Partial Purification and Some Properties of an Aromatic-amino-acid and an Aspartate Aminotransferase in *Brevibacterium linens* 47

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An L-aromatic-amino-acid aminotransferase (AT-IA) and an L-aspartate aminotransferase (AT-IB) were partially purified from *Brevibacterium linens* 47 grown on L-phenylalanine as sole nitrogen source and some properties were examined. Both enzymes were active with L-aromatic amino acids and L-aspartate. AT-IA showed 6 to 12 times higher affinity and slightly higher $V_{\max}$ for the aromatic amino acids than for aspartate. AT-IB had higher affinity for tryptophan and tyrosine than for aspartate. However, this enzyme showed 6 to 10 times higher $V_{\max}$ for the latter than for the aromatic amino acid substrates. Both enzymes had similar pH (8.5–9.0) and temperature (37–40 °C) optima, but they differed in their molecular weights (126 000 for IA and 81 000 for IB) and markedly in their thermostability. At 50 °C, AT-IB was 14 times more rapidly inactivated and its inactivation rate also increased more rapidly (z-value, 7.9 °C) as the temperature increased than AT-IA (z-value, 15.5 °C). The cofactor pyridoxal-5'-phosphate was tightly bound to both enzymes. Two enzymes co-eluted on DEAE-Trisacryl M column chromatography at pH 7.5 and could be separated by chromatography on a hydroxyapatite (HA-Ultrogel) column at the same pH. Chromatography on hydroxyapatite of AT-I from cells grown on L-phenylalanine and on ammonium sulphate revealed that AT-IA was present only in phenylalanine grown cells. AT-IB was present in both extracts at similar levels, suggesting that it is constitutive. The inducibility of IA suggested that it has an *in vivo* catabolic role. AT-IA is probably the key enzyme for the utilization of the aromatic amino acids as sole nitrogen sources in *B. linens* 47.

INTRODUCTION

Aminotransferases in general have broadly overlapping substrate specificities and can catalyse *in vitro* the reversible transamination reactions, their role *in vivo* being usually determined by enzyme synthesis regulation (repressibility or inducibility) as in prokaryotic cells and/or by cellular organization, i.e. occurrence mainly in particular organs or organelles, as in higher organisms and eukaryotic micro-organisms (Jensen & Calhoun, 1981). L-Aromatic-amino-acid:2-oxoglutarate aminotransferase (EC 2.6.1.57), also active for L-aspartate, is a ubiquitous enzyme in micro-organisms, and catalyses the last step of phenylalanine and tyrosine biosynthesis or the first step of aromatic amino acid catabolism. L-Aspartate:2-oxoglutarate aminotransferase (EC 2.6.1.1) is also widespread in nature, and in animal and plant cells occurs both as mitochondrial and cytosolic isoenzymes. In micro-organisms, both enzymes have been extensively studied in *Escherichia coli* (Mavrides & Orr, 1975; Gelfand & Steinberg, 1977; Powell & Morrison, 1978) and to a lesser extent in *Klebsiella aerogenes* (Paris & Magasanik, 1981*a, b*), *Pseudomonas aeruginosa* (Whitaker *et al.*, 1982), *Flavobacterium* spp. (Beschle *et al.*, 1982) and *Saccharomyces cerevisiae* (Kradolfer *et al.*, 1982; Yagi *et al.*, 1982). With the *E. coli*

Abbreviations: MVL medium, mineral salts/vitamins/lactate medium; PLP, pyridoxal-5'-phosphate.

enzymes, considerable overlap of substrate specificity has been observed and they also share some similarities in their physical properties such as molecular weights and isoelectric points (Mavrides & Orr, 1975; Powell & Morrison, 1978). Thus in *E. coli*, there has been considerable confusion on the identity and non-identity of these two enzymes (Collier & Kohlhaw, 1972; Mavrides & Orr, 1975), and it is only recently that their non-identity has been unequivocally established by the sequential mutagenesis approach (Gelfand & Steinberg, 1977) and subsequent enzymological studies (Powell & Morrison, 1978). Moreover, in spite of their probable importance in the organic nitrogen metabolism of bacteria there has been relatively little investigation of these enzymes other than in *E. coli*.

