Biochimica et Biophysica Acta, 638 (1981) 305–312
Elsevier/North-Holland Biomedical Press

BBA 48179

ACTIVATION-DEACTIVATION REACTIONS IN THE ATPase ENZYME IN *RHODOSPIRILLUM RUBRUM* CHROMATOPHORES

LUIT SLOOTEN and ADRIAAN NUYTEN

Vrije Universiteit Brussel, Faculty of Sciences, Laboratory of Biophysics, Pleinlaan 2, 1050 Brussels (Belgium)

(Received April 3rd, 1981)

Key words: ATPase activation; Proton gradient; Mg^{2+} effect; Chromatophore; Bacterial photosynthesis; (R. rubrum)

(1) The ATPase enzyme in untreated chromatophores from *Rhodospirillum rubrum* is in a low-activity state (designated as E^o). It can be activated by application of a transmembrane $\Delta\tilde{\mu}_{H^+}$ generated by light-induced electron transport, or by application of acid-base jumps. (2) After rapid dissipation of the light-induced $\Delta\tilde{\mu}_{H^+}$, the active state of the ATPase enzyme decays (in the absence of added substrates or products) to a low-activity state (designated as E'), with a half-time of the order of 2–4 min. This state differs from E^o in that E' (but not E^o) can be rapidly reactivated by addition of substrate, but only when the Mg^{2+} concentration is kept below 20–30 μM. Since this is characteristic of an activated enzyme containing tightly bound ADP (Slooten, L. and Nuyten, A. (1981) Biochim. Biophys. Acta 638, 313–326), it is suggested that release of endogenous, tightly bound ADP is one of the factors involved in activation of the ATPase enzyme.

Introduction

Activation phenomena in the onset of ATP hydrolysis and ATP synthesis have been studied in some detail in mitochondria and chloroplasts. In submitochondrial particles, activation of ATP synthesis [1] as well as ATP hydrolysis [2] was attributed to an energy-dependent displacement of an ATPase-inhibitor protein. Membrane energization also results in exchange of nucleotides bound at a tight binding site (or sites) of the ATPase enzyme [3]. In chloroplasts, also, activation of phosphorylation was ascribed to energy-dependent conformational changes leading to displacement of an endogenous ATPase-inhibitor protein [4], and exchange of tightly bound nucleotides [4,5]. Similarly, activation of ATPase in chloroplasts (after treatment with dithiol reagents to inactivate the endogenous ATPase-inhibitor protein [6,7]) was associated with release of tightly bound nucleotides [8], and deactivation (in the course of hydrolysis) with reappearance of tightly bound ADP [9]. Very low concentrations of ADP added before the onset of hydrolysis caused a severe inhibition of the reaction [10].

Relatively little is known about activation processes in photosynthetic bacteria. Light activation of ATPase was observed in *Rhodopseudomonas capsulata* chromatophores. One of the factors involved in light activation was depletion of endogenous ADP [11]. However, an experimental difficulty with this organism is that the active state of the ATPase enzyme is light dependent, not just light triggered [11]. This is in contrast to the situation in *Rhodospirillum rubrum* chromatophores. Here, the active state of the ATPase enzyme, once generated, is maintained for some time during a subsequent dark period in the presence of MgATP [12,13], as well as in the absence of substrates. The latter point is described in the present article, which deals with the mechanism of activation and deactivation of the ATPase enzyme in the absence of substrates.

Abbreviations: CCCP, carbonyl cyanide *m*-chlorophenylhydrazone; Mes, 4-morpholineethanesulfonic acid; SDS, sodium dodecyl sulfate; BChl, bacteriochlorophyll.

Methods

R. rubrum, strain S1, was grown as described previously [14]. The cells were broken by sonication [15]. The media used for sonication, and for washing and storage of the chromatophores [14] were modified in that glycylglycine and KCl were present throughout at 20 and 50 mM, respectively; the pH was adjusted to 8.0 with NaOH.

Where indicated, 'choline chloride chromatophores' were used. These were prepared in the same way, except that KCl was replaced by 30 mM choline chloride.

Rates of ATPase were estimated by colorimetric determination of P_i. The assay medium contained (unless otherwise indicated) 0.2 M sucrose, 50 mM KCl, 0.1 mM EDTA, 20 mM glycylglycine and NaOH to pH 8.0. The reaction volume was 2 ml and the bacteriochlorophyll concentration, 19–25 μM. The reaction was performed in a stirred spectrophotometer cuvette, thermostatically maintained at 20°C, and was stopped after 2 min by addition of 1 ml phosphate reagent. The reagent was as described earlier [16] except that it contained, in addition, 3.5 M SDS (British Drug House, specially pure). The detergent prevents precipitation of proteins and eliminates the need for trichloroacetic acid precipitation step [17]. After 2 min of color development, the extinction was read in a cuvette with a 3 cm path length, at 785 nm. This was an isosbestic point for a transient absorbance change due to bacteriochlorophyll degradation.

