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## BBA Report

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# Conformational change with temperature of ATPase from *Bacillus stearothermophilus*

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SUMMARY

The graphs showing the relationships between the logarithms of the fluorescence intensities of ATPase (EC 3.6.1.3) from *Bacillus stearothermophilus* and of 8-anilino-1-naphthalenesulfonic acid bound to the enzyme and the reciprocal of temperature both exhibit sharp breaks at 50 °C. Similar relationships are observed in the presence of ATP (ADP) and $Mg^{2+}$. The results suggest that a thermal transition in the conformation of the enzyme may occur at 50 °C, both in the absence and presence of ATP(ADP) and $Mg^{2+}$.

The thermodynamic quantities for some enzymes[1–4] including ATPase (EC 3.6.1.3)[1] of *Bacillus stearothermophilus* suggested that conformational changes of the enzyme proteins may occur at 50–55 °C. Since the growth of the bacterium becomes rapid above 50 °C, such a conformational change in the enzyme protein of the organism seems to be of physiological significance. The present study was undertaken in the hope of finding a thermal transition in the conformation of the ATPase protein, by measuring the fluorescence spectra of the protein and of 8-anilino-1-naphthalenesulfonic acid (ANS) bound to the protein at different temperatures.

ATPase was purified as described previously[1]. 0.2 ml of ATPase solution in 20 mM Tris–HCl buffer (pH 8.0) and 2.8 ml of a solution containing the additions to the buffer indicated in the text were mixed at each temperature, and the fluorescence spectrum

Abbreviation: ANS, 8-anilino-1-naphthalenesulfonic acid.

of the mixture was measured with a Hitachi recording spectrofluorimeter, MPF-2A. Fluorescence intensities of ATPase and of ANS were not corrected for instrument response over the wavelength region studied and were expressed as relative fluorescence against 1 $\mu$M tryptophan in aqueous solution and 1 $\mu$M quinine sulfate in 0.025 M $H_2SO_4$, respectively.

The fluorescence spectrum of the enzyme exhibited an emission maximum at 335 nm, when excited at 280 nm (Curve A in Fig. 1). The protein contained 1.6 g tryptophan/100 g protein, as determined by the Goodwin and Morton[5] method. The

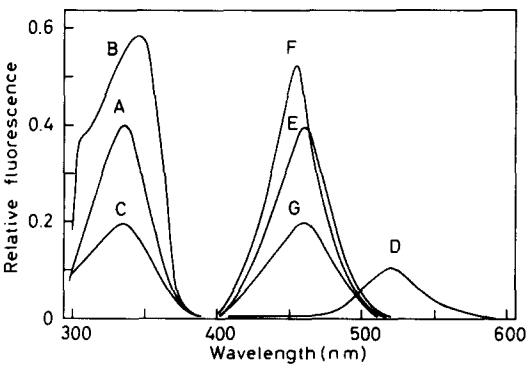


Fig. 1. Fluorescence spectra of ATPase and ANS under various conditions at 30 °C. Native enzyme (A), inactivated enzyme (B), and native enzyme in the presence of ATP and $Mg^{2+}$ (C); ANS (1 $\mu$M) only (D), ANS mixed with native enzyme (E), ANS with inactivated enzyme (F) and ANS with native enzyme in the presence of ATP and $Mg^{2+}$ (G). Final concentrations of the protein (native or inactivated), ANS, ATP and $Mg^{2+}$ ($MgCl_2$) were 0.7 $\mu$M, 8 $\mu$M, 5 mM and 3 mM, respectively. In the study of ATPase or ANS fluorescence, the slit widths were set at 12 or 14 mm, and the light emitted was passed through a Toshiba UV-29 or −39 filter to a photomultiplier, respectively.

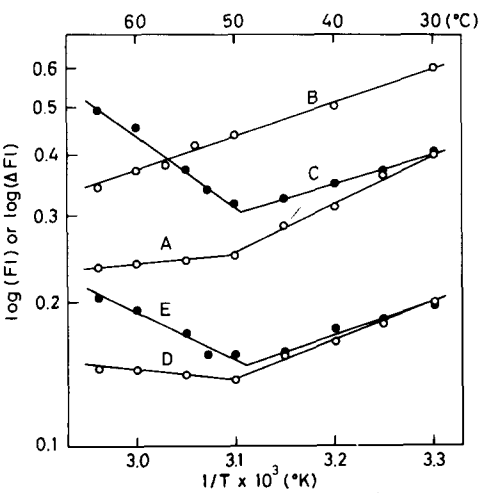


Fig. 2. Effects of temperature on the fluorescence intensities of ATPase and of ANS bound to the enzyme under various conditions. Fluorescence intensities (*FI*) of native enzyme (A), inactivated enzyme (B) and ANS mixed with native enzyme (C). Decreases in fluorescence intensities ($\Delta FI$) of native enzyme (D) and ANS bound to native enzyme (E) on addition of ATP and $Mg^{2+}$.

ultraviolet fluorescence spectrum of the enzyme may be attributed to the tryptophan residues of the protein. The fluorescence intensity of the enzyme decreased with an increase in temperature, although the shape of the spectrum remained unchanged. When the logarithm of the fluorescence intensity ($\log(F1)$) at 335 nm is plotted *versus* the reciprocal of temperature ($1/T$), two straight lines intersecting at 50 °C can be fitted to the points (Curve A in Fig. 2). The fluorescence of ribonuclease A or Bence–Jones protein decreased with increasing temperature in general, but a sharp rise in fluorescence intensity was observed over a narrow range of temperature[6,7]. Such fluorescence changes have been considered ascribable to a thermal transition in the protein conformation. The fluorescence spectrum of ATPase inactivated by treatment at 85 °C for 5 min was different from that of the native protein (Curve B in Fig. 1), and exhibited two emission maxima at 305 and 345 nm, which may be ascribed to the tyrosine and tryptophan residues of the protein, respectively. The graph showing the relationship between $\log(F1)$ and $1/T$ at 345 nm was almost linear (Curve B in Fig. 2). The results obtained in the present study may therefore indicate that a thermal transition in the conformation of the enzyme occurs at 50 °C.

