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ENERGY TRANSDUCTION IN PHOTOSYNTHETIC BACTERIA

IV. LIGHT-DEPENDENT ATPase IN PHOTOSYNTHETIC MEMBRANES FROM *RHODOPSEUDOMONAS CAPSULATA*

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SUMMARY

ATPase activity of photosynthetic membrane fragments from the bacterium *Rhodopseudomonas capsulata* can be stimulated by continuous illumination under conditions of active cyclic electron flow. The activation corresponds to an increase in the maximum velocity of the reaction and does not affect the apparent K_m for ATP (0.11 mM). No stimulation in the light is observed in the presence of classical uncouplers or oxidized 2,6-dichlorophenolindophenol (DCIP), which, *per se*, stimulate ATPase in the dark. It is demonstrated, however, that oxidized DCIP acts as an uncoupler of bacterial photophosphorylation.

The effect of light is elicited after a few minutes of preillumination, or in a much shorter time if an ADP trapping system is supplied. Activation does not occur if ADP is added during the preillumination (apparent K_m for inhibition by ADP = 1 μM). The effect of ADP is not related to competitive inhibition with ATP, which can be observed at higher concentrations (apparent K_i = 0.26 mM). ADP, however, is not effective if added after some minutes of preillumination.

INTRODUCTION

Photosynthetic membranes from higher plant chloroplasts do not hydrolyze ATP in the dark: ATPase activity can, however, be elicited, in the light, in the presence of Ca^{2+} (ref. 1) ("light-dependent ATPase"), or in the dark after preillumination in a system containing a thiol reagent and Mg^{2+} (refs 2 and 3) ("light-triggered ATPase"). A cofactor for electron flow is required during the light treatment in both instances. These two types of activity, as well as those present in the dark in membranes mildly digested with trypsin[4,5] or preincubated with high concentrations of dithiothreitol[6], are all catalyzed by the same coupling factor protein, CF_1 (ref. 7).

By contrast, chromatophores from non-sulfur photosynthetic purple bacteria can, in the dark, catalyze the hydrolysis of ATP[8]; this activity, regarded as the reversal

Abbreviations: DCIP, 2,6-dichlorophenolindophenol; FCCP, carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone; PMS, phenazine methosulfate; TMPD, tetramethyl-*p*-phenylenediamine.

of the last step of photophosphorylation, has been demonstrated to depend on the presence of a coupling factor protein bound to the membrane[9].

The first attempts to find an effect of light on ATPase of *Rhodospirillum rubrum* chromatophores similar to that found in chloroplasts, gave only equivocal or negative results[10]. Subsequently, Horiuti *et al.*[11] reported that light could affect ATPase in this bacterial system in two opposite ways, depending on the experimental conditions used: light-dependent inhibition was observed in the absence of any cofactor, while a stimulation was obtained in the presence of $4 \cdot 10^{-4}$ M phenazine methosulfate (PMS). The latter effect, however, was ascribed to a change in the redox conditions of the assay due to a direct non-enzymatic photoreduction of the dye.

In this paper we present evidence for a light-promoted activation of ATPase in *Rhodopseudomonas capsulata* chromatophores, caused by a direct interaction of the enzyme with cyclic electron flow. The light-activated ATPase is dependent on Mg^{2+}, insensitive to uncouplers (while activity in the dark is stimulated), completely sensitive to oligomycin and not stabilized in the dark by thiol reagents. ADP can inhibit light activation by a mechanism unrelated to competitive inhibition with ATP.

MATERIALS AND METHODS

Rhodopseudomonas capsulata (strain St. Louis, American Type Culture Collection) was grown anaerobically at 30 °C in the medium described by Ormerod, *et al.*[12]. The procedure for the preparation of chromatophores has been described previously: the buffer used was 0.05 M glycylglycine, pH 7.2, containing 2.5 mM $MgCl_2$[13]. The particles were stored in darkness at −20 °C in the presence of the same buffer containing 50 % glycerol.

