Biochimica et Biophysica Acta 809 (1985) 57–65
Elsevier

BBA 41813

Transient stimulation of light-triggered ATP hydrolysis by preillumination of chloroplasts in the presence of ATP

Tatiana Shigalowa [a,*], Ute Lehmann [b], Martina Krevet [a] and Heinrich Strotmann [a]

[a] *Botanisches Institut II (Biochemische Pflanzenphysiologie) der Universität Düsseldorf, Universitätsstr. 1, 4000 Düsseldorf, and* [b] *Botanisches Institut der Tierärztlichen Hochschule, Bünteweg 17, 3000 Hannover (F.R.G.)*

(Received March 25th, 1985)

Key words: ATP hydrolysis; Adenine nucleotide binding; Thylakoid membrane permeability; (Spinach chloroplast)

Chloroplasts which were preilluminated in the presence of dithiothreitol and ATP + Mg^{2+} show a 1.5–5-fold higher initial velocity of ATP hydrolysis in the subsequent dark than chloroplasts which were preilluminated in the absence of ATP. The half maximal time of ATP preincubation is about 1 min, the half maximal ATP concentration 15 μM. Nucleotide specificity with regard to the diastereomers of adenosine 5′-(*O*-1-thiotriphosphate) is the same as in nucleotide binding to CF_1 and as the substrate specificity in ATP hydrolysis. ADP abolishes the stimulating effect of ATP if simultaneously present in the light. ATP-stimulated ATP hydrolysis generates a lower transmembrane ΔpH than normal ATP hydrolysis and the relaxation of the flash-induced electrochromic absorption change is accelerated by ATP-preincubation in the light. These results suggest that the ATP pretreatment causes an increase of thylakoid membrane permeability. The target for ATP is most likely the ATPase itself. Dithiothreitol activation in the presence of ATP might induce a reversible dislocation of CF_1 relative to CF_0 resulting in transitory membrane leakage. Reversibility is demonstrated by the finding that the stimulated rate of ATP hydrolysis is normalized after about 1 min. Moreover ATP/P_i exchange which exhibits a lag under these conditions recovers up to the control rate after 1 min.

Introduction

ATP hydrolysis can be induced by preillumination of broken chloroplasts in the presence of thiol compounds ('light-triggered ATP hydrolysis') [1–4]. Chloroplast energization is needed for the transfer of the membrane ATPase from an inactive to a catalytically active state [5–7]. This reaction is accompanied with release of tightly bound ADP from CF_1, and precedes both ATP hydrolysis [8] and ATP synthesis [9–11]. Rebinding of ADP, on the other hand, causes deactivation of the activated enzyme [8,12–14]. Thiol modulation strongly accelerates the hydrolyzing and to some extent also the ATP synthesizing direction, in particular at low protonmotive force [15,16]. Modulation, which is most probably due to reduction of a disulfide bond in γ subunit of CF_1 [16,17], creates a reversible proton-translocating ATPase. Accordingly, a $\Delta\bar{\mu}_H$ is generated by inward proton transport, while ATP is cleaved [18–21].

In intact chloroplasts an active reversible ATPase is induced by illumination in the absence of thiol compounds [22–23]. Their role is probably

* Present address: Lomonossow University, Faculty of Biology, Section Plant Physiology, Moscow, U.S.S.R.

Abbreviations: ATPαS, adenosine 5′-(*O*-1-thiotriphosphate); FCCP, carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone; PMS, phenazine methosulfate; Tricine, *N*-[2-hydroxy,1,1-bis(hydroxymethyl)ethyl]glycine; Chl, chlorophyll.

fulfilled by the endogenous thioredoxin system [23–25]. Thus, the modulated enzyme may be the catalytically active ATPase occurring in vivo, whereas the unmodulated enzyme, which is essentially capable of synthesizing ATP, may be a property of broken chloroplasts only.

The usual method for measurement of ATP hydrolyzing activity of broken chloroplasts includes preillumination for several minutes in the presence of dithiothreitol followed by the addition of ATP in the dark. However, ATP may also be added before the onset of illumination. While a marginal ATP hydrolysis is observed in the light (2–5 μmol/mg Chl per h) the activity increases dramatically after turning off the light.

We have observed that the initial rate of dark ATP hydrolysis under those conditions is much higher than in the cases where ATP is injected together with or briefly after the light/dark transition. This stimulatory effect of ATP preincubation in the light is studied in the present paper.

