Effect of Azide on the ATPase Activity of Isolated CF_1

Ignat B. Minkov* and Heinrich Strotmann

Institut für Biochemie der Pflanzen, Heinrich Heine Universität Düsseldorf, Universitätsstraße 1, D-4000 Düsseldorf 1, Bundesrepublik Deutschland

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The effect of azide on Mg^{2+}- and Ca^{2+}-dependent ATPase of differently activated CF_1 isolated from spinach chloroplasts was studied. It is shown that Mg^{2+}-ATPase activity is sensitive towards azide irrespective of the applied method of enzyme activation. Heat- or trypsin-activated Ca^{2+}-ATPase is also inhibited, whereas methanol- and octylglucoside-stimulated or DTT-activated Ca^{2+}-ATPase is not affected by azide. Preincubation of the DTT-activated enzyme with low concentrations of Mg^{2+} induces azide susceptibility of the Ca^{2+}-dependent ATPase.

Introduction

In energy transducing membranes (plasma membrane of bacteria, inner membrane of mitochondria and thylakoid membrane of chloroplasts) the utilization of a transmembrane electrochemical proton gradient for phosphorylation of ADP is catalyzed by a reversible proton translocating ATPase (F_0F_1). Although the structure and function of this protein complex have been extensively investigated, the enzymatic mechanism and regulation of the enzyme are still a matter of debate [1].

Important information has been obtained by employing specific inhibitors which can be subdivided into those interacting with F_0 and those affecting F_1. An ubiquitous F_0 inhibitor is the carbodiimide DCCD [2], a reagent which binds covalently to a specific glutamyl or aspartyl residue, respectively, of the proteolipid [3]. Among chloroplast F_1 inhibitors there are ribose-modified ADP analogues [4], anthraquinone derivatives [5], the naturally occurring chalcone derivative phlorizin [6], the flavonoid quercetin [7, 8], the cyclic tetrapeptide tentoxin [9, 10] and the chemically undefined natural product Dio 9 [11]. These compounds inhibit photophosphorylation, H^+-transport coupled ATP hydrolysis and ATP-P_i exchange of isolated thylakoids as well as ATP hydrolysis catalyzed by isolated CF_1.

Another interesting ATPase inhibitor is azide. The ATP hydrolyzing activity of mitochondrial [12–15] and *E. coli* F_1 [16] was found to be much more sensitive towards azide than the ATP synthesizing activity of the membrane vesicles. Azide does not inhibit photophosphorylation and it is a weak inhibitor of light-triggered ATP hydrolysis and ATP-P_i exchange [17], but it has a very specific effect on the regulation of the ATP hydrolyzing activity of the membrane-associated CF_0CF_1 [18]. Moreover, azide is a strong inhibitor of methanol-activated ATP hydrolysis catalyzed by thylakoids, a reaction which is not associated with proton pumping, and affects the sulfite-stimulated ATPase activity of thylakoids as a competitive inhibitor versus sulfite [19]. Azide inhibits the Mg^{2+}-dependent ATPase of methanol-activated isolated CF_1, but fails to inhibit Ca^{2+}-ATPase activity [17].

The diverse effects of azide on chloroplast ATPase are difficult to fit into a conclusive concept. It seems that the membrane-bound and the isolated CF_1 behave differently towards azide, and that the procedure of activation affects the susceptibility of isolated CF_1 to azide. The latent CF_1 can be activated by a variety of treatments including heat, trypsin, high concentrations of DTT, detergents and alcohols resulting in Ca^{2+}- or Mg^{2+}-dependent ATPase activities [1]. In this study the relationship between azide inhibition and the type of

Abbreviations: CF_0CF_1, chloroplast H^+-translocating ATPase; CF_1, hydrophilic sector of the chloroplast H^+-translocating ATPase ("coupling factor 1"); DTT, dithiothreitol; EDTA, ethylenediaminetetraacetic acid; PEP, phosphoenolpyruvate; Tris, tris(hydroxymethyl)-aminomethane.

* *Permanent address:* Department of Animal and Human Physiology, Sofia University, 8 Dr. Tzankov Boul., Sofia/Bulgaria.

Reprint requests to Prof. H. Strotmann.

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CF_1 activation as well as the role of the divalent metal ion in azide inhibition was investigated.

