PHOTO-POTENTIATION OF ADENOSINE[1] TRIPHOSPHATE HYDROLYSIS

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In 1960 Petrack and Lipmann (1) reported on a photohydrolysis of adenosine triphosphate (ATP) which was catalyzed by spinach chloroplasts and by extracts of blue-green algae. In addition to light the reaction required a sulfhydryl compound and a redox cofactor such as flavin mononucleotide (FMN) or phenazine methosulfate (PMS). The dephosphorylation rates reported for the reaction approached 200 μmoles/hr/mg chlorophyll. Avron (2) also found in chloroplasts a light-dependent ATPase which required PMS. The rates of ATP hydrolysis reported were very low although extremely high light intensities (200,000 lux) were employed. Avron did not observe the enhancement of the rate of photohydrolysis by added sulfhydryl compounds as reported by Petrack and Lipmann. A dark ATPase activity has also been reported in chloroplasts by Wessels and Baltscheffsky (3).

Petrack and Lipmann found the photohydrolysis of ATP required relatively high concentrations of sulfhydryl compounds. We have confirmed this result using spinach chloroplasts but also noticed that the extent of hydrolysis was not linear with time but increased exponentially until a constant rate was achieved. The progress of the reaction as a function of time is shown in figure 1. Neither preincubation with ATP in the dark

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or in the light nor preincubation with cysteine in the dark or in the light significantly reduced the "lag" period. Figure 1 also shows the results of extinguishing the light after the ATPase activity has developed. Two reaction vessels were used, one kept in continuous light for 40 minutes

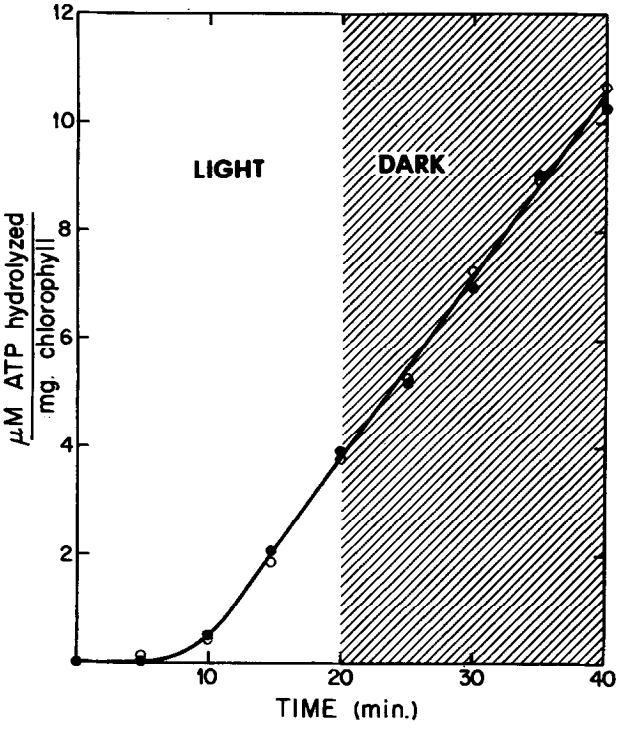

Figure 1. Dephosphorylation in light and darkness. Inorganic phosphate formed in vessel kept in continuous light is indicated by O—O—O; that formed in vessel transferred to darkness at 20 minutes is indicated by ●—●—●. Chloroplasts were prepared essentially as described by Hill and Walker (4). The complete reaction mixture contained the following per ml.: Tris (pH 8.0), 75 μmoles; cysteine, 40 μmoles; $MgCl_2$, 7.5 μmoles; FMN, 0.15 μmoles; ATP, 2 μmoles; phospho-enol pyruvate, 2 μmoles; excess pyruvic kinase and chloroplasts containing 50 μg chlorophyll. The reaction was carried out at 12-15°C and aliquots assayed for inorganic phosphate by the methods of Fiske and Subba Row (5).

and one returned to darkness after 20 minutes' illumination. The rate in the darkened vessel was identical to that occurring in the vessel kept in continuous light. The hydrolysis reaction per se does not require light.