Methods

Broken chloroplasts from spinach leaves were prepared as in Ref. 26. For measurement of ATP hydrolysis the chloroplasts were incubated at 20°C in a medium containing 25 mM Tricine buffer (pH 8.0)/50 mM NaCl/5 mM $MgCl_2$/10 mM dithiothreitol/50 μM PMS. The reactions were carried out in test tubes which were inserted in a water bath. The chloroplasts were illuminated for 2 min with white light at an intensity of 300 $W \cdot m^{-2}$. (γ-^{32}P)ATP at the indicated concentrations was either present from the beginning or added together with turning off the light. 0.2 ml samples were taken at certain intervals and deproteinized with 0.05 ml 3 M $HClO_4$. In these samples ^{32}P-labeled inorganic phosphate was determined according to Ref. 27. Radioactivity was measured by liquid scintillation counting in 'Ready-solv EP' scintillator (Beckman). (γ-^{32}P)ATP was prepared as described in Ref. 28 and purified as in Ref. 29. In some experiments (8-^{14}C)ATP (Amersham-Buchler) instead of ^{32}P-labeled ATP was used as substrate. In these cases the deproteinized extracts were neutralized by KOH and subjected to TLC on PEI-Cellulose plates [30]. The nucleotide spots were scraped out and radioactivity was counted in 'Unisolve 1' scintillator (Koch-Light Laboratories).

9-Aminoacridine fluorescence was measured in a fluorimeter constructed and kindly made available to us by Dr. E. Weis [31] using a thermostated cuvette (20°C) with magnetic stirring device. Fluorescence was excited at 405 nm (10 $W \cdot m^{-2}$), the red actinic light (filter RG 630, Schott) was 300 $W \cdot m^{-2}$. In addition to the components listed above, the medium contained 5 μM 9-aminoacridine.

Electrochromic absorbance changes were measured as in Ref. 32. These experiments were conducted together with Dr. R. Wagner in the Institute of Biophysics, University of Osnabrück.

Levels of tightly bound nucleotides were measured by the quenching technique described in Ref. 33 and which was combined with the so-called hexokinase accessibility method [34]. The combined method allows to determine also the pattern of the bound nucleotides briefly after the quench. Chloroplasts were incubated in a medium as described above with 10 μM [8-^{14}C]ATP added either before or after preillumination. Quenching was performed by addition of 0.2 ml quench solution to 0.5 ml reaction mixture. The quench solution contained 175 μM FCCP, 17.5 mM ADP, 35 mM glucose and 975 U/ml hexokinase (type F-300, Sigma). 10 s after the quench an aliquot (0.2 ml) was deproteinized with 0.05 ml 3 M $HClO_4$, neutralized and subjected to TLC as above. From the relative radioactivity in the ATP spot, the amount of bound ATP was calculated. The rest of the quenched reaction volume was washed thrice as described [26]. From the radioactivity retained by the membranes (bound ADP + ATP) and the bound ATP the proportion of bound ADP was calculated.

Results

Fig. 1 shows time-course of ATP hydrolysis by preilluminated chloroplasts when ATP was added either at light-dark transition or at the beginning of the light period. In the former case the reaction is linear over 3 min, whereas in the latter a higher initial velocity is followed by a lower rate which corresponds to the one obtained in the control. The transitory stimulation of ATP hydrolysis by preillumination in the presence of ATP is 2.6-fold

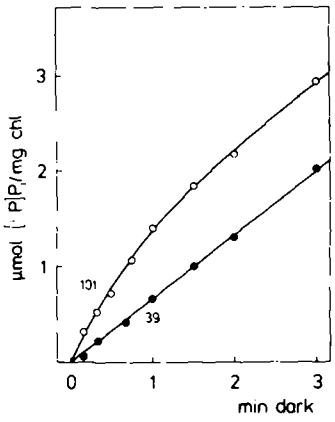

Fig. 1. Kinetics of light-triggered ATP hydrolysis. [^{32}P]ATP (0.5 mM) was added either before illumination (2 min) (○) or together with turning off the light (●). The chlorophyll concentration was 30.0 µg/ml.

in this experiment. In various experiments factors between 1.5 and 5 were observed. Both the ATP-stimulated and the unstimulated reaction are inhibited by phlorizin (not shown), a specific inhibitor of the thylakoid-ATPase [4]. There is no significant difference in the concentrations for half-maximal inhibition (approx. 0.4 mM). Hence the two reactions are catalyzed by the same enzyme.

Fig. 2 shows rates of ATP hydrolysis as a function of ATP concentration when ATP was injected before and 5 and 20 s after preillumination. While no significant difference between the latter two series is observed, V_{max} as well as the apparent K_m is increased when ATP was present during the previous light activation. A similar increase of K_m(ADP) was observed in photophosphorylation when the capacity of the reaction was increased by increasing the light intensity [13] or when chloroplasts were partially uncoupled [51].

