyceric acid and various simple sugar phosphates. Subsequent "secondary" reactions utilize the same or similar photochemically produced cofactors to accomplish further reduction and transformation of intermediate compounds from the cycle to various secondary products of photosynthesis. All such reactions which occur in the chloroplast and utilize such photochemically produced cofactors may properly be considered as photosynthetic reactions[2,3]. Such substances as starch granules in the chloroplasts have long been recognized as products of photosynthesis. The recognition of other constituents of the chloroplasts as possible direct products of photosynthesis has recently emerged as a result of a more detailed understanding of the nature of the carbon reduction reactions.

The light-accelerated incorporation of ^{14}C into amino acids[4-6] and proteins[7] was observed during the earliest studies of photosynthesis with $^{14}CO_2$. Similar observations have been reported from other laboratories[8-11].

Nichiporovich[8] has presented and reviewed evidence that the synthesis of proteins in the chloroplasts of higher plants is greatly accelerated during photosynthesis. Moreover, he pointed out that the accelerated protein synthesis appears to utilize intermediates of photosynthetic carbon reduction since the proteins were labeled when $^{14}CO_2$ was administered but not when [^{14}C] carbohydrates were supplied. Sissakian[12] reported that protein can be synthesized in

isolated chloroplast from non-protein nitrogen. Recently, Heber[13] reported that spinach chloroplasts, isolated following a period of photosynthesis with $^{14}CO_2$, had incorporated carbon 14 into the soluble protein of these chloroplasts more rapidly than it was incorporated into the soluble cytoplasmic proteins.

One type of evidence for the photosynthesis of proteins in chloroplasts would be the demonstration of kinetic precursor-product relationships for a sequence of compounds beginning with CO_2 and ending with protein. At the same time, all pools of intermediate compounds should be shown to exist and to be formed in the chloroplast during photosynthesis in vivo. Such a demonstration became experimentally conceivable with the development of methods for studying quantitatively pool sizes and rates of turnover of intermediate compounds during steady-state photosynthesis[14,15].

By means of these methods, it has already been possible to show that in Chlorella pyrenoidosa there are at least two pools of amino acids, one of which is rapidly labeled with ^{14}C during photosynthesis with $^{14}CO_2$. It was shown that the maximum rate of labeling of certain amino acids such as alanine was achieved as soon (five minutes) as the presumed precursors (in this case the intermediates of the carbon reduction cycle) were saturated with $^{14}CO_2$. No other labeled compounds in the cell except the intermediates of the carbon reduction cycle and phosphoenolpyruvic acid, were saturated with $^{14}CO_2$ by this time, so the required precursor-product relationship was indicated.

Although pyruvic acid, the presumed intermediate compound between phosphoenolpyruvic acid and the carbon skeleton of alanine was not isolated, the rapid labeling of alanine shows that the pool size of

pyruvic acid must have been very small and that pyruvic acid was consequently quickly saturated with ^{14}C. A similar relation was shown for aspartic acid, the precursors of which may be presumed to be phosphoenolpyruvic acid and CO_2. The situation with glutamic acid and glutamine was more complex due to the fact that some labeling of glutamic acid in the less active pool, assumed to be outside the chloroplast, occurs probably as the result of some "leakage" of small labeled compounds such as acetate from the chloroplast to the cytoplasm. Nevertheless, the data published suggest that the primary chloroplastic pool of glutamic acid is rapidly labeled and eventually becomes saturated with carbon 14.

Given the demonstration of photosynthetically formed pools of amino acids in the chloroplast, the next step in the study of protein photosynthesis is to relate the labeling of protein with ^{14}C during photosynthesis in the presence of $^{14}CO_2$ to that of the labeled amino acids in the active pools.

At the same time, we have tried to obtain information which might help to resolve the question of the origin of the carbon 14 skeletons of protein-bound amino acids. Bidwell compared the products of $^{14}CO_2$ photosynthesis in several algae, including Chlorella pyrenoidosa[16]. The specific radioactivities of the bound amino acids were found to be closely similar to one another whereas the specific radioactivities of the free amino acids and carbohydrates varied greatly among the organisms. Bidwell postulated that protein synthesis might take place in these organisms directly from immediately assimilated carbon atoms and not from soluble amino acids, in the same manner as has been postulated for higher plant tissues[17-18]. The existence of more than one pool of

the same compound could also explain discrepancies between labeling of total pools of free compounds and pools of bound amino acids.

EXPERIMENTAL

The experimental methods relating to maintenance of the algae (Chlorella pyrenoidosa) under steady-state photosynthesis and the analysis of the labeling of compounds with ^{14}C has been described in this journal[14,15]. These same methods have been used in these studies except as noted.

Steady-state apparatus

The apparatus for maintaining the algae under conditions of steady-state photosynthesis with $^{14}CO_2$ has been modified from that described previously[14,15]. The new apparatus has been modified to include automatic and independent pH control, density control, and volume control, as described elsewhere in this journal[19]. Control of pH is achieved by automatic addition of 0.1 N NH_4OH, when the pH of the algae suspension tends to drop below the control value of 6 due to exchange of hydrogen ions for NH_4^+ ions. The medium used in this experiment was modified from that described elsewhere[19] by substituting 2.0 mM KH_2PO_4 for 1.0 mM KH_2PO_4 plus 1.0 mM K_2HPO_4.

