V-ATPase of Thermus thermophilus Is Inactivated during ATP Hydrolysis but Can Synthesize ATP*

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The ATP hydrolysis of the V₁-ATPase of Thermus thermophilus have been investigated with an ATP-regenerating system at 25 °C. The ratio of ATPase activity to ATP concentration ranged from 40 to 4000 μM; from this, an apparent Kₘ of 240 ± 24 μM and a Vₘₐₓ of 5.2 ± 0.5 units/mg were deduced. An apparent negative cooperativity, which is frequently observed in case of F₁-ATPases, was not observed for the V₁-ATPase. Interestingly, the rate of hydrolysis decayed rapidly during ATP hydrolysis, and the ATP hydrolysis finally stopped. Furthermore, the inactivation of the V₁-ATPase was attained by a prior incubation with ADP-Mg. The inactivated V₁-ATPase contained 1.5 mol of ADP/mol of enzyme.

Difference absorption spectra generated from addition of ATP-Mg to the isolated subunits revealed that the A subunit can bind ATP-Mg, whereas the B subunit cannot. The inability to bind ATP-Mg is consistent with the absence of Walker motifs in the B subunit.

These results indicate that the inactivation of the V₁-ATPase during ATP hydrolysis is caused by entrapping inhibitory ADP-Mg in a catalytic site.

Light-driven ATP synthesis by bacteriorhodopsin-V₁, V₃-ATPase proteoliposomes was observed, and the rate of ATP synthesis was approximately constant. ATP synthesis occurred in the presence of an ADP-Mg of which concentration was high enough to induce complete inactivation of ATP hydrolysis of V₃, V₁-ATPase. This result indicates that the ADP-Mg-inhibited form is not produced in ATP synthesis reaction.

V₃, V₁-ATPases and F₃, F₁-ATPases constitute two subclasses of the ATPase/ATP synthase superfamily (1, 2). V₃, V₁-ATPases are present in the membranes of lysosomes (3), clathrin-coated vesicles (4), chromaffin granules (5), and the central vacuoles of yeast (6). They are responsible for vacuolar acidification, which plays an important role in a number of cellular processes (1). V₃, V₁-ATPases are also found in the plasma membranes of most archaia (7–9) and some kinds of eubacteria (10–12). Several studies indicate that the physiological role of V₃, V₁-ATPases of some archaia and the thermophilic eubacterium Thermus thermophilus is ATP synthesis coupled to proton flux across the plasma membranes (7, 9, 13–15).

V₃, V₁-ATPases consist of two functional assemblies, a peripheral V₃ moiety and a membrane integrated V₁ moiety, which are counterparts of the F₃ and F₁ moiety of the F₃, F₁-ATPase (1, 15–17). The peripheral V₁ moiety is composed of two major subunits, A and B, and other minor subunits. Both structural analysis and sequence homology indicate an evolutionary relationship between V₃, V₁-ATPases and F₃, F₁-ATPase and that the A and B subunit of V₃, V₁-ATPase are homologous to the β and α subunit of F₃, F₁-ATPase (2). The A subunit of V₃, V₁-ATPases contains the Walker motifs (1), which are critical for nucleotide binding (18, 19). Labeling of the A subunit by 2-azido-[³²P]ATP correlates well with inactivation of ATPase activity, with complete inactivation observed upon modification of a single A subunit per complex (20). These findings indicate that the catalytic site of the V₃, V₁-ATPase is located on the A subunit. On the other hand, the B subunit of V₃, V₁-ATPases lacks Walker motifs. A recent study reported that the B subunit in the V₃, V₁-ATPase of clathrin-coated vesicles was modified by 3-O-(4-benzoylbenzoyladenosine 5'-triphosphate (21). However, any direct evidence for the nucleotide binding to the isolated B subunit of the V₃, V₁-ATPase has not been reported yet.