In *Brevibacterium linens* 47 we have separated two activities of aromatic-amino-acid aminotransferase (AT-I and AT-II) on DEAE-Trisacryl M column chromatography and on polyacrylamide gel electrophoresis (C.-W. Lee & M. J. Desmazeaud, unpublished results). During the purification of AT-I for further characterization, it was further resolved into two activities. We describe here the partial purification and characterization of these two activities.

METHODS

Organism and culture conditions. *Brevibacterium linens* 47 was used throughout this investigation and is described elsewhere (Boyaval *et al.*, 1984). Mineral salts/vitamins/lactate (MVL) medium contained per litre: KH_2PO_4 , 0.24 g; K_2HPO_4 , 1.7 g; CaCl_2 , 25 mg; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.2 g; $\text{FeSO}_4 \cdot \text{H}_2\text{O}$, 5 mg; NaCl, 10 g; sodium DL-lactate (60%, w/v syrup; Sigma), 16.6 g; and 5 ml of the vitamin solution of Bousfield (1972). The nitrogen source was either L-phenylalanine (12 mM) for 'induced' cells or ammonium sulphate (12 mM) for 'non-induced' cells. The medium was adjusted to pH 7.3 with 0.1 M-NaOH and autoclaved at 121 °C for 20 min, except for the vitamin solution which was added aseptically after sterilization by filtration (pore size 0.2 µm; Schleicher & Schüll, Dassel, FRG) to the sterile medium. The organism was grown in 200 ml batches of medium in one litre conical flasks inoculated with 1% (v/v) of a 24 h grown culture in the same medium. Cultures were incubated at 26 °C on a reciprocal shaker (100 r.p.m.) to late exponential phase (48 to 72 h). Stock cultures were maintained on brain heart infusion agar (Difco) slants and plates. Cell growth was followed by the optical density at 650 nm.

Preparation of crude extracts. Cells were harvested by centrifugation at 10000 g for 10 min at 4 °C and washed twice in 50 mM-potassium phosphate buffer, pH 7.5 at 4 °C. Then 1 g (wet wt) of cell paste was resuspended in 5 ml 50 mM-potassium phosphate buffer, pH 7.5, containing 2 mM-2-mercaptoethanol, 1 mM-EDTA and 0.1 mM-pyridoxal-5'-phosphate (PLP) ('supplemented' buffer), and cells were disrupted by ultrasonic treatment of five periods of 60 s at 0.7 A (Ultrasonic, Meaux, France) at 0 °C. Cell debris and unbroken cells were removed by centrifugation at 4 °C for 20 min at 33000 g. The supernatant contained 1.5 to 2.0 mg proteins ml⁻¹ and was termed the crude extract.

