Plant Physiol. (1967) 42, 991-996

Enhanced Dark CO_2 Fixation by Preilluminated Chlorella pyrenoidosa and Anacystis nidulans[1]

Robert K. Togasaki[2] and Martin Gibbs[3]

Department of Biochemistry, Cornell University, Ithaca, New York 14805

Received March 22, 1967.

Summary. The products of short time photosynthesis and of enhanced dark $^{14}CO_2$ fixation (illumination in helium prior to addition of $^{14}CO_2$ in dark) by *Chlorella pyrenoidosa* and *Anacystis nidulans* were compared. Glycerate 3-phosphate, phosphoenolpyruvate, alanine, and aspartate accounted for the bulk of the ^{14}C assimilated during enhanced dark fixation while hexose and pentose phosphates accounted for the largest fraction of isotope assimilated during photosynthesis. During the enhanced dark fixation period, glycerate 3-phosphate is carboxyl labeled and glucose 6-phosphate is predominantly labeled in carbon atom 4 with lesser amounts in the upper half of the C_6 chain and traces in carbon atoms 5 and 6. Tracer spread throughout all the carbon atoms of photosynthetically synthesized glycerate 3-phosphate and glucose 6-phosphate. During the enhanced dark fixation period, there was a slow formation of sugar phosphates which subsequently continued at 5 times the initial rate long after the cessation of $^{14}CO_2$ uptake. To explain the kinetics of changes in the labelling patterns and in the limited formation of the sugar phosphates during enhanced dark CO_2 fixation, the suggestion is made that most of the reductant mediating these effects did not have its origin in the preillumination phase.

It is concluded that a complete photosynthetic carbon reduction cycle operates to a limited extent, if at all, in the dark period subsequent to preillumination.

A current concept of photosynthesis postulates that a reductant generated in the light mediates the assimiliation of CO_2 through the "light independent" reactions of the reductive pentose phosphate cycle. If this is correct, then it should be possible to separate the overall process into 2 steps: the generation of the reductant in the light phase and its utilization in a subsequent dark period. Calvin and Benson (1, 2) showed that *Chlorella* and *Scenedesmus* illuminated in an atmosphere of 100 % N_2 (preillumination) were able to assimilate briefly $^{14}CO_2$ in the subsequent dark period at a rate higher than that of an unilluminated sample. From analysis of the products of this enhanced dark $^{14}CO_2$ fixation, which included labeled sugars, they concluded that both carboxylation of an accumulated CO_2 acceptor and reduction of some of the subsequently labeled glycerate 3-P to the carbohydrate level took place. They postulated the existence of a long lived reductant that survived into the dark period. Bassham and Kirk (3) have reported a rapid and parallel incorporation of tracer into glycerate-3-P and hexose monophosphate during enhanced dark $^{14}CO_2$ fixation following a period of photosynthesis. These workers envisaged the generation of long lived reducing power along with ribulose 1,5-diP during the period of illumination and the conversion of $^{14}CO_2$ to carbohydrate in the dark through the reactions of the photosynthetic carbon reduction cycle, mediated by this reducing power.

Gaffron and co-workers (4, 5) investigated the existence of enhanced dark $^{14}CO_2$ fixation in preilluminated *Scenedesmus obliquus*. However, they found 95 % of the assimilated tracer to be in the carboxyl carbons of glycerate 3-P and P-enolpyruvate. Hence, they argued that enhanced dark CO_2 fixation represented only the carboxylation of an accumulated CO_2 acceptor.

In a series of papers, Tamiya, Miyachi, and co-workers (6, 7) studied the effect of various oxidants upon the level of enhanced dark $^{14}CO_2$ fixation during preillumination, and also during the subsequent dark period. They concluded that the agent responsible for enhanced dark CO_2 fixation was a reductant and designated it as "R". Subsequent investigations have not clarified the nature of "R" nor the mode of its operation.

The exact nature of enhanced dark CO_2 fixation by preilluminated algae remains uncertain. In this report, the labeled products of enhanced

[1] Supported by funds from the National Science Foundation and from the United States Air Force through the Air Force Office of Scientific Research Development Command under contract No. AF 49(638)785.

[2] Present address: Department of Biology, Harvard University, Cambridge, Massachusetts 02138.

