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Structure, expression, and function of kynurenine aminotransferases in human and rodent brains

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Abstract

Kynurenine aminotransferases (KATs) catalyze the synthesis of kynurenic acid (KYNA), an endogenous antagonist of *N*-methyl-D-aspartate and alpha 7-nicotinic acetylcholine receptors. Abnormal KYNA levels in human brains are implicated in the pathophysiology of schizophrenia, Alzheimer's disease, and other neurological disorders. Four KATs have been reported in mammalian brains, KAT I/glutamine transaminase K/cysteine conjugate beta-lyase 1, KAT II/aminoadipate aminotransferase, KAT III/cysteine conjugate beta-lyase 2, and KAT IV/glutamic-oxaloacetic transaminase 2/mitochondrial aspartate aminotransferase. KAT II has a striking tertiary structure in N-terminal part and forms a new subgroup in fold type I aminotransferases, which has been classified as subgroup Iε. Knowledge regarding KATs is vast and complex; therefore, this review is focused on recent important progress of their gene characterization, physiological and biochemical function, and structural properties. The biochemical differences of four KATs, specific enzyme activity assays, and the structural insights into the mechanism of catalysis and inhibition of these enzymes are discussed.

Keywords

Kynurenic acid; Pyridoxal 5′-phosphate; Kynurenine; Kynurenine aminotransferase; Aminotransferase; Tryptophan metabolism; Cysteine conjugate beta-lyase; Neurodegeneration

Tryptophan catabolism and KYN pathway

Of the dietary tryptophan not used in protein synthesis, approximately 99% is metabolized along the kynurenine (KYN) pathway (from tryptophan, via KYN and quinolinic acid (QUIN), to nicotinamide adenine dinucleotide (NAD). An alternative pathway is the conversion of tryptophan to serotonin and then to melatonin [1] (Fig. 1). Kynurenic acid (KYNA) is a metabolite in the KYN pathway, and its formation from tryptophan is a complicated process with a number of enzymes involved. The overall process leading to the formation of KYNA includes oxidation of tryptophan to formylkynurenine, hydrolysis of formylkynurenine to

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KYN, and transamination of KYN to a side chain keto acid intermediate, and intramolecular cyclization of the intermediate to KYNA. The enzymes directly catalyzing the reaction from KYN to KYNA are KATs.

KYNA and its possible physiological functions

KYNA, an intermediate in the tryptophan catabolic pathway, acts as a noncompetitive glutamate receptor antagonist and is believed to play a role in modulating glutamate-mediated neurotransmission through interaction with glutamate receptors [2]. In mammalian brains, glutamate is the major excitatory neurotransmitter, acting through both ligand-gated ion channels (ionotropic) and G protein-coupled (metabotropic) receptors. Activation of these receptors is responsible for basal excitatory synaptic transmission and for many of the forms of synaptic plasticity, such as long-term potentiation and long-term depression, which underlie learning and memory [3,4]. However, any events or processes that substantially increase the activity of glutamate receptors often induce the death of neurons, considered to take place during ischemia, trauma, hypoxia, hypoglycemia, and hepatic encephalopathy [5]. Failure to maintain physiological concentrations of brain KYNA could be one of the causative factors leading to neuropathological conditions. Indeed, deficiency or over-accumulation of KYNA has been linked to a number of neurodegenerative diseases and psychotic disorders in humans (Table 1; see also recent reviews in [6,7]). The alterations of KYNA levels in those disorders are inconsistent perhaps due to multiple factors implicated from status of the diseases to the specificity of the specimen measured. Comprehensive analyses of the KYN pathway with each individual disease and even corresponding subtypes of these diseases should be carefully considered. For example, although the two branches of the KYN pathway, KYNA and QUIN branches, are acutely segregated in the brain, persistent down-regulation of the QUIN branch may with time increase the bioavailability of KYN for the synthesis of KYNA [6,7]. The resulting benefit of enhanced neuroprotection would then have to be weighed against the cognitive side effects, which can be expected as a consequence of increased inhibition of *N*-methyl-D-aspartate (NMDA) and α7-nicotinic acetylcholine receptors (nAChR) [6].

KYNA is also the antagonist of the α7 nAChR [25–28]. KYNA level changes in brains may therefore affect the physiological functions related to α7-nicotinic acetylcholine neurotransmission [29–32]. Postnatal KAT II$^{-/-}$ mice had transient lower hippocampal KYNA levels and higher spontaneous locomotor activity than wild-type (WT) mice. Golgi staining of cortical and striatal neurons revealed enlarged dendritic spines and a significant increase in spine density in KAT II$^{-/-}$ mice but not in WT controls [33]. At 21 postnatal days, α7 nAChR activity induced by exogenous application of agonists to CA1 stratum radiatum interneurons was ~65% higher in mKAT II$^{-/-}$ than WT mice [27]. However, the compensatory increase of Kat1 and Kat3 may be responsible for the normalization of KYNA levels in the adult KAT II$^{-/-}$ mouse brain [34]. KYNA also affects extracellular glutamate and dopamine levels in various regions of the rat brain [35–38], possibly by antagonizing presynaptic α7 nAChRs or by increasing acetylcholine release [39].