Materials and Methods

Preparation of CF_1

CF_1 was isolated from spinach chloroplasts as described by Binder *et al.* [20]. The protein was stored as an ammonium sulfate suspension (50% saturation) in a solution containing 50 mM Tris-HCl, pH 8.0, 1 mM ATP and 2 mM EDTA at +4 °C. For activation an aliquot of 1–2 ml was centrifuged. The precipitated protein was dissolved in 0.3 ml 2 mM Tris-HCl, pH 8.0 + 80 μM EDTA and desalted by passage through a Sephadex G-50 column.

Activation procedures

(1) *Heat-activation* was carried out by treatment of CF_1 (0.5–1 mg/ml) for 4 min at 62 °C in a medium containing 30 mM Tris-HCl, pH 8.0, 40 mM ATP, 2 mM EDTA, 10 mM DTT [21].

(2) *DTT-activation* was achieved by incubation of CF_1 (0.5–0.6 mg/ml) for 3 h at room temperature in a medium containing 50 mM Tris-HCl, pH 8.0, 50 mM DTT and 2.5 mM EDTA [22]. For the experiments in which the effect of divalent cations (Mg^{2+} or Ca^{2+}) was studied, the EDTA concentration was reduced to 60 μM.

(3) For *octylglucoside-preactivation* the enzyme (0.5–0.6 mg/ml) was kept for 6 min at 36 °C in a medium which contained 50 mM Tris-HCl, pH 8.0, 0.5 mM EDTA, 2.5 mM ATP and 50 mM octylglucoside [23].

(4) For *trypsin-activation* CF_1 (0.5–0.6 mg protein) was preincubated for 5 min in medium consisting of 50 mM Tris-HCl, pH 8.0, 2 mM or 60 μM EDTA and trypsin [24]. Trypsin activity was stopped by adding soybean trypsin inhibitor. The proportion CF_1 : trypsin : trypsin inhibitor protein was 1 : 2 : 4 by weight.

Measurements of enzyme activity

ATPase activity was measured in a reaction medium containing 50 mM Tris-HCl, pH 8.0, 50 μM EDTA, 1 mM ATP and 1 mM $MgCl_2$ or 1 mM $CaCl_2$. The temperature was 35 °C. Octylglucoside-dependent Mg^{2+}-ATPase activity was measured in standard reaction medium supplemented with 50 mM octylglucoside; octylglucoside-dependent Ca^{2+}-ATPase activity was measured in the presence of 50 mM octylglucoside, 8 mM $CaCl_2$ and 4 mM ATP. For determination of methanol-stimulated ATPase activity [25] the latent CF_1 was incubated in standard reaction medium containing additional 20% (Ca^{2+}-ATPase) or 30% methanol (Mg^{2+}-ATPase), respectively.

Samples of the reaction mixture were taken after different times, deproteinized and analyzed for inorganic phosphate as in ref. [26].

Sulfite-dependent Mg^{2+}-ATPase activity was determinded by spectrophotometric registration of the decrease of NADH concentration at 340 nm in standard reaction medium supplemented with 1 mM PEP, 10 units/ml pyruvatkinase, 5 units/ml lactate dehydrogenase and 0.2 mM NADH.

Heat activated Ca^{2+}-ATPase activity was determined as increase of hydrogen ion concentration measured by a pH glass electrode in unbuffered standard reaction medium [27].

Results

Isolated CF_1 was subjected to different treatments of activation. The characteristics of the ATPase reaction, notably its specificity for Ca^{2+} or Mg^{2+}, are known to depend on the method of activation [1]. Table I compares ATPase activities in the absence and presence of 0.1 mM NaN_3. Irrespective of the procedure of enzyme activation, the resulting Mg^{2+}-dependent ATPase activity is always sensitive towards azide, whereas the Ca^{2+}-ATPase is either inhibited or not affected by azide depending on the type of activation. Heat- and trypsin-activated Ca^{2+}-ATPase is inhibited, while DTT-activated and octylglucoside- or methanol-stimulated Ca^{2+}-ATPase is virtually insensitive towards azide in a concentration range up to 0.1 mM (Fig. 2). Azide inhibition of DTT-activated Ca^{2+}-ATPase found in some experiments (cf. Tables II and III), did not exceed 15% even at concentrations > 0.1 mM.

