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Substrate specificity and structure of human aminoadipate aminotransferase/kynurenine aminotransferase II

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Synopsis

KAT (kynurenine aminotransferase) II is a primary enzyme in the brain for catalysing the transamination of kynurenine to KYNA (kynurenic acid). KYNA is the only known endogenous antagonist of the *N*-methyl-D-aspartate receptor. The enzyme also catalyses the transamination of aminoadipate to α-oxoadipate; therefore it was initially named AADAT (aminoadipate aminotransferase). As an endotoxin, aminoadipate influences various elements of glutamatergic neurotransmission and kills primary astrocytes in the brain. A number of studies dealing with the biochemical and functional characteristics of this enzyme exist in the literature, but a systematic assessment of KAT II addressing its substrate profile and kinetic properties has not been performed. The present study examines the biochemical and structural characterization of a human KAT II/AADAT. Substrate screening of human KAT II revealed that the enzyme has a very broad substrate specificity, is capable of catalysing the transamination of 16 out of 24 tested amino acids and could utilize all 16 tested α-oxo acids as amino-group acceptors. Kinetic analysis of human KAT II demonstrated its catalytic efficiency for individual amino-group donors and acceptors, providing information as to its preferred substrate affinity. Structural analysis of the human KAT II complex with α-oxoglutaric acid revealed a conformational change of an N-terminal fraction, residues 15-33, that is able to adapt to different substrate sizes, which provides a structural basis for its broad substrate specificity.

Keywords

aminoadipic acid; crystal structure; kynurenic acid (KYNA); kynurenine; kynurenine aminotransferase (KAT); neurodegenerative disease

INTRODUCTION

Aminotransferase, capable of catalysing the transamination of kynurenine to KYNA (kynurenic acid), has commonly been termed KAT (kynurenine aminotransferase). KYNA is the only known endogenous antagonist of the NMDA (*N*-methyl-D-aspartate) subtype of

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glutamate receptors [1-4]. KYNA is also the antagonist of the α7-nicotinic acetylcholine receptor [5-8]. The level of KYNA is altered in several neurodegenerative diseases, including Huntington's disease [9,10], Alzheimer's disease [11], schizophrenia [12-14] and acquired immunodeficiency syndrome dementia [15]. Because KYNA must be produced by KAT-catalysed kynurenine transamination, aminotransferases, responsible for catalysing kynurenine to KYNA, have been considered to be targets for maintaining and regulating physiological concentrations of brain KYNA.

In humans, rats and mice, four enzymes, KAT I, II, III and IV, are considered to be involved in KYNA synthesis in the central nervous system [16-21]. Of these, KAT I and KAT II have been extensively studied and the crystal structures of hKAT I (human kynurenine aminotransferase I) and hKAT II (human kynurenine aminotransferase II) are now available [16,17,19,22-25]. It has been reported that KAT I has a broad substrate specificity and displays maximum activity at relatively basic conditions. This poses critical questions regarding the contribution of KAT I to brain KYNA production [16,17]. On the other hand, KAT II is identical to AADAT (aminoadipate aminotransferase) and catalyses the transamination of kynurenine and aminoadipate and is localized in the soluble cytoplasm [16]. The enzyme has been cloned from both rats and humans and its presence in the brain has been confirmed by Northern and Western blotting [26-29]. Previous studies on KAT II/AADAT have been focused on its activity on aminoadipate in peripheral organs, especially in the liver and the kidney, and on its role in brain KYNA production [30-32].

Although there have been a number of studies addressing the biochemical characterization of KAT II/AADAT, the overall substrate specificity and kinetic properties have not been clearly established for KAT II from any species. In the present study, we determined its overall substrate profile, kinetic parameters and the complex structure of hKAT II with α-oxoglutaric acid. The present study shows a novel biochemical characterization of hKAT II.

MATERIALS AND METHODS

Expression, purification and activity assay of recombinant hKAT II

Protein expression and purification—Recombinant hKAT II coding sequence (GenBank® Nucleotide Sequence accession number NM_182662) was cloned into an Impact™-CN plasmid (New England Biolabs) for expression of a fusion protein containing a chitin-binding domain. hKAT II was purified by affinity purification, DEAE-Sepharose, hydroxyapatite and gel-filtration chromatography. The purified recombinant hKAT II was concentrated to 10 mg · ml^{-1} of protein in 10 mM phosphate buffer (pH 7.4) using a Centricon YM-30 concentrator (Millipore) [24].

Activity assay—The KAT activity assay was based upon methods described previously [33]. Briefly, a reaction mixture (50 μl final volume) containing 10 mM L-kynurenine, 2 mM α-oxoglutarate, 40 μM PLP (pyridoxal 5′-phosphate) and 2 μg of the protein sample was prepared in 100 mM potassium phosphate buffer (pH 7.4). The mixture was incubated at 37° C for 15 min, and the reaction was stopped by the addition of 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 4°C, was analysed by HPLC, with UV detection performed at 330 nm for both kynurenine and KYNA.