Structural similarity and sequence homology of the major subunits of V₃, V₁-ATPases and F₃, F₁-ATPases lead to the hypothesis that the mechanisms of ATP hydrolysis and ATP synthesis by V₃, V₁-ATPases are almost identical to those of F₃, F₁-ATPases. Nevertheless, the enzymatic properties of V₃, V₁-ATPases and F₃, F₁-ATPases are different (1). Whereas azide inhibits ATP hydrolysis by F₃, F₁-ATPases by stabilizing the inhibitory ADP-Mg-F₃, F₁-ATPase complex (22–24), it does not inhibit ATPase activity of V₃, V₁-ATPases (1, 10).

Precise understanding of V₃, V₁-ATPases would allow the comparison to F₃, F₁-ATPases and the elucidation of the common essential mechanism for the coupling of proton translocation across a membrane with ATP formation. However, several problems, such as the difficulty of obtaining a large amount of pure enzyme from vacuolar membranes and an unstable V₁ moiety (17), have limited our investigation of enzymatic properties of V₃, V₁-ATPases.

T. thermophilus, originally isolated from a hot spring in Japan, is thermophilic, obligatory aerobic, Gram-negative, and chemoheterotrophic eubacterium (25). Its respiratory chain may include energy coupling Site I (26). This bacterium has a large amount of the V₃, V₁-ATPase on the plasma membrane, instead of F₃, F₁-ATPase (15).

In contrast to eukaryotic equivalents, the V₁ moiety of T. thermophilus is easily detached from the membranes using chloroform treatment and ATPase-active stable complex can be obtained in large amounts (10). Throughout this manuscript, the V₁ moiety from T. thermophilus is referred to V₁-ATPase.

The V₁-ATPase consists of four kinds of subunit with ap-
ent molecular sizes of 66 (A or α), 55 (B or β), 30 (γ), and 11 (δ) kDa, which are present in a stoichiometry of A₂B₂γδ. Similar to its eukaryotic counterparts, the V₁-ATPase also shows enzymatic properties different from those of F₁-ATPases, such as low specific activity, high $K_m$ values, and resistance to azide inhibition (10). We previously reported a specific activity of the V₁-ATPase of about 0.1 units/mg of protein at 55 °C in the absence of an ATP-regenerating system.

In this report, we demonstrate the particular kinetic behaviors of the V₁-ATPase of *T. thermophilus* in the presence of ATP regenerating system, the nucleotide binding properties of the isolated A and B subunits, and ATP synthesis of a V₁V₁-ATPase co-reconstituted with bacteriorhodopsin for the first time.

**EXPERIMENTAL PROCEDURES**

**Materials—** V₁-ATPase and V₂V₁-ATPase were prepared from *T. thermophilus* plasma membranes using the previously described methods (10, 15). Bacteriorhodopsin (bR) is prepared from Halobacterium halobium (27). [*γ-32P]ATP was purchased from NEN Life Science Products. Radiolabeled ATP (9.25MBeq/ml) was diluted with nonradioactive ATP to the desired specific activity. Egg yolk phosphatidylcholine (type XVI-E) was purchased from Sigma. Other chemicals were purchased from Nacarai Corp.

**Construction of Overexpression Systems of the A and B Subunits and Purification of the Products—** The DNA fragments of the A and B subunits were PCR amplified using Es Taq™ polymerase (Takara) to minimize errors. The sequence of the amplified DNA fragments was confirmed using ABI 373A sequencer. The obtained DNA fragments was transformed into competent JM 103, the expression of subunit genes was induced by addition of 0.4 mm isopropyl-1-thio-β-D-galactopyranoside (IPTG).

