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THERMAL PROPERTIES OF FRUCTOSE-1,6-DIPHOSPHATE ALDOLASE FROM THERMOPHILIC BACTERIA

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SUMMARY

1. Fructose-1,6-diphosphate aldolase (EC 4.1.2.13) from *Bacillus stearothermophilus* is purified to a state in which it is homogeneous, both ultracentrifugally and electrophoretically.

2. The enzyme has a molecular weight of 60 600 and contains two atoms of Zn. Its activity is strongly inhibited by EDTA at 65°. These results may indicate that the thermophile aldolase is of the yeast type.

3. The enzyme is activated by K^+ and, to a lesser extent, by Na^+. The optimum pH is 8.5–8.6 in a Tris or borate buffer.

4. The amino acid composition is almost similar to that of the muscle-type aldolase and contains 30% of the α-helical conformation.

5. The enzyme exhibits a maximum activity at 70° and is stable on exposure up to 45°. Slight inactivation is observed on treatment of the enzyme at 55–65°, and exposure to 75° for 30 min results in almost complete inactivation.

6. Thermodynamic quantities for the enzyme suggest that a structural change in the enzyme molecule occurs at the transition temperature, 50–53°. The relationship between the inhibition of the activity by EDTA and temperature may suggest that the change occurs around the active site of the enzyme.

INTRODUCTION

In a previous study[1] a membrane-bound enzyme, ATPase (EC 3.6.1.3), was isolated and purified from thermophilic bacteria, and some properties, especially thermal, were examined. Thermodynamic quantities for the purified enzyme, calculated for the temperature range 30–65°, suggested that a structural change in the enzyme molecule may occur at the transition temperature, 55°. A similar structural change of the enzyme has also been suggested for the enzyme in the membrane-bound state[2].

These findings stimulated the authors to investigate the thermal properties of a

Abbreviations: ORD, optical rotatory dispersion. CD, circular dichroism.

soluble enzyme of the same bacteria and to examine whether a structural change at a transition temperature such as that observed with the ATPase occurs with a soluble enzyme or not. Fructose-1,6-diphosphate aldolase (EC 4.1.2.13) has already been purified by THOMPSON AND THOMPSON[3], and some properties have been presented. The enzyme was then chosen to be studied along such a line of approach. The purification procedure of the enzyme according to them, however, had been unsuccessful with the cells used in the present study. The enzyme was purified by another procedure. Described herein are some properties, especially thermal, of the enzyme thus purified.

MATERIALS AND METHODS

Microorganism and cultures

The thermophilic bacteria used in the present study were *Bacillus stearothermophilus* NCA 2184 kindly donated by Professor C. E. Georgi, Nebraska University, and cultured in the medium described previously[1]. Mass cultures in a 200-ml medium were kindly supplied by the Central Research Laboratories, Ajinomoto Co.,

Purification of the aldolase

The cells (200 g wet weight) were subjected to the lysozyme treatment according to the procedure described previously[1], and the undisrupted cells and membrane ghosts were removed by centrifugation at 25 000 × *g* for 15 min.

The supernatant thus obtained was diluted with 10 mM Tris–maleate buffer (pH 7.0) so as to obtain a solution of about 30 mg protein per ml. The protein fraction precipitated during 40–60% saturation with $(NH_4)_2SO_4$ was dissolved in 10 mM Tris–maleate buffer (pH 7.0), and dialyzed against the same buffer at 4° overnight.

The protein solution thus obtained was diluted with a concentrated Tris–maleate buffer (pH 7.0) so as to obtain a solution containing 20–23 mg protein per ml and 40 mM Tris–maleate. To this solution were added magnesium acetate and polyethylene glycol (No. 6000, Nihon Rikagaku Co.) in final concentrations of 10 mM and 8%, respectively, and stirred for 30 min at room temperature. After removal of the precipitates by centrifugation, magnesium acetate and polyethylene glycol were added to the resulting supernatant in final concentrations of 10 mM and 13%, respectively. The precipitates formed were removed by centrifugation, and the pH of the supernatant was adjusted to 6.2 with 1 M acetic acid. After removal of the precipitates by centrifugation magnesium acetate and polyethylene glycol were added to the resulting supernatant in final concentrations of 20 mM and 22%, respectively, and the pH was adjusted to 5.3 with 1 M acetic acid. The precipitates were collected by centrifugation, and dissolved in 10 mM phosphate buffer (pH 7.0).

