THE EFFECTS OF 8-METHYL LIPOIC ACID ON THE EVOLUTION OF OXYGEN AND REDUCTION OF CARBON DIOXIDE DURING PHOTOSYNTHESIS*

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Kinetic studies of transient changes in the pattern of ^{14}C-labeled compounds formed during photosynthesis with $^{14}CO_2$ have given much information about the operation of the carbon reduction cycle in vivo. Such changes accompanying the change from light to dark (Calvin and Massini, 1952; Bassham et al., 1956) and the sudden change from high levels of CO_2 pressure to very low levels of CO_2 pressure (Wilson and Calvin, 1955) provided an important part of the evidence on which the carbon reduction cycle of photosynthesis was formulated (Bassham et al., 1954). Introduction of a chemical inhibitor capable of interferring with electron transport from the photochemical stage of photosynthesis to the carbon reduction pathway could be a tool for further studies of this type.

It has been suggested (Bassham, 1963) that disulfide compounds such as lipoic acid or some enzyme bound disulfides might function in the electron transport from substances at the potential of reduced PPNR to the site of carbon reduction. We therefore chose an analog of lipoic acid in the hope of interferring with such a function. Also, we hoped to obtain information about a possible reductive carboxylation reaction in vivo in the light.

Chlorella pyrenoidosa, grown in continuous culture (Bassham and Calvin, 1957) was harvested and resuspended in a mineral nutrient solution described

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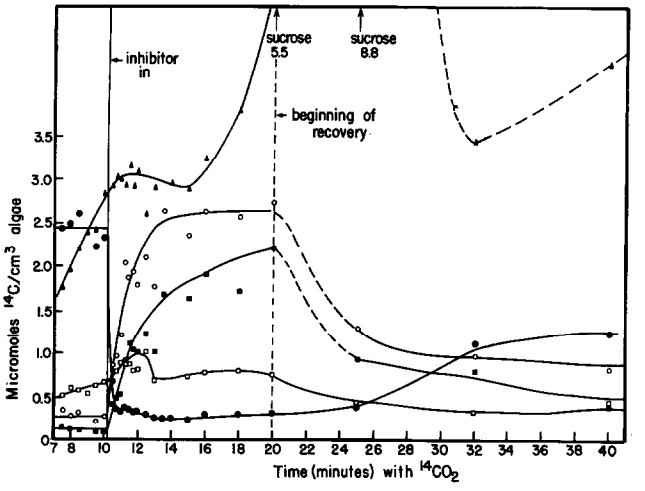
elsewhere (Bassham and Kirk, 1963). 80 ml of a 2% (wet packed volume/ suspension volume) suspension of algae were placed in the steady state apparatus (Bassham and Kirk, 1963). However, the pH control (automatic addition of 0.1 N NH_4OH) was set at 5.0. Air, containing 2% CO_2, was passed through the vessel which was illuminated from one side by an incandescent lamp, est. 33,000 lux incident, and from the other side by a bank of alternate white and blue fluorescent lamps, est. 8000 lux incident. After one hour of photosynthesis, the system was closed and $^{14}CO_2$ was introduced. The rates of oxygen evolution and carbon dioxide and $^{14}CO_2$ uptake were continuously observed (Bassham and Kirk, 1960; Smith et al., 1961).

Previous studies (Bassham and Kirk, 1960) showed that phosphoglyceric acid (PGA) and the sugar phosphates of the carbon reduction cycle become "saturated" with radiocarbon after some 5 min of photosynthesis in the presence of $^{14}CO_2$ of constant specific radioactivity. After 8 min, 6 samples of the algae suspension were taken at 30 sec intervals into weighed tubes containing methanol to give a final concentration of 80% methanol, to stop the enzymic reactions. 10 mg of 8-methyl lipoic acid in 200 µl of ethanol was then injected into the algae suspension. Control experiments showed that 200 µl of ethanol alone cause no measureable change in photosynthesis rate or labeling pattern.