The algae were placed in the steady-state apparatus in a 2.3 to 2.5% suspension (wet packed volume/suspension volume) 24 hrs prior to the experiment. They were allowed to photosynthesize and grow under steady-state rates for this 24 hr period with the exception of an 8 hr period of darkness which ended 7 hrs prior to the introduction of $^{14}CO_2$. At the start of the experiment, the gas circulating system was closed and a stopcock opened connecting the system with a 5 liter flask which contained 2% CO_2 in air labeled with ^{14}C to a specific radioactivity of about 7 or 7.5 μcuries per μmole. The mixing of this

$^{14}CO_2$ and $^{12}CO_2$ with the 2% $^{12}CO_2$ already in the small system (effective volume 435 cc) resulted in a final specific radioactivity of 6 to 7 μcuries per μmole. The 80 ml of 2.0% algae suspension contained the equivalent of 1.6 cm^3 of wet packed algae. The rates of O_2 and of CO_2 uptake were approximately 12 μmoles per cm^3 of algae per min. The percentage CO_2 in the system declined from 2.1% to 0.32% during the first 5 hrs duration of the experiment. Approximately 2 ml samples of the algae suspension were taken into weighed test tubes containing 8 ml of methanol by means of the solenoid-operated sampling valve. The resulting 80% methanolic mixture has been found to stop the reactions of photosynthesis within a second at room temperature[1].

The procedures for the analysis of soluble compounds by chromatography and radioatuography are as previously reported[1,2,15] except where recently modified[14].

Protein extraction

The procedure for protein extraction from Chlorella pyrenoidosa was as follows: The killed algae suspension, after standing for 20 min, was centrifuged at 2000 g for 20 min at 5°C. The supernatant solution was set aside for determination of protein by use of the micro-tannic method[10]. Since only 2 to 4% as much protein was found in this supernatant solution as in the alkaline extract (see below) no further study was made of this small amount of protein. The sediment was mechanically mixed for 15 min with 400 μl of 1.0 N NaOH in a tube which was then put into liquid nitrogen until the mixture was frozen. The frozen material was lyophilized in a dessicator over H_2SO_4 overnight. Water (2 ml) was added

to the dried sample, giving a solution which was 0.2 N in NaOH. After 30 min at room temperature with occasional mechanical agitation, the protein was extracted. The sample was then centrifuged at 2000 g for 20 min at 15°C. The sediment was washed with 1 ml of 0.2 N NaOH solution and centrifuged again at 2000 g for 20 min.

The supernatant solutions from the last two centrifugations were combined and the total volume of 3 ml was made 0.5 N in perchloric acid. After standing for 30 min at 0 to 2°C, the protein was centrifuged at 2000 g for 20 min at 0 to 2°C. The precipitate was washed once with cold 0.5 N perchloric acid and centrifuged again. The washed precipitate of protein, designated protein A, was dissolved in 3 ml of 0.2 N NaOH and the total radioactivity was determined. The protein content of an aliquot portion of this solution was determined by the micro-tannic method[20].

The protein was again precipitated with perchloric acid in the same manner as above. The washed precipitate was placed with 6 N HCl in an ampule which was cooled, without freezing the contents. Vacuum was applied to the ampule to remove dissolved gases and the ampule was sealed and heated at 110°C for 22 hr to hydrolyze the protein. After the ampule was opened, the HCl was removed by twice adding water and distilling in vacuum in a dessicator over P_2O_5 and KOH.

After nearly complete removal of the HCl, the hydrolyzed protein A was taken up in 0.5 ml of H_2O and the solution was passed through an ion exchange column (cation exchange resin, H^+ form). Subsequently the column was eluted with 1 ml of 4 N NH_4OH and the eluate from the column was evaporated in a dessicator over H_2SO_4 overnight. The dried residue was dissolved in 60 or 80 µl of water. An aliquot sample was diluted and its ^{14}C total radioactivity was

determined.

Another aliquot sample (20 or 40 µl) of the hydrolyzed protein was placed on Whatman no. 1 or no. 2 filter paper, previously washed with 0.5% oxalic acid solution, for two dimensional paper chromatography.[15] The paper was developed in the long direction with "semi-stench"[21] for 26 to 28 hrs, and in n-butanol:propionic acid:water in the second direction for 22-24 hrs. Location of radioactive compounds was accomplished by radioautography with single emulsion X-ray film[14].

The supernatant solutions from the perchlorate precipitations were collected for the determination of their proteins (protein B). Protein B was precipitated by tannic acid[22] using 10 mg tannic acid per mg of protein. The precipitate was washed twice with water and hydrolyzed in the same manner as the protein A fraction. The hydrolysate was chromatographed as described above.