DEAE-cellulose column chromatography. The protein solution thus obtained was passed through a column of DEAE-cellulose previously equilibrated with 25 mM phosphate buffer (pH 7.0). The column was washed with 25 mM and then with 40 mM phosphate buffer (pH 7.0), of twice the volume of the column. The enzyme was eluted with 80 mM phosphate buffer (pH 7.0). The elution pattern is shown in Fig. 1A.

DEAE-Sephadex A-50 column chromatography. The fractions shown covered with a black bar in Fig. 1A were collected and brought to 70% saturation with respect to $(NH_4)_2SO_4$. The precipitates were suspended in 50 mM phosphate buffer (pH 7.0), and dialyzed against the same buffer at 4° overnight. After centrifugation, the super-

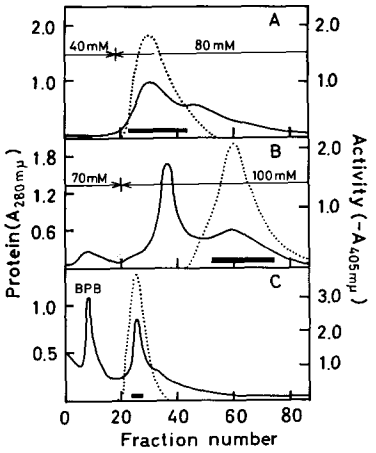


Fig. 1. Elution patterns of the aldolase on DEAE-cellulose, on DEAE-Sephadex and on polyacrylamide. A. Chromatography on DEAE-cellulose. B. Chromatography on DEAE-Sephadex A-50. C. Electrophoresis on polyacrylamide gel. Column dimensions :(A) 8 cm × 15 cm and (B) 2 cm × 20 cm. Volumes collected for each tube (ml): (A) 15, (B) 5 and (C) 5. Amounts of the protein charged: (A) 3 g, (B) 200 mg and (C) 60 mg. Bromophenol blue (BPB) was used as a tracking dye of the leading ion in the electrophoresis (C). ———, protein ($A_{280\,m\mu}$); · · · · ·, aldolase activity ($-\Delta A_{405\,m\mu}$/10 min).

natant was layered onto a column of DEAE-Sephadex A-50 previously equilibrated with 50 mM phosphate buffer (pH 7.0). The column was washed with 50 mM and then with 70 mM phosphate buffer (pH 7.0), of twice the volume of the column. The enzyme was eluted with 100 mM phosphate buffer (pH 7.0). The elution pattern is shown in Fig. 1B.

Polyacrylamide gel electrophoresis. The fractions shown covered with a black bar in Fig. 1B were collected, concentrated through a collodion bag, and dialyzed against 25 mM Tris–glycine buffer (pH 6.7). The sample (20 ml) containing 60 mg protein was then purified by electrophoresis on a polyacrylamide gel using a preparative disc-electrophoretic apparatus (Toyo Kagaku Sangyo Co.). The elution pattern is shown in Fig. 1C. The fractions shown covered with a black bar in the figure were collected, concentrated through a collodion bag, and dialyzed against 20 mM Tris–HCl buffer (pH 8.0) at 4° overnight.

A purification scheme giving the recovery and specific activity at each stage is presented in Table I.

Assay of the activity

The activity of the enzyme in a crude state was measured as follows. To 0.9 ml of 6 mM fructose 1,6-diphosphate in 20 mM potassium phosphate buffer (pH 7.4) was added 0.1 ml of the enzyme solution (5–100 μg aldolase). After 10 min of incubation at 65°, the reaction was stopped by adding 4 ml of 8% $HClO_4$. The amount of fructose 1,6-diphosphate that was decomposed was determined according to the procedure of ROE *et al.*[4] by measuring the absorbance at 405 mμ. Fructose 1,6-diphosphate prepared from Baker's yeast according to the procedure of NEUBERG AND LUSTIG[5] was used (the purity, about 60%).

