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Purification and characterization of aspartate aminotransferase from the halophile archaebacterium *Haloferax mediterranei**

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Aspartate aminotransferase from the archaebacterium *Haloferax mediterranei* was purified and found to be homogeneous. An average M_r of 66000 was estimated. The native halophilic transaminase exhibited no maximum absorption at 410 nm, which indicates that the apo form is obtained by our purification procedure, and the molar absorption coefficient at 275 nm in 3.5 M-KCl (pH 7.8) was found to be 78.34 $mM^{-1} \cdot cm^{-1}$. Plots of titration data show that 1 mol of halophilic aspartate aminotransferase binds 2 mol of pyridoxal 5′-phosphate. The halophilic transaminase behaved as a dimer with two similar subunits and had a maximum activity in the pH range 7.6–7.9 and at 65 °C in 3.5 M-KCl. By differential scanning calorimetry, the denaturation temperature of the halophilic holo- and apo-transaminase was determined to be 78.5 and 68.0 °C respectively at 3.3 M-KCl (pH 7.8). At low salt concentration the halophilic transaminase was inactivated, following first-order kinetics. The K_m values for 2-oxoglutarate and L-aspartate, in 3 M-KCl (pH 7.8), were 0.75 mM and 12.6 mM respectively.

INTRODUCTION

Haloferax mediterranei (strain R-4, A.T.C.C. 33500) is an extreme halophile archaebacterium able to grow on carbohydrates, polyalcohols, organic acids and some amino acids, and has features very different from other known Halobacteriaceae species (Rodriguez-Valera *et al.*, 1983; Muriana *et al.*, 1987). We have recently shown (F. J. G. Muriana, M. C. Alvarez-Ossorio & A. M. Relimpio, unpublished work) that *H. mediterranei* contains a halophilic aspartate aminotransferase the synthesis of which is increased by L-aspartate when the latter is used as the principal energy source.

Aspartate aminotransferase (AspAT; EC 2.6.1.1), a vitamin B-6-dependent enzyme, preferentially catalyses the interconversion of dicarboxylic amino and oxo acids. The mechanism of action of this enzyme, based on the three-dimensional structures of several mammalian isoenzymes, together with spectroscopic, kinetic, X-ray and other data, has been described in detail (Christen & Metzler, 1985). The enzyme has been isolated from a variety of sources, including mammalian organs (Christen & Metzler, 1985), plants (Reynolds *et al.*, 1981; Christen & Metzler, 1985), a yeast (Yagi *et al.*, 1982), a green alga (Lain-Guelbenzu *et al.*, 1990) and bacteria (Christen & Metzler, 1985; Lee & Desmazeaud, 1985). Also, in addition to evolutionary considerations, a study of AspAT from a heat-stable archaebacterium *Sulfolobus solfataricus* has been described (Marino *et al.*, 1988). It was therefore of interest to study this particular enzyme from halophile bacteria and compare it with AspATs from other sources; also, elucidation of the molecular structure of the halophilic transaminase should provide important information on the structure–function relationship and on the evolutionary process in micro-organisms adapted to extreme salt concentrations.

In the present study we have purified AspAT from *H. mediterranei* and investigated some molecular and kinetic properties of the protein.

EXPERIMENTAL

Micro-organism and growth conditions

H. mediterranei strain R-4 isolated from seawater-evaporation ponds near Alicante (Spain) was grown aerobically in liquid shake culture as previously described (Rodriguez-Valera *et al.*, 1983).

Enzyme purification

Unless otherwise stated, the purification process was carried out at 15 °C. The freshly harvested bacteria were resuspended in 50 mM-sodium phosphate buffer, pH 7.8, containing 3.5 M-KCl. The cells were broken by pressure shearing followed by ultrasonic disintegration as we reported previously (Muriana *et al.*, 1987). Cell debris was removed by centrifugation at 167000 *g* for 30 min. The supernatant was dialysed against 50 mM-sodium phosphate buffer, pH 6.6, containing 1.73 M-$(NH_4)_2SO_4$, and then centrifuged as above. Solid $(NH_4)_2SO_4$ was added to the supernatant to a final concentration of 2.4 M. After being stirred for 2 h, the suspension was centrifuged as above and the supernatant (crude extract) was collected. The halophilic AspAT showed a slight interaction with the cofactor, because by simple dialysis the apoenzyme was resolved from pyridoxal 5′-phosphate. Therefore the apo form of the native protein was obtained by our purification method.