More than 10% of the total soluble protein was the recombinant A subunit after induction, about 5% in case of the recombinant B subunit (see Fig. 5). Both subunits were purified by the same method. About 10 g of cells were obtained from an overnight culture, suspended in 50 ml of buffer containing 50 mM Tris-SO₄ (pH 8.0), 50 mM NaCl, and 0.1 mM EDTA, and disrupted by sonication. The membrane fraction and cell debris were removed by centrifugation, and the supernatant was applied onto a DEAE Sephacel column (2 × 10 cm) equilibrated with Buffer A (50 mM Tris-Cl (pH 8.0) and 0.1 mM EDTA). The column was washed with 200 ml of Buffer A, and the proteins were eluted with linear NaCl gradient (0–0.5 M). Fractions containing the recombinant subunits after SDS-PAGE analysis (15% of acrylamide gels). The fractions were combined, and solid ammonium sulfate was added to a final concentration of 1.2 M and stirred for 1 h. After precipitation by centrifugation, the supernatant was applied onto a butyl-tosopearl column (Tosoh Corp.; 2 × 10 cm). Proteins were eluted with a reverse ammonium sulfate gradient (1.2–0 M). Fractions containing the subunit were combined, and proteins were precipitated by ammonium sulfate. The precipitate was dissolved with a minimum volume of Buffer A, and it was applied on a Sephacryl S-300 gel permeation column (1 × 50 cm) equilibrated with Buffer A plus 50 mM Na₂SO₄. The column was eluted with the same buffer and fractions containing the subunits were chosen after SDS-PAGE analysis (15% of acrylamide gels). The purified subunits were stored at 4 °C until use.

**Analytical Methods—** The protein concentrations of V₁-ATPase were determined by measurement of absorbance at 280 nm using a factor of 0.59 for the absorbance of 1 mg/ml of protein. The factor of 0.59 was determined by quantitative total amino acid analysis and spectral data. Unless otherwise specified, ATPase activity was measured at 25 °C with an enzyme-coupled ATP-regenerating system. The reaction mixture contained 50 mM Tris-Cl (pH 8.0), 100 mM KCl, different concentrations of ATP-Mg, 2.5 mM phosphoenolpyruvate, 50 μg/ml pyruvate kinase, 50 μg/ml lactate dehydrogenase, and 0.2 mM NADH in a final volume of 1.2 ml. Typically, the reaction was started by addition of the enzyme dissolved in 50 mM Tris-SO₄ (pH 8.0), 50 mM Na₂SO₄, 0.2 mM EDTA to 1.2 ml of the assay mixture, and the rate of ATP hydrolysis was measured as the rate of oxidation of NADH determined by the rapid inactivation was only observed at high concentrations of ATP. The inactivation almost disappeared and the rate of ATP hydrolysis almost constant during turnover at 30 μM ATP.
ATP (Fig. 1, a and b, trace 0.03 mM). The V_oV_1-ATPase also exhibited similar activation and inactivation phases in hydrolyzing 0.01–4 mM ATP-Mg (Fig. 1c). The time-dependent changes were analyzed using a simple sequential model for the activation and inactivation processes, and the apparent first order rate constants for each process were calculated by non-linear regression fitting. The apparent rate constants were plotted against the ATP concentrations. As shown in Fig. 2, the rate constants of inactivation exhibited a monophasic dependence on ATP concentration, and the half-maximum rate of inactivation was attained at 140 mM ATP. The associated maximum inactivation rate constant was 0.0055 s⁻¹. We calculated the ATP concentration for the half-maximum rate of activation with the same method. It was 70 mM ATP, and the associated maximum activation rate constant was 0.65 s⁻¹ (Fig. 2a).

By analogy to the inactivation of F_1-ATPases (24, 31, 32), we suspected that the inactivation was caused by entrapment of inhibitory ADP at a catalytic site. To investigate the effect of ADP for the inactivation, we assayed the ATPase activity of V_1-ATPase by the measurement of inorganic phosphate with or without the ATP regenerating system. When 50 μg/ml pyruvate kinase and 2 mM phosphoenolpyruvate were present in the assay mixture, the hydrolysis of ATP proceeded linearly up to 4 min after addition of enzyme. Then, the rate of hydrolysis gradually decelerated 5 min after the reaction was started. On the other hand, in the absence of the ATP-regenerating system, the deceleration of the rate of hydrolysis occurred more rapidly, and the hydrolysis completely stopped at 6 min (Fig. 3a). The apparent rate constant of inactivation of the V_1-ATPase under these conditions was about 3 × 10⁻³ s⁻¹ in the presence of an ATP-regenerating system, and about 7 × 10⁻³ s⁻¹ in the absence of an ATP regenerating system. These values are in the same order of magnitude as the value deduced in Fig. 2a. Fig. 3b shows the effect of ADP in the assay mixture on the rate of hydrolysis of ATP. Various amounts of ADP were added to the assay mixtures in the absence of a regenerating system, and the hydrolysis of 4 mM ATP was assayed. Increasing ADP concentrations in the assay mixtures led to a decrease of the rate of ATP hydrolysis.

