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Characterization of Aspartate Aminotransferase Isoenzymes from Leaves of *Lupinus albus* L. cv Estoril

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Two aspartate aminotransferase (EC 2.6.1.1) isoenzymes (AAT-1 and AAT-2) from *Lupinus albus* L. cv Estoril were separated, purified, and characterized. The molecular weight, pI value, optimum pH, optimum temperature, and thermodynamic parameters for thermal inactivation of both isoenzymes were obtained. Studies of the kinetic mechanism, and the kinetics of product inhibition and high substrate concentration inhibition, were performed. The effect of some divalent ions and irreversible inhibitors on both AAT isoenzymes was also studied. Native PAGE showed a higher molecular weight for AAT-2 compared with AAT-1. AAT-1 appears to be more anionic than AAT-2, which was suggested by the anion exchange chromatography. SDS-PAGE showed a similar sub-unit molecular weight for both isoenzymes. The optimum pH (between 8.0 and 9.0) and temperature (60-65ºC) were similar for both isoenzymes. In the temperature range of 45-65ºC, AAT-2 has higher thermostability than AAT-1. Both isoenzymes showed a high affinity for keto-acid substrates, as well as a higher affinity to aspartate than glutamate. Manganese ions induced an increase in both AAT isoenzymes activities, but no cooperative effect was detected. Among the inhibitors tested, hydroxylamine affected both isoenzymes activity by an irreversible inhibition mechanism.

Keywords: Aspartate aminotransferase, Isoenzymes, Molecular weight, Kinetic constants, Inhibition, Activation

Introduction

Aspartate aminotransferase (AAT, EC 2.6.1.1) is a pyridoxal 5-phosphate dependent enzyme that catalyses the reversible transfer of an amino group from aspartate to 2-oxoglutarate in order to form oxaloacetate and glutamate. In plants, AAT plays an important role in numerous metabolic processes, and is the major source of aspartate in cells. AAT is related to ammonia assimilation in the glutamine synthetase/glutamate synthase (GS/GOGAT) cycle, the transfer of intercellular carbon from mesophyll cells to bundle sheath cells in C_4 plants, and the transfer of the reducing equivalent across mitochondrial, chloroplast, and peroxisomal membranes through a malate-aspartate hydrogen shuttle (Vance and Gantt, 1992; Wadsworth, 1997).

AAT exists as multiple isoenzymes in different plant species that are associated with different subcellular compartments cytosol, mitochondria, plastids, and glyoxisomes. Some of their amino acid sequences were deduced from cDNAs (Jones *et al*., 1990; Turano *et al*., 1992; Gregerson *et al*., 1994; Jones *et al*., 1994; Winefield *et al*., 1994; Taniguchi *et al*., 1995; Wilkie *et al*., 1995).

However, the existence of multiple isoenzymes is not yet completely understood. Whether different isoenzymes have specific metabolic roles, or if they represent only a redundancy as a result of an evolutionary process, is unknown (Wadsworth, 1997). So, it is important to carry out a complete characterization and study of the kinetic and enzymatic properties of the AAT isoenzymes from a single plant source. Moreover, AAT isoenzymes can be used as biochemical markers for the identification of different populations of *Lupinus* (Cabral, 1990).

Three AAT isoenzymes were detected in leaves from *Lupinus albus* L. cv Estoril. In this work, two of them (designated AAT-1 and AAT-2) were separated, purified, and characterized. The following properties for both isoenzymes were determined: molecular weight, pI value, optimum pH, and optimum temperature. The thermal inactivation characterization was studied, and the thermodynamic parameters were obtained for both isoenzymes. Kinetic studies, product inhibition, and high substrate concentration inhibition kinetics were also performed using *steady-state* methods, and the respective kinetic constants were obtained.

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The effect of some divalent ions, Ca^{2+}, Mg^{2+}, Mn^{2+}, and inhibitor substances was also tested with both AAT isoenzymes.

Materials and Methods

Plant material White lupine seeds (*Lupinus albus* L. cv Estoril) were germinated in moist cottonwool at room temperature for five days. Seedlings were transplanted to a nutrient solution and maintained for 5 weeks in a growth chamber at 18ºC and 55% humidity with 14/10 h dark/light periods.