[3] Present address: Department of Biology, Brandeis University, Waltham, Massachusetts 02154.

dark $^{14}CO_2$ fixation and photosynthetic $^{14}CO_2$ fixation by *Chlorella pyrenoidosa* and *Anacystis nidulans* are compared, as well as the distribution of tracer within the glucose-6-P and glycerate-3-P formed under each condition.

Materials and Methods

Plant Materials. *Chlorella pyrenoidosa* and *Anacystis nidulans* were grown as described in an earlier paper (8). Cells were washed twice with 2 mM K_2SO_4, and resuspended in 20 mM potassium phosphate (pH 7.5) with a final cell density of 10 mg wet weight per ml. Prior to each experiment, the cell suspension was aerated in darkness for at least 16 hours to reduce endogenous reserves.

Experimental Apparatus and Protocol. The apparatus for the preillumination experiments consisted of 3 parts. The first vessel for pretreatment was a round flat glass illumination vessel, lollipop shaped, encased in a plexiglass constant temperature bath, and it was equipped with a tube for introducing helium gas. The second vessel for $^{14}CO_2$ fixation was a darkened 125 ml separatory funnel that carried a rubber stopper equipped with a tube for introducing helium and another tube for the outlet. One ml of solution containing 30 μmoles of $NaH^{14}CO_3$ and 1200 μc of radioactivity was placed inside the funnel just prior to the experiment. The third vessel for the termination of the reaction was a darkened round bottom flask containing 200 ml of absolute ethyl alcohol maintained at 78°.

In the preillumination experiments, a cell suspension of 50 ml was illuminated in the lollipop for 30 minutes under helium. Then the light was turned off and the content of the lollipop was transferred into the second vessel. The second vessel was stoppered and shaken manually. Then the gas outlet was closed, the stopcock opened, and the cell suspension under pressure of helium gas was forced into the ethyl alcohol in the third vessel. The third vessel was stoppered and kept at 78° in darkness for 5 more minutes, and then cooled in an ice bath. The entire operation was carried out in a dark room. For a control, a cell suspension was kept dark in the lollipop under helium for 30 minutes prior to exposure to $NaH^{14}CO_3$ in the dark in the second vessel.

In the photosynthesis experiments, a cell suspension was illuminated in the lollipop for 30 minutes under air instead of helium, and then the $NaH^{14}CO_3$ solution was injected into the lollipop. The entire content of the lollipop was eventually transferred directly into the third vessel to terminate the reaction.

The light sources were 2 spot lamps each producing 4000 ft-c of white light.

Fractionation of the Products of $^{14}CO_2$ Fixation. Products of $^{14}CO_2$ fixation were fractionated into 4 fractions, designated as insoluble, ether soluble, trichloroacetic acid insoluble, and water soluble.

The components of the water soluble fraction were separated on a Dowex 1-8 × chloride column. Eleven fractions each containing ^{14}C were observed in the elution pattern. The major components of 10 fractions were identified.

Determination of Distribution of Tracer in Glucose 6-P and Glycerate 3-P. Samples from fraction 4 containing glucose 6-P and from fraction 6 containing glycerate 3-P were treated with potato acid phosphatase and the resultant glucose and glyceric acid were degraded as described earlier (9).

Table I. *Fractionation of the Products of CO_2 Fixation*

The cell suspension in 80 % (v/v) ethyl alcohol was centrifuged and the residue was extracted at 78° twice with 80 % ethyl alcohol, 3 times with 20 % (v/v) ethyl alcohol, and twice with 100° water. The final residue was designated as insoluble fraction. The supernatant fractions were combined, reduced in volume under vacuum, and treated at 4° with 50 % trichloroacetic acid until a final concentration of 5 % was reached. The mixture was centrifuged and the residue was re-extracted with cold 5 % trichloroacetic acid. The final residue was designated as trichloroacetic acid insoluble fraction. The supernatant fractions were combined and extracted with several portions of ethyl ether until the water phase appeared neutral to pH paper. The extracted ether soluble fractions were combined, brought to near dryness under an air stream, and redissolved in a small portion of absolute ethyl alcohol. The final neutral aqueous phase (water soluble fraction) contained the phosphorylated compounds, amino acids, sugars, and organic acids. The total cpm ($\times 10^{-6}$) incorporated in each experiment was: I) 6.2; II) 5.0; III) 6.3; IV) 10.1.

