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MINI-REVIEW

Regulatory Proteins of F_1F_0-ATPase: Role of ATPase Inhibitor

Tadao Hashimoto,[1] Yukuo Yoshida,[1] and Kunio Tagawa[1]

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Abstract

An intrinsic ATPase inhibitor inhibits the ATP-hydrolyzing activity of mitochondrial F_1F_0-ATPase and is released from its binding site on the enzyme upon energization of mitochondrial membranes to allow phosphorylation of ADP. The mitochondrial activity to synthesize ATP is not influenced by the absence of the inhibitor protein. The enzyme activity to hydrolyze ATP is induced by dissipation of the membrane potential in the absence of the inhibitor. Thus, the inhibitor is not responsible for oxidative phosphorylation, but acts only to inhibit ATP hydrolysis by F_1F_0-ATPase upon deenergization of mitochondrial membranes. The inhibitor protein forms a regulatory complex with two stabilizing factors, 9K and 15K proteins, which facilitate the binding of the inhibitor to F_1F_0-ATPase and stabilize the resultant inactivated enzyme. The 9K protein, having a sequence very similar to the inhibitor, binds directly to F_1 in a manner similar to the inhibitor. The 15K protein binds to the F_0 part and holds the inhibitor and the 9K protein on F_1F_0-ATPase even when one of them is detached from the F_1 part.

Key Words: F_1-ATPase; F_1F_0-ATPase; mitochondria; ATPase inhibitor; 9K protein; 15K protein; oxidative phosphorylation; membrane potential.

Introduction

ATP synthase found in mitochondria, chloroplasts, and bacteria is located in the membranes and catalyzes ATP synthesis coupled to respiratory chain-linked transport of protons. The enzyme can also create a proton gradient across the membrane using energy released by hydrolysis of ATP. In anaerobic

[1]Department of Physiological Chemistry, Medical School, Osaka University, 4-3-57 Nakanoshima, Kita-ku, Osaka 530, Japan.

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bacteria, the ATP-hydrolyzing reaction of the enzyme is specially important for regulating the intracellular pH, transporting nutrients, and supplying energy for the movement machinery. ATP synthase consists of two main parts; a hydrophilic F_1-part carrying catalytic sites (F_1-ATPase) and a hydrophobic F_0-part that is involved in proton translocation. Thus, ATP synthase is called F_1F_0-ATPase.

The subunit structure of bacterial F_1F_0-ATPase has been clarified: the enzyme consists of eight different subunits with a stoichiometry of $\alpha_3\ \beta_3\ \gamma\ \delta\ \varepsilon a\ b_2 c_{10}$ (Kagawa *et al.*, 1979; Fillingame, 1981; Futai and Kanazawa, 1983). Mitochondrial F_1F_0-ATPase was originally thought to have a structure similar to the bacterial enzyme. However, later the mitochondrial enzyme was found to contain extra subunits, such as oligomycin sensitivity-conferring protein (OSCP) (MacLennan and Tzagoloff, 1968), F_6 protein (Fessenden-Raden, 1972), and F_B protein (Lam *et al.*, 1967). The OSCP and F_6 protein are required for the interaction of the F_1-part with the F_0-part. F_B protein, a subunit of F_0, is reported to be necessary for the energy-coupling reactions of F_1F_0-ATPase.

An intrinsic ATPase inhibitor is considered to be a regulatory factor of mitochondrial F_1F_0-ATPase. This protein has been isolated from beef heart (Pullman and Monroy, 1963; Horstman and Racker, 1970), rat liver (Cintron and Pedersen, 1979), rat skeletal muscle (Yamada and Huzel, 1988), and yeast (Satre *et al.*, 1975; Ebner and Maier, 1977; Hashimoto *et al.*, 1981) mitochondria, and the amino acid sequences of the inhibitors in yeast (Matsubara *et al.*, 1981; Dianoux and Hoppe, 1987) and in beef heart (Frangione *et al.*, 1981) have been determined. The inhibitor protein forms an equimolar complex with purified F_1- or F_1F_0-ATPase and completely inhibits the enzyme activity (Gómez-Fernandez and Harris, 1978; Hashimoto *et al.*, 1981). This inhibitor protein is considered to bind to the β subunit of the enzyme (Klein *et al.*, 1980; Jackson and Harris, 1983; Beltran *et al.*, 1988) and to be released upon energization of mitochondrial membranes (Schwerzmann and Pedersen, 1981; Klein and Vignais, 1983; Power *et al.*, 1983; Husain and Harris, 1983). Schwerzmann and Pedersen (1986) postulated four distinct activity states of membrane-bound F_1F_0-ATPase, in which the affinity of the enzyme for the inhibitor protein differs depending on the membrane potential. In their model, the enzyme functions in either the synthesis or hydrolysis of ATP, only when the inhibitor is released. We recently found that mutant mitochondria that do not contain the inhibitor protein synthesize ATP at the same rate as normal mitochondria, and that even in the mutant mitochondria F_1F_0-ATPase does not hydrolyze ATP when the membrane potential is maintained (Hashimoto *et al.*, 1990a). In the present review, we describe the properties of the ATPase inhibitor and

related proteins with reference to energization and deenergization of the membrane.