A typical assay for measuring ATPase activity contained, in a final volume of 1 ml: Tris–acetate, pH 8, 100 μmoles; $MgCl_2$, 5 μmoles (unless otherwise specified); and particles containing from 30 to 50 μg of bacteriochlorophyll. When an ATP-regenerating system was used, 5 μmoles of phosphoenolpyruvate and 30 units of pyruvate kinase were added. All components, except ATP, were incubated in the dark or in the light for 5 min at 30 °C; the reaction was started by the addition of 0.1 ml of 0.05 M ATP using an automatic device (Selecta pipette). 0.2 ml of 25 % trichloroacetic acid was added to stop the reaction; after centrifugation the inorganic phosphate present in the deproteinized supernatant was assayed using the colorimetric procedure described by Taussky and Shorr[14].

Uncouplers and inhibitors were added in small amounts of ethanolic solution; suitable control experiments using equal amounts of ethanol were performed routinely.

In the experiments where ATPase was assayed using γ-labeled ATP, the $^{32}P_i$ hydrolyzed was measured as described by Horio *el al.*[15].

Synthesis of [γ-^{32}P]ATP

ATP labeled in the terminal phosphate group was synthetized using the ATP–$^{32}P_i$ exchange reaction of *Rps. capsulata* chromatophores in the light The procedure used was essentially as described by Horio *et al.*[15]. Labeled ATP was eluted from a charcoal column (2 cm × 2 cm) using 60 % ethanol containing 1 % NH_3. The radioactive fractions were pooled, lyophilized and adjusted to the proper concentration and specific activity with carrier ATP. The ATP concentration of the

preparation was assayed spectroscopically and enzymatically. The specificity of the label in the γ-position was tested using the glucose–hexokinase reaction; after completion of the reaction, practically all the radioactivity was found to be no longer charcoal adsorbable in 5 % trichloroacetic acid, confirming the transfer of the phosphate group to glucose[16]. Contamination by labeled inorganic phosphate, as judged by isobutanol–benzene extraction, was about 3 %.

Carotenoid absorption change

Fast changes in carotenoid absorption spectra were measured in the laboratory of Dr A. R. Crofts[17], University of Bristol, using the single beam rapid responding spectrophotometer described previously; the measuring wavelength was 530 nm.

EXPERIMENTAL RESULTS

Light-promoted activation of ATPase

Rps. capsulata chromatophores possess an ATPase active in the dark, which is considerably enhanced if the reaction is carried on in the light. The conditions under which the increase in activity takes place are summarized in Table I; the activation is observed only in the presence of 0.2 mM succinate (in an aerobic assay), *i.e.* only when the proper redox conditions for cyclic electron flow are established, and is completely inhibited by low concentration of antimycin A. Although dark ATPase is only partially sensitive to oligomycin (maximum inhibition observed, 50–55 %), in the light, the per cent inhibition is markedly increased due to the complete sensitivity of the light-enhanced activity to the antibiotic. The activation phenomenon can always be observed with excellent reproducibility; the light to dark activity ratio varies slightly in different preparations (from 1.5 to 2.5).

The metal dependence of ATPase in the two conditions was examined: maximum activity is obtained in the presence of Mg^{2+} or Mn^{2+}, lower rates being observed with Ca^{2+}, Co^{2+} or Fe^{2+} (ref. 18). No difference in this respect has been observed under light or dark conditions except for Ca^{2+}, in the presence of which no light effect was obtained. Similarly, no difference could be detected in the pH dependence of the reaction[18].

TABLE I

EFFECT OF SODIUM SUCCINATE, ANTIMYCIN A, AND OLIGOMYCIN ON DARK AND LIGHT ATPASE

Conditions of assays as described in Materials and Methods.

Additions	*Activity (μmoles P_i/h per mg bacteriochlorophyll)*	
	Dark	*Light*
None	52.6	55.5
Sodium succinate (0.2 mM)	53.2	111.2
Sodium succinate (0.2 mM) + antimycin A ($6 \cdot 10^{-7}$ M)	53.7	61.8
Sodium succinate (0.2 mM) + oligomycin (10 μg/ml	25.4	30.8

The properties of the light and dark ATPase were better defined kinetically using isotope γ-labeled ATP (Fig. 1): the K_m value for ATP (in the presence of 5 mM $MgCl_2$) is 0.11 mM, both in the light and in the dark, in good agreement with the value previously reported for *Rh. rubrum* chromatophores[15]. The light activation is reflected only in an increase in the maximum velocity.

