PN 1303

Light and dark stages in the hydrolysis of adenosine triphosphate by chloroplasts

In recent years, there have been a number of reports by PETRACK AND LIPMANN[1] and AVRON[2] and from our own laboratory[3], upon the existence of a light-activated hydrolysis of ATP in chloroplasts of higher plants. Interest has been focused upon this process because of the possibility that the photohydrolysis of ATP may represent a reversal of the photophosphorylation mechanism. It has now been discovered that the elevated rates of the hydrolysis of ATP depend upon actinic light in an indirect manner; a separation of the requirements for ATP hydrolysis into light and dark stages has been achieved.

Light-induced ATP synthesis and hydrolysis for chloroplast membrane preparations are shown in Table I. For activation of "cyclic" phosphorylation, Mg^{2+} and an electron carrier such as PMS are required[4]. The photophosphorylation rate is elevated about 3-fold further by the presence of a reduced sulfhydryl compound such as lipoic acid. Light-induced hydrolysis of ATP is shown in the lower part of Table I. In the presence of the complete system, ATP hydrolysis in the light is seven times higher than the dark reaction. Light-activated ATPase requires the presence of lipoic acid and Mg^{2+}, but not PMS. The role of PMS and the nature of the light requirement for activation of ATP hydrolysis was further examined by changing the period of illumination, since the experiments suggested that some product or condition was produced in the light which could enormously promote the rate of ATP hydrolysis.

The effect of a prior period of illumination on light-induced ATPase is summarized in Table I (column 3) and Fig. 1. It was discovered that a 300-sec preillumination period carried out in the presence of lipoic acid, PMS and Mg^{2+}, was the optimum condition for activating ATP hydrolysis in the dark. In the presence of the complete system, subsequent ATP hydrolysis in the dark underwent a remarkable activation above the all-dark reaction, and lipoic acid, PMS, and Mg^{2+} were all critically required in the preillumination phase (*cf.* numbers in parentheses in Table I).

These results suggested that it would be of interest to assess more precisely the time of preillumination required to induce the elevated dark hydrolysis of ATP, and also to examine the dark decay of the process. In these experiments, the rate of ATP hydrolysis was followed over a 15-min time course as before under two different conditions. In the first, the time period of the preillumination phase was varied between 0 and 400 sec (Fig. 1, open circles). After these various intervals, the light was turned off and ATP was immediately added to the reaction system. The elevated ATP hydrolysis in the dark became maximal around 300 sec ($t_{\frac{1}{2}} = 70$ sec). In the second series, chloroplasts were preilluminated for 300 sec, the light was turned off and after varying time intervals (decay time) ATP was injected, and the subsequent hydrolysis over a 15-min period assayed. The curve shown (Fig. 1, solid points) for the decay of ATP-splitting activity was obtained ($t_{\frac{1}{2}} = 47$ sec). Apparently the growth and decay of the conditions leading to elevated ATP hydrolysis are both exponential, and have an unexpectedly long life time.

These results distinguish clearly between the light and dark stages in the hydrolysis of ATP in chloroplasts. Light-activated ATPase in chloroplasts is a misleading term since it may imply that the action of light is directly to stimulate ATP break-

Abbreviation: PMS, phenazine methosulfate.

down, rather it would appear that the function of light is to promote the production of some condition which favors the subsequent hydrolysis of ATP in the dark at rates many times faster than the all-dark reaction. This phenomenal increase in ATP breakdown under conditions of preillumination which depends upon the presence of Mg^{2+} and a thiol compound in addition to photosynthetic electron-transport activity, may be explained on two bases. The results dictate either the formation of an intermediate in the light which is subsequently required during the hydrolysis and is expended by ATP during the dark stages of the reaction, or that illumination leads to some permeability or structural changes (which are known to occur under these conditions). The studies carried out to date on the presence of energy-linked intermediates in photophosphorylation have been limited to only a few investigations. HIND AND JAGENDORF[6] were able to separate photophosphorylation into light and dark stages, showing that a condition or substance was produced in the light having a short half-life, which subsequently could lead to the net formation of ATP from ADP and phosphate in the dark; also HINKSON *et al.*[7] have obtained evidence for the presence of a rapidly labelled [^{32}P]intermediate. If the results of the present investigation are the consequence of the light-induced production of intermediates related to the photophosphorylation mechanism, then the life time of these intermediates would appear to be prolonged under conditions of our experiments; it may

