A Light-triggered ATP-Pi Exchange Activity in Chloroplasts

by

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Isolated chloroplasts which are capable of catalysing ATP formation at rapid rates, have been reported to lack ATPase or ATP-Pi exchange activities (Avron and Jagendorf, 1959; Avron, 1960). Recently, however, conditions were described under which ATPase activity can be induced in chloroplasts (Petrack et al., 1965; Avron, 1962). It has been assumed that the ATPase reactions studied represent a reversal of the latter stages of ATP production during photophosphorylation. If that were the case it would be expected that the conditions which induce an ATPase reaction may also induce the appearance of other reactions which depend on a reversal of these stages, such as the ATP-Pi exchange reaction. The conditions recently developed (Petrack et al., 1965; Hoch and Martin, 1963; Marchant and Packer, 1963) for the thiol and magnesium dependent ATPase, are particularly suitable for such a study, since light was required for triggering the enzymatic activity but not for its further maintainance (Bennun and Avron, 1964).

METHODS

Once washed chloroplasts and chloroplast fragments were prepared from lettuce (Lactuca sativa var. romaine) leaves, as previously described (Avron, 1960).

ATP-Pi exchange was assayed at 22°C in a reaction mixture containing: Tris-HCl, pH 7.8, 20 mM; NaCl, 20 mM; phosphate buffer, pH 7.8, 2 mM (containing about 2×10^6 cpm of P^{32}); $MgCl_2$, 3.7 mM; PMS*, 0.003 mM; ATP, 6 mM; DTT, 15 mM, and chloroplasts containing 60 µg of chlorophyll, in a total volume of 1.0 ml. Routinely, the reaction mixture was illuminated for 2 min. at 70,000 lux in the absence of ATP. ATP was added immediately after the light was turned off, and the reaction allowed to proceed in the dark for 10 minutes. It was terminated by the addition of trichloroacetic acid to a final concen-

*The abbreviations used are: DTT, dithiothrietol; PMS, phenazine methosulfate; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea.

tration of 3%. ATP^{32} content was then assayed by the isobutanol-benzene extraction procedure, as previously described (Avron, 1960).

ATPase activity was assayed in the following reaction mixture: Tris-HCl, pH 7.8, 20 mM; NaCl, 20 mM; $MgCl_2$, 7.4 mM; PMS, 0.03 mM; ATP, 6 mM; DTT, 25 mM, and chloroplasts containing 60 µg of chlorophyll, in a total volume of 1.0 ml. The experimental procedure was identical to that used in the ATP-Pi exchange assay. Inorganic phosphate was determined as described (Berenblum and Chain, 1938). The method was somewhat modified to include the addition of H_2O_2, in an amount equivalent to the DTT present to avoid prereduction of the phosphomolybdate complex by the DTT.

RESULTS AND DISCUSSION

Illumination of chloroplasts under the conditions described in Methods induced ATP-Pi exchange activity which proceeded linearly in the dark for at least 20 minutes. In the absence of the light induction there was hardly any exchange in the dark (Table I). DTT was used as the dithiol reagent since it proved to be more effective than cysteine. Anaerobic conditions which are required when reduced lipoic acid is used (Petrack et al., 1965) were not necessary with DTT.

The results of a component study are shown in Table I. As in photophosphorylation (Avron, 1960) and light-triggered ATPase (Petrack et al., 1965), there was an absolute requirement for magnesium ions for the operation of the light-triggered ATP-Pi exchange reaction. The amount of magnesium required for optimal activity was similar in all three reactions. Phenazine methosulfate which is required in photophosphorylation was also needed to activate ATP-Pi exchange. However, the effectiveness of PMS varied from one experiment to another. In order to differentiate between the requirements for the induction and for the reaction itself, components were added either to the light or to the dark stage. Phosphate and ATP were not required during the inductive light stage (Table I). The presence of magnesium ions during the light stage gave optimal results. However, some activity was retained when magnesium was added to the dark stage alone. DTT and PMS had to be present during the light stage.

In the absence of ATP there was no formation of ATP^{32}. Moreover, when ATP was substituted by ADP there was no ATP^{32} formation. These results eliminate the possibility that the ATP-Pi exchange was the result of an ATPase which hydrolyzed ATP, followed by the dark synthesis of ATP^{32}. The ATP-Pi exchange activity like photophosphorylation was particle-bound, since most of the activity remained in chloroplast fragments.

The decay of the light effect could be observed when ATP was added to preilluminated

Table I

Light-triggered ATP-Pi exchange activity, a component study

Components present in Light + Dark	Components present in Dark	Rate of ATP-Pi exchange activity
		per cent of control
Mg, PMS, DTT, Pi	ATP	100
Mg, PMS, DTT	ATP, Pi	87
Mg, PMS, -	ATP, Pi	3
Mg, - DTT	ATP, Pi	18
-, PMS, DTT	ATP, Pi	1
Mg, PMS, DTT	-, Pi	0
-, -, -	ATP, Pi, Mg, PMS, DTT	2
Mg, PMS, -	ATP, Pi, DTT	7
Mg, -, DTT	ATP, Pi, PMS	7
-, PMS, DTT	ATP, Pi, Mg	20
-, PMS, -	ATP, Pi, Mg, DTT	6
Mg, PMS, DTT	ADP, Pi	2
-, -, -	ADP, Pi, Mg, PMS, DTT	1
Mg, PMS, 70 mM cystein	ATP, Pi	58

Components were present during both light and dark or only during the dark stage as indicated. For assay conditions see Methods.