Alternatively, ATPase was measured with a glass electrode [18] at room temperature and under a nitrogen atmosphere in order to prevent 'drift' due to CO_2 uptake. In this case, the glycylglycine concentration in the above medium was lowered to 0.2–0.6 mM, depending on the sensitivity required, and 20 mM NaCl was added instead. The reaction volume was 5 ml and the bacteriochlorophyll concentration 13 μM. Addition of $MgCl_2$ and/or ATP in the course of a pH measurement sometimes caused extensive baseline transients. The duration of these transients was shortened (a) by the inclusion of EDTA in the assay medium, and (b) by the use of a stock solution consisting of equal amounts of glycine and $MgCl_2$, buffered at pH 8.5–9.0 as required. Blank experiments were routinely included. In the blanks the chromatophores were replaced by an equivalent amount of buffer, since the response time of the electrode depends upon the buffering capacity of the medium. The time resolution of the technique is illustrated in Fig. 1. (The details of this experiment will be discussed elsewhere.)

In both setups, actinic light was provided by a 100 W tungsten-iodine lamp, and was focused onto the cuvette after passing through 2 cm of water and through a Balzer interference filter with maximum transmission at 800 nm. The ATPase reaction rate itself was measured in the dark.

Bacteriochlorophyll was estimated using an in vivo

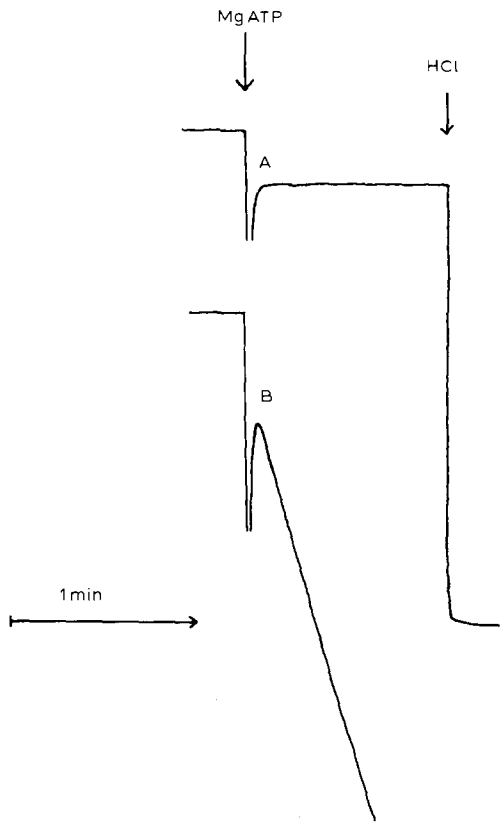

Fig. 1. Recorder tracings of ATP hydrolysis as measured with the pH technique. Trace A, no chromatophores; trace B, chromatophores present. MgATP (traces A and B) and 90 nmol HCl (trace A) were added at the indicated times. The buffer capacity was the same in traces A and B. In trace B, MgATP was added 25 s after a light trigger (duration 15 s) given in the presence of nigericin. CCCP was added just after the light trigger. Other details as in Fig. 5A.

extinction coefficient of 140 $mM^{-1} \cdot cm^{-1}$ [19]. The Mg^{2+} concentrations were calculated using the pK_a values for ATP and the stability constants for the Mg^{2+} complexes of ATP as given by Phillips et al. [20], corrected for an ionic strength of 75 mM. The stability constants for the K^+ and Na^+ complexes of ATP were taken from Ref. 21. From these data, an overall stability constant of $20.2 \cdot 10^3$ M^{-1} for Mg^{2+} complexation with ATP was calculated. The conditional stability constant for the Mg-EDTA complex, $2.66 \cdot 10^6$ M^{-1}, was calculated from Ref. 22.

Nigericin was a gift from Eli Lilly Laboratories, Indianapolis. Valinomycin, oligomycin, ADP and ATP were from Sigma. All other reagents were analytical grade.

Results

Fig. 2 shows experiments in which ATP hydrolysis in the dark was started by simultaneous addition of ATP, $MgCl_2$ and CCCP. A light trigger was given just before the onset of hydrolysis (open circles), or was omitted (solid circles). Uncoupler stimulation of dark-activated ATPase was much more extensive after than before light activation. This is why most experiments were done in the presence of uncoupler. Light activation was inhibited by addition of uncoupler before, instead of after illumination (e.g., triangle in Fig. 2).

