UCRL-16790

University of California

Ernest O. Lawrence Radiation Laboratory

PHOTOSYNTHESIS AND DARK METABOLISM IN ISOLATED CHLOROPLASTS, LEVELS OF CARBON CYCLE INTERMEDIATE COMPOUNDS AND OF ADP, ATP, AND OF PYROPHOSPHATE

TWO-WEEK LOAN COPY

This is a Library Circulating Copy which may be borrowed for two weeks. For a personal retention copy, call Tech. Info. Division, Ext. 5545

DISCLAIMER

This document was prepared as an account of work sponsored by the United States Government. While this document is believed to contain correct information, neither the United States Government nor any agency thereof, nor the Regents of the University of California, nor any of their employees, makes any warranty, express or implied, or assumes any legal responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by its trade name, trademark, manufacturer, or otherwise, does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof, or the Regents of the University of California. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof or the Regents of the University of California.

Submitted to Biochemical and
Biophysical Research Communications

UCRL-16790

UNIVERSITY OF CALIFORNIA
Lawrence Radiation Laboratory
Berkeley, California

AEC Contract No. W-7405-eng-48

PHOTOSYNTHESIS AND DARK METABOLISM IN ISOLATED CHLOROPLASTS. LEVELS OF CARBON CYCLE INTERMEDIATE COMPOUNDS AND OF ADP, ATP, AND OF PYROPHOSPHATE

J. A. Bassham, R. G. Jensen, Martha Kirk and Susan Gelb

April 1966

UCRL-16790

PHOTOSYNTHESIS AND DARK METABOLISM IN ISOLATED CHLOROPLASTS. LEVELS OF CARBON CYCLE INTERMEDIATE COMPOUNDS AND OF ADP, ATP, AND OF PYROPHOSPHATE*

J. A. Bassham, R. G. Jensen,** Martha Kirk and Susan Gelb
Lawrence Radiation Laboratory, University of California
Berkeley, California

In kinetic studies with isolated spinach chloroplasts and radioactive bicarbonate and phosphate, during successive periods of light, dark and light, we have found further evidence for the light activation of the carboxylation and diphosphatase steps of the photosynthetic carbon reduction cycle (Pedersen et al., 1966A). Levels of ATP, ADP, and pyrophosphate decline rapidly in the dark and increase rapidly in the light. These studies became possible as the result of recent development of techniques of preparing and incubating isolated chloroplasts which photosynthesize a wide range of products from $H^{14}CO_3^-$ and $H^{32}PO_4^{-2}$, without the addition of substrate levels of intermediate compounds, cofactors, or enzymes (Jensen and Bassham, in preparation) and at rates of 30 to 70 μmoles CO_2/mg chlorophyll·hr.

Experimental. The chloroplast isolation method reported by Walker (1964) has been considerably modified to give the following conditions. The standard isolation and reaction solution contains the following (given as molarity in the final solution) with the pH adjusted to 7.6: 0.33 M sorbitol; 0.06 M NaCl; 2×10^{-3} M (ethylene, dinitrilo) tetraacetic acid, dipotassium salt; 1×10^{-3} M $MnCl_2$; 1×10^{-3} M $MgCl_2$; 5×10^{-4} M K_2HPO_4; 2×10^{-3} M $NaNO_3$; 5×10^{-3} M $Na_4P_2O_7 \cdot 10\ H_2O$; and 2×10^{-3} M sodium isoascorbate.

Fifty g of spinach leaves are washed, chilled, and the midribs removed. They are placed in a chilled Waring Blendor with 100 ml of the chilled stan-

---

*The work described in this paper was sponsored, in part, by the U. S. Atomic Energy Commission.
** NIH Postdoctoral Fellow, 1965-1966.

dark solution and blended for only 5 seconds at high speed. The slurry is poured and pressed through 6 layers of cheesecloth, and the resulting juice is centrifuged for 50 seconds at 2000 x g. The resulting pellet is resuspended in the chilled standard solution until used. Carbon dioxide fixation and phosphate incorporation are carried out in 1.5 ml of chloroplast suspension (0.27 mg chlorophyll) in a round-bottom flask (diameter 4 cm) stoppered with a serum cap. The flask is mounted on a shaker rack in a bath (20°C) with a transparent bottom through which light from a bank of 20 watt Nu-Lite Ultralux fluorescent lamps illuminates the reaction flask with an intensity of 1800 foot candles.