The activity of the purified enzyme was measured as follows. To 0.9 ml of 4 mM

fructose 1,6-diphosphate (cyclohexylammonium salt, Boehringer-Mannheim Co.) and 120 mM potassium acetate in 50 mM Tris–HCl buffer (pH 8.6) was added 0.1 ml of the enzyme solution (0.3 μg aldolase). After 10 min of incubation, the reaction was stopped by adding 1 ml of 10% trichloroacetic acid, and the triose formed was measured according to the procedure of DOUNCE *et al.*[6].

In either of the two assay conditions, a linearity of the reaction with respect to time was obtained.

Measurement of protein

Protein concentration was determined by means of either the biuret method[7] during the purification of the enzyme, or the method of LOWRY *et al.*[8], using crystalline

TABLE I

SUMMARY OF DATA OBTAINED AT DIFFERENT STAGES IN PURIFICATION OF THE ALDOLASE

Stage	*Total protein (mg)*	*Total activity (μmoles triose per min)*	*Specific activity (μmoles triose per min per mg protein)*	*Recovery (%)*
Extract	18 400	20 200	1.1	100
40–60% $(NH_4)_2SO_4$ ppt.	13 400	16 100	1.2	80
8% polyethylene glycol (pH 7.0) supernatant	7 500	14 200	1.9	71
13% polyethylene glycol (pH 6.2) supernatant	4 920	13 700	2.8	67
22% polyethylene glycol (pH 5.4) ppt.	3 120	12 500	4.0	62
DEAE-cellulose	258	5 160	20	26
DEAE-Sephadex A-50	60	2 700	45	13
Electrophoresis on polyacrylamide gel	31	1 710	55	8.8

bovine serum albumin as a standard, with the purified enzyme. For the analyses of the elution patterns of proteins on DEAE-cellulose, on DEAE-Sephadex and on polyacrylamide gel, the 280-mμ absorbance was measured.

Ultracentrifugal analysis

A sedimentation velocity experiment was carried out at 20° in a Hitachi ultracentrifuge, UCA-1, with phase-plate Schlieren optics. The molecular weight was estimated by means of the short column method of YPHANTIS[9]. Measurement was performed at a speed of 25 866 rev./min at 25° for 12 h in a Spinco ultracentrifuge, Model E. The value of 0.75 ml/g was assumed for a specific volume of the thermophile aldolase.

Disc-electrophoretic analysis

Analytical and preparative polyacrylamide gel electrophoreses were both carried out in a Tris–glycine buffer (pH 8.3) according to the procedure of ORNSTEIN[10] and DAVIS[11]. For the analysis, the sample containing 40 μg protein in 0.5 M sucrose was layered onto the gel, and electrophoresis was carried out at 4° for 15 min at 4 mA/cm. The protein was stained with Amido black 10B.