Ammonia, an uncoupler of photosynthetic phosphorylation (6), exerts a dual effect on this ATP hydrolysis. Figure 2 demonstrates the effect of increasing concentration of ammonium chloride on the hydrolysis in the dark following illumination. The results of adding ammonia after illumination and before dark incubation is shown in the upper trace. The ATPase ac-

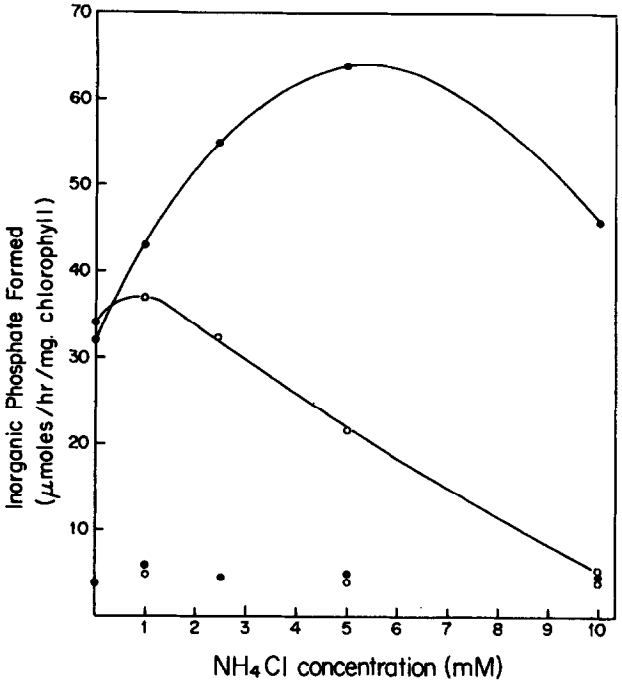

Figure 2. Effect of ammonia on ATP hydrolysis and on potentiation of the ATPase activity. The solid dots (●—●—●) represent rates obtained with ammonia in the dark phase only while the open circles (o—o—o) show rates obtained with ammonia in both the dark and light phases. The lowest points are dark controls. Reaction mixtures were the same as described for figure 1 with the addition of ammonium chloride as indicated.

tivity, developed in the light without ammonia, is enhanced about twofold by addition of 5 mM ammonia at the start of the dark period. Quite contrary results are obtained, however, if ammonia is added at the start of illumination. The lower curve shows the inhibition of hydrolysis when ammonia is present during both light and dark phases. The potentiation of the ATPase is therefore inhibited by ammonia. The rate of non-potentiated dark hydrolysis is unaffected by ammonia (lower points).

When the requirements for potentiation of ATP hydrolysis were examined more closely, it became apparent that two ATPase activities were involved. If ATP was omitted during the light phase, ammonia did not elicit an increased rate during the following dark period. The results in Table I (expt. 1) show that the first ATPase activity (system I) was not dependent upon ATP for potentiation and, once formed, was not affected by ammonia. The second ATPase activity (system II) required ATP (other nucleotides have not been examined) for development and the final hydrolytic rate was accelerated by ammonia.

The second experiment showed that the systems could be further differentiated by the requirement for a redox cofactor during illumination. The complete mixture in the light with ammonia added in the dark contains both system I and II. If ATP alone was omitted, a rate was obtained which was that of ATPase I (independent of ammonia in the dark); further omission of either FMN or cysteine resulted in complete inhibition of this activity. If ATP was present during the light phase but FMN omitted, the rate obtained in the presence of ammonia was that of system II, further omission of cysteine also prevented this activity from developing. Note that the sum of the rates of systems I and II when assayed separately was very close to that of the complete system.

Hence both ATPase activities require light and cysteine for potentiation, in addition one activity requires FMN (I) and one requires ATP (II). Both activities are prevented from developing by ammonia, but the rate of system II is enhanced by ammonia once the activity is formed.

Table I

Potentiation mixture	ATP Hydrolysis Rate (μmoles/hr/mg.chlorophyll) $-NH_4^+$	$+NH_4^+$ (2.5 mM)
Experiment 1		
Complete	36	91
-ATP	20	20
Experiment 2		
Complete		51
-FMN		35
-Cysteine		1
-ATP	14	
-ATP, -FMN	0	

The complete potentiation and reaction mixtures were as described for Fig. 1 with the exception of 5 mM ATP and the omission of phospho-enol pyruvate and kinase in Experiment 1. A ten-minute illumination period (16°) was followed by a twenty-minute dark assay period (23°).

The mechanism by which the activities are induced in chloroplasts is not readily apparent. The inhibition of ATPase development by ammonia suggests that the site of potentiation is an intermediate in photosynthetic phosphorylation which is bypassed (or has a very short lifetime) during electron transport in the presence of ammonia.

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