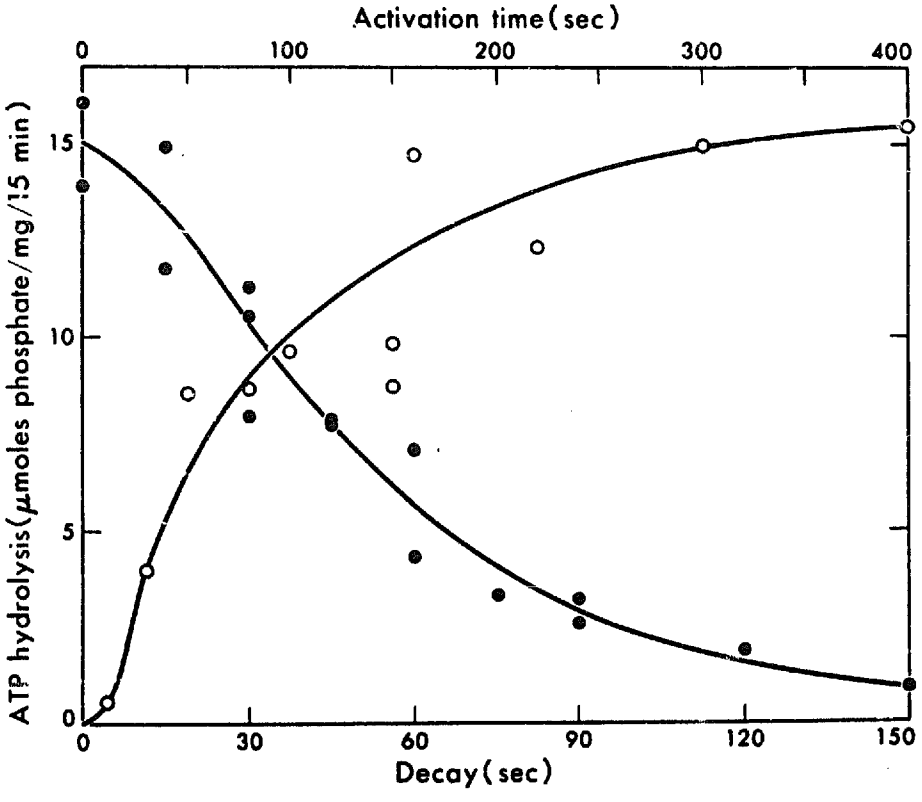

Fig. 1. Light induction and dark decay of ATP hydrolysis. Conditions for ATP hydrolysis as in Table I. Dark decay: for each point on the curve an initial 5-min illumination of the reaction mixture was made; ATP was then added in the dark after the intervals shown on the abscissa and the system was incubated for 15 min for assay of dark ATP hydrolysis. The dark reaction without preillumination (0.7 μmole/mg chlorophyll/15 min) was deducted from readings before plotting. Light-induction: for each point on the curve a different initial period of preillumination was given; ATP was then added immediately upon extinguishing the light and the system was incubated for 15 min for assay of dark ATP hydrolysis.

TABLE I

PHOTO-INDUCED HYDROLYSIS AND SYNTHESIS OF ATP IN CHLOROPLASTS

The reaction mixture contained: Tris buffer (33 mM, pH 8.0), PMS (20 μM), ascorbate (0.75 mM), chloroplasts (50 μg chlorophyll/ml), reduced lipoic acid (5 mM), and $MgCl_2$ (5 mM). Substrate concentrations: hydrolysis, ATP (2.5 mM); for synthesis, ADP (2 mM) + P_i (2 mM). ATP hydrolysis or synthesis was estimated over a 15-min period commenced 40 sec after the addition of ATP or ADP, respectively, by estimation of the change in P_i concentration using a phosphomolybdic acid reduction method with $SnCl_2$ as reducing agent[5]. Assays were carried out in test tubes 11 cm from a 150-W General Electric reflector flood in a thermostatic bath at 27°. Spinach chloroplasts were prepared in 0.5 M sucrose, 0.1 M Tris (pH 8.0) (*cf.* ref. 4), washed once in the same medium and twice in 35 mM NaCl, 5 mM Tris (pH 8.0) to remove "soluble" proteins. Lipoic acid was reduced using $NaBH_4$ and adjusted to pH 8.0 after removing excess reagent at low pH. L = assay with continuous illumination; L → D = assay in dark following 5 min preillumination; D = dark reaction only. Figures in parentheses = factors omitted during the 5 min preillumination only.

	μmoles phosphate/mg chlorophyll/15 min		
	L	*D*	*L → D*
ATP synthesis			
Complete system	−9.5	0.0	
Lipoic acid absent	−3.3	—	
PMS absent	−1.8	—	
$MgCl_2$ absent	−1.5	—	
ATP hydrolysis			
Complete system	+3.0	+0.42	+16.0
Lipoic acid absent	+0.9	—	+ 0.8 (1.42)
PMS absent	+6.2	—	+ 1.3 (0.7)
$MgCl_2$ absent	+0.1	—	+ 0.7 (0.4)

be that the reduced sulfhydryl compound functions in this way. Alternatively, the results may be explicable on the basis of permeability considerations since remarkable changes in the light-scattering properties of chloroplasts have been observed under the identical conditions of the experiments and which have similar chemical requirements and rise and decay times to those reported here for ATP hydrolysis[3].

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