Azide-sensitive Mg^{2+}- or Ca^{2+}-dependent ATPases show the following common characteristics of inhibition: (1) Azide has no significant effect on the initial rate, but inhibits ATP hydrolysis with progression of the reaction (Fig. 1). The result suggests that either a slow-forming inhibitory complex is generated [28] or that manifestation of

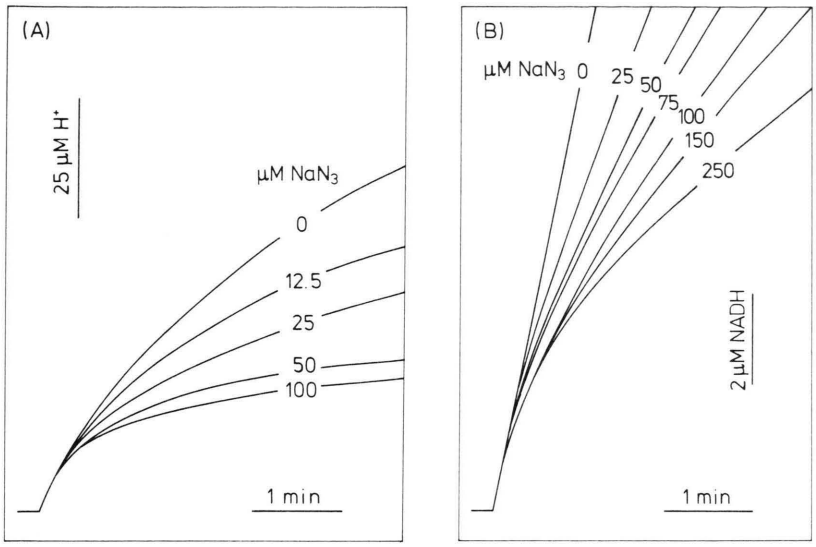

Fig. 1. Influence of azide on ATPase activity of heat-activated CF_1. (A) Ca^{2+}-ATPase activity, measured by a pH electrode; (B) SO_3^{2-}-dependent Mg^{2+}-ATPase activity, measured by NADH consumption in a coupled enzymatic assay. The protein concentration during the reaction was 5.7 μg ml^{-1} (A) and 1.9 μg ml^{-1} (B), respectively.

Table I. Inhibition of differently activated CF_1-ATPase by NaN_3.

Procedure of activation	Cation in reaction medium	Type of activity	ATPase activity (μmol P_i mg protein^{-1} in 5 min) control	+ 0.1 mM NaN_3
DTT	Ca^{2+}		18.9	19.4
Octylglucoside	Ca^{2+}	OG-dependent	10.0	10.7
	Mg^{2+}	OG-dependent	31.0	18.5
No activation	Mg^{2+}	OG-dependent	25.2	5.1
Trypsin	Ca^{2+}		12.7	5.3
No activation	Ca^{2+}	20% MeOH-dependent	7.1	8.2
	Mg^{2+}	30% MeOH-dependent	15.6	9.1
heat	Ca^{2+}		13.8	5.0
	Mg^{2+}	SO_3^{2-}-dependent	5.2[a]	2.7[a]

[a] Given in μmol NADH oxidized mg protein^{-1} in 1 min (measured by coupled enzymatic assay).

Table II. Effect of ADP and Mg^{2+} preincubation on azide inhibition of DTT-activated Ca^{2+}-ATPase.

Preincubation	NaN_3 in reaction medium [μM]	Activity (μmol P_i mg protein^{-1} in 3 min)
–	–	10.6
–	100	9.2
5 μM ADP	–	10.3
5 μM ADP	100	8.8
7.5 μM $MgCl_2$	–	7.8
7.5 μM $MgCl_2$	100	4.8
5 μM ADP + 7.5 μM $MgCl_2$	–	9.2
5 μM ADP + 7.5 μM $MgCl_2$	100	4.9

Table III. Effect of P_i and Mg^{2+} preincubation on azide inhibition of DTT-activated Ca^{2+}-ATPase.