Inhibition of F_1-ATPase by ATPase Inhibitor

Bovine F_1-ATPase exhibits two types of kinetics of ATP hydrolysis (Grubmeyer *et al.*, 1982; Cross *et al.*, 1982). A substoichiometric amount of ATP binds tightly to only the first catalytic site and is hydrolyzed slowly (uni-site hydrolysis). Under these conditions, the release of the products, ADP and P_i, is the rate-limiting step. However, a substrate amount of ATP binds to the second and third catalytic sites and promotes the release of the products, resulting in up to one million-fold increase in the rate of ATP hydrolysis (multi-site hydrolysis). The *E. coli* enzyme is reported to have similar catalytic properties (Duncan and Senior, 1985; Noumi *et al.*, 1986). These observations indicate the cooperative interaction of the three catalytic sites on the β subunit during ATP hydrolysis and support the binding change mechanism of ATP synthesis (Boyer *et al.*, 1982). Several synthetic reagents are known to inhibit F_1-ATPase by binding to a catalytic site of the enzyme. Adenosine triphosphopyridoxal binds to a single catalytic site of *E. coli* F_1-ATPase and completely inhibits uni-site as well as multi-site hydrolysis of ATP (Noumi *et al.*, 1987). Fluorosulfonylbenzoyl inosine, an affinity probe for the catalytic site of F_1F_0-ATPase, modifies Tyr-345 of a single β subunit of bovine F_1-ATPase, resulting in complete inhibition of the enzyme activity (Bullough and Allison, 1986). A nonhydrolyzable analogue of ATP, adenylylimidodiphosphate, which binds to a single high-affinity site of the enzyme, also inhibits the activity completely (Cross and Nalin, 1982).

The intrinsic ATPase inhibitor binds to the β subunit of mitochondrial F_1-ATPase (Klein *et al.*, 1980; Jackson and Harris, 1983, 1986, 1988; Beltran *et al.*, 1988). This protein binds to purified F_1-ATPase in an equimolar ratio in the presence of ATP and Mg^{2+} and completely inhibits the enzyme activity (Gómez-Fernandez and Harris, 1978; Hashimoto *et al.*, 1981). The ε subunit of *E. coli* F_1-ATPase has also been reported to bind to the β subunit of the enzyme (Lötscher *et al.*, 1984; Bragg and Hou, 1986; Tozer and Dunn, 1987). Thus, the binding of the inhibitor to one of the β subunits probably interferes with the cooperativity of the three catalytic sites of F_1-ATPase. The inhibitor protein seems to have an affinity to the enzyme that is hydrolyzing ATP. Indeed, it binds to the enzyme–substrate complex but not to the ATP-free enzyme. The inactivated enzyme–inhibitor complex contains 1 mol each of ATP and ADP, indicating that two of the three catalytic sites are occupied by the substrate and product, respectively (Klein *et al.*, 1981; Hashimoto *et al.*, 1981).

Energy-Dependent Release of ATPase Inhibitor from F_1F_0-ATPase

ATPase inhibitor was first isolated from beef heart mitochondria as a potent inhibitor of isolated F_1-ATPase (Pullman and Monroy, 1963), but initially it was not clear whether the inhibitor was involved in oxidative phosphorylation *in vivo* (Horstman and Racker, 1970). Asami *et al.* (1970) showed that the inhibitor protein inhibits only ATP hydrolysis or ATP-driven energy transfer, not oxidative phosphorylation in submitochondrial particles. Van de Stadt *et al.* (1973) found that submitochondrial particles with low ATPase activity, which bind the inhibitor protein, become active when incubated with a respiratory substrate. These observations suggested that the ATPase inhibitor is released or at least displaced (Dreyfus *et al.*, 1981) from the binding site when the enzyme becomes functional. Its actual release from the phosphorylating enzyme was confirmed by direct measurement of the inhibitor protein (Schwerzmann and Pedersen, 1981; Klein and Vignais, 1983; Power *et al.*, 1983).

