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Modulation of the Chloroplast ATPase by Tight ADP Binding. Effect of Uncouplers and ATP

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Abstract

Inactivation of the membrane-bound ATPase by tight ADP binding was studied under nonenergized conditions. The energy state of the system was controlled either by omitting $MgCl_2$, preventing ATP hydrolysis, or by addition of an uncoupler which dissipates the $\Delta\bar{\mu}_{H^+}$. In the absence of Mg^{2+}, ATP prevents the inactivation of the enzyme by ADP, in a competitive manner. This effect of ATP resembles that of GDP with Mg^{2+} present. In the presence of nigericin, Mg^{2+}, and ATP, inactivation occurs after a 10–15-sec interval, during which the enzyme is able to hydrolyze ATP at a relatively rapid rate. The degree of inactivation is proportional to the level of bound ADP detected. This behavior is different from that of the coupled ATPase (no uncoupler added), where inactivation is attained only upon exhaustion of the ATP by its hydrolysis, despite the finding that ADP binds tightly to the active ATPase at all stages of the reaction. Higher levels of tightly bound ADP were detected in the presence of an uncoupler. We suggest that the interval during which the enzyme becomes inactive is that required for the enzyme to generate and bind ADP, and to change from the active to the inactive conformation. These results support the mechanism suggested previously for the modulation of the ATPase by tight nucleotide binding.

Key Words: Chloroplasts; ATPase; uncoupler; nigericin; tightly bound nucleotides; P_i–ATP exchange; ATP hydrolysis; enzyme modulation; ATP synthetase; tightly bound ADP.

Introduction

The chloroplast ATP synthetase is a latent ATPase which can be activated by energization in the presence of thiol reagents. The activated enzyme catalyzes the hydrolysis of ATP and a P_i–ATP exchange reaction in the dark (see Shavit, 1980). The hydrolysis of ATP is coupled to proton uptake by the thylakoid membranes, which results in the formation of a $\Delta\bar{\mu}_{H^+}$ (Carmeli,

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1970; Bakker-Grunwald and Van Dam, 1973; Davenport and McCarty, 1981). The P_i–ATP exchange reaction is inhibited by uncouplers, present during the activation stage or in the following dark reaction period (Carmeli and Avron, 1967; Rienits, 1967). In contrast, the effect of uncouplers on ATP hydrolysis is more complex. The rate of the ATPase is stimulated by low concentrations of an uncoupler, added either before the activation step or during the reaction itself (Rienits, 1967; Carmeli, 1969). At higher uncoupler concentrations, the initial transient stimulation is followed by a strong inhibition of the ATPase rate.

The chloroplast ATPase contains tightly bound nucleotides. The sites of binding of nucleotides in CF_1 appear to reside in the α and β subunits (Magnusson and McCarty, 1976). Exchange of these nucleotides with medium nucleotides occurs upon energization (Harris and Slater, 1975; Strotmann *et al.,* 1976; Gräber *et al.,* 1977). Several investigators have suggested recently (Bar-Zvi and Shavit, 1980a,b; Schumann and Strotmann, 1980; Dunham and Selman, 1981) that modulation of the ATPase occurs via the binding of ADP to the tight binding site(s) on the ATPase. The conversion of the activated ATPase to its inactive conformation involves conformational changes in the enzyme structure concomitant with the tight binding of ADP. We have also shown that when the active enzyme conformation hydrolyzes ATP it also contains tightly bound ADP (Bar-Zvi and Shavit, 1980b). However, as long as ATP is present, this form of the enzyme does not decay to yield the inactive conformation. The enzyme decays to its inactive form when energy derived from ATP hydrolysis (as $\Delta\bar{\mu}_{H^+}$) is no longer available (Bar-Zvi and Shavit, 1980a).

In the present study, we controlled the energy state of the system by addition of a suitable concentration of an uncoupler or by the presence of Mg^{2+} which is required for hydrolysis but not for the binding of nucleotides. The conversion of the active enzyme conformation to its inactive state was correlated with the tight binding of ADP, in the presence of ATP under nonhydrolytic conditions, either in the absence of Mg^{2+} or in the presence of an uncoupler that rapidly dissipates the $\Delta\bar{\mu}_{H^+}$. These experiments indicate that the conversion of the active enzyme to its inactive form containing tightly bound ADP is primarily controlled by the energetic state of the system. When hydrolysis of ATP is prevented, inactivation appears to be controlled by the ratio of ATP to ADP and their association to the tight nucleotide binding sites on the enzyme.

