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EFFECT OF ADP ON ATPase FROM A STRAIN OF *BACILLUS STEAROTHERMOPHILUS*

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Summary

Bacillus stearothermophilus ATCC 12016 was unable to grow at temperatures below 40°C. On incubating the bacteria at the temperatures, ATP in cells disappeared, ADP was accumulated and ATPase (EC 3.6.1.3) was inactivated. When the purified ATPase was incubated at the temperatures for 1 h with 0.17 mM ADP in the presence of $MgCl_2$, the enzyme was completely inactivated. The inactivated enzyme was reactivated on dilution or dialysis or on warming at 65°C. During the incubation of the enzyme sample, the absorbance spectrum of the enzyme changed. On further incubating the sample over 1.5 h, the second step of spectral change occurred together with the change of the circular dichroism and the dissociation into a lower molecular weight species of the protein. When the enzyme was treated with ADP-$MgCl_2$ at 65°C, the inactivation and conformational change of the enzyme was not observed.

Introduction

It was shown that *Bacillus stearothermophilus* ATCC 7953, one of the obligate thermophilic bacteria, died at temperatures below 35°C, at which the organism was unable to grow [1]. Recent studies have revealed that another strain of *B. stearothermophilus*, (ATCC 12016), exhibited no growth at temperatures below 40°C, but the organism exhibited no death at such temperatures. During the studies, it has been found that no change of the activity of ATPase (EC 3.6.1.3) was observed with *B. stearothermophilus* ATCC 7953 on incubating at temperatures below 35°C, while the enzyme of the strain ATCC 12016 was inactivated on incubating the organism at temper-

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atures below 40° C. The ATPase purified from the strain 12016 was stable at 0—4° C for 1 week [2]. The inactivation of the enzyme, when the strain ATCC 12016 was incubated at temperatures below 40° C, may be ascribable to some factor(s) other than cold inactivation. The present study, therefore, deals with the cause of the inactivation of the thermophile ATPase from *B. stearothermophilus* ATCC 12016, when incubated at temperatures below 40° C.

Materials and Methods

Microorganism and culture. B. stearothermophilus ATCC 12016 (NCA 2184) was aerobically cultured at 65° C in a peptone medium, as described previously [2]. The culture in a middle logarithmic phase was used in the following experiments.

Preparation and assay of ATPase. The culture was centrifuged, and from the cells thus harvested was prepared a purified or membrane-bouns ATPase, according to the procedure as described previously [2]. The purified enzyme was homogeneous, as judged from the disc-electrophoretic pattern of the protein.

The culture in a middle logarithmic phase was transferred to 4 or 30° C and aerobically incubated for the periods as indicated in the text. The cells collected by centrifugation were disrupted in a French pressure cell and centrifuged at 5000 × *g* for 10 min. The cell-free extracts thus obtained were divided into two portions. A portion was subjected to the determination of ATP and ADP. Another portion was centrifuged at 25 000 × *g* for 20 min, and the membrane fraction was subjected to the ATPase assay. The ATPase activity determined with the membrane fraction was expressed in mmol P_i formed in 10 min per g wet weight cells.

The assay of the enzyme in a purified or membrane-bound state was carried out at 65° C, according to the procedure as described previously [2].

Assay of ATP and ADP. To the cell-free extracts was added 2% (final concentration) $HClO_4$, and the precipitates were removed by centrifugation. After the supernatant was neutralized with 0.5 M KOH, the amounts of ATP and ADP were determined chromatographically on DEAE-Sephadex A-25, according to the procedure of Caldwell [3].

Spectroscopic observation. The solution containing the purified ATPase and ADP (or AMP) in 3 ml of 20 mM Tris · HCl buffer (pH 8.0) was pipetted into the sample and reference cuvettes. After temperature equilibration, 0.1 ml of $MgCl_2$ solution or water was added to the sample or reference cuvette, respectively, and the difference spectrum was measured in a Hitachi recording spectrophotometer, EPS-3T. The final concentrations of the enzyme, ADP (AMP) and $MgCl_2$ were 3.0 μM, 0.17 mM and 0.17 mM, respectively.