The relationship between the release of the inhibitor protein and the onset of phosphorylation has been studied extensively by Pedersen's group (Schwerzmann and Pedersen, 1981; Cintron *et al.*, 1982; Schwerzmann and Pedersen, 1986) and Harris's group (Power *et al.*, 1983; Husain and Harris, 1983; Lippe *et al.*, 1988a,b). Harris's group reported that release of the inhibitor from submitochondrial particles was directly related with the onset of oxidative phosphorylation: increase in the phosphorylation rate was parallel with the degree of release of ATPase inhibitor, although several minutes was required to attain the maximum rate. On the other hand, Pedersen's group, also using submitochondrial particles, observed a brief lag between the start of phosphorylation and release of the inhibitor. During this lag phase, most protein molecules of the inhibitor remained associated with the particles, but they were gradually released when respiration had proceeded for a few minutes. Summarizing these properties of the inhibitor protein, Schwerzmann and Pedersen (1986) proposed a model for regulation of F_1F_0-ATPase by the inhibitor in which the enzyme changes its binding affinity to the inhibitor protein. Their model implies that the inhibitor protein acts to slow down conformational change of the enzyme, which may explain the delay in release of inhibitor protein observed after onset of ATP synthesis in submitochondrial particles containing the inhibitor. These observations strongly suggest that the ATPase inhibitor is released from its binding site on F_1F_0-ATPase to allow phosphorylation of ADP and that it is not involved in the phosphorylation reaction.

Very recently we constructed mutant yeast strains with no inhibitor protein by transformation of cells with the *in vitro*-disrupted gene (Hashimoto *et al.*, 1990a). The rate of ATP synthesis in the mutant mitochondria is comparable to that in normal mitochondria, and neither normal nor

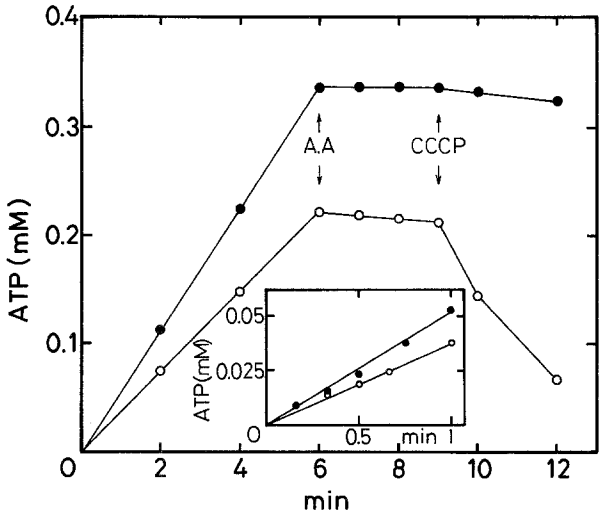

Fig. 1. ATP synthesis and hydrolysis by normal and ATPase inhibitor-deficient mitochondria. Mitochondria (0.6 mg) were incubated at 25°C in medium containing 0.3 M mannitol, 5 mM potassium phosphate, pH 6.5, 5 mM succinate, 0.1% bovine serum albumin, and 1 mM ADP in a final volume of 2 ml. At the times indicated, adenine nucleotides in the medium were measured by HPLC. Antimycin A (2 nmol) and CCCP (2 nmol) were added as indicated. (●) Control mitochondria; (○) ATPase inhibitor-deficient mitochondria. The inset shows an initial phase of the ATP synthesis by control (●) and ATPase inhibitor-deficient (○) mitochondria.

mutant mitochondria show any lag phase in phosphorylation. Addition of antimycin A to respiring mitochondria blocked ATP synthesis, but did not cause rapid hydrolysis of ATP in mutant or normal mitochondria. The ATP-hydrolyzing activity of the mutant mitochondria was greatly induced by deenergization of membranes, while that of normal mitochondria remained latent (Fig. 1). Even in the inhibitor-deficient mitochondria, F_1F_0-ATPase did not function to hydrolyze ATP unless the membrane potential was dissipated. These observations indicate that the ATPase inhibitor is released from and binds to F_1F_0-ATPase upon energization and deenergization, respectively, of mitochondrial membranes. Thus, it is concluded that mitochondrial F_1F_0-ATPase does not hydrolyze ATP when the membrane potential is maintained, and that the ATPase inhibitor binds to the enzyme to prevent ATP hydrolysis only when the membrane potential is dissipated.