Effect of pH and temperature on hKAT II—To determine the effect of the buffer pH on hKAT II activity, a buffer mixture consisting of 100 mM phosphate and 100 mM boric acid was prepared, and the pH of the buffer was adjusted to pH 6.0, 7.0, 8.0, 9.0, 10.0 and 11.0. The buffer mixture was selected to maintain a relatively consistent buffer composition and ionic strength, but would have sufficient buffering capacity for a relatively broad range of pH values.

A typical reaction mixture containing 10 mM kynurenine, 2 mM α-oxoglutarate and 2 μg of hKAT II was prepared using the buffer mixture at different pHs. The reaction mixture was incubated and analysed in the hKAT II activity assay as described above. To determine the effect of temperature on hKAT II-catalysed kynurenine transamination, the typical reaction mixture [final volume of 50 μl in 100 mM phosphate buffer (pH 7.4)] was incubated at temperatures ranging from 10-80°C for 15 min and the product formed in the mixture was analysed as described above for the hKAT II activity assay.

Substrate specificity—To test the transamination activity of other amino acids, an assay mixture containing 15 mM amino acid, 20 mM glyoxylate or α-oxobutyrate (for glycine), 40 μM PLP, 100 mM potassium phosphate buffer (pH 7.4) and 2 μg of enzyme was prepared in a total volume of 50 μl. The mixture was incubated for 15 min at 37°C. The product was quantified based on the detection of the OPT (*O*-phthaldialdehyde thiol)-amino-acid product conjugate by HPLC, with electrochemical detection performed after their corresponding reaction mixtures were derivatized using an OPT reagent [34]. In a kinetic study for amino-acid substrates, the typical reaction mixture specified in the KAT activity assay [50 μl final volume prepared in 100 mM phosphate buffer (pH 7.4)] containing various concentrations (0.47-30 mM) of the amino acids which showed detectable transaminase activity with hKAT II and 30 mM glyoxylate was used. The mixture was incubated for 15 min at 37°C. To determine the substrate specificity for α-oxo acids, 16 individual α-oxo acids were tested for their ability to function as the amino-group acceptor for hKAT II. In the assays, the typical reaction mixture stated above also contained a different α-oxo acid at various concentrations (0.47-60 mM) and 15 mM kynurenine, and the rate of KYNA production was determined by performing the KAT activity assay as described above. The kinetic parameters were calculated by fitting the Michaelis-Menten equation to the experimental data using the enzyme kinetics module in SigmaPlot 8.2 (SPSS).

The effect of other amino acids on KAT enzyme activity—Analysis of the substrate specificity revealed that a number of amino acids could serve as the amino-group donor for hKAT II. To determine the effect of other amino acids on hKAT II-catalysed KYNA production, a different amino acid (final concentration of 5 mM) was incorporated into the typical reaction mixture (total volume of 50 μl) which contained 5 mM kynurenine, 20 mM glyoxylate and 2 μg of hKAT II (pH 7.4) and the enzyme activity was assayed in the same manner as described above for the KAT activity assay.

Crystal structure of the hKAT II complex with α-oxoglutaric acid

The crystals were grown using the hanging-drop vapour diffusion methods described previously [24]. The crystals of the enzyme-α-oxoglutaric acid complex were obtained by soaking the crystals in 2.5 mM α-oxoglutaric acid in the crystallization buffer for 3 days. Individual hKAT II complex crystals were cryogenized using 25 % (v/v) glycerol in the crystallization buffer as a cryoprotectant solution. Diffraction data for the crystal were collected at the Brookhaven National Synchrotron Light Source beam line X29A [λ = 1.0908 AÅ (1 Å = 0.1 nm)]. All data were indexed and integrated using HKL-2000 software (HKL Research), and scaling and merging of the diffraction data were performed using the program SCALEPACK [35]. The parameters of the crystals and data collection are listed in Table 1. The structure of hKAT II was determined using the molecular-replacement method using the hKAT II structure (Protein Data Bank code 2QLR). The program Molrep [36] 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 [37] and graphic sessions for model building using the program O [38]. Solvent molecules were automatically added and refined with ARP/wARP [39] together with Refmac 5.2.

Superposition of the structures was done using Lsqkab [40] in the CCP4 suite, and Figures were generated using PyMOL [41].

RESULTS

Biophysical properties of hKAT II

On the basis of SDS/PAGE analysis, very high purity of the isolated hKAT II was achieved. The isolated hKAT II was stable under freezing conditions (results not shown). No noticeable decrease in specific activity was observed when aliquots of hKAT II were stored at -80°C during a period of 6 months, which allowed us to analyse critically the substrate specificity and kinetic properties of hKAT II using aliquots of the enzyme from the same batch of highly purified hKAT II. The enzyme showed maximum activity at approx. 50°C and had a broad optimum pH range from pH 7-9 (Figure 1), which is similar to those reported previously [16, 17].