Expt No	Organism	Expt condition	Time	Water soluble	Ether soluble	Trichloroacetic acid insoluble	Insoluble
				%	%	%	%
I	*Anacystis*	Photosynthesis	1 Min	88.0	0.9	1.2	9.9
II	*Anacystis*	Dark CO_2 fixation	8 Min	98.2	0.6	0.3	0.9
III	*Chlorella*	Photosynthesis	15 Sec	93.0	4.9	0.4	2.0
IV	*Chlorella*	Dark CO_2 fixation	1 Min	99.0	0.7	0.0	0.6

* Data are expressed as percent of total ^{14}C incorporated.

Results and Discussion

Roughly 98 % of ^{14}C assimilated during enhanced dark $^{14}CO_2$ fixation by *Chlorella* and *Anacystis* resided in the water soluble fraction (table I). The same fraction also accounted for the bulk of ^{14}C assimilated during photosynthesis. Therefore, the other fractions were not treated further and an analysis of the water soluble fraction was considered to be equivalent to an analysis of all products of enhanced dark $^{14}CO_2$ fixation and of photosynthesis.

With *Anacystis*, 91 % of the tracer assimilated during enhanced dark $^{14}CO_2$ fixation resided in glycerate 3-P, P-enolpyruvate, aspartate, and alanine. The sugar phosphates accounted for 8 %. In contrast, the products of short time photosynthesis showed lesser amounts in the 4 carboxyl-containing compounds (33 %) and a higher percentage (60 %) of incorporated tracer in the sugar phosphates (fig 1). Experiments with *Chlorella* showed an identical pattern.

Similarity of products of enhanced dark $^{14}CO_2$ fixation is taken as evidence that similar processes were operating in both the green and blue-green algae. The presence of only a few percent of isotope in fractions containing carbohydrates is interpreted as indicating that very little reductant formed during the preillumination phase survived into the dark phase. Confirmatory evidence for this conclusion is obtained from comparison of the distribution patterns of ^{14}C within the glycerate 3-P and glucose 6-P formed during enhanced dark $^{14}CO_2$ fixation with that synthesized during a brief period of photosynthesis (tables II and III). Photosynthetically synthesized glycerate 3-P and glucose 6-P showed the expected spreading of tracer into all carbon atoms (9, 10). In sharp contrast, glycerate 3-P formed during the enhanced dark phase remained carboxyl labeled and glucose 6-P from the same experiments had very little isotope in the C-5 and C-6 positions. Indeed, the hexose monophosphate had an unusually extreme asymmetrical isotopic distribution, with C-4 accounting for approximately 75 to 80 % of the tracer, followed by C-3, then by C-1 and C-2, with little tracer in C-5 and C-6. The very uneven distribution of tracer in glucose 6-P formed during enhanced dark $^{14}CO_2$ fixation may resemble the tracer distribution of the compound formed in an early period of photosynthesis. Therefore, enhanced dark CO_2 fixation may reflect a slowed down version of photosynthetic $^{14}CO_2$ fixation. We consider the intra- and intermolecular distributions of ^{14}C as evidence establishing that the photosynthetic carbon reduction cycle functions very little in the dark period following preillumination. Therefore, the bulk of enhanced dark CO_2 fixation represents the carboxylation of ribulose 1,5-diP accumulated during the preillumination period.