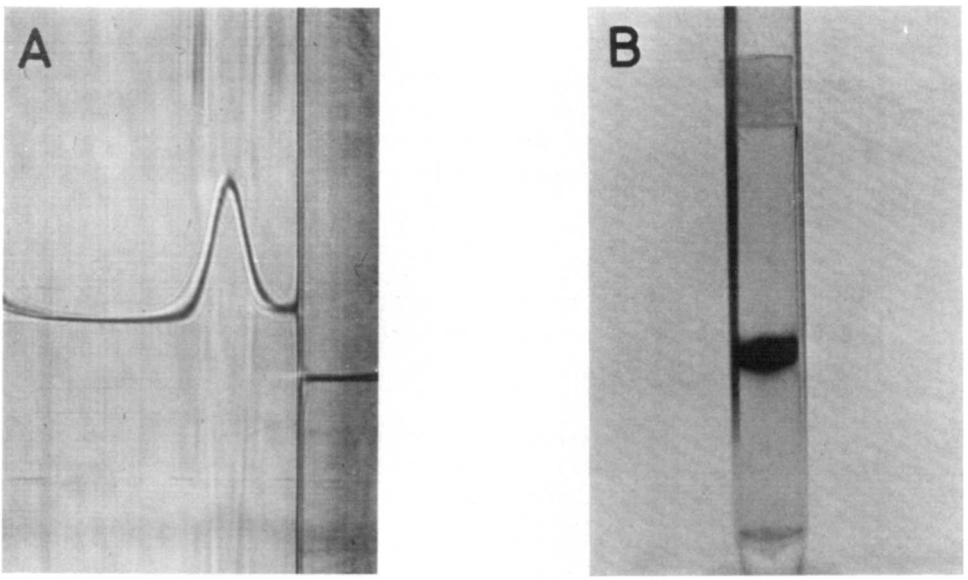


Fig. 2. Homogeneity of the aldolase. A. Sedimentation pattern of the aldolase. Protein concentration was 0.4% in 100 mM phosphate buffer (pH 7.0). The photograph was taken 42 min after reaching full speed, 60 000 rev./min. B. Disc-electrophoretic pattern of the aldolase.

Amino acid analysis

The hydrolysis of the protein was carried out at 110° with 6 M HCl under N_2. Analysis was carried out in a Hitachi liquid chromatographic apparatus, 034-0004.

Optical rotatory dispersion (ORD) and circular dichroism (CD)

ORD was measured in a Jasco ORD/CD-5 recording spectropolarimeter with a quartz cell of either 1.0-cm or 0.1-mm path length. CD was measured with a CD attachment to the Jasco instrument.

Determination of metal

The analysis of the metals of the aldolase was carried out in a Hitachi atomic absorption spectrophotometer, 207.

RESULTS

Homogeneity of the aldolase

The thermophile aldolase purified in the present study was homogeneous, both ultracentrifugally and electrophoretically, as shown in Fig. 2. The sedimentation coefficient ($s_{20,w}$) was 4.1, and the molecular weight was estimated to be 60 600 ± 1300, which was slightly smaller than those reported with the yeast-type aldolase (67 500–70 000)[12,13], but far smaller than those of the muscle-type aldolase (120 000–160 000)[14–18].

The enzyme was found to contain 2 atoms of Zn per mole of the enzyme molecule.

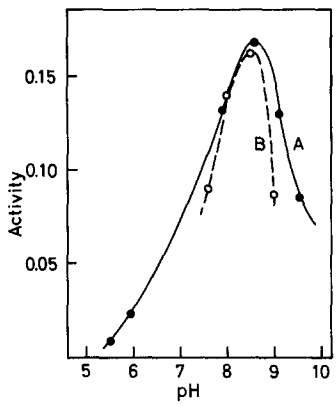


Fig. 3. Effect of pH on the aldolase activity. The activity was expressed in μmoles of triose formed per 10 min. The reaction temperature was 65°. A, 50 mM Tris–HCl buffer containing 120 mM potassium acetate; B, 50 mM potassium borate buffer containing 50 mM potassium acetate.

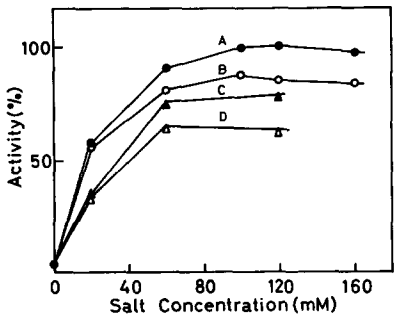


Fig. 4. Effects of Na^+ and K^+ on the aldolase activity. The activities were represented as a percentage against the activity in the presence of 120 mM potassium acetate. The reaction was carried out at 65°. A, potassium acetate; B, KCl; C, sodium acetate; D, NaCl.