The crude extract was loaded on to a column (1.6 cm × 40 cm) of Sepharose CL-4B equilibrated with 50 mM-sodium phosphate buffer, pH 6.6, containing 2.4 M-$(NH_4)_2SO_4$. Elution was carried out with a decreasing linear gradient of the phosphate buffer containing 2.4–0.9 M-$(NH_4)_2SO_4$. Fractions (10 ml each) were collected at a flow rate of 50 ml/h. Those with AspAT activity were pooled, concentrated by ultrafiltration, extensively dialysed against the phosphate buffer containing 2.4 M-$(NH_4)_2SO_4$, and loaded on a DEAE-cellulose column (1.5 cm × 6 cm) equilibrated with the same buffer. The halophilic transaminase was eluted again with a decreasing linear gradient of the phosphate buffer

Abbreviations used: AspAT, aspartate aminotransferase.

* Dedicated to the memory of Dr. Luis Catalina García de Longoria, who was director of the Department for Vegetal Biochemistry Research at Seville (Instituto de Agrobiología y Recursos Naturales, C.S.I.C.), where most of these experiments were performed.

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but containing 2.4–0.5 M-$(NH_4)_2SO_4$. Active fractions, collected at a flow rate of 15 ml/h, were pooled, concentrated by ultrafiltration, extensively dialysed against 50 mM-sodium phosphate buffer, pH 7.8, containing 3.5 M-KCl, and loaded on a Sephadex G-200 column (2.6 cm × 100 cm) equilibrated with the same buffer. Elution was performed with the equilibration buffer at a flow rate of 15 ml/h. Active fractions were pooled and dialysed against the phosphate buffer containing 3.5 M-KCl. This halophilic AspAT preparation was examined for purity by using the methods described below.

Assay of enzyme activity and protein determination

The halophilic AspAT activity was measured at 25 °C by monitoring the oxidation of NADH at 340 nm in a reaction mixture (1 ml) containing 50 mM-sodium phosphate buffer, pH 7.8, 3.1 M-KCl, 10 mM-2-oxoglutarate, 0.3 mM-NADH, 60 units of malate dehydrogenase from pig heart in 20% (v/v) glycerol, 0.05 mM-pyridoxal 5′-phosphate, 100 mM-L-aspartate, and the appropriate amount of enzyme. One unit of activity was defined as the amount of enzyme catalysing the conversion of 1 μmol of substrate/min under the specified conditions.

Protein was determined by the procedure of Lowry *et al.* (1951), with crystalline BSA as standard.

Gel electrophoresis

PAGE was carried out as described by Jovin *et al.* (1964). SDS/PAGE was performed according to the method of Weber & Osborn (1969).

Analytical ultracentrifugation

Sedimentation-equilibrium and sedimentation-velocity experiments were performed at 20 °C on a Beckman model E analytical centrifuge equipped with schlieren optics. The corrected sedimentation coefficient was calculated as described by Pundak & Eisenberg (1981).

Determination of diffusion coefficient

The diffusion coefficient of halophilic transaminase was determined by the method of Andrews (1965), and the corrected value as described by Pundak & Eisenberg (1981).

Determination of Stokes radius

The Stokes radius (r_S) of halophilic transaminase was determined by following general methods (Siegel & Monty, 1966).

Determination of frictional coefficient and frictional ratio

The frictional coefficient (f) of halophilic transaminase was calculated according to the method previously described (Brewer *et al.*, 1974).

M_r studies

Gel filtration under native conditions was carried out at 15 °C on a column (1.6 cm × 90 cm) of Sephadex G-200 (fine grade) (Belew *et al.*, 1978). The M_r of the native enzyme was also determined by PAGE, by sedimentation equilibrium, from the Stokes radius and from the diffusion coefficient value (Andrews, 1965; Siegel & Monty, 1966; Brewer *et al.*, 1974). Studies under dissociating conditions were performed by SDS/PAGE.