Light activation was saturated within 2 s after the onset of continuous illumination (Fig. 3, open circles). The electron-transport inhibitor, antimycin A, caused a 13-fold reduction in the initial rate of light activation (solid circles). In this respect, activation of ATPase is almost equally sensitive to antimycin A as is the rate of phosphorylation (Ref. 23 and unpublished data). By contrast, the final extent of light activation of the ATPase enzyme was less than 20% inhibited by antimycin A. This is presumably connected with the fact that antimycin A caused only

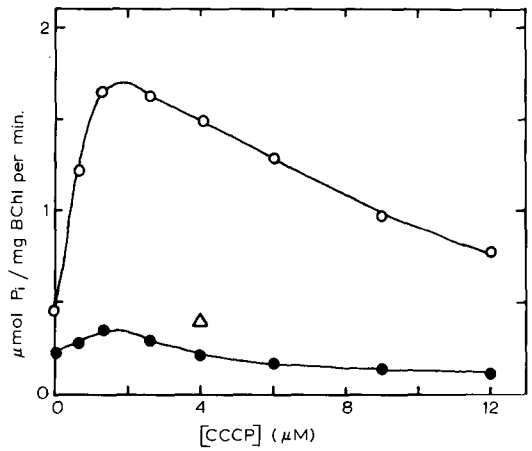

Fig. 2. Uncoupler dependence of ATP hydrolysis in *R. rubrum* chromatophores. The measurements were done with the P_i method. o———o, chromatophores were illuminated for 10 s in 1.3 ml medium (see Methods). Hydrolysis was started just after the switching off of the light by addition of ATP, $MgCl_2$ and CCCP dissolved in 0.7 ml medium. Final concentrations were 0.7 mM ATP, 8.8 mM $MgCl_2$ and CCCP as indicated. •———•, as o———o except that no light was given. △, as o———o, but CCCP was added before, instead of after illumination.

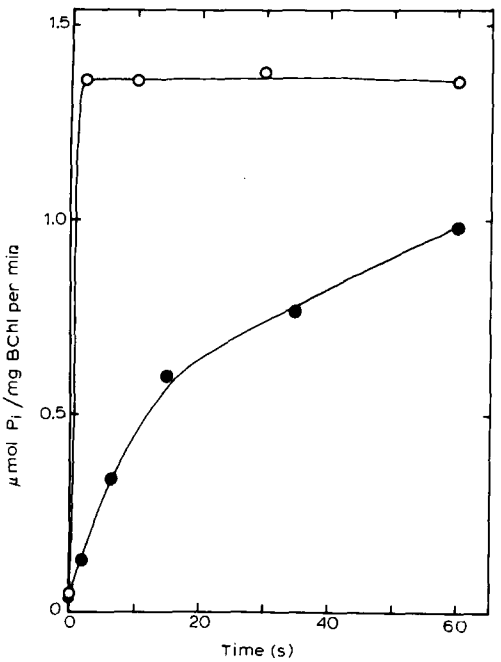

Fig. 3. Kinetics of light activation of the ATPase enzyme in the presence or absence of antimycin (P_i method). o———o, chromatophores were illuminated for the indicated times in 1.3 ml medium. The 800 nm interference filter was replaced by a far-red cut-off filter (Schott RG 715) in these measurements. Hydrolysis was started just after the switching off of the light by addition of 0.7 ml medium containing ATP, $MgCl_2$ and CCCP. Final concentrations were, 1.2 mM ATP, 9.3 mM $MgCl_2$ and 4.2 μM CCP. •———•, as o———o except that 0.3 μM antimycin A was added before lightactivation.

about 50% inhibition in the extent of light-induced proton uptake (not shown), in agreement with earlier data concerning the effect of antimycin A on the steady-state $\Delta\tilde{\mu}_{H^+}$ in continuous light [23].

Activation of ATPase could also be achieved by acid-base jumps (Table I). A pH jump of 3.5 units (from 5.0 to 8.5) caused a significant stimulation of ATPase with respect to the control (Expts. 1 and 2). The effect was saturated after 7 min incubation in the acid stage (not shown). By itself, a K^+-diffusion potential (positive inside) did not cause a significant stimulation of ATPase (Expt. 3). Control experiments gave the same, negative results when the time interval between application of the diffusion potential and the onset of hydrolysis was varied (not shown). However, a K^+-diffusion potential applied in the base stage enhanced the stimulation of ATPase caused by a pH jump (Expt. 4). Under these conditions, acid-base activation was equally effective as light activation (Expt. 6). The enhancement of acid-base activation by K^+ was largely dependent on the presence of valinomycin (Expt. 5).