Effect of pH on the activity

As shown in Fig. 3, the enzyme exhibits a maximum activity at pH 8.5–8.6 in a 50 mM Tris–HCl buffer or 50 mM borate buffer. The optimum pH value obtained for the present thermophile aldolase was considerably higher than those reported with the thermophile aldolase purified by THOMPSON AND THOMPSON[3] (pH 7.3 in 50 mM borate buffer and pH 7.5 in 50 mM phosphate buffer) and with the yeast-type aldolase (pH 7.2–7.8)[12,13,19,20].

Effects of K^+ and Na^+ on the activity

The yeast-type aldolase has been shown to be activated by K^+ (refs. 13, 14). As suggested from the value of the molecular weight and from the fact that the enzyme contains Zn atoms, the thermophile aldolase may be classified with a yeast-type aldolase. The effect of K^+ on the thermophile aldolase activity was then examined. As shown in Fig. 4, the aldolase was remarkably activated by potassium acetate (120 mM), about 20 times the activity without K^+, and to a lesser extent, by 100 mM KCl (about 17 times). A yeast-type aldolase has been shown to be activated by potassium acetate more than by KCl (ref. 13). As shown in Fig. 4, the thermophile aldolase was also activated by Na^+, although lesser than by K^+. Here again, the enzyme was more activated by sodium acetate than by NaCl.

Amino acid composition

The amino acid composition of the thermophile aldolase is presented in Table II. As shown in the table, the proline content was low, as in the case of the ATPase of the same bacteria[1], and the amount of tyrosine was far smaller than that reported with protease produced from *Bacillus thermoproteolyticus* (12.54 g/100 g protein)[21]. As shown in the table, the amino acid composition of the enzyme was considered to be almost similar to those reported with the aldolase from mammalian muscle[22,23], al-

TABLE II

AMINO ACID COMPOSITION OF THE ALDOLASE

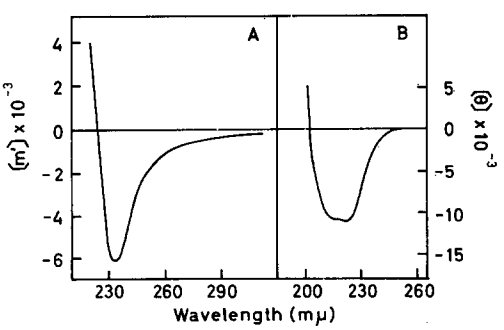
Amino acid	*Amino acid residues in protein (g/100 g protein)*		
	Thermophile aldolase	*Muscle aldolase**	
		(a)	*(b)*
Aspartic acid	9.70	8.63	9.7
Threonine	4.66	5.37	7.1
Serine	3.67	4.48	6.57
Glutamic acid	14.17	12.95	11.4
Proline	3.29	4.58	5.71
Glycine	5.85	4.43	5.61
Alanine	7.25	7.81	8.56
Valine	7.94	5.64	7.40
Methionine	3.14	1.41	1.17
Isoleucine	7.08	5.92	7.87
Leucine	8.48	10.11	11.5
Tyrosine	3.07	4.45	5.31
Phenylalanine	4.14	2.59	3.06
Lysine	8.55	8.09	9.54
Histidine	2.41	3.85	4.21
Arginine	6.67	5.74	6.33

* The values for muscle aldolase were cited from ref. 22(*a*) and 23(*b*).

though slight differences were observed in the contents of some amino acids, such as methionine. It may therefore be concluded that the thermophile aldolase exhibits no peculiarity in the amino acid composition.

ORD and CD profiles of the enzyme

The ORD profile of the thermophile aldolase exhibited a prominent Cotton effect in the ultraviolet region, and a trough at 232 mμ, as shown in Fig. 5A. The value



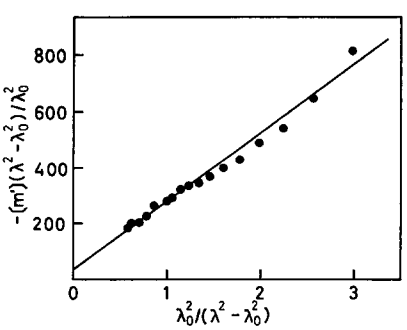


Fig. 5. ORD and CD profiles of the aldolase. The protein concentrations were 20.2 mg/ml (ORD) and 2.6 mg/ml (CD), both in 20 mM Tris–HCl buffer (pH 8.5). A. ORD. B. CD.