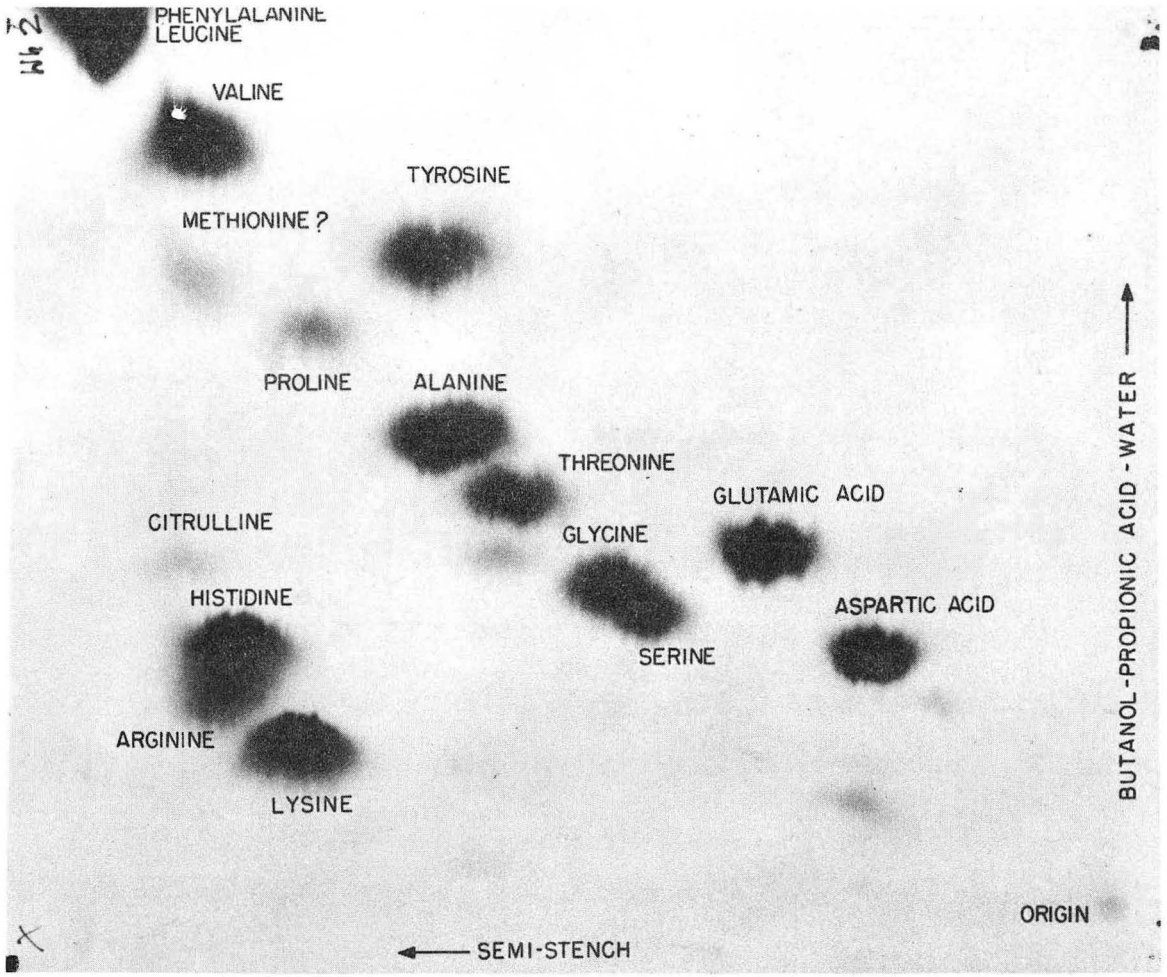
The radioautograph of ^{14}C-labeled amino acids obtained by hydrolysis of protein A is shown in Fig. 1. The radioactive areas of the paper chromatograms corresponding to the darkened areas of the film were cut out and their ^{14}C content was determined by means of a semi-automatic double GM tube-scaler-printout device[23].

Amino acid estimation

The amino acids from the protein hydrolysate were eluted from the paper chromatograms after determination of their ^{14}C content. The amounts of amino acids were determined by developing a color with ninhydrin solution and measuring the extinction as described elsewhere in this journal[19].

RESULTS

The amounts of protein recovered from Chlorella pyrenoidosa



ZN-3901

Fig. 1. Radioautograph of ^{14}C-labeled amino acids obtained by hydrolysis of protein A.

during a two hour steady state experiment are shown in Table I. A cm^3 of wet packed algae centrifuged according to our techniques has a dry weight of about 160 mg. Therefore, the mean value of 45.6 mg extracted protein represented about 27% of the dry weight. Direct estimation of the fraction B protein gave a mean value of about 11 mg compared to the 14 mg obtained by difference.

The rates of uptake of CO_2 and evolution of oxygen and the apparent rate of uptake of ammonium ion during a 5 hr photosynthesis experiment (no. 53) is shown in Table II. The "NH_4^+" uptake is based upon the rate of addition of $^{15}NH_4^+$ required to maintain the pH at the 6 during the course of / experiment. The assumption that this represents NH_4^+ uptake rate only could be in error if the algae secrete acid into the medium by processes unrelated to ammonium ion uptake. (An uptake of ammonium ion equal to about 2 μmoles/min·cm^3 algae would be equivalent to 6 or 7 μmoles of CO_2/min·cm^3 algae, or approximately 50% of the total photosynthetic CO_2 uptake.)

After 3 hrs the rate of ammonium ion uptake appears to have dropped considerably and the metabolism of the algae may have shifted somewhat toward fat synthesis. Such a shift during the course of the experiment is also indicated by the changing ratio of O_2/CO_2. Thus it appears that we may not have achieved perfectly steady-state conditions over the entire course of the experiment, but for the first 3 hrs conditions appear to have been reasonably close to steady-state. This tendency for the metabolism to change has been observed frequently and may be due to some residual rhythm following the harvesting of the algae

TABLE I

AMOUNTS OF PROTEIN EXTRACTED FROM CHLORELLA

Sample No.	Time of PS min	PROTEIN extracted by 0.2 N NaOH mg/g algae	PROTEIN precipitated by 0.5 N $HClO_4$ Protein A
1	5	49.9	31.6
2	10	48.2	32.8
3	15	44.9	32.9
4	20	46.3	31.3
5	30	46.0	31.1
6	45	44.5	31.1
7	60	42.4	29.1
8	90	44.2	32.3
9	120	43.9	31.5
Mean Value	-	45.6	31.5

TABLE II

PHOTOSYNTHESIS RATES

Time	μmoles/min/cm^3 algae			
	O_2	CO_2	O_2/CO_2	"NH_4^+"
24 min	13.09	12.63	1.04	
30-100 min				2.19
2 hr, 15 min	14.55	13.78	1.06	
100-185				2.10
3 hr, 45 min	13.35	12.04	1.10	
3 hr-5 hr				1.21

from the continuous culture apparatus and its resuspension in a different nutrient in the steady-state apparatus. In any event, the data bearing on the question of relation between free amino acid pools and bound amino acid pools was mostly obtained during the first 3 hrs under steady-state conditions.