Enzyme assays. Both AT-IA and AT-IB were active for the substrate pair L-phenylalanine:2-oxoglutarate, and their activities in column eluates were conveniently assayed as L-phenylalanine:2-oxoglutarate aminotransferase. The reaction mixture contained in a final volume of 1.0 ml: Tris/HCl buffer (pH 8.5), 100 µmol; 2-oxoglutarate, 10 µmol; PLP, 0.05 µmol; and enzyme, 0.3 to 5 milliunits (mU). After preincubation for 5 min at 37 °C, the reaction was started by the addition of 10 µmol L-phenylalanine and stopped after 10 min with 0.5 ml 2 M-NaOH. A_{320} was measured against a blank in which enzyme was omitted. To calculate the enzyme activity, a molar absorption coefficient (ϵ_M) of 17500 M⁻¹ cm⁻¹ for phenylpyruvate was used (Dawson *et al.*, 1969). For the enzyme assay with alternative aromatic amino acids as amino group donors, the amino group donor and acceptor were added at similar concentrations. The activities of tyrosine aminotransferase and tryptophan aminotransferase were measured at 330 nm, and the following values of ϵ_M were respectively applied for the conversion of A_{330} into enzyme activities: 19500 M⁻¹ cm⁻¹ for 4-hydroxybenzaldehyde formed from 4-hydroxyphenylpyruvate in alkali (Diamondstone, 1966), and 15000 M⁻¹ cm⁻¹ for indolpyruvate (Collier & Kohlhaw, 1972). Aspartate aminotransferase activity was assayed by a modification of the method of Sizer & Jenkins (1962). The reaction mixture contained in a final volume of 1.0 ml: Tris/HCl buffer (pH 8.5), 100 µmol; 2-oxoglutarate, 10 µmol; NADH, 100 µg; malate dehydrogenase (EC 1.1.1.37), 2 U; and enzyme. The reaction was started by the addition of 10 µmol L-aspartate, and carried out at 37 °C. The decrease in A_{340} was followed with a Cary 219 recording spectrophotometer (Varian Associates Inc., Palo Alto, Calif., USA). The ϵ_M value of 6200 M⁻¹ cm⁻¹ was assumed for NADH in calculating the enzyme activity.

Definition of enzyme unit and specific activity. One unit of aminotransferase is defined as that amount which produces 1 µmol of corresponding 2-oxo acid min⁻¹ under the conditions described. Specific activity is expressed as milliunits (mU; nmol min⁻¹) mg protein⁻¹.

Protein assay. Protein concentrations after each purification step were determined by the 'standard' method of Bradford (1976), using crystalline bovine serum albumin (Serva Feinbiochemica, Heidelberg, FRG) as a standard, while those in column effluents were monitored by A_{280} .

Enzyme purification. All steps were carried out at 2 to 4 °C unless indicated otherwise. Freshly prepared 20% (w/v) streptomycin sulphate solution, which had been adjusted to pH 7.0 with 1 M-NaOH, was added to crude extracts with constant stirring to a final concentration of 0.5% (w/v). After standing for 15 min, insoluble material was removed by centrifugation. Ammonium sulphate fractionation of the supernatant was carried out by adding solid ammonium sulphate, and protein precipitated between 50 and 75% saturation was collected by centrifugation. The precipitate was dissolved in a minimum volume of 'supplemented' 0.05 M-buffer and passed through a Sephadex G-25 column (bed volume, 90 ml; diameter, 2.4 cm), equilibrated previously with the same buffer. Eluates were applied to a column (bed volume, 70 ml; diameter, 2.5 cm) of DEAE-Trisacryl M (Industrie Biologique Française, Clichy, France), pre-equilibrated with the 'supplemented' 0.05 M-buffer. Proteins were eluted with a linear gradient of NaCl (0 to 0.4 M in a total volume of 400 ml of the starting buffer). Fractions of 3.4 ml were collected and those contained in the major peak (AT-I) of the activity were pooled and concentrated by addition of solid ammonium sulphate (0.56 g ml⁻¹). The precipitate collected by centrifugation was dissolved in a minimal volume of 5 mM-potassium phosphate buffer, pH 7.5 and desalted through a Sephadex G-25 column. Active fractions were applied to an hydroxyapatite (HA-Ultrogel; IBF) column (bed volume, 26 ml; diameter, 1.6 cm) previously equilibrated with 5 mM-potassium phosphate buffer, pH 7.5. After washing with 83 ml of the starting buffer, a linear gradient was applied from 5 to 200 mM-potassium phosphate buffer, pH 7.5 in a total volume of 140 ml. Active fractions were pooled and concentrated by ammonium sulphate precipitation as described above. Protein precipitate was redissolved in 1.0 ml of 'supplemented' 0.1 M-buffer and applied to a Sephacryl S-200 superfine (Pharmacia) gel filtration column (bed volume, 96.2 ml; diameter, 1.4 cm; void volume, 54.5 ml). Fractions of 0.68 ml were collected at 15.7 ml h⁻¹ and portions analysed for enzyme activity. Active fractions were pooled and concentrated by Immersible CX-10 (molecular weight cut, 10⁴ daltons; Millipore) Portions (2.5 ml) of concentrated preparation were passed through a small Sephadex G-25 column (PD-10; Pharmacia) pre-equilibrated with 50 mM-potassium phosphate buffer, pH 7.5, and used for further characterization.

