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Biochemical and structural characterization of mouse mitochondrial aspartate aminotransferase, a newly identified kynurenine aminotransferase-IV

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Synopsis

Mammalian mitochondrial aspartate aminotransferase (mAspAT) is recently reported to have kynurenine aminotransferase (KAT) activity and plays a role in the biosynthesis of kynurenic acid (KYNA) in rat, mouse and human brains. This study concerns the biochemical and structural characterization of mouse mAspAT. In this study, mouse mAspAT cDNA was amplified from mouse brain first stand cDNA and its recombinant protein was expressed in an *Escherichia coli* expression system. Sixteen keto acids were tested for the co-substrate specificity of mouse mAspAT and fourteen of them were shown to be capable of serving as co-substrates for the enzyme. Structural analysis of mAspAT by macromolecular crystallography revealed that the cofactor binding residues of mAspAT are similar to those of other KATs. The substrate binding residues of mAspAT are slightly different from those of other KATs. Our data provide a biochemical and structural basis towards understanding the overall physiological role of mAspAT *in vivo* and insight into controlling the levels of endogenous KYNA through modulation of the enzyme in the mouse brain.

Keywords

aspartate aminotransferase; crystal structure; keto acid; kynurenic acid; kynurenine; kynurenine aminotransferase

Introduction

Kynurenic acid (KYNA) is the only known endogenous antagonist of the *N*-methyl-D-aspartate (NMDA) subtype of glutamate receptors [1-4]. KYNA is also the antagonist of the α 7-nicotinic acetylcholine receptor [5-8]. In mammalian brains, glutamate is the major

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Author contribution

QH participated in the design of the study, carried out the experiment, performed analysis and wrote the manuscript. HR carried out the experiment and performed analysis. TC participated in the design of the study and helped to draft the manuscript. DAT participated in the design of the study and helped to draft the manuscript. JL participated in the design of the study, carried out the experiment, and wrote the manuscript.

The atomic coordinates and structure factors (codes: 3HLM, 3PD6 and 3PDB) have been deposited in the Protein Data Bank, Research Collaboratory for Structural Bioinformatics, Rutgers University, New Brunswick, NJ (<http://www.rcsb.org>).

excitatory neurotransmitter and acts through both ligand-gated ion channels and G-protein-coupled receptors, which are collectively called glutamate receptors [9]. Activation of these receptors is responsible for basal excitatory synaptic transmission and many forms of synaptic plasticity such as long-term potentiation and long-term depression, which are thought to underlie learning and memory [9,10]. However, any event or process leading to a sudden or chronic increase in the activity of glutamate receptors often induces the death of neurons [11]. Consequently, a mechanism capable of preventing glutamate receptors from being overly stimulated seems essential for maintaining the normal physiological condition of the brain [12,13]. Brain KYNA levels are abnormal in the progression of some neurological and psychiatric disorders (see review in [14]), which suggest that variations in brain KYNA, acting as an endogenous modulator of glutamatergic and cholinergic neurotransmission, may be functionally significant.

In addition to the roles KYNA plays in the central nervous system, KYNA has been identified as an agonist for the previously “orphaned” receptor GPR35 [15]. More recently, a study has demonstrated that KYNA is implicated in the regulation of leukocyte binding on the endothelium due to activation of GPR35 by KYNA [16].