The distribution of ^{14}C among the several bound amino acids following different times of photosynthesis with $^{14}CO_2$, and the total amounts of the bound amino acids in both protein A and B are shown in Table III. The acid hydrolysis method used results in the conversion of asparagine to aspartic acid and of glutamine to glutamic acid as well as loss of certain amino acids, particularly tryptophane, cysteine, cystine and arginine.

The sum of radiocarbon found in all bound amino acids from Table III is compared in Fig. 2 with the total radiocarbon found in the protein A and protein B fractions before hydrolysis. Some loss of radioactivity no doubt results from the decomposition of unstable compounds during hydrolysis, and other losses are usually encountered during chromatography. Probably there are some non-protein compounds which follow the protein during the isolation procedure, though the relatively small amounts of carbon 14 in unknown spots on the two-dimensional chromatograms of the hydrolyzed protein suggests that this is a minor contribution. In any event, the maximum slope of the total bound amino acid ^{14}C-labeling curve is only a little more than 1 μmole/cm^{3}algae/min. The specific radioactivities of the pools of free amino acids and of bound amino acids are shown for glycine, alanine, threonine, serine, aspartic acid and glutamic acid in Figs. 3 through 8, respectively. The figure for bound aspartic acid includes both bound aspartic acid and bound asparagine while that for bound glutamic acid includes both bound glutamic acid and bound glutamine. This is a consequence of the fact that the acid hydrolysis of the protein hydrolyzes

TABLE III

AMOUNT OF ^{14}C AND TOTAL C IN BOUND AMINO ACIDS PER CM^3 ALGAE

	μmoles ^{14}C in bound amino acids/cm^3 algae							μmoles amino acid per cm3 aglae			μmoles ^{12}C in amin acid per cm3 alga
Time:	10 min	30 min	1 hr	2 hr	3 hr	4 hr	5 hr	A	B	A + B	
Amino Acid											
Glycine	.17	.92	2.0	4.2	7.1	9.1	9.5	31.4	1.8	33.2	66.4
Serine	.11	.76	1.7	4.4	6.3	8.5	9.9	15.7	1.8	17.5	52.5
Alanine	.38	2.5	4.8	11.3	17.1	22.3	27.4	40.9	6.9	47.8	143.4
Threonine	.05	.90	2.5	6.3	9.6	15.1	16.5	13.1	2.8	15.9	63.6
Asparagine + Aspartic	.38	2.1	4.1	9.7	15.2	20.4	24.3	38.0	5.4	43.4	173.6
Glutamine + Glutamic	.09	1.3	3.6	10.5	15.9	24.6	29.8	35.2	8.0	43.2	216.0
Tyrosine	.40	1.3	2.4	6.4	9.2	12.8	15.5	7.2	1.5	8.7	78.3
Proline	—	.26	1.1	4.3	8.7	12.7	16.0	11.8	7.2	19.0	95.0
Valine + Methionine	.36	2.0	4.0	13.7	18.0	22.9	25.2	23.4	2.6	26.0	130.0
Phenylalanine + Leucine	.47	6.0	11.8	23.5	36.8	24.5	19.1	33.4	2.6	36.0	288.0
Cystine, Arginine + Histidine	.22	1.4	3.5	9.5	15.7	21.0	24.5	7.7	1.7	9.4	56.4
Lysine	.29	1.7	3.6	8.6	13.4	17.0	21.5	8.7	1.7	10.4	62.4
X_1	.03	.05	.12	.33	.33	.87	.73	6.7	—	6.7	21.0
X_2	.01	.06	.13	.20	.43	.57	.85	.51	—	.51	(40)
X_4	—	—	.10	.26	.64	.54	.72	1.8	—	1.8	
SUM	2.96	21.3	45.4	113.2	174.4	212.9	241.5	275.5	44.0	319.5	1,486.6