Polyacrylamide slab gel electrophoresis. The procedure of Davis (1964) was followed except that the concentration of the running gel buffer was 0.375 M. Electrophoresis was for 4 h at 150 V at 4 °C. The gel was stained for protein with Coomassie brilliant blue R-250 (Serva) or for the phenylalanine aminotransferase activity by the procedure of Ryan *et al.* (1972). The activity staining mixture contained per ml: L-phenylalanine, 2 mg; 2-oxoglutarate, 1 mg; NAD, 2 mg; phenazine methosulphate, 50 µg; nitroblue tetrazolium, 0.5 mg; glutamate dehydrogenase, 0.1 U; PLP, 0.1 mg; and 0.1 M-Tris/HCl buffer, pH 8.5. In blanks, 2-oxoglutarate or the amino acid was omitted.

Estimation of molecular weights. The same column of Sephacryl S-200 used for the last step of purification, was equilibrated with 'supplemented' 0.1 M-buffer and calibrated with the following molecular weight markers: cytochrome *c*, 12500; chymotrypsinogen A, 25000; hen egg albumin, 45000; bovine serum albumin, 68000; lactate dehydrogenase, 140000; and catalase, 240000.

Thermal inactivation kinetics. Concentrated enzyme preparation after step 5 (Table 1) was used, after passing through a Sephadex G-25 column which was pre-equilibrated with 0.1 M-potassium phosphate buffer, pH 7.5. Portions of 1.0 ml of diluted enzyme solution in glass test tubes (6 × 50 mm) were pre-incubated in a water bath at the desired temperature (± 0.2 °C). The increase in temperature of the stirred enzyme solution was monitored and the sampling (50 µl) at zero heating time was done when it attained the desired temperature. Thereafter, 50 µl samples were removed and immediately transferred into test tubes containing 950 µl cold 50 mM-potassium phosphate buffer, pH 7.5, which were kept in an ice bath until all samples were taken. Residual activity was assayed as phenylalanine aminotransferase, and expressed as percentage of the activity at zero heating time.

Thermodynamic parameters of thermal inactivation were obtained graphically by plotting log (k'/T) versus $1/T$ according to Eyring's absolute reaction rate equation (Aylward & Haisman, 1969):

$$k' = K \frac{kT}{h} e^{(-\Delta H^*/RT)} e^{(\Delta S^*/R)}$$

where k' is the specific rate constant; K , transmission coefficient; k , Boltzman's constant; h , Planck's constant; T , absolute temperature; R , gas constant; ΔH^* , change in enthalpy for the activation process of thermal inactivation of the enzyme; ΔS^* , change in entropy for the same process. In practice, k' was obtained by $2.303/D$ (D , decimal reduction time), ΔH^* by $-2.303R \times \text{slope}$, and ΔS^* from $2.303R[\text{intercept} + \log(Kk/h)]$, K being taken as unity (Aylward & Haisman, 1969). The free energy of activation for the thermal inactivation of the enzyme was calculated from the equation: $\Delta F^* = \Delta H^* - T\Delta S^*$.