Isoenzyme extraction, separation and purification Crude extract was obtained by collecting leaves from five different plants, maceration in the presence of 2% (w/w) insoluble polyvinylpolypyrollidone (PVP) with a 10 mM Tris-HCl buffer (pH 8.0) that contained 0.2 mM pyridoxal 5-phosphate (PLP) and 5 mM 2-mercaptoethanol. Then it was centrifuged for 30 min at 20,000 *g* and filtered through 0.2 μm membranes. All of the procedures were performed below 4ºC.

The enzyme extract that was obtained was subjected to a temperature fractionation (4 min at 45ºC) and salt fractionation with ammonium sulfate (25-80%). This was followed by centrifugation (20,000 *g* for 15 min) and filtration through 0.2 μm membranes. In order to obtain separated fractions of AAT-1 and AAT-2, the enzyme extract was subjected to a purification sequence of gel exclusion chromatography (Sephacryl S-200 HR, *Pharmacia*, Uppsala, Sweden), and anion exchange chromatography (Mono-Q 5/5, *Pharmacia*), following the procedure described in Griffith and Vance (1989) and modified by Martins (1998).

AAT isoenzymes were eluted using a linear salt gradient, 100 to 300 mM NaCl in 20 mM Tris-HCl (pH 8.0), that contained 2 mM 2-mercaptoethanol. AAT-1 and AAT-2 were separately eluted at 200 and 150 mM NaCl, respectively. Fractions that contained either isoenzyme were pooled and desalinized with an Amicon membrane (microcon-10). The separation and purification of AAT isoenzymes was confirmed by SDS-PAGE.

AAT activity and protein measurements **Assay 1**-AAT activity was assayed by the coupled malate dehydrogenase method that monitored the disappearance of NADH at 340 nm (Rej and Hørder, 1983). This enzyme assay was used routinely and in most determinations performed in the forward reaction, purification procedures, optimum pH and temperature, thermal inactivation, and kinetic studies. One International Unit is defined as the amount of enzyme that catalyses the oxidation of 1 μmol NADH per min at 25ºC ($\varepsilon = 6.3 \times 10^2$ L mol^{-1} mm^{-1}). **Assay 2**-AAT activity was measured by the decrease in absorbance at 260 nm, due to the oxaloacetate consumption (Hatch, 1973). One International Unit is defined as the amount of enzyme that catalyses the utilization of 1 μmol of oxaloacetate per min at 25ºC ($\varepsilon = 1.2 \times 10^2$ L mol^{-1} cm^{-1}). This enzyme assay was used in optimum pH determinations that were performed in the reverse reaction, kinetic studies, and effect of divalent ions and inhibition studies. **Assay 3**-AAT activity in the forward reaction was assayed by the coupled glutamate dehydrogenase method (GDH, EC 1.4.1.3) (Turano *et al.*, 1990). This assay was used on oxaloacetate product inhibition studies. **Assay 4**-AAT activity in the reverse reaction was determined by the decrease in absorbance at 340 nm, due to NADH oxidation that involved the enzyme glutamate dehydrogenase (GDH, EC 1.4.1.3). This assay was used on high substrate concentration inhibition studies with oxaloacetate.

Soluble protein was determined by the Lowry method (Lowry *et al*., 1951) with bovine serum albumin as the standard. During the purification process, the protein content evaluation was done by measuring the absorbance at 280 nm.

