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A LIGHT- AND CYSTEINE-ACTIVATED ATP–P_i EXCHANGE REACTION IN CHLOROPLASTS AND ITS RELATIONSHIP TO ATPase AND PHOTOPHOSPHORYLATION

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SUMMARY

1. 'Broken' chloroplasts from spinach if illuminated for a period in the presence of cysteine and phenazine methosulphate develop an ATP–P_i exchange activity which can be observed in the dark. The conditions giving rise to ATP–P_i exchange activity are similar to those giving rise to the thiol-activated light-triggered ATPase.

2. ATP is not necessary during illumination for development of ATP–P_i exchange activity, but the activity declines if a period of time elapses between illumination and addition of ATP. This is accompanied by a similar decline in the cysteine-activated light-triggered ATPase.

3. The ATP–P_i exchange and ATPase show the same dependence on ATP concentration and are both inhibited by added ADP.

4. Both reactions are inhibited by Dio-9.

5. Desaspidin, 4-octyl-2,6-dinitrophenol and carbonyl cyanide 4-trifluoromethoxyphenylhydrazone, added immediately after illumination, inhibit the ATP–P_i exchange. The ATPase is initially stimulated under these conditions and then inhibited. If present during illumination, desaspidin and octyldinitrophenol inhibit the ATPase.

6. It is concluded that the ATP–P_i exchange reaction and the ATPase are activities of the same enzyme complex in the chloroplast and that this is probably part of the terminal enzyme system of photophosphorylation.

INTRODUCTION

Cysteine and some other sulphydryl compounds have been shown to participate in the activation by light of an ATPase[1,2] in chloroplasts. The activation requires the presence of Mg^{2+} and an electron carrier such as phenazine methosulphate (PMS) during the light treatment. Although light is required for the activation, the ATPase

Abbreviations: FCCP, carbonyl cyanide 4-trifluoromethoxyphenylhydrazone; PMS, phenazine methosulphate.

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remains active in the dark following illumination[2–4]. The behaviour of the ATPase can thus be studied both during illumination and in the subsequent dark period.

The thiol compounds necessary for the activation of the ATPase bring about a diminished yield of ATP if present during cyclic photophosphorylation with PMS (refs. 1–3), and PETRACK *et al.*[2] proposed a mechanism in which the thiol compounds act essentially as uncouplers. It was suggested that illumination brings about the formation of a high-energy intermediate which is utilized for the synthesis of ATP from ADP and P_i during photophophorylation. It was proposed that the ATPase activity could arise because the high-energy compound can be continually reformed from ATP by a reversal of the reactions leading to ATP formation, and that the presence of the thiol, or rather the results of its interaction with chloroplasts in the light, permits the continual breakdown of the high-energy compound.

In the dark the stability of the ATPase is dependent upon the continued presence of ATP. If an interval of time elapses between the light phase and addition of the ATP, the ATPase activity rapidly diminishes and within 2 min returns to the low level found in chloroplasts prior to activation[2,3]. PACKER AND MARCHANT[3] have proposed that the energy from the hydrolysis of the ATP can be utilized in the dark to maintain the chloroplast in an activation state that is generated in the light and is characterized by enhanced light-scattering properties. The increased light scattering, brought about in the light, is rapidly reversed in the dark unless a thiol compound is present during the illumination and ATP is present in the dark[3,4]. Inhibition of the ATPase by ADP is accompanied by a rapid drop in light scattering by the activated chloroplast suspension[3].

The relationship of this cysteine-activated, 'light-triggered' ATPase and the terminal enzymes of phosphorylation have been discussed by many authors[2–5]. The ATPase shows a number of properties (pH optimum, sensitivity to inhibition and uncouplers of photophosphorylation) that are consistent with the conclusion that it represents a portion of the terminal enzymes of the photophosphorylation process. However, photophosphorylation can proceed at rates of about 1000 μmoles ATP formed per h per mg chlorophyll whereas rates of only 100–140 μmoles ATP hydrolysed per h per mg chlorophyll are observed for the thiol- and light-activated ATPase. This discrepancy points to a difference between uncoupler-induced ATPase in mitochondria and thiol- and light-activated ATPase in chloroplasts, and suggested that the latter reaction, unlike the former, might be associated with an ATP–P_i exchange reaction. Chloroplasts have been shown not to have an ATP–P_i exchange activity[6,7] either in the light or the dark. This paper is concerned with an ATP–P_i exchange that can be demonstrated in chloroplasts and its relationship to the cysteine-activated 'light-triggered' ATPase. During the course of this work CARMELI AND AVRON[8] published a preliminary report also describing an ATP–P_i exchange activity in chloroplasts after illumination in the presence of dithioerythreitol.