In another experiment the time of addition of ATP in the light was varied, while the preillumination time was kept constant (2 min). The initial rate of subsequent dark ATP hydrolysis is plotted as a function of preincubation time in Fig. 3. Maximum acceleration required preincubation with ATP in the range of minutes with a half-time of about 1 min. In this experiment ^{14}C-labeled ATP was used as substrate and the rate of ATP hydrolysis was calculated from decrease of [^{14}C]ATP concentration, determined after TLC separation. Since the commercial [^{14}C]ATP con-

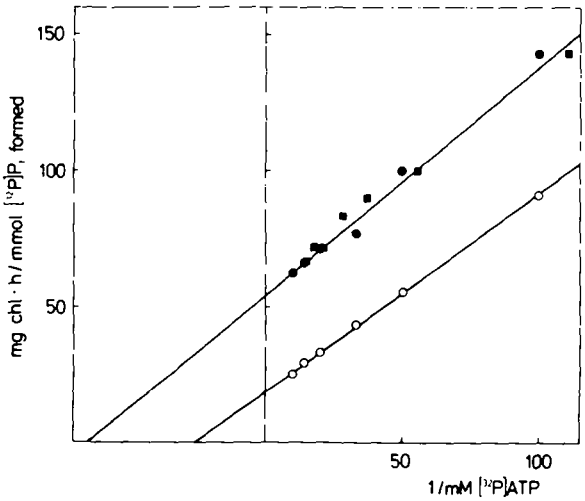

Fig. 2. Light-triggered ATP hydrolysis as a function of ATP concentration. [^{32}P]ATP at varying concentrations was added either before illumination (○), 5 s (●) or 20 s (■) after turning off the light. The chlorophyll concentration was 37.0 µg/ml. The calculated apparent K_m and V_{max} values are 39 µM, 54 µmol per mg Chl per h (○) and 13 µM, 19 µmol per mg Chl per h (●, ■), respectively.

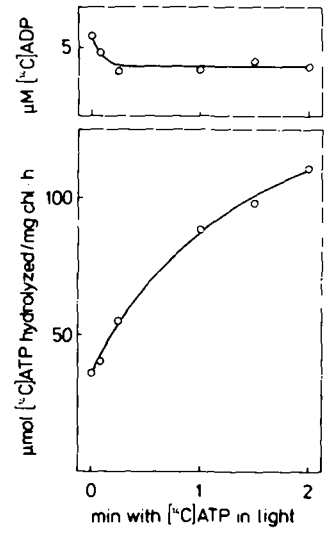

Fig. 3. Rate of light-triggered ATP hydrolysis as a function of preincubation time with ATP in the light. Chloroplasts (26.8 µg chlorophyll/ml) were preilluminated for 2 min. [^{14}C]ATP (51.4 µM) was added at the indicated times before turning off the light. For measurement of initial rates of dark ATP hydrolysis, samples were taken 0, 15 and 30 s after turning off the light. The samples were analyzed for labeled AMP, ADP and ATP by TLC as described in Methods. The upper part of the figure shows actual concentrations of [^{14}C]ADP at the light/dark transition.

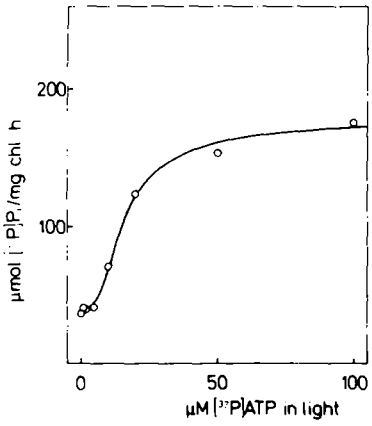

Fig. 4. Rate of light-triggered ATP hydrolysis as a function of ATP concentration during preillumination. [^{32}P]ATP at the indicated concentrations was added before illumination (2 min). Together with turning off the light, labeled ATP was supplemented to 100 µM in each of the samples. Rates of [^{32}P]P_i release were calculated from aliquots taken at 0, 15 and 30 s in the dark. The chlorophyll concentration was 25.2 µg/ml.

tained some contaminating [^{14}C]ADP, the concentration of [^{14}C]ADP and the light/dark transition was also determined. At preincubation times up to 20 s the concentration of [^{14}C]ADP decreased from 6 to 4 µM due to photophosphorylation with endogenous P_i. The phosphate content available to ATP synthesis with excess ADP of our chloroplast preparations was 0.4 µmol per mg Chl.

TABLE I

EFFECT OF Mg^{2+} ON LIGHT-TRIGGERED ATP HYDROLYSIS

For this experiment chloroplasts were isolated in a medium without $MgCl_2$ and washed once in a hypotonic medium containing 1 mM EDTA instead of $MgCl_2$. The assay medium also contained 1 mM EDTA. $MgCl_2$ (5 mM) and [^{32}P]ATP (50 µM) were added at the times indicated. The chlorophyll concentration was 27.5 µ/ml, the illumination time was 2 min. For determination of the rates of ATP hydrolysis, aliquots were taken at 0, 10, 20, 40, 60 s in the dark.