Preincubation	NaN_3 in reaction medium [μM]	Activity (μmol $[^{32}P]P_i$ mg protein^{-1} in 3 min)
–	–	5.9
–	50	5.2
10 μM $MgCl_2$	–	5.8
10 μM $MgCl_2$	50	2.7
1 mM P_i	–	6.2
1 mM P_i	50	6.2
10 μM $MgCl_2$ + 1 mM P_i	–	4.5
10 μM $MgCl_2$ + 1 mM P_i	50	2.8

azide inhibition requires catalytic turnover. The latter explanation is supported by the finding that incubation of the activated enzyme with azide prior to substrate addition does not increase the extent of inhibition (not shown). (2) Inhibition is saturated at azide concentrations between 50 and 100 μM (Fig. 2). (3) Even at saturating azide concentrations no complete inhibition is observed (Fig. 2). Similar characteristics apply to azide inhibition of other F_1-ATPases and were particularly elaborated for F_1 isolated from beef heart mitochondria [13, 28].

The above results suggest a relationship between the procedure of activation, divalent metal ion specificity and azide susceptibility of the ATPase. Photophosphorylation and ATP hydrolysis catalyzed by CF_0CF_1 *in situ* depend on the presence of Mg^{2+}. One role of the divalent metal ion as a cofactor is the formation of ATP- or ADP-metal ion complexes which are the true substrates of the CF_0CF_1-ATPase [29]. In isolated CF_1, the free divalent cation is furthermore an inhibitor of the ATPase competitive with the ATP-metal ion complex. As free Ca^{2+} is less inhibitory than free Mg^{2+}, optimal activity of the trypsin- or heat-activated CF_1 is found with Ca^{2+} as cofactor although Ca-ATP is a weaker substrate than Mg-ATP [30]. The strong inhibitory action of free Mg^{2+} (or Mn^{2+}) is overcome by weak acid anions like bicarbonate, maleate or phosphate, and the affinity for

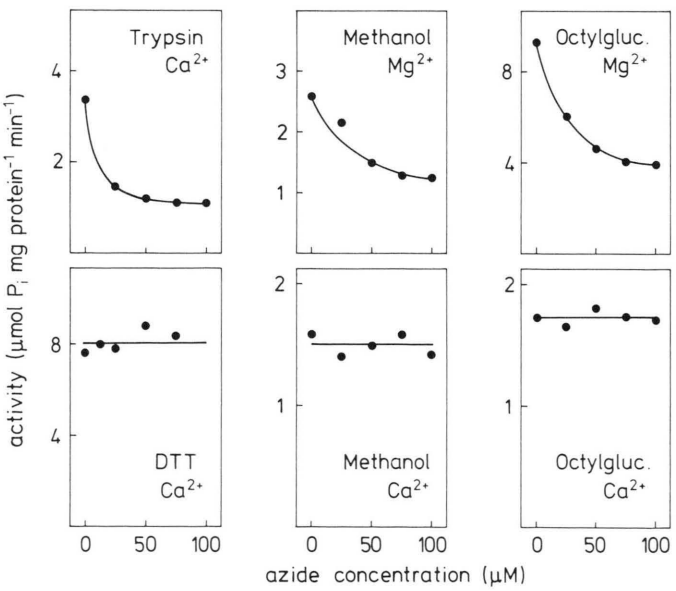

Fig. 2. ATPase activity of differently activated CF_1 as affected by azide. Trypsin and DTT activation was performed as described in Materials and Methods. Methanol-stimulated Mg^{2+}-ATPase was measured with 30%, methanol-stimulated Ca^{2+}-ATPase with 20% methanol present in the reaction medium. For measurement of octylglucoside-dependent Mg^{2+}-ATPase activity, latent CF_1 was incubated in reaction medium containing 25 mM octylglucoside; Ca^{2+}-dependent activity was likewise assayed in the presence of 25 mM octylglucoside, but with CF_1 preactivated for 6 min in the presence of 25 mM octylglucoside. The rates were measured between 1 and 2 min after start of the reaction by addition of ATP.

Mg-ATP (or Mn-ATP) is increased in the presence of these anions [31, 32]. Similarly, sulfite changes the heat-activated CF_1-ATPase from Ca^{2+}- to Mg^{2+}-specificity [33]. Hence the type of activation and the presence of certain anions have a large effect on the properties of the catalytic binding sites for free metal ions and the ATP-metal ion complex, respectively.