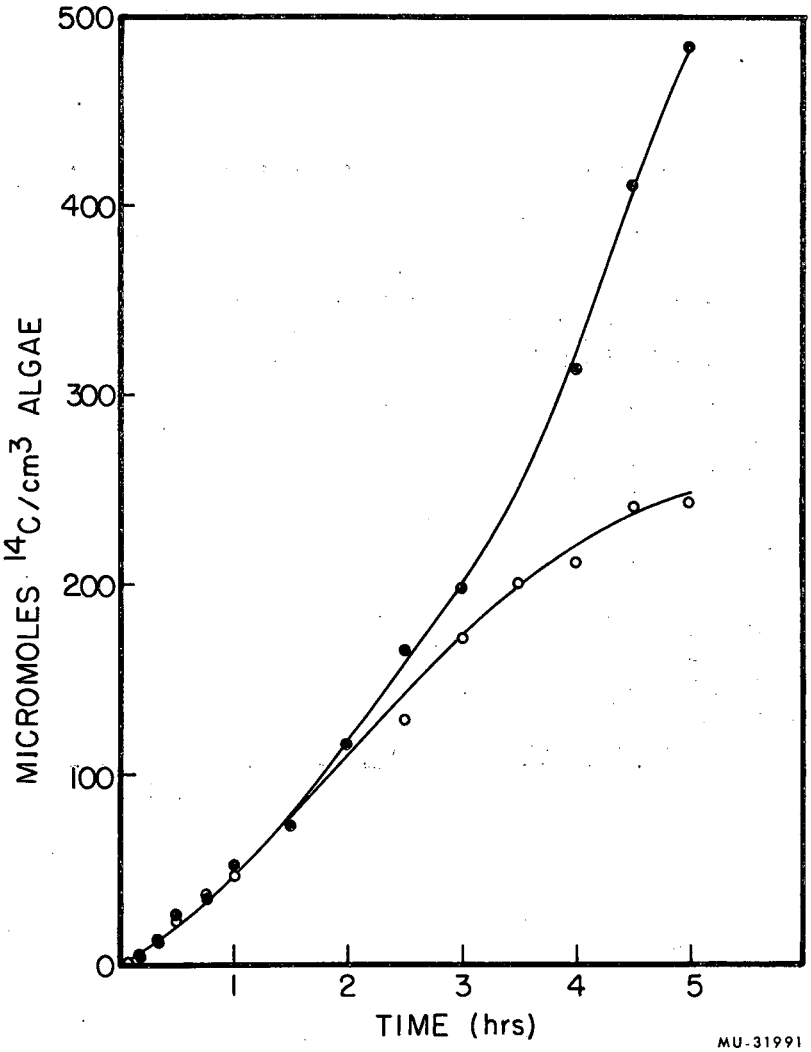


Fig. 2. Labeling of protein during photosynthesis in Chlorella pyrenoidosa. Closed circles represent the sum of protein fraction A and protein faction B. Open circles represent sum of all radioactivity found in amino acids on two-dimensional paper chromatogram after hydrolysis of fraction A and fraction B.

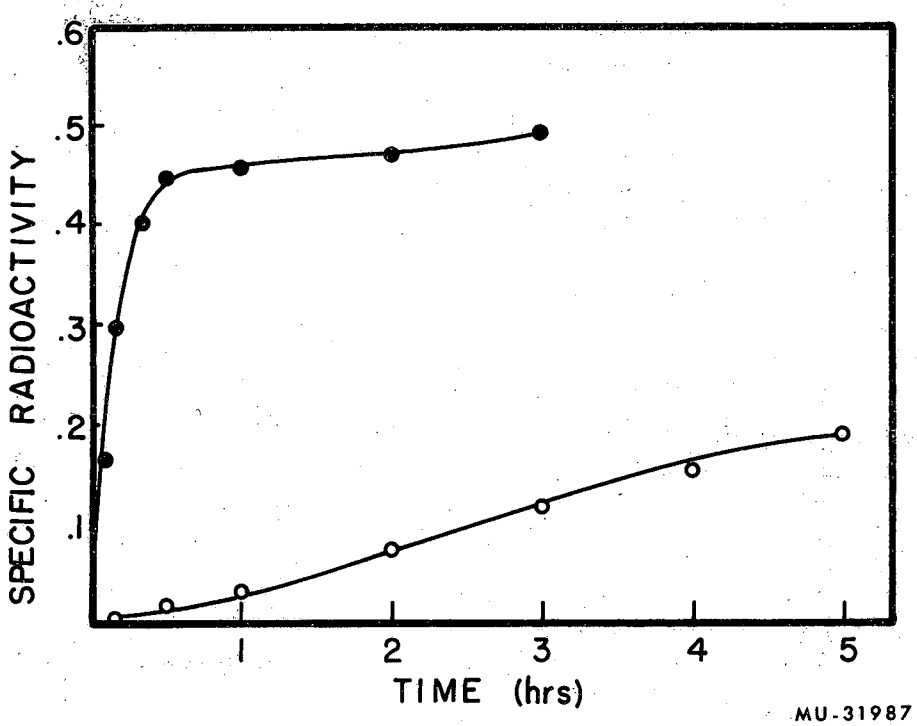


Fig. 3. Specific radioactivity of the free pool of alanine and of alanine obtained by hydrolysis of protein fractions A and B. Closed circles represent free amino acid, and open circles represent amino acids from hydrolyzed protein. Specific radioactivity is here defined as the μmoles of ^{14}C divided by the total μmoles of carbon found in the amino acids.

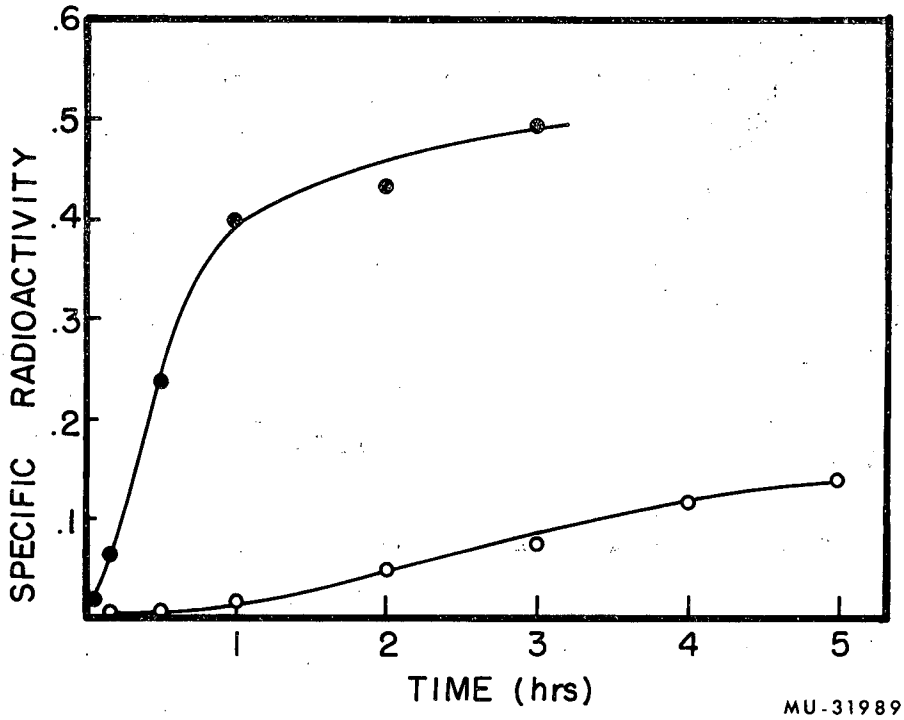


Fig. 4. Specific radioactivity of the free pool of glutamic acid and of glutamic acid obtained by hydrolysis of protein fractions A and B. Closed circles represent free amino acid, and open circles represent amino acids from hydrolyzed protein.