RESULTS

Comparison of chromatographic profiles of phenylalanine aminotransferase activity in crude extracts

When crude extracts of 'induced' and 'non-induced' cells were chromatographed on a DEAE-Trisacryl M column, two activity peaks (AT-I and AT-II) were obtained (Fig. 1). The level of

Fig. 1

Fig. 2

Fig. 1. Aminotransferase activity profiles of crude extracts of *B. linens* 47. Enzyme activity was assayed as L-phenylalanine:2-oxoglutarate aminotransferase. Extracts were prepared from cells grown on MVL medium containing L-phenylalanine (●) or ammonium sulphate (○) as sole nitrogen sources, and 40 mg proteins of each extract were chromatographed on DEAE-Trisacryl M (a). The activity of AT-I from each extract was collected and re-chromatographed on HA-Ultrogel (b). Linear gradients (---) of NaCl or potassium phosphate buffer, pH 7.5 were as indicated.

Fig. 2. Polyacrylamide slab gel electrophoresis of AT-IA (a, c) and AT-IB (b, d). Enzyme preparations obtained after step 5 (Table 1) were used: (a, b) protein staining; (c, d) activity staining for the L-phenylalanine:2-oxoglutarate aminotransferase as described in Methods; O, origin; TD, tracking dye front (bromophenol blue).

AT-I of 'induced' cells was 5 to 10 times higher than that of 'non-induced' cells, while the activity of AT-II was similar in both extracts. AT-I of 'induced' cells was separated into two activities (AT-IA and AT-IB) by chromatography on an HA-Ultrogel column (see below). To determine whether the higher level of enzyme activity of 'induced' cells was due to the increase of the level of AT-IA or AT-IB alone or both, AT-I from 'induced' and 'non-induced' cells were chromatographed on HA-Ultrogel and activity profiles were compared. When AT-I, collected from DEAE-Trisacryl M column chromatography of the cell extract of 'induced' cells, was re-chromatographed on HA-Ultrogel, two activity peaks were eluted (AT-IA and AT-IB in eluting order). By contrast, AT-I from 'non-induced' cells gave only one activity peak comparable to AT-IB of 'induced' cells, which suggested that the activity AT-IB was present in both extracts. No significant difference was observed in the activity level of AT-IB in both extracts. The results showed that AT-IA was an inducible enzyme whilst AT-IB appears to be a constitutive enzyme.

Separation and partial purification of the aromatic-amino-acid aminotransferase (AT-IA) and the aspartate aminotransferase (AT-IB) in B. linens 47

AT-IA and AT-IB, which could be classified respectively as an aromatic-amino-acid aminotransferase and an aspartate aminotransferase for the reasons given below, were enriched respectively about 80- and 54-fold for their primary activities by the purification steps shown in Table 1.

Table 1. Summary of partial purification of an aromatic-amino-acid aminotransferase (AT-IA) and an aspartate aminotransferase (AT-IB) from *B. linens* 47

Procedure	Total protein (mg)	Total activity* (mU)				Specific activity* (mU mg protein ⁻¹)				Yield (%)
		Phe	Tyr	Trp	Asp	Phe	Tyr	Trp	Asp	
1. Crude extract	638	51040	15310	31260	24880	80	24	49	39	100†
2. Streptomycin sulphate and (NH ₄) ₂ SO ₄ fractionation, 50–75% saturation	163	42360	12710	26895	22005	260	78	165	135	83†
3. DEAE-Trisacryl column chromatography	20.2	28381	8484	18241	14665	1405	420	903	726	55.6†
4. HA-Ultrogel column chromatography										
AT-IA	5.4	21244	6210	13716	10978	3934	1150	2540	2023	41.6†
AT-IB	13.3	1929	878	1450	11843	145	66	109	890	47.6‡
5. Sephacryl S-200 gel filtration										
AT-IA	1.79	11658	3167	6847	5298	6513	1769	3825	2960	22.8†
AT-IB	3.55	1150	533	845	7331	324	150	238	2065	29.5‡

* The amino group acceptor in the assay mixture was 10 mM-2-oxoglutarate.

† Yield for the activity of phenylalanine aminotransferase.

‡ Yield for the activity of aspartate aminotransferase.