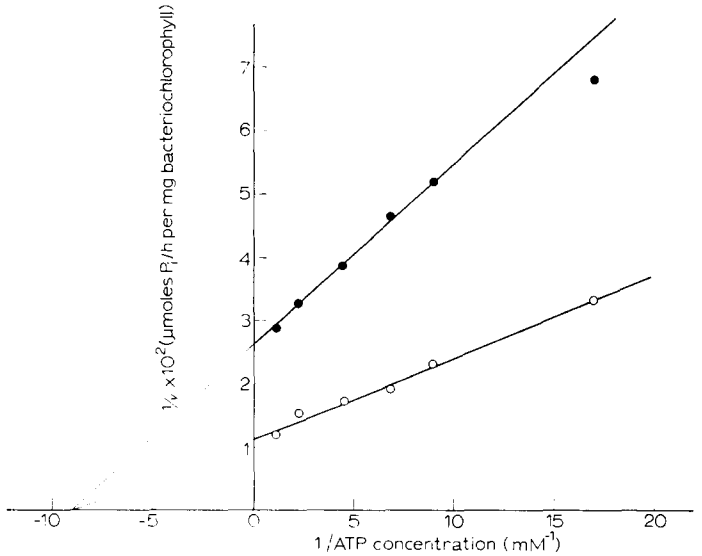

Fig. 1. Reciprocal plot of the velocity of ATPase reaction *vs* ATP concentration. ○, reaction performed in light; ●, reaction performed in dark.

The absolute requirement of light for a high rate of ATP hydrolysis is illustrated in Fig. 2: if illuminated assays are transferred to the dark, the rate of the ATPase reaction falls immediately to the dark level. Addition of 5 mM dithiothreitol failed to stabilize the activated rate in the postillumination period. It should be also observed that the course of the reaction in the light is not completely linear with time, while linearity is maintained in the dark during the same time span.

Effect of ADP

A study of the kinetics of the activation process was attempted with experiments similar to those described above: however, no enhancement could be observed when particles, already hydrolyzing ATP, were transferred from dark to light. In fact, as was established subsequently, a preillumination period of 3–5 min in the absence of ATP is required in order to promote the activation process. After preillumination the particles maintain the capability of being activated immediately by light for a considerable time. Centrifugation of preilluminated chromatophores demonstrated that this property is intrinsic in the membranes and is not a consequence of the accumulation of a photoproduct in the assay medium. The requirement of preillumination can, however, be eliminated if an ATP-regenerating system is added to the assay mixture; under these conditions the light activation occurs within 1 min (Table II). This set of data, as a whole, suggests that ADP — endogenous, and possibly exogenous or derived from the hydrolysis of ATP — can inhibit the light activation

process very effectively. This hypothesis was confirmed by the observation that addition of ADP in the μM concentration range during the preillumination period blocks the activation process (Fig. 3).

However, as one could argue from the data reported in Fig. 2, concentrations of ADP higher than these and derived from ATP hydrolysis are present already after