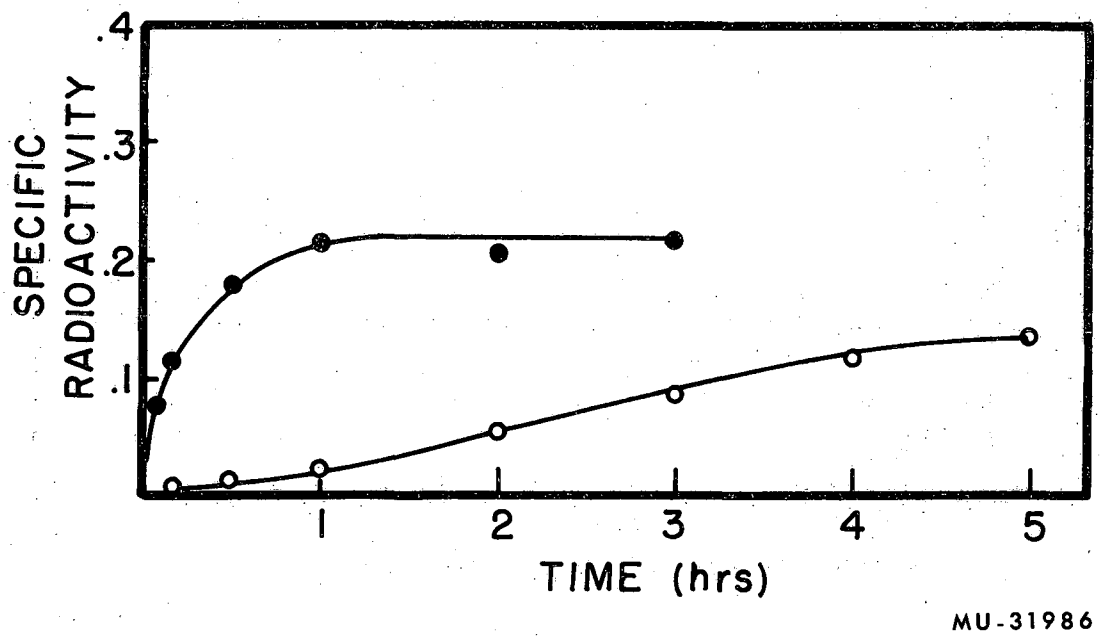


Fig. 5. Specific radioactivity of the free pool of aspartic acid and of aspartic acid obtained by hydrolysis of protein fractions A and B. Closed circles represent free amino acid, and open circles represent amino acids from hydrolyzed protein.

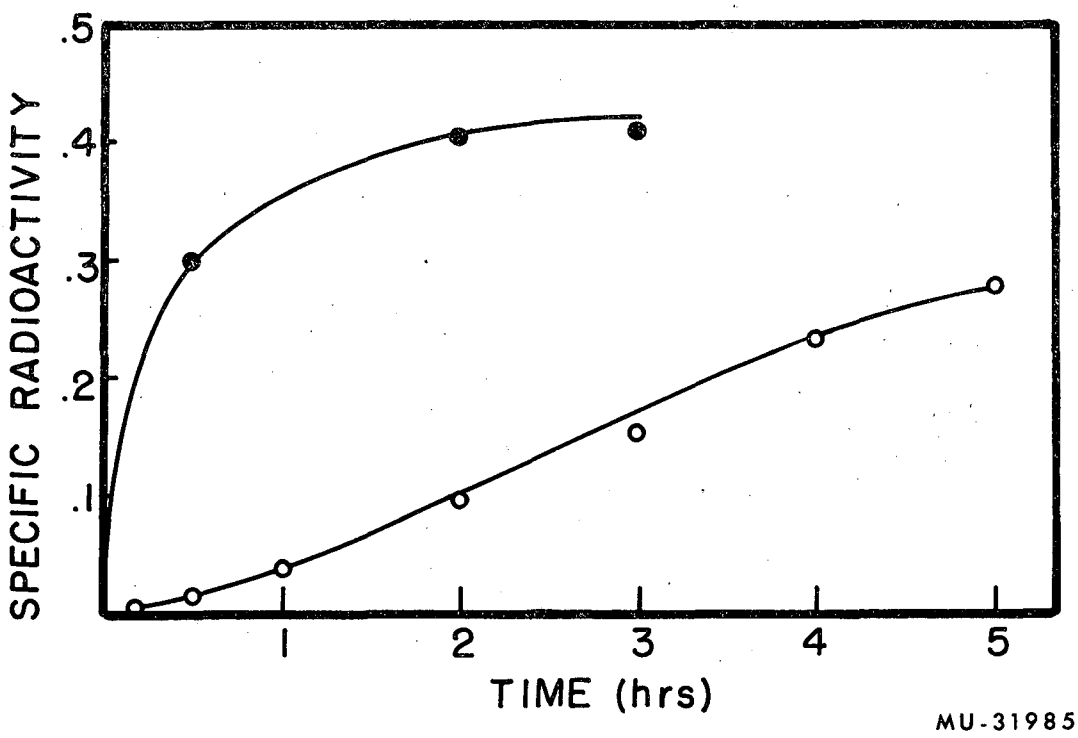


Fig. 6. Specific radioactivity of the free pool of threonine and of threonine obtained by hydrolysis of protein fractions A and B. Closed circles represent free amino acid, and open circles represent amino acids from hydrolyzed protein.