TABLE I

ACID-BASE TRIGGERED ATPase

The measurements were done with choline cloride chromatophores (see Methods) using the P_i method. Hydrolysis took place in the presence of 1.2 mM ATP, 9.3 mM $MgCl_2$ and 4.2 μM CCCP. The reaction was started by adding these components dissolved in 0.44 ml of a mixture of the same composition as that already present. Errors indicate the range of duplicate measurements. Pretreatment: (expts. 1 and 6) chromators were added to 1.56 ml neutralized mixture, pH 8.5, containing 30 mM choline chloride. Hydrolysis was started 1 min later. In Expt. 6, and 10-s light trigger was given just prior to the onset of hydrolysis. (Expts. 2, 4 and 5) chromatophores were added to 0.56 ml 'acid solution', pH 5.0, containing 30 mM choline chloride. After 10 min the pH was brought to 8.5 by addition of 1.0 ml 'base solution' containing 30 mM of either choline chloride (Expt. 2) or KCl (Expt. 4 and 5). Hydrolysis was started 5 s later. (Expt. 3) chromatophores were added to 0.56 ml neutralized mixture, pH 8.5, containing 30 mM choline chloride. After 1 min, 1.0 ml neutralized mixture containing 30 mM KCl was added. Hydrolysis was started 5 s later. Medium compositions: all solutions contained 0.2 M sucrose, 0.1 mM EDTA, salts as indicated, and were brought to pH with NaOH. In addition acid solution contained 20 mM Mes, base solution contained 20 mM glycylglycine, and neutralized mixture contained 7.2 mM Mes and 13.8 mM glycylglycine. Valinomycin (0.2 nmol) was added just after chromatophores, unless indicated otherwise.

Experiment		ATPase (μmol P_i/mg BChl per min)
No.	Pretreatment	
1	Dark control	0.28 ± 0.03
2	Δ pH	0.46 ± 0.03
3	$\Delta\psi$	0.32 ± 0.01
4	Δ pH + $\Delta\psi$	1.19 ± 0.05
5	Δ pH + $\Delta\psi$ [a]	0.57 ± 0.03
6	Light control	1.16 ± 0.03

[a] Valinomycin omitted.

In the experiments described below, chromatophores were illuminated in the presence of valinomycin (an electrophoretic K^+ carrier [24,25]) or nigericin (a K^+/H^+ antiporter [24,25]); in either case, 4.2–6 μM CCCP (an electrophoretic H^+ carrier) was added immediately after the switching off of the light. As a result, the light-induced transmembrane electrochemical proton gradient ($\Delta\tilde{\mu}_{H^+}$) decayed to zero within 20 s after CCCP addition, as indicated by measurements of light-induced proton uptake, light-induced 9-aminoacridine fluorescence quenching, and light-induced carotenoid absorbance changes (not shown).

Fig. 4 shows the Mg^{2+} dependence of ATP hydrolysis. As described earlier [12], hydrolysis is partly inhibited by high concentrations of $MgCl_2$. At the optimal concentration of $MgCl_2$ the concentration of free Mg^{2+} was 20–30 μM, depending on whether ATP was present at 0.3 or 1.2 mM (the Mg^{2+} concentrations were calculated using the stability constants given in Methods). In experiments where hydrolysis started just after a 10-s light trigger (Fig. 4, open circles), the activity was at least twice that when no light trigger was given (triangles). The solid circles show experiments in which hydrolysis started 3 min after a light trigger; the light-induced $\Delta\tilde{\mu}_{H^+}$ was dissipated immediately after the light trigger by addition of uncoupler, as explained above. The dark interval between the light trigger and the onset of hydrolysis caused a partial inhibition (cf. open circles); however, this inhibition was manifest only when hydrolysis was carried out at inhibitory concentrations of $MgCl_2$. This indicates that the sensitivity of the light-activated enzyme toward Mg^{2+} increases during a dark period between the light trigger and the onset of hydrolysis. (Note that $MgCl_2$ was not present during preincubation.)

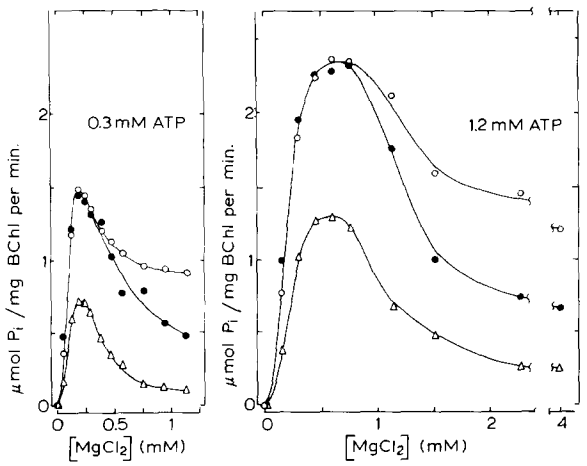

Fig. 4. $MgCl_2$ dependence of ATP hydrolysis catalyzed by nonactivated (△) and light-activated (○, ●) ATPase (P_i method). (○, ●) Chromatophores were illuminated for 10 s in 1.3 ml medium supplemented with 0.2 nmol valinomycin. 0.7 ml medium containing CCCP (final concentration 4.2 μM) was added just after the switching off of the light. The indicated concentrations of ATP and $MgCl_2$ were added along with CCP (○———○), or 3 min after CCCP (●———●). (△) As ○———○ except that no light was given.