Samples of the algae suspension were taken at times shown in the Figs. All algal samples were subsequently extracted, concentrated and analyzed by two-dimensional paper chromatography and radioautography as described previously (Bassham and Calvin, 1957). The amount of ^{14}C in each compound was determined by means of the automatic spot counter (Moses and Lonberg-Holm, 1962).

The course and degree of inhibition with several levels of 8-methyl lipoic acid and with lipoic acid are indicated in Table I.

Figs. 1,2 and 3 show the amount of ^{14}C-labeled compounds obtained from the samples taken during the course of experiment 3 (see Table I). As in previous studies with the steady state apparatus (Bassham and Kirk, 1960;



Figs. 1,2 and 3. Effects of 8-methyl lipoic acid on labeling of compounds with ^{14}C during photosynthesis with $^{14}CO_2$.

Fig. 1.
- ▲ Sucrose
- ○ Fructose-1,6-diphosphate
- □ "Glucose diphosphates"
- ■ Sedoheptulose-1,7-diphosphate
- ● 3-Phosphoglyceric acid

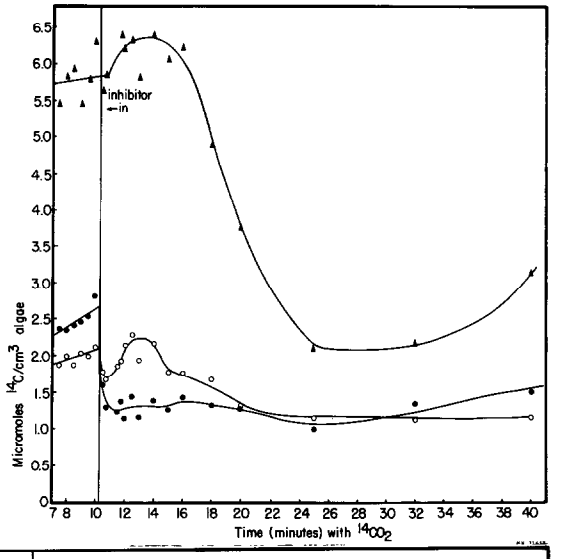


Fig. 2.
- ▲ Glucose-6-phosphate
- ● Sedoheptulose-7-phosphate
- ○ Fructose-6-phosphate

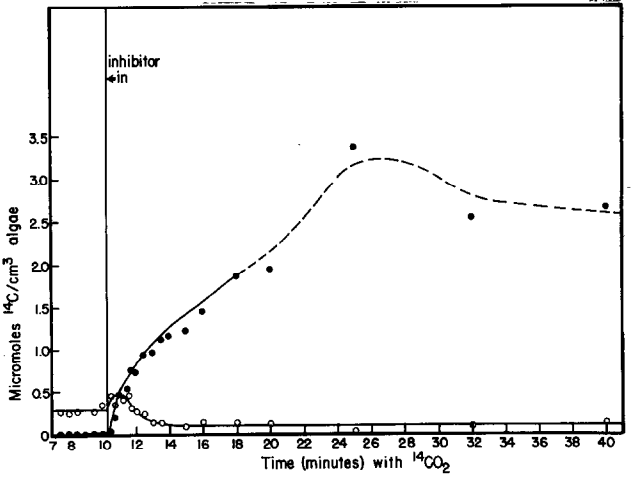


Fig. 3.
- ● Glycolic acid
- ○ Ribulose-1,5-diphosphate

Table I. INHIBITION OF PHOTOSYNTHESIS IN Chlorella pyrenoidosa BY 8-METHYL LIPOIC ACID AND BY LIPOIC ACID.