Materials and Methods

Thylakoid chloroplasts were isolated from fresh market lettuce leaves as described (Bar-Zvi and Shavit, 1980a). Chlorophyll concentration was deter-

mined as described (Arnon, 1949). [2-^{3}H] ADP and [2,8-^{3}H]ATP were purchased from Amersham, and [γ-^{32}P]ATP was synthetized by photophosphorylation and purified as described (Bar-Zvi and Shavit, 1980a). PEI-cellulose[2] sheets were obtained from Macherey-Nagel Co.

ATP Hydrolysis and P_i–ATP exchange

Chloroplasts were activated by illumination for 2 min with a strong light beam (80,000 Lux) filtered through a water layer and constant stirring, in an activation mix of 0.9 ml containing the following components (μmol): tricine (pH 8.0), 20; KCl, 50; $MgCl_2$, 10; dithiothreitol (DTT), 10; phenazine methosulfate, 0.033; and chloroplasts containing 50–120 μg chlorophyll. ATP hydrolysis was initiated by the addition of [γ-^{32}P]ATP (1–3 $\times$ 10^3 cpm/nmol) at the indicated concentrations either with or without an uncoupler, as indicated. Total volume of assay mix was 1 ml. Uncoupler dissolved in methanol was added in a maximal volume of 50 μl (5%). P_i–ATP exchange was initiated by the addition of 0.1 ml of a mix containing ATP and 5 μmol $^{32}P_i$ (2–4 $\times$ 10^6 cpm). Reactions were terminated by the addition of trichloroacetic acid to a final concentration of 3%. The rates of P_i–ATP exchange and ATP hydrolysis, without uncouplers added, were constant during the time periods measured. $^{32}P_i$ released or esterified was determined by the isobutanol–xylene extraction method (Lindberg and Ernster, 1956).

Nucleotide Binding

Binding of nucleotides was done essentially as described (Shavit and Strotmann, 1980). Binding was initiated by addition of the labeled nucleotide (containing 1–2 $\times$ 10^5 cpm/nmol) at the indicated concentration to thrice washed chloroplasts activated as described above, and quenched after the time indicated by addition of 5 mM of unlabeled ATP.

The analysis of nucleotides bound during the hydrolysis of [^{3}H]ATP was done as follows: chloroplast pellets obtained after addition of the quencher were washed three times (Shavit and Strotmann, 1980) and finally resuspended in 20 μl of the washing medium. To 10 μl of this suspension, 5 μl of perchloric acid (12%) was added. After centrifugation for 2 min at 12,000 $\times$ *g*, 5 μl of the supernatant solution were loaded on PEI-cellulose sheets. One microliter of a standard solution containing unlabeled ATP, ADP, and AMP was applied to the same spots. The sheets were dried and then washed in a large volume of distilled water for 15 min. After drying, the sheets were

[2]Abbreviations: CF_1, chloroplast factor 1; CF_0, the membrane portion of chloroplast ATP synthetase; tricine, tris-(hydroxymethyl)methylglycine; CHL, chlorophyll; PEI-cellulose, polyethyeleneimine cellulose.

developed with 0.2 M NH_4HCO_3. Spots containing adenine nucleotides were detected by ultraviolet light and cut out, and nucleotides were extracted by soaking in a solution of 0.7 M $MgCl_2$. One hour later, radioactivity was determined after addition of a scintillation cocktail (toluene–Triton X-100 base).

Results

Activated membrane-bound ATPase, in the presence of Mg^{2+}, will completely hydrolyze the ATP present in the reaction mix. Upon completion of the reaction, the active enzyme–ADP complex undergoes a conformational change to yield the inactive enzyme form which contains ADP, tightly and irreversibly bound. In the absence of Mg^{2+}, no hydrolysis of ATP occurs, and while the enzyme may bind ATP, it remains in its active conformation (Fig. 1). The tight binding of ADP and the inactivation of the ATPase occur also in the absence of Mg^{2+}. Without Mg^{2+}, ATP is able to prevent inactivation of the ATPase by competing with ADP for the tight nucleotide binding site(s). Competition between ATP and ADP for the nucleotide tight binding site(s) on the ATPase is shown in Fig. 2.