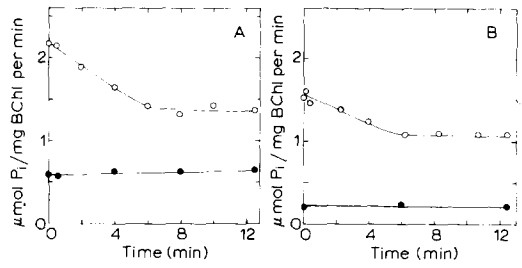

Fig. 5. Kinetics of the dark decay of the light-induced active state of the ATPase enzyme (pH method). ○———○, chromatophores were illuminated for 15 s in 4.5 ml medium in the presence of 0.1 μM nigericin. CCCP (final concentration 6 μM) dissolved in 0.5 ml medium was added just after the switching off of the light. Hydrolysis was started at the indicated time after addition of CCCP by addition of 0.31 mM $MgCl_2$ and 1.2 mM ATP (Fig. 5A), or 1.3 mM $MgCl_2$ and 0.21 mM ATP (Fig. 5B). Rates were determined 9 s later from the slopes of the recorder tracings. Each point is from one experiment. ●———●, as ○———○, except that no light was given.

Experiments with the pH technique (Figs. 5 and 6) provided more insight into the role of Mg^{2+}. Fig. 5 shows initial rates of ATP hydrolysis. The open circles show experiments in which the dark interval between a light trigger and the onset of hydrolysis was varied as indicated. Again, the light-induced $\Delta\tilde{\mu}_{H^+}$ was dissipated immediately after the light trigger (i.e., at t = 0). The data indicate that a slow partial decay of the light-induced active state takes place in the absence of substrates. In five different batches of chromatophores, the half-time of the decay varied between 1.4 and 4.2 min, and the final extent of the inhibition varied between 30 and 65%. Within a given batch of chromatophores, the results obtained during treatment of ATPase with 10 μM Mg^{2+} (Fig. 5A) were similar to those with 1 mM Mg^{2+} (Fig. 5B) (the concentration of MgATP was 0.2 mM in either case).

Fig. 6 shows the kinetics of ATP hydrolysis as observed during the same experiments. In experiments with the light-activated ATPase enzyme, hydrolysis started either immediately after (open circles) or 12.5 min after the light trigger (solid circles). The deactivation caused by the 12.5 min dark interval between the light trigger and the onset of hydrolysis was reversed within about 20 s after addition of substrates when the reaction was measured at 10 μM Mg^{2+} (Fig. 6A, circles). This was not the case

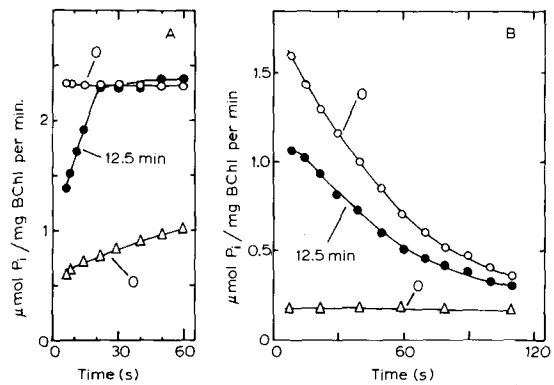

Fig. 6. Mg^{2+} inhibition of substrate-dependent reactivation of the ATPase enzyme (pH method). ○———○, chromatophores were illuminated for 15 s in 4.5 ml medium in the presence of 0.1 μM nigericin. CCCP (6 μM) dissolved in 0.5 ml medium was added just after the switching off of the light. $MgCl_2$ and ATP were added along with CCCP. ●———●, as ○———○ except that $MgCl_2$ and ATP were added 12.5 min after CCCP. △———△, as ○———○ except that no light was given. Concentrations of $MgCl_2$ and ATP in A and B were as in Fig. 5A and B, respectively. Hydrolysis started at t = 0.

when the reaction was measured at 1 mM Mg^{2+} (Fig. 6B, circles). Thus, Mg^{2+} inhibited a substrate-dependent reactivation of the enzyme; in fact, it caused a further deactivation of the enzyme in the course of hydrolysis, in agreement with earlier observations [7,12]. This will be discussed elsewhere [26]. In the presence of 10 μM Mg^{2+}, hydrolysis catalyzed by the light-activated and then dark preincubated enzyme (Fig. 6A, solid circles) accelerated much faster than that catalyzed by the nonactivated enzyme (triangles), suggesting that different processes are involved in these two (re)activation reactions. This means that the light-inactivated enzyme did not return to the 'nonactivated' state during the 12.5 min dark period between the light trigger and the onset of hydrolysis.