KYNA may serve as an endogenous anti-excitotoxic agent in modulating glutamatergic neurotransmission in ways that may affect neuroprotection and neuronal vulnerability such as in Huntington's disease. The KAT II$^{-/-}$ mouse is an excellent model to test this hypothesis because the neuronal vulnerability of the mice is enhanced to an intrastriatal injection of QUIN, an NMDA receptor agonist. However, this lesion enlargement could be prevented when the striatal KYNA deficit in mutant mice is neutralized by timely pharmacological interventions, suggesting that endogenous KYNA controls the vulnerability of striatal neurons to QUIN [40,41].

KYNA has been identified as an endogenous ligand of an orphan G protein-coupled receptor (GPR35) that is predominantly expressed in immune cells [42]. The tryptophan metabolic pathway is activated during inflammatory conditions, such as viral invasion, bacterial lipopolysaccharide, or interferon stimulation [43,44]. The activation of tryptophan metabolism causes a reduced plasma tryptophan level and an elevated KYNA concentration [1]. Therefore, this receptor–ligand pair may also play a role in immunological regulation.

In addition to above roles, KYNA is also involved in the control of the cardiovascular function by acting at rostral ventrolateral medulla of the central nervous system [45]. Spontaneously hypertensive rat (SHR), the most widely used animal model for studying genetic hypertension, is associated with abnormally low KYNA levels, due to a missense mutation in KAT I, in an area of the central nervous system that influences physiological blood pressure [46,47].

Brain KATs

KYNA is produced enzymatically by irreversible transamination of KYN, the key intermediate in the KYN pathway. Because KYNA passes through the blood brain barrier poorly, it must be produced in the brain [48]. In human, rat, and mouse, four proteins arbitrarily named KATs I, II, III, and IV, have been considered to be involved in KYNA synthesis in the central nervous system [34,49–54]. Based on the intimate relationship between abnormal brain KYNA concentrations and neurodegenerative diseases or psychotic disorders, enzymes involved in brain KYNA synthesis have been considered as potential targets for regulating brain KYNA concentration [1,7,41,51,55–61]. Although the involvement of KAT enzymes in brain KYNA production has been discussed, their specific contributions in brain KYNA synthesis remain to be established.

Gene characterization

KAT I/glutamine transaminase K (GTK)/cysteine conjugate beta-lyase (CCBL) 1, KAT II/aminoadipate aminotransferase (AADAT), KAT III/CCBL2, and KAT IV/glutamic-oxaloacetic transaminase (GOT) 2/mitochondrial aspartate aminotransferase (ASAT) have been reported in mouse, rat, and human brains. Their gene names, protein names, total residues, chromosome loci, GenBank accession numbers, and cellular targeting are listed in Table 2.

Among the four human KATs, human KAT I and KAT III share the highest sequence identity (51.7%). Both mouse and human KAT III genes are composed of 14 exons (isoform a) (Fig. 2) [34]. The first exon is a 5′-terminal UTR sequence. Exon 2 encodes 33 amino acid residues in human KAT III gene, 34 amino acids in mouse KAT III gene, which probably corresponds for the leader sequence for mitochondrial targeting. KAT III shares similar intron–exon boundaries with KAT I. Exon 2 can be alternatively spliced in KAT III, having an isoform b lacking the alleged leader sequence, and probably skipped in KAT I (Fig. 2). In NCBI database (URL: http://www.ncbi.nlm.nih.gov/sites/entrez?Db=gene&Cmd=retrieve&dopt=full_report&list_uids=883&log$=databasead&logdbfrom=nuccore#), three transcript variants are listed for human KAT I gene, two of them encode the same isoform a, and one encodes 372 amino acid isoform b. Human isoform b lacks a key residue, Tyr101, for substrate binding and residues Gly99Gly100Tyr101 for PLP binding, so its KAT activity has yet to be confirmed. However, there is a human KAT I protein in the database: AK297995 with 516 amino acid residues, 94 more residues than its isoform a. If exon 2 was not skipped in KAT I, it would only encode 32 amino acid residues; thus, a further confirmation of the sequence AK297995 is needed.

The genes of KAT I and KAT III are phylogenetically distant from those of KAT II and KAT IV. It is well established that KAT IV/mitochondrial ASAT targets mitochondria [64–72]. KAT

II is also considered to be a mitochondrial protein [63,73,74]. Indeed, the probability of export to mitochondria is predicted as 0.9865 (URL: http://ihg2.helmholtz-muenchen.de/ihg/mitoprot.html). The predicted cleaved sequence is the first 22 amino acids at the N-terminal end. However, the crystal structure [75,76] of human KAT II shows that a major main chain conformational change involves residues 16–31, which is not only involved in substrate binding but also in shielding the substrate-binding pocket from bulk solvents. Residues 1–16 fold well with remainder of the protein and do not seem to be dispensable. In addition, the recombinant protein lacking the mitochondrial targeting sequence (residues 1–22) did not show any transaminase activity (Han and Li, unpublished data). Taken together, these data suggest that KAT II behaves very differently from other mitochondrial KATs that do not require the mitochondrial targeting sequence for activity [54,77,78]. Consequently, if KAT II actually targets mitochondria, it must use a very different mechanism.