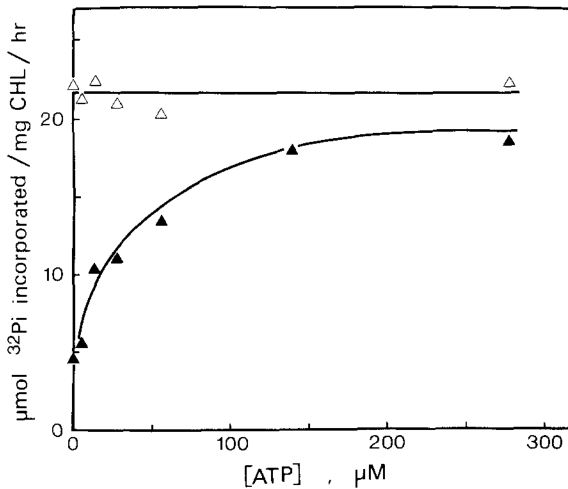

Fig. 1. Effect of ATP on the inhibition of the P_i–ATP exchange by ADP. Chloroplast membranes were activated as described in Materials and Methods, except that $MgCl_2$ was omitted and KCl, 100 mM, and EDTA, 0.2 mM, were added. ATP alone (△) or together with 5.6 μM ADP (▲) were added immediately after activation. $^{32}P_i$, ATP, and $MgCl_2$ (5, 5, and 10 mM, respectively) were added after 1 min. During the incubation of ATP without Mg^{2+}, 0.5–2% of the ATP added was hydrolyzed.

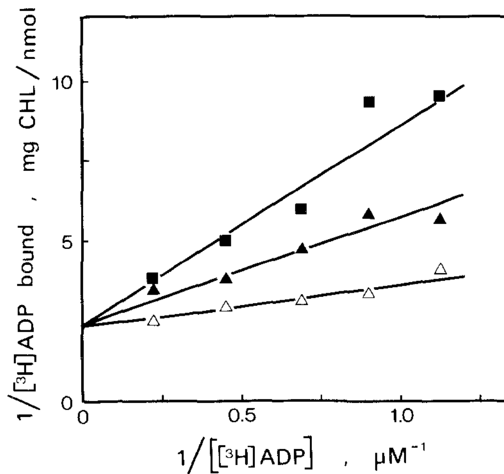

Fig. 2. Inhibition of [^{3}H]ADP binding by ATP. Chloroplast membranes were washed three times with a solution lacking $MgCl_2$, as described in Materials and Methods, and then activated as described in Fig. 1. [^{3}H]ADP, at the indicated concentration, was added alone (△), or together with 14 (▲) or 36 (■) μM ATP, 10 sec after activation. After 1 min in the dark, 5 mM ATP and 10 mM $MgCl_2$ were added.

To study the conversion of the active ATPase to its inactive conformation, in the presence of ATP and Mg^{2+}, we controlled ATP hydrolysis by adding an uncoupler, which rapidly collapses the $\Delta\bar{\mu}_{H^+}$. The time course of ATP hydrolysis in the presence of nigericin is given in Fig. 3. At 200 μM ATP and 1 μM nigericin (Fig. 3A), a concentration high enough to efficiently inhibit ATP formation and P_i–ATP exchange, the initially stimulated rate of the ATPase (see Fig. 4A) is followed by relaxation toward a lower steady-steady velocity. However, at lower ATP concentrations the initially stimulated rates of ATPase (although dependent upon the substrate concentration) are not maintained for more than 10–15 sec. Hydrolysis of ATP stops even though only a fraction of the ATP added was hydrolyzed. This behavior is also observed with 200 μM ATP when the concentration of nigericin is raised (Fig. 3B) and with 5 mM ATP and 0.1 mM nigericin (not shown). Thus, inactivation of the ATPase in the presence of ATP, Mg^{2+} and an uncoupler depends on the concentrations of both the substrate and the uncoupler. As shown in Fig. 4, the order of addition of the uncoupler and ATP does not affect the inactivation pattern of the ATPase. Addition of nigericin after 15 sec, while ATP is being hydrolyzed, induces the transient enhancement in the rate of hydrolysis followed by inactivation of the ATPase (Fig. 4A) in a manner similar to the inactivation pattern observed when nigericin is added together with ATP. Preincubation of the activated enzyme with nigericin for 30 sec