Regulatory Factors of Yeast F_1F_0-ATPase

9K Protein and 15K Protein

F_1-ATPase binds to the ATPase inhibitor in the presence of ATP and Mg^{2+}, but the complex formed gradually dissociates in the absence of ATP

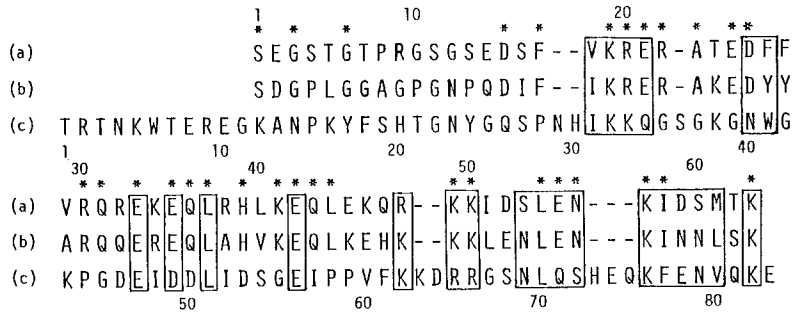

Fig. 2. Comparison of amino acid sequences of yeast ATPase inhibitor and 9K and 15K proteins. (a) ATPase inhibitor (Matsubara *et al.*, 1981), (b) 9K protein (Matsubara *et al.*, 1983), and (c) 15K protein (Yoshida *et al.*, 1984). Identical and similar amino acid residues in the three proteins are enclosed in solid boxes. Asterisks indicate identical amino acid residues in the ATPase inhibitor and 9K protein. Dashes indicate gaps, making alignment highly homologous. Reproduced by permission of the publisher from (Tagawa *et al.*, 1986).

and Mg^{2+}. In contrast, F_1F_0-ATPase bound to mitochondrial membranes is inactive and is not activated even in the absence of external ATP and Mg^{2+}, suggesting the existence in mitochondria of some factor that stabilizes the enzyme–inhibitor complex. In fact, a protein fraction obtained from yeast mitochondria was found to stabilize the inactivated F_1F_0-ATPase–inhibitor complex, although it did not stabilize the F_1-ATPase–inhibitor complex (Hashimoto *et al.*, 1983). This fraction contained two proteins, designated as 9K protein and 15K protein from their apparent molecular weights on SDS-PAGE (Hashimoto *et al.*, 1984). On SDS-PAGE, the 9K protein and 15K protein were hardly distinguishable from the yeast ε and δ subunits, respectively, of F_1-ATPase, but their sequences in the amino terminal regions differed from those of the ε and δ subunits (Okada *et al.*, 1986). The concerted actions of these two proteins greatly accelerates and stabilizes the binding of the ATPase inhibitor to F_1F_0-ATPase (Hashimoto *et al.*, 1984). The amino acid sequence of the 9K protein (Matsubara *et al.*, 1983) has similarities to those of yeast ATPase inhibitor (Matsubara *et al.*, 1981) and 15K protein (Yoshida *et al.*, 1984) especially in their carboxyl terminal regions (Fig. 2). The ATPase inhibitor and the two stabilizing factors are present in mitochondria in equimolar ratios to F_1-ATPase, probably forming a regulatory substructure of F_1F_0-ATPase (Okada *et al.*, 1986).

Properties of 9K Protein

The 9K protein, like the inhibitor protein, binds to F_1-ATPase forming an equimolar complex with the enzyme in the presence of ATP and Mg^{2+} (Hashimoto *et al.*, 1987). The apparent dissociation constant of the enzyme–9K protein complex is 3.7×10^{-6} M, which is comparable to that of the

enzyme–inhibitor complex (4.2×10^{-6} M). Formation of a complex with the 9K protein decreases the ATP-hydrolyzing activity of the enzyme 40%, whereas formation of a complex with the ATPase inhibitor results in complete inhibition of its activity. When added to F_1-ATPase simultaneously, the bindings of the 9K protein and ATPase inhibitor are competitive and the sum of their bindings does not exceed 1 mol per mole of enzyme. However, when added successively, with more than 10 min between their additions, the one bound first is not displaced by the other. Thus, the remaining ATPase activity of the enzyme–9K complex is insensitive to external ATPase inhibitor, and conversely, the F_1-ATPase activity inhibited by the inhibitor protein is not reactivated by the addition of 9K protein. These observations seem to be inconsistent with the conclusion described above that the 9K protein acts as a stabilizer of the inactivated F_1F_0-ATPase–inhibitor complex, but, as described below, the function of the 15K protein may provide a clue to this problem.