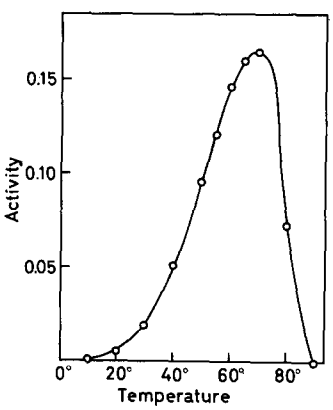
Fig. 6. The Moffitt–Yang plot. The plot was obtained from the ORD data presented in Fig. 5A.

of $[m']$ at this wavelength was -6030 degree. As shown in Fig. 5B, the CD profile of the enzyme exhibited two troughs at 212 and 222 mμ, and the value of Θ at 222 mμ was $-11\,200$ degree·cm^2·decimole^{-1}. The results clearly indicate the existence of α-helical conformation in the aldolase molecule. From the values of $[m']_{232\ m\mu}$ and $\Theta_{222\ m\mu}$ for complete random coiled and α-helical structure[24–26], the α-helix content of the aldolase was estimated to be 31.0 and 27.8% from the ORD and CD data, respectively. From the Moffitt–Yang plot for the ORD of the aldolase (Fig. 6), the values of a_0 and b_0 were, respectively, estimated to be $+33$ and $-245°$. The λ_0 was assumed to be 212 mμ. The α-helix content of the aldolase estimated from the $b_0{}^H$ of the Moffitt–Yang plot was 32.6%.

When the thermophile aldolase was exposed to 30–70°, no change in the ORD profile of the enzyme was observed. This suggests that no conformational change of the enzyme occurred in this temperature range. The addition of substrate, 5 mM fructose 1,6-diphosphate, also caused no change in the ORD profile of the enzyme between 30 and 70°.

Effect of temperature on the activity

As shown in Fig. 7, the aldolase activity gradually increased with increasing the temperatures, and a maximum activity was observed at about 70°, which was slightly



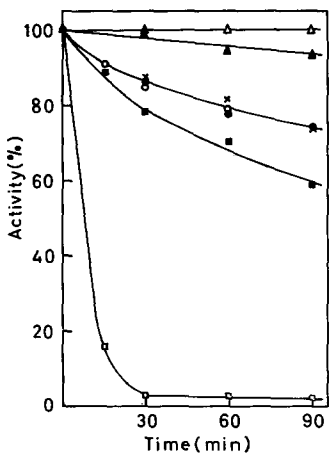


Fig. 7. Effect of temperature on the aldolase activity. The activity was expressed in μmoles triose formed in 10 min.

Fig. 8. Thermal inactivation of the aldolase at various temperatures. △—△, 45°; ▲—▲, 50°; ○—○, 55°; ●—●, 65°; ×—×, 65° in the presence of 1 mM fructose 1,6-diphosphate; ■—■, 70°; □—□, 75°. The activity was expressed as per cent against the activity before heat treatment.

higher than the optimum temperature for the cell growth (65°). When the temperature was further increased above 70°, the activity rapidly decreased, and was completely lost at 90°.

The thermophile aldolase was stable at 4° for 1 month. As shown in Fig. 8, the aldolase was stable on exposure to 45° for 1.5 h, and the enzyme lost only about 5% of its original activity after exposure of the sample to a temperature of 50° for 1.5 h.

On exposing the sample to a temperature of 55–65° for 1.5 h, the enzyme lost about 25% of its original activity, and exposure to a temperature of 70° resulted in a loss of 40% activity. When the enzyme was exposed to a temperature of 75°, the activity was almost completely lost after 30 min.

When the enzyme was exposed to a temperature of 65° in the presence of 1 mM fructose 1,6-diphosphate, any protective effect of the substrate from thermal inactivation of the enzyme, as observed with glucose-6-phosphate isomerase (EC 5.3.1.9) of the same bacteria[27], was not observed with the aldolase.