In Fig. 7, the dark time between the light trigger and the start of the reaction was again varied, but now CCCP was added at the onset of hydrolysis. The deactivation of the enzyme due to a dark interval after a light trigger could be reversed by a second light trigger given just prior to the onset of hydrolysis (open circles).

Baltscheffski et al. [13] reported that light activation of ATPase in *R. rubrum* chromatophores is dependent on the presence of phosphate. Since they worked with micromolar concentrations of ATP, we repeated some of the above experiments using 1.5–3 μM MgATP. Some results, obtained in the absence of uncoupler, are shown in Fig. 8. Without a light trigger (trace 3), the ATPase activity was about equal to the oligomycin-insensitive ATPase activity (traces 1 and 2). A light trigger caused a period of increased activity (trace 4); a second light trigger, given after the rate of the reaction had slowed down nearly to that of oligomycin-insensitive ATPase, had very little effect. At that time the enzyme was inactivated, for after a second addition of ATP at 3 μM, hydrolysis did not accelerate until a third light trigger had been given. However, the light-induced active state of the enzyme was largely retained in a 4 min dark period between the light trigger and addition of MgATP (trace 5).

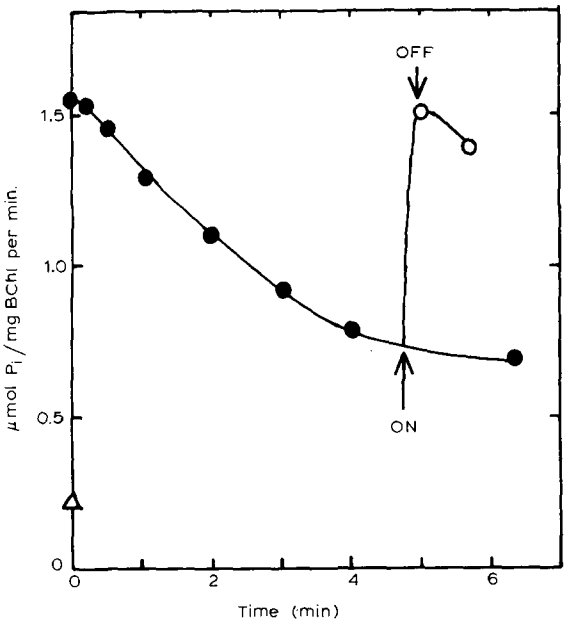

Fig. 7. Energy-dependent reactivation of the ATPase enzyme (P_i method). •———•, chromatophores were illuminated for 10 s in 1.3 ml medium. The light was switched off at $t = 0$. Hydrolysis was started at the indicated times by addition of 0.7 ml medium containing ATP, $MgCl_2$ and CCCP (final concentrations: 1.2 mM, 9.3 mM and 4.2 μM, respectively). Each point is from one experiment. o———o, as •———• except that an additional 10-s light trigger was given during the indicated time.

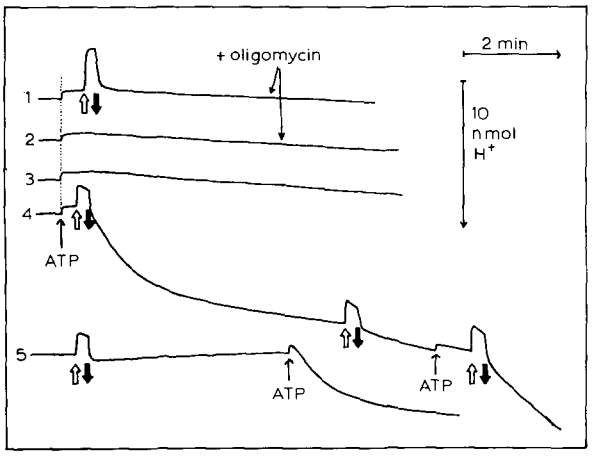

Fig. 8. Light activation of ATPase at low substrate concentrations. (pH method). The medium (Methods) was supplemented with 3 mM $MgCl_2$. Nigericin was present at 0.2 μM. Where indicated, additions of ATP at 3 μM were made. Where indicated, oligomycin was present at 5 μg/ml. The light was switched on and off at the time indicated by the thick upward and downward-pointing arrows, respectively.