The shaking flask is preilluminated for 8 minutes. Then 22.5 μmoles of $NaH^{14}CO_3^-$ (9.5 μC) and 20 μC of carrier-free ^{32}P-labeled phosphate are added. Small samples (50 μl) of the chloroplast suspension were removed at the times indicated in the figures and immediately filled in 80% methanol. The resulting mixture was analyzed by two-dimensional paper chromatography, radioautography, and dual-channel scintillation counting (Pedersen et al., 1966A and 1966B).

Results. The labeling of 3-phosphoglyceric acid (PGA), ribulose-1,5-diphosphate (RuDP), and dihydroxyacetone phosphate (DHAP) with ^{32}P and ^{14}C are shown in Figures 1, 2, and 3. From Figures 1 and 2, it can be seen that the formation of PGA by the carboxylation of RuDP continues for about 2 minutes of darkness. The fact that the carboxylation apparently then ceases, in spite of a measurable remaining pool of RuDP, is consistent with our conclusion based on in vivo studies (Pedersen et al., 1966A) that there is a light activation of the carboxylation reaction of photosynthesis, and that this activation decays in about 2 minutes of darkness. When the light is turned on once again, the level of RuDP rises very rapidly due to the phosphorylation of sugar monophosphates by the photosynthetically formed ATP.

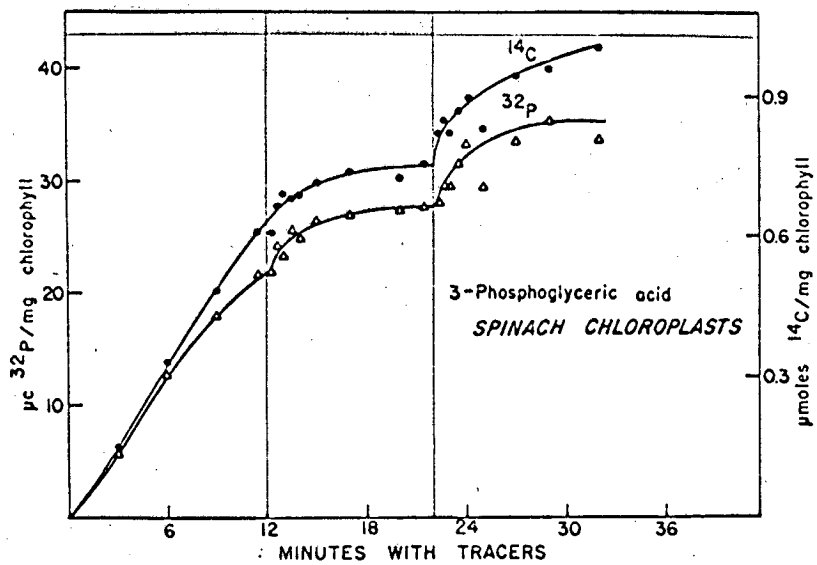

Fig. 1. Levels of ^{32}P and ^{14}C in 3-phosphoglyceric acid during photosynthesis and dark period in isolated spinach chloroplasts. The ^{14}C content of the photosynthetic intermediates has been converted to μmoles by dividing the measured radioactivity by the known specific activity of the $H^{14}CO_3^-$. Phosphorous radioactivity is expressed directly in μC.

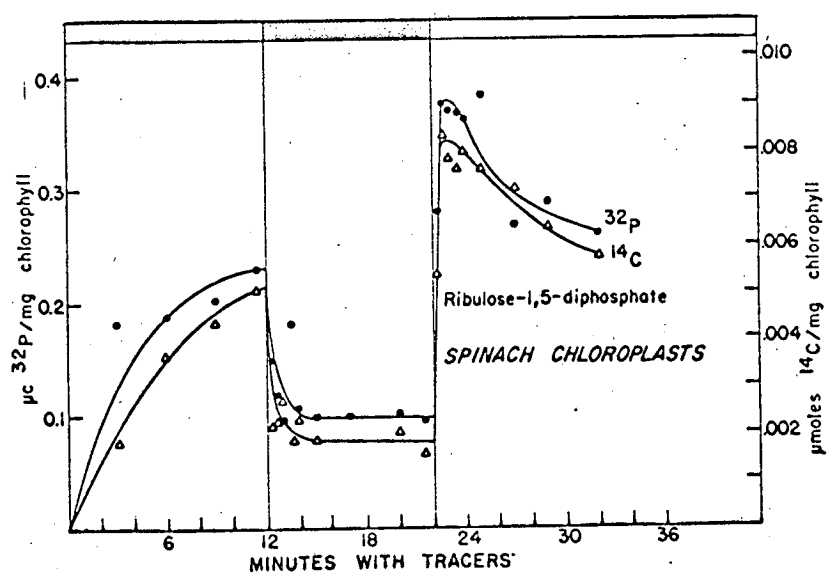

Fig. 2. Levels of ^{32}P and ^{14}C in ribulose-1,5-diphosphate during photosynthesis and dark period in isolated spinach chloroplasts.