Circular dichroism measurement. Circular dichroism (CD) measurement was made with a Jasco ORD/UV-5 recording spectropolarimeter with a CD attachment, using a quartz cell of 2 or 5 mm path length. The sample contained 1.1 μM the purified ATPase, 0.1 mM ADP (AMP) and 0.1 mM $MgCl_2$ in 20 mM Tris · HCl buffer (pH 8.0).

Ultracentrifugal analysis. Ultracentrifugal analysis was carried out in a Hitachi ultracentrifuge, UCA-1, with phase plate Schlieren optics at 20 or

65° C. The sample for analysis contained 10.7 μM the purified ATPase with or without 5 or 50 mM ADP (AMP) and 5 or 50 mM $MgCl_2$ in 20 mM Tris · HCl buffer (pH 8.0), respectively. The sample was incubated for the periods indicated in the text before the analysis.

Measurement of viable count. Viable count of a culture incubated at temperatures below 40° C was carried out according to the procedure as described previously [1].

Measurement of protein. Protein concentration was determined by the method of Lowry et al. [4], using crystalline bovine serum albumin as standard.

Results

ATPase activity of the bacteria incubated at temperatures below 40° C

When *B. stearothermophilus* ATCC 12016 exhibiting a maximum growth rate at 65° C was incubated at temperatures below 40° C, no growth of the organism was observed. Unlike *B. stearothermophilus* ATCC 7953 (ref. 1), no change in viable count of the culture of the strain 12016 was observed at such temperatures. When the strain 12016 was aerobically incubated in a culture medium at 4° C (or 30° C), the ATPase activity of the organism, when expressed in mmol P_i formed in 10 min per g wet weight cells, gradually decreased and attained to a constant level (40—50% of the original activity) after 4—5 h of incubation (Fig. 1). The thermophile ATPase, either in a purified or membrane-bound state, obtained from the bacteria grown at 65° C was stable at 0—4° C [2]. The decrease in the enzymatic activity of the ATPase of the bacteria, when incubated at temperatures below 40° C, may be considered to be due to some factor(s) other than the cold inactivation, which has been reported with mesophile ATPases [5—9].

When the bacteria were aerobically incubated at 4 or 30° C, the amount of ATP in cells decreased, and concurrently that of ADP increased (Fig. 1). After 3 h of incubation, ATP completely disappeared, and the amount of ADP attained to a constant level. As shown in Fig. 1, the ATPase activity of the bacteria began to decrease after 1 h of incubation, and reached a constant level (50% of the original activity) after 4—5 h of incubation. The results shown

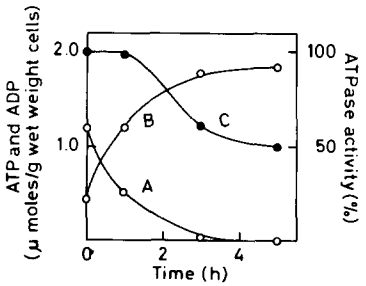


Fig. 1. Changes of the contents of ATP (A) and ADP (B) and ATPase activity (C) of the bacteria incubated at 30° C. The enzyme activity with the bacteria before the incubation at 30° C was 1.0 mmol of P_i formed in 10 min per g wet weight cells.

in the figure seem to suggest some relationship between the ADP concentration and ATPase activity of the bacteria. As the ATPase activity was assayed with the membrane fraction, the decrease in the ATPase activity of the bacteria may not be ascribable to the ADP inhibition of the enzyme. A possibility that the enzyme in cells may have been inactivated by ADP during the incubation of the bacteria at 30° C or below may be considered. The change in the activity of the purified ATPase on incubation with ADP was then examined.