Thermodynamic quantities for the aldolase

The relationship between the reaction velocity and substrate concentrations expressed in the Lineweaver–Burk plot was linear in the temperature range 30–70°. This indicates that the aldolase reaction obeys the Michaelis–Menten's theory. The values of Michaelis constants (K_m) and maximum velocities (v_{max}) estimated at 30 and 65° were $0.2 \cdot 10^{-4}$ and $2.1 \cdot 10^{-4}$ M and 5 and 42 μmoles of the triose formed per min per mg protein, respectively.

Curve A in Fig. 9 shows the relationship between temperature (T) and K_m. The graph had a discontinuity of slope and approximated to two straight lines meeting at an angle. This may indicate a change from one value of the enthalpy change (ΔH) for formation of the ES complex to another at the transition temperature, 50°. The values of ΔH below and above 50°, assuming that K_m is equal to the dissociation constant of the ES complex[28], were calculated to be −5900 and −23 000 cal/mole, respectively (Table III). The values of the entropy change (ΔS) below and above 50° were then calculated to be 2 and 51 cal per mole per degree, respectively. The results may indicate that a structural change of the aldolase molecule on formation of the ES complex was different at temperatures below and above 50°.

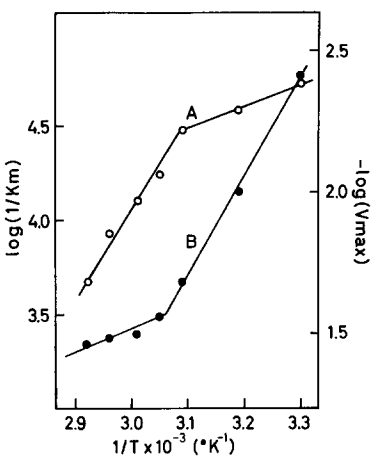


Fig. 9. Effect of temperature on K_m and v_{max} for the aldolase. A, log $(1/K_m)$; B, log (v_{max}).

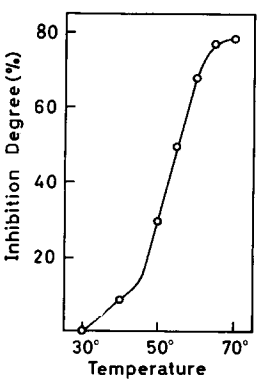


Fig. 10. Relationship between temperature and degree of inhibition of the aldolase activity by $1 \cdot 10^{-5}$ M EDTA.

Similarly, the values of ΔH and ΔS for activation of the ES complex below and

TABLE III

THERMODYNAMIC QUANTITIES FOR THE ALDOLASE

Quantity	*Temperature:*				
	30°	*40°*	*50°*	*60°*	*65°*
For formation of the ES complex					
ΔH (cal/mole)	−5 900	−5 900	−5 900	−23 000	−23 000
ΔF (cal/mole)	−6 500	−6 600	−6 600	−6 300	−6 100
ΔS (cal/mole per degree)	2	2	2	50	50
For activation of the ES complex					
ΔH (cal/mole)	16 000	16 000	16 000	3 300	3 300
ΔF (cal/mole)	9 000	8 800	8 600	8 600	8 700
ΔS (cal/mole per degree)	22	22	22	−16	−16

above the transition temperature, 53°, were calculated from Curve B in Fig. 9 to be 16 000 and 3300 cal/mole and 22 and −16 cal/mole per degree, respectively.

Effect of temperature on the EDTA inhibition of the activity

The activity of the thermophile aldolase was completely inhibited by $1 \cdot 10^{-4}$ M EDTA at 65°. The activity at 30°, on the other hand, was not inhibited by the same concentration of EDTA. This may indicate that the molecular structure, especially near the Zn-binding site, of the enzyme at 65° is different from that at 30°.