Table 2. Kinetic parameters of partially purified aminotransferases AT-IA and AT-IB from *B. linens* 47

The buffer used was 0.1 M-Tris/HCl, pH 8.5. Assay conditions are given in the text. The constants were determined from Lineweaver-Burk plots of the activities at various substrate concentrations, except for the $V_{\max}$ values marked by †, which are the activities at the indicated substrate concentrations.

Amino group donor (mM)	Amino group acceptor*	Apparent K_m (mM)		$V_{\max}$ (mU mg protein ⁻¹)	
		IA	IB	IA	IB
L-Phenylalanine	2-Oxoglutarate	1.3	6.2	6714	485
L-Phenylalanine (10)	Pyruvate	ND	ND	728†	31†
DL-Phenylalanine (10)	2-Oxoglutarate	ND	ND	5393†	272†
L-Tyrosine	2-Oxoglutarate	1.4	1.6	4040	343
L-Tyrosine (1)	Pyruvate	ND	ND	308†	23†
DL-Tyrosine (0.5)	2-Oxoglutarate	ND	ND	423†	38†
L-Tryptophan	2-Oxoglutarate	0.7	0.6	4780	273
L-Tryptophan (10)	Pyruvate	ND	ND	600†	37†
DL-Tryptophan (10)	2-Oxoglutarate	ND	ND	2625†	165†
L-Aspartate	2-Oxoglutarate	8.4	3.8	3356	2785

ND, Not determined.

* Amino group acceptors were added at 20 mM in assay mixtures.

Electrophoretic studies. When 20 µg of the enzyme proteins of AT-IA and AT-IB (after step 5) were electrophoresed on a polyacrylamide slab gel at pH 8.9, protein staining showed one major band ($R_F = 0.87$) and two minor bands for AT-IA, and one major band ($R_F = 0.84$) and three minor ones for AT-IB. Activity staining of duplicate gel showed one activity band for each enzyme preparation, which corresponded to the major protein bands (Fig. 2). These activity bands were absent in blanks in which either 2-oxoglutarate or the amino acid substrate was omitted from the complete staining mixture.

Substrate specificity and kinetic parameters. Both AT-IA and AT-IB were active for L-phenylalanine, L-tyrosine, L-tryptophan and L-aspartate (Table 2) and were completely inactive

Fig. 3

Fig. 3. Estimation of the molecular weights of AT-IA and AT-IB by gel filtration on Sephacryl S-200. The standard proteins (○) used were: 1, cytochrome *c*; 2, chymotrypsinogen A; 3, hen egg albumin; 4, bovine serum albumin; 5, lactate dehydrogenase; 6, catalase.

Fig. 4

Fig. 4. Dependence on pH of activity of *B. linens* 47 aminotransferases AT-IA (filled symbols) and AT-IB (open symbols). Enzyme activity was assayed as L-phenylalanine:2-oxoglutarate aminotransferase. The following buffers were used: pH 6.0–8.0, potassium phosphate (▲, △); pH 7.5–9.0, Tris/HCl (●, ○); pH 8.6–10.5, glycine/NaOH (■, □). 100% activity corresponds to approximately 300 mU ml⁻¹ and 200 mU ml⁻¹ for AT-IA and AT-IB, respectively.

for D-isomers. For all aromatic amino acids, no inhibition of activity for L-amino acid was observed by the D-isomer, when the latter was present at the same concentration as that of L-isomer. AT-IA showed a higher apparent K_m value for aspartate than for the aromatic amino acids, and the lowest V_{max} for aspartate. Therefore, this enzyme can justly be classified as an L-aromatic-amino-acid aminotransferase (EC 2.6.1.57). Though AT-IB showed higher affinity for tryptophan and tyrosine than for aspartate, its 6- to 10-fold higher V_{max} for aspartate compared to the aromatic amino acids suggested its classification as an aspartate aminotransferase (EC 2.6.1.1). For both enzymes oxalacetate was a good amino group acceptor for the aromatic amino acids (data not shown), but pyruvate acted at a much lower rate (Table 2).