Effect of ADP on the ATPase activity

The purified enzyme (1.1 μM) was incubated with ADP (up to 10 mM) at 4 or 30° C for 5 h, and the sample was assayed after dilution with 20 mM Tris · HCl buffer (pH 8.0). No change in the enzyme activity was observed. The enzyme incubated with low concentrations of ADP (0.17 mM) in the presence of $MgCl_2$ (0.17 mM) at 30° C was diluted with 100 volumes of Tris buffer containing 0.17 mM ADP-$MgCl_2$ and then the activity was measured at 65° C in the presence of 0.17 mM ADP. As shown by curve A in Fig. 2, the enzyme lost the activity almost completely after 1 h of incubation. Since the enzyme was not inhibited by 0.17 mM ADP at 65° C, the enzyme may have been inactivated on incubating with 0.17 mM ADP-$MgCl_2$ at 30° C for 1 h. When the enzyme sample incubated with ADP-$MgCl_2$ at 30° C for 1 h was diluted with 100 volumes of 20 mM Tris · HCl buffer (pH 8.0) and assayed in the absence of ADP, no change in the activity of the enzyme was observed for 1 h of incubation, and since then the activity gradually decreased (curve B). The results suggest that the ADP-$MgCl_2$-inactivated enzyme may have been reactivated on dilution of the sample. As shown by curve C, the extent of reactivation of the ADP-$MgCl_2$-treated ATPase was greater on dialysis than on dilution. Similar results were observed with up to 5 mM ADP-$MgCl_2$ and at temperatures below 40° C. When the enzyme treated with 10 mM ADP-$MgCl_2$ was diluted with 100 volumes of Tris buffer and assayed in the absence of ADP, the activity was found to decrease rapidly as shown by curve D in Fig. 2.

When increasing concentration of $MgCl_2$ was added to the enzyme solution containing a fixed amount of ADP, the enzyme was inactivated to an increasing

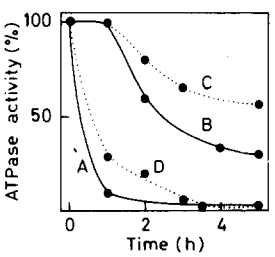


Fig. 2. Effect of ADP on the purified ATPase activity. (A) The enzyme (1.1 μM) incubated with 0.17 mM ADP and 0.17 mM $MgCl_2$ in 20 mM Tris · HCl buffer (pH 8.0) at 39° C was diluted with 100 volumes of Tris buffer containing 0.17 mM ADP-$MgCl_2$ and assayed in the presence of 0.17 mM ADP. (B) The enzyme treated with 0.17 mM ADP-$MgCl_2$ at 30° C was diluted with 100 volumes of Tris buffer and assayed in the absence of ADP. (C) The enzyme treated with 0.17 mM ADP-$MgCl_2$ at 30° C was dialyzed against Tris buffer at 4° C overnight and assayed in the absence of ADP. (D) The enzyme treated with 10 mM ADP-$MgCl_2$ at 30° C was diluted with 100 volumes of Tris buffer and assayed in the absence of ADP. The assay mixture contained 0.03 μM ATPase.

extent, and a maximum inactivation was observed when the molar ratio of $MgCl_2$/ADP was more than unity. The results may indicate that the thermophile ATPase was inactivated by the binding of ADP-$MgCl_2$ (1 : 1) complex to the protein.

When the enzyme was incubated with 10 mM ADP-$MgCl_2$ at 4° C, no change in the activity was observed for at least 5 h. When the enzyme was treated with 0.17 mM ADP-$MgCl_2$ at 65° C and was diluted with 100 volumes of Tris buffer containing 0.17 mM ADP-$MgCl_2$ and assayed in the presence of 0.17 mM ADP, the activity showed no change even after 5 h of incubation of the sample.

When the membrane-bound ATPase (10 mg protein membrane/ml) was incubated with 5 mM ADP-$MgCl_2$ at 30° C and diluted with 100 volumes Tris buffer and assayed in the absence of ADP, no change in the enzyme activity was observed for 1 h of incubation. Since then the activity gradually decreased and attained to a constant level (50—60% of the original activity) after 5 h of incubation. When the membrane sample treated for 5 h was dialyzed against Tris buffer at 4° C and assayed in the absence of ADP, the activity was restored to 90% of the original activity.

Effect of ADP on the absorption spectrum of ATPase

When the purified ATPase (3.0 μM) was incubated with 0.17 mM ADP at 30° C, no spectral change of the solution was observed even after 4 h of incubation. Addition of $MgCl_2$ (0.17 mM) to the solution, however, caused a slow spectral change of the mixture. Fig. 3 shows the change in the differece spectrum of the enzyme-ADP solution between in the presence and absence of $MgCl_2$. The spectrum exhibited a trough at 264 nm and a small peak at 283 nm. The difference spectrum of ADP between in acidic and neutral solution exhibited a trough at 262 nm (curve C in Fig. 3). Essentially no spectral change was observed with a mixture of 0.17 mM ADP and 0.17 mM $MgCl_2$ at 30° C for 5 h. The spectral change of the mixture of enzyme-ADP on addition of $MgCl_2$ may be due to the state change of ADP (the 264-nm trough) and the conformational change of the enzyme (the 283-nm peak).