Amino acid analysis

Halophilic protein samples (40 μg) were hydrolysed *in vacuo* at 110 °C for 24 h, 48 h and 72 h in 6 M-HCl and analysed on a modular automatic amino acid analyser equipped with a Hewlett–Packard 3390 A integrator and with an Aminex A-9 (Bio-Rad Laboratories) resolve column. Tryptophan was measured spectrophotometrically (Beaven & Holiday, 1952).

Isoelectric focusing

Halophilic AspAT samples were electrofocused in LKB PAG plates (pH range 3.5–9.5 or 4.0–5.0) in an LKB Multiphor unit and were stained and destained, all according to the manufacturer's recommended procedure.

Absorption coefficient and fluorescence spectra

The u.v.-absorption and fluorescence emission spectra were obtained on a Beckman DU-7 spectrophotometer and a Perkin–Elmer fluorescence spectrophotometer model 650–40 respectively at 25 °C.

Titration with pyridoxal 5′-phosphate

Purified halophilic AspAT (8.5 nmol in 1 ml) was titrated by the addition of pyridoxal 5′-phosphate in 5 μl portions containing 0.5 nmol of pyridoxal 5′-phosphate. The fluorescence was monitored at 532 nm (excitation wavelength 396 nm) to monitor free coenzyme.

Thermodynamic parameters

Activation energies and thermodynamic quantities were estimated from the Arrhenius and transition-state relationships (Laidler & Peterman, 1979).

Differential scanning calorimetry

Calorimetric measurements were made on a Microcal MC-2 calorimeter. The calorimetric data were analysed as previously described (Pfeil, 1986).

RESULTS

Purification of halophilic AspAT

The enzyme from *H. mediterranei* was purified to apparent homogeneity from cytosolic extracts of lysed cells. The purification involved $(NH_4)_2SO_4$ precipitation, fractionation on Sepharose CL-4B and DEAE-cellulose with a decreasing concentration gradient of $(NH_4)_2SO_4$, and gel-permeation chromatography on Sephadex G-200.

A typical purification scheme is summarized in Table 1. The enzyme, which is obtained in the apo form, was purified about 100-fold with a 27% final yield. The purified enzyme was judged to be homogeneous by the criteria of native and SDS/PAGE. The enzyme also sedimented and unfolded thermally (Fig. 1, apo) as a single symmetrical peak during the sedimentation-velocity and calorimetric experiments respectively.

M_r and subunit structure

The M_r of the native halophilic AspAT was calculated to be 66000 (±3000) by exclusion chromatography, 68200 (±2000) by standardized non-denaturing gel electrophoresis, 65600 (±3000) by sedimentation equilibrium and 64200 (±2500) by diffusion equilibrium. The purified halophilic enzyme, when submitted to SDS/PAGE, gave a single protein band corresponding to an apparent M_r of 32500 (±1000). Also, a single band corresponding to pI 6.46 was obtained when analysed on an isoelectric-focusing polyacrylamide gel.

Amino acid composition

The amino acid composition of the halophilic transaminase is reported in Table 2. The predominant residues are aspartic acid/asparagine, glutamic acid/glutamine, alanine and glycine.

Table 1. Purification of AspAT from *H. mediterranei*

For experimental details see the text.

Step	Total protein (mg)	Total activity (munits)	Sp. activity (munits/mg)	Yield (%)	Purification (fold)
Crude extract	3603	75663	16	100	1
1.73 M-$(NH_4)_2SO_4$ supernatant	2425	75175	31	99	2
2.4 M-$(NH_4)_2SO_4$ supernatant	1825	73026	40	96	2.5
Sepharose CL-4B	208	70723	341	93	21.3
DEAE-cellulose	80	47668	594	63	37.1
Sephadex G-200	14	20440	1533	27	95.8