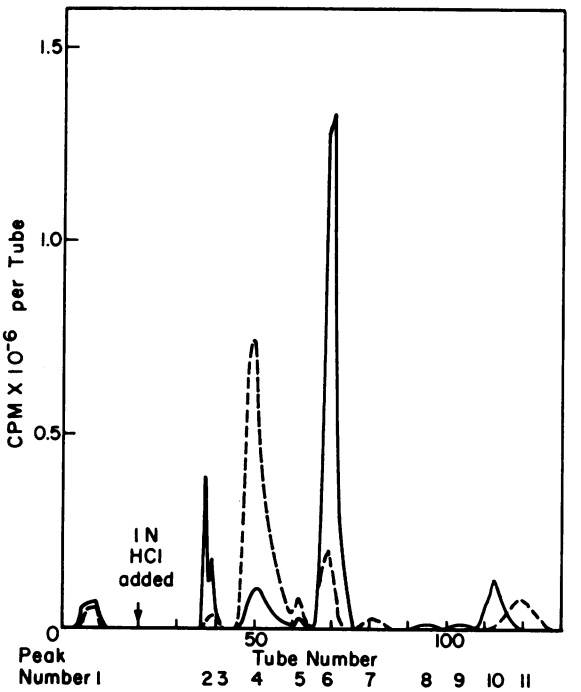

FIG. 1. Separation of components of the water soluble fraction obtained from *Anacystis*. The components of the water soluble fraction (see table I) were separated by gradient elution with HCl on a 1 × 40 cm Dowex 1-8× chloride column. Gradient elution was carried out by connecting a 1000 ml separatory funnel containing 0.1 N HCl to a 125 ml mixing flask containing water. Ten μmoles each of unlabeled glucose 6-P, glycerate 3-P, and fructose-1,6-diP, were added to the sample. The sample was applied to the resin bed, followed by 100 ml of water, and then by the HCl gradient. Aliquots of 0.05 ml were taken from the 5 ml fractions in each tube and its tracer content determined. Aliquots were also assayed enzymically for the added carrier.

The solid line represents the labeled products from cells exposed to $NaH^{14}CO_3$ for 16 minutes in the dark following preillumination. The dashed line represents the products of 1 minute of photosynthetic $^{14}CO_2$ fixation. Movement of radioactivity in high voltage electrophoresis, and in paper chromatography with at least 2 different solvents, was used to identify the components of each fraction. In addition, samples of peak 11 were treated with ribulose-1,5-diP carboxylase and $NaH^{12}CO_3$ and the major labeled product of the reaction was found to be glycerate 3-P. A sample from peak 10 was treated with 2,4-dinitrophenyl-hydrazine. The product was found to be identical to the 2,4-dinitrophenyl-hydrazine derivative of authentic pyruvate. The components of each peak were: 1) Alanine, with traces of other neutral amino acids and some sugars, 2) Aspartic acid with a trace of glutamic acid, 3) Mostly malic acid, 4) Glucose 6-P with some fructose 6-P and pentose monophosphate, 5) Dihydroxyacetone-P with a small amount of pentose monophosphate, 6) Glycerate 3-P, 7) Unidentified, 8) Sedoheptulose-1,7-diP, 9) Fructose 1,6-diP, 10) Phosphoenolpyruvate, and 11) Ribulose 1,5-diP.

The occurrence of small amounts of isotope in the sugar phosphates formed during the dark phase is considered next. If the formation of labeled sugar phosphates during enhanced dark $^{14}CO_2$ fixation was mediated by a reductant surviving into the dark phase from the preillumination period, the movement of assimilated tracer into the sugar phosphates should diminish during an increasing dark period. On the other hand, if formation of labeled sugar phosphates was mediated by reactions unrelated to preillumination, different kinetics might be expected. To examine these possibilities more closely, preilluminated cells of *Anacystis* were exposed to $NaH^{14}CO_3$ in the dark for different time periods. Subsequently distribution of tracer among the water soluble compounds and tracer distribution within glucose 6-P were determined at each time point. Figure 2 illustrates the time course of ^{14}C incorporation into the water soluble compounds during enhanced dark $^{14}CO_2$ fixation by *Anacystis*. Tracer content of the glycerate 3-P and P-enolpyruvate increased parallel to total $^{14}CO_2$ uptake for the initial 4 minutes, and then declined. The percentage distribution of isotope extrapolated to zero time was 96 % for glycerate 3-P, again suggesting that the carboxylation of ribulose-1,5-diP was the prime route of carbon fixation during the enhanced dark $^{14}CO_2$ fixation. Since $^{14}CO_2$ uptake ceased after 4 minutes, it is quite clear that the tracer content of the other fractions increased with time, at the expense of glycerate 3-P and P-enolpyruvate. The tracer content of fraction 5 (triose-P) showed a slow linear increase for 4 minutes, followed by a constant level throughout the rest of the time periods. The tracer content of fraction 4 (hexose and pentose monophosphates) increased slowly for the first 2 minutes, and then increased linearly at 5 times the initial rate for the remaining time intervals. Fructose and sedoheptulose diphosphates increased in

Table II. *Distribution of ^{14}C in Glycerate 3-P Formed During Photosynthesis and Enhanced Dark $^{14}CO_2$ Fixation*