We have observed that preincubation of DTT-activated CF_1 with a low concentration of Mg^{2+} inhibited the subsequently assayed Ca^{2+}-ATPase activity. For example, preincubation with 10 μM Mg^{2+} resulted in about 10% inhibition of Ca^{2+}-ATPase activity. This type of inhibition is probably not due to competition at the catalytic site since Mg^{2+} was diluted upon transfer of pretreated CF_1 into assay medium where the excess of Ca^{2+} over Mg^{2+} was 600-fold. The effect of Mg^{2+}-pretreatment of DTT-activated CF_1 on azide sensitivity of subsequently assayed Ca^{2+}-ATPase activity was studied in some detail. While the untreated control was not affected, the Mg^{2+}-pretreated enzyme was strongly inhibited by azide (Fig. 3a), indicating that the preincubation with Mg^{2+} has caused acquisition of azide susceptibility of the azide-resistant DTT-activated Ca^{2+}-dependent enzyme. The same type of experiment conducted with azide-susceptible trypsin-activated CF_1 is shown in Fig. 3b. The trypsin-activated Ca^{2+}-ATPase was not inhibited by Mg^{2+} pretreatment but the initial rate was slightly increased. The relative inhibition of Ca^{2+}-ATPase by azide was unchanged.

In vivo CF_0CF_1 is activated by energization of the thylakoids by light. In order to obtain an active ATP hydrolyzing enzyme in the subsequent dark period, preillumination must be carried out in the presence of DTT (thiol modulation) which replaces the natural thioredoxin system [22]. Upon addition of micromolar concentrations of ADP, however, the enzyme is rapidly deactivated as a result of tight ADP binding [34] to one of the catalytic sites located in the β subunits of CF_1 [35]. Deactivation and tight binding of ADP, respectively, are strongly decelerated by inorganic phosphate [34, 36]. In a previous paper [18] we have shown that this particular effect of phosphate is abolished by azide in a competitive manner.

As DTT-activated isolated CF_1 may have similar characteristics as thiol-modulated CF_0CF_1 *in situ*, the influence of preincubation with ADP ± Mg^{2+} or phosphate ± Mg^{2+} on Ca^{2+}-ATPase activity and azide inhibition of DTT-activated isolated CF_1 was investigated. In contrast to thiol-modulated CF_0CF_1 *in situ* preincubation of DTT-activated isolated CF_1 with a low concentration of

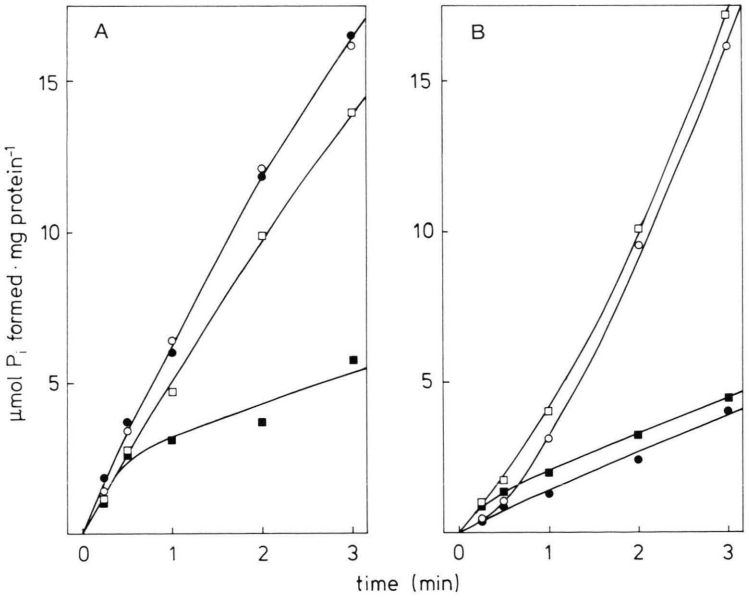

Fig. 3. Effect of Mg^{2+} preincubation on Ca^{2+}-ATPase activity of DTT- (A) and trypsin-activated CF_1 (B). Open circles: no Mg^{2+} preincubation, azide absent; filled circles: no Mg^{2+} preincubation, 50 μM (A) or 100 μM azide present (B); open squares: preincubation with 10 μM (A) or 50 μM Mg^{2+} (B), azide absent; filled squares: preincubation with 10 μM (A) or 50 μM Mg^{2+} (B), 50 μM (A) or 100 μM azide present (B). The preincubation time was 3 min.