Furthermore, 1 μM V_1-ATPase was preincubated with various concentrations of ADP-Mg for 120 min at 25 °C, and the residual ATPase activities were measured in the presence of 4 mM ATP-Mg. As shown in Fig. 3c, the extent of inactivation of ATP Synthesis of V-type H⁺-ATPase
the V1-ATPase was dependent on the concentration of added ADP-Mg.

These results strongly suggest that the mechanism of inactivation is similar to that of the ADP-Mg inhibition observed for F1-ATPase.

Kinetics of ATP Hydrolysis by V1-ATPase—Because the ATP hydrolysis by V1-ATPase did not proceed linearly over time, it is difficult to define the rate at a given ATP concentration. In the present study, we plotted the maximum rate of ATP hydrolysis at each ATP concentration (Fig. 4). The ATP concentration ranged from 40 to 4000 μM. The kinetic data were also plotted in V/S versus V form (inset, Eadie-Hofstee plot). From this plot, an apparent K<sub>m</sub> of 240 ± 24 μM and an apparent V<sub>max</sub> of 5.2 ± 0.5 units/mg were deduced. An apparent negative cooperativity that is frequently observed for FoF<sub>1</sub>- or F<sub>1</sub>-ATPase (31, 33–41, 43) was not observed for the V<sub>1</sub>-ATPase here.

Binding of ATP to the Isolated Subunits—The kinetics features of inactivation of V<sub>1</sub>-ATPase during the ATPase reaction described above are similar to those recently reported for the mutant F<sub>1</sub>-ATPase, which lacks nucleotide binding at the noncatalytic sites (32). In that case, turnover-dependent inactivation was explained as the failure to recover from the ADP inhibited state due to the inability of nucleotide binding at noncatalytic sites. If the V<sub>1</sub>-ATPase lacks nucleotide binding to the noncatalytic subunit (subunit B), the similarity of V<sub>1</sub>-ATPase to mutant F<sub>1</sub>-ATPase is well understood. The lack of Walker motifs of B subunit of V<sub>1</sub>-ATPase further underlined this idea. Thus, in an attempt to characterize nucleotide binding to the isolated subunits of V1-ATPase, we constructed overexpression systems for the A and B subunits and purified the products. We could successfully obtain large amounts of these subunits (Fig. 5). The isolated recombinant A and B subunits migrated as single protein bands with little contamination.

Difference absorption spectra have been used to probe the binding of adenine nucleotides to the F<sub>1</sub>-ATPase and its isolated subunits (29). When these proteins bind ADP or ATP, difference spectra are induced by a red shift of the absorption maximum accompanied by a slight decrease of the magnitude. The difference absorption spectra induced by the interaction of ATP with the isolated A or B subunits showed significantly different profiles (Fig. 6). Upon addition of ATP-Mg to the isolated A subunit, a trough at 260 nm and a peak at 280 nm were observed, and the magnitudes of the peak and the trough depended on the amount of the added ATP. Saturation was
observed when the concentration of ATP reached about an equimolar concentration to the A subunit (Fig. 6, inset). In contrast, neither a trough nor a peak was observed for the B subunit (Fig. 6b).