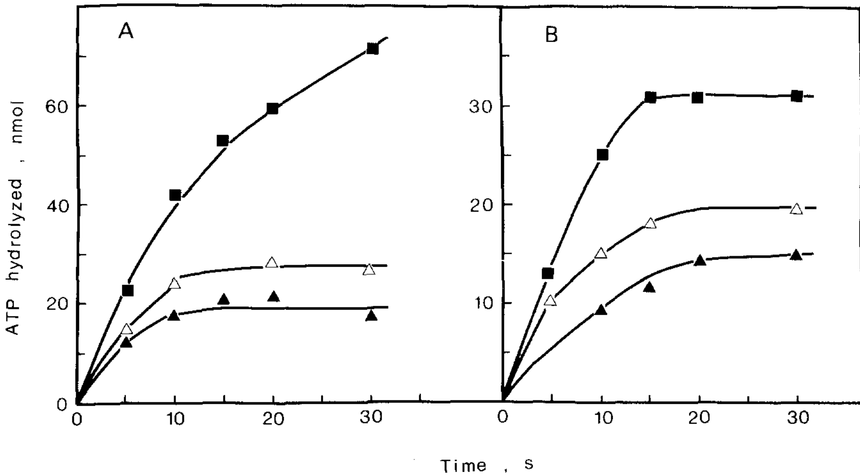

Fig. 3. Effect of ATP and nigericin on the inactivation of the ATPase. [γ-^{32}P]ATP, as indicated, was added to activated chloroplast membranes [(A) 48 and (B) 58 μg chlorophyll] immediately after activation together with 1 (A) and 10 (B) μM nigericin. The reaction was quenched at the time indicated. ATP concentration, μM: (▲) 50; (△) 100; (■) 200.

before addition of ATP does not preclude hydrolysis of ATP for several seconds before inactivation of the enzyme occurs (Fig. 4B). The initial transient enhancement in the rate of the ATPase depends on the concentrations of ATP and uncoupler and was not observed here at 50 μM ATP. Other uncouplers, such as gramicidin D, carbonylcyanide *p*-trifluoromethoxyphe-

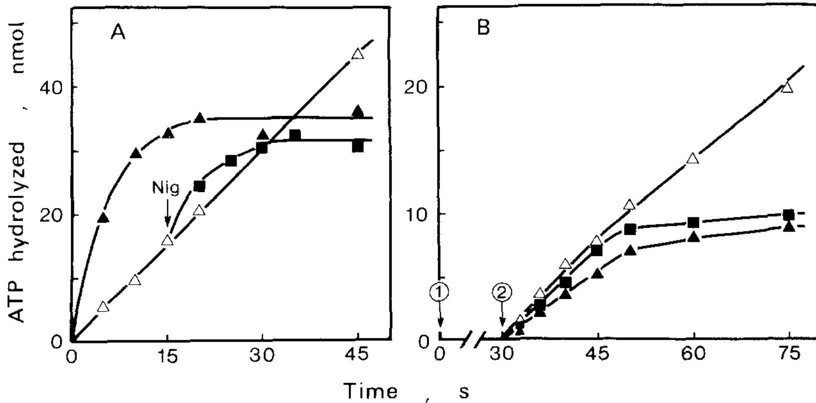

Fig. 4. Inactivation of the ATPase by addition of nigericin at different stages of the reaction. (A) [γ-^{32}P]ATP (200 μM) was added alone (△) or with 10 μM nigericin (Nig) (▲) to activated chloroplasts (54 μg chlorophyll). ■, ATP was added immediately after activation and nigericin 15 sec later. (B) The following additions were made to activated chloroplasts (63 μg chlorophyll) immediately after activation (1) or 30 sec later (2): ■, 10 μM nigericin (1) and 50 μM [γ-^{32}P]ATP (2); △, 20 μl methanol (1) and [γ-^{32}P]ATP (2); ▲, nothing (1) and [γ-^{32}P]ATP + nigericin (2).