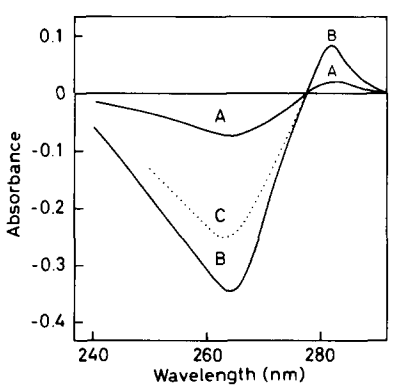


Fig. 3. Changes of the absorbance spectrum of the mixture of ATPase and ADP on addition of $MgCl_2$. The difference spectrum of the mixture of 3.0 μM ATPase and 0.17 mM ADP between in the presence and absence of 0.17 mM $MgCl_2$ was observed 5 (A) and 60 min (B) after the addition of $MgCl_2$ at 30° C. Difference spectrum of 0.1 mM ADP between in acidic (0.1 M HCl) and 0.1 M phosphate buffer (pH 7.0) was observed at 30° C (C).

The spectral change of the enzyme-ADP solution induced by $MgCl_2$ attained to a constant level about 1 h after the addition of $MgCl_2$ (Fig. 4). On further incubation of the mixture, the second step of spectral change of both the enzyme and ADP commenced about 1.5 h after the addition of $MgCl_2$ and completed after 4—5 h of incubation. Similar spectral change of the mixture of the enzyme, ADP and $MgCl_2$ was observed at temperatures below 40°C. The amount of the spectral change of the enzyme on addition of ADP-$MgCl_2$ decreased on increasing the incubation temperature over 40°C (curve A in Fig. 6). At 65°C no spectral change was observed even after 5 h of incubation. When ADP of the concentrations above 0.17 mM was used, spectral measurement became difficult, because of a strong absorbance of ADP in the ultraviolet region.

When AMP was used instead of ADP, no spectral change of the enzyme was observed even after 3 h of incubation at 30°C. When $5 \cdot 10^{-5}$ M NaN_3, a potent inhibitor of the thermophile ATPase [2], was present in the mixture of the enzyme, ADP and $MgCl_2$, no spectral change of the mixture was observed at 4 or 30°C.

When the enzyme treated with 0.17 mM ADP-$MgCl_2$ for 55 min at 30°C was warmed at 65°C, the absorbance spectrum of the sample which had changed during the treatment at 30°C was found to revert to the original one as shown by curves C and D in Fig. 4. The activity of the enzyme lost during the incubation at 30°C was almost completely recovered when the spectrum of the enzyme reverted to the original one. When the enzyme treated with 0.17 mM ADP-$MgCl_2$ at 30°C for 4 h was warmed at 65°C, the absorption spectrum which had changed during the incubation at 30°C reverted partially, and the activity was also found to be recovered to 60—80% of the original activity.

Effect of ADP on the CD spectrum of ATPase

The CD spectrum of the purified ATPase exhibited two troughs at 212 and 222 nm (curve A in Fig. 5), indicating the presence of α-helix conformation as

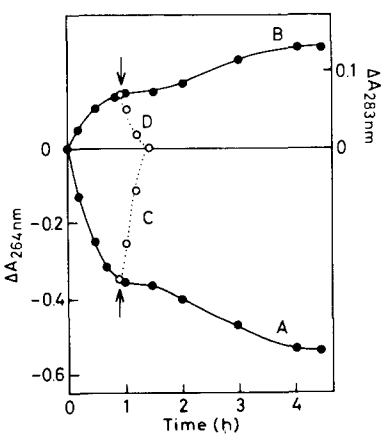
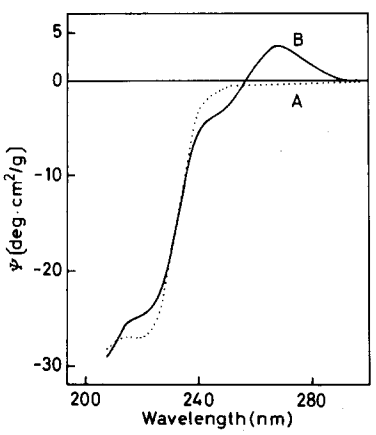