Additions before illumination	Additions after illumination	Rate of dark ATP hydrolysis (µmol [^{32}P]P_i/ mg Chl per h)
ATP	–	0.0
ATP	Mg^{2+}	22.8
ATP, Mg^{2+}	–	58.8
–	ATP, Mg^{2+}	22.2

TABLE II

EFFECT OF THE DIASTEREOMERS OF ATPαS ON LIGHT-TRIGGERED ATP HYDROLYSIS

ATP and the thiophosphate analogs, respectively, at a concentration of 25 µM were added either before or after illumination (2 min). [^{32}P]ATP (50 µM) was always injected together with turning off the light. The chlorophyll concentration was 30.0 µg/ml. Aliquots for determination of the initial rate of ATP hydrolysis were taken after 10, 20, 30, 40 and 60 s in the dark.

Nucleotide	Rate of ATP hydrolysis (µmol [^{32}P]P_i/mg Chl per h)		
	Added before illumination	Added after illumination	Ratio of the rates
ATP	96.0	33.4	2.9
ATPαS, A	40.0	18.4	2.2
ATPαS, B	27.3	32.8	0.8

Between 20 s and 2 min to further change in [^{14}C]ADP concentration was observed, whereas the rate of ATP hydrolysis was still accelerated in this range. The result excludes the possibility that an increase of the ATP/ADP ratio or phosphate potential, respectively, at the light/dark transition might be responsible for the observed stimulation of ATP hydrolysis.

Fig. 4 shows rates of ATP hydrolysis as a function of ATP concentration during preillumination. Here the ATP concentration was supplemented to 100 µM in every sample at the end of the light period. The diagram indicates a sigmoidal concentration curve, half maximum acceleration being attained at about 15 µM ATP. The requirement of both ATP and Mg^{2+} in the light for the observed stimulation is demonstrated by the results of Table.I.

Nucleotide specificity was studied by employing the two diastereomers of ATPαS *. The A form of ATPαS exhibiting S configuration [35] has been found to replace ATP in light-triggered ATP hydrolysis, while the B form was no substrate [36]. Since the B form was only a weak competitive inhibitor in ATP hydrolysis, this isomer or its Mg^{2+} complex, respectively, was concluded not to

* These compounds were kindly supplied to us by Prof. F. Eckstein.

TABLE III

EFFECT OF ADP ON LIGHT-TRIGGERED ATP HYDROLYSIS

ADP and [^{32}P]ATP (each 50 μM) were added either before or after illumination. The chlorophyll concentration was 24.9 μg/ml. Aliquots for determination of initial rates of ATP hydrolysis were taken after 0, 15, 30, 60 and 120 s in the dark.

Addition before illumination	Addition after illmination	Rate of dark ATP hydrolysis (μmol [^{32}P]P_i/mg Chl per h)	% inhibition by ADP
ATP	-	104.4	
ATP, ADP	-	46.2	55.7
-	ATP	51.6	
-	ATP, ADP	32.2	37.6

be recognized by the active site of chloroplast ATPase. Moreover, light-induced tight binding of ADP and ATP by membrane-associated CF_1, was more effectively inhibited in a competitive manner by the A forms of ATPαS and ADPαS than by the corresponding B forms [36]. Likewise stimulation of dark ATP hydrolysis by preillumination of chloroplasts is attained with the A form of ATPαS, but not with the B form (Table II). As a superimposed effect, the A isomer in contrast to the B isomer causes substrate competition, which lowers the activity of ATP hydrolysis in comparison with the control.

Table III shows a considerable decrease of the rate of dark ATP hydrolysis when ADP was present together with ATP in the light. Inhibition of the catalytic reaction is also observed when ADP is added together with ATP after preillumination. The absolute as well as the relative reduction of the rates under these two circumstances (55.7% and 37.6%, respectively), however, indicate a superimposed partial abolition of the light-dependent stimulatory effect of ATP by equimolar concentration of ADP.

ATP hydrolysis in light-triggered, dithiothreitol-modulated chloroplasts is controlled by $\Delta\tilde{\mu}_H$, which is generated by the H^+-translocating ATPase reaction itself, as was convincingly shown by uncoupler studies [4–6]. As a possible explanation of the observed ATP-dependent stimulation, an increase of membrane permeability for protons could be taken into account. Therefore the effect of uncouplers on the stimulated and the unstimulated ATPase reaction was investigated. In Fig. 5 the uncoupler NH_4Cl was employed. The relative acceleration by NH_4Cl is much smaller in ATP-treated than in untreated chloroplasts as is demonstrated by the decrease of the ratio of the rates on increasing the uncoupler concentration.