KYNA is produced enzymatically by the irreversible transamination of kynurenine, the key intermediate in the tryptophan catabolic pathway. In humans, rats and mice, four proteins arbitrarily named KAT I, II, III and IV, have been considered to be involved in KYNA biosynthesis in the central nervous system [17-23]. KAT I is identical to glutamine transaminase K (GTK) and cysteine conjugate beta-lyase (CCBL) 1; KAT II is identical to aminoadipate aminotransferase (AADAT); KAT III is identical to CCBL 2; and KAT IV is identical to glutamic-oxaloacetic transaminase (GOT) 2 or mitochondrial aspartate aminotransferase (mAspAT). These proteins are all pyridoxal 5-phosphate (PLP)-dependent enzymes. Among the individual mammalian KATs, KAT I [20,24-26] and KAT III [23] share similar genomic structure and high sequence identity [21] and have been assigned to subgroup I γ in fold type I aminotransferases [14,27]. KAT II is unique in swapping the catalytically essential N-terminal region [28-30]. A further sequence phylogenetic analysis revealed that KAT II and KAT II homologs form a separate lineage [31]. These indicate that KAT II and its homologs actually form a new subgroup in fold type I aminotransferases, designated as subgroup I α , the eighth subgroup in the fold type [14]. Based on the sequence information, KAT IV/mAspAT has been assigned to subgroup I α [27]. AspATs from different sources have been used as a model for studying the mechanism of catalysis for aminotransferases and hundreds of AspAT structures are available in the Protein Data Bank (PDB). Among these, the only mitochondrial AspAT structures available are from the chicken [32]; there are none from mammals or humans. It has been reported that mAspAT/KAT IV plays a major role in KYNA synthesis in mouse brain [22]. A recent biochemical test supports this assertion [33], indicating that mAspAT/KAT IV could be a primary target for brain KYNA regulation. Here, we report the crystal structure of mouse mAspAT in its PLP form, as well as its structures in complex with substrate kynurenine and co-substrate oxaloacetate. We also provide a kinetic characterization of mAspAT with 12 co-substrates. Determination of the three-dimensional structure of mouse mAspAT may contribute to the rational design of selective inhibitors that are of intense medical interest with respect to a number of human pathological conditions, in which the brain KYNA level is abnormal.

Experimental

Expression and purification recombinant mAspAT

mAspAT coding sequence (GenBank acc no, NM_010325) was amplified from a mouse brain cDNA pool and cloned into an ImpactTM-CN plasmid (New England Biolabs) for expression of a fusion protein containing a chitin-binding domain. Transformed *E. coli* cells

were cultured and harvested as the start materials for affinity purification. Further purification of the recombinant mAspAT was achieved by ion exchange (Q-sepharose) and gel filtration chromatographies. The purified recombinant mAspAT was concentrated to 10 mg ml⁻¹ protein in 10 mM phosphate buffer (pH7.5) using a Centricon YM-30 concentrator (Millipore) [33].

KAT Activity assay

KAT activity assay was based on previously described methods [20,23,29]. Briefly, a reaction mixture of 100 μ L, containing 5 mM L-kynurenine, 2 mM α -ketoglutarate, 40 μ M PLP, and 5 μ g of recombinant protein, was prepared using 100 mM potassium phosphate buffer (pH 7.5). This reaction mixture is identified hereafter as the typical reaction mixture. The mixture was incubated for 15 min at 38°C and the reaction stopped by adding an equal volume of 0.8 M formic acid. The supernatant of the reaction mixture, obtained by centrifugation at 15,000 *g* for 10 min at room temperature, was analyzed for the product, KYNA, by high-performance liquid chromatography (HPLC) with ultraviolet detection at 330 nm.

Co-substrate specificity

To determine the substrate specificity for α -keto acids, 16 α -keto acids were individually tested for their ability to function as an amino group acceptor for mouse mAspAT. Each of the 16 α -keto acids was assayed at 2 mM in the presence of 5 mM kynurenine in the 100 μ L typical reaction mixture and the rate of KYNA production was determined as described in the KAT activity assay. The kinetic study for α -ketoacid substrates of mouse mAspAT is based on a previously used method [23].

mouse mAspAT Crystallization

The crystals were grown by the hanging-drop vapor diffusion method with the volume of reservoir solution at 500 μ L and the drop volume at 2 μ L, containing 1 μ L of protein sample and 1 μ L of reservoir solution. The optimized crystallization buffer consisted of 20% PEG 4000, 100 mM ammonium sulfate, and 6% glycerol. mAspAT: kynurenine complex crystals were prepared by co-crystallizing the enzyme in the presence of 2 mM kynurenine, and mAspAT: oxaloacetate complex crystals in the presence of 2 mM oxaloacetate (previously neutralized by NaOH).