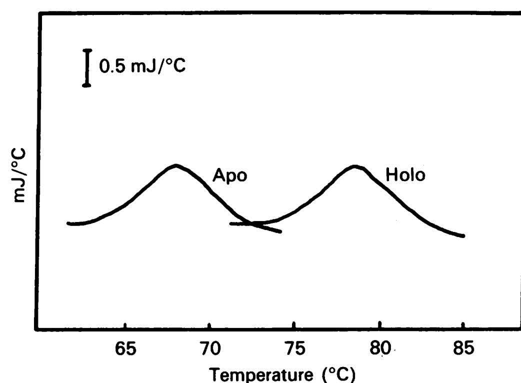

Fig. 1. Excess specific heat capacity of the apo and holo forms of the halophilic AspAT as a function of temperature

Protein concentration was 0.8 mg/ml in 0.05 M-sodium phosphate buffer, pH 7.8, containing 3.5 M-KCl.

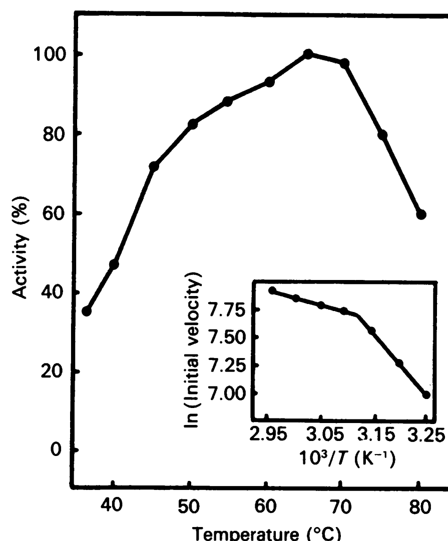

Fig. 2. Dependence on temperature of activity of the halophilic AspAT

An Arrhenius plot of the results in the range 35–65 °C is shown in the inset.

Table 2. Amino acid analysis of halophilic AspAT

Amino acid	Composition (mol/100 mol)	Proposed no. of residues/subunit*
Asx	12.8	38
Thr	4.1	12
Ser	4.9	15
Glx	12.0	36
Pro†	–	–
Gly	8.6	26
Ala	9.3	28
Val	4.2	13
Met	5.6	17
Ile	5.0	15
Leu	4.2	13
Tyr	2.4	7
Phe	3.9	12
Lys	3.9	12
His	3.1	9
Arg	5.1	15
Trp	–	7
CyS†	–	–

* A subunit M_r of 33000 was assumed.
† Not determined.

Absorption and fluorescence spectra

The u.v.-absorption spectrum of pure halophilic AspAT displayed a maximum at 275 nm when measured at 3.5 M-KCl, pH 7.8, with a molar absorption coefficient of 78.34 $mM^{-1} \cdot cm^{-1}$ ($A_{280}^{0.1\%} = 1.19$). The A_{280}/A_{260} ratio was 1.64. However, the halophilic enzyme exhibited no maximum absorption at 410 nm.

The halophilic apotransaminase recovers its full activity in 10 min when incubated with 0.05 mM-pyridoxal 5′-phosphate at 25 °C (in the presence of 10 mM-2-oxoglutarate the rate of re-activation is higher; results not shown).

The fluorescence emission spectra of the native halophilic apoenzyme are almost the same when excited at either 275 or 280 nm. Plots of titration data show that 1 mol of halophilic AspAT binds 2 mol of pyridoxal 5′-phosphate, i.e. a 1:1 coenzyme/subunit stoichiometry.

Effects of pH and temperature

The pH-dependence of halophilic transamination between L-aspartate and 2-oxoglutarate was examined in 0.15 M-Mes/NaOH or Tris/HCl buffers containing 3.5 M-KCl from pH 5.6 to pH 9.1 at 25 °C. Maximum activity is observed at pH 7.6–7.9. However, when halophilic enzyme is kept for a long time, a pH of 6.7 is more suitable for the maintenance of maximum activity, especially if the medium contains $(NH_4)_2SO_4$ (results not shown).

At pH 7.8, the halophilic AspAT exhibited maximal activity at about 65 °C in the presence of 3.5 M-KCl (Fig. 2). An Arrhenius plot is shown in the inset in Fig. 2. Table 3 summarizes the

Table 3. Arrhenius behaviour and thermodynamic activation parameters for AspAT from *H. mediterranei* and some mammalian sources

The results for the mammalian enzymes are taken from Rej & Vanderlinde (1981). Key: s, soluble (cytosolic); m, mitochondrial.