As seen in Figure 3, there is a transient peak in DHAP label just after the light was turned on again. Our interpretation of this peak is that during the first minute of light there is a rapid reduction of PGA to DHAP and phosphoglyceraldehyde which are in equilibrium with the fructose and sedoheptulose diphosphates, whose conversion to monophosphates is not yet catalyzed, (Pedersen et al., 1966A). After 1 minute, light-activated diphosphatase activity increases, resulting in a transitory drop in the levels of these three sugar phosphates. The total ^{32}P-labeling of fructose and sedoheptulose diphosphates (not shown) followed a curve very similar to that of dihydroxyacetone phosphate.

Figure 4 shows the changes in labeled ATP, ADP and pyrophosphate (PP_i) during light-dark and light. There is not a complete labeling of these cofactors during the light periods; thus the ^{32}P-labeling represents only approximates of the total concentration of these cofactors. The levels of labeled ATP, ADP and PP_i all decline in the dark and rise in the light. The drop in level of labeled ATP and subsequent rise in the light might have been expected since photophosphorylation ceases during the dark period and the supply of ATP is used up to some extent during the light-dark transition through residual operation of the carbon reduction cycle. The change in the level of ADP was surprising, since in in vivo experiments (Pedersen et al., 1966A) the ADP level changed in the opposite direction. The simplest explanation of these changes in ATP and ADP from light to dark to light is that there is an active adenylate kinase catalyzing the equilibrium (Eq. 1):

$$2\ ADP \rightleftharpoons AMP + ATP \qquad (1)$$

$$ATP \longrightarrow ADP + P_i \qquad (2)$$

$$\overline{\qquad\qquad\qquad\qquad\qquad\qquad}$$

$$ADP \longrightarrow AMP + P_i \qquad (3)$$

This reversible adenylate kinase reaction together with the conversion of ATP to inorganic phosphate and ADP by reactions of the carbon cycle (Eq. 2)

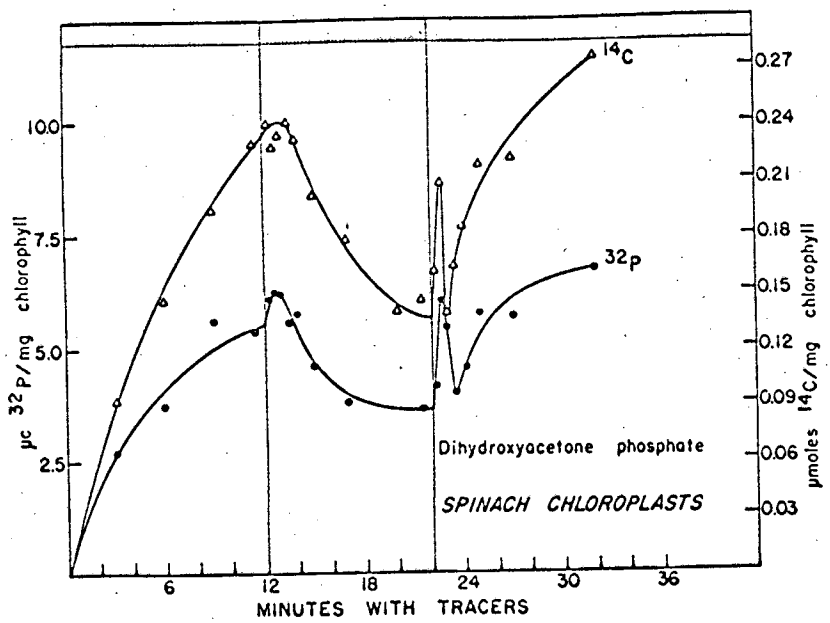

Fig. 3. Levels of ^{32}P and ^{14}C in dihydroxyacetone phosphate during photosynthesis and dark period in isolated spinach chloroplasts.