Discussion

The results of Fig. 2 indicate that ATP hydrolysis with high substrate concentrations and zero or suboptimal uncoupler concentrations is rate limited at different levels, depending on whether or not the enzyme has been activated. In the light-activated enzyme, the rate-limiting step is the dissipation of the ATPase-induced $\Delta\tilde{\mu}_{H^+}$ (open circles); in the nonactivated enzyme, the rate-limiting step is the turnover capacity of the enzyme. The uncoupler sensitivity of light activation (Fig. 2, triangle) suggests that a transmembrane $\Delta\tilde{\mu}_{H^+}$ is required for activation of ATP hydrolysis. This is confirmed by the effect of acid-base jumps (Table I), and especially by the fact that a K^+-diffusion potential, positive inside, enhanced activation of ATPase by an acid-base jump. The K^+-diffusion potential applied in these experiments was at least 106 mV if we assume that the internal K^+ concentration of the chromatophores (prepared and stored in choline chloride) did not exceed 0.3 mM (cf. Refs. 27 and 28). This potential may have been insufficiently large to activate the enzyme by itself. Alternatively, an additional requirement for activation of ATPase may be an acidic pH of the chromatophore interior.

The light-induced $\Delta\tilde{\mu}_{H^+}$ decayed within 15–20 s in the dark in the presence of CCCP and valinomycin or nigericin (not shown). The decay of the light-induced high-activity state of the ATPase enzyme was much slower under the same conditions (Fig. 5). This indicates that activation of ATP hydrolysis involves an energy-dependent, slowly reversible conformational change (or changes) of the enzyme.

The data pertaining to the destabilization of the light-activated enzyme in the dark (Figs. 4–7) can be explained with the following scheme:

$$E^o \Rightarrow E \leftrightarrows E' \qquad (1)$$

in which E^o and E represent the nonactivated and activated ATPase enzyme, respectively; open arrows indicate energy-dependent reactions. When the membrane is deenergized, E is (in the absence of added ATP, ADP or $MgCl_2$) transformed into the state E′ in a slow reaction ($t_{1/2}$ 1.4–4.2 min; see Fig. 5 and Results). Like E^o, the form E′ exhibits a low activity but unlike E^o, it is (re)activated rapidly after addition of substrate (within 20 s under the conditions used in Fig. 6A). Mg^{2+} at above 20–30 μM inhibits this substrate-dependent reactivation of E′ (Figs. 4 and 6B). E′ can also be transformed back into E by illuminating the chromatophores once more (Fig. 7).

In the accompanying paper [26], evidence is presented suggesting that the transition $E \rightarrow E'$ corresponds with the rebinding of endogenous ADP, liberated in the light, so that the activation and deactivation reactions may be written as:

$$E^o_{ADP} \Rightarrow E + ADP \rightleftarrows E_{ADP} \qquad (2)$$

where E_{ADP} has replaced E′ of Eqn. 1.

The data of Figs. 5 and 6 indicate that after light activation, even prolonged dark periods do not restore the enzyme to the nonactivated conformation (E^o_{ADP} in Eqn. 2). ADP addition after a light trigger is equally ineffective in this respect (see Ref. 26). So the question arises as to how the nonactivated conformation is produced at all. A related question is in what respect E^o differs from E. At present we can only speculate about these questions. Thus, membrane energization may lead to displacement of an endogenous ATPase-inhibitor protein, in analogy with the situation in chloroplasts [4] and mitochondria [1,2]. An endogenous ATPase inhibitor has indeed been isolated from *R. rubrum* chromatophores [29]. In submitochondrial particles it has been shown that rebinding of the ATPase inhibitor, after its energy-dependent release, is specifically dependent on hydrolysis of MgATP (not on the hydrolysis products) [30]. The mitochondrial F_1-ATPase is similarly deactivated slowly in the course of MgATP hydrolysis; again this is not due to product accumulation [31]. We assume, therefore, that an MgATP -dependent deactivation of the ATPase enzyme, resulting in the formation of E^o_{ADP}, occurs in the dark during preparation of the chromatophores, either in whole cells or in the sonicated suspension.

In contrast with the results obtained by Baltscheffski et al. [13], light activation of ATPase did not require phosphate, irrespective of whether hydrolysis was assayed with millimolar or micromolar concentrations of MgATP. Light activation in the experiments of Baltscheffski et al. [13] was expressed as a decrease in K_m (from 5 to 1.3 μM) rather than an increase in V. In our experiments, light activation

does result in an increase in V (the apparent K_m of light-activated ATPase was 30–40 μM, depending on the Mg^{2+} concentration; Slooten, L., unpublished data). It seems likely that Baltscheffski et al. [13] obtained the ATPase enzyme in a form which, by our standards, would be considered activated, and that the events associated with P_i-dependent light activation of the enzyme are different from those studied here.