Parameter	Halophilic	Human (s)	Human (m)	Human (sera)	Pig (s)
Arrhenius slope (K^{-1}) (between 35 and 45 °C)	−5882	−6280	−6290	−6080	−6150
Arrhenius slope (K^{-1}) (between 50 and 65 °C)	−1204	–	–	–	–
E_a (kJ·mol^{-1}) (between 35 and 45 °C)	48.9	52.3	52.3	50.6	51.1
E_a (kJ·mol^{-1}) (between 50 and 65 °C)	10.0	–	–	–	–
$\Delta H^{\ddagger}_{37\,°C}$ (kJ·mol^{-1})	46.3	49.7	49.7	47.9	48.6
$\Delta H^{\ddagger}_{65\,°C}$ (kJ·mol^{-1})	7.2	–	–	–	–
$\Delta G^{\ddagger}_{37\,°C}$ (kJ·mol^{-1})	57.8	60.2	60.2	–	–
$\Delta G^{\ddagger}_{65\,°C}$ (kJ·mol^{-1})	60.7	–	–	–	–
$\Delta S^{\ddagger}_{37\,°C}$ (J·mol^{-1}·K^{-1})	−37.1	−33.9	−33.9	–	–
$\Delta S^{\ddagger}_{65\,°C}$ (J·mol^{-1}·K^{-1})	−158.3	–	–	–	–

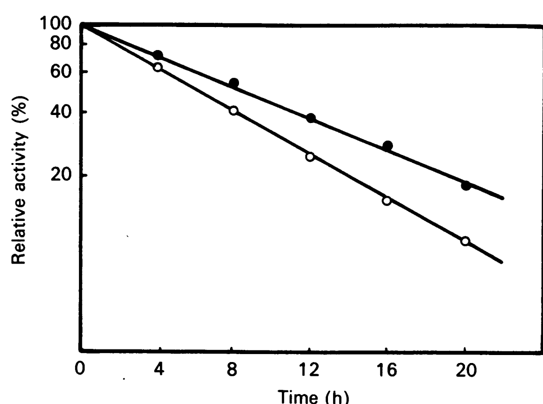

Fig. 3. Effect of salt concentration on the stability of the halophilic AspAT

Enzyme samples were dialysed against 0.05 M-sodium phosphate buffer, pH 7.8, containing 1.3 M-NaCl (○) or -KCl (●). The activities of portions removed at various time intervals were measured.

Arrhenius behaviour and thermodynamic activation parameters of AspAT from the halophile and other sources.

The halophilic enzyme was found to be stable up to 50 °C for several hours (results not shown). At 70 °C it preserved 50 % of its activity after being heated for 60 min in the presence of 2-oxoglutarate, after 9 min in the presence of L-aspartate, and after 7 min without substrates.

Thermal denaturation of the halophilic AspAT was not reversible. The denaturation temperature of the holo form was found to be 78.5 °C at 3.3 M-KCl (pH 7.8) (Fig. 1, holo), the real or calorimetric enthalpy ΔH_{cal} was estimated to be 277.9 kJ·mol^{-1}, and the van't Hoff enthalpy ΔH_{vH} was 791.6 kJ·mol^{-1}. The halophilic apo-transaminase exhibited a denaturation temperature of 68.0 °C at 3.3 M-KCl (pH 7.8) (Fig. 1, apo), the respective ΔH_{cal} and ΔH_{vH} values being 181.2 and 989.1 kJ·mol^{-1}.

Effect of salt

The inactivation of halophilic AspAT at 1.3 M concentration of NaCl and KCl was studied in phosphate buffer, pH 7.8, at room temperature. The inactivation followed first-order kinetics (k' for NaCl = 0.111 h^{-1}; k' for KCl = 0.085 h^{-1}) (Fig. 3).