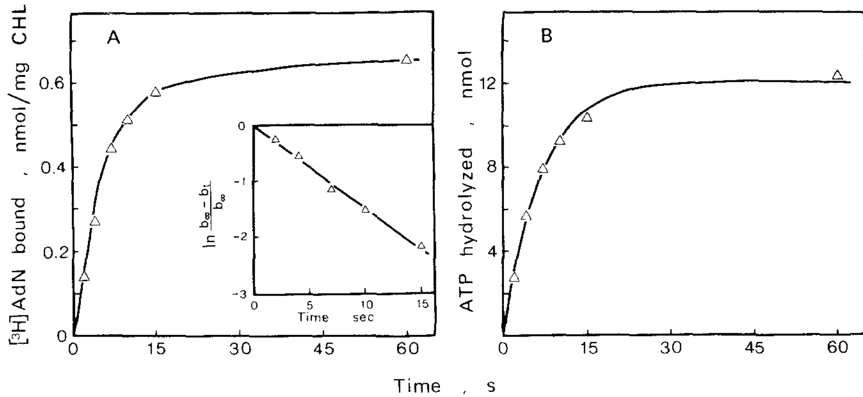

Fig. 5. Tight binding of nucleotides during uncoupled ATP hydrolysis. Nigericin (10 μM) was added to activated chloroplast membranes (69 μg chlorophyll) immediately after activation with 50 μM [^{3}H]ATP (A) or 50 μM [γ-^{32}P]ATP (B). Reactions were quenched after the indicated time. Inset, data plotted as $\ln(b_\infty - b_t)/b_\infty$ versus time. b_∞, maximal level of binding after 60 sec, and b_t, level of binding at time t. AdN, adenine nucleotide.

nylhydrazone (FCCP), and ammonium chloride gave similar results (not shown).

The time course of tight nucleotide binding occurring during the transient phase of uncoupled ATP hydrolysis is given in Fig. 5. Binding is nearly complete after 15 sec and follows first-order kinetics with respect to nucleotide concentration (Fig. 5A and inset). In Fig. 5B the determined values of P_i released by hydrolysis of ATP are shown, as well as a theoretical curve calculated from the determined rate constant for tight nucleotide binding (0.15 sec^{-1}), assuming that the nucleotide-free enzyme is fully active while the enzyme–nucleotide complex is inactive. The curve fits the experimental points best when k_{cat} is assumed to be 40 sec^{-1} (corresponding to a V_{max} of 94 μmol ATP hydrolyzed per milligram chlorophyll per hour). As shown in

Table I. Nucleotides Tightly Bound to the Activated Membrane-Bound ATPase[a]

Additions	Quenching time (sec)	Bound [^{3}H]nucleotides (nmol/mg chlorophyll)			
		AMP	ADP	ATP	Total
None	3	0.001	0.008	0	0.009
	60	0.001	0.196	0.030	0.227
Nigericin, 10 μM	3	0.009	0.202	0.044	0.255
	60	0.019	0.778	0.234	1.031

[a]Chloroplasts containing 106 μg chlorophyll were activated as described in Materials and Methods. [^{3}H]ATP, 35 μM, was added 10 sec after activation with or without nigericin, as indicated. Binding was quenched by the addition of unlabeled ATP, 5 mM. Tightly bound nucleotides were analyzed as described in Materials and Methods.

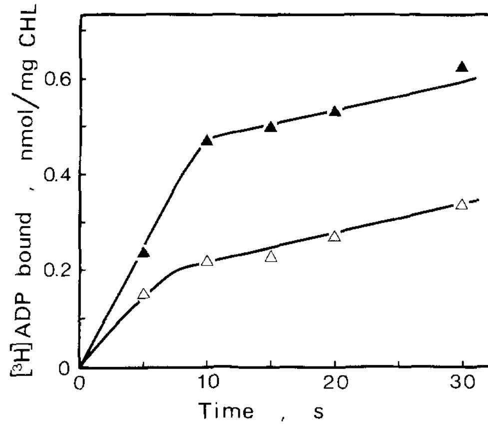

Fig. 6. Effect of nigericin on the time course of [^{3}H]ADP binding. 5.3 μM [^{3}H]ADP was added alone (△) or with 10 μM nigericin (▲) to activated chloroplasts (128 μg chlorophyll) immediately after activation. Binding was quenched at the indicated time by addition of 5 mM ATP.