Source of PGA		C-1	C-2	C-3	% Recovery
		COOH	α	β	
Chlorella	% Of C-1	100	15.5	9.3	
Photosynthesis	% Of total	82	12.5	7.5	102
15 Seconds	Sp act*	9.5			
Chlorella	% Of C-1	100	0.08	0.06	
Dark fixation	% Of total	95.0	0.07	0.06	95.1
1 Minute	Sp act*	14.0			
Anacystis	% Of C-1	100	11.7	2.3	
Photosynthesis	% Of total	92	10.8	2.2	105
1 Minute	Sp act*	7.7			
Anacystis	% Of C-1	100	0.16	0.58	
Dark fixation	% Of total	102	0.17	0.58	103
8 Minutes	Sp act*	11.7			

* Specific activity (sp act) is expressed as mμcuries per mg of carbon.

Table III. *Distribution of ^{14}C in Glucose 6-P Formed during Enhanced Dark CO_2 Fixation and Photosynthesis by* Anacystis *and* Chlorella

Source of G6P		C-1	C-2	C-3	C-4	C-5	C-6	% Recovery
Anacystis	% Of C-4	1.2	2.4	12.2	100	0	...**	
Dark fixation,	% Of total	0.9	1.9	9.5	79	0	...	92
4 Minutes	Sp act*				0.18			
Anacystis	% Of C-4	2.6	5.2	10.0	100	0	5.2	
Dark fixation,	% Of total	2.1	4.2	8.3	81	0	4.2	99.8
8 Minutes	Sp act*				0.17			
Anacystis	% Of C-4	2.5	2.8	4.0	100	0	0	
Dark fixation,	% Of total	2.1	1.4	3.3	82.3	0	0	89.1
16 Minutes	Sp act*				1.31			
Anacystis	% Of C-4	17.0	13.3	78	100	6.9	11.7	
Photosynthesis,	% Of total	6.8	5.4	31.2	40	2.8	4.7	90.9
1 Minute	Sp act*				7.45			
Chlorella	% Of C-4	18.2	24.4	42.5	100	13.0	14.5	
Photosynthesis,	% Of total	6.4	8.7	15.0	35.2	4.6	5.2	75
15 Seconds	Sp act*				0.42			
Chlorella	% Of C-4	2.8	6.6	27.2	100	0.8	0.4	
Dark fixation,	% Of total	1.6	5.0	20.7	76.00	0.6	0.3	102
1 Minute	Sp act*				0.54			

* Specific activity (sp act) is expressed as mμcuries per mg of carbon.

** Sample lost.

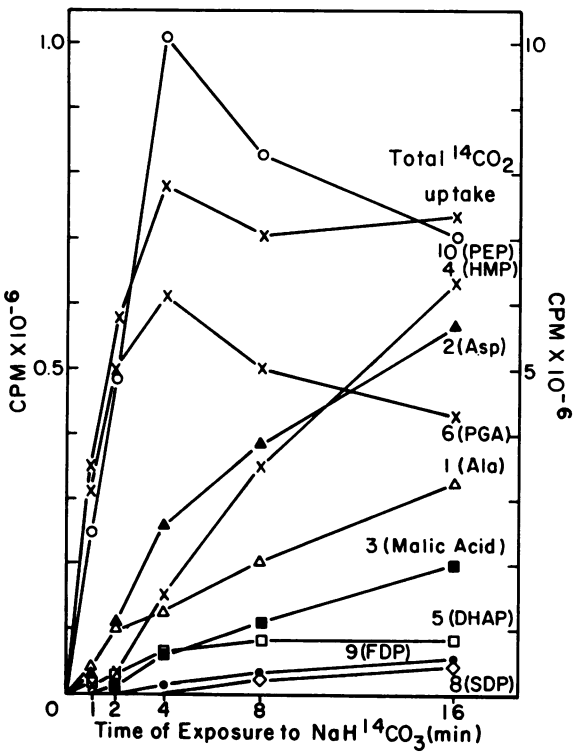

FIG. 2. Time course of ^{14}C incorporation into products during enhanced dark $^{14}CO_2$ fixation by *Anacystis*. Cell concentration was 10 mg wet weight per ml. After 30 minutes of preillumination, 50 ml of cell suspension was exposed to 1 ml of NaH $^{14}CO_3$ (1200 μc, 30 μmoles) in darkness for a given time period. The water soluble products were isolated and separated on a Dowex 1-8× chloride column. Total radioactivity in each fraction is plotted against the time of exposure of the cells to NaH $^{14}CO_3$ in dark. Total $^{14}CO_2$ uptake and glycerate 3-P corresponds to the scale on right. The rest of the materials correspond to the scale on left. $^{14}CO_2$ uptake is equivalent to 3 % of the total $^{14}CO_2$ available.