Biochemical properties

The KAT catalyzed transamination reaction involves two steps, KYN to a side chain keto acid intermediate and intramolecular cyclization of the intermediate to KYNA (Fig. 3). The first step is mediated by KAT enzymes. The enzymatic product, however, is unstable and undergoes rapid intramolecular cyclization to form KYNA. All four mammalian KATs show transamination activity towards KYN leading to the formation of KYNA.

KAT I/GTK/CCBL1—Information about KAT I has been reviewed by Cooper [79]. KAT I is an enzyme with multiple functions, including CCBL activity and transaminase activity with many amino acids. Other functions suggested for KAT I included involvement in salvaging *α*-keto acids derived from essential amino acids [80], closing the last step of the methionine salvage pathway [79] and mediating the toxicity of the sulfur-containing fragments released from halogenated alkene-derived cysteine *S*-conjugates [81,82], which may contribute to the nigral cell damage observed in Parkinson's disease [83,84]. As an aminotransferase, KAT I has broad amino acid specificity [52,80,84]. In general, the enzyme is most active with large neutral/aromatic/sulfur-containing amino acids, including glutamine, phenylalanine, leucine, KYN, tryptophan, methionine, tyrosine, histidine, cysteine, aminobutyrate, *S*-(1,1,2,2-tetrafluoroethyl)-L-cysteine, and *S*-(1,2-dichlorovinyl)-L-cysteine. The enzyme has detectable activity with *Se*-methylselenocysteine, 5-*S*-cysteinyl-dopamine, 5-*S*-cysteinyDOPA, asparagines, glycine, alanine, arginine, serine, and lysine [52,84]. KAT I can use many *α*-keto acids as its amino group acceptors. Sixteen *α*-keto acids, *α*-ketoglutarate, *α*-ketocaproic acid, phenylpyruvate, *α*KMB, mercaptopyruvate, indo-3-pyruvate, *α*-ketovalerate, *α*-ketoleucine, *α*-ketobutyrate, *p*-hydroxyphenylpyruvate, *α*-ketoadipate, glyoxylate, oxaloacetate, *α*-ketovaline, *α*-ketoisoleucine, and pyruvate, have been tested in the KAT activity assay. All of them demonstrated the capacity to act as amino group acceptors, but five *α*-keto acids, *α*-ketoglutarate, *α*-ketoiosleucine, indo-3-pyruvate, *α*-ketoadipate, and *α*-ketovaline, have very low activity. Based on the catalytic efficiency (k_{cat}/K_m) and specific activity, human KAT I is efficient in catalyzing the transamination reaction of *α*-ketocaproic acid, *α*-ketoleucine, glyoxylate, *α*-ketovalerate, *α*KMB, mercaptopyruvate, *α*-ketobutyrate, and *p*-hydroxy-phenylpyruvate. Although the activity of KAT I using oxaloacetate, phenylpyruvate, or pyruvate as an amino group acceptor was detectable, the specific activity with these three *α*-keto acids was so low that they are unlikely to be physiological co-substrates for human KAT I [52].

KAT II/AADAT—Like KAT I, KAT II is also a multifunctional aminotransferase. Initially, the enzyme was named AADAT when it was partially purified from rat liver in 1969 [85]. Tobes and Mason later confirmed that AADAT was identical to rat KAT II [86,87]. The KAT II gene was subsequently isolated by RT-PCR from rat kidney [88] and by EST assembly from mouse [89] and human [63]. Recombinant mouse KAT II is reactive to both aminoadipate and

KYN and prefers ketoglutarate as an amino group acceptor [88]. Recombinant human KAT II has shown activity towards aminoadipate and α-ketoglutarate [63]. Human KAT II is able to catalyze the transamination reaction for a number of amino acids. Kinetic analysis of human KAT II revealed that the enzyme is efficient in catalyzing the transamination of aminoadipate, KYN, methionine, and glutamate, and is less efficient in catalyzing tyrosine, phenylalanine, tryptophan, leucine, 3-HK, glutamine, alanine, and aminobutyrate. Human KAT II can use many α-keto acids as its amino group acceptors. Sixteen α-keto acids have been tested in the KAT activity assay; all of them showed the capability to act as amino group acceptors. Human KAT II is efficient in catalyzing the transamination of α-ketoglutarate, α-ketocaproic acid, phenylpyruvate, and αKMB, and less efficient in catalyzing mercaptopyruvate, indo-3-pyruvate, α-ketovalerate, α-ketoleucine, α-ketobutyrate, *p*-hydroxyphenylpyruvate, α-ketoadipate, glyoxylate, oxaloacetate, α-ketovaline, α-ketoisoleucine, and pyruvate, listed here in order of decreasing catalytic efficiency [90]. The KYNA level in KAT $II^{-/-}$ mice is low in the brain of young mice but progresses to normal as they reach adulthood [33]. KAT II is also involved in lysine metabolism by catalyzing the transamination of aminoadipate to α-ketoadipate [90–94].