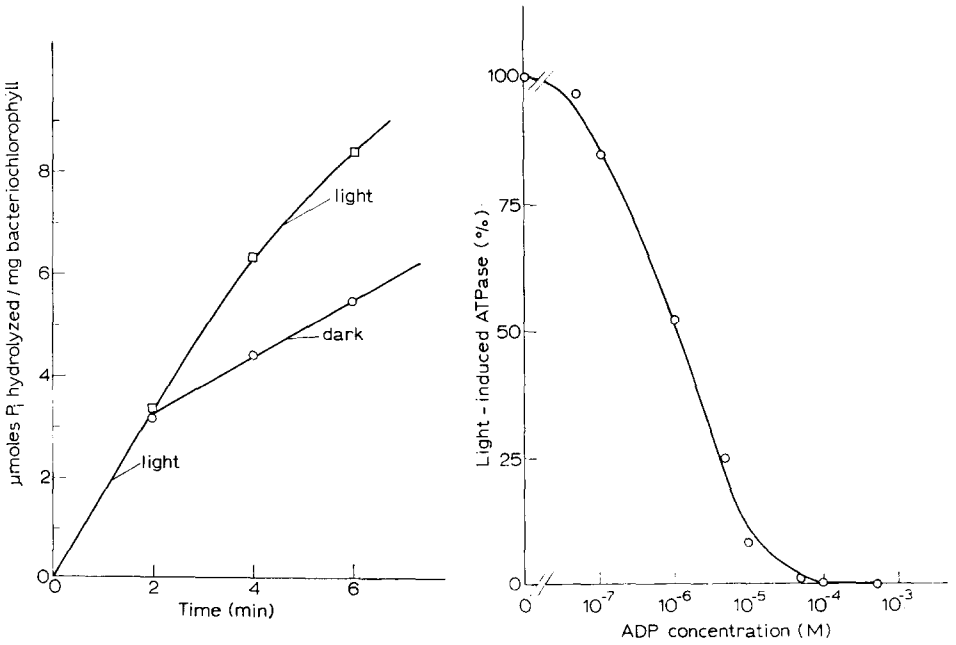

Fig. 2. Time course of ATPase reaction. Assay mixtures were preilluminated for 5 min and the reaction started by addition of ATP in the light; after 2 min part of the assays were transferred to dark. □, continuous light; ○, transferred to dark.

Fig. 3. Inhibition of light-activated ATPase by addition of ADP before preillumination. The rate of ATPase, in the absence of ADP in the light and in the dark was 90 μmoles/h per mg bacteriochlorophyll and 49 μmoles/h per mg bacteriochlorophyll, respectively. Light-dependent ATPase is defined as the difference between these two rates.

TABLE II

LACK OF REQUIREMENT OF A PREILLUMINATION TIME FOR LIGHT ACTIVATION OF ATPASE IN THE PRESENCE OF AN ATP-REGENERATION SYSTEM (PHOSPHOENOLPYRUVATE–PYRUVATE KINASE)

Conditions of assay	*Activity (μmoles P_i/h per mg bacteriochlorophyll)*	
	+ ATP-regenerating system	*− ATP-regenerating system*
Dark	60.2	41.6
Light*	130	105
Light**	127	53

* Assays preilluminated for 5 min before the reaction was started by addition of ATP.
** Assays kept in dark for 5 min before the reaction was started by addition of ATP in the presence of light.

TABLE III

EFFECT OF ADP ON ATPASE ACTIVITY IN THE LIGHT: INFLUENCE OF THE TIME OF ADDITION IN RELATION TO PREILLUMINATION

All samples were preilluminated for 5 min and 10^{-5} M ADP added at the indicated time. ATPase reaction was started by addition of ATP. For photophosphorylation measurements the reaction was started by simultaneous addition of $^{32}P_i$ (10 mM) and ADP (final concentration 2 mM); esterified phosphate was measured as described previously[13].

Conditions	*ATPase activity (μmoles/h per mg bacteriochlorophyll)*	*Photophosphorylation (μmoles/h per mg bacteriochlorophyll)*
No additions (non-preilluminated)	27.1	188
No additions (preilluminated)	26.8 *	140
10^{-5} M ADP, before preillumination	26.4 *	—
10^{-5} M ADP, before preillumination	27.1	234
10^{-5} M ADP, after 3.5 min preillumination	49.6	—
10^{-5} M ADP, after 5 min preillumination	64.5	—
10^{-5} M ADP, 2 min after addition of ATP	76.6	—

* ATPase reaction performed in the dark.

1 or 2 min from the start of the reaction, but do not affect the rate of ATP hydrolysis in the light. In fact, as demonstrated in Table III, complete inhibition of light activation by ADP is observed only if the nucleotide is added at the beginning of the preillumination period. It is evident, therefore, that the effect of preillumination, although readily reversed if the conditions of the assay are shifted to the dark, cannot be suppressed by addition of ADP after preillumination.

This effect of ADP should not be confused with competitive inhibition. In fact, with substrate inhibitor concentration ratios such as those used in these experiments (from 10 to 100), a negligibly small degree of inhibition should be expected, if a K_i value for ADP comparable to the K_m for ATP is assumed. These qualitative conclusions were more rigorously tested in kinetic experiments. A Dixon plot for the inhibition of dark ATPase by ADP is shown in Fig. 4: a pure competitive mechanism is observed, similar to that already reported for other phosphorylating systems[1]. The measured K_i for ADP is 0.26 mM. A completely different pattern is observed in the light; under these conditions a markedly non-linear behaviour is evident at low ADP concentrations (10–50 μM), followed by linear competitive inhibition at higher levels. Again the extrapolated K_i value is 0.26 mM (Fig. 5). It is evident, therefore, that in the light a different mechanism is superimposed upon the normal competitive inhibition phenomenon.