MATERIALS AND METHODS

'Broken' chloroplasts were prepared from market spinach (*Spinacia oleracea*). After washing with tap and distilled water the spinach was cooled to approx. 0° and used immediately or stored at 0° overnight. The preparative manipulations were carried out at 0° in a cold room. Approx. 50 g of small leaves were dispersed in 135 ml

of 0.8 M sucrose, containing 0.02 M Tris–HCl (pH 8.0), 0.01 M NaCl and 2 mM EDTA, using either a Waring blendor for 15 sec or a mortar with acid-washed, EDTA-treated sand. The suspension was strained through cheese cloth and the fluid centrifuged at 1000 × *g* for 3–5 min. The supernatant fluid was centrifuged at 3000 × *g* for 5 min, the pellet resuspended in 135 ml of grinding medium (from which EDTA was omitted) and the chloroplasts centrifuged down at 3000 × *g* for 5 min. The pellet was resuspended in 135 ml of 0.01 M NaCl containing 0.04 M Tricine–NaOH buffer (pH 8.0). After 20 min the broken chloroplasts were recovered by centrifugation at 10000 × *g* for 15 min. The pellet was resuspended in 135 ml of 0.01 M NaCl–0.04 M Tricine–NaOH buffer (pH 8.0) and the chloroplasts recovered by centrifugation at 10000 × *g* for 15 min. The chloroplasts were resuspended in 5–10 ml of 0.01 M NaCl–0.04 M Tricine–NaOH buffer (pH 8.0). The chloroplasts were used within 2 h of preparation. Chlorophyll was measured by the method of ARNON[9].

Incubations for assay of ATPase, ATP–P_i exchange and photophosphorylation were carried out in a cylindrical vessel (1.3 cm internal dia.) jacketed at 25° and the contents were stirred vigorously by magnetic stirring. The gas phase was air. The vessel was illuminated by two diametrically opposed 150-W photoflood lamps, each separated from the reaction vessel by glass cylinders 13 cm long, through which water was passed. Light of 100000 lux reached the cell from each lamp. All measurements of the ATPase and ATP–P_i exchange were made in the dark following illumination of the reaction mixture. The standard conditions used were as follows. The reaction mixture during illumination contained NaCl, PMS, ascorbate, Tricine–NaOH buffer (pH 8.0), $MgCl_2$, cysteine and chloroplasts. Immediately at the end of the illumination period, ATP was added and, in the case of exchange measurements, P_i containing ^{32}P added 20 sec later. Samples (1 ml) were removed into 1 ml of 10 % trichloroacetic acid using an all-glass syringe. The experiments were carried out in a darkened room. The final concentrations in the standard reaction mixture were NaCl (35 mM), $MgCl_2$ (5 mM), Tricine–NaOH buffer (pH 8.0, 100 mM), ascorbate (0.75 mM), PMS (20 μM), cysteine (80 mM), ATP (2.5 mM), $^{32}P_i$ (20 mM) and chloroplasts containing 30–60 μg chlorophyll/ml. The pH during illumination and during the dark was pH 8.0. The standard reaction mixture was varied as occasion demanded and the variations are noted in legends to tables and figures. Unless otherwise specified the standard reaction conditions were used. The ascorbate, PMS and cysteine solutions were made up freshly daily.

The ATPase activity was measured as P_i liberated in the presence of ATP during the first 5 min after ATP addition. The rate of hydrolysis appeared constant for at least 10 min with ATP at an initial concentration of 2.5 mM and for approx. 5 min with an ATP concentration of 0.5–1.0 mM. P_i was determined according to HORWITT[10].

The ATP–P_i exchange was measured as [^{32}P]ATP formed from $^{32}P_i$ during the first 5 min after $^{32}P_i$ addition. Measurement was made of ATP hydrolysis and the rate of exchange calculated from the rate of increase of specific activity of the [^{32}P]-ATP. The increase of specific activity was almost linear over the first 5 min of the reaction. Under the conditions used the specific activity of the [^{32}P]ATP did not exceed 3 % of that of the $^{32}P_i$. [^{32}P]ATP was measured as the radioactivity remaining in the aqueous phase after removal of unreacted $^{32}P_i$ as [^{32}P]phosphomolybdate into isobutanol–benzene[10]. Radioactivity was measured with a gas-flow, thin-end-window Geiger–Müller apparatus.