Data collection and processing

Individual mouse mAspAT crystals were cryogenised using 20% glycerol in the crystallization buffer as a cryo-protectant solution. Diffraction data of mouse mAspAT crystal were collected at the Brookhaven National Synchrotron Light Source beam line X29A ($\lambda = 1.0908$ Å). Data were collected using an ADSC Q315 CCD detector. All data were indexed and integrated using HKL-2000 software; scaling and merging of diffraction data were performed using the program SCALEPACK [34]. The parameters of the crystals and data collection are listed in Table 1.

Structure determination

The structure of mouse mAspAT was determined by the molecular replacement method using the published chicken mAspAT (PDB code, 7aat) [32]. The program Molrep [35] was employed to calculate both cross-rotation and translation functions of the model. The initial model was subjected to iterative cycles of crystallographic refinement with the Refmac 5.2 [36] and graphic sessions for model building using the program Coot [37]. Solvent molecules were automatically added and refined with ARP/wARP [38] together with Refmac 5.2.

Analysis of biochemical data and crystal structure

The kinetic parameters of the recombinant enzyme towards different α -keto acids were calculated by fitting the Michaelis–Menten equation to the experimental data using the Enzyme Kinetics Module for SigmaPlot (SPSS Science, Chicago, IL, USA). Superposition of structures was done using LSQKAB [39] in the CCP4 suite. Figures were generated using PyMOL [40]. Protein and substrate interaction were also analyzed using PyMOL [40].

Results

Co-substrate specificity of mouse mAspAT

Mouse mAspAT was tested for KAT activity towards sixteen α -keto acids using 5 mM kynurenine as the amino group donor. Fourteen of them had detectable activity (Fig. 1). Table 2 illustrates the enzyme kinetic parameters towards each α -keto acid, including K_m and k_{cat}/K_m . Based on kinetic analysis, mouse mAspAT amino group acceptors with K_m values less than 4 mM include phenylpyruvate, oxaloacetate, hydroxyphenylpyruvate, α -ketoglutarate, mercaptopyruvate and indo-3-pyruvate.

Overall Structure

The structures of mouse mAspAT were determined by molecular replacement and refined to 2.50 Å resolution for the mAspAT PLP form, 2.40 Å resolution for the mAspAT: kynurenine complex, and 2.40 Å resolution for the mAspAT: oxaloacetate complex. Final models contain 4 × 401 amino acid residues each and yield a crystallographic R value of 18.1% and an R_{free} value of 23.4% for its PLP form, 17.7% and 19.5% for the mAspAT: oxaloacetate complex, and 17.8% and 18.9% for the mAspAT: Kynurenine complex (Table 1). There are four protein molecules in an asymmetric unit, which form two biological homodimers. The residues of the four subunits in mAspAT structures are numbered 30 (A) – 430 (A) for chain A, 30 (B) – 430 (B) for chain B, 30 (C) – 430 (C) for chain C, and 30 (D) – 430 (D) for chain D. The results of the refinement are summarized in Table 1. The statistics on Ramachandran plot as defined with PROCHECK [41] is also shown in table 1. An overview of the monomer structure model is shown in Fig. 2a. The structure has an N-terminal arm (Residues 30–42), large (Residues 76–348) and small (residues 43–75, 349–430) domains. The residue Asp243 interacts with the pyridine nitrogen of the cofactor, whose structural and functional conservation in fold-type I of the PLP-dependent enzymes, indicates its importance for catalysis. Comparison analysis suggests that mAspAT share similar cofactor binding sites with the other three KATs.