Table I, during the hydrolysis of [^{3}H]ATP with or without nigericin, most of the nucleotide retained as tightly bound to washed chloroplasts is ADP. The level of bound ADP with uncoupler present is much higher than that without uncoupler. After 60 sec, inactivation of the ATPase occurred both with and without the uncoupler. Without uncoupler, most of the ATP added is hydrolyzed and ADP binds to the tight nucleotide binding site(s). With the uncoupler, only a fraction of the ATP is hydrolyzed, but the level of bound ADP is about four times higher than that obtained after 3 sec. Although the level of bound ATP is about one-third of that of ADP, it is nevertheless higher than that observed without the uncoupler. Binding of [^{3}H] ADP, in the absence of ATP, is shown in Fig. 6. The time course of ADP binding is not affected by the uncoupler, but with nigericin the level of bound ADP was nearly doubled. This high level of bound ADP is also obtained if the order of

Table II. Levels of Bound [^{3}H]ADP upon Addition of the Uncoupler at Different Stages of the Reaction[a]

Nigericin addition	[^{3}H]ADP bound, nmol/mg chlorophyll
—	0.266
With [^{3}H]ADP	0.671
With the quencher	0.712
30 sec after the quencher	0.699

[a] [^{3}H]ADP, 5 nmol, was added to activated chloroplasts containing 75 μg chlorophyll, immediately after activation. Binding was quenched by the addition of 5 μmol ATP, 30 sec later. Nigericin (10 μM) was added as indicated.

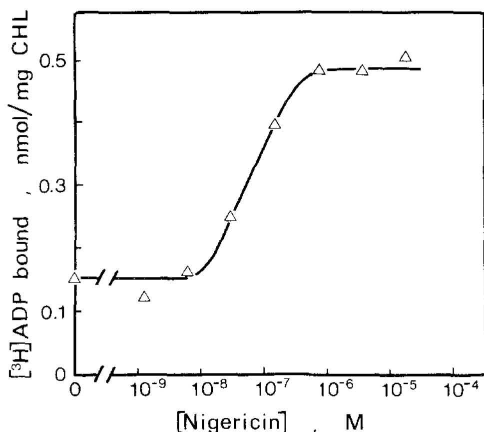

Fig. 7. Effect of uncoupler on the level of tightly bound [^{3}H]ADP. [^{3}H]ADP was added to activated chloroplast membranes (130 μg chlorophyll) 10 sec after activation, together with the indicated concentrations of nigericin. Binding was quenched 30 sec later by the addition of 5 mM unlabeled ATP.

addition of the uncoupler was varied (Table II). Addition of the uncoupler together with [^{3}H]ADP (as in Fig. 6) or together with the unlabeled ATP (quencher) 30 sec after [^{3}H]ADP or 30 sec after addition of the quencher results in the same high level of bound labeled nucleotide. As shown in Fig. 7 the level of bound ADP depends on the concentration of nigericin. Binding is stimulated by nigericin in the concentration range 10^{-8}–10^{-6} M, at which the binding reaches its maximal level. The dissociation of the ligand from the enzyme during the washing of the chloroplast pellets is probably prevented by the uncoupler.

Discussion

In the absence of $\Delta\bar{\mu}_{H^+}$, the inactivation of the ATPase by tight binding of ADP to noncatalytic site(s) is relatively rapid and involves changes in the conformation of the enzyme. However, when the enzyme hydrolyzes ATP, inactivation is observed only after complete hydrolysis of the substrate and collapse of $\Delta\bar{\mu}_{H^+}$, although tight binding of ADP occurs at all stages of the reaction (Bar-Zvi and Shavit, 1980b).