ADP did not affect the activity of the Ca^{2+}-ATPase (Table II). Pretreatment with phosphate slightly increased to ATPase activity (Table III). The two pretreatments, however, did not change the inhibitory action of azide. Like in Fig. 3a, preincubation with Mg^{2+} induced azide-sensitivity of the DTT-activated Ca^{2+}-ATPase. However, azide inhibition was not altered by the simultaneous presence of ADP or phosphate during pretreatment.

Discussion

The reported effects of azide on isolated CF_1 are rather different from those on CF_0CF_1 *in situ*. In thylakoids neither $\Delta\tilde{\mu}_{H^+}$-coupled phosphorylation nor coupled ATP hydrolysis is affected by azide, but the ATPase reaction is inhibited by azide when gradient formation is prevented by addition of an uncoupler [17, 18]. These results led Wei *et al.* [17] to suggest that membrane energization generates a conformation of the enzyme in which the binding site for azide is hidden.

During cleavage of ATP by thylakoid vesicles, a proton gradient is necessary to maintain the active state of light-triggered ATPase [37, 38]. The gradient counteracts tight binding of the reaction product ADP, a process which is known to cause deactivation of the thiol-modulated ATPase [34]. On the other hand, tight ADP binding and deactivation of the ATPase are decelerated by inorganic phosphate [34, 36]. This effect is specifically abolished by azide which competes with phosphate [18]. In accordance with the observation that the ATPase activity of DTT-activated isolated CF_1 is not regulated by ADP and phosphate, no interference between azide, ADP and phosphate was found (Table II and III).

The effectiveness of azide on isolated CF_1 depends on the procedure of activation and the divalent metal ion employed as cofactor. The molecular mechanisms of the different types of activation are not yet fully understood. Activation by detergents like octylglucoside [39] or by alcohols [40] was found to be related to displacement of ε subunit, a polypeptide of CF_1 which is considered to be an ATPase inhibitor. Activation by heat or trypsin was likewise traced back to dissociation (or digestion) of ε subunit [41]. Later studies, however, suggested that trypsin activation is related with clipping of α subunit to a slightly smaller α′ [42]. Heat or DTT treatment, on the other hand, was proposed to cause a change of the conformation or position of subunit γ [42]. DTT furthermore effects reduction of a disulfide bridge in γ subunit [43].

The multiple changes of primary, tertiary and quarternary structures of CF_1 caused by the diverse activating procedures may explain the non-uniform metal ion specificity and azide susceptibility, two properties which seem to be related with each other. The Mg^{2+}-dependent activity is highly sensitive towards azide, whereas the azide effect on Ca^{2+}-dependent activity is ambiguous. The most remarkable case is the DTT-activated enzyme which is per se resistant to azide, but gains azide sensitivity by pretreatment with Mg^{2+}.

Altogether six divalent cation binding sites were detected on CF_1 by employing Mn^{2+} as a probe in EPR binding studies. They could be classified as one tight and five loose sites in the absence, or two tight and four loose binding sites in the presence of nucleotides [30]. Extended subsequent work suggested cooperative interaction of two [44] or three [45] high affinity sites which are proposed to be involved in the linkage of ATP to the catalytic sites [45]. The functional role of the other binding sites is obscure. Unfortunately those binding studies have been carried out with latent CF_1; hence nothing is known about possible changes of affinity, specificity and cooperativity caused by the different activating treatments.