Substrate study of hKAT II

hKAT II was tested for activity towards 24 amino acids and 16 oxo acids. Of the 24 amino acids tested, hKAT II was able to catalyse transamination of 20 of the amino acids. The specific activity of hKAT II towards individual amino acids under the conditions applied is shown in Figure 2. This substrate screening assay determined that hKAT II has a very broad substrate specificity. Kinetic analysis of hKAT II resulted in the determination of the catalytic efficiency of the enzyme towards individual amino-group donors and acceptors (Table 2), which provided a basis to determine its primary amino-group donors and acceptors and for speculation regarding its primary physiological functions. On the basis of the kinetic data, hKAT II should be equally efficient in catalysing the transamination of aminoadipate, kynurenine, methionine and glutamate (Table 2). All 16 α-oxo acids tested were capable of serving as amino-group acceptors. Of these, α-oxoglutarate, α-oxocaproic acid, phenylpyruvate and α-KMB (α-oxo-γ-methiol-butyric acid) were highly efficient as amino-group acceptors for hKAT II (Table 2). Although glyoxylate showed a relatively low affinity for hKAT II, it did not inhibit hKAT activity at high concentrations and its glycine-OPT complex was easily resolved from all other amino acid-OPT complexes during HPLC analysis with electrochemical detection. Therefore glyoxylate has been used as the primary amino-group acceptor for screening of the amino-group donors of hKAT II and kinetic analysis of the enzyme in the presence of different amino-group donors. KYNA displayed an absorbance peak with a λ_{max} of approx. 330 nm and was easily resolved from kynurenine during reverse-phase separation; therefore kynurenine was used as the primary amino-group donor during screening of amino-group acceptors and their kinetic analysis.

Effects of other amino acids on hKAT II-catalysed kynurenine transamination

On the basis of the K_m of hKAT II with different amino acids, it seems apparent that some amino-acid substrates are likely to inhibit hKAT II activity towards kynurenine. When each of the 23 amino acids (at 5 mM concentration) was incorporated with 5 mM kynurenine into the kynurenine/hKAT II/α-oxo acid reaction mixture, aminoadipate, asparagine, glutamate, histidine, cysteine, lysine, 3-hydroxy-kynurenine and phenylalanine were all shown to decrease the rate of KYNA formation, but none of them inhibited more than 35 % of KAT activity (Figure 2).

Overall structure

The crystal structure of the hKAT II-α-oxoglutaric acid complex was determined by molecular replacement and refined to a resolution of 2.48 Å. The final model yields a crystallographic R value of 21.5 % and an R_{free} value of 24.4 % with ideal geometry (Table 1). There are four

protein molecules that form two biological homodimers in an asymmetric unit and the protein residues are numbered 1(A)-425(A) for molecule A, 1(B)-425(B) for molecule B, 1(C)-425 (C) for molecule C, and 1(D)-425(D) for molecule D. Pro^{18} (A) to Gly^{29} (A), Arg^{20} (B) to Gly^{29} (B), and Arg^{20} (D) to Arg^{28} (D) have not been included in the final hKAT II-α-oxoglutaric acid complex model because they are highly disordered. To distinguish the residues from the two subunits of a biological dimer in the description of the substrate interaction, residues from the opposite subunit have been labelled with an asterisk (*). The results of the refinement are summarized in Table 1.

The structural basis of the broad substrate specificity of hKAT II

We were able to model α-oxoglutaric acid in one of the four subunits. In the other three subunits, the equivalent position is occupied by glycerol. Inspection of the active centre of the hKAT II crystal structure revealed that the substrate lies near the PLP molecule that forms LLP (lysine pyridoxal-5′-phosphate) with Lys^{263}. Several residues, including Tyr^{142} and Asn^{202} from one subunit, and Gly^{39}* and Tyr^{74}* from the opposite subunit, define the substrate-binding site and contact the α-oxoglutaric acid molecule. The ring of Tyr^{74}* has a weak hydrophobic interaction with α-oxoglutaric acid, and its hydroxy group forms a weak hydrogen bond with the O_2 atom of α-oxoglutaric acid (Figure 3). The interactions of glycerol and the hKAT II active-centre residues include the formation of hydrogen bonds between glycerol and residues Tyr^{142}, Gln^{118} and Tyr^{233} and hydrophobic interactions between glycerol and residues Tyr^{74}* and Leu^{19}* from the opposite subunit (Figure 4). Upon superposing of the hKAT II subunit B (with α-oxoglutaric acid) and hKAT II subunit D (with glycerol) on to the hKAT II-kynurenine structure (Figure 5), we identified the residues within 5Å of the ligands. α-Oxoglutaric acid, glycerol and kynurenine basically occupy similar positions, but α-oxoglutaric acid has fewer interactions with the enzyme than kynurenine. The carboxylic group of the α-oxoglutaric acid substrate does not form salt bridges with the guanidinium group of Arg^{399}. PLP forms LLP with Lys^{263} when the subunit binds glycerol or α-oxoglutaric acid, and forms PMP (pyridoximine 5′-phosphate) when the protein binds kynurenine. Most residues within 5Å have the same conformation, except for a few residues in the N-terminal fragment (residues 15-33) (Figure 5). When the protein molecule binds glycerol, this N-terminal fragment moves towards the centre to interact with glycerol. However, when it binds kynurenine, the N-terminal fragment moves away from the centre and leaves room for kynurenine. Also, when it binds α-oxoglutaric acid, the N-terminal fragment seems to move further away to leave more space for the substrate. In summary, the finding that the N-terminal fragment is disordered simply indicates that the N-terminal fragment is highly flexible and is moved upon binding of the substrate.