Function of 15K Protein

The 15K protein also exists in an equimolar ratio to F_1F_0-ATPase in yeast mitochondria. The 15K protein binds to F_1F_0-ATPase but not to F_1-ATPase. Its binding to F_1F_0-ATPase gives a saturation curve, forming an equimolar complex with the enzyme (Hashimoto *et al.*, 1990b). The dissociation constant of the enzyme–15K protein complex is estimated to be 1.4×10^{-5} M. This protein has no activity to inhibit the enzyme. The ATPase inhibitor and 9K protein bind competitively to F_1F_0-ATPase in the presence of ATP and Mg^{2+} as they do to F_1-ATPase, and both proteins can bind simultaneously to the F_1F_0-ATPase in the presence of the 15K protein, although the ATP-hydrolyzing activity of the enzyme–ligand complex is greatly influenced by the order of additions of the ATPase inhibitor and 9K protein: When the ATPase inhibitor is added first, the enzyme is completely inhibited, but when the 9K protein is added first, it is only partially inhibited, even after the binding of 1 mol of ATPase inhibitor. These results suggest that the catalytic subunit of F_1F_0-ATPase binds either the ATPase inhibitor or the 9K protein, and that the 15K protein functions to prevent release of unbound ligands from the F_1 part of the enzyme.

As described above, the mitochondrial F_1F_0-ATPase activity is reversible in nature and is inhibited by binding of ATPase inhibitor only when the membrane potential is lost. If the 9K protein can act competitively with the ATPase inhibitor *in situ* when the membrane potential is increased, it is conceivable that the 9K protein facilitates release of the ATPase inhibitor from its binding site on the enzyme. Interchange between the inhibitor and 9K protein on the catalytic subunit of the enzyme may occur upon energization and deenergization of membranes.

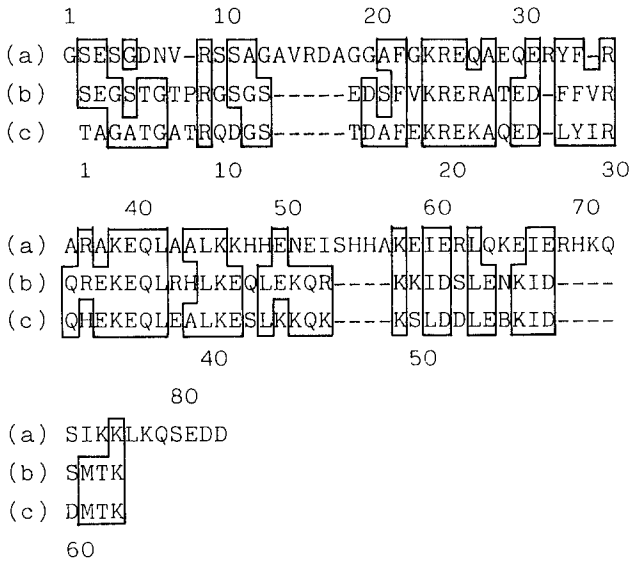

Fig. 3. Comparison of amino acid sequences of (a) beef ATPase inhibitor (Frangione *et al.*, 1981), (b) *S. cerevisiae* inhibitor (Matsubara *et al.*, 1981), and (c) *C. utilis* inhibitor (Dianoux and Hoppe, 1987). Identical and similar amino acid residues are enclosed in solid boxes. Dashes indicate gaps, making alignment highly homologous.

ATPase Inhibitor and ε Subunit of *E. coli* F_1-ATPase

The primary structures of the ATPase inhibitors from *Saccharomyces cerevisiae* (Matsubara *et al.*, 1981), *Candida utilis* (Dianoux and Hoppe, 1987), and beef heart (Frangione *et al.*, 1981) have been reported. As shown in Fig. 3, the sequences of these three inhibitors are very similar. Their secondary and tertiary structures also resemble each other. The α-helical contents of the inhibitors from *S. cerevisiae* (Tagawa *et al.*, 1986), *C. utilis* (Dianoux and Hoppe, 1987), and beef heart (Frangione *et al.*, 1981) mitochondria are 80, 60, and 50%, respectively. The helical region of the ATPase inhibitor of *S. cerevisiae* (from Arg-30 to Arg-48) contains characteristic repeats of basic, acidic, and hydrophobic amino acid residues and these are each expected to be clustered on the surface of the three-dimensional molecule (Matsubara *et al.*, 1983). In this connection it is noteworthy that Jackson and Harris (1986) suggested the importance of a hydrophobic patch structure, which is present on one side of the α-helix of the bovine inhibitor protein, for its binding to F_1-ATPase. This characteristic feature of the inhibitor protein is highly conserved in the three inhibitor proteins, suggesting that these structures are suitable for formation of a stable complex with the β subunit of F_1-ATPase.