KAT III/CCBL2—Recently proposed as a novel member of the mammalian KAT family, KAT III has been identified in mouse, rat, and human [34]. The biochemical properties of KAT III have been further determined in mice [54]. Its presence in mouse, rat, and human brains has been confirmed at mRNA level [34] and by protein activity assay (Han and Li, unpublished data). The enzyme showed activity towards a number of amino acids, including some aromatic amino acids (phenylalanine, KYN, tryptophan, 3-HK, tyrosine, and histidine), sulfur-containing amino acids (methionine and cysteine), and other amino acids (glutamine, asparagine, serine, alanine, aminobutyrate, and lysine). Kinetic data revealed that mouse KAT III is efficient in catalyzing the transamination of glutamine, histidine, methionine, phenylalanine, asparagine, cysteine, and KYN. Mouse KAT III has detectable KAT reactivity to 13 α-keto acids. Based on kinetic analysis, glyoxylate, α-ketocaproic acid, phenylpyruvate, α-ketobutyrate, αKMB, α-ketovalerate, indo-3-pyruvate, *p*-hydroxyphenylpyruvate, mercaptopyruvate, and oxaloacetate are good amino group acceptors for mKAT III, and pyruvate, phenylpyruvate, and α-ketoglutarate are poor co-substrates for the enzyme.

KAT IV/GOT2/mitochondrial ASAT—The KAT activity of ASAT was first reported in *E. coli* [95], and later it was found that mitochondrial ASATs in mouse, rat, and human had KAT activity; therefore, mammalian mitochondrial ASAT was named KAT IV [53]. KAT IV was considered a major player for the formation of KYNA in mouse, rat, and human brains. In addition to its role in KYNA production, mitochondrial ASAT (also called GOT2) catalyzes the reversible transamination of oxaloacetate to aspartate in conjunction with the conversion of glutamate to α-ketoglutarate [96]. The enzyme has a number of specific roles in astrocytes and neurons in brain [69,97–100]. Firstly, it has a role in the entry of glutamate into the tricarboxylic acid cycle, and in resynthesis of intramitochondrial glutamate from tricarboxylic acid cycle intermediates [99,101–105]; secondly, it has a key role in the synthesis of neurotransmitter glutamate in brains [106,107]; and thirdly, it is an essential component of the malate–aspartate shuttle, which is considered the most important mechanism for transferring reducing equivalents from the cytosol into mitochondria in brain [69,103,108–111]. KAT IV was also reported to have cysteine conjugate beta-lyase activity [112]. Mouse KAT IV shows high transamination activity towards glutamate, aspartate, phenylalanine, tyrosine, and cysteine, and detectable activity towards tryptophan, 3-HK, methionine, KYN, and asparagine. It can use α-ketoglutarate, phenylpyruvate, αKMB, indo-3-pyruvate, hydroxyphenylpyruvate, mercaptopyruvate, α-ketocaproic acid, oxaloacetate, α-ketobutyrate, pyruvate, and glyoxylate as amino group acceptors. It also shows detectable activity towards other co-substrates,

including α-ketovalerate, α-ketoleucine, α-ketoadipate, α-ketovaline, and α-ketoisoleucine (Han and Li, unpublished data).

Differences in substrate specificity, pH profiles, inhibition of four KATs—All four KATs show activity with the aromatic amino acids KYN, phenylalanine, tyrosine, and tryptophan, and with the sulfur-containing amino acid, methionine. KATs I and III display very similar amino acid substrate profiles. Both show high activity with glutamine and essentially no detectable activity to glutamate, which contrasts with KAT II and IV that show high activity with glutamate and extremely low or undetectable activity to glutamine. KAT II is unique in having AADAT activity.

Human KAT II [90] and mouse KAT IV (Han and Li, unpublished data) have their highest activity at pH 7 and pH 8, respectively, and both have a broad optimal pH range (pH 7.0–9.0). However, the optimal pH range for KAT I is reported as being considerably higher in the literature. For example, the optimal pH range of human brain KAT I [19,50,113,114] and placenta KAT I [115] is pH 9.5–10. This led to a conclusion that KAT I had a very limited contribution to brain KYNA production under physiological conditions [49–51]. However, a lower optimal pH range was also reported for insect cell-expressed human KAT I (pH 7.5–9.0) [52], rat liver KAT I (pH 6.5) [74], and human heart KAT I (pH 8–9) [116], which indicates that human KAT I can efficiently catalyze the transamination of KYN under physiological conditions. Analysis of human KAT I activity under different pH conditions revealed that Tris amine, a commonly used buffer for KAT I assays in the literature, greatly inhibits KAT I around neutral pH conditions, but shows no inhibition at basic pH condition [52]. Therefore, the earlier reports of a higher optimal pH profile for KAT I (9.5–10) were probably due to Tris-mediated inhibition of the enzyme around pH 8 [52,117]. Mouse KAT III displays optimal activity around pH 9.0–10.0 [54], which is close to the pH optima reported for mammalian brain “KAT I” in the literature [19,50,113,114], but apparently different from the pH optima (7.5–9.0) of recombinant human KAT I [52]. Since KAT I and KAT III share similar substrate profile, the isolated brain “KAT I” with high optimal pH might actually be KAT III.