DISCUSSION

AADAT was first detected in rat liver [42] and its potential function in liver lysine degradation has been well described [43]. It was reported that the same aminotransferase from liver and kidney had both KAT activity and AADAT activity [44-46] and the true identity of rat KAT II and AADAT was confirmed by Tobes and Mason [45].

KAT II has been considered to be the principal enzyme responsible for the synthesis of KYNA in the rodent and human brain [17,18]. Mice in which KAT II was knocked out by genomic manipulation showed a substantial decrease in brain KYNA levels and displayed phenotypic changes that were in line with this chemical deficit [7,47,48]. In the present study, kinetic analysis shows a better overall catalytic efficiency of hKAT II towards kynurenine than towards other amino-group donors, except for aminoadipate. Although hKAT II has a relatively high K_m for kynurenine, its catalytic efficiency in the kynurenine to KYNA pathway is enhanced by its relatively high catalytic-centre activity towards kynurenine and also by the irreversible

nature of the kynurenine to KYNA pathway. The enzymatic product of kynurenine transamination is an α-oxo acid intermediate that is unstable and undergoes rapid intramolecular cyclization to KYNA. Consequently, no direct enzymatic product accumulates during KAT-catalysed kynurenine transamination and the equilibrium always proceeds towards KYNA production. Therefore the transamination of kynurenine to KYNA is one of the major functions of hKAT II.

Although most of the previous studies on KAT II have emphasized its function in KYNA biosynthesis because of the important role of KYNA in the central nervous system, the enzyme has a higher catalytic efficiency for aminoadipate than for kynurenine (Table 2). Human liver has been shown to have AADAT activity [49]. In rats, AADAT activity has been found in liver and kidney tissues [42,44-46,50-52]. In humans, lysine is the main source of aminoadipate. Lysine, an essential amino acid in humans, is degraded when there is an excess present beyond that required for protein synthesis. The aminoadipate pathway is the major route for the catabolism of lysine and occurs in the liver [27,53,54]. From the enzyme kinetic parameters in this paper, we showed that aminoadipate was the best amino-acid substrate for hKAT II. The fact that α-oxoadipate is not a good amino-group acceptor for the enzyme suggests that the AADAT enzyme reaction does not tend to be reversible, and favours the direction from aminoadipate to α-oxoadipate, one of the reactions involved in lysine degradation (Figure 6). An additional argument for a primary role of hKAT II in the aminoadipate to α-oxoadipate pathway comes from the comparison of hKAT II with its homologues from some micro-organisms. The KAT II or AADAT present in lower eukaryotes and certain bacteria is involved primarily in lysine biosynthesis (the reversal of lysine catabolism) because it catalyses the transamination reaction using α-oxoadipate as an amino acceptor to yield aminoadipate [55-58]. The kinetic constant (K_m) of a yeast KAT II homologue is 14 mM for α-oxoadipate and 20 mM for aminoadipate [59]. The homologue enzyme from *Thermus thermophilus* catalyses efficiently the transamination from α-oxoadipate to aminoadipate with negligible activity in the reverse reaction [55]. These results show that the KAT II homologues in yeast and *T. thermophilus* favour the transamination direction from α-oxoadipate to aminoadipate, the reaction involved in lysine biosynthesis. In contrast, the affinity of hKAT II for aminoadipate (K_m = 0.9 mM) is much greater than its affinity for oxoadipate (K_m = 20.9 mM), which indirectly supports a role for hKAT II in lysine degradation.

Aminoadipate occurs naturally in the brain [53,60]. The compound is known to be a selective toxin for astrocytes and related glial cells because injection of a racemic mixture of aminoadipate induced transient degenerative changes in non-neuronal cells [61]. Its toxicity to glial cells has been further demonstrated by a number of subsequent *in vivo* or *in vitro* studies [62-69]. Since KAT II is present in the astrocyte-like cells of mammalian brains [28,70,71], it raises a reasonable argument for the role of KAT II in the neutralization of aminoadipate in the mammalian brain. On the other hand, many previous studies have shown that a high concentration of aminoadipate inhibits glutamate transport [72,73], blocks glutamine synthetase [74], prevents the uptake of glutamate into synaptic vesicles [75] and functions as an NMDA receptor agonist [76]. These effects can contribute to an increased excitatory tone because synaptic glutamate concentrations are elevated. Therefore the hyperactive behaviour observed in *katII*$^{-/-}$ mice [47] raised an interesting question as to whether the aminoadipate level is increased in the knockout mouse brain and consequently contributes to the pathological mechanisms of the abnormal behaviour. High levels of aminoadipate in serum and/or urine have also been observed in many cases with neurological and other disorders [77-87].