For further discussion of the interrelationship between divalent cations and azide effects, results on other F_1 type ATPases may be relevant. F_1 from *E. coli* possesses both Mg^{2+}- and Ca^{2+}-dependent ATPase activities. In general agreement with our results on CF_1, the Mg^{2+}-ATPase was found to be 200-fold more sensitive towards azide than the Ca^{2+}-ATPase. F_1 of an *E. coli* mutant in which serine-174 of the β subunit was replaced by phenylalanine showed a decreased sensitivity of Mg^{2+}-ATPase and an increased sensitivity of Ca^{2+}-ATPase against azide, suggesting that the area around serine-174 in β subunit may constitute the azide binding site [46]. On the other hand, mutants defective in α subunit were reported to be insensitive to azide [47], suggesting that α subunit may also be involved in azide binding. F_1 from beef heart mitochondria showed synergistic inhibition

of Mg^{2+}-ATPase activity by azide and Mg^{2+} [28]. As the initial rate was less affected than the steady state rate (similar to our results on CF_1), the authors proposed that azide binding is a slow reaction which requires a bound Mg^{2+} to form an enzyme-Mg^{2+}-azide complex with decreased catalytic activity. A similar interpretation may also explain our results on CF_1. However, although Mg^{2+} seems to induce azide susceptibility of the DTT-activated Ca^{2+}-ATPase, Mg^{2+} is not necessary when other methods of activation (*e.g.* trypsin or heat treatment) are employed.

A result which may give a key to a more precise understanding of azide inhibition, was reported by Noumi *et al.* [48]. These authors found that the Mg^{2+}-ATPase of *E. coli*-F_1 was strongly inhibited by azide when multi-site conditions (high substrate concentration) were employed, but was not at all affected under conditions which permitted uni-site catalysis only. This result led them to propose that the inhibitory effect of azide on steady state ATP hydrolysis may be due to lowering of catalytic cooperativity. Application of this idea to CF_1 gives rise to the interesting speculation that the different types of artificial activation may result in enzymes with different degrees of steady state catalytic cooperativity which can be discriminated by azide. Treatments which yield azide-insensitive Ca^{2+}-ATPases might create active enzymes that work in a uni-site mode, whereas the azide-susceptible ATPases might represent multi-site ATPases. The DTT-activated enzyme, on the other hand, might switch from uni-site to multi-site catalysis upon preincubation with Mg^{2+}.

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[1] H. Strotmann and S. Bickel-Sandkötter, Annu. Rev. Plant Physiol. **35,** 97–120 (1984).
[2] R. B. Beechey, C. T. Holloway, I. G. Knight, and A. M. Robertson, Biochem. Biophys. Res. Commun. **23,** 75–80 (1965).
[3] W. Sebald, J. Hoppe, and E. Wachter, in: Function and Molecular Aspects of Biomembrane Transport (E. Quagliariello, F. Plamieri, S. Papa, and M. Klingenberg, eds.), pp. 63–74, Elsevier/North Holland, Amsterdam 1979.
[4] G. Onur, G. Schäfer, and H. Strotmann, Z. Naturforsch. **38c,** 49–59 (1982).
[5] H. Strotmann, K. Brendel, K. S. Boos, and E. Schlimme, FEBS Lett. **145,** 11–15 (1982).
[6] S. Izawa, G. D. Winget, and N. E. Good, Biochem. Biophys. Res. Commun. **22,** 223–226 (1966).
[7] Y. Mukohata, S. Nabakayashi, and M. Higashida, FEBS Lett. **85,** 215–218 (1978).
[8] V. Shoshan, Y. Shahak, and N. Shavit, Biochim. Biophys. Acta **591,** 421–433 (1980).
[9] J. A. Steele, T. F. Uchytil, and R. D. Durbin, Biochim. Biophys. Acta **459,** 347–350 (1977).
[10] J. A. Steele, T. F. Uchytil, and R. D. Durbin, Biochim. Biophys. Acta **504,** 136–141 (1978).
[11] R. E. McCarty, R. J. Guillory, and E. Racker, J. Biol. Chem. **240,** 4822–4823 (1965).
[12] P. Pedersen, Biochem. Biophys. Res. Commun. **64,** 610–616 (1975).
[13] E. A. Vasilyeva, I. B. Minkov, A. F. Fitin, and A. D. Vinogradov, Biochem. J. **202,** 15–23 (1982).
[14] G. A. Vigers and F. D. Zigel, Biochem. Biophys. Res. Commun. **30,** 83–89 (1968).
[15] I. Moyle and P. Mitchell, FEBS Lett. **56,** 55–61 (1975).
[16] H. Kobayashi, M. Maeda, and Y. Anraku, J. Biochem. **81,** 1071–1077 (1977).
[17] J. Wei, B. Howlett, and A. T. Jagendorf, Biochim. Biophys. Acta **934,** 72–79 (1988).
[18] I. B. Minkov and H. Strotmann, Biochim. Biophys. Acta **973,** 7–12 (1989).
[19] E. M. Larson and A. T. Jagendorf, Biochim. Biophys. Acta **973,** 67–77 (1989).
[20] A. Binder, A. Jagendorf, and E. Ngo, J. Biol. Chem. **253,** 3094–3100 (1978).
[21] S. Lien and E. Racker (1971), Methods Enzymol. **23,** 547–555 (1971).
[22] R. E. McCarty and E. Racker, J. Biol. Chem. **243,** 129–137 (1968).
[23] U. Pick and S. Bassilian, Biochemistry **21,** 6144–6152 (1982).
[24] D. W. Deters, E. Racker, N. Nelson, and H. Nelson, J. Biol. Chem. **250,** 1041–1047 (1975).
[25] H. Sakurai, K. Shinohara, T. Hisabori, and K. Shinohara, J. Biochem. **90,** 95–102 (1981).
[26] H. Strotmann, in: Proceedings of the IInd International Congress on Photosynthesis Research (G. Forti, M. Avron, and A. Melandri, eds.), **Vol. 2,** pp. 1319–1328, Dr. W. Junk publishers, The Hague 1972.
[27] B. Chance and M. Nishimura, Methods Enzymol. **10,** 641–645 (1967).
[28] S. G. Daggett, T. A. Tomaszek Jr., and S. M. Schuster, Arch. Biochem. Biophys. **236,** 815–824 (1985).
[29] S. Bickel-Sandkötter, Biochim. Biophys. Acta **809,** 117–124 (1985).
[30] Y. Hochman and C. Carmeli, Biochemistry **20,** 6287–6292 (1981).
[31] N. Nelson, H. Nelson, and E. Racker, J. Biol. Chem. **247,** 6506–6510 (1972).
[32] Y. Hochman and C. Carmeli, Biochemistry **20,** 6293–6297 (1981).