Tryptophan, glutamine, phenylalanine, or cysteine (2 mM) show inhibition of human KAT I activity [52,113]. A very high concentration (32 mM) of methionine, histidine, tyrosine, or leucine also decrease human KAT I activity significantly [52]. Based on human KAT I crystal structure and activity assays, 3-indolepropionic acid and DL-indole-3-lactic acid demonstrated high levels of inhibition [117]. Although 5 mM aminoadipate, asparagine, glutamate, histidine, cysteine, lysine, 3-HK, and phenylalanine decrease human KAT II activity, none of them inhibit more than 35% of the KAT activity when they are used at an equimolar concentration with KYN [90]. Quisqualate is an inhibitor of KAT II [50,53], but it also inhibits KAT IV [53]. Recently, (*S*)-4-ethylsulfonylbenzoylalanine was developed as the first potent and selective inhibitor of rat KAT II [118,119], but it did not efficiently inhibit human KAT II [118]. The presence of 5 mM cysteine, glutamine, histidine, methionine, leucine, and phenylalanine can significantly decrease mouse KAT III activity [54]. Apparently, its inhibition by most proteinogenic amino acids is very similar to that of human KAT I. However, mouse KAT III is greatly inhibited by 5 mM methionine, but not by 5 mM tryptophan, while human KAT I is greatly inhibited by 2 mM tryptophan, but not by 2 mM methionine [52,54]. Similarly, mouse KAT I is greatly inhibited by 5 mM tryptophan, but not by 5 mM methionine (Han and Li, unpublished data). Mouse KAT IV-catalyzed KYNA production is diminished by aspartate at an equimolar concentration with KYN, whereas other three mouse KATs are not affected by this amino acid (Han and Li, unpublished data). Thus, the specific activity or contribution of KAT IV in KYNA production in a crude sample can be estimated based on the differences in the rate of KYNA production between reaction mixtures in the presence and absence of aspartate, respectively.

The purpose of comparing four different KATs is to find their unique characteristics and develop a sensitive and specific method to assay each individual KAT. The assay method that was previously reported for KAT I using pyruvate as a co-substrate at pH 9.5 [50] could also detect activity of other three KATs, because they can all use pyruvate as an amino group acceptor and have activity at the applied pH range [52,54] (Han and Li, unpublished data). In addition, pyruvate is not a preferred co-substrate of KAT I enzymes. The commonly used KAT II assay [50] is also problematic because, under the same assay conditions, KAT I and III have detectable activity and KAT IV is very active [52,54] (Han and Li, unpublished data). Therefore, one needs to be cautious when predicting which KAT being affected in clinic and pharmacological studies if one does not have a specific assay for each individual KAT. The significant differences in optimal pH, specific inhibitors, and unique substrates of four KATs, which may be used to develop specific activity assays for each individual KAT, are summarized in Table 3.

Structural properties

The crystal structures of human KAT I [120] and its homologues, glutamine-phenylpyruvate aminotransferase from *Thermus thermophilus* HB8 [121], KAT from a mosquito, *Aedes aegypti* [122], and KAT from yeast [123], have been determined. The crystal structures of human KAT II [75,76] and its homologues from *T. thermophilus* [124] and *Pyrococcus horikoshii* [125] have also been solved. Mouse KAT III crystal structures have been determined recently [54]. Although there have been no substrate-bound crystal structures available for rat, mouse, or human KAT IV, the ligand-bound chicken mitochondrial ASAT crystal structure and mouse mitochondrial KAT IV unbound structure (PDB code, 3hlm) have been available for studies dealing with its catalytic mechanism and molecular modeling [126]. In addition, some striking structural features of KAT I and II have been reviewed [127].

KAT catalyzed transamination reaction—Like all known aminotransferases, KAT enzymes conduct Ping-pong bi-bi reactions to catalyze the reaction from amino acid 1 and keto acid 2 to amino acid 2 and keto acid 1, respectively. The interaction of amino acid 1 with the KAT-PLP form of the enzyme produces the KAT–amino acid 1 complex, which then leads to covalent alteration of the complex to produce its KAT + PMP form and the first product (keto acid 1). Then, the keto acid 1 is released and a second substrate keto acid 2 adds to the enzyme. The amino group, originally transferred to KAT-PLP to form KAT + PMP, is then transferred to keto acid 2, forming amino acid 2 and regenerating the KAT-PLP form. Finally, amino acid 2 is released and the cycle is complete [128] (Fig. 4).