Molecular weight and pI measurements The molecular weights of native AAT isoenzymes were measured by native-PAGE and gel exclusion chromatography (Sephacryl S-200 HR, *Pharmacia,* 86 × 2,6 cm) using the experimental conditions previously described. Native-PAGE was performed in a vertical system (Midget Electrophoresis Unit LKB 2050) with refrigeration in 7.5% polyacrylamide and 2.6% BIS gel slabs ($102 \times 82 \times 0.75$) at constant 60 mA and maximum 250 volt in a 100 mM Tris-glycine buffer (pH 8.3). SDS-PAGE was performed with 10% polyacrylamide and 2.6% BIS gel slabs with 1% SDS, using the same running conditions. The samples that were used were prepared in a 100 mM Tris-HCl buffer that contained 2.5% (w/v) SDS and 5.0% (v/v) β-mercaptoethanol. Staining was done using a solution that contained 0.5% (w/v) Coomassie Blue R-250, 40% (v/v) methanol, and 7% (v/v) acetic acid. Polyacrylamide gel isoelectric focusing (IEF) was performed in a horizontal system Multiphor II (LKB-*Pharmacia*) with refrigeration in 5% polyacrylamide and 3% BIS gel slabs ($250 \times 125 \times 0.5$) in a pH gradient between 3.5 and 10. The pI and molecular weights of native isoenzymes and sub-units were calculated using commercial standard proteins (*Pharmacia*) by the fitting of appropriate curves and interpolation.

Optimum pH, optimum temperature, and thermal inactivation Optimum pH was determined in assay buffers that ranged from pH 5.0 to 10.5 in both forward and reverse reactions. Optimum temperature was determined by heating 1 mL aliquots of the enzyme extract in glass tubes in a water bath for 1 min at temperatures between 20 to 65ºC. This was immediately followed by reading the enzyme activity in a spectrophotometer with temperature-controlled cells. Thermal inactivation studies were performed by heating 1 mL aliquots of an enzyme extract in glass tubes in a water bath from 40 to 65ºC for different periods of time, cooled in ice immediately after treatment, and then centrifuged (10 min at 10,000 *g*) in order to clarify the extract.

The thermal inactivation parameters (rate constants, k, and half-lives, $t_{1/2}$) were obtained graphically by plotting the enzymatic activity versus time at different temperatures, and fitting a first-order equation. The activation energy (E_a) was calculated graphically, according to the Arrhenius equation. The thermodynamic parameters, enthalpy of activation ($\Delta H^{\ddagger}$), free energy of activation ($\Delta G^{\ddagger}$), and entropy of activation ($\Delta S^{\ddagger}$) for the thermal denaturation of both isoenzymes were calculated using the Eyring equation, $k = (k_b T/h)\exp(-\Delta G^{\ddagger}/RT)$(where k_b is the Boltzmann constant and h the Plank constant) and the relationships $\Delta H^{\ddagger} = E_a - RT$ and $\Delta G^{\ddagger} = \Delta H^{\ddagger} - T\Delta S^{\ddagger}$.

Kinetic studies Kinetic constants K_m and V_{max} and the apparent inhibition constants for both isoenzymes were calculated using the

direct linear plot methodology that is applied to the *ping-pong bi-bi* kinetic mechanism (Henderson, 1992). In the inhibition studies the inhibition pattern was deduced from the Lineweaver-Burk plots (Dixon and Webb, 1979).

In the direct-linear plot method, we made a primary plot of v versus the concentration of one substrate at a constant concentration of the second substrate (with non-saturating conditions for both substrates). By doing several of these primary plots at different concentrations of the second substrate, we were able to calculate the same number of apparent constants K_m^{app} and V_{max}^{app}. These values were then used to plot a straight line in a secondary graph of $1/V_{max}^{app}$ versus the reciprocal of the second substrate concentration, using a weighted least squares technique (each point is given a weight equal to $1/\sigma^2$, where σ is the standard deviation). From the slope and the intercept of this line, the kinetic parameters K_m and V_{max} are calculated. An identical procedure was used for the product inhibition and high substrate concentration inhibition studies. The experimental data were replotted according to the slope method (slope from primary plot versus inhibitor concentration) in order to calculate the apparent inhibition constants.

Effect of divalent ions Calcium chloride, magnesium chloride, and manganese chloride were used in a range of concentrations between 0.5 and 6.0 mM in a 100 mM Tris-HCl buffer at pH 7.8. Enzymatic activity was determined with both AAT isoenzymes for the reverse reaction, using the enzymatic assay 2 with a constant oxaloacetate concentration (0.2 mM). A control assay with no divalent ions in the solution was performed in all of the cases. The samples that contained the enzyme were incubated with solutions that contained the different ions for 10 min at 37ºC. The enzyme assay was then performed after 2 h at room temperature. For the study of the kinetics of the manganese activation, the enzymatic activity values were obtained using a range of manganese concentrations between 2 and 6 mM, varying the glutamate concentrations (10, 15, 20, 30, and 40 mM) with a constant concentration of oxaloacetate (0.2 mM).