Effect of uncouplers

We have already reported that the uncouplers 2,4-dinitrophenol and carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone (FCCP) stimulate dark ATPase of *Rps. capsulata* photosynthetic membranes[19], in a way similar to that previously described for other tightly coupled systems. It was therefore of interest to investigate the relationship existing between light-dependent activation and stimulation by uncouplers. The experiments shown in Table IV demonstrate that FCCP is unable to stimulate further the activity of ATPase in the light. However, if during the pre-

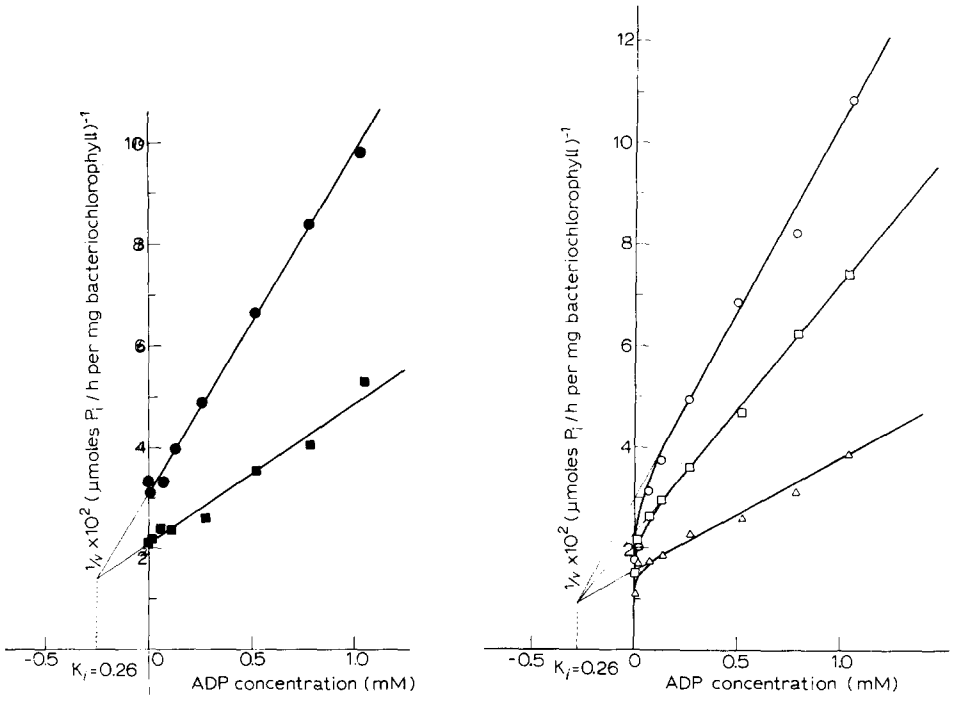

Fig. 4. Linear competitive inhibition of ADP with ATP in ATPase reaction measured in the dark. ■, 0.426 mM ATP; ●, 0.213 mM ATP.

Fig. 5. Biphasic pattern of inhibition by ADP in ATPase reaction, measured in the light. The experiments were performed after 5 min preillumination in the presence of the indicated amounts of ADP. ○, 0.213 mM ATP; □, 0.426 mM ATP; △, 0.852 mM ATP.

TABLE IV

EFFECT OF FCCP AND DINITROPHENOL ON ATPASE ACTIVITY ASSAYED IN THE DARK OR IN THE LIGHT

Additions	*Activity (μmoles P_i/h per mg bacteriochlorophyll)*	
	Dark	*Light*
Expt I		
None	40	98
FCCP ($1 \cdot 10^{-7}$ M)	67.5	93.5
ADP ($5 \cdot 10^{-5}$ M)	43.5	48
ADP ($5 \cdot 10^{-5}$ M) + FCCP ($1 \cdot 10^{-7}$ M)	67.2	67.5
Expt II		
None	44	109
2,4-Dinitrophenol ($2 \cdot 10^{-3}$ M)	70.5	71
Antimycin A ($6 \cdot 10^{-7}$ M)	45.2	46.3
2,4-Dinitrophenol ($2 \cdot 10^{-3}$ M) + antimycin A ($6 \cdot 10^{-7}$ M)	71.3	72
ADP ($5 \cdot 10^{-5}$ M)	—	44
ADP ($5 \cdot 10^{-5}$ M) + 2,4-dinitrophenol ($2 \cdot 10^{-3}$ M)	—	70.5

illumination period ADP is present at a concentration which keeps ATPase at the dark level, FCCP is now able to circumvent the effect of ADP and to stimulate the activity.