Transmembrane proton gradients were measured by 9-aminoacridine fluorescence quenching. Although the calculated absolute values are too high compared with other methods [37], 9-aminoacridine traces may give a reliable relative measure of ΔpH [38]. The light-induced ΔpH signal is reverted within about 30 s after turning

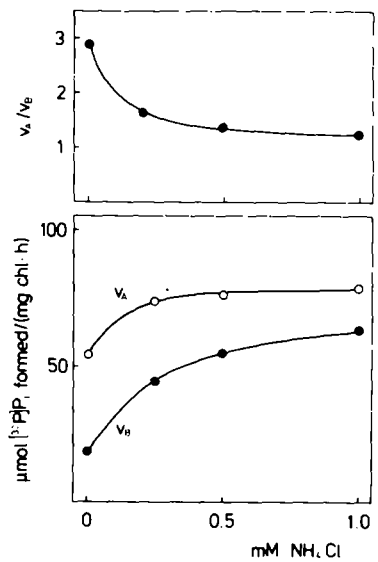

Fig. 5. Light-triggered ATP hydrolysis as affected by the uncoupler NH_4Cl. [^{32}P]ATP (50 μM) was added either before (○) or after illumination (2 min) (●). NH_4Cl at the indicated concentrations was added together with turning off the light. ATP hydrolysis was followed in samples taken after 5, 15, 25, 40 and 60 s in the dark. The given rates represent initial velocities. The chlorophyll concentration was 28.5 μg/ml.

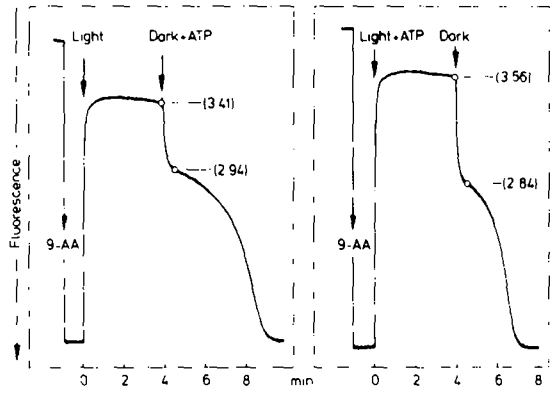

Fig. 6. 9-aminoacridine fluorescence traces indicating changes in light-induced and ATPase-induced transmembrane ΔpH. ATP (50 μM) was added either before illumination or together with turning off the light. The numbers in parenthesis are calculated ΔpH values [38]. The chlorophyll concentration was 25.7 μg/ml.

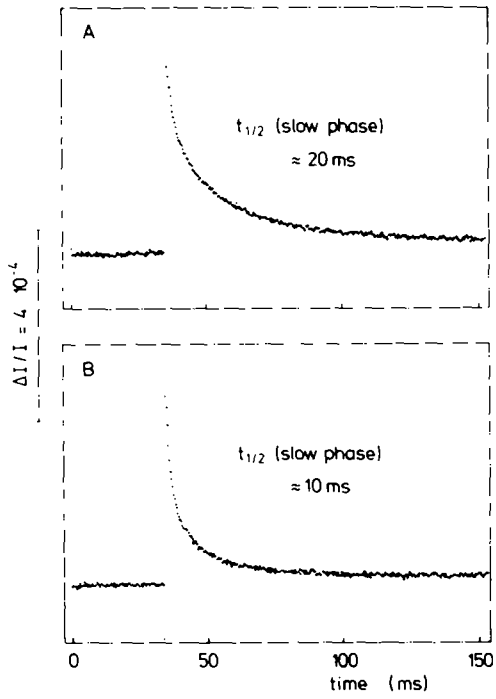

Fig. 7. Flash-induced absorbance changes at 522 nm measured 30 s after preillumination (2 min). In (A) ATP (50 μM) was added together with turning off the light, in (B) ATP was present during preillumination. The signals of 15×100 μs flashes (2 Hz) were sampled. The assay medium contained 50 μM pyocyanine instead of PMS, the chlorophyll concentration was 25 μg/ml.

off the light when no ATP is present in the medium (not shown). When ATP was present during preillumination or added together with turning off the light, a transitory inward proton translocation coupled with ATP hydrolysis takes place in the dark until the substrate ATP is exhausted (Fig. 6). Since the rate of ATP hydrolysis is higher when ATP was present in the previous light, a faster relaxation of ATP-induced ΔpH is observed. On the other hand, no increase, but decrease of the extent of dark ΔpH is evident under these conditions, indicating an apparent slip of proton coupling of the ATPase reaction.

In Fig. 7 the electrochromic absorbance change at 522 nm is compared in chloroplasts which were preilluminated in the absence or presence of ATP. The absorbance change was induced by 100 μs light flashes 30 s after the preillumination period. Preillumination in the presence of dithiothreitol itself accelerated the relaxation of the electrochromic signal compared with unmodulated chloroplasts [32]. However, when ATP was present during the light, the relaxation time was still reduced by a factor of about 2.