Inhibition studies The effect of hydroxylamine, iodoacetate, iodoacetamide, and 3-bromopropionic acid on the AAT isoenzymes was studied using constant concentrations of each substance in the range of 0.25 to 6.0 mM in a 100 mM Tris-HCl buffer at pH 7.8. The enzymatic activity was determined for both AAT isoenzymes for the reverse reaction, using the enzymatic assay 2 with a constant oxaloacetate concentration (0.2 mM). A control assay with no inhibitor solution was performed in all of the cases. The samples that contained the enzyme were incubated with the different inhibitor solutions for 10 min at 37ºC. The enzyme assay was then performed after 2 h at room temperature. For the subsequent hydroxylamine assays, two concentrations were used (0.25 and 0.5 mM), varying the glutamate concentrations (10, 15, 20, 30, and 40 mM) with a constant concentration of oxaloacetate (0.2 mM).

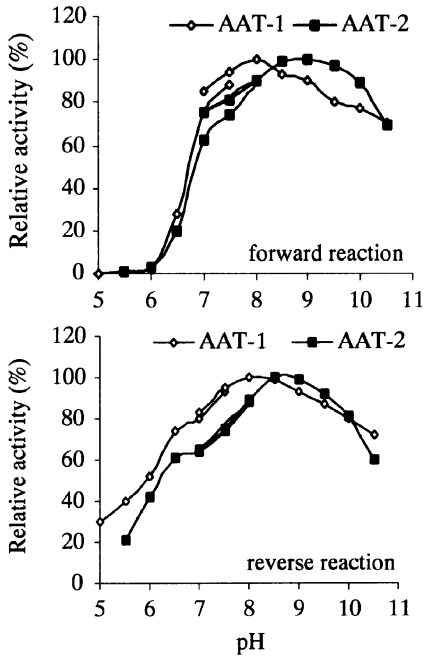

Fig. 1. Effect of pH on the activity of AAT-1 and AAT-2 isoenzymes. For each isoenzyme two different buffers were always used: one in the low pH range and another in the high pH range. Some overlapping in intermediate values occur to confirm that the behaviour of both buffers remained consistent.

Results and Discussion

The molecular weight and pI values (Table 1) are very similar to those that were found by other authors and in other plant species (Reynolds *et al.*, 1981; Griffith and Vance, 1989; Turano *et al.*, 1990; Yagi *et al.*, 1993). SDS-PAGE shows a single band for each isoenzyme, indicating a similar sub-unit molecular weight. Native-PAGE showed that AAT-2 has a higher native molecular weight, and is less anionic than AAT-1. The different values for the native molecular weights that were obtained by PAGE and gel exclusion chromatography reflects the differences in electrical charges between these isoenzymes, due to a different amino-acid composition, but with a similar molecular conformation, which is usual with isoenzymes. In fact, the ion-exchange chromatography shows that AAT-1 is eluted at a higher salt concentration, which agrees with its lower pI value.

Optimum pH was determined for AAT-1 and AAT-2 in the forward and reverse reactions (Fig. 1). Both isoenzymes show similar activity curves that are stable at a broad range of pH values. In the forward reaction, where L-aspartate and 2-oxoglutarate are substrates, the optimum pH for AAT-1 and

Table 1. Molecular weight and pI of aspartate aminotransferases, AAT-1 and AAT-2.

	Gel exclusion (Da)	Native-PAGE (Da)	SDS-PAGE (Da)	pI
AAT-1	88,000 ± 820	112,000 ± 7,000	52,200 ± 1,200	5.54 ± 0.07
AAT-2	92,000 ± 940	136,000 ± 5,000	54,800 ± 1,300	5.79 ± 0.09

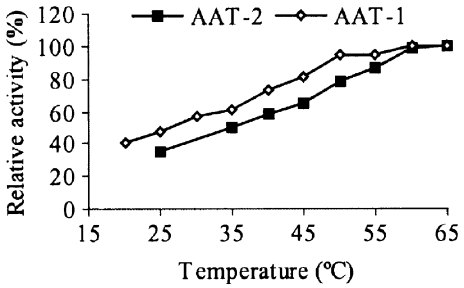

Fig. 2. Effect of temperature on the activity of AAT-1 and AAT-2 isoenzymes.