Similarly dinitrophenol stimulates ATPase in the dark or in experimental conditions which do not allow light activation (in the presence of antimycin A or ADP). In the light dinitrophenol inhibits ATPase; the rate, however, is always equal to that observed in the dark in the presence of the same amount of this compound. These effects indicate that dinitrophenol, in addition to its uncoupling action on chromatophores, might also be an inhibitor of electron flow and consequently block light activation.

Effect of DCIP

It was reported by Bose and Gest[10], and subsequently by Horiuti *et al.*[11], that oxidized DCIP stimulates dark ATPase in *Rh. rubrum* membranes, the stimulation being reversed by ascorbate. The latter authors suggested that the stimulation reflected a dependency of ATPase activity on the redox state of the membrane. In view of the possibility that the light-dependent activation be related to light-induced changes in the redox conditions of the electron carrier proteins, the effects of DCIP and ascorbate were carefully studied.

The effect of DCIP, oxidized or reduced by ascorbate, on ATPase and photophosphorylation is shown in Fig. 6 and Table V. Oxidized DCIP stimulates ATPase and inhibits phosphorylation in a symmetrical pattern up to a concentration of 10^{-4} M;

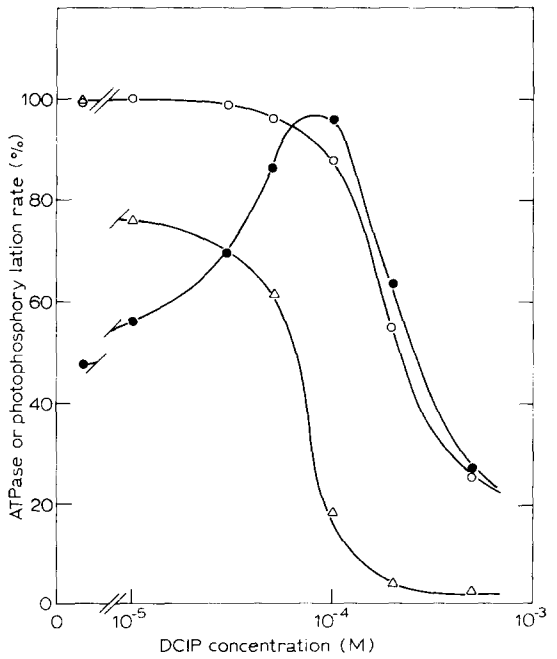

Fig. 6. Rates of photophosphorylation and dark ATPase as a function of the concentration of added oxidized DCIP. 100% activity of ATPase, corresponding to the uncoupled rate in the presence of FCCP ($2 \cdot 10^{-7}$ M), was 93 μmoles P_i hydrolyzed/h per mg bacteriochlorophyll; phosphorylation rate in the absence of oxidized DCIP was 110 μmoles/h per mg bacteriochlorophyll. ○—○, ATPase + FCCP; ●—●, ATPase − FCCP; △—△, photophosphorylation.

at higher concentrations ATPase is inhibited. No additional stimulation is observed in the presence of FCCP. These results are practically coincident with those described by Horio *et al.*[8] in *Rh. rubrum*. However, in partial disagreement with these authors, in our experience ascorbate could not effectively reverse the inhibition of the activity due to excess dye ($>10^{-4}$ M).