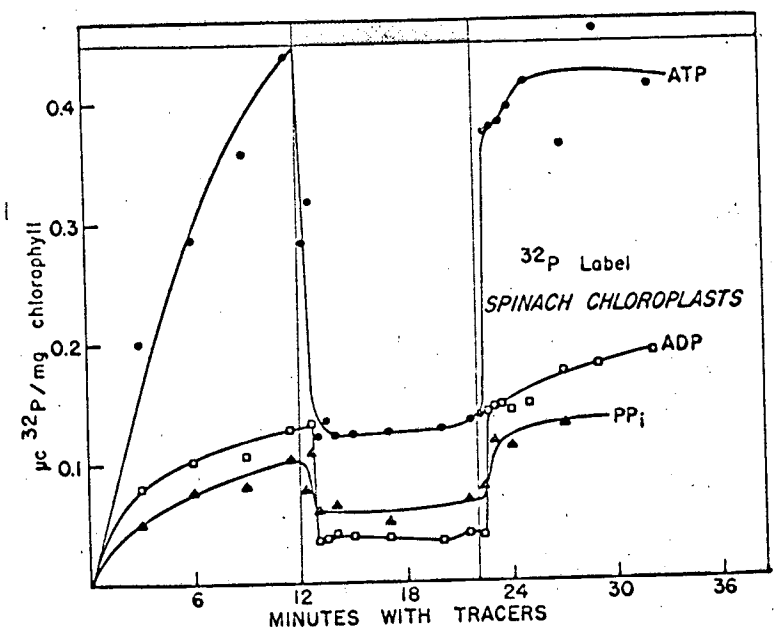

Fig. 4. Levels of ^{32}P in ATP, ADP, and pyrophosphate during photosynthesis and dark period in isolated spinach chloroplasts.

converts ADP to AMP and inorganic phosphate (Eq. 3).

The fall in the level of pyrophosphate, PP_i, in the dark, and its subsequent rise in the light show once again, with isolated chloroplasts, a phenomenon which we have reported earlier with *in vivo* photosynthesis in *Chlorella pyrenoidosa* (Pedersen *et al.*, 1966A). In other experiments during *in vivo* photosynthesis (Pedersen *et al.*, 1966B), it was found that the addition of methyl octanoate caused an inhibition of photophosphorylation, while at the same time producing a transitory rise in the level of PP_i. A possible conclusion, based upon that single observation, could have been that methyl octanoate blocks some step prior to the formation of ATP, and causes the accumulation of an intermediate from which pyrophosphate is derived.

Recently, Baltscheffsky and von Stedingk (1966) have arrived at such a conclusion based upon work with isolated chromatophores from *Rhodospirillum rubrum*. In their experiments, oligomycin, said to inhibit the final ATP-forming reaction, caused a slight stimulation in uptake of the inorganic phosphate in the absence of added nucleotide. The light-induced phosphate disappearance was believed to correspond to the appearance of PP_i.

In our studies we found that methyl octanoate inhibited other reactions of the photosynthetic carbon reduction cycle. Therefore, we suggested that another explanation for the PP_i changes with the addition of methyl octanoate might be that utilization of PP_i or its unstable precursor by photosynthesis was blocked to such an extent that its level could momentarily increase, even it it is derived from a decreasing amount of ATP.

In short, pyrophosphate, or its precursor, is formed either parallel to, or from, ATP produced by photophosphorylation. It is consumed by reactions, possibly metabolic, which can occur in the dark. Formation and utilization occur in isolated chloroplasts as well as in whole cells.

Since the chloroplasts were prepared and incubated in unlabeled 5×10^{-3} M PP_i ($Na_4P_2O_7 \cdot 10\ H_2O$, see Experimental), the sudden changes in labeled PP_i would seem to require that there be no equilibration between metabolic PP_i and PP_i in the medium. Perhaps a more plausible explanation of the data would be that the labeled PP_i is formed from a precursor, during or after the chloroplast killing.

References

Baltscheffsky, H., and von Stedingk, L.-V., Biochem. Biophys. Res. Commun. 22, 722 (1966).

Pedersen, T. A., Kirk, M., and Bassham, J. A., Physiol. Plantarum, 19, 219 (1966A).

Pedersen, T. A., Kirk, M., and Bassham, J. A., Biochim. Biophys. Acta, 112, 189 (1966B).

Walker, D. A., Biochem. J. 92, 22C (1964).