The ε subunits of *E. coli* and chloroplast are reported to resemble the ATPase inhibitor of mitochondria (Smith and Sternweis, 1977; Nelson *et al.*, 1972). Recently Dunn *et al.* (1987) analyzed the uni-site and multi-site hydrolyses of ATP and found that the ε subunit reduces the rate of product release in uni-site hydrolysis of ATP. In multi-site hydrolysis, the specific activity of the enzyme containing the ε subunit is much less than that of the ε-depleted enzyme. Woods *et al.* (1987) confirmed this effect of the ε subunit by measuring the exchange reaction of oxygen between inorganic phosphate and water. These observations indicate that the ε subunit reduces the cooperative interaction between the three catalytic subunits, which is required to reduce the affinity of the catalytic site for ADP and inorganic phosphate. Similar observations on the catalytic properties of F_1-ATPase have been observed in mitochondria: Galante *et al.* (1981) reported that the inhibitor inhibits the net hydrolysis of ATP more strongly than the ATP–P_i exchange reaction. Gómez-Puyou *et al.* (1983), from studies on the rates of the ATP–P_i exchange reaction and ATP hydrolysis, postulated that the role of the inhibitor protein is to inhibit product release in hydrolysis of ATP. These observations suggest that the mechanism of inhibition of ATP hydrolysis by the inhibitor protein is similar to that by the ε subunit of the *E. coli* enzyme. However, Kuki *et al.* (1988) found that a truncated ε subunit carrying 80 amino acid residues of its amino terminal can form an active enzyme complex, but does not inhibit ATP hydrolysis. The inhibitory activity of the truncated subunit is, however, observed when its amino acid residues extend to Ala-93, suggesting that a peptide between residues 80–93 is essential for the inhibitory activity of the subunit. The carboxyl-terminal region (residues 121–138), which is similar to the sequence of mitochondrial ATPase inhibitor, is not involved in the inhibitory action of the subunit, suggesting that the mechanism of inhibition by the ε subunit is not directly related to that of the mitochondrial ATPase inhibitor. But the native ε subunit of the bacterial enzyme seems to have two functional domains: one for the binding of F_1 to F_0, and the other for its inhibitory action on the enzyme. Mitochondrial ATPase inhibitor and 9K protein found in yeast have partially similar sequences to that of the bacterial ε subunit (Matsubara *et al.*, 1983). Conceivably the ATPase inhibitor, 9K protein, and bacterial ε subunit are derived from a common ancestral gene and have diverged with different functions in the process of evolution (Matsubara *et al.*, 1983).

Concluding Remarks

During the last ten years, much information has been accumulated about the protein sequences of various subunits of F_1F_0-ATPase. The sequences

of all eight subunits of *E. coli* F_1F_0-ATPase have been deduced from the nucleotide sequences of their genes (Futai and Kanazawa, 1983). Walker *et al.* (1985) found that the α, β, and γ subunits of bovine F_1-ATPase have sequences similar to the corresponding subunits of the *E. coli* enzyme and that the bovine δ subunit is similar to the ε subunit of the *E. coli* enzyme. Ovchinnikov *et al.* (1984) found that the sequence of bovine OSCP is similar to that of the δ subunit of *E. coli* F_1-ATPase. Thus, the relationship of the subunits in *E. coli* and mitochondrial F_1-ATPases is now fairly clear, and suggests that the machinery of oxidative phosphorylation is common to bacteria and mitochondria, differing only in regulatory proteins. Mitochondrial ATPase inhibitor resembles the ε subunit of *E. coli* F_1-ATPase in its effect in inhibiting ATP hydrolysis by the purified enzyme. However, an essential difference between the ATPase inhibitor and the bacterial ε subunit is that the former is released from the enzyme complex upon energization of membranes, whereas the latter is essential for oxidative phosphorylation. F_1F_0-ATPase has been reported to catalyze both synthesis and hydrolysis of ATP, even when a high membrane potential is maintained (Schwerzmann and Pedersen, 1986). However, as shown in Fig. 1, when the membrane potential is high, F_1F_0-ATPase does not hydrolyze ATP either in the presence or in the absence of the ATPase inhibitor, and in normal mitochondria, in which the ATPase inhibitor inhibits the enzyme activity, it does not hydrolyze ATP even when the membrane potential is lost. Hirata *et al.* (1986) reported that an applied voltage of -180 mV was sufficient to prevent the generation of electric current dependent on ATP hydrolysis of F_1F_0-ATPase that was incorporated into a planar phospholipid bilayer. These observations strongly suggest that the direction of the catalytic reaction of F_1F_0-ATPase is determined by the membrane potential, not by the action of the inhibitor protein.

Experiments on binding of the ATPase inhibitor together with 9K and 15K proteins to F_1F_0-ATPase suggest that 9K protein is a counterpart ligand to the ATPase inhibitor and that the 15K protein facilitates the binding of both the ATPase inhibitor and 9K protein to F_1-ATPase. Thus, we conclude that the mitochondrial ATPase inhibitor does not take part in ATP synthesis by F_1F_0-ATPase, but inhibits the enzyme activity to hydrolyze ATP when the membrane potential is lost, and that the 9K protein and 15K protein regulate the binding of the inhibitor to the enzyme in response to energization and deenergization of membranes.