Fig. 10 shows the relationship between temperature and inhibition degree of the activity by $1 \cdot 10^{-5}$ M EDTA. At this concentration of EDTA, about 80% of the activity was inhibited at 65°. As clearly shown by the curve, the degree of inhibition became gradually larger with increasing in temperature up to 45°, but an abrupt increase of the degree of inhibition was observed above 45°.

When the enzyme was incubated with $1 \cdot 10^{-5}$ M EDTA at 65° for 5 min and $1.2 \cdot 10^{-5}$–$2.0 \cdot 10^{-5}$ M $ZnSO_4$ was then added to the solution, only 63% of its original activity was found to be restored. This may indicate that EDTA was rather firmly bound to the Zn atoms of the enzyme at 65°. The enzyme was incubated with $1 \cdot 10^{-5}$ M EDTA at 65° for 5 min, rapidly cooled to 30°, and then the activity was measured at 30°. No inhibition of the activity was observed. The result suggests that EDTA bound to the Zn atoms at 65° was easily released at 30° by adding the substrate.

DISCUSSION

Fructose-1,6-diphosphate aldolase from thermophilic bacteria has been reported by THOMPSON AND THOMPSON[3] to be a muscle-type aldolase, because the activity is not inhibited by chelating reagents such as α,α'-dipyridyl and 8-hydroxyquinoline. The results obtained in the present study, however, suggest that the thermophile aldolase is not of the muscle type but of the yeast type. The molecular weight of the thermophile aldolase (mol. wt. 60 600) was similar to those so far reported with the yeast-type aldolases (mol. wt. 67 500–70 000)[12,13], but far different from those with the muscle-type aldolases (mol. wt. 120 000–160 000)[14–18]. In addition, the thermophile aldolase was found to contain Zn atoms in the molecule, and the

activity was strongly inhibited by EDTA. Recently, FREEZE AND BROCK[29] reported that the thermostable aldolase from *Thermus aquaticus* resembles a yeast-type aldolase, except in the case of its extreme heat stability[29].

Although the thermophile aldolase may be classified with a yeast-type aldolase, some properties of the thermophile enzyme were different from the yeast-type aldolases in the corresponding properties. The molecular weight of the thermophile enzyme was lower than those of the yeast-type enzymes. The yeast-type aldolases have been shown to contain only one atom of Zn or Co in one molecule of the enzyme[30], while the thermophile enzyme contains two atoms of Zn. Furthermore, the optimum pH for the thermophile enzyme (pH 8.5–8.6) was considerably higher than those reported with the yeast-type enzymes (pH 7.2–7.8)[12,13,19,20]. These properties by means of which the thermophile enzyme differed from those of the yeast-type enzymes may be due to a specific protein structure exhibiting the thermostability of the enzyme.

Thermodynamic quantities for the thermophile aldolase calculated in the temperature range 30–70° may indicate that a structural change of the enzyme molecule occurs at the transition temperature, 50–53°. The ORD profile of the enzyme exhibited no change in this temperature range, both in the presence and absence of the substrate. The structural change suggested with the enzyme, therefore, may occur around the active site of the enzyme molecule, without any gross change of the enzyme conformation.

The atoms of Zn or Co contained in a yeast-type aldolase are considered to act as an active site of the enzyme[30,31]. The Zn atoms of the thermophile aldolase are considered also to act as an active site of the enzyme, because the activity was inhibited by EDTA. The relationship between the inhibition of the activity by EDTA and temperature observed in the present study suggests that the molecular structure near the Zn-binding site(s) of the enzyme was gradually changed with increasing the temperature and a great change in the structure occurred above the transition temperature. A structural change of the thermophile aldolase molecule suggested from the thermodynamic quantities for the enzyme, therefore, is considered to be due to such a structural change around the Zn-binding site(s) of the molecule.

From the results on the amino acid composition and α-helix content of the thermophile aldolase it may be considered that the thermostability of the enzyme is due to some factor other than an extraordinary protein structure of a peculiar amino acid composition and protein conformation. Such non-peculiarity in the amino acid composition or protein structure has been reported with the ATPase[1] and glucose-6-phosphate isomerase[27], and therefore may be observed with almost all the enzymes of the bacteria.

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