In the absence of Mg^+, when hydrolysis of ATP does not occur, inactivation of the enzyme by ADP binding is prevented by ATP, due to competition with ADP for the tight nucleotide binding site on the ATPase

(Figs. 1 and 2). Thus, without energization resulting from ATP hydrolysis, ATP is able to bind to the tight nucleotide binding site(s) and prevent decay of the enzyme to its inactive conformation.

In the presence of Mg^{2+} and continuous hydrolysis of ATP, the effect of ATP on the inactivation of the enzyme by ADP does not appear to be due to simple competition between the two nucleotides for the nucleotide binding site (but see Carmeli and Lifshitz, 1972). However, ADP was reported to be a competitive inhibitor with ATP during steady-state ATP hydrolysis (Bennun and Avron, 1965; Dunham and Selman, 1981). The ATPase from *R. rubrum* is inhibited by ADP when Mg^{2+} is present and by ATP in its absence (Slooten and Nuyten, 1981). In contrast, the chloroplast ATPase is not inactivated by ATP, either with or without Mg^{2+}. Moreover, even in the absence of Mg^{2+}, ATP prevents inactivation of the ATPase by ADP binding (Figs. 1 and 2). In addition to the formation of the Mg^{2+}–ATP complex, which serves as the substrate in the hydrolytic reaction, Mg^{2+} may also interact directly with the ATPase. Soluble CF_1 contains bound Mg^{2+} which is required for hydrolysis of ATP (Hochman and Carmeli, 1981). The effect of ATP without Mg^{2+} is similar to that observed with GDP and Mg^{2+} (Shavit *et al.*, 1981). Both nucleotides compete with ADP for binding and stabilize the active enzyme conformation. The effect of ATP is not mimicked by its nonhydrolyzable analog, adenylyl imidodiphosphate (AMP-PNP) which inactivates the ATPase (Shavit *et al.*, 1981).

Inactivation of the ATPase in the presence of ATP, Mg^{2+}, and an uncoupler depends both on the ATP and uncoupler concentrations. At high ATP and low uncoupler concentrations the ATPase remains in its active conformation (Fig. 3A), although the uncoupler decreases the $\Delta\bar{\mu}_{H^+}$ created by ATP hydrolysis (Bakker-Grunwald, 1974). At higher uncoupler concentrations most of the ATPase molecules decay to their inactive conformation upon binding of ADP (Figs. 3B and 5) and the decay is not prevented by increasing the ATP to ADP ratio. As in the case of ATP hydrolysis without uncoupler (coupled ATPase), the nucleotide found tightly bound during uncoupled ATPase is mainly ADP (Table I). However, unlike the coupled ATPase, the degree of inactivation is proportional to the level of bound ADP (Fig. 5), indicating that binding of ADP results in the immediate inactivation of the enzyme. Inactivation of the ATPase by added ADP (without ATP) was also correlated with the level of bound ADP (Bar-Zvi and Shavit, 1980a; Schumann and Strotmann, 1980; Dunham and Selman, 1981). Thus, a direct correlation between bound ADP and inactivation of the ATPase is found only under nonenergized conditions, and inactivation must occur after dissipation of the $\Delta\bar{\mu}_{H^+}$. The dissipation of the $\Delta\bar{\mu}_{H^+}$ by an uncoupler is rather rapid (<1 sec), while inactivation is completed only after 10–15 sec. This interval may be necessary for the enzyme to generate and bind ADP and to undergo a change

in conformation yielding the inactive enzyme–ADP complex. This conclusion is also supported by the independence of the initial transient hydrolytic phase (10–15 sec) from the concentrations of ATP and uncoupler and from the order of addition of ATP and uncoupler (Figs. 3 and 4).

The higher levels of bound nucleotide, detected on washed chloroplast membranes after incubation with [^{3}H]ATP or [^{3}H]ADP, in the presence of an uncoupler (Tables I and II), must be due to tighter binding induced by the uncoupler rather than to the exposure of additional binding site(s) on the ATPase. Binding of ADP indeed follows pseudo-first-order kinetics with respect to the nucleotide (Fig. 5), and addition of the uncoupler only changes the level of bound ADP but not the time course of binding (Fig. 6). Also, addition of the uncoupler after the quencher increases the level of bound ADP detected (Table II).