Photophosphorylation measurements were carried out in the same apparatus used for the ATPase and ATP–P_i exchange studies. The concentrations of reactants were as given above except that cysteine was absent and ADP (2.0 mM) replaced ATP. The ATP yield was measured as [^{32}P]ATP formed during the first 60 sec of illumination.

Carrier-free $H_3{}^{32}PO_4$ was purchased from Philips–Duphar. It was heated with 0.5 M HCl for 1 h at 100°, then brought to pH 5–7 with NaOH. Some samples were shaken with charcoal and filtered prior to neutralization. ATP (disodium salt) was purchased from Sigma Chemical Co. It contained approx. 1.0 % ADP. ADP (trisodium salt) was from Boehringer and Soehne. PMS and cysteine·HCl were British Drug Houses laboratory reagents. Tricine (*N*-tris(hydroxymethyl)methylglycine), Grade A, was from Calbiochem.

The atractyloside was kindly provided by Professor N. SPRIO, Dio-9 by N.V. Nederlandse Gist- and Spiritus Fabriek, carbonyl cyanide 4-trifluoromethoxyphenylhydrazone (FCCP) by Dr. P. G. HEYTLER, 4-octyl-2,6-dinitrophenol by Dr. H. C. HEMKER and desaspidin by Dr. I. RUNEBERG.

RESULTS

In Table I results are set out showing that during the incubation of $^{32}P_i$ with ATP in the dark after illumination of chloroplasts in the presence of cysteine and PMS ATP becomes labelled with ^{32}P. Treatment with 1 M HCl at 100° for 10 min prior to extraction of P_i with molybdate transforms all of the radioactivity from an organic form to P_i. Incubation with hexokinase and glucose prior to acid treatment stabilizes about 90 % of the radioactivity in the organic form. These results indicate that the terminal phosphorus of ATP is the major source of radioactivity in the organic phosphate fraction in the 'exchange' experiment. This conclusion was confirmed by

TABLE I

INCORPORATION OF $^{32}P_i$ INTO ATP BY CHLOROPLASTS ACTIVATED BY ILLUMINATION IN PRESENCE OF CYSTEINE AND PMS

Exchange and photophosphorylation incubations carried out under standard conditions (see METHODS) except $^{32}P_i$ was 2 mM ($6.2 \cdot 10^5$ counts/min·μmole). The incubations (5 min for exchange and 1 min for photophosphorylation) were terminated by the addition of $HClO_4$ to 5 %. Cysteine, ADP and ATP were then added to appropriate tubes to make all tubes approximately equivalent in concentration of these substances. Samples of the deproteinized supernatant were brought to pH 8 with KOH and $KClO_4$ removed at 0°. Samples (1 ml) were treated as designated and the radioactivity remaining after removal of $^{32}P_i$ was determined in the usual way. The values quoted are corrected for values in a non-incubated control. The incubation with hexokinase was carried out in the presence of glucose (30 mM) and EDTA (3 mM) for 20 min at 25°.

Treatment of deproteinized supernatant	*Radioactivity remaining after removal of $^{32}P_i$ (counts/min·ml)*	
	Exchange	*Photophosphorylation*
Nil	8050	53 050
1 M HCl at 100° for 10 min	23	36
Hexokinase, followed by 1 M HCl at 100° for 10 min	7284	49 448

adsorption on and elution from Dowex 1. Approx. 90 % of the radioactivity present in the organic phosphate fraction of the sample was found in the ATP fraction.

Conditions necessary for activation of ATP–P_i exchange and ATPase

Table II illustrates the conditions necessary to elicit an ATP–P_i exchange in chloroplasts in comparison with those required for the ATPase. It is clear that illumination in presence of cysteine is required to produce the exchange activity. ATP is not necessary during illumination, and cannot be replaced by ADP. A period of 20 sec was allowed to elapse between extinguishing the light (and adding ATP) and the addition of $^{32}P_i$, in order to allow any 'X_E' to be dissipated. According to HIND AND JAGENDORF[12] X_E would have a half life of under 1.0 sec at pH 8.0 and 25°. In fact, control experiments failed to show any formation of [^{32}P]ATP from X_E, ATP and $^{32}P_i$ under the conditions of these experiments.