Kinetic constants

The halophilic transaminase shows a rate that depends hyperbolically on the concentration of both substrates and a bi-substrate ping-pong mechanism for the enzyme reaction. The data were analysed by a direct-linear-plot method (Eisenthal & Cornish-Bowden, 1974), giving K_m values of 0.75 mM for 2-oxoglutarate and 12.6 mM for L-aspartate at 3 M-KCl (pH 7.8).

Sedimentation velocity

The average value $s_{20,w} = 3.44 \pm 0.06$ S was found.

Diffusion coefficient

The average value of $D_{20,w}$ was $4.85 \times 10^{-7} \pm 0.04 \times 10^{-7}$ cm^{-2}/s.

Stokes radius

The average value, in phosphate buffer containing 3.5 M-KCl (pH 7.8), of r_S was 3.58 (±0.02) nm.

Frictional coefficient and frictional ratio

The value of the frictional coefficient (f) was 8.77×10^{-8} g·s^{-1}, and the ratio f/f_0 was 1.34.

DISCUSSION

In this paper we present data on the purification and characterization of the main physicochemical and catalytic properties of AspAT from the halophile archaebacterium *H. mediterranei*. To our knowledge, it is the first transaminase that has been purified to apparent homogeneity from a halophile source since the first report of the existence of this enzyme activity in archaebacteria from the Dead Sea in 1976 (Mevarech *et al.*, 1976).

The Sepharose/$(NH_4)_2SO_4$ fractionation method can handle large protein quantities (Mevarech *et al.*, 1976), has a good separation yield and thus was the method of choice for the crude stage. Two additional chromatographic methods, ion-exchange and gel-filtration chromatography, were sufficient to achieve complete purification. The procedure was carried out entirely at high ionic strength, which was necessary to maintain the native enzyme, and at room temperature to avoid possible cold-lability (Pundak & Eisenberg, 1981; De Medicis *et al.*, 1982).

It may be noted that the reconstituted halophilic holotransaminase shows a specific activity of 1.5 units/mg, which is two orders lower than that of other non-halophilic AspATs (124–350 units/mg) (Jenkins, 1985). These differences may be explained on the basis of their dissociation constants (K_d) for pyridoxal 5′-phosphate, because high specific activities are a feature of non-halophilic AspATs with low K_d (0.44–0.001 μM) (Jenkins & Fondan, 1985), and our own experiments indicate that the K_d for

halophilic AspAT is close to 7.0 μM. (F. J. G. Muriana, M. C. Alvarez-Ossorio & A. M. Relimpio, unpublished work). The foregoing agrees with previous studies on brain 4-aminobutyrate aminotransferase, where a K_d value of 1.6 μM and a specific activity of 17.6 units/mg have been described (John & Fowler, 1976). Indeed, the equilibrium

$$\text{Holoenzyme} \rightleftharpoons \text{apoenzyme} + \text{coenzyme}$$

has been related to several regulation systems of pyridoxal 5'-phosphate-dependent enzymes, such as brain glutamate decarboxylase and 4-aminobutyrate aminotransferase, both of which have high K_d values (Cooper *et al.*, 1978).

The halophilic AspAT from *H. mediterranei* is a dimer of two subunits identical in M_r (33000), which is smaller than those previously described (45000–65000) (Yagi *et al.*, 1982; Christen & Metzler, 1985; Kuramitsu *et al.*, 1985; Lee & Desmazeaud, 1985; Marino *et al.*, 1988; Lain-Guelbenzu *et al.*, 1990). This is common to other halophilic enzymes (Keradjopoulos & Holldorf, 1979).

The amino acid composition of the halophilic AspAT (Table 2) is very similar to that of the cellular bulk proteins (Rodriguez-Valera *et al.*, 1983). The preponderance of acidic over basic residues in this enzyme is reflected in the isoelectric point (6.46), which is lower than that of the mitochondrial isoenzymes from pig and chicken heart (Christen & Metzler, 1985) rabbit liver (Kuramitsu *et al.*, 1985), yeast (Yagi *et al.*, 1982) and the archaebacterium *S. solfataricus* (Marino *et al.*, 1988).