The relatively high uncoupler concentrations required to inactivate the ATPase, as compared to those required to inhibit ATP synthesis and P_i–ATP exchange, may reflect the higher energetic requirement of these latter reactions, where $\Delta\bar{\mu}_{H^+}$ is necessary for both formation of the phosphate anhydride bond and maintenance of the ATPase in its active conformation. To maintain the ATPase in its active conformation, a localized proton transfer, which is inhibited at high uncoupler concentration, may suffice. It is also possible that uncouplers may interact with both the CF_1 and CF_0 components of the ATP synthetase (Shoshan *et al.,* 1980). Our results support the mechanism we have proposed for the modulation of the ATPase by tight nucleotide binding (Bar-Zvi and Shavit, 1980a, 1982).

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References

Arnon, D. I. (1949). *Plant Physiol.* **24,** 1–15.

Bakker-Grunwald, T. (1974). *Biochim. Biophys. Acta* **368,** 386–392.

Bakker-Grunwald, T., and Van Dam, K. (1973). *Biochim. Biophys. Acta* **292,** 808–814.

Bar-Zvi, D., and Shavit, N. (1980a). *FEBS Lett.* **119,** 68–72.

Bar-Zvi, D., and Shavit, N. (1980b). *Proceedings of the 5th International Congress on Photosynthesis* (Akoyunoglou, G., ed.), Vol. 2, Balaban Int. Sci. Serv., Philadelphia, pp. 801–810.

Bar-Zvi, D., and Shavit, N. (1982). *Biochim. Biophys. Acta.* **681,** 451–458.

Bennun, A., and Avron, M. (1965). *Biochim. Biophys. Acta.* **109,** 117–127.

Carmeli, C. (1969). *Biochim. Biophys. Acta* **189,** 256–266.
Carmeli, C. (1970). *FEBS Lett.* **7,** 297–300.
Carmeli, C., and Avron, M. (1967). *Eur. J. Biochem.* **2,** 318–326.
Carmeli, C., and Lifshitz, Y. (1972). *Biochim. Biophys. Acta* **267,** 86–95.
Davenport, J. W., and McCarty, R. E. (1981). *J. Biol. Chem.* **256,** 8947–8956.
Dunham, K. R., and Selman, B. R. (1981). *J. Biol. Chem.* **256,** 212–218.
Gräber, P., Schlodder, E., and Witt, H. T. (1977). *Biochim. Biophys. Acta* **461,** 426–440.
Harris, D. H., and Slater, E. C. (1975). *Biochim. Biophys. Acta* **387,** 335–348.
Hochman, Y., and Carmeli, C. (1981). *Biochemistry* **20,** 6287–6292.
Lindberg, O., and Ernster, L. (1956). *Method. Biochem. Anal.* **3,** 1–24.
Magnusson, R. P., and McCarty, R. E. (1976). *Biochem. Biophys. Res. Commun.* **70,** 1283–1289.
Rienits, K. G. (1967) *Biochim. Biophys. Acta* **143,** 595–605.
Schumann, J., and Strotmann, H. (1980). *Proceedings of the 5th International Congress on Photosynthesis* (Akoyunoglou, G., ed.), Vol. 2, Balaban Int. Sci. Serv., Philadelphia, pp. 881–892.
Shavit, N. (1980). *Ann. Rev. Biochem.* **49,** 111–138.
Shavit, N., Aflalo, C., and Bar-Zvi, D. (1981). In *Energy Coupling in Photosynthesis* (Selman, B. R., and Selman-Reimer, S., eds.), Elsevier/North-Holland, Amsterdam, pp. 197–207.
Shavit, N., and Strotmann, H. (1980). *Methods Enzymol.* **69,** 321–326.
Shoshan, V., Shahak, Y., and Shavit, N. (1980). *Biochim. Biophys. Acta* **591,** 423–433.
Slooten, L., and Nuyten, A. (1981). *Biochim. Biophys. Acta* **633,** 313–326.
Strotmann, H., Bickel, S., and Huchzermeyer, B. (1976). *FEBS Lett.* **61,** 194–198.