TABLE II

CONDITIONS FOR THE ACTIVATION OF ATP–P_i EXCHANGE AND ATPase IN CHLOROPLASTS

Standard conditions were used during illumination and dark periods except for the omissions and additions noted in the table. $^{32}P_i$, 20 mM ($1.6 \cdot 10^5$ counts/min·μmole). Illumination for 3 min. Chlorophyll concentrations, 27.7 and 29.6 μg/ml, in Expts. 1 and 2, respectively. The rates given are analyses made on 4 samples taken during the first 4 min after extinguishing the light. Photophosphorylation rate in both experiments, 1200 μmoles ATP synthesised per h per mg chlorophyll.

Expt. No.	*Conditions*	*ATP–P_i exchange (μmoles [^{32}P]ATP per h per mg chlorophyll)*	*ATPase (μmoles P_i liberated per h per mg chlorophyll)*
1	Complete system	13.5	137
	No ATP	−0.8	0
	ATP present during illumination	14.0	143
	ADP replacing ATP	−0.1	6
	No illumination	0.0	9
	No cysteine	0.0	14
2	Complete system	8.0	104
	Cysteine added after light	0.0	14
	PMS added after light	5.7	27
	Mg^{2+} added after light	8.7	104
	Mg^{2+} omitted completely	0.0	0

The results of Table II indicate a partial dependence of both the exchange reaction and the ATPase on the presence of PMS during the light phase. Values similar to those with PMS added after illumination were obtained when PMS was omitted altogether from the reaction mixture. PETRACK *et al.*[2] comment that slight ATPase activity could be obtained with fresh unwashed chloroplasts in the absence of added PMS, probably due to the presence of endogenous redox carriers which are active in an atmosphere of air.

Under the conditions of the experiments reported here no requirement for Mg^{2+} during illumination could be observed. This is in contrast to the findings of PETRACK *et al.*[2] and PACKER AND MARCHANT[3] for the development of ATPase activity. CARMELI AND AVRON[8] report that Mg^{2+} is necessary during illumination of lettuce

chloroplasts to elicit both ATPase and ATP–P_i exchange activities. The reason for the differences between the observations reported here and by others in this respect is not known.

The level of ATP–P_i exchange and ATPase achieved under standard conditions appeared to be independent of the concentration of the chloroplast suspension in the reaction mixture between 20 and 80 μg chlorophyll/ml.

Not all thiol components are effective in bringing about ATPase and ATP–P_i exchange activities. 2,3-Dimercaptopropanol (1–20 mM) and thioglycollate (5 mM) were ineffective. Lipoic acid (10 mM) was active. Dithioerythreitol (5 mM) was very effective in activating the ATP–P_i exchange.

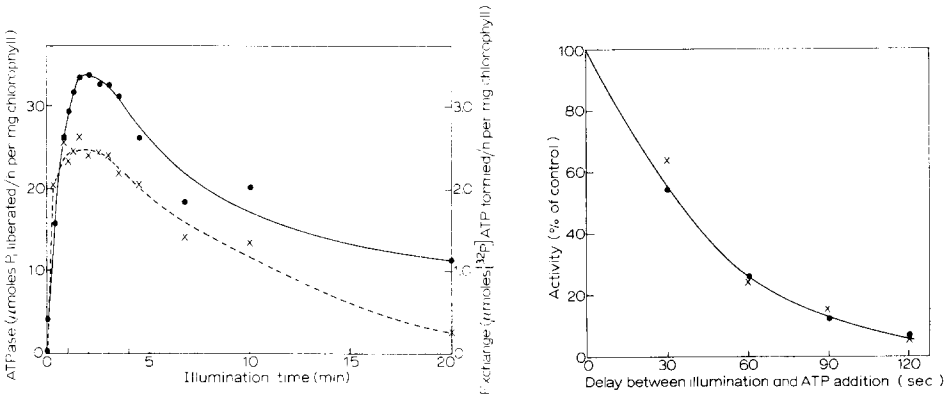


Fig. 1. Effect of duration of illumination on ATP–P_i exchange and ATPase activities. Reactions were carried out under standard conditions. $^{32}P_i$, 2 mM ($1.56 \cdot 10^6$ counts/min·μmole). Chloroplasts, 50 μg chlorophyll/ml. Samples were removed from reaction mixture during illumination, pipetted immediately into a solution of ATP with or without $^{32}P_i$ and incubated in the dark for 5 min for the measurement of ATPase or ATP–P_i exchange. × ---×, exchange; ●—●, ATPase.