Active site of mouse mAspAT

Residual electron density clearly revealed the presence of covalently bound PLP in the cleft situated at the interface of the subunits in the biological dimer of the PLP form of the structure. The C4A atom of PLP is covalently attached to the NZ atom of Lys279 through the formation of an internal Schiff base, and the internal aldimine gives rise to residue LLP279, represented as sticks in Fig. 3. The PLP pyridine ring is stacked between residues Ala245 and Trp162 by hydrophobic interactions. The side chains of Tyr246 and Asp243 are hydrogen bonded to O3 and N1 of the pyridoxal, respectively. The phosphate moiety of PLP is interacting with Thr135, S133, Ser276, Arg287, and the Tyr96 from the other subunit.

Substrate Recognition and Catalysis

Inspection of the crystal structure of the mAspAT: kynurenine complex revealed that the substrate lies near the N1 atom of PMP, but kynurenine and the cofactor do not form an external aldimine. Several residues, including Ile44, Thr135, Trp162, Asn215, Arg287, and Arg407 from one subunit, and Tyr96, Arg313, Ser317, and Asn318 from the other subunit,

define the substrate-binding site and contact the kynurenine molecule. The carboxylic group of the kynurenine substrate forms a salt bridge with the guanidinium group of Arg407. The salt bridge is fixed by hydrogen-bonding interactions with the side chain of Asn215 and Gly65 at both sides of the salt bridge. The ring of Tyr96 (B) has a weak hydrophobic interaction with the phenyl ring of kynurenine in this complex structure (Fig. 4a).

mAspAT binds oxaloacetate in the similar manner in the crystal structure of the mAspAT: oxaloacetate complex as it binds kynurenine in the structure of mAspAT: kynurenine complex described above. As the oxaloacetate molecule is smaller than kynurenine, there are fewer protein residues that interact with oxaloacetate. The Thr135 and Arg287 residues from one subunit, and Asn318 from another subunit, all of which interact with kynurenine, are more than 4 Å distance away from oxaloacetate and consequently are not in close contact with it (Fig. 4b).

Conformational change

Upon superposing all the subunits (11 monomers except chain A in the PLP form) in three structures onto chain A in the structure of mAspAT PLP form, we identified that the large domains were well superposed onto one another and the small domains were not, which suggests that conformational changes occur in the small domain (Fig. 2a). The conformational changes of the small domain cause Ile44 and Arg407 to move and interact with the substrate. This small domain conformational change not only facilitates substrate binding but is also effective for shielding the substrate-binding pocket from bulk solvents. Carefully checking the conformation of substrate binding residues, we determined that there were no significant side chain conformational changes of the substrate binding residues except for a slight change in residue Arg313. It has been reported that by binding substrates, other AspATs and other aminotransferases change their conformations from an open to a closed form [14,23,32,42-47], which involves a large-scale conformational change (domain-domain rotation). It seems that mouse mAspAT uses same mechanism in substrate binding and catalysis.

Discussion

Mouse mAspAT is a major aminotransferase involved in KYNA production in the brain; therefore, it can be considered as a potential regulatory target for maintaining physiological concentrations of brain KYNA. We report herein the co-substrate specificity and crystal structures of mouse mAspAT and its complexes with kynurenine and oxaloacetate. The enzyme can use a number of α -keto acids as co-substrates for KAT activity. The structures of mAspAT PLP form and its complexes with kynurenine and oxaloacetate provide an important molecular basis for a comprehensive understanding of the substrate binding and enzyme catalysis in mAspAT, making it possible to work with structure and ligand-based design of the inhibitors of this enzyme.