The absorption spectrum of the native halophilic AspAT (apo form) shows a maximum at 275 nm when it is measured at high salt concentration. The molar absorption coefficient agrees with the data reported for other non-halophilic transaminases (Yagi *et al.*, 1982). The emission spectrum results mainly from tryptophan fluorescence, because a peak at 337 nm was detected at high salt concentration. It has been claimed that the exposure of tryptophan, and probably other aromatic residues, might be a general property of halophilic enzymes (Leicht *et al.*, 1978).

The halophilic AspAT has a well-defined optimum pH around 7.6–7.9, which is in complete agreement with those previously reported for the transaminases from other sources (Christen & Metzler, 1985; Kuramitsu *et al.*, 1985), but significantly lower than the optimum pH values found for the transamination reaction in yeast (Yagi *et al.*, 1982), *Pseudomonas striata* (Yagi *et al.*, 1976) and *Brevibacterium linens* 47 (Lee & Desmazeaud, 1985).

The halophilic transaminase shows a remarkable gradient of thermophilicity, the optimum temperature being 65 °C when measured at 3.5 M-KCl. $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ values for the halophilic enzyme are lower than the corresponding values for the mammalian enzymic reaction (Table 3). These differing contributions of enthalpic and entropic energies to $\Delta G^{\ddagger}$ may be due to the structuring of water molecules around exposed hydrophobic amino acid residues during catalysis (Low *et al.*, 1973). The thermal stability of the halophilic AspAT is significantly greater in the presence of 2-oxoglutarate than that of most well-studied transaminases from mesophiles and similar to the remarkable resistance to heat-inactivation of the truly thermophilic transaminase from *S. solfataricus* (Marino *et al.*, 1988). Moreover, differential-scanning-calorimetry studies confirm a reduction in thermal stability of the apo form with respect to the holo form (Fig. 1), which suggests that the thermolability of the halophilic AspAT is more probably due to detachment of the cofactor or changes in configuration limited to the active site than to the fragility of second and higher structures of the enzyme as a whole.

The stabilization of the halophilic AspAT also apparently requires high concentrations of salt effective for salting-out. The halophilic transaminase not only tolerates salt for its stability, but also requires high salt concentration for its activity. It is probably not a coincidence that KCl, the more abundant intracellular salt (Eisenberg & Wachtel, 1987), is also the best activator at concentrations approaching physiological conditions.

The apparent K_m of halophilic AspAT for L-aspartate (12.6 mM) at high salt concentration is exceptionally different from those reported for other sources (0.11–4.2 mM) (Christen & Metzler, 1985; Lee & Desmazeaud, 1985; Lain-Guelbenzu *et al.*, 1990). On the other hand, the apparent K_m value for 2-oxoglutarate (0.75 mM), also at high salt concentration, is of the same order as those reported for soya-bean root nodules (Ryan *et al.*, 1972), chicken and pig heart, rat liver and *Bacillus subtilis* (Christen & Metzler, 1985), but is slightly higher than those for the AspATs from yeast (Yagi *et al.*, 1982), rabbit liver (cytosolic; Kuramitsu *et al.*, 1985), *Chlamydomonas reinhardtii* (Lain-Guelbenzu *et al.*, 1990), *Ps. striata* (Christen & Metzler, 1985) and *S. solfataricus* (Marino *et al.*, 1988).

Under conditions in which the M_r and enzyme activity of halophilic AspAT remain unchanged, the corrected sedimentation coefficient $s_{20,w}$ (3.44 S) is lower than, whereas the corrected diffusion coefficient $D_{20,w}$ (4.85×10^{-7} cm$^2 \cdot$s^{-1}) seems to be similar to, those observed for a variety of different AspATs from non-halophilic systems (Christen & Metzler, 1985). It is likely that the decrease in $s_{20,w}$ is the result of changes in the incompletely understood interactions of the halophilic enzyme with the medium (i.e. with water and salt), rather than from significant conformational changes (Pundak & Eisenberg, 1981).

In conclusion, the various studies surveyed here demonstrate that the properties of AspAT from *H. mediterranei*, which pertain to the halophilic character, are common to most other halophilic enzymes. Conceivably, the difference in molecular and catalytic features between the halophilic and non-halophilic AspATs is due to their differences in structure.

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