It has been demonstrated by several laboratories[20-22] that in spinach chloroplasts oxidized DCIP acts as an uncoupler of photophosphorylation at concentrations higher than 10^{-4} M. The effect of oxidized DCIP on energy conservation of *Rps. capsulata* photosynthetic membranes was directly tested by examining its action on the extent and on the half-time of decay of the carotenoid spectral shift induced by a Xenon flash. As shown in Fig. 7, the decay of the signal is accelerated about 10-fold as a consequence of the addition of 10^{-4} M DCIP; the decay becomes slower again if the dye is reduced with $0.5 \cdot 10^{-3}$ M ascorbate. The extent of the signal is lowered to about 55 % of the original value by the same dye concentration and is restored on

TABLE V

REVERSAL OF STIMULATION OF DARK ATPASE BY DCIP BY ADDITION OF SODIUM ASCORBATE

Additions	*Activity (μmoles P_i/h per mg bacteriochlorophyll)*
None	39.6
DCIP (10^{-4} M)	82.9
DCIP (10^{-4} M) + sodium ascorbate ($1.2 \cdot 10^{-4}$ M)	72.9
DCIP (10^{-4} M) + sodium ascorbate ($2.5 \cdot 10^{-4}$ M)	68
DCIP (10^{-4} M) + sodium ascorbate ($1.5 \cdot 10^{-3}$ M)	52

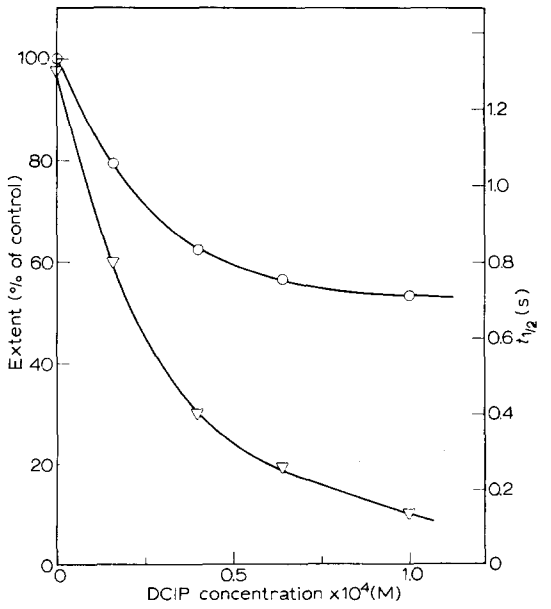

Fig. 7. Effect of oxidized DCIP on the extent (○—○) and the half-time (▽—▽) of decay of the spectral carotenoid shift induced by a Xenon flash (200 μs).

addition of ascorbate. Therefore, little doubt exists that oxidized DCIP acts in this respect as a typical uncoupler[17]. In line with this conclusion also are other observations on the effect of DCIP on light-dependent ATPase: additions of various amounts of oxidized dye do not significantly stimulate the ATPase rate in the light; moreover, as in the case of FCCP and dinitrophenol, DCIP can overcome the inhibition of ADP (Table VI). We were unable to simulate the effect of DCIP with other dyes in the same redox potential span (such as oxidized tetramethyl-*p*-phenylenediamine (TMPD) or ferricyanide). We believe, therefore, that most of the stimulation by DCIP is due to its uncoupling action in the oxidized state and cannot be considered as evidence of a redox dependency of coupled ATPase. PMS, which was reported to affect, in association with ascorbate, *Rh. rubrum* ATPase in a way similar to DCIP, does not interact with *Rps. capsulata* membranes. The results described here do not therefore completely disprove Horio's suggestion for a redox-dependent coupling between ATPase and the electron transport chain, although they severely limit its experimental basis.

TABLE VI

EFFECT OF DCIP ON ATPASE ACTIVITY PERFORMED IN THE DARK OR IN THE LIGHT, IN THE PRESENCE OR IN THE ABSENCE OF ADP

Additions	*Activity (μmoles P_i/h per mg bacteriochlorophyll)*	
	Dark	*Light*
None	48	125
DCIP (10^{-4} M)	92	130
ADP ($5 \cdot 10^{-6}$ M)	—	80
ADP ($5 \cdot 10^{-6}$ M) + DCIP (10^{-4} M)	—	147
ADP (10^{-5} M)	52	58
ADP (10^{-5} M) + DCIP (10^{-4} M)	96.5	136

DISCUSSION

Light-promoted ATPase of *Rps. capsulata* differs in many aspects from the light-triggered Mg^{2+}-ATPase of higher plant chloroplasts[23]. To summarize, bacterial ATPase is strictly dependent upon continuous illumination (it should therefore be defined more properly as "light-dependent" Mg^{2+}-ATPase); it is not stabilized in the dark by ATP or thiol reagents and is not stimulated by uncouplers.