Among the crystal structures of KAT enzymes [75,76,90,117,120–123,129], different intermediate structures have been reported, i.e., PLP form (internal aldimine, with or without substrate binding), external aldimine, and PMP form (with or without substrate binding). Two ligands in one active center have been seen in a human KAT I complex structure [117], which provides evidence that the catalytic center has room to bind two ligands simultaneously. These two ligands could represent two different substrates that interact through both hydrophobic interaction and a hydrogen bond. The hydrogen bond could be used in proton transfer during the transamination reaction. This proton transfer could help the two substrates shift their positions resulting in the binding of the incoming substrate and effectively replacing the product position. Therefore, it seems that the release of the first product from the enzyme active center requires the addition of the second substrate. These different intermediate structures help draw a whole picture for the KAT catalyzed transamination reaction. In addition, a number of KAT enzymes show different conformational changes between two subunits when they are bound with ligands, i.e., indole-3-acetate bound human KAT I [117], the KAT complex of mosquito with cysteine [129], and KYN bound mouse KAT III [54]. Similar mechanisms were previously documented in the aromatic amino acid aminotransferase complex of the

Paracoccus denitrificans with 3-phenylpropionate [130] and the alanine glyoxylate aminotransferase complex of *A. aegypti* with alanine [131]. Based on these data, it is reasonable to suggest that the two subunits of aminotransferases are not synchronized during the amino acid/keto acid transamination.

Overall structure—All four KATs belong to the alpha family of PLP-dependent enzymes, where they have been assigned to the fold type I group [132–136]. As observed for the majority of other members of this group, KATs invariably function as homodimers, hosting two active sites at the inter-subunit interface. The structure has a large domain and a small domain. The large domain contains an alpha/beta/alpha type fold, and the small domain comprises the C-terminal part and a small fragment of the N-terminal part, some of them also have an N-terminal arm (Fig. 5).

In KATs, an Asp residue interacts with the pyridine nitrogen of the cofactor, which is structurally and functionally conserved in the fold type I PLP-dependent enzyme family and which indicates its importance for catalysis. Fold type I aminotransferases were further classified into seven subgroups, Iα, Iβ, Iγ, Iδ, Iφ, Iλ, and Iω, and KAT I and IV have been assigned to Iγ and Iα, respectively [138]. KAT III is highly similar to KAT I in terms of primary sequence and crystal structure; therefore, it can be assigned to Iγ, too [54]. KAT II is unique in swapping the catalytically essential N-terminal region. The N-terminal region of KAT II bears a substrate-recognition helix and associated loop, which buds from the large domain of each subunit and protrudes towards the facing subunit, where it provides multiple inter-subunit contacts and essential residues for shaping the PLP-binding site. In contrast, the N-terminal regions of KAT I, III, and IV participate in building up the small domain and structuring the active site of the same subunit, i.e., they do not show the swapping observed in KAT II. Because of its striking feature of the N-terminal tertiary structure, KAT II does not belong to any existing subgroups. Therefore, it was proposed as a new subgroup in fold type I aminotransferases [76]. A further sequence phylogenetic analysis revealed that KAT II and KAT II homologs form a separate lineage [124]. This indicates that KAT II and its homologs actually form a new subgroup in fold type I aminotransferases, which we have designated a subgroup Iε, the eighth subgroup for KAT II.

Ligand-binding sites—The ligand-binding sites of KAT I and KAT II have been reviewed [127]. The crystal structures of human KAT I in complex with phenylalanine or indole-3-acetate [117,120], human KAT II in complex with KYN [76], mouse KAT III structure in complex with KYN [54], and chicken mitochondrial ASAT (86% sequence identical to human KAT IV) structures [126,139] allowed the identification of a number of residues that are crucial for ligand binding in four KAT enzymes. All three KAT enzymes have similar binding sites for the cofactor, PLP (Fig. 6).

The substrate α-carboxyl moiety forms a salt-bridge with a structurally conserved Arg, which drives the correct positioning of the substrate α-amino group just above the PLP C4A catalytic center. The presence of this Arg residue is a strictly conserved hallmark of all members of the aminotransferase superfamily whose structures have been determined so far [138]. Specifically, the recognition of the substrate side chain is achieved by different structural determinants in different KATs. A remarkable structural trait of human KAT I consists of a hydrophobic pocket or a crown of aromatic residues formed by several residues, including LLP, Tyr63* (the asterisk represents residues from opposite subunit of the enzyme dimer), His279*, Phe278*, Tyr101, and Phe125, in which the substrate side-chain is perfectly nested. Mouse KAT III has a similar aromatic crown consisting of LLP, Tyr160, Tyr312*, and Tyr98*, which seems less hydrophobic. Human KAT I, mouse KAT III, and the mosquito KAT are highly similar in terms of both primary sequence and 3-dimensional structure, and they belong to same Iγ subgroup of fold type I aminotransferases. The substrate profiles of these three KAT

enzymes are essentially the same, including aromatic amino acids (KYN, phenylalanine, tryptophan, tyrosine, and histidine), sulfur-containing amino acids (methionine and cysteine) and other amino acids (glutamine and aminobutyrate). However, they exhibit quite different catalytic efficiencies to the above amino acids. Human KAT I is more efficient in the transamination of certain hydrophobic amino acids (such as leucine and tryptophan), mouse KAT III is more efficient in the transamination of relatively hydrophilic amino acids or the amino acids with a hydrogen bond forming group (such as histidine, cysteine and methionine), and the efficiency of the mosquito KAT is somewhere in between [52,54,78]. The available crystal structures of human KAT I [117,120], mosquito KAT [122,129], and mouse KAT III [54] allow us to identify the substrate binding residues. There are two variable binding residues in the active centers of these three enzymes (Table 4). Tyr residue at the active site might enhance the binding of relatively hydrophilic substrates, or the substrates that can form hydrogen bond, while the corresponding Phe residue at the same position might better interact with relatively hydrophobic substrates. These structural differences among the three enzymes might explain their difference in term of catalytic efficiency to different substrates.