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References

Asami, K., Juntti, and Ernster, L. (1970). *Biochim. Biophys. Acta* **205**, 307–311.
Beltran, C., Gómez-Puyou, A., and Tuena de Gómez-Puyou, M. (1988). *Biochem. Biophys. Res. Commun.* **152**, 867–873.
Boyer, P. D., Kohlbrenner, W. E., McIntosh, D. B., Smith, L. T., and O'Neal, C. C. (1982). *Ann. N.Y. Acad. Sci.* **402**, 65–83.
Bragg, P. D., and Hou, C. (1986). *Arch. Biochem. Biophys.* **244**, 361–372.
Bullough, D. A., and Allison, W. S. (1986). *J. Biol. Chem.* **261**, 14171–14177.
Cintron, N. M., and Pedersen, P. L. (1979). *J. Biol. Chem.* **254**, 3439–3443.
Cintron, N. M., Hullihen, J., Schwerzmann, K., and Pedersen, P. L. (1982). *Biochemistry* **21**, 1878–1885.
Cross, R. L., and Nalin, C. M. (1982). *J. Biol. Chem.* **257**, 2874–2881.
Cross, R. L., Grubmeyer, C., and Penefsky, H. S. (1982). *J. Biol. Chem.* **257**, 12101–12105.
Dianoux, A. C., and Hoppe, J. (1987). *Eur. J. Biochem.* **163**, 155–160.
Dreyfus, G., Gómez-Puyou, A., and Tuena de Gómez-Puyou, M. (1981). *Biochem. Biophys. Res. Commun.* **100**, 400–406.
Duncan, T. M., and Senior, A. E. (1985). *J. Biol. Chem.* **260**, 4901–4907.
Dunn, S. D., Zadorozny, V. D., Tozer, R. G., and Orr, L. E. (1987). *Biochemistry* **26**, 4488–4493.
Edner, E., and Maier, K. L. (1977). *J. Biol. Chem.* **252**, 671–676.
Fessenden-Raden, L. M. (1972). *J. Biol. Chem.* **247**, 2351–2357.
Fillingame, R. H. (1981). *Curr. Top. Bioenerg.* **11**, 35–106.
Frangione, B., Rosenwasser, F., Penefsky, H. S., and Pullman, M. E. (1981). *Proc. Natl. Acad. Sci. USA* **78**, 7403–7407.
Futai, M., and Kanazawa, H. (1983). *Microbiol. Rev.* **47**, 285–312.
Galante, Y. M., Wong, S. Y., and Hatefi, Y. (1981). *Biochemistry* **20**, 2671–2676.
Gómez-Fernandez, J. C., and Harris, D. A. (1978). *Biochem. J.* **176**, 967–975.
Gómez-Puyou, A., Ayala, G., Muller, U., and Tuena de Gómez-Puyou, M. (1983). *J. Biol. Chem.* **258**, 13673–13679.
Grubmeyer, C., Cross, R. L., and Penefsky, H. S. (1982). *J. Biol. Chem.* **257**, 12092–12100.
Hashimoto, T., Negawa, Y., and Tagawa, K. (1981). *J. Biochem.* **90**, 1151–1157.
Hashimoto, T., Yoshida, Y., and Tagewa, K. (1983). *J. Biochem.* **94**, 715–720.
Hashimoto, T., Yoshida, Y., and Tagawa, K. (1984). *J. Biochem.* **95**, 131–136.
Hashimoto, T., Yoshida, Y., and Tagawa, K. (1987). *J. Biochem.* **102**, 685–692.
Hashimoto, T., Mimura, H., Yoshida, Y., Ichikawa, N., and Tagawa, K. (1990a). In Bioenergetics: Molecular Biology, Biochemistry and Pathology (Kim, C. H. and Ozawa, T. eds) Plenum Publishing Corporation (in press).
Hashimoto, T., Yoshida, Y., and Tagawa, K. (1990b). *J. Biochem.*, in press.
Hirata, H., Ohno, K., Sone, N., Kagawa, Y., and Hamamoto, T. (1986). *J. Biol. Chem.* **261**, 9839–9843.
Horstman, L., and Racker, E. (1970). *J. Biol. Chem.* **245**, 1336–1344.
Husain, I., and Harris, D. A. (1983). *FEBS Lett.* **160**, 110–114.
Jackson, P. J., and Harris, D. A. (1983). *Biosci. Rep.* **3**, 921–926.
Jackson, P. J., and Harris, D. A. (1986). *Biochem. J.* **235**, 577–583.
Jackson, P. J., and Harris, D. A. (1988). *FEBS Lett.* **229**, 224–228.
Kagawa, Y., Sone, N., Hirata, H., and Okamoto, H. (1979). *J. Bioenerg. Biomembr.* **11**, 39–78.
Klein, G., and Vignais, P. V. (1983). *J. Bioenerg. Biomembr.* **15**, 347–362.
Klein, G., Satre, M., Dianoux, A. C., and Vignais, P. V. (1980). *Biochemistry* **19**, 2919–2925.
Klein, G., Lunardi, J., and Vignais, P. V. (1981). *Biochim. Biophys. Acta* **636**, 185–192.
Kuki, M., Noumi, T., Maeda, M., Amemura, A., and Futai, M. (1988). *J. Biol. Chem.* **263**, 17437–17442.