2',3'-O-(2,4,6-Trinitrophenyl)-ATP is known to have a significantly higher affinity for the nucleotide binding sites of F₁-ATPase and to give a clear difference spectrum in the visible wavelength region upon binding (29, 42, 43). The difference spectrum was induced by addition of 2',3'-O-(2,4,6-trinitrophenyl)-ATP to the isolated A subunit, but was not induced in case of the B subunit (data not shown). These results suggest that the isolated A subunit can bind 1 mol of ATP-Mg per enzyme, but the isolated B subunit cannot bind ATP-Mg. The ability and inability of ATP binding of the A and B subunit are consistent with the presence and absence of the Walker motifs in these subunits.

Analysis of Bound Nucleotide of V₁-ATPase—To analyze bound nucleotide, the V₁-ATPase was preincubated with or without 1 mM ADP-Mg for 60 min, and free nucleotides were removed with a Sephadex G-50 centrifuge column. The enzyme was denatured with perchloric acid, and the amount of released adenine nucleotide was quantified by anion exchange high performance liquid chromatography. The analysis revealed that endogenous ADP on the purified V₁-ATPase preparation was less than 0.1 mol per mol of enzyme, whereas ADP-Mg-preincubated V₁-ATPase contained 1.5 mol of ADP per mol of the enzyme.

T. thermophilus V₁V₁-ATPase Synthesizes ATP Coupled to Proton Flux—ATP synthesis was started by the addition of 2 mM MgSO₄ to the assay mixture. The aliquots of 50 μl were taken at indicated time, and 50 μl of 4% trichloroacetic acid were added. After neutralization by the addition of 20 μl of 2 x potassium-phosphate buffer (pH 7.5), ATP content was measured. a, shown are time courses of ATP synthesis in the presence (○) or absence (●) of 20 μM carbonyl cyanide p-trifluoromethoxyphenylhydrazone in the reaction mixture. Inset, light-driven proton pumping of V₁V₁-ATPase-bR proteoliposomes. a, analysis of the V₁V₁-ATPase by 15% of SDS-PAGE. Lane 1, molecular size standards (97, 66, 30, 45, 21, and 14 kDa); lane 2, 10 μg of V₁V₁-ATPase; lane 3, 20 μg of V₁V₁-ATPase.
After preillumination for 15 min to generate a stable transmembrane pH gradient, the ATP synthesis reactions were started by addition of MgSO₄ (indicated as time 0 in Fig. 7). The synthesized ATP was measured with the luciferin-luciferase assay. ATP synthesis proceeded with a constant rate for 40 min after starting the reaction. The constant rate of ATP synthesis was found to be 0.67 μmol of ATP mg⁻¹min⁻¹, which is 3–4 times larger than the one of F₄F₅-ATPase from thermophilic bacterium, PS3 (30). The time-dependent inhibition by ADP observed in case of ATP hydrolysis was not observed in ATP synthesis.

DISCUSSION

Inactivation of V₁-ATPase May Be Caused by Entrapment of Inhibitory ADP-Mg in a Catalytic Site—The results presented clearly show three distinct phases of ATP hydrolysis by the V₁-ATPase in the presence of an ATP regenerating system (Fig. 1, a and b). The initial lag apparently shows the presence of initial inhibited species of V₁-ATPase. The inhibited enzyme changed rapidly to an active form. The cause of this initial inhibition is not clear at present. The activated enzyme was re-inactivated during turnover. The apparent half-maximum rate of the inactivation was attained at 140 μM ATP, which reflects the affinity of the ATP binding site for the inactivation. The presence of ADP-Mg in the assay mixture increased the rate of turnover-dependent inactivation.

The specific activity of V₁-ATPase is 5.2 unit/mg protein at 25 °C in the presence of an ATP-regenerating system, but we previously reported that the specific activity of V₁-ATPase was about 0.1 unit/mg protein at 55 °C in the absence of ATP regenerating system (10). Probably, the low specific activity was due to the turnover-dependent inactivation of the V₁-ATPase. In addition, contaminant ADP in the ATP solution could increase the rate of inactivation, so that ATP hydrolysis almost stopped 1–2 min after starting the reaction.

Previously, a similar slow inactivation of yeast V-ATPase was reported by Khibak et al. (44). They showed sulfite eliminates the inactivation, but in our case, 33 mM Na₂SO₃ apparently inhibited the initial rate of the ATP hydrolysis of T. thermophilus V₁-ATPase (data not shown).