The fluorescence spectrum of ANS in aqueous solution, when excited at 365 nm (30 °C), exhibited an emission maximum at 520 nm (Curve D in Fig. 1). The emission maximum of ANS, when excited in the presence of the enzyme, was shifted to 460 nm, and the intensity was considerably increased (Curve E). This may indicate that ANS was bound to the enzyme. When the temperature of a mixture of the enzyme and ANS was raised, the fluorescence intensity at 460 nm changed without any change in the shape of the spectrum. The graph showing the relationship between $\log(F1)$ and $1/T$ exhibited a sharp break at 50 °C (Curve C in Fig. 2). The fluorescence spectrum of ANS bound to the inactivated enzyme was different from that bound to the native protein (Curve F in Fig. 1), and the intensity was not appreciably changed with increasing temperature. Gally and Edelman[7] reported that enhancement of the fluorescence of ANS bound to Bence–Jones protein was observed during the thermally induced molecular transition of the protein. A thermal transition of the ATPase may therefore be accompanied by alteration not only in the local environment of the tryptophan residues but also in the ANS-binding property (the hydrophobic state) of the protein.

When either ATP or $Mg^{2+}$ alone was added to the enzyme or ANS bound to the enzyme at 30 °C, no change in the fluorescence spectrum of the protein or ANS was observed. On addition of increasing concentrations of ATP or $Mg^{2+}$ to the enzyme solution containing 3 mM $Mg^{2+}$ or 5 mM ATP, respectively, the fluorescence intensity of the protein decreased without any change in the shape of the spectrum. The fluorescence intensity of ANS bound to the enzyme also decreased on addition of both ATP and $Mg^{2+}$ at 30 °C without any change in the shape of the spectrum. The fluorescence intensity of the enzyme or of ANS reached a constant value in the presence of more than 5 mM ATP and 3 mM $Mg^{2+}$ (Curve C or G in Fig. 1). Similar spectral changes of the enzyme and of ANS were observed on addition of ADP, competitive inhibitor of the enzyme[1], but AMP, adenosine or pyrophosphate caused no spectral change. GTP and CTP were hydrolyzed by the enzyme with the relative rates of 90 and 16% of the rate with ATP, respectively[1]. 6 mM GTP and

CTP also decreased the fluorescence intensity of the enzyme or ANS bound to the enzyme. The decrease in the fluorescence intensity ($\Delta F1$) of the enzyme in the presence of GTP or CTP was 98 or 17%, respectively, of that caused by ATP, and the value of $\Delta F1$ of ANS bound to the enzyme in the presence of GTP or CTP was 97 or 21% of that caused by ATP, respectively. The fluorescence intensity of the inactivated enzyme or ANS bound to the inactivated protein was not changed on addition of ATP(ADP) and $Mg^{2+}$. These results suggest that the decrease in fluorescence intensity of ATPase or ANS bound to the enzyme on addition of ATP (ADP) and $Mg^{2+}$ may be due to the conformational change of the protein on the formation of the enzyme–$Mg^{2+}$–ATP(ADP) complex.

The graph showing the relationship between $\log(\Delta F1)$ of the protein or ANS bound to the protein on addition of 6 mM ATP(ADP) and 3 mM $Mg^{2+}$ and $1/T$ (Curves D or E in Fig. 2) may indicate that the thermal transition in the protein conformation may occur at 50 °C even in the presence of ATP (ADP). ATP was rapidly hydrolyzed in the presence of $Mg^{2+}$ at high temperatures. The spectral changes observed with ATP at the temperatures might therefore be those caused by ADP and $Mg^{2+}$. The fluorescence intensity of the enzyme at 335 nm measured immediately after addition of ATP and $Mg^{2+}$ at 65 °C was the same as that measured after 30 min of incubation. The conformational change of the enzyme caused by ATP may therefore be the same as that caused by ADP.

In conclusion, thermophile ATPase may exhibit a thermal transition in the local environment of tryptophan residues and in the hydrophobic state of the protein at 50 °C, both in the absence and presence of ATP (ADP) and $Mg^{2+}$.

REFERENCES

1 Hachimori, A., Muramatsu, N. and Nosoh, Y. (1970) *Biochim. Biophys. Acta* 207, 426–437
2 Sugimoto, S. and Nosoh, Y. (1971) *Biochim. Biophys. Acta* 235, 210–221
3 Muramatsu, N. and Nosoh, Y. (1971) *Arch. Biochem. Biophys.* 144, 245–252
4 Howard, R.L. and Becker, R.R. (1970) *J. Biol. Chem.* 245, 3186–4194
5 Goodwin, T.W. and Morton, R.A. (1946) *Biochem. J.* 40, 628–632
6 Gally, J.A. and Edelman, G.M. (1962) *Biochim. Biophys. Acta* 60, 499–509
7 Gally, J.A. and Edelman, G.M. (1965) *Biochim. Biophys. Acta* 94, 175–182

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