In contrast, the aromatic hydrophobic pocket observed in human KAT I and KAT III is largely absent in the structures of human KAT II and chicken mitochondrial ASAT (human KAT IV homolog). The equivalent positions of KYN side chain binding of human KAT II are occupied by polar or neutral amino acids, including Ile19, Arg20, Leu40, and Tyr74 from one subunit, and Tyr142*, Leu293*, and Ser143* from the opposite subunit. Additionally, the substrate-binding pocket in KAT II appears sensibly wider, mainly owing to the absence of a residue equivalent to human KAT I Trp18 and to the substitution of KAT I Phe 278 with Leu 293 [76]. The equivalent positions of substrate side chain binding of the chicken mitochondrial ASAT are occupied by Trp133, Ile15, Tyr67*, and Arg284*, revealed by a complex structure with 2-methyl-L-aspartic acid [139]. KYN might occupy the same position with 2-methyl-L-aspartic acid; however, the binding site of KYN with KAT IV needs to be determined.

Conformational change—It is well known that by binding substrates, ASAT changes its conformation from the open form to the closed form [126,139–144]. This substrate-mediated conformational change is also observed in other aminotransferases [121,130,145]. The mosquito KAT:cysteine and mosquito KAT:glutamine structures show that the protein subunits are crystallized as closed forms when they bind substrates. It was proposed that approach of cysteine or glutamine to the built-in cavity, which is exposed to the solvent region, induces the movement of small domain towards the large domain. By doing so, the enzyme encapsulates the substrate within the cavity to produce a Michaelis complex [129]. However, similar conformational changes have not been observed in subgroup Iγ KATs, human KAT I, and mouse KAT III, and the new subgroup Iε aminotransferase, human KAT II. A peculiar feature of human KAT II is a pronounced conformational change that affects the enzyme upon substrate binding, resulting in the correct structuring of the active site. When the protein molecule binds glycerol, the N-terminal stretch encompassing residues Asn15–Met33 moves towards the center to interact with glycerol; when it binds KYN, the stretch moves away from the center and leaves room for KYN; when it binds α-ketoglutaric acid the stretch seems to move further away to leave more space for the substrate. In addition to the slippage of this N-terminal stretch, a repositioning of a set of residues, including Ile19, Arg20, and Tyr74 of the primary subunit and Tyr142* and Gln118* of the secondary subunit in the functional homodimer, is observed upon binding of KYN (Fig. 7c). However, *T. thermophilus* AADAT shows a similar conformational change (the small domain shift) to other ASATs [126,139–144] or mosquito KAT [129] upon binding a substrate, which drives the rearrangement of the active center residues. Therefore, it was proposed that substrate specificity in human KAT II and *T. thermophilus* for AADAT is achieved through an induced-fit mechanism, which, by properly structuring the active site of the enzyme, ultimately controls the recognition of substrates [90,124,127]. In contrast, less conformational changes take place during catalysis

in human KAT I and mouse KAT III than in human KAT II. The conformational changes of human KAT I [117,120] are characterized by a largely pre-formed active site that allows the recognition of a broader set of amino acids and an N-terminal fragment movement. In human KAT I, there is a shift of the N-terminal α-helix (residues Pro17–Glu27) upon binding of a large ligand. As a result, Trp18 is brought into contact with the ligand. In addition to this N-terminal conformational change, residue Tyr101 is also repositioned (Fig. 7a). This conformational change of the N-terminal α-helix is not only for certain substrate binding, but also for plugging the active center and preventing the solvents from entering the catalytic cavity during the transamination reaction. Mouse KAT III conformational change involves residues Ser51–65, which forms the first N-terminal α-helix. By binding the substrate KYN or glutamine, this N-terminal α-helix moves towards and interacts with the substrates (the interaction between Trp54 and a substrate). This conformational change also involves some side-chain movements, including Tyr160 that shifts toward the substrate and forms a hydrogen bond with a substrate, and Tyr312* that turns away from the center and leaves room for the substrate binding and still keeps interactions with the substrates. Residues Gln71 and Tyr98* also have side chain rearrangement (Fig. 7b). By comparison with KAT I and III binding sites, it seems that mouse KAT III has more conformational changes than does human KAT I.

KAT structure-based drug design—Based on the complex structure of human KAT I and indole-3-acetic acid, two binding moieties of the inhibitor, an indole ring and an α-carboxylate, were revealed. Six chemicals with both binding moieties were further tested for their ability to inhibit hKAT I activity. Among them, 3-indolepropionic acid and DL-indole-3-lactic acid displayed the highest level of inhibition, and, therefore, it was proposed that these two inhibitors were promising candidates for future studies [117]. The structural information about the steric requirement for KYN homing displayed by the active sites of human KAT II and some empty space left in the structure of the human KAT II:KYN complex was exploited for the design of KAT II inhibitors [76]. The anthranilic moiety was proposed as a hot spot for the chemical modification of substrate-like, highly selective KAT II inhibitors. As a result, (*S*)-4-ethylsulfonylbenzoylalanine was developed as the first potent and selective inhibitor of rat KAT II [118,119].