In the case of mitochondrial F₁-ATPase, Jault and Allison (34) indicated that three kinetics phases were present at low ATP concentration. An initial burst phase decelerated rapidly to a slow intermediate phase, which, in turn, gradually accelerated to a final steady-state rate. They postulated that the transition of the initial burst phase to the slow intermediate phase was caused by accumulation of inhibitory ADP-Mg at a catalytic site and that the transition of the intermediate phase to the final steady state was caused by binding of ATP to noncatalytic sites, which promoted the dissociation of inhibitory ADP-Mg from the affected catalytic site (31, 34). Recently, Matsui et al. (32) observed a rapid and nearly complete turnover-dependent inactivation of a mutant F₁-ATPase, which lacks the ability of nucleotide binding at noncatalytic sites. The mutant F₁-ATPase was also completely inactivated by prior incubation with stoichiometric ADP-Mg; thus, they concluded that the entrapment of ADP-Mg in a catalytic site caused the turnover-dependent inactivation. Interestingly, they also observed the ATP concentration dependence of the rate of inactivation and found that the half-maximum rate of inactivation was attained at 5 μM ATP, which coincides with one of the two Kᵣ values, about 4 μM, obtained from initial rate analysis (32).

In this study, we observed a similar inactivation of V₁-ATPase during turnover of ATP hydrolysis. The Kᵣ of 240 μM determined for the hydrolysis of 40–4000 μM ATP may be comparable to the apparent Kᵣ of 140 μM for inactivation. Furthermore, nearly complete inactivation of V₁-ATPase was attained by prior incubation of V₁-ATPase with ADP.

The analogy of the inactivation of F₁-ATPases leads us to postulate that the inactivation of V₁-ATPase is caused by the entrapment of inhibitory ADP-Mg on the catalytic sites. The role of the noncatalytic nucleotide binding sites of F₁-ATPase during ATP hydrolysis is the release of inhibitory ADP bound at the catalytic sites. It was shown that a mutant of F₁-ATPase that lacked the ability of nucleotide binding on the noncatalytic sites exhibited strong inhibition by ADP (32). The strong inactivation of the V₁-ATPase seems similar to that of the mutant of F₁-ATPase. Actually, the B subunit of the V₁-ATPase does not have a region homologous to the Walker motifs A and B. As expected, the interaction of ATP and the isolated B subunit was not observed in measurements of difference spectra. Taken together, we prefer the view that the noncatalytic B subunit in the T. thermophilus V₁-ATPase does not bind nucleotide or has only a very weak affinity for nucleotides. The results obtained suggest that the inactivation of V₁-ATPase was due to the failure of binding of ATP to noncatalytic sites.

Endogenous ADP on V₁-ATPase was less than 0.1 mol per mol of enzyme, whereas 1.5 mol of ADP per mol of the enzyme was bound to V₁-ATPase after the preincubation with 1 mM ADP-Mg for 1 h. Because the loaded nucleotides could not be removed by centrifugation elutions, this nucleotide is thought to bind the catalytic site with high affinity.

The mechanism of initial activation of V₁-ATP is unknown. Unlike the irreversibly turnover-dependent inactivated enzyme, the initial inhibited enzyme is rapidly activated by the binding of ATP. Furthermore, the initial activation occurred at a lower ATP concentration range than the turnover dependent inactivation. These results clearly show that the initial inhibited form is not identical to the irreversible inactivated form. Further studies will be necessary to clarify the characteristics of the bound adenine nucleotides and the initial inhibited form.