Fig. 2. Decay of ATP–P_i exchange and ATPase activities in the dark in absence of added ATP. Reactions were carried out under standard conditions. $^{32}P_i$, 20 mM($1.58 \cdot 10^5$ counts/min·μmole). Chloroplasts, 42.6 μg chlorophyll/ml. Each value in the figure was calculated from analyses from 4 samples taken during the first 4 min of incubation following ATP addition. ×, exchange; ●, ATPase.

The period of illumination is critical for both the ATPase and ATP–P_i exchange activities (Fig. 1). Maximum activities were obtained after 1.5–3.0 min in different experiments. Prolonged illumination at the high light intensities used in these studies apparently brought about inactivation of the systems responsible for the ATPase and ATP–P_i exchange (*cf.* AVRON[13]). Unless otherwise mentioned, a standard illumination time of 2 min was used in experiments reported in this paper.

If an interval of time is permitted to elapse between the end of illumination and addition of ATP, the ATPase diminishes[2,3]. In Fig. 2 it can be seen that the ATP–P_i exchange behaves in an identical fashion. CARMELI AND AVRON[8] in a similar experiment (using chloroplasts from lettuce leaves) obtained somewhat different results in that the decay of ATPase activity was less marked than that of ATP–P_i exchange activity and also that the decay in both activities was much slower than reported here.

Effect of concentrations of P_i, ADP and ATP on the activity of the exchange enzyme system

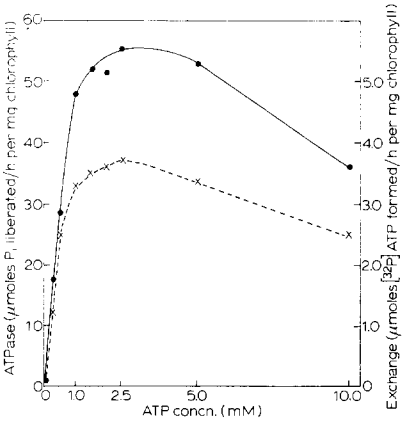
Variations of the rate of ATP–P_i exchange are shown as a function of P_i concentration (Table III), ATP concentration (Fig. 3) and of concentration of added ADP (Fig. 4). The behaviour of the ATPase is also examined in the latter two instances. The amount of P_i needed for maximum activity of the ATP–P_i exchange system is similar to that required for maximum rates of cyclic photophosphorylation[14]. The rates of the ATP–P_i exchange and the ATPase change in very similar manner with variations in ATP concentration (Fig. 3). Both activities are inhibited by added ADP, the ATPase being more affected than the exchange (Fig. 4). Inhibition by ADP has

TABLE III

EFFECT OF P_i UPON ATP–P_i EXCHANGE ACTIVITY

Standard conditions were used during illumination and dark periods. Initial P_i concentration determined by analysis. Specific activity of $^{32}P_i$, $1.63 \cdot 10^5$ counts/min·μmole. Illumination for 3 min. Chlorophyll concentration, 59.2 μg/ml. Rates of exchange based on analysis made on 4 samples taken during the first 90 sec after extinguishing the light.

Initial P_i concn. (mM)	*μmoles $[^{32}P]ATP$ formed per h per mg chlorophyll*
1.25	17.5
3.05	19.6
6.25	22.7
11.3	25.7
22.9	26.4



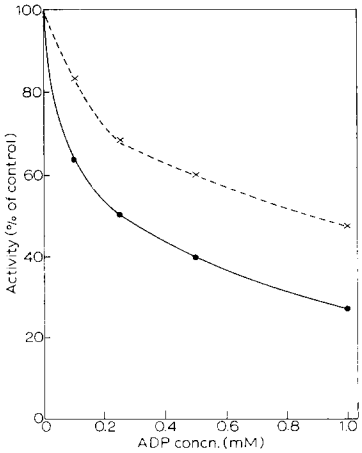


Fig. 3. Influence of ATP concentration upon ATP–P_i exchange and ATPase activities. Reactions carrried out under standard conditions. Illumination for 2 min. Chloroplasts, 37.8 μg chlorophyll per ml. $^{32}P_i$, 20 mM ($3.2 \cdot 10^4$ counts/min·μmole). Values calculated from analyses made on single samples taken after 5 min dark incubation. ×---×, exchange; ●—●, ATPase.