Molecular weight estimation of the enzymes. The apparent molecular weights of the partially purified aminotransferases (after step 5) were estimated by gel filtration on a Sephacryl S-200 column to be 126 000 and 81 000 for AT-IA and AT-IB, respectively (SD 4000, two runs; Fig. 3). For linear correlation between log (mol. wt) and elution volumes with Sephacryl S-200, it was important that the molarity of the elution buffer was greater than 0.05 M.

pH and temperature optima. Both enzymes had a pH optimum of 8.5 to 9.0 (Fig. 4) for the L-phenylalanine:2-oxoglutarate aminotransferase activity. AT-IB showed a less symmetrical pH profile than AT-IA, and retained higher relative activity at lower pH values. With glycine/NaOH buffers at pH 8.5 and 9.0, the activity was nearly completely inhibited for both enzymes. The reason is not yet clear. Both enzymes also showed similar temperature dependence of the activity and had an optimum at 37 to 40 °C at pH 8.5. Plotting the logarithm of the activities between 0 to 30 °C against the reciprocal of the absolute temperature gave straight lines, from which the activation energy was calculated to be 47.7 kJ mol⁻¹ and 52.5 kJ mol⁻¹ for AT-IA and AT-IB, respectively (Fig. 5).

Stability and thermal inactivation. Both partially purified enzymes could be stored at 4 °C in 'supplemented' 0.1 M-potassium phosphate buffer, pH 7.5, containing 0.02% sodium azide for at least six months without noticeable loss of activity. Thermal inactivation experiments at several temperatures (37 to 55 °C) were carried out with the enzyme preparations obtained after step 5. AT-IA showed biphasic inactivation kinetics, while AT-IB showed monophasic kinetic curves (Fig. 6). The z-values, defined as the increase in temperature necessary to produce a 10-fold

Fig. 5. Dependence on temperature of activity of *B. linens* 47 aminotransferases AT-IA (■) and AT-IB (□). Activity was assayed as L-phenylalanine:2-oxoglutarate aminotransferase. Arrhenius plots of the results in the range 0–30 °C are given in the inset.

Fig. 6. Thermal inactivation curves of AT-IA (a) and AT-IB (b). Enzymes were in 0.1 M-potassium phosphate buffer, pH 7.5 and the activity was assayed as L-phenylalanine:2-oxoglutarate aminotransferase. Protein concentrations in the enzyme solutions were 40 $\mu\text{g ml}^{-1}$ and 80 $\mu\text{g ml}^{-1}$ for IA and IB, respectively. 100% residual activity corresponds to 260 mU ml^{-1} and 25 mU ml^{-1} for IA and IB, respectively. Experimental details are described in Methods.

Table 3. *Thermodynamic constants of the thermal inactivation of aminotransferases AT-IA and AT-IB from B. linens 47*

The data are given for 50 °C.

Enzyme	$10^4 \times$ Inactivation rate (s^{-1})	ΔF^* (kJ mol^{-1})	ΔH^* (kJ mol^{-1})	ΔS^* ($\text{J mol}^{-1} \text{K}^{-1}$)
AT-IA	7.5	98.40	120.30	68
AT-IB	106.6	91.70	238.90	456

increase in the rate of inactivation (k'), were calculated in the temperature range examined to be 15.5 °C and 7.9 °C for AT-IA and AT-IB, respectively. This reflects a considerable difference between the two enzymes. Thermodynamic constants for the activation process of the thermal inactivation of the two enzymes were obtained by plotting the results of Fig. 6 according to Eyring's absolute reaction rate equation (see Methods), and data obtained at 50 °C are shown in Table 3. In spite of the similar values of the activation energy (ΔF^*) of thermal inactivation obtained for the two enzymes, the inactivation rate of AT-IB was 14 times higher than that of AT-IA.