The symbols are: Ala, alanine; Asp, aspartic acid; PEP, P-enolpyruvate; HMP, hexose monophosphate (this fraction from the experiments of 8 and 16 mins contained some labeled pentose monophosphates); DHAP, dihydroxyacetone-P; FDP, fructose-1,6-diP; SDP, sedoheptulose-1,7-diP and PGA, glyceric acid 3-P.

tracer content linearly after a lag period of 2 and 4 minutes, respectively.

To account for these kinetic data, we envisage that the initial slow formation of carbohydrate is mediated by a reductant that had its origin in the preillumination period while the subsequent more rapid reduction of glycerate 3-P is driven by a reducing power generated during the enhanced dark $^{14}CO_2$ fixation phase of the experiments. A reasonable mechanism to account for the kinetics of changes of sugar phosphate labeling starts with the assumption that there is considerable movement of the glycerate 3-P between the photosynthetic apparatus and the cytoplasm (11). The time required for labeled glycerate 3-P formed in the carboxylation reaction to equilibrate with cytoplasmic glycerate 3-P and for tracer to enter the triose-P pool could account for the lag observed in the labeling of the C_5, C_6, and C_7 sugar phosphates. The reducing power would be supplied by the glycolytic breakdown of reserve carbohydrates. Kandler and Liesenkotter (12) have showed that in *Chlorella* glycolysis is inhibited during photosynthesis but not in a subsequent dark period, resulting in a drop in the level of sugar phosphates. If following preillumination, a similar series of reactions was occurring in *Anacystis*, then substrate phosphorylation produced ATP and DPNH generated in the cytoplasm might, in turn, mediate the reduction of glycerate 3-P released from the photosynthetic apparatus into the cytoplasm.

These transient changes are now considered with respect to the extremely unsymmetrical distribution of tracer within the glucose 6-P formed during the dark periods (table III). A comparable finding was observed in the polyglucan isolated from the blue-green alga, *Tolypothrix tenuis*, after exposure to $^{14}CO_2$ in the dark for 1 hour (13). The limited spreading of tracer from C-4 into the upper half of the C_6 chain was explained in terms of incomplete isotopic equilibration by triose-P isomerase and of the transketolase-transaldolase catalyzed reactions (13). The complete absence of isotope in C-5 and C-6 coupled to the observation that we could not detect ^{14}C in the ribulose 1,5-diP despite the presence of labeled pentose monophosphate lends strong support to the idea that the most likely site for the break in the photosynthetic carbon reduction cycle is the apparent absence of a kinase reaction forming ribulose 1,5-diP in the dark. However, there was a continuous supply of ATP during the entire experimental period as evidenced by the kinetics of the labeling of the sugar phosphates. It would seem that the kinase enzyme does not have access to ATP which is produced by reactions located outside of the photosynthetic apparatus. Earlier, Pederson, Kirk, and Bassham (14) came to this conclusion when investigating light-dark transients during photosynthesis in *Chlorella*. Alternatively, it may be that the hexose and pentose phosphates formed in the cytoplasm cannot penetrate the photosynthetic apparatus. Studies with the whole chloroplast isolated from higher plants showed that ribose 5-P and fructose 1,6-diP but not fructose 6-P and glucose 6-P can cross the double membrane and enter the photosynthetic carbon reduction cycle (15). An "absolute" light-dark switch controlling the activity of ribulose 5-P kinase is unlikely since at least 2 groups have demonstrated sufficient ribulose 5-P kinase in extracts of photosynthetic organisms kept in darkness to catalyze the observed rates of CO_2 fixation (16, 17). However, preliminary experiments in this laboratory have shown that the catalytic activity of ribulose 5-P kinase is about

2-fold higher in illuminated leaves over that found in the dark control (E. Latzko, unpublished observations). Most likely ATP produced in the chloroplast during the light phase is consumed rapidly in kinase and hydrolytic reactions (8, 14) and that ATP other than that produced by photophosphorylation cannot be utilized by the kinase reactions of the photosynthetic carbon reduction cycle. We are not aware of any unequivocal evidence demonstrating that ATP can penetrate the entire photosynthetic apparatus which retains their outer double membrane and their stroma.