There are four KATs in human and rodent brains [14]. The identification of a number of residues that are crucial for ligand binding in the four KAT enzymes were facilitated by the crystal structures in this report, specifically: human KAT I in complex with phenylalanine or indole-3-acetate [24,25]; human KAT II in complex with KYN [28]; mouse KAT III structure in complex with KYN [23]; and mouse mAspAT in complex with KYN. The substrate α -carboxylate moiety forms a salt-bridge with a structurally conserved Arg and forms a hydrogen bond with a structurally conserved Asn (Fig. 5, the residue with black background). The presence of this Arg residue is a strictly conserved hallmark of all those members of the aminotransferase superfamily whose structures have been determined to date [27]. The recognition of the substrate side chain is achieved specifically by different structural determinants in different KATs. The remarkable structural traits involved in

substrate side chain binding of human KAT I, human KAT II, and mouse KAT III have been reviewed previously [14]. KAT I and III have an aromatic hydrophobic pocket which is largely absent in the mouse mAspAT structure. In contrast, the active site of mAspAT tends to resemble KAT II, which may explain why mAspAT and KAT II have similar co-substrate specificity [29].

The identity of AspAT with KAT was first reported in *Escherichia coli* [48] and when later mAspAT in mice, rats, and humans was found to have KAT activity, it was named KAT IV [22]. It has been shown that mouse mAspAT has high transamination activity towards glutamate and aspartate, and has detectable activity towards phenylalanine, tyrosine, cysteine, tryptophan, 3-HK, methionine, kynurenine, and asparagine [33]. In this study, we demonstrated that mouse mAspAT could use α -ketoglutarate, phenylpyruvate, α KM B, indo-3-pyruvate, hydroxyphenylpyruvate, mercaptopyruvate, α -ketocaproic acid, oxaloacetate, α -ketobutyrate, pyruvate, and glyoxylate as amino group acceptors. Mouse mAspAT was found to be a major player for the formation of KYNA in the mouse brain [22], and this finding is supported by a recent study [33]. However, the inhibition study of mouse mAspAT shows that aspartate, glutamate, glutamine, phenylalanine, tyrosine, cysteine, tryptophan and histidine can competitively inhibit its KAT activity [33]. Since aspartate, glutamate and glutamine are the most abundant proteinogenic amino acids in mouse brains [49], and the specific KAT activity of mouse mAspAT is the lowest of the four mKATs [33], the contribution of mouse mAspAT to KYNA formation in mouse brains would be limited unless kynurenine was highly sequestered from these abundant amino acids. Biochemically, mouse mAspAT primarily catalyzes the reversible transamination of oxaloacetate to aspartate in conjunction with the conversion of glutamate to α -ketoglutarate [50]. The enzyme has a number of specific roles in astrocytes and neurons in brains [51-55]. Firstly, it has a role in the entry of glutamate into the tricarboxylic acid cycle, and the re-synthesis of intramitochondrial glutamate from tricarboxylic acid cycle intermediates [53,56-60]; secondly, it has a key role in the synthesis of the neurotransmitter glutamate in brains [61,62]; and thirdly, it is an essential component of the malate-aspartate shuttle, which is considered to be the most important mechanism for transferring reducing equivalents from the cytosol into mitochondria in brain tissue [55,58,63-66]. All of these functions of mAspAT are related to glutamate or aspartate. Therefore, mAspAT may need to co-localize with these two amino acids, which may limit its ability to catalyze the formation of KYNA. Nevertheless, a considerable portion of the total KAT activity in mouse brain crude proteins seems to be attributable to mAspAT [33], and moreover an earlier report has also indicated that mAspAT was majorly responsible for this KAT activity [22]. Accordingly, the specific contribution of mAspAT in brain KYNA biosynthesis has yet to be substantiated. A gene knock-out study in animal models could address this question definitively.