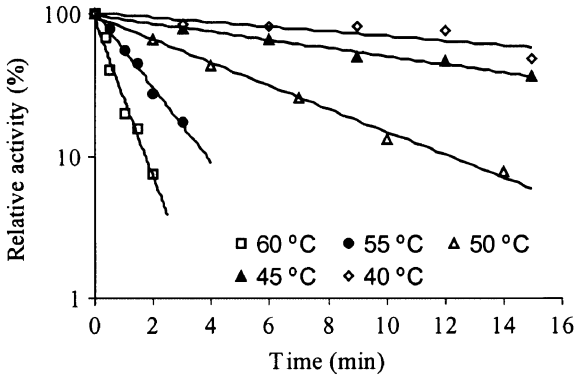

Fig. 3. Inactivation curves obtained for AAT-1.

AAT-2 was 7.5-8.5 and 8.5-9.5, respectively. In the reverse reaction in which L-glutamate and oxaloacetate are substrates, optimum pH for AAT-1 and AAT-2 was 7.5-8.5 and 8.5-9.0, respectively. Other studies showed only small differences in pH optimum values, according to different sources of the AAT isoenzymes (Reynolds *et al*., 1981; Griffith and Vance, 1989; Turano *et al*., 1990; Yagi *et al*., 1993). For AAT isoenzymes from rice bran, Yagi *et al.* (1993) found some differences on optimum pH curves between the forward and reverse reactions. The forward reaction appears to be less pH dependent than the reverse reaction, although both isoenzymes showed a similar behavior with rapidly decreasing enzymatic activity above pH 8.5.

The enzyme activity increased with the temperature going up to the highest temperature that was investigated, 65°C (Fig. 2). These results are in agreement with the knowledge that AAT are thermostable enzymes (Turano *et al*., 1991; Cubellis *et al*., 1993). The thermal inactivation of both isoenzymes follows first-order kinetics, as can be seen by a linear representation in a semi-logarithmic plot (Fig. 3). The inactivation and thermodynamic parameters are presented in Tables 2 and 3, respectively. For the range of 40-65°C, AAT-2 has higher thermostability compared with AAT-1, as shown by the obtained values of $t_{1/2}$, E_a, $\Delta S^{\ddagger}$, $\Delta H^{\ddagger}$, and $\Delta G^{\ddagger}$. Those values indicate that heat mainly affects the apoenzyme stability. This causes a loss of enzymatic activity and seems to be related to the cystein content of the isoenzymes (Cubellis *et al*., 1993), and can result from different sub-cellular localization (Wadsworth, 1997).

Table 2. Thermal inactivation parameters obtained for AAT-1 and AAT-2.

T (°C)	AAT-1			AAT-2		
	$t_{1/2}$ (min)	k (min^{-1})	r^2 ($p<0.05$)	$t_{1/2}$ (min)	k (min^{-1})	r^2 ($p<0.05$)
40	18.69	0.016	0.749	-	-	-
45	9.98	0.029	0.984	85.96	0.003	0.924
50	3.55	0.082	0.992	41.17	0.007	0.981
55	1.17	0.264	0.992	6.63	0.041	0.862
60	0.50	0.567	0.979	2.13	0.124	0.942
65	-	-	-	0.27	0.757	0.989

Table 3. Thermodynamic parameters of heat inactivation of AAT-1 and AAT-2.

	E_a (kcal mol^{-1})	T (°C)	$\Delta G^{\ddagger}$ (kcal mol^{-1})	$\Delta H^{\ddagger}$ (kcal mol^{-1})	$\Delta S^{\ddagger}$ (cal mol^{-1} K^{-1})
AAT-1	38.42	40	23.49	37.78	45.64
		45	23.50	37.77	44.86
		50	23.21	37.76	45.03
		55	22.81	37.75	45.51
		60	22.67	37.74	45.24
AAT-2	58.20	45	24.93	57.57	102.59
		50	24.79	57.56	101.41
		55	24.03	57.55	102.15
		60	23.67	57.54	101.65
		65	22.82	57.53	102.64

Table 4. Kinetic constants for AAT-1 and AAT-2.