Fig. 4. Ansorbance change with time of the mixture of ATPase, ADP and $MgCl_2$. The absorbance change of the mixture (3.0 μM ATPase, 0.17 mM ADP and 0.17 mM $MgCl_2$) at 30°C was observed at 264 (A) and 283 nm (B). The mixture incubated at 30°C for 55 min was warmed at 65°C at the time indicated by an arrow and the absorbance change was also observed at 264 (C) and 283 nm (D).

suggested previously [2]. On addition of ADP (up to 0.1 mM) to the enzyme (1.1 μM), no change in the CD spectrum of the solution was observed even after 5 h of incubation at 30°C. When $MgCl_2$ (0.1 mM) was added to the solution, no change in the CD spectrum of the mixture was observed for 1 h of incubation at 30°C. About 1.5 h after the addition of $MgCl_2$, the CD spectrum of the mixture commenced to change, and a new peak and a trough appeared at 268 and 245 nm, respectively, together with the change in the value of the specific ellipticity (ψ) at 212 and 222 nm (curve B in Fig. 5). The change in the CD spectrum of the mixture completed after 4—5 h of incubation. When a mixture of 0.1 M ADP and 0.1 M $MgCl_2$ was incubated at 30°C for 4 h, essentially no change of the CD spectrum of the mixture was observed. As suggested from the peak and trough positions, therefore, the change in the CD spectrum at 245 nm may be due to the state change of ADP and those at 212, 222 and 268 nm to the change of the enzyme conformation; the ordered structure (212 and 222 nm) and the state of tryptophan residues (268 nm). The CD measurement of the sample with more than 0.1 mM ADP was difficult, because of a strong absorbance of ADP in the ultraviolet region.

Similar CD spectral change of the mixture of the enzyme, ADP and $MgCl_2$ was observed at temperatures below 40°C. The amount of the CD spectral change of the enzyme on addition of ADP-$MgCl_2$ (the value of ψ at 268 nm) decreased on increasing the temperature, and no change of the CD spectrum of the mixture was observed at 65°C (Fig. 6).

On incubation of the enzyme with 0.1 mM AMP in the presence of $MgCl_2$ at 30°C, no change in the CD spectrum of the mixture was observed after 5 h of incubation.



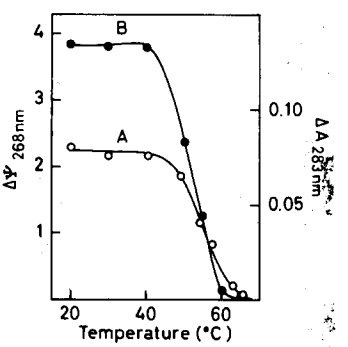


Fig. 5. Change of the CD spectrum of the mixture of ATPase and ADP on addition of $MgCl_2$. The spectra of the mixture (1.1 μM ATPase and 0.1 mM ADP) were observed before (A) and 5 h (B) after the addition of 0.1 mM $MgCl_2$ at 30°C, respectively.

Fig. 6. Changes of the absorbance and CD spectra of the enzyme incubated with ADP-$MgCl_2$ at different temperatures. (A) The enzyme (3.0 μM) was incubated with 0.17 mM ADP-$MgCl_2$ for 1 h at different temperatures and the changes of absorbance at 283 nm were read. (B) The enzyme (1.1 μM) was incubated with 0.1 mM ADP-$MgCl_2$ for 5 h at different temperatures and the changes of the $\psi_{268\,nm}$ value were read.