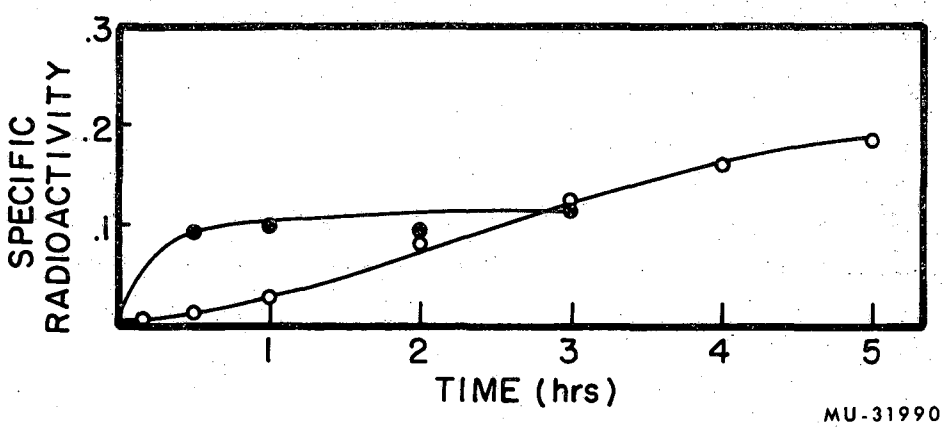


Fig. 7. Specific radioactivity of the free pool of serine and of serine obtained by hydrolysis of protein fractions A and B. Closed circles represent free amino acid, and open circles represent amino acids from hydrolyzed protein.

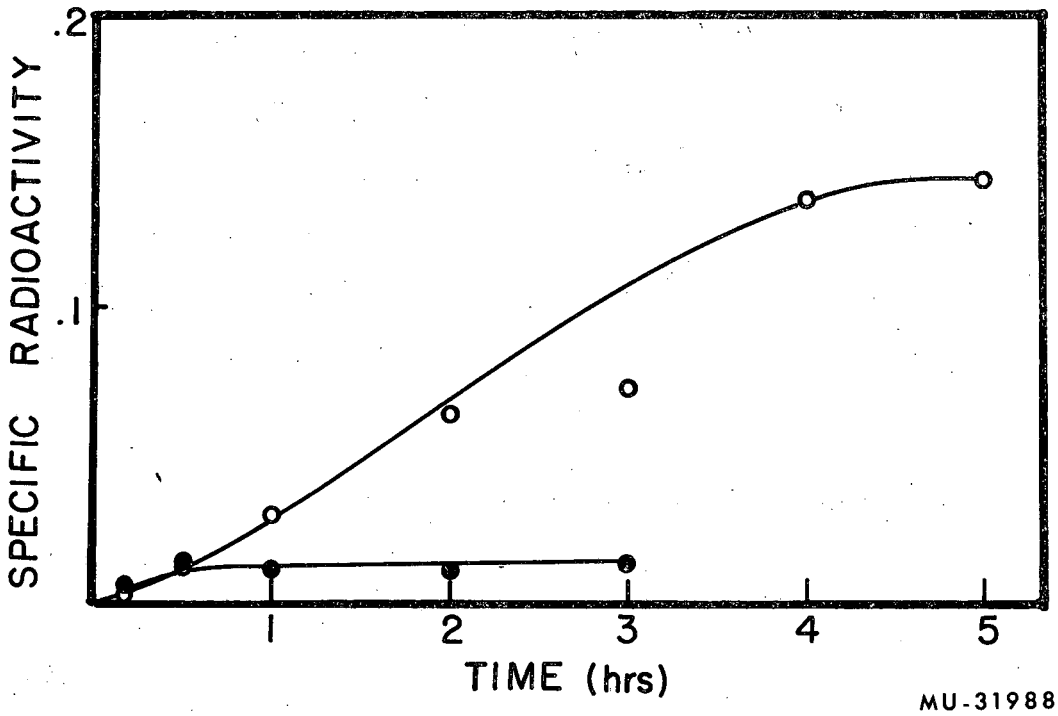


Fig. 8. Specific radioactivity of the free pool of glycine and of glycine obtained by hydrolysis of protein fractions A and B. Closed circles represent free amino acid, and open circles represent amino acids from hydrolyzed protein.

the amide groups.

DISCUSSION

In the curves for free amino acids we see the previously described[2] saturation of actively turning over pools of "primary" amino acids separated from other inactive pools of the same amino acids. These pools which we believe to be photosynthetically formed and located in the chloroplast, typically constitute 40-50% of the total amino acid pools and therefore "saturate" at a level which gives an average specific radioactivity of 0.2-0.5. However, we see that in the case of serine in this experiment, saturation occurred at about 0.1 specific radioactivity for the entire pool of serine while for glycine this "saturation" occurs at a specific radioactivity for the total glycine pool of only about .015. This is another way of saying that the actively turning over pool of glycine, presumed to be in the chloroplast, is extremely small. This result explains why we were led to suggest the possibility of the formation of bound glycine moieties of protein from a non-amino acid precursor[2]. However, from the present data, it can be seen that the protein bound glycine can all arise from the small and actively turning over pool of glycine; indeed, for all of the bound and free amino acids for which labeling rates have been compared, the maximum rate of labeling of the bound amino acid is achieved only by the time the actively turning over pool of the free amino acid is saturated with ^{14}C. We regard this as conclusive evidence for the conversion of amino acids of the actively turning over free pools to the bound amino acids of the proteins during photosynthesis.