	K_m (mM)		V_{max} (U mg^{-1})	
	AAT-1	AAT-2	AAT-1	AAT-2
L-Aspartate	2.26 ± 0.05	3.3 ± 0.2	2.967 ± 0.034	0.153 ± 0.005
2-Oxoglutarate	0.27 ± 0.01	0.61 ± 0.08	1.145 ± 0.016	0.129 ± 0.013
L-Glutamate	37 ± 3	40 ± 4	0.149 ± 0.010	0.035 ± 0.003
Oxaloacetate	0.10 ± 0.01	0.06 ± 0.01	0.054 ± 0.004	0.013 ± 0.001

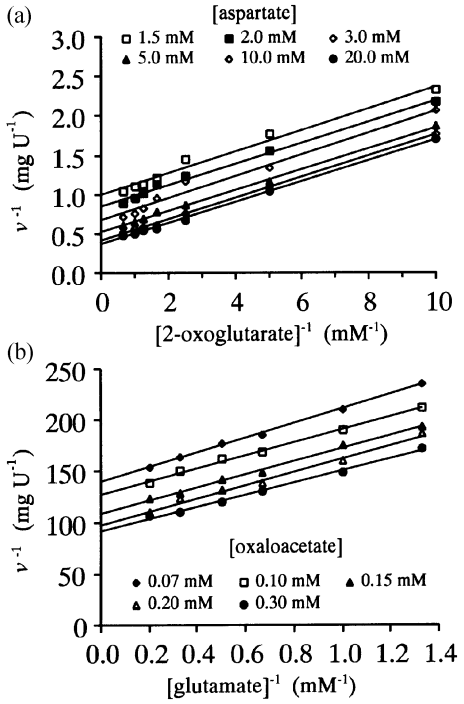

Fig. 4. Examples of the characteristic patterns of primary plots. (a) AAT-1 in forward reaction; [2-oxoglutarate] varying from 0.1 to 1.5 mM. (b) AAT-2 in reverse reaction obtained with; [glutamate] varying from 1 to 10 mM.

Kinetic studies, product inhibition, and high substrate concentration inhibition Kinetic characterization was performed, and the results were compared with other known AAT isoenzymes. The K_m and V_{max} values for each substrate (oxaloacetate, glutamate, 2-oxoglutarate, and aspartate) were individually determined for AAT-1 and AAT-2 (Table 4). Both isoenzymes showed a high affinity for keto-acid substrates, and a higher affinity to aspartate than glutamate. These results agree with other AAT isoenzymes that were studied from different sources (Reynolds *et al.*, 1981; Griffith and Vance, 1989; Turano *et al.*, 1990; Yagi *et al.*, 1993).

The reaction mechanism for both AAT-1 and AAT-2 was determined from the double reciprocal plots of the initial velocity against the concentration of one substrate at a fixed concentration of the other substrate. This procedure was followed for all of the substrates. Examples of these plots, both in the forward and in the reverse reactions, are shown in Fig. 4 (*a* and *b*). We found an increase in the apparent V_{max} values with an increase in aspartate concentrations. This resulted in a system of parallel lines on the double reciprocal plots (primary plots) for both AAT-1 and AAT-2 isoenzymes, which is consistent with a *ping-pong bi-bi* kinetic mechanism (Dixon and Webb, 1979).

However, an unequivocal conclusion about the kinetic mechanism can only be taken by an agreement between the results from the use of primary plots at non-saturating

Table 5. Product inhibition. Inhibition pattern and inhibition constants for AAT-1 and AAT-2 at non-saturating concentrations of substrates.