Its linkage with the high-energy state of the membrane is not as evident as in chloroplasts although its absolute dependence upon cyclic electron flow could reflect such a relationship.

The activation by light corresponds to an increase of the apparent V of the reaction. The finding that light-dependent ATPase is more sensitive to oligomycin than the dark activity suggests that the reaction elicited by light could be catalyzed by tightly coupled sites, which, in the dark, cannot interact with water at all; the dark activity should accordingly be related to loosely coupled or totally uncoupled sites. Alternatively, it can be assumed that some ATPase activity of tightly coupled sites is evident also in the dark (and is only partially sensitive to oligomycin) and that light is affecting the turnover number of the enzyme.

Whichever of these two assumptions is correct, the activity observed in the light corresponds to the maximum rate observable in the presence of optimal concentration of uncouplers. This rate can be taken as a measure of the maximum turnover of the enzyme in the absence of any limiting factor, such as inhibitory effects of the high-energy state of the membrane or the back-pressure of the proton motive force. This observation leads also to the conclusion that, in the light, the active site of the enzyme can interact with water more readily than in the dark.

The influence of ADP on ATPase can be separated into two components: a competitive inhibition, characterized by a rather high K_i value and common to dark and light ATPase, and a specific inhibitory effect which is fully effective at concentrations of about 50 μM and which is related only to the light-dependent activity. Only the latter effect takes place, therefore, in a concentration range comparable with the K_m for ADP in photophosphorylation (10^{-5} M). The simplest explanation of the inhibition of ATPase by low concentrations of ADP could be the reincorporation into ATP of hydrolyzed phosphate by photophosphorylation, *i.e.* a net light-dependent ADP–ATP exchange reaction. Such a phenomenon has been well documented in chloroplasts[23].

Although photophosphorylation undoubtedly plays some role at high concentrations of ADP ($5 \cdot 10^{-4}$ M), the mechanism of inhibition of light activation by ADP cannot be explained only in these simple terms. In fact, (a) ADP at μM concentration blocks light activation if present during the preillumination treatment, but fails to be effective when is accumulated as a reaction product of ATPase (Fig. 2); (b) ADP (10^{-5} M) completely inhibits light activation only if added at the beginning of the preillumination and is decreasingly less effective if supplied later; it has no effect when added together with ATP or when the reaction of ATP hydrolysis has already started (see Table II). ADP cannot readily reverse the effect brought about by light which is, however, immediately reversed in the dark and accordingly cannot be accounted for by permanent damage of the membrane caused by illumination.

As a working hypothesis the effect of ADP may be considered as a specific effect on the ATPase enzyme. According to this interpretation the effect of light is visualized as a conformational change induced by the simultaneous exhaustion of endogenous ADP during preillumination (or brought about by the phosphoenolpyruvate–pyruvate kinase system) and by the energization of the membrane by light. On the basis of the effect of the uncouplers it has to be assumed also that the limiting step of ATPase reaction is coincident under light conditions or in the uncoupled state. Although at present we have no direct proof of a conformational change of the coupling factor in *Rps. capsulata*, experimental evidence obtained in other laboratories on spinach chloroplasts supports this concept[16,24,25]. However, further studies are needed in order to establish the relevance of these observations to the phenomena described in this paper.

NOTE ADDED IN PROOF (Received June 29th, 1972)

While this paper was in the press, a publication has appeared by Carmeli and Lifshitz[26], in which experiments very relevant to the present work are described. These authors demonstrated that the decay in the dark of light-triggered ATPase of lettuce chloroplasts is greatly accelerated by ADP (apparent K_m = 1.1 μM) and that

the effect of ADP is inhibited by phosphate or arsenate. ATP also inhibited competitively the effect of ADP with an apparent K_m much lower than that for ATPase activity. The authors proposed that the conformation and activity of chloroplast ATPase is modulated by ADP and phosphate which behave as allosteric effectors. Since their results are largely coincident with those described in the present paper it can be suggested that the phenomena observed could be of general significance and that conformational changes might play a role in the function and (or) regulation of ATPase of photophosphorylating membranes.

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