Biochemical analysis indicates that hKAT II is a multifunctional aminotransferase. Kinetic analysis of the enzyme towards different amino acids and oxo acids showed that the enzyme was efficient in catalysing the transamination of a number of other amino acids, as well as kynurenine and α-aminoadipate, and could use many α-oxo acids as amino-group acceptors.

The characterization of multiple substrates for hKAT II suggests that the enzyme might play a role in the metabolism of other oxo acids and amino acids, which requires further study. It also raises a fundamental question regarding the structural basis of its broad substrate specificity. A previous study [24] showed that a major conformational change of residues 16-31 in hKAT II is involved in substrate binding and for shielding the substrate-binding pocket from bulk solvents. The hKAT II-α-oxoglutaric acid structure shows that this N-terminal fraction is very flexible and it has different conformations when the protein subunit binds to different ligands. This N-terminal fraction moves close to the active centre when the protein subunit binds a small ligand, and moves away from the centre when the subunit binds a large ligand. In the hKAT II-α-oxoaglutarate structure we did not find any other large-scale conformational changes, such as domain-domain rotation, which is involved in binding substrates in other aminotransferases [88-94]. So the conformational change of part of the N-terminal residues is the main mechanism which adapts to accommodate various substrates of different sizes in the active centre.

In summary, there have been a number of previous studies [16,17,44-46] dealing with the biochemical characterization of KAT II/AADAT, which emphasize either the KAT activity of the enzyme or its AADAT function. The present study, however, produces an overall picture regarding the substrate profiles of hKAT II, its catalytic efficiency for different amino-group donors and acceptors and its structural basis of substrate binding and catalysis. Our results suggest that hKAT II is likely to play primary roles in both the kynurenine to KYNA and aminoadipate to α-oxoadipate pathways. In addition, the enzyme may be involved in the regulation of other amino acids and α-oxo acids. Therefore it will be interesting to study the overall functions of hKAT II *in vivo* in the future, particularly in order to use the available *Kat II* gene-deficient mice to test any possible hypotheses.

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Abbreviations used

AADAT, aminoadipate aminotransferase; KAT, kynurenine aminotransferase; hKAT II, human kynurenine aminotransferase II; KYNA, kynurenic acid; LLP, lysine pyridoxal-5′-phosphate; NMDA, *N*-methyl-D-aspartate; OPT, *O*-phthaldialdehyde thiol; PLP, pyridoxal 5′-phosphate.

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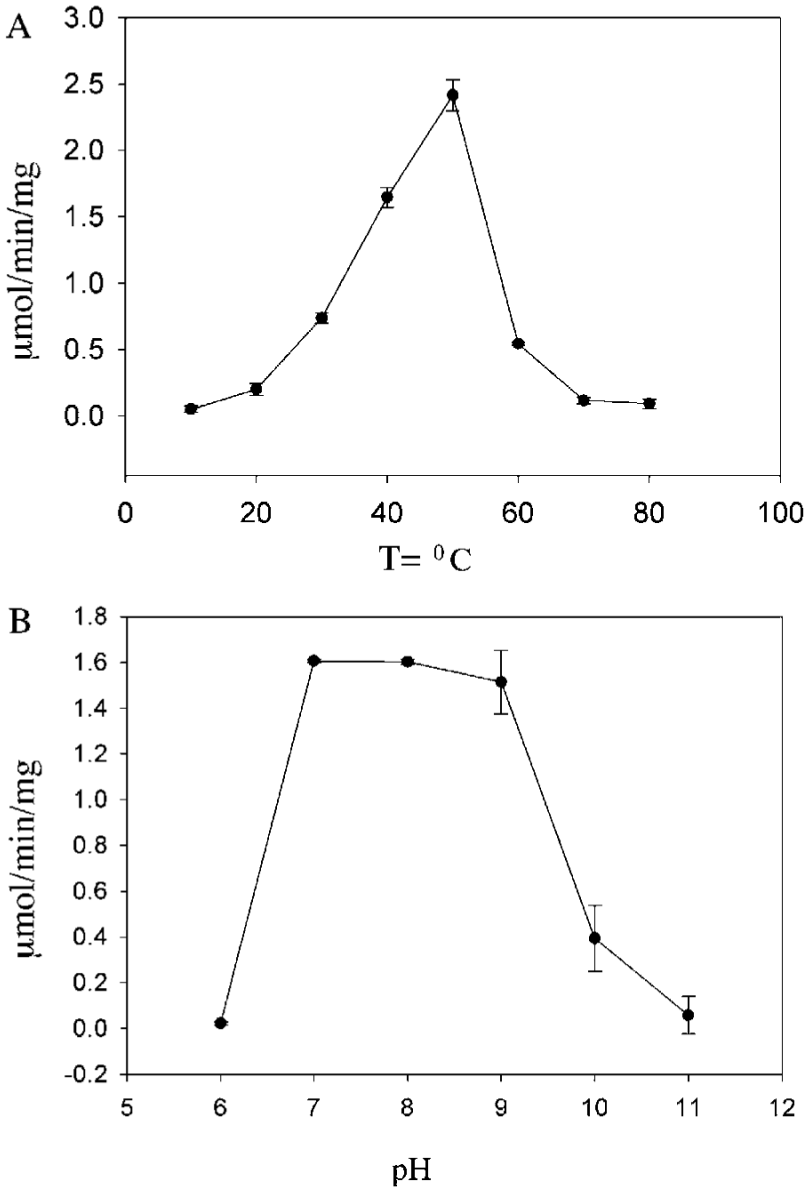

Figure 1. Effect of pH and temperature on enzyme activity

The activities of recombinant hKAT II at different temperatures (A) and pH values (B). T, temperature. Results are means ± S.D. (*n*=2).