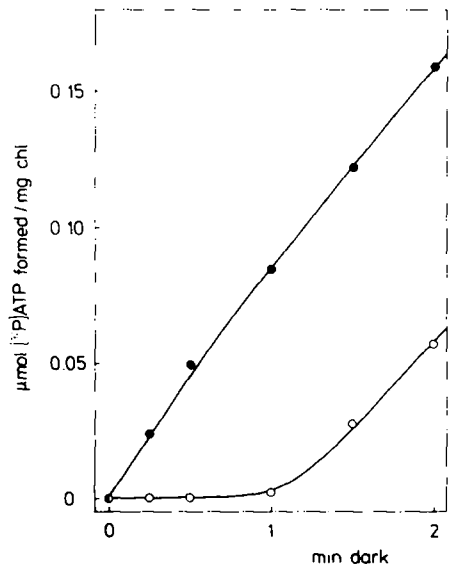

Fig. 8. Kinetics of light-triggered ATP/P_i exchange. ATP (2.5 mM) was added either before (○) or 15 s after preillumination (550 $W \cdot m^{-2}$) (●). In both cases [^{32}P]P_i (5 mM) was provided after 15 s in the dark. The chlorophyll concentration was 25 μg/ml. The deproteinized samples were analyzed for ^{32}P-labeled organic phoshate as in Ref. 27.

TABLE IV

INCORPORATION OF ^{14}C-LABELED ADENINE NUCLEOTIDES UPON ADDITION OF [^{14}C]ATP

[^{14}C]ATP (10 μM) was added either before or after illumination for 2 min at 210 $W \cdot m^{-2}$. At the indicated times in the dark the reaction was quenched and the samples were analyzed as described in Methods. The chlorophyll concentration was 97.2 μg/ml. The percentages are of maximal incorporation (1.60 nmol/mg Chl).

Time in the dark (s)	[^{14}C]ATP added after illumination (nmol [^{14}C]AdN bound/mg Chl)				[^{14}C]ATP added before illumination (nmol [^{14}C]AdN bound/mg Chl)			
	ATP	%	ADP	%	ATP	%	ADP	%
0	0.00	–	0.00	–	0.56	35.0	0.01	0.6
5	0.19	11.9	0.38	23.8	0.59	36.9	0.11	6.9
10	0.25	15.6	0.48	30.0	0.61	38.1	0.19	11.9
60	0.49	30.6	0.68	42.5	0.66	41.3	0.51	31.9

The results of Figs. 5–7 suggest that preillumination in the presence of ATP increases the proton permeability of the thylakoid membrane. This conclusion is supported by measurements of light-triggered ATP/P_i exchange reaction. ATP/P_i exchange is the result of simultaneous ATP hydrolysis and resynthesis of ATP, which is driven by $\Delta\tilde{\mu}_{H^+}$ generated during hydrolysis reaction [39]. ATP/P_i exchange is a quite sensitive measure of coupling, since a slight increase of membrane permeability may cause ΔpH to drop below the energetic threshold for ATP resynthesis. Fig. 8 demonstrates that the rate of ATP/P_i exchange is linear if the substrates ATP and P_i were added after preillumination. However, the reaction shows a lag of about 1 min, if ATP was added before preillumination. The duration of the lag phase corresponds with the phase of stimulation of ATP hydrolysis as shown in Fig. 1.

Activation of ATPase by preillumination of the chloroplasts is related with release of tightly bound ADP [8] from a site which is located in the β subunit of CF_1 [40]. Deactivation of the enzyme, on the other hand, is induced by rebinding of ADP to this site [8,12,14]. The same nucleotide binding site is also able to bind ATP as was demonstrated by competition experiments [36] and localization studies [41]. When ATP instead of ADP is bound, no deactivation of the enzyme occurs [42,43]. In context with these results, levels of tightly bound ADP and ATP were followed at light/dark transitions. When the chloroplasts were preilluminated in the absence of medium nucleotides, endogenous tightly bound nucleotides are released [26], so that the CF_1 molecules are essentially free from bound ADP and ATP. Upon addition of [^{14}C]ATP, incorporation of labeled ATP and ADP takes place (Table IV). After 5 s reaction in the dark, 12% of the sites are containing ATP, 24% have a tightly bound ADP and 64% of the sites are still free. When [^{14}C]ATP was added before illumination, more than 1/3 of the sites are already filled with labeled ATP when the light is turned off, but no labeled ADP is present. After 5 s in the dark the ratio of ATP-containing sites to ADP-containing sites to free sites is 37 : 7 : 56 under these conditions. However, with continuing dark time the differences in the patterns of bound nucleotides progressively disappear.