Effect of ADP on the ultracentrifugal pattern of ATPase

When the enzyme (10.7 μM) was incubated with 5 mM ADP-$MgCl_2$ at 20°C for 1 h, the sedimentation pattern of the sample exhibited one peak of sedimentation coefficient ($s_{20,w}$) of 12.0 S. The value of $s_{20,w}$ of the native enzyme at the same protein concentration was 12.0 S. The enzyme, when treated with 0.17 mM ADP-$MgCl_2$ for 1 h at 30°C, lost activity. The 12.0-S component of the sample as described above may not be the native enzyme. When the enzyme was incubated with ADP-$MgCl_2$ for 1.5 h, a component 4.5 S appeared besides a 10.5-S component (Fig. 7A), and after 4 h of incubation only the 4.5-S component was observed. As in the case of the 12.0-S component, the 10.5-S component cannot be considered to be the native enzyme, because the sample containing the component exhibited no enzymatic activity. When the enzyme was treated with 5 mM ADP-$MgCl_2$ for 4 h at 20°C and then dialyzed against 20 mM Tris · HCl buffer (pH 8.0), the sample thus obtained exhibited two components of 11.8 and 4.5 S. The 11.8-S component may be the native enzyme. On treating the enzyme with 50 mM ADP-$MgCl_2$ at 20°C, the enzyme was dissociated into the 4.5 S component even after 1 h of incubation. The enzyme, when treated with 1% sodium dodecyl sulfate at 20°C for 1 h, was splitted into the 1.4- and 1.7-S components (Fig. 7B). The components may be considered to be the subunits of the protein. The 4.5-S component may still be an assemble molecule of the subunits of the enzyme.

When the enzyme was treated with 5 mM ADP-$MgCl_2$ for 5 h at 20°C, no change in the ultracentrifugal pattern of the enzyme was observed. The sedimentation pattern of the enzyme treated with 50 mM ADP-$MgCl_2$ at 65°C for 1 h exhibited a large peak of 12.7 S and a small peak of 5.0 S. The profile of the enzyme treated with 5 mM ADP-$MgCl_2$ at 65°C for 3 h exhibited one peak of 12.5 S. The s value of the native enzyme alone at 65°C was estimated to be 13.0 S. No or less dissociation of the enzyme into a lower molecular species (the 4.5-S component) seemed to occur on incubation of the enzyme with ADP-$MgCl_2$ at 65°C.

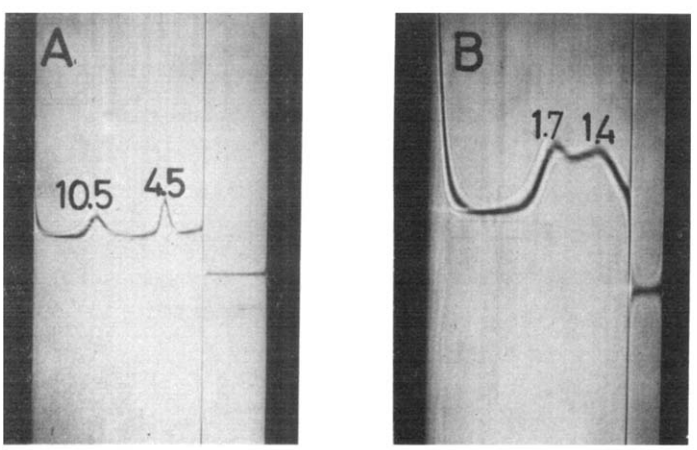


Fig. 7. Sedimentation patterns of ATPase under various conditions. (A) The enzyme (10.7 μM) was incubated with 5 mM ADP-$MgCl_2$ at 20°C for 1.5 h. (B) The enzyme was incubated with 1% sodium dodecyl sulfate at 20°C for 1 h. The photographs were taken 22 (A) and 120 min (B) after reaching full speed, 60 000 rev./min. Numerals on the figures refer to the $s_{20,w}$ values.

Discussion

The ATPase from *B. stearothermohilus* ATCC 12016 was reversibly inactivated on incubating with ADP-$MgCl_2$ (0.17—5 mM) for 1 h at temperatures below 40° C. Such inactivation of the enzyme may be due to the conformational change of the enzyme to the 12.0 S component exhibiting no enzymatic activity, which may be accompanied by a change of the absorbance spectrum of the protein. When the enzyme was incubated with ADP-$MgCl_2$ for more than 1 h, the inactivation of the enzyme became less reversible, and the 12.0 S component may be converted to a mixture of the 10.5-S and 4.5-S components and finally to the 4.5 S component only. This step of conformational change of the enzyme may be accompanied by the changes of the absorbance and CD spectra. The 10.5 S component may be an intermediate in the 12.0 S to 4.5 S transition. The conformational change of the enzyme with ADP-$MgCl_2$ may be initiated by a combination of ADP-$MgCl_2$ to amino acid residues of the enzyme through hydrogen bonding. Such hydrogen bond formation may cause a gradual conformational change of the protein, which may result in further formation of hydrogen bonding.