In addition to the aforementioned roles played by mAspAT as an aminotransferase, mAspAT is identical to a long-chain fatty acid binding protein (FABP) [67-71]. In mammalian tissues, it was suggested that FABPs be involved in at least a substantial portion of overall fatty acid uptake, the first step for the involvement of fatty acids in cellular metabolism. In mice, KAT IV/mAspAT has the lowest KAT specific activity among the four reported KATs [33], but it seems to be a primary contributor for the brain KAT activity in mouse brains [22,33]. As quantity may compensate for low efficiency, this may indicate a significantly high mAspAT protein level. This relative abundance of mAspAT may enhance its function in long-chain fatty acid binding. As targeting fatty acid oxidation has been proposed as a therapeutic strategy for treating insulin resistance and the rate of fatty acid oxidation is largely affected by fatty acid availability and its uptake into cells, one potential approach to treating insulin resistance is to decrease fatty acid uptake into heart or skeletal muscle [72]. Inhibiting the ability of mAspAT to bind a fatty acid, could lead to the

reduction of cellular fatty acid uptake. Studying the mechanism of long-chain fatty acid binding of mAspAT will help elucidate the protein inhibition or inactivation. Although molecular-modelling studies of the crystal structure of mAspAT suggest that the identified pocket within the larger domain of the enzyme might accommodate the typical long-chain fatty acid [73], whether this pocket serves as a fatty acid-binding site remains to be elucidated. A future study of co-crystallization of mouse mAspAT and a long-chain fatty acid could identify the binding site and provide the basis for investigating the inhibition of the fatty acid binding.

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Abbreviations

KAT	kynurenine aminotransferase
αKMB	α -keto- γ -methiobutyric acid
KYNA	kynurenic acid
mAspAT	mitochondrial aspartate aminotransferase
LLP	lysine-pyridoxal-5'-phosphate
PLP	pyridoxal-5'-phosphate
PMP	pyridoximine 5'-phosphate

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Figure 1. Transamination activity of mouse mAspAT towards different α -ketoacids

Purified recombinant mouse mAspAT was incubated with each of 16 α -ketoacids at 2 mM in the presence of 5 mM kynurenine in a typical reaction mixture (100 mM phosphate, pH 7.5). The activity was quantified by the amount of KYNA produced in the reaction mixture.

Figure 2. Overall structure

A, Cartoon representation of the structure of mAspAT monomer. N-terminal arm (pink), small domain (blue) and large domain (green) are shown in different colors. **B**, Upon superposing all the subunits (11 monomers except chain A in the PLP form) in the three structures onto chain A in the structure of mAspAT PLP form, all the monomers are shown in ribbon. The fragment with significant conformation changes is indicated by an arrow.

The PLP cofactor and the amino acid residues within a 4 Å distance of PLP are shown in the figure. The residues are colored in teal (subunit A) and green (subunit B). Hydrogen bond lengths are labeled with the unit of Å.

Figure 4. Substrate binding site

A, Stereo view of the kynurenine binding site in the mAspAT: kynurenine complex structure. The kynurenine substrate (KYN) and the protein residues within 4 Å distance of the kynurenine substrate are shown. The $2F_o - F_c$ electron density map covering the kynurenine is shown contoured at the 0.7 Sigma level. *B*, Stereo view of the oxaloacetate (OAA) binding site in the mAspAT: oxaloacetate complex structure. The oxaloacetate substrate (Kyn) and the protein residues within 4 Å distance of the oxaloacetate substrate are shown. The $2F_o - F_c$ electron density map covering the oxaloacetate is shown contoured at the 0.9 sigma level.

W 18	QGFP 35-8	Y* 63	F 125	N 185	FH* 278-9	R 395	Human KAT I
IR* 19-20	GGLP* 38-41	Y* 74	Y 142	N 202	LH* 293-4	R 399	Human KAT II
W 54	QGFP 71-4	Y* 98	Y 160	N 219	YH* 312-3	R 430	Mouse KAT III
IL 44-5	VGAY 64-67	Y* 96	W 162	N 215	R* 313	IN* 317-8	Mouse KAT IV /mAspAT

Figure 5. Comparison of the residues implicated in substrate binding of four selected KATs
The Arg and Asn residues which bind the substrate α -carboxylate moiety are indicated with white lettering on black background.