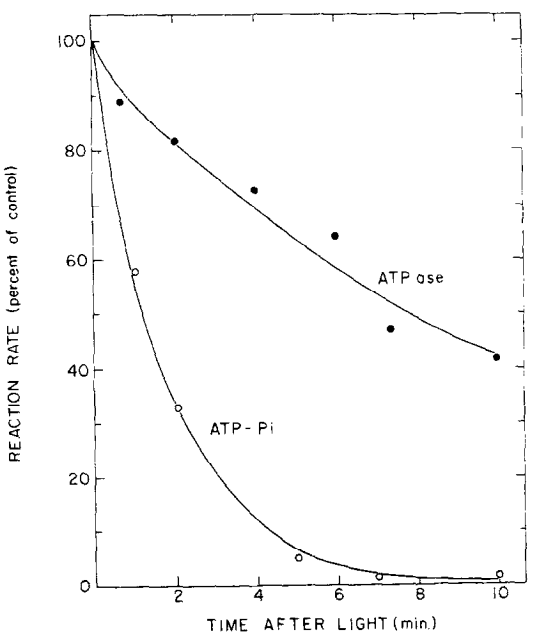

Figure 1: Decay of the light effect in ATP-Pi exchange and ATPase activities. ATP was added to preilluminated chloroplasts at the times indicated after the light was turned off. For assay conditions, see Methods.

chloroplasts at various time intervals after the light was turned off (Fig. 1). The half life of the decay of the light effect in inducing ATP-Pi exchange, or ATPase activity was of the order of several minutes. This is much longer than the half life of several seconds in the decay of the light effect which makes possible ATP formation in the dark in chloroplasts (Hind and Jagendorf, 1963). The half time of the decay of the light effect seems also longer in the ATPase induced by DTT than in the ATPase which was induced by reduced lipoic acid (Petrack et al., 1965). The disparity between the half time of decay of the light effect in ATP-Pi exchange on one hand and photophosphorylation on the other hand suggests that: (a) the decays which are being measured do not reflect the same conditions, or (b) the dithiol reagents render more stability to the light induced conditions.

Several uncouplers and inhibitors of photophosphorylation were tested for their effect on both the light-triggered ATPase and the light-triggered ATP-Pi exchange reaction when added to the light (and present in the dark) stage, or when added only to the dark stage (Table II).

Table II

Effect of inhibitors and uncouplers on the light-triggered ATP-Pi exchange and ATPase activity in chloroplasts

Inhibitor	Concentration	Rate of ATP-Pi exchange		Rate of ATPase activity	
	M	Light + Dark	Dark	Light + Dark	Dark
		per cent of control			
Atebrin	7×10^{-6}	87	47	112	110
	8×10^{-6}	18	20	-	-
	6×10^{-5}	1	3	7	16
Octyl-guanidine	10^{-6}	68	64	81	94
	8×10^{-6}	4	4	8	20
Phloridzin	10^{-4}	76	59	45	51
	5×10^{-4}	9	6	7	24
NH_4Cl	10^{-4}	84	96	82	98
	2×10^{-4}	42	20	-	-
	5×10^{-4}	10	12	75	67
	5×10^{-3}	0	3	13	38
DCMU	10^{-6}	110	100	114	110
	10^{-5}	102	98	100	118
Arsenate	10^{-3}	48	33	*95	*104
	10^{-2}	5	7	*91	*72

*ATPase activity was assayed by measuring P^{32} released from ATP^{32}.

Inhibitors were present during both light and dark or only at the dark stage as indicated. For assay conditions look under Methods.

Atebrin, octyl-guanidine and phloridzin inhibited both of the light-triggered reactions under all conditions. Ammonium chloride inhibited the ATP-Pi exchange activity somewhat stronger than the ATPase activity. The reactions were inhibited independent of whether NH_4Cl was added during the light or the dark stage. This result is in contradiction to the reported lack of inhibition of ATPase activity by NH_4Cl when added to the dark stage (Petrack et al., 1965). Arsenate which acts as a competitive inhibitor of phosphate in photophosphorylation (Avron and Jagendorf, 1959) inhibited the ATP-Pi exchange reaction (Table II). This should be expected if arsenate competed with phosphate in the exchange with ATP. However, such competition should not exist in the hydrolysis of phosphate from ATP. Indeed the results in Table II show that arsenate did not inhibit the ATPase activity. There was no inhibition of the ATPase and the ATP-Pi exchange reactions by DCMU. This is in agreement with the observation that DCMU does not inhibit photophosphorylation catalyzed by PMS (Jagendorf and Avron, 1959). The inhibition of the light-triggered ATPase and ATP-Pi exchange reactions by uncouplers and inhibitors of photophosphorylation indicates a direct relation between the three reactions.

The rate of ATP-Pi exchange reaction varied between 9 to 17 µmoles Pi^{32} exchanged with ATP per mg chlorophyll per hr. The rate of the ATPase activity under similar conditions was much higher and varied between 100-170 µmoles Pi released from ATP per mg chlorophyll per hour. If we assume that a common intermediate, produced from ATP, is involved in the ATPase and ATP-Pi exchange reactions, two pathways would be available for the further reaction of such an intermediate. One would lead to eventual breakdown (i.e. ATPase), and the other to recombination with inorganic phosphate leading to the resynthesis of ATP (i.e. exchange) On these assumptions the results would indicate a greater tendency of the intermediate to break down under the conditions used.

In conclusion, chloroplasts were shown to possess a light-triggered ATP-Pi exchange activity under the same conditions which produce a light-triggered ATPase activity. Both light-triggered reactions were directly related to photophosphorylation by similarity in cofactor requirements and by their response to uncouplers and inhibitors of photophosphorylation.

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