The absorption spectrum of H-meromyosin has been reported to change rapidly on mixing the protein with ATP or its analogues in the presence of Mg^{2+} [10]. The spectral change (small) of the protein has been considered to be due to the formation of the ES complex during the enzyme reaction. On the other hand, the conformational change of the thermophile ATPase, when mixed with ADP-Mg^{2+}, was very slow but large in quantity, and the change was observed only at temperatures lower than the optimum temperature for the enzyme activity, 65° C. The conformational change of the thermophile ATPase induced by ADP-Mg^{2+} may be quite different in property from that of H-meromyosin. The conformational change of the thermophile ATPase was observed with ADP, but not with AMP, and the spectral change of the mixture of the enzyme, ADP and Mg^{2+} was stongly inhibited by NaN_3, an inhibitor of the enzyme. These results may indicate that ADP-Mg^{2+} may bind to the active site or its neighbour of the enzyme. As suggested from the drastic change of the enzyme conformation, a possibility that more than 1 mol of ADP may bind to the site(s) other than the active center of the enzyme cannot be excluded.

The ATPase from *Streptococcus faecalis* has been reported to be cold labile only in the presence of ADP [11]. The change in the physical properties of the enzyme, however, has not been reported. Penefsky and Warner [5] reported that incubation of beef heart mitochondrial ATPase at 0° C leads to a loss of enzymatic activity and partial dissociation of the molecule, and suggested that the postulated lability of hydrophobic bond in protein [12] may provide an attractive possible explanation for the nature of the cold-labile forces which are involved in maintaining the native conformation of the enzyme. Unlike mesophile ATPases [5—9,11], the thermophile ATPase was inactivated with ADP only in the presence of Mg^{2+} and in the temperature range, 0—40° C. The nature of ADP-Mg^{2+} labile forces of the thermophile ATPase may not be hydrophobic. When the incubation temperature of the enzyme with ADP-Mg^{2+} increased over 40° C, the amount of the conformational change became decreased, and at 60—65° C no change was observed. As reported previously [13],

the enzyme exhibited a conformational change with temperature at 50°C. The extent of the conformational change of the enzyme with ASD-MG^{2+} at a temperature may depend on the conformation of the enzyme at the temperature. If the thermal transition of the protein conformation of the thermophile ATPase may be related to the thermophilicity or thermostability of the enzyme, the bondings ascribable to the nature of ADP-Mg^{2+} lability may also be related to the thermal property of the enzyme.

When the bacterium was incubated at temperatures below 40°C, the amount of ADP increased from 0.4 to 1.9 μmol/g wet weight cells. If the volume of 1 g wet weight cells is assumed to be 1 ml (actually the volume must be smaller than 1 ml), the ADP content of the bacterium is estimated to increase "from 0.4 to 1.9 mM". Glutamine synthetase (EC 6.3.1.2) from the same bacterium was shown to necessitate Mg^{2+} of the concentrations above 5 mM to exhibit the activity [13]. It may therefore be suggested that the bacterium contains at least 5 mM free Mg^{2+}. The purified enzyme (0.9 μg/ml) exhibited an activity of 0.9 μmol P_i formed in 10 min per mg protein, and the bacterium actively growing exhibited an activity of 1 mmol P_i formed in 10 min per g wet weight cells. The enzyme concentration may be calculated to be about 4 μM. The concentration of ADP in intact cells, therefore, seems to be enough to inactivate the ATPase at temperatures below 40°C. When the ATPase was treated with ADP-$MgCl_2$ for a long period the inactivation of the enzyme became less reversible. The membrane-bound enzyme, however, appeared to be almost completely recovered even after a long period of incubation with ADP-$MgCl_2$. The inactivation of the enzyme in intact cells may be reversibly recovered, when the bacterium incubated at low temperatures is transferred into a fresh culture medium at 65°C. The ADP-Mg^{2+}-induced inactivation of the ATPase in *B. stearothermophilus* ATCC 12016 may be related to the inability of the organism to grow at temperatures below 40°C.

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