Product inhibitor	Isoenzyme	K_i (mM)	
		with respect to aspartate[a]	with respect to 2-oxoglutarate[b]
Oxaloacetate	AAT-1	0.030 ± 0.001 (mixed inhibition)	0.005 ± 0.001 (competitive inhibition)
	AAT-2	0.024 ± 0.001 (mixed inhibition)	0.026 ± 0.001 (competitive inhibition)
Glutamate	AAT-1	10.5 ± 0.3 (competitive inhibition)	11.1 ± 0.4 (mixed inhibition)
	AAT-2	30 ± 2 (competitive inhibition)	16 ± 1 (mixed inhibition)

[a]aspartate is the variable substrate and 2-oxoglutarate concentration is held constant at 0.2 mM.
[b]2-oxoglutarate is the variable substrate and aspartate concentration is held constant at 2.0 mM.

Table 6. Inhibition constants obtained with high substrate concentration for AAT-1 and AAT-2.

	K_i with respect to 2-oxoglutarate[a]	K_i with respect to oxaloacetate[b]
AAT-1	40 ± 3 mM	0.52 ± 0.03 mM
AAT-2	38 ± 3 mM	1.15 ± 0.02 mM

[a]aspartate is the variable substrate (at non-saturating concentrations) and 2-oxoglutarate is the high concentration substrate.
[b]glutamate is the variable substrate (at non-saturating concentrations) and oxaloacetate is the high concentration substrate.

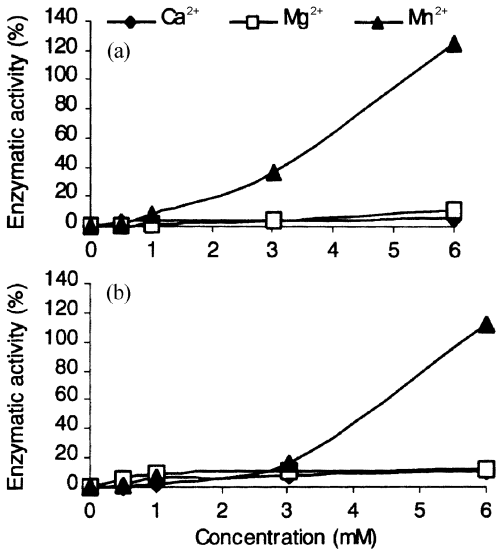

Fig. 5. Effect of Ca^{2+}, Mg^{2+} and Mn^{2+} on the enzymatic activity of (a) AAT-1 and (b) AAT-2 isoenzymes.

concentrations for substrates, as well as both product and high substrate concentration inhibition studies (Dixon and Webb, 1979; Segel, 1993; Palmer, 1995). Turano *et al.* (1990) also used product inhibition studies to distinguish this from the other two-substract, two-product kinetic mechanism. For the specific experimental conditions used in this work, the observed pattern of both product and high substrate concentration inhibition was also consistent with a *ping-pong bi-bi* kinetic mechanism. The double reciprocal plots of the initial velocity against substrate concentration at different product concentrations (at fixed concentration of the other) showed characteristic inhibition patterns-competitive (glutamate in relation to aspartate and oxaloacetate in relation to 2-oxoglutarate) and mixed (glutamate in relation to 2-oxoglutarate and oxaloacetate in relation to aspartate) (Table 5).

Both isoenzymes showed similar inhibition patterns in high-substrate concentration inhibition studies. A competitive inhibition pattern was observed for the high keto-acid concentration (2-oxoglutarate and oxaloacetate), but no inhibition was found by the high amino-acid concentration (aspartate and glutamate). The different values for the kinetic