Discussion

Transitory stimulation of dark ATP hydrolysis following preillumination in the presence of ATP may be attributed to partial release of the ATPase reaction from its control by the transmembrane proton motive force, as was demonstrated by measurements of the chemical (Fig. 6) and the electrical potential difference (Fig. 7), the action of the uncoupler ammonium chloride (Fig. 5) and the kinetics of ATP/P_i exchange (Fig. 8). Stimulation requires ATP pretreatment with a half-time of about 1 min. The low time constant initially led us to suggest that light-induced phosphorylation of a thylakoid membrane protein catalyzed by protein kinase might be responsible for the increase of membrane permeability. The corresponding experiments gave, however, no support for such a

presumption. In accordance with published results [44,45], at least 7 polypeptides were found to incorporate ^{32}P from added γ-^{32}P-labeled ATP, but there was no significant difference between light- and dark-treated chloroplasts under the employed experimental conditions (presence of dithiothreitol). Protein phosphorylation was dependent on illumination only when dithiothreitol was omitted from the medium, i.e., conditions which permit only a slight activation of light-triggered ATPase.

Several results suggest that the ATPase complex itself is the target for ATP in the observed alteration of membrane permeability. Nucleotide specificity with regard to the two diastereomers of ATPαS is the same as for ATP hydrolysis and nucleotide binding to membrane-associated CF_1. ADP, which effectively competes with ATP in binding to CF_1 [36,42,43], abolishes ATP-induced stimulation of ATP hydrolysis, and the half maximal concentration of ATP required for stimulation is in the micromolar range as for binding to CF_1. Binding studies directly demonstrated that tightly bound ATP is present on membrane if illuminated together with ATP (Table IV). It is known that those bound nucleotides are exclusively related with the CF_1 sector of the thylakoid-ATPase [26,52].

Dithiothreitol modulation results in reduction of a disulfide bond in γ-subunit of isolated [46,47] or membrane-bound CF_1 [16,17]. In chloroplasts an energy-dependent conformational change of the enzyme greatly enhances the accessibility of this bond to dithiothreitol [48]. In energized membranes the sensitive group in γ-subunit is modified by *N*-ethylmaleimide [16,49], but this modification is prevented by ATP (or ADP + P_i [49,50], indicating a change of the position of the sensitive group in γ-subunit induced by nucleotide binding on β-subunit. Although there is no direct proof by this study, it seems possible that dithiothreitol treatment in the presence of ATP might result in a conformation of the ATPase complex which is different from the normal modulated state. One may imagine that such a treatment causes a slight dislocation of CF_1 relative to CF_0, so that a proton leak or bypass is opened.

An important feature of this kind of uncoupling is reversibility, which was demonstrated by the normalization of the rate of ATP hydrolysis after 1 min and the recovery of ATP/P_i exchange after passing through an initial lag of 1 min. Restoration of coupling matches with an accomodation of the pattern of bound nucleotides to the one observed in the control.

Acknowledgements

T.S. was recipient of a scholarship from the Deutsche Akademische Austauschdienst. The work was supported by Deutsche Forschungsgemeinschaft. The authors are indebted to Dr. E. Weis (Düsseldorf) and Dr. R. Wagner (Osnabrück) for their advice and help in some experiments and to Mrs. Karin Kiefer for here excellent technical assistance.

References

1 Petrack, B. and Lipmann, F. (1961) Symposium on Light and Life (McElroy, W.D. and Glass, B., eds.), pp. 621–630, The John Hopkins Press, Baltimore, MD
2 Carmeli, C. and Avron, M. (1967) Eur. J. Biochem. 2, 318–326
3 McCarty, R.E. and Racker, E. (1968) J. Biol. Chem. 243, 129–137
4 Carmeli, C. (1961) Biochim. Biophys. Acta 189, 256–266
5 Bakker-Grunwald, T. and Van Dam, K. (1974) Biochim. Biophys. Acta 347, 290–298
6 Bakker-Grunwald, T. (1974) Biochim. Biophys. Acta 368, 386–392
7 Harris, D.A. and Crofts, A.R. (1978) Biochim. Biophys. Acta 502, 87–112
8 Schumann, J. and Strotmann, H. (1981) in Photosynthesis II. Photosynthetic Electron Transport and Photophosphorylation (Akoyunoglou, G., ed.), pp. 223–230, Balaban International Science Services, Philadelphia, PA
9 Gräber, P., Schlodder, E. and Witt, H.T. (1977) Biochim. Biophys. Acta 461, 426–440
10 Schlodder, E. and Witt, H.T. (1981) Biochim. Biophys. Acta 635, 571–584
11 Strotmann, H. (1984) in Advances in Photosynthesis Research (Sybesma, C., ed.), Vol. II, pp. 477–484, Martinus Nijhoff/Dr. W. Junk Publishers, Dordrecht The Netherlands.
12 Dunham, K. and Selman, B.R. (1981) J. Biol. Chem. 256, 212–218
13 Bickel-Sandkötter, S. and Strotmann, H. (1981) FEBS Lett. 125, 188–192
14 Bar-Zvi, D. and Shavit, N. (1982) Biochim. Biophys. Acta 681, 451–458
15 Mills, J.D. and Mitchell, P. (1982) FEBS Lett. 144, 63–67
16 Ketcham, S.R., Davenport, J.W., Warncke, K. and McCarty, R.E. (1984) J. Biol. Chem. 259, 7286–7293