Fig. 4. Effect of ADP upon ATP–P_i exchange and ATPase activities. Conditions as for Fig. 4 except 1.0 mM ATP was used. Control values without addition of ADP were: ATP–P_i exchange, 4.74 μmoles per h per mg chlorophyll; ATPase, 37.7 μmoles per h per mg chlorophyll. ×---×, exchange; ●—●, ATPase.

also been reported by PACKER AND MARCHANT[3]. The measurements of the effect of ADP on the ATPase activity were corrected for an increase in P_i which occurred when ADP was added to the reaction mixture in the absence of ATP. This correction was 26 % of the P_i liberated in the presence of 1.0 mM ATP and 1.0 mM ADP.

The effects of some inhibitors and uncoupling agents

It is clear from Table IV that the chloroplast is insensitive to atractyloside, which inhibits the phosphorylation of added ADP by mitochondria[15]. The inhibitory effect of sucrose is possibly related to its effects on the conformational changes of chloroplasts in the light and dark[12]. The antibiotic Dio-9, introduced by GUILLORY[16] as an uncoupler and inhibitor of mitochondrial oxidative phosphorylation and shown by MCCARTY, GUILLORY AND RACKER[17] to inhibit photophosphorylation and the Ca^{2+}-dependent, trypsin-activated ATPase of VAMBUTAS AND RACKER[18], is also a potent inhibitor of the two light-induced reactions. The concentration found effective is similar to those used by MCCARTY, GUILLORY AND RACKER[17].

The other three substances listed in Table IV—FCCP (ref. 19), desaspidin[20] and 4-octyl-2,6-dinitrophenol[21]—have been shown to be uncouplers of photophosphorylation. Under the conditions of the experiment shown in Table IV, all three compounds inhibited the exchange reaction, whether added during illumination or in the dark. The effects on the ATPase varied. FCCP stimulated the ATPase in low concentration and inhibited slightly in higher concentration. Desaspidin and octyldinitrophenol, on the other hand, inhibited strongly, particularly when added during the illumination.

Since these results are based on a single analysis made at the end of a 5-min incubation, a more extensive study of the kinetics of the ATPase in the presence of these uncouplers was undertaken in the experiment the results of which are summarized in Fig. 5. In the absence of uncouplers, essentially linear kinetics were obtained. In the presence of all uncouplers, the activity decreased with time. When

TABLE IV

SENSITIVITY OF ATP–P_i EXCHANGE AND ATPASE TO SOME INHIBITORS AND UNCOUPLERS OF PHOTOPHOSPHORYLATION

Standard conditions were used, modified by addition of the listed substances either just prior to illumination (*L*) or at the end of illumination (*D*). Illumination for 2 min for exchange and 1 min for photophosphorylation. Dark incubation for 5 min. $^{32}P_i$ (2 mM) added immediately after illumination. Activities are quoted as percentage activity of untreated controls and based on measurements made at the end of a 5-min incubation and assuming linear kinetics.

Addition	*Rates of activity*				
	Exchange		*ATPase*		*Photophosphorylation*
	L	*D*	*L*	*D*	
Atractyloside (50 μg/ml)	100	100	105	100	95
Sucrose (0.67 M)	54	46	47	76	
Dio-9 (2 μg/ml)	14	12	8	8	
FCCP (0.5 μM)	85	73	125	119	85
FCCP (1 μM)	54	54	90	90	40
Desaspidin (2 μM)	0	5	5	67	
4-Octyl-2,6-dinitrophenol (20 μM)	12	10	27	40	

the uncouplers were added at the end of the illumination, the ATPase was initially stimulated followed by an inhibition. Both the initial stimulation and subsequent inhibition were particularly marked with desaspidin. Only in the case of FCCP was the initial stimulation seen when the uncoupler was present during the illumination. Only inhibition was seen with the other uncouplers.