V₁V₅-ATPase Can Synthesize ATP in Co-reconstituted Liposomes—Several findings indicate that the physiological role of V₁V₅-ATPases in some archa and T. thermophilus is the synthesis of ATP coupled to a proton flux (7–10, 13, 15, 25, 26). The results in this study give direct evidence for the ability of T. thermophilus V₁V₅-ATPase to synthesize ATP coupled to proton flux. This is the first report of ATP synthesis with a reconstituted proteoliposome of a V₁V₅-ATPase. We used the V₁V₅-ATPase-hR co-reconstituted proteoliposomes because a steady pH potential is attained by light-induced proton pumping. The reaction mixture for ATP synthesis contained 2 mM ADP-Mg, which is sufficient to induce complete inactivation of the V₁V₅-ATPase for ATP hydrolysis. However, ATP synthesis continued for up to 40 min indicates that ADP-Mg-induced inactivation does not occur under ATP synthesis conditions. It is possible that the membrane potential and/or the pH gradient protects V₁V₅-ATPase from ADP-Mg inactivation.

The particular characteristics of T. thermophilus V₁V₅-ATPase, where ATP hydrolysis-turnover-dependent inactivation occurs under ATP hydrolysis condition but ATP synthesis is not inhibited in the presence of a proton motive force, are thought to be favorable for physiological ATP synthesis. When the proton motive force is close to zero for the cell, the hydrolysis of intracellular ATP may be inhibited by the generation of the inactivated species of the V₁V₅-ATPase, so that a rapid decrease of intracellular ATP is avoided.

During the preparation of this report, an interesting paper by Bald et al. was published (45). They reconstituted the mutant F₁F₅-ATPase, which lacks nucleotide binding to the noncatalytic site, into liposomes with hR and examined light-driven ATP synthesis. Contrarily to the quickly inhibition of
ATP hydrolysis activity, the mutant F_{0}F_{1}-ATPase synthesized ATP at nearly constant rate up to 60 min. This results further reinforces the similarity of the V_{0}V_{1}-ATPase to the mutant F_{0}F_{1}-ATPase, which lacks nucleotide binding to the noncatalytic subunit.