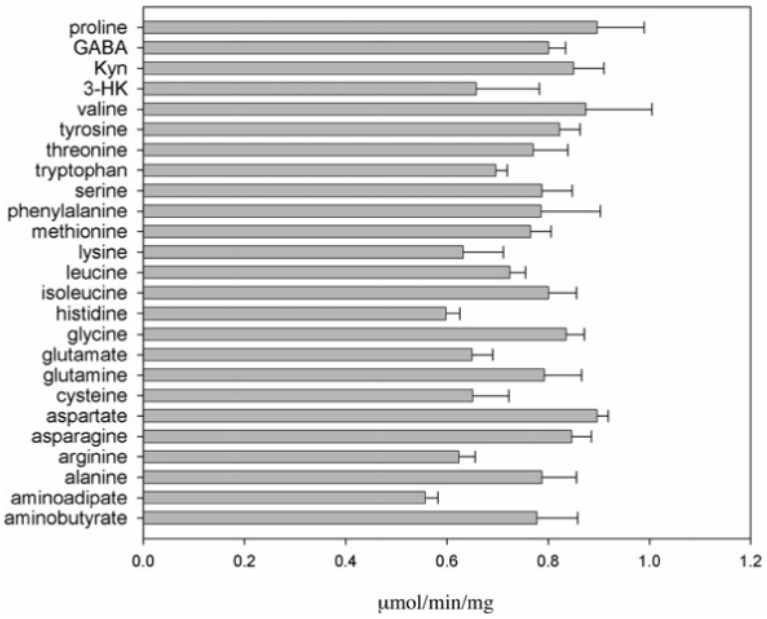

Figure 2. Effect of other amino acids on hKAT II/KAT activity

The activities were quantified by measuing the amount of KYNA produced in the reaction mixtures. The rate of KYNA production in the hKAT II-containing reaction mixtures is shown. Results are means ± S.D. (*n*=4). 3-HK, 3-hydroxy-kynurenine; GABA, γ-aminobutyric acid; Kyn, kynurenine.

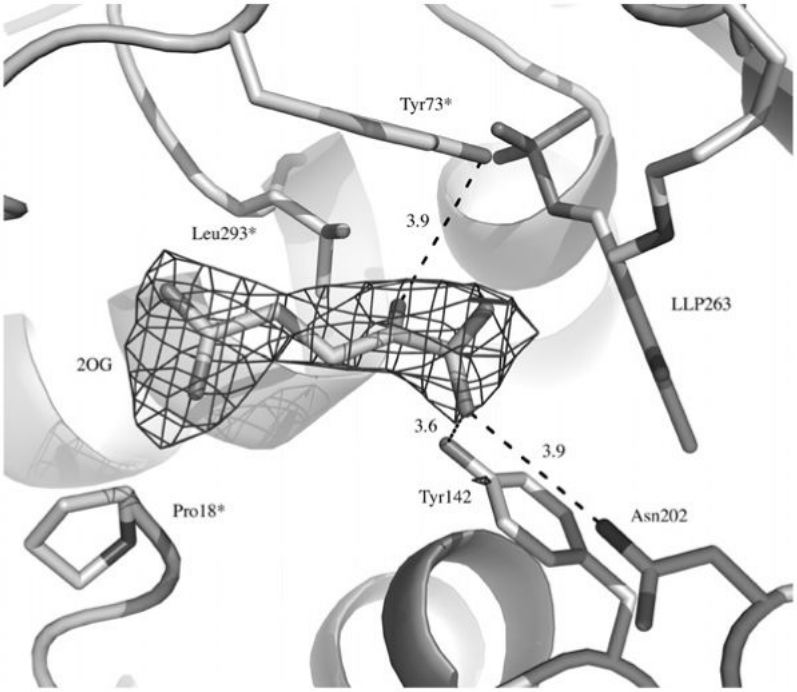

Figure 3. *α*-Oxoglutaric acid binding site

α-Oxoglutaric acid (2OG) and the protein residues within a distance of 4 AÅ of 2OG are shown (distances are stated in AÅ). The 2*F*o-*F*c electron-density map covering 2OG is contoured at the 1.1σ level. The Figure was generated by PyMOL. * indicates residues on the opposite subunit.