Finally, enhanced dark CO_2 fixation was observed with preilluminated cells of *Porphyridium cruentum* and *Tolypothrix tenuis* (unpublished observations). Since the phenomenon is observed in green, blue-green and red algae, it appears to be a common property of all photosynthetic algae. Whether or not leaves of higher plants have this property is unknown.

Literature Cited

1. Calvin, M. and A. A. Benson. 1947. The path of carbon in photosynthesis. Science 107: 476–80.
2. Benson, A. A. and M. Calvin. 1950. Respiration and photosynthesis. J. Exptl. Botany 1: 63–68.
3. Bassham, J. A. and M. Kirk. 1963. Synthesis of compounds from $^{14}CO_2$ by *Chlorella* in the dark following preillumination. In: Studies on Microalgae and Photosynthetic Bacteria, (special issue) Plant Cell Physiol. 493–503.
4. Fager, E. W., J. L. Rosenberg, and H. Gaffron. 1950. Intermediates in photosynthesis. Federation Proc. 9: 535–42.
5. Gaffron, H. and E. W. Fager. 1951. The kinetics and chemistry of photosynthesis. Ann. Rev. Plant Physiol. 2: 87–114.
6. Miyachi, S., S. Izawa, and H. Tamiya. 1955. Effect of oxygen on the capacity of carbon dioxide fixation by green algae. J. Biochem. 42: 221–44.
7. Miyachi, S. 1960. Effect of some sulfhydryl reagents on light-induced CO_2-fixation. Plant Cell Physiol. 1: 117–30.
8. Togasaki, R. K. and M. Gibbs. 1963. Preillumination experiments in *Chlorella pyrenoidosa* and *Anacystis nidulans*. In: Studies on Microalgae and Photosynthetic Bacteria. (special issue) Plant Cell Physiol. 505–11.
9. Havir, E. A. and M. Gibbs. 1963. Studies on the reductive pentose phosphate cycle in intact and reconstituted chloroplast systems. J. Biol. Chem. 238: 3184–87.
10. Gibbs, M. and O. Kandler. 1957. Asymmetric distribution of ^{14}C in sugars formed during photosynthesis. Proc. Natl. Acad. Sci. U. S. 43: 446–51.
11. Urbach, W., M. A. Hudson, W. Ullrich, K. A. Santarius, and U. Heber. 1965. Verteilung und Wanderung von Phosphoglycerat zwischen den Chloroplasten und dem Cytoplasma während der Photosyntheses. Z. Naturforsch. 20b: 890–98.
12. Kandler, O. and I. Haberer-Liesenkotter. 1963. Über den Zusammenhang Zwischen Phosphathaushalt und Photosynthese. V. Regulation der Glykolyse durch die Lichtphosphorylierung bie *Chlorella*. Z. Naturforsch. 18b: 718–30.
13. Cheung, W. Y. and M. Gibbs. 1966. Dark and photometabolism of sugars by a blue green alga: *Tolypothrix tenuis*. Plant Physiol. 41: 731–37.
14. Pederson, T. A., M. Kirk, and J. A. Bassham. 1966. Light dark transients in levels of intermediate compounds during photosynthesis in air-adapted *Chlorella*. Physiol. Plantarum 13: 219–31.
15. Bamberger, E. S. and M. Gibbs. 1965. Effect of phosphorylated compounds and inhibitors on CO_2 fixation by intact spinach chloroplasts. Plant Physiol. 40: 919–26.
16. Peterkofsky, A. and E. Racker. 1961. The reductive pentose phosphate cycle. III. Enzyme activities in cell-free extracts of photosynthetic organisms. Plant Physiol. 36: 409–14.
17. Latzko, E. and M. Gibbs. 1966. Enzyme patterns of the reductive pentose phosphate cycle. Plant Physiol. 41: lvi–lvii.
18. Santarius, K. A. and U. Heber. 1965. Changes in the intracellular levels of ATP, ADP, AMP and Pi and regulatory function of the adenylate system in leaf cells during photosynthesis. Biochim. Biophys. Acta 102: 39–54.