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REFERENCES

1. Forgac, M. (1989) Physiol. Rev. 69, 765–796
2. Gogarten, J. P., Kibak, H., Taiz, L., Bowman, E. J., Bowman, B. J., Manolson, M. F., Poole, R. J., Date, T., Oshima, T., Konishi, J., Denda, K., and Yoshida, M. (1989) Biochim. Biophys. Acta 102, 1379–1387
3. Arai, K., Simaya, K., Hiratani, N., and Ohkuma, S. (1992) J. Biol. Chem. 267, 837–841
4. Arai, H., Terres, G., Pink, S., and Forgac, M. (1988) J. Biol. Chem. 263, 8796–8802
5. Moriyama, Y., and Nelson, N. (1989) J. Biol. Chem. 264, 3577–3582
6. Uchida, E., Ohsumi, Y., and Anraku, Y. (1985) J. Biol. Chem. 260, 1090–1095
7. Isotani, K. (1986) J. Bacteriol. 167, 837–841
8. Konishi, J., Wakagi, T., Oshima, T., and Yoshida, M. (1987) J. Biochem. 102, 1379–1387
9. Namba, T., and Mukohata, Y. (1987) J. Biochem. 102, 591–598
10. Yokoyama, K., Oshima, T., and Yoshida, M. (1990) J. Biol. Chem. 265, 21946–21950
11. Kakinuma, Y., and Igarashi, K. (1990) FEBS Lett. 241, 97–101
12. Honer, Z. U., Bentrup, K., Ubbink-Kok, T., Lolkema, J. S., and Konings, W. N. (1997) J. Bacteriol. 179, 1274–1279
13. Mukohata, Y., Isayama, M., and Fujikawa, A. (1986) J. Biochem. 101, 1–8
14. Lübben, M., and Schafer, G. (1988) J. Bacteriol. 171, 6106–6116
15. Yokoyama, K., Akahane, Y., Ishii, N., and Yoshida, M. (1994) J. Biol. Chem. 269, 12248–12253
16. Bowman, B., Dschida, J. W., Harriss, T., and Bowman, J. E. (1989) J. Biol. Chem. 264, 15606–15612
17. Moriyama, Y., and Nelson, N. (1987) J. Biol. Chem. 262, 14725–14729
18. Yoshida, M., and Amano, T. (1995) FEBS Lett. 359, 1–5
19. Abrahams, J. P., Leslie, A. G., Lutter, R., and Walker, J. E. (1994) Nature 370, 621–628
20. Zhang, J., Vasilyeva, E., Feng, Y., and Forgac, M. (1995) J. Biol. Chem. 270, 15494–15500
21. Vasilyeva, E., and Forgac, M. (1997) J. Biol. Chem. 272, 12775–12782
22. Muneyuki, E., Makino, M., Kamata, H., Kagawa, Y., Yoshida, M., and Hirata, H. (1993) Biochim. Biophys. Acta 1144, 62–68
23. Vasilyeva, E. A., Minkov, I. B., Tritin, A. F., and Vinogradov, A. D. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 21571–21578
24. Jault, J. M., Dou, C., Grolsky, A. N., Matsui, T., Yoshida, M., and Allison, S. W. (1996) J. Biol. Chem. 271, 28818–28824
25. Oshima, T., and Imabori, K. (1974) Int. J. Syst. Bacteriol. 24, 102–112
26. McKay, A., Quitter, J., and Jones, C. W. (1982) Arch. Microbiol. 131, 43–50
27. Oesterhelt, D., and Stoeckenius, W. (1973) Proc. Natl. Acad. Sci. U. S. A. 70, 2853–2857
28. Yokoyama, K., Hisabori, T., and Yoshida, M. (1989) J. Biol. Chem. 264, 21837–21841
29. Hisabori, T., Muneyuki, E., Odaka, M., Yokoyama, K., Mochizuki, K., and Yoshida, M. (1990) J. Biol. Chem. 265, 4551–4556
30. Richard, P., Pitard, B., and Rigaud, J. L. (1995) J. Biol. Chem. 270, 21571–21578
31. Jault, M. J., and Allison, S. W. (1994) J. Biol. Chem. 269, 313–325
32. Matsui, T., Muneyuki, E., Honda, M., Allison, S. W., Dou, C., and Yoshida, M. (1997) J. Biol. Chem. 272, 8215–8222
33. Boyer, P. D. (1993) Biochim. Biophys. Acta 1140, 215–250
34. Jault, J. M., and Allison, S. W. (1995) J. Biol. Chem. 268, 1558–1566
35. Ebel, R. E., and Lardy, H. A. (1975) J. Biol. Chem. 250, 191–196
36. Gresser, M. J., Myers, J. A., and Boyer, P. D. (1982) J. Biol. Chem. 257, 12030–12038
37. Cross, R. L., Grubmeyer, C., and Penefsky, H. S. (1982) J. Biol. Chem. 257, 12101–12105
38. Wong, S. Y., Matsuoka-Yagi, A., and Hafezi, Y. (1984) Biochemistry. 23, 5004–5010
39. Muneyuki, E., and Hirata, H. (1988) FEBS Lett. 234, 455–458
40. Matsuda, C., Muneyuki, E., Endo, H., Yoshida, M., and Kagawa, Y. (1994) Biochim. Biophys. Acta 1188, 108–116
41. Kato, Y., Sasa, T., Muneyuki, E., and Yoshida, M. (1995) Biochim. Biophys. Acta 1231, 275–281
42. Muneyuki, E., Hisabori, T., Sasa, T., Mochizuki, K., and Yoshida, M. (1996) Biochim. Biophys. Acta 1268, 23325–23333
43. Muneyuki, E., Hisabori, T., Allison, W. S., Jault, J. M., Sasa, T., and Yoshida, M. (1994) Biochim. Biophys. Acta 1188, 108–116
44. Kibak, H., Eckhout, V. D., Cultler, T., Taiz, L. S., and Taiz, L. (1993) J. Biol. Chem. 268, 23325–23333
45. Bald, D., Amano, T., Muneyuki, O., Pitard, B., Rigaud, J.-L., Kreup, J., Hisabori, T., Yoshida, M., and Shibata, M. (1996) J. Biol. Chem. 273, 865–870