Evidence Supporting the Theory of Two Sites of Photophosphorylation in Green Plants*

by

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An acceptance of the reductive pentose phosphate cycle of photosynthetic CO_2 fixation by green plants leads to the conclusion that for each mole of CO_2 fixed 3 moles of ATP and 2 moles of NADPH are consumed (Calvin and Bassham, 1957). Careful studies from several labroatories have demonstrated that isolated chloroplasts produce stoichiometric amounts of ATP:NADPH (Arnon, et al., 1958; Keister, et al., 1961; Turner, et al., 1962). Evidently one site of photophosphorylation is located in the non-cyclic electron transfer chain between water and the reduction of NADP but this electron transfer sequence doesn't supply sufficient ATP to operate the reductive pentose phosphate cycle. With the definitive demonstration of cyclic photophosphory-

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lation in the presence of PMS** without isotopic oxygen exchange (Krall, et al., 1961) an electron flow sequence was evident which could theoretically supply the additional ATP. However it is difficult to determine from data in the literature whether cyclic electron transfer catalyzes photophosphorylation at the same site as non-cyclic electron transfer and/or involves an additional site(s). This paper presents data which indicates that cyclic photophosphorylation involves at least two sites of phosphorylation one of which is not associated with the non-cyclic electron transfer phosphorylation site(s). These experiments have been described briefly (Black, 1967).

METHODS

Once-washed chloroplast fragments were prepared from fresh spinach leaves and photophosphorylation and NADP reduction were assayed by methods previously employed (Turner, et al., 1962). The ATPase activity described by Petrack and Lipmann, 1961, was assayed using DTT as the thiol reagent and preilluminating the chloroplasts for 5 minutes prior to the addition of ATP in the dark. The routine procedure employed for treatment of chloroplasts with n-heptane consisted of adding 0.1 ml of n-heptane to 1.0 ml of suspended chloroplasts and shaking 10 seconds with a Burrell "wrist-action" shaker (130-140 strokes/min). Treated chloroplasts were stored in an ice-bath and aliquots removed for assays at the indicated intervals.

RESULTS AND DISCUSSION

Treatment of chloroplasts with a non-polar solvent such as n-heptane

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**Abbreviations used are: DTT, dithiothreitol; and PMS, phenazine methosulfate.

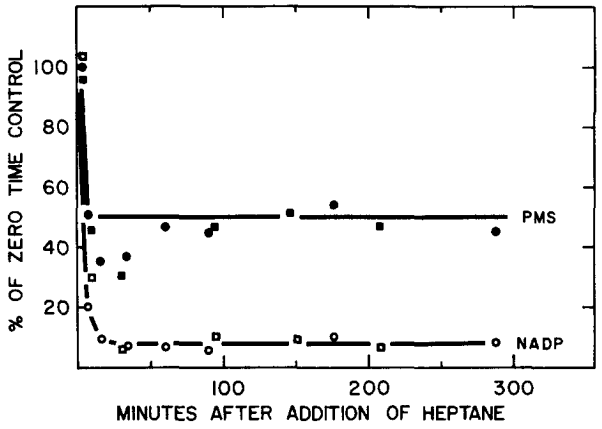

Figure 1: Selective decay of photophosphorylation activity in spinach chloroplasts following n-heptane treatment.

The respective photophosphorylation activities in zero time controls were: ● PMS 400; o NADP 112; ■ PMS 290; and ◘ NADP 141 μmoles of ATP formed/mg chlorophyll/hr. The circles and squares indicate separate experiments with a 10-fold difference in n-heptane concentration (.01 or 0.1 ml of n-heptane/ml of chloroplasts).

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results in a rapid and selective decay of photophosphorylation activity (Fig. 1). With PMS as the catalyst, cyclic photophosphorylation decays partially and then remains relatively constant for several hours. With longer periods, 24 hours, of storage the activity decays. The stepwise decay of cyclic photophosphorylation is in sharp contrast to the almost complete, 90-95%, decay of photophosphorylation coupled to NADP reduction (Fig. 1). The initial decay kinetics appear to be similar for both cyclic and non-cyclic photophosphorylation.

Under similar experimental conditions as those of Figure 1, the reduction of NADP was examined. As seen in Table I there is an increase in NADPH

Table I

NADP REDUCTION FOLLOWING n-HEPTANE TREATMENT OF SPINACH CHLOROPLASTS

Minutes After Treatment	Control Chloroplasts	Heptane Treated Chloroplasts
	μmoles NADPH/mg chlorophyll/hr.	
0	141	---
2.5	---	140
15	---	197
34.5	---	203
64.5	---	130
128	---	120
191	---	38
220	---	31
226	140	---

formation with about the same kinetics of increase as the kinetics of decay of the associated photophosphorylation. NADP reduction continues at a rate at least equivalent to the control rate for about 2 hours and then decays, Table I. Complete decay requires several days.

Heptane treatment of chloroplasts also affects other chloroplast activities which may be associated with the formation of ATP. For example in Table II one can observe that in heptane treated chloroplasts the light-triggered, thiol, and magnesium dependent ATPase (Petrack and Lipmann, 1961) has decay

Table II

EFFECT OF n-HEPTANE TREATMENT ON THE LIGHT-TRIGGERED, THIOL, AND MAGNESIUM DEPENDENT ATPase* OF CHLOROPLASTS

Minutes After Treatment**	Control Chloroplasts	Heptane Treated Chloroplasts
	μmoles of Pi released/mg chlorophyll/hr.	
8	170	70
18	157	67
86	207	29
185	186	19
202	169	27
243	152	11

*ATPase activity assayed by the release of Pi from ATP.

**Time at the end of the illumination period.

kinetics somewhat similar to the decay kinetics of photophosphorylation coupled to NADP reduction. The time involved in completion of the experimental procedures of the ATPase assay make comparisons with Fig. 1 difficult. In Fig. 1, the total time of each experiment is one minute but in Table II the chloroplasts are illuminated 5 minutes and then incubated 10 minutes in the dark with ATP. The times in Table II are at the end of the illumination period.

These experiments have demonstrated that cyclic photophosphorylation can be inhibited in a stepwise fashion while non-cyclic electron flow is still operative. The experiments can be interpreted as indicating that non-cyclic photophosphorylation occurs at one site which is common with cyclic photo-

phosphorylation. In addition to this non-cyclic site of photophosphorylation, during cyclic electron flow, another site is present which is not activated by non-cyclic electron flow. Thus chloroplasts can be prepared which function in both cyclic and non-cyclic electron flow but which only synthesize ATP during cyclic electron flow. The light-triggered, thiol, and magnesium dependent ATPase activity of spinach chloroplasts appears to be more related to the non-cyclic electron flow phosphorylation site than to the cyclic electron flow site.

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