Acknowledgements

Thanks are due to Professor C. Sybesma for stimulating discussions and critical reading of the manuscript. This research was supported by Grant No. 22.0016.78 from the Belgian Fonds voor Kollektief Fundamenteel Onderzoek (F.K.E.O.).

References

1 Harris, D.A., Von Tscharner, V. and Radda, G.K. (1979) Biochim. Biophys. Acta 548, 72–84
2 Van De Stadt, R.J., De Boer, B.L. and Van Dam, K. (1973) Biochim. Biophys. Acta 292, 338–349
3 Harris, D.A., Radda, G.K. and Slater, E.C. (1977) Biochim. Biophys. Acta 459, 560–572
4 Harris, D.A. and Crofts, A.R. (1978) Biochim. Biophys. Acta 502, 87–102
5 Gräber, P., Schlodder, E. and Witt, H.T. (1977) Biochim. Biophys. Acta 461, 426–440
6 Nelson, N., Nelson, H. and Racker, E. (1972) J. Biol. Chem. 247, 7657–7662
7 Bakker-Grunwald, T. and Van Dam, K. (1974) Biochim. Biophys. Acta 347, 290–298
8 Shoshan, V. and Selman, B.R. (1979) J. Biol. Chem. 254, 8801–8807
9 Bar-Zvi, D. and Shavit, N. (1980) FEBS Lett. 119, 68–72
10 Carmeli, C. and Lifshitz, Y. (1972) Biochim. Biophys. Acta 267, 86–95
11 Melandri, B.A., Baccarini-Melandri, A. and Fabbri, E. (1972) Biochim. Biophys. Acta 275, 383–394
12 Edwards, P.A. and Jackson, J.B. (1976) Eur. J. Biochem. 62, 7–14
13 Baltscheffski, M. and Lundin, A. (1978) in Cation Fluxes across Membranes (Packer, L. and Mukohata, Y., eds.), pp. 209–218, Academic Press, New York
14 Slooten, L. and Branders, C. (1979) Biochim. Biophys. Acta 547, 79–90
15 Slooten, L. and Sybesma, C. (1976) Biochim. Biophys. Acta 449, 565–580
16 Tausski, H. and Shorr, E. (1953) J. Biol. Chem. 202, 675–685
17 Dulley, J.R. (1975) Anal. Biochem. 67, 91–96
18 Nishimura, M., Ito, T. and Chance, B. (1962) Biochim. Biophys. Acta 59, 177–182
19 Clayton, R.K. (1963) in Bacterial Photosynthesis (Gest, H., San Pietro, A. and Vernon, L.P., eds.), pp. 495–500, Antioch Press, Yellow Springs, OH
20 Phillips, R.C., George, P. and Rutman, R.J. (1963) Biochemistry 2, 501–508
21 Melchior, N.C. (1954) J. Biol. Chem. 208, 615–627
22 Skoog, D.A. and West, D.M. (1969) Fundamentals of Analytical Chemistry, 2nd edn., p. 345, Holt, Rinehart and Winston, London
23 Melandri, B.A., Casadio, R. and Baccarini-Melandri, A. (1977) in Proceedings of the 4th International Congress on Photosynthesis (Hall, D.O., Coombs, J. and Goodwin, T.W., eds.), pp. 601–609, The Biochemical Society, London
24 Pressman, B.C., Harris, E.J., Jagger, W.S. and Johnson, J.H. (1967) Proc. Natl. Acad. Sci. U.S.A. 58, 1949–1956
25 Jackson, J.B., Crofts, A.R. and Von Stedingk, L.V. (1968) Eur. J. Biochem. 6, 41–54
26 Slooten, L. and Nuyten, A. (1981) Biochim. Biophys. Acta 638, 313–326
27 Baccarini-Melandri, A., Casadio, R. and Melandri, B.A. (1977) Eur. J. Biochem. 78, 389–402
28 Symons, M., Nuyten, A. and Sybesma, C. (1979) FEBS Lett. 107, 10–14
29 Johansson, B.C. and Baltscheffski, M. (1975) FEBS Lett. 53, 221–224
30 Gomez-Fernandez, J.C. and Harris, D.A. (1978) Biochem. J. 176, 967–975
31 Chernyak, B.V., Chernyak, V.Ya., Gladysheva, T.B., Kozhanova, Z.E. and Kozlov. I.A. (1981) Biochim. Biophys. Acta 635, 552–570