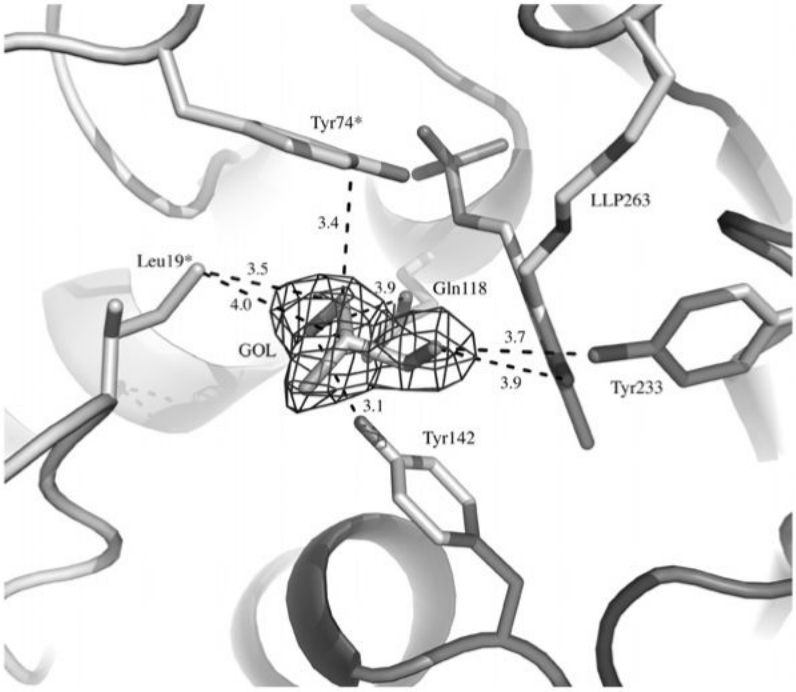

Figure 4. Glycerol-binding site in the active centre

Glycerol (GOL) and the protein residues within a 4 Å distance of GOL are shown (distances are stated in Å). The 2*F*o-*F*c electron-density map covering GOL is contoured at the 1.1σ level. The Figure was generated by PyMOL. * indicates residues on the opposite subunit.

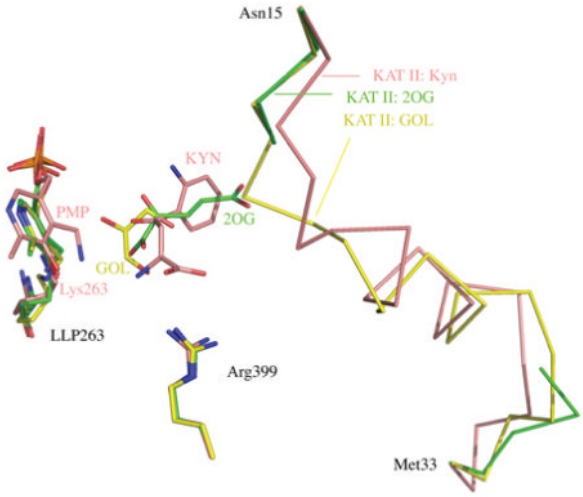

Figure 5. Superposition of the hKAT II subunit B (with *α*-oxoglutaric acid, green) and hKAT II subunit D (with glycerol, yellow) on to the hKAT II-kynurenine structure (pink)

The major protein conformation changes involved in ligand binding are shown in ribbon form (residues 15-33). GOL, glycerol; Kyn, kynurenine; LLP263, PLP formed with Lys^{263}; 2OG, *α*-oxoglutaric acid, PMP, pyridoximine 5′-phosphate.