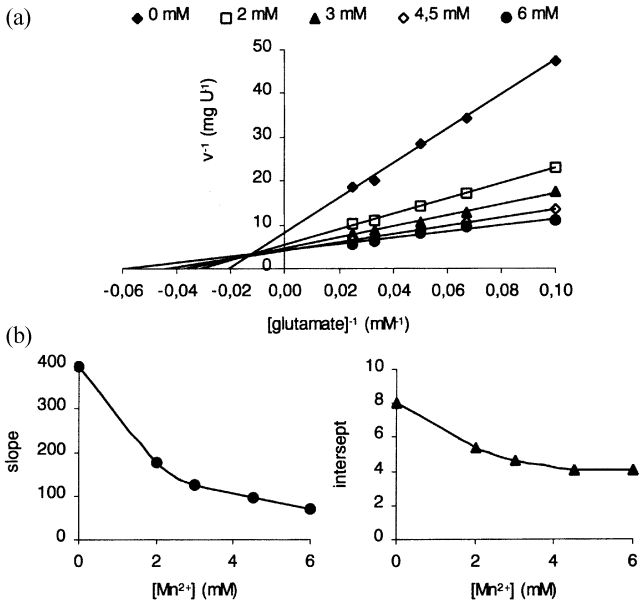

Fig. 6. Kinetic of activation of AAT-1 isoenzyme by Mn^{2+}. (a) Primary plot at different Mn^{2+} concentration. (b) Secondary plots of slope and intersept versus Mn^{2+} concentration.

constants that were obtained for both isoenzymes are presented in Table 6. The competitive inhibition-type behavior that was found in the previous work for these isoenzymes with some structural analogues (Martins, 1998; Martins *et al.*, 2001) agrees with these results with respect to the kinetic mechanism. Also, the *ping-pong bi-bi* kinetic mechanism suggests a single binding site in the enzyme, which is characteristic of the pyridoxal dependent enzymes (Palmer, 1995).

Effect of divalent ions At the range of the assayed concentrations, the enzymatic activity of AAT isoenzymes was only slightly increased by Ca^{2+} and Mg^{2+}. Manganese ions (Mn^{2+}) caused a considerable increase in the enzymatic activity of both AAT isoenzymes (Fig. 5). The kinetic activation by manganese was also studied. The fitting of the Michaelis-Menten equation to the activity values for all Mn^{2+} concentrations (with r^2 always above 0.980 at a significance level of 95%) indicates that no cooperative effects exist. The primary and secondary plots that were obtained with AAT-1 are presented in Fig. 6. The non-linear pattern of the secondary plots that were obtained for both isoenzymes indicates a dependence of the slope and the intercept in respect to the manganese concentration. The fitting to the Michaelis-Menten equation and the primary and secondary plots are both in agreement with a non-essential type activation mechanism (Dixon and Webb, 1979).

Inhibition studies The effect of hydroxylamine, iodoacetate, iodoacetamide, and 3-bromopropionic acid on the enzymatic activity of AAT-1 and AAT-2 isoenzymes is presented in Fig.

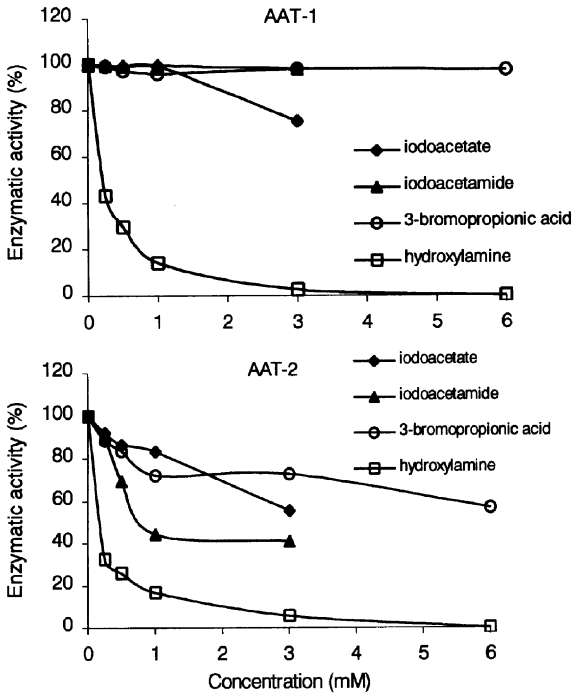

Fig. 7. Effect of hydroxylamine, iodoacetate, iodoacetamide and 3-bromopropionic acid on the enzymatic activity of AAT-1 and AAT-2 isoenzymes.

7. All of these substances cause a decrease in enzymatic activity, although there is a more pronounced effect by hydroxylamine, which is a site-specific inhibitor. A Michaelis-Menten plot that was obtained with hydroxylamine for both isoenzymes is indicative of an irreversible inhibition mechanism (Palmer, 1995).

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