Effect of pyridoxal-5'-phosphate. The activities of the final enzyme preparations AT-IA and AT-IB were diminished by only 13% and 24%, respectively, in the absence of PLP. When the enzymes were dialysed against 1000 vols 50 mM-potassium phosphate buffer, pH 7.5, at 4 °C for 16 h, AT-IA and AT-IB lost, respectively, only 55% and 67% of the initial activities. Thus, the cofactor appeared to be tightly bound to the proteins. More than 90% of the initial activities were restored by the addition of 0.05 mM-PLP to the dialysed enzyme solutions.

DISCUSSION

In earlier work with *Brevibacterium linens* 47, two activities (AT-I and AT-II) of aromatic-amino-acid aminotransferase were separated on DEAE-Trisacryl M column chromatography of cell extracts (C.-W. Lee & M. J. Desmazeaud, unpublished results). From the comparison of cell extracts of 'induced' and 'non-induced' cells by the same chromatography, we concluded that AT-I was an inducible enzyme having a minimum level (Fig. 1) which was also observed in cell extracts of 'non-induced' cells. In the present study, it was shown that AT-I was composed of an inducible component and a constitutive one (respectively IA and IB). Because no activity of IA was observed in extracts of 'non-induced' cells on HA-Ultrogel chromatography, it is now clear that the minimum level of AT-I on DEAE-Trisacryl column chromatography was in fact the level of the constitutive enzyme IB which co-eluted with IA from the DEAE-Trisacryl column. Thus we have shown that extracts of *B. linens* 47 contain three distinct aromatic-amino-acid aminotransferases. Two enzymes, AT-IA and AT-IB, were partially purified and some properties were examined. Based on the kinetic parameters, AT-IA was classified as an L-aromatic-amino-acid aminotransferase (EC 2.6.1.57) and AT-IB as an L-aspartate aminotransferase (EC 2.6.1.1). The enzymes were most readily distinguished by their heat stability: inactivation rates at 50 °C were $7.5 \times 10^{-4} \text{ s}^{-1}$ versus $106.6 \times 10^{-4} \text{ s}^{-1}$, respectively, for IA and IB. The entropy changes (ΔS^* , Table 3) involved in the inactivation process suggested that the thermolability of AT-IB was due more probably to the fragility of second and higher structures as a whole of the enzyme rather than to a simple detachment of the cofactor or minor changes in configuration limited to the active site. In fact, the results of the dialysis of the enzymes showed that the cofactor PLP was tightly bound for both enzymes. AT-IA was 5- to 10-fold induced by the growth of cells on L-phenylalanine as sole nitrogen source. This strongly suggests a catabolic role of the enzyme *in vivo*. Similarly, an inducible aromatic aminotransferase (AT-3) has been identified in extracts of *Pseudomonas aeruginosa* (Whitaker *et al.*, 1982). In *Escherichia coli* and *Klebsiella aerogenes*, no inducible aromatic-amino-acid aminotransferase has been identified. In *E. coli*, the original transaminase A of Rudman & Meister (1953) is now known to consist of at least two components (Collier & Kohlhaw, 1972; Mavrides & Orr, 1975; Gelfand & Steinberg, 1977; Powell & Morrison, 1978): a tyrosine-repressible aromatic-amino-acid aminotransferase and a tyrosine-nonrepressible aspartate aminotransferase. The ability of *E. coli* to grow on L-tyrosine as sole nitrogen source (Silbert *et al.*, 1963) thus appears to be due to the activity of the latter enzyme which is also active for the aromatic amino acids (Powell & Morrison, 1978). However, *E. coli* cannot utilize L-tryptophan as sole nitrogen source in the presence of glucose, because of the great sensitivity of tryptophanase to the carbon catabolite repression (Botsford & DeMoss, 1971). In *K. aerogenes*, the aminotransferase I, a constitutive enzyme, has been shown to be responsible for the utilization of the aromatic amino acids as sole sources of nitrogen (Paris & Magasanik, 1981a, b).

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