17 Arana, J. and Vallejos, R. (1982) J. Biol. Chem. 257, 1125–1127
18 Carmeli, C. (1970) FEBS Lett. 7, 297–300
19 Bakker-Grunwald, T. and Van Dam, K. (1973) Biochim Biophys. Acta 292, 808–814
20 Carmeli, C. Lifshitz, Y. and Gepshtein, A. (1975) Biochim. Biophys. Acta 376, 249–258
21 Schreiber, U. and Rienits, K.G. (1982) FEBS Lett. 141, 287–291
22 Mills, J.D. and Mitchell, P. (1982) Biochim. Biophys. Acta 679, 75–83
23 Shahak, Y. (1982) Plant Physiol. 70, 87–91
24 McKinney, D.W., Buchanan, B.B. and Wolusiuk, R.A. (1978) Photochemistrty 17, 794–795
25 Mills, J.D., Mitchell, P. and Schürmann, P. (1980) FEBS Lett. 144, 63–67
26 Strotmann, H. and Bickel-Sandkötter, S. (1977) Biochim. Biophys. Acta 460, 126–135
27 Avron, M. (1960) Biochim. Biophys. Acta 40, 257–272
28 Avron, M. (1961) Anal. Biochem. 2, 535–543
29 Magnusson, R.P., Portis, A.R. and McCarty, R.E. (1976) Anal. Biochem. 72, 653–657
30 Goody, R.S. and Eckstein, F. (1971) J. Am. Chem. Soc. 93, 6252–6257
31 Weis, E. (1981) Planta 151, 33–39
32 Junge, W. and Witt, H.T. (1968) Z. Naturforsch. 23b, 244–254
33 Strotmann, H., Bickel-Sandkötter, S. and Shoshan, V. (1979) FEBS Lett. 101, 316–320
34 Rosen, G., Gresser, M., Vinkler, C. and Boyer, P.D. (1979) J. Biol. Chem. 254, 10654–10661
35 Eckstein, F. (1979) Acc. Chem. Res. 12, 204–210
36 Strotmann, H., Bickel-Sandkötter, S., Edelmann, K., Eckstein, F., Schlimme, E., Boos, K.S. and Lüstorff, J. (1979) Biochim. Biophys. Acta 545, 122–130
37 McCarty, R.E. (1976) in Encyclopedia of Plant Physiology (Stocking, C.R. and Heber, U., eds.), Vol. III, pp. 347–376, Springer, Berlin
38 Schuldiner, S., Rottenberg, H. and Avron, M. (1972) Eur. J. Biochem. 25, 64–70
39 Davenport, J.W. and McCarty, R.E. (1981) J. Biol. Chem. 256, 8947–8954
40 Czarnecki, J.J., Abbott, M.S. and Selman, B.R. (1982) Proc. Natl. Acad. Sci. USA 79, 7744–7748
41 Abbott, M.S., Czarnecki, J.J. and Selman, B.R. (1984) J. Biol. Chem. 259, 12271–12278
42 Schumann, J. (1981 in Energy Coupling in Photosynthesis (Selman, B.R. and Selman-Reimer, S., eds.), pp. 223–230, Elsevier/North-Holland, Amsterdam
43 Bar-Zvi, D. and Shavit, N. (1982) J. Bioenerg. Biomembr. 14, 467–478
44 Horton, P. (1983) FEBS Lett. 152, 47–52
45 Bennett, J. (1983) Biochem. J. 212, 1–13
46 Nalin, C.M. and McCarty, R.E. (1984) J. Biol. Chem. 259, 7275–7280
47 Moroney, J.V., Fullmer, C.S. and McCarty, R.E. (1984) J. Biol. Chem. 259, 7281–7285
48 Moroney, J.V. and McCarty, R.E. (1982) J. Biol. Chem. 257, 5915–5920
49 McCarty, R.E. and Fagan, J. (1972) Biochemistry 12, 1503–1507
50 McCarty, R.E., Portis, A.R. and Magnusson, R.P. (1974) Proceedings of the IIIrd International Congress on Photosynthesis (Avron, M., ed.), pp. 975–984, Elsevier, Amsterdam
51 Vinkler, C. (1981) Biochem. Biophys. Res. Commun. 99, 1095–1000
52 Harris, D.A. and Slater, E.C. (1975) Biochim. Biophys. Acta 387, 335–348