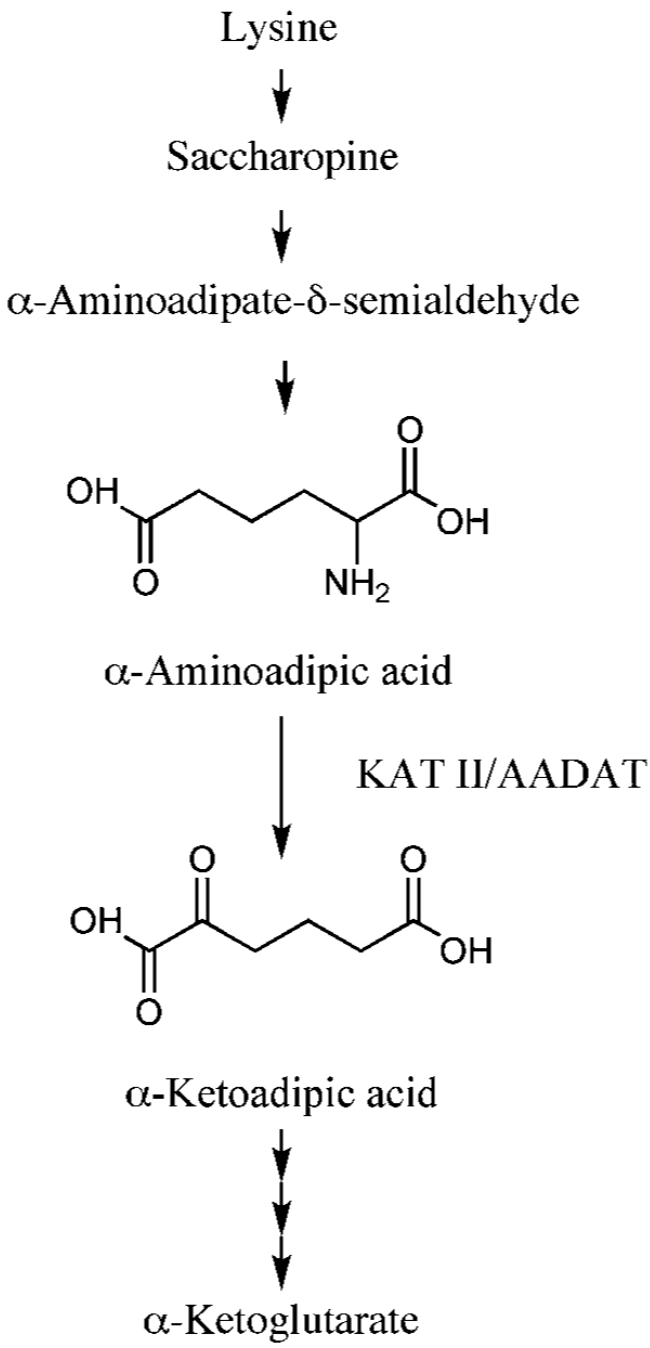

Figure 6. Lysine catabolism pathway in mammals

Table 1

Data collection and refinement statistics of the hKAT II complex with *α*-oxoglutaric acid

GOL, glycerol; 2OG, *α*-oxooglutaric acid; RMS, root mean square.

Crystal data	
Space group	P1
Unit cell	
a (Å)	60.9
b (Å)	72.1
c (Å)	109.1
α (°)	90.0
β (°)	100.7
γ (°)	93.9
Data collection	
X-ray source	Brookhaven National Laboratory-X29
Wavelength (Å)	1.0809
Resolution (Å)*	2.48 (2.55-2.48)
Total number of reflections	193082
Number of unique reflections	63835
R-merge*	0.11 (0.30)
Redundancy*	3.4 (2.7)
Completeness (%)*	89.6 (59.7)
Refinement statistics	
R-work (%)	21.5
R-free (%)	24.4
RMS bond lengths (Å)	0.020
RMS bond angles (°)	1.857
Number of ligand or cofactor molecules	4 LLP 1 2OG 7 GOL
Number of water molecules	828
Average B overall (Å^2)	33.9

* The values in parentheses are for the highest resolution shell.

Table 2

Kinetic parameters of hKAT II towards amino acids and *α*-oxo acids

The K_m and k_{cat} for oxo acids were derived by using various concentrations of individual oxo acids in the presence of 10 mM kynurenine; for amino acids, the results were derived by using various concentrations of individual amino acids in the presence of 30 mM glyoxylate. 3-HK, 3-HK, 3-hydroxy-kynurenine; α-KMB, α-oxo-γ-methiol-butyric acid. Results are means±S.E.M. (*n*=2).

	K_m (mM)	k_{cat} (min^{-1})	k_{cat}/K_m ($min^{-1} \cdot mM^{-1}$)
Amino-acid substrates			
Aminoadipate	0.9±0.1	179.4±5.7	196.2
Kynurenine	4.7±0.8	585.3±39.9	125.9
Methionine	1.7±0.5	206.4±17	123.8
Glutamate	1.6±0.4	185.0±14.8	118.7
Tyrosine	1.8±0.2	131.5±5.2	74.4
Phenylalanine	5.2±1.7	327.1±46.2	63.4
Tryptophan	4.3±0.4	254.0±8.6	58.7
Leucine	5.1±3.0	285.0±70.4	56.1
3-HK	3.8±1.0	102.1±12.2	26.8
Glutamine	8.1±2.9	95.5±16.6	11.8
Alanine	19.4±9.2	173.6±43.2	9.0
Aminobutyrate	17.8±4.3	157.2±19.4	8.8
Oxo acid substrates			
α-Oxoglutarate	1.2±0.4	460.1±49.8	374.5
α-Oxocaproic acid	1.5±0.1	289.7±8.1	188.0
Phenylpyruvate	1.8±0.2	284.0±13	156.9
α-KMB	2.4±0.7	256.2±31.9	105.2
Mercaptopyruvate	2.8±0.5	215.5±12.6	77.7
Indo-3-pyruvate	1.4±1.2	89.1±32.5	64.6
α-Oxovalerate	3.4±0.3	159.3±4.9	47.0
α-Oxoleucine	3.3±0.8	150.5±13.6	45.4
α-Oxobutyrate	12.7±2.1	208.9±12.5	16.4
Hydroxyphenylpyruvate	1.5±1.1	23.6±9.5	16.2
α-Oxoadipate	20.9±5	290.3±30	13.9
Glyoxylate	18.0±3.7	218.4±18.4	12.1
Oxaloacetate	16.8±12.4	93.9±51.6	5.6
α-Oxovaline	12.9±2.9	55.1±4.6	4.3
α-Oxoisoleucine	14.2±3.8	55.2±6.9	3.9
Pyruvate	9.7±5.6	21.8±6.4	2.3