Lam, K. W., Warshaw, J. B., and Sanadi, D. R. (1967). *Arch. Biochem Biophys.* **119**, 477–484.
Lippe, G., Sorgato, M. C., and Harris, D. A. (1988a). *Biochim. Biophys. Acta* **933**, 1–11.
Lippe, G., Sorgato, M. C., and Harris, D. A. (1988b). *Biochim. Biophys. Acta* **933**, 12–21.
Lötscher, H. R., de Jong, C., and Capaldi, R. A. (1984). *Biochemistry* **23**, 4134–4140.
MacLennan, D. H., and Tzagoloff, A. (1968). *Biochemistry* **7**, 1603–1610.
Matsubara, H., Hase, T., Hashimoto, T., and Tagawa, K. (1981). *J. Biochem.* **90**, 1159–1165.
Matsubara, H., Inoue, K., Hashimoto, T., Yoshida, Y., and Tagawa, K. (1983). *J. Biochem.* **94**, 315–318.
Nelson, N., Nelson, H., and Racker, E. (1972). *J. Biol. Chem.* **247**, 7657–7662.
Noumi, T., Taniai, M., Kanazawa, H., and Futai, M. (1986). *J. Biol. Chem.* **261**, 9196–9201.
Noumi, T., Tagaya, M., Miki-Takeda, K., Maeda, M., Fukui, T., and Futai, M. (1987). *J. Biol. Chem.* **262**, 7687–7692.
Okada, Y., Hashimoto, T., Yoshida, Y., and Tagawa, K. (1986). *J. Biochem.* **99**, 251–256.
Ovchinnikov, Y. A., Modyanov, N. N., Grinkevich, V. A., Aldanova, N. A., Kostetsky, P. V., Trubetskaya, O. E., Hundal, T., and Ernster, L. (1984). *FEBS Lett.* **175**, 109–112.
Power, J., Cross, R. L., and Harris, D. A. (1983). *Biochim. Biophys. Acta* **724**, 128–141.
Pullman, M. E., and Monroy, G. C. (1963). *J. Biol. Chem.* **238**, 3762–3769.
Satre, M., de Jerphanion, M. B., Huet, J., and Vignais, P. V. (1975). *Biochim. Biophys. Acta* **387**, 241–255.
Schwerzmann, K., and Pedersen, P. L. (1981). *Biochemistry* **20**, 6305–6311.
Schwerzmann, K., and Pedersen, P. L. (1986). *Archiv. Biophys. Biochem.* **250**, 1–18.
Smith, J. B., and Sternweis, P. C. (1977). *Biochemistry* **16**, 306–311.
Tagawa, K., Hashimoto, T., and Yoshida, Y. (1986). In Methods in Enzymology (Fleisher, S. and Fleischer, B. eds) vol. 126, pp. 504–512, Academic Press, New York.
Tozer, R. G., and Dunn, S. D. (1987). *J. Biol. Chem.* **262**, 10706–10711.
Van de Stadt, R. J., de Boer, B. L., and Van Dam, K. (1973). *Biochim. Biophys. Acta* **292**, 338–349.
Walker, J. E., Fernley, I. M., Gay, N. J., Gibson, B. W., Northrop, F. D., Powell, S. J., Runswick, M. J., Saraste, M., and Tybulewicz, L. J. (1985). *J. Mol. Biol.* **184**, 677–701.
Woods, J. M., Wise, J. G., Senior, A. E., Futai, M., and Boyer, P. D. (1987). *J. Biol. Chem.* **262**, 2180–2186.
Yamada, E., and Huzel, N. J. (1988). *J. Biol. Chem.* **263**, 11498–11503.
Yoshida, Y., Wakabayashi, S., Matsubara, H., Hashimoto, T., and Tagawa, K. (1984). *FEBS Lett.* **170**, 135–138.