[33] A. N. Mal'yan and E. A. Akulova, Biokhimiya **43,** 1206–1211 (1978).
[34] J. Schumann and H. Strotmann, in: Photosynthesis II. Electron Transport and Photophosphorylation (G. Akoyunoglou, ed.), pp. 881–892, Balaban International Science Services, Philadelphia, Pa. 1981.
[35] J. M. Zhou, Z. Xue, Z. Du, T. Melese, and P. D. Boyer, Biochemistry **27,** 5129–5135 (1988).
[36] K. Dunham and B. R. Selman, J. Biol. Chem. **256,** 10044–10049 (1981).
[37] T. Bakker-Grunwald and K. van Dam, Biochim. Biophys. Acta **292,** 808–814 (1973).
[38] S. Kleefeld, D. Lohse, A. R. Mansy, and H. Strotmann, Biochim. Biophys. Acta **1019,** 11–18 (1990).
[39] F. Yu and R. E. McCarty, Arch. Biochem. Biophys. **238,** 61–68 (1985).
[40] M. L. Richter, W. J. Patrie, and R. E. McCarty, J. Biol. Chem. **259,** 7371–7373 (1984).
[41] N. Nelson, H. Nelson, and E. Racker, J. Biol. Chem. **247,** 7657–7662 (1972).
[42] J. V. Moroney and R. E. McCarty, J. Biol. Chem. **257,** 5910–5914 (1982).
[43] J. Schumann, M. L. Richter, and R. E. McCarty, J. Biol. Chem. **260,** 11817–11823 (1985).
[44] A. E. Haddy, W. D. Frasch, and R. R. Sharp, Biochemistry **24,** 7926–7930 (1985).
[45] C. Carmeli, J. Y. Huang, D. M. Mills, A. T. Jagendorf, and A. Lewis, J. Biol. Chem. **261,** 16969–16975 (1986).
[46] K. Takeda, J. Miki, H. Kanazawa, T. Tsuchiya, and M. Futai, J. Biochem. **97,** 1401–1407 (1985).
[47] J. G. Wise, L. R. Latchney, A. M. Ferguson, and A. E. Senior, Biochemistry **23,** 1426–1432 (1984).
[48] T. Nuomi, M. Maeda, and M. Futai, FEBS Lett. **213,** 381–384 (1987).