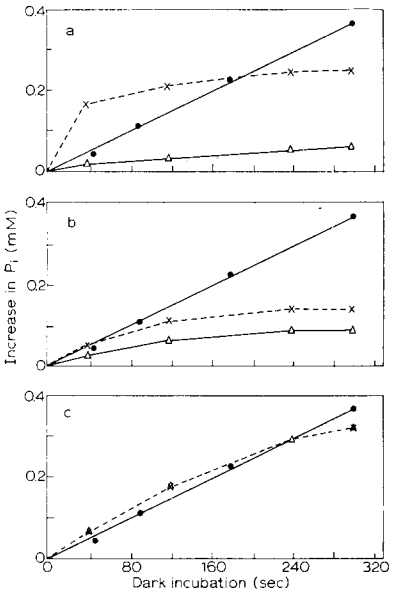


Fig. 5. Effect of desaspidin, octyldinitrophenol and FCCP upon ATPase activities. Standard reaction conditions were used. Illumination for 2 min. Chlorophyll, 63.8 μg/ml. (a) desaspidin, 2 μM; (b) octyldinitrophenol, 20 μM; (c) FCCP, 1 μM. ●—●, control in absence of inhibitor; ×---×, inhibitor added at end of light phase; △---△, inhibitor present during light phase.

DISCUSSION

The data presented provide strong evidence that the ATP–P_i exchange and the ATPase activities present in chloroplasts after illumination in the presence of cysteine are part of the same enzyme complex. The results of previous workers[2–5] have suggested that the ATPase activity is a property of the terminal enzyme system of photophosphorylation working in reverse. If this is true, it follows that the ATP–P_i exchange is also a property of the phosphorylation enzymes of the photophosphorylation apparatus of the chloroplast. This view is strongly supported by the inhibition of both the ATPase and ATP–P_i exchange by Dio-9 recorded in Table IV, and by phlorizin[8]. Both of these substances inhibit photophosphorylation at a point close to the participation of ADP and P_i (refs. 17, 22).

The effects of the uncouplers of photophosphorylation used here, *viz.* desaspidin, octyldinitrophenol and FCCP, are not as simple as Dio-9 and require more detailed discussion. In mitochondrial systems uncouplers of oxidative phosphorylation, *e.g.* 2,4-dinitrophenol, inhibit the active ATP–P_i exchange which is normally present and at the same time induce a powerful ATPase[23]. These findings are easily understood in terms of either the chemical hypothesis[24] or the chemiosmotic hypothesis[25] of

oxidative phosphorylation. In the former the uncoupler causes the breakdown of a high-energy intermediate (A ~ C) and in the latter it allows dissipation of a trans-membrane proton potential. Since, according to these two hypotheses, A ~ C or the membrane potential can be built up by utilization of the energy of the added ATP, the observed effect is that in the presence of uncouplers the hydrolysis of ATP can proceed unhindered. On the other hand, the ATP–P_i exchange reaction, which requires A ~ C or the membrane potential, is inhibited.

The data of Fig. 5 illustrate that the uncouplers when added in the dark following illumination in the presence of cysteine initially stimulate the ATPase, although they inhibit ATP–P_i exchange (Table IV). This could be explained in terms of the mechanisms discussed for mitochondria. The inhibition of the chloroplast ATPase that follows the initial stimulation can be understood in terms of the unique requirements for the maintenance of ATPase and ATP–P_i exchange in chloroplasts. It is clear from the experiments reported here and by others that the continued availability of ATP is necessary for the maintenance of the ATPase and ATP–P_i exchange activities. PACKER AND MARCHANT[3] suggest that the ATP supplies energy necessary to maintain the chloroplasts in a particular conformational state (characterized in part by the ATPase and produced by illumination in the presence of thiol). In the dark, lack of ATP allows the reversion of the chloroplast to its state prior to illumination (in which ATPase is virtually absent). An uncoupler, in terms discussed above, would produce a situation in which the energy from ATP breakdown, in the form of a high-energy compound or trans-membrane proton potential, is not available to maintain the particular state of the chloroplasts giving rise to ATPase. Thus, the initial stimulation of the ATPase caused by breakdown of A ~ C or dissipation of the membrane potential would be followed by a reversion of the chloroplast towards its state prior to illumination, with little or no ATPase or exchange activity. An uncoupler present during illumination, by bringing about dissipation of energy derived from illumination, would interfere with development of the conformational state associated with the ATPase and ATP–P_i exchange activity. Thus one would expect that an uncoupler that was effective in preventing the conformational change when present during illumination would also be most effective in stimulating the ATPase when added at the end of the illumination. Desaspidin at 2 μM is a case in point. Apparently, at the concentration employed, FCCP uncoupled insufficiently during the light phase to prevent the conformational change, and its slight uncoupling activity was also reflected in the slight initial stimulation of the ATPase activity.

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