Exp.	Conditions	Time (min)	μmoles/min·cm^3 algae (wet pack) O_2 evolution	CO_2 uptake
1	initial	before	10.9	8.2
	after 1 mg 8-Me lipoic acid	2-10	7.8	5.5
2	initial	before	8.8	7.6
	after 5 mg 8-Me lipoic acid	2-8	1.3	1.3
		8-12	recovering	
		12-20	7.0	4.9
3	initial	before	12.9	10.8
	after 10 mg 8-Me lipoic acid	0-20	0	0
		20-26	recovering	
		26-40	4.2	2.9
4	initial	before	10.2	7.6
	after 10 mg lipoic acid	3-10	0.2	0.3
		14-20	5.4	4.3
		26-40	8.7	7.0

Smith et al., 1961) the specific activity of the $^{14}CO_2$ has been maintained at a constant measured value during the course of the experiment. The amount of ^{14}C found experimentally in each compound has been converted to the μmoles of carbon in the compound in the active reservoir by dividing the amount of ^{14}C by the ratio $^{14}C/(^{12}C + ^{14}C)$ in the CO_2 administered. This result has been further converted to give the μmoles of carbon in the compound per cm^3 of wet packed algae.

Fig. 1 shows a sudden, large drop in the level of PGA and a sudden rise in the levels of fructose-1,6-diphosphate and sedoheptulose-1,7-diphosphate upon introduction of the inhibitor. The rate of decrease of the PGA pool during the first 15 sec with the inhibitor may be calculated as $(2.4 - 0.7)/0.25 = 6.8$ micromoles of ^{14}C/min/cm^3 of algae. This rate may be compared with the rate of uptake of carbon dioxide from Table I, which was 10.8 micromoles per min. The rate of increase in sucrose, which is accelerated once the fructose diphosphate has increased, occurs in the

absence of any appreciable increase in uridine diphosphoglucose (not shown but included in and similar to the "glucose diphosphates").

Changes in the levels of some of the sugar monophosphates are shown in Fig. 2. Labeled fructose and sedoheptulose monophosphates decrease rapidly at first, then undergo a small positive transient, before continuing to decline gradually. Glucose-6-phosphate undergoes a gradual but large decrease in radioactivity during the time that sucrose increases.

Fig. 3 shows the labeling of ribulose-1,5-diphosphate and of glycolic acid. Considering the great change in phosphoglyceric acid and the complete inhibition of photosynthesis, it is surprising that ribulose diphosphate changes in concentration by only a small amount during the same period. The great increase in glycolic acid labeling does not in this case follow any increase in the level of ribulose-1,5-diphosphate, as was the case in earlier studies (Wilson and Calvin, 1955) in which the CO_2 pressure was suddenly lowered. It may be that the disulfide compound accelerates the oxidation of the glycolaldehyde thiamine pyrophosphate compound (2-(1,2-dihydroxyethyl)-thiamine pyrophosphate) formed during the transketolase reaction (Calvin and Bassham, 1962). Such an oxidation has been postulated to account for the stimulation of glycolate labeling accompanying an increase in O_2 tension (Bassham and Kirk, 1962).

In terms of the photosynthetic carbon reduction cycle as usually expressed (Bassham et al., 1954) the more immediate effects of the addition of inhibitor seem to suggest either a stimulation in the reduction of PGA (which is difficult to rationalize with the apparent inhibition of CO_2 uptake and O_2 evolution) or perhaps more plausibly, an inhibition of carboxylation reactions leading to the formation of PGA.

In the latter case, since ribulose-1,5-diphosphate neither accumulates nor disappears it is necessary to postulate that its formation from ribulose-5-phosphate is also inhibited. Inhibition of this reaction (presumably catalyzed by a kinase) may be somehow related to inhibition of

the conversion of fructose and sedoheptulose diphosphates to their respective monophosphates (presumably through the action of phosphatases).

Other explanations of the inhibitor effects (such as inhibition of an *in vivo* reductive carboxylation of hexose and heptose diphosphates) are possible, but their serious consideration must await further experimental evidence. The immediacy and magnitude of effects caused by 8-methyl lipoic acid and lipoic acids, indicate important and primary roles for disulfide groups in the primary reactions for carbon reduction during photosynthesis.

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