This conclusion appears to be in agreement with the results of studies reported by Hellebust and Bidwell[24] who found that protein-bound serine and glycine were derived from "photosynthate" by a route bypassing

the bulk of soluble pools of these amino acids in wheat leaves. As we have shown here, the pools of these amino acids at the photosynthetic site can be very small compared with the total soluble pool, a possibility recognized by Hellebust and Bidwell. What is perhaps surprising is that results from two such different photosynthetic organisms should exhibit such similiarity in regard to actively-turning over pool sizes of specific amino acids.

It is somewhat disturbing that the total rate of labeling of bound amino acids isolated from the protein is never more than about 1.2 μmoles of $^{14}C/min/cm^3$ algae, if we remember that the synthesis of free amino acids from $^{14}CO_2$ may be as high as 3.5-6 μmoles/min/cm^3 algae[2]. A number of factors may combine to cause this apparent discrepancy. First the recovery of protein cannot be complete though probably the sum of fraction A and fraction B protein accounts for some 60-80% of the total protein which we might estimate to be some 40% of the dry weight, or 64 mg. Most of the free amino acids for which we have not drawn curves appear to saturate with ^{14}C more slowly than the "primary" amino acids as a consequence of their being formed from the primary amino acids. Thus the carbon 14 must find its way through a number of more slowly saturating pools before it can be incorporated into the protein. We have from time to time seen indication of the formation of labeled peptides during photosynthesis and these may not be precipitated by the treatment used in the isolation of protein. Since the pool sizes of these peptides may be substantial, the flow of ^{14}C through the peptide pools may delay its entry into the protein. Finally, we may expect that the synthesis of other non-protein compounds including the nucleic acids will account for a substantial portion of the labeled amino acid utilization.

Nevertheless, the rate of flow of ^{14}O into free amino acids is so much more than we have found the rate into protein to be in this study that we must consider the possibility of the free amino acids being used in part for the synthesis of non-nitrogenous compounds.

At the same time, synthesis of protein from non-photosynthetically formed amino acids, as found by Hellebust and Bidwell, would utilize the nitrogen released by the incorporation of photosynthetically formed amino acids into non-nitrogenous compounds.

Further investigation of the quantitative aspects of these pathways and of the peptide pool sizes will be required to obtain a more complete picture of the relation of free amino acid pool labeling and bound amino acid pool labeling.

ACKNOWLEDGEMENT

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

1. J.A. BASSHAM, A.A. BENSON, L.D. KAY, A.Z. HARRIS, A.T. WILSON AND M. CALVIN, J. Am. Chem. Soc., 76 (1954) 1760.

2. J.A. BASSHAM AND M. CALVIN, The Path of Carbon in Photosynthesis, Prentice-Hall, Englewood Cliffs, New Jersey, 1957.

3. M. CALVIN AND J.A. BASSHAM, The Photosynthesis of Carbon Compounds, W.A. Benjamin, Inc., New York, 1962.

4. M. CALVIN AND A.A. BENSON, Science, 107 (1948) 476.

5. W. STEPKA, A.A. BENSON AND M. CALVIN, Science, 108 (1948) 304.

6. M. CALVIN, J.A. BASSHAM, A.A. BENSON, V. LYNCH, C. OUELLET, L. SCHOU, W. STEPKA AND N. TOLBERT, Symp. Soc. Exptl. Biol., 5 (1951) 284.

7. S. ARONOFF, A.A. BENSON, W.Z. HASSID AND M. CALVIN, Science, 105 (1947) 664.

8. A.A. NICHIPOROVICH, First Geneva Conference on Peaceful Uses of Atomic Energy, paper 697, 1955.

9. T.F. ANDREYEVA, Doklady Akad. Nauk SSSR, 785 (1951) 1033.

10. N.R. VOSKRENSKAYA, Doklady Akad. Nauk SSSR, 932 (1953) 911.

11. L.A. NEZGOVOROVA, Fiziol. Rastenii, Akad. Nauk SSSR, 6 (1959) 451.

12. N.M. SISSAKIAN, Proceedings of the Second United Nations Conference on the Peaceful Uses of Atomic Energy, Vol. 25, part 2, p. 159.

13. U. HEBER, Nature, 195 (1962) 91.

14. J.A. BASSHAM AND MARTHA KIRK, Biochim. et Biophys. Acta, 43 (1960) 447.

15. D.C. SMITH, J.A. BASSHAM AND MARTHA KIRK, Biochim. et Biophys. Acta, 48 (1961) 299.

16. R.G.S. BIDWELL, Can. J. Bot., 35 (1957) 945.

17. F.C. STEWARD, R.G.S. BIDWELL AND E.W. YEMM, J. Exptl. Bot., 9 (1958) 11.

18. F.C. STEWARD, R.G.S. BIDWELL AND E.W. YEMM, Nature, 178 (1956) 734.

19. J.A. BASSHAM AND M. KIRK, submitted for publication.

20. W. MEJBAUM-KATZENELLENBOGEN, Acta Biochim. Polon., 2 (1955) 279.

21. G.J. CROWLEY, V. MOSES AND J. ULLRICH, J. Chromatog., in press.

22. B. MORAWIECKA, AND W. MEJBAUM-KATZENELLENBOGEN, Clin. Chim. Acta, 7 (1962) 722.

23. V. MOSES AND K.K. LONBERG-HOLM, Anal. Biochem., 5 (1963) 11.

24. J.A. HELLEBUST AND R.G.S. BIDWELL, Can. J. Bot., 41 (1963) 985.