Table 1

Data collection and refinement statistics

Crystal Data	mAspAT	mAspAT:OAA	mAspAT:KYN
Space Group	P21212		
Unit Cell			
a (Å)	76.8	77.9	282.4
b (Å)	87.4	87.4	78.1
c (Å)	284.3	282.4	87.5
$\alpha=\beta=\gamma$ (°)	90.0	90.0	90
Data collection			
X-ray source	BNL ^a -X29		
Wavelength (Å)	1.0809		
Resolution (Å) ^b	2.50 (2.59-2.50)	2.40 (2.49-2.40)	2.40 (2.49-2.40)
Total number of reflections	751, 633	730, 307	585, 440
Number of unique reflections	67, 188	76, 929	76, 708
R-merge ^b	0.12 (0.42)	0.12 (0.32)	0.11 (0.34)
Redundancy ^b	12.8 (3.8)	10.3 (3.7)	8.1 (2.3)
Completeness (%) ^b	92.4 (54.9)	92.3 (55.3)	93.9 (60.4)
Refinement statistics			
R-work (%)	18.1	17.7	17.8
R-free (%)	23.4	19.5	18.9
RMS Bond lengths (Å)	0.026	0.016	0.019
RMS Bond angles (°)	1.982	1.597	1.725
No. of ligand or cofactor molecules	4 LLP ^c 6 GOL ^c	2 LLP 5 GOL 1 OAA ^c 7 BME ^c 2 PMP ^c	2 LLP 13 GOL 1 KYN ^c
No. of water molecules	689	721	831
Average B overall (Å ²)	39.1	28.3	29.1
Statistics on Ramachandran plot (%)			
Most favored regions	89.1	91.6	91.4
Additional allowed regions	10.4	8.1	8.3
Generously allowed regions	0.2	0.0	0.1
Disallowed regions	0.3	0.3	0.3

^a Brookhaven National Laboratory^b The values in parentheses are for the highest resolution shell^c GOL: glycerol; KYN: kynurenine; BME: β -mercaptoethanol

Table 2
Kinetic parameters of mouse mAspAT towards α -keto acids

The activities were measured as described in the “Experimental” section. The K_m and k_{cat} for α -keto acids were derived by using varying concentrations (0.2 to 50 mM) of individual α -keto acids in the presence of 15 mM of kynurenine. The parameters were calculated by fitting the Michaelis–Menten equation to the experimental data using the Enzyme Kinetics Module of SigmaPlot. Results are means $\pm$ SE.

	K_m mM	k_{cat} min ⁻¹	k_{cat}/K_m min ⁻¹ mM ⁻¹
Phenylpyruvate	0.7 $\pm$ 0.4	37.7 $\pm$ 6.9	57.8
Oxaloacetate	0.9 $\pm$ 0.4	19.2 $\pm$ 2.6	21.1
Hydroxyphenylpyruvate	1.6 $\pm$ 0.7	24.7 $\pm$ 5.5	15.7
α -ketoglutarate	2.4 $\pm$ 0.6	32 $\pm$ 2.7	13.4
Mercaptopyruvate	3.2 $\pm$ 0.7	26.1 $\pm$ 1.6	8.1
indo-3-pyruvate	3.6 $\pm$ 0.3	29.1 $\pm$ 1.1	8
α KMB	5.7 $\pm$ 0.5	24.8 $\pm$ 0.7	4.3
Glyoxylate	4.2 $\pm$ 0.8	11.4 $\pm$ 1.1	2.7
Pyruvate	8.3 $\pm$ 1.0	22.1 $\pm$ 0.9	2.7
α -ketocaproic acid	10.4 $\pm$ 1.4	24.6 $\pm$ 1.2	2.4
α -ketobutyrate	42.2 $\pm$ 14.2	41.3 $\pm$ 7.9	1
α -ketovalerate	10.9 $\pm$ 1.5	7.5 $\pm$ 0.4	0.7