Conclusion

A number of studies concerning the identification and functional characterization of KATs in rat, mouse and human have been undertaken. The researches involving KATs have greatly contributed to our understanding of tryptophan degradation and complexity in brains. Towards this end, four individual KATs have been implicated in brain KYNA biosynthesis, i.e., KAT I/GTK/CCBL1, KAT II/AADAT, KAT III/CCBL2, and KAT IV/GOT2/mitochondrial ASAT. The research progress in the identification and biochemical and structural characterization of mammalian KATs is discussed in this review. In summary, all four KAT enzymes are multi-functional enzymes and share many amino acid and α-ketoacid substrates. Therefore, they most likely have overlapping biological functions. The biochemical characterization of the four KAT enzymes provided a substrate profile for each individual KAT, which provides an essential basis to design a specific activity assay for each KAT. Because it is essentially impossible to have single assay conditions (KYN and α-ketoacid combination) to test the specific KAT activity of any given KAT, we propose that a specific inhibitor can be used to distinguish the four KATs. Crystallographic studies of these enzymes from different organisms, including human, have revealed distinctive structural traits of four KAT enzymes. KAT II is considered as a new subgroup, Iε in fold type I aminotransferases. A striking feature of KAT II concerns domain swapping of the N-terminal regions. Different conformational changes during catalysis create divergent active sites in the four KAT enzymes and affect substrate specificity. Structural investigations have provided insight into enzyme-specific inhibition and contributed to the

development of specific inhibitors of human KAT I and KAT II, which are of interest for the treatment of Alzheimer's disease, schizophrenia, and other neurological disorders.

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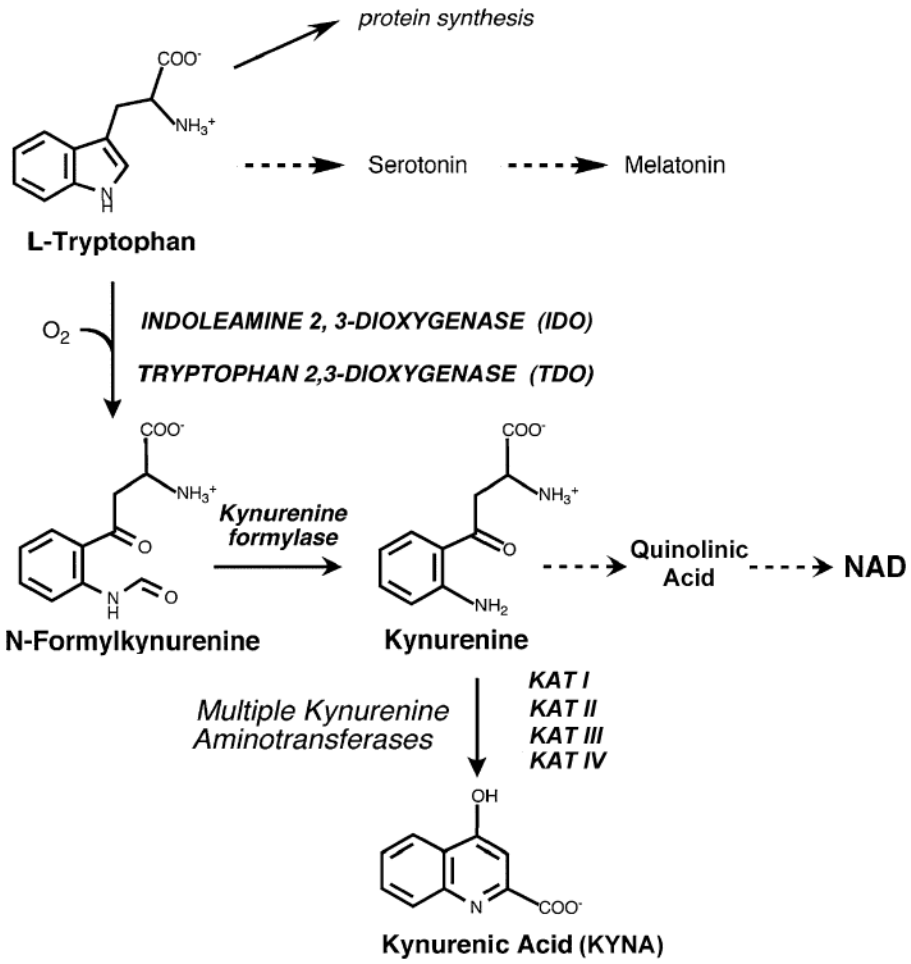

Fig. 1.

Tryptophan catabolism in humans and mammals. *IDO* Indoleamine 2,3-dioxygenase, *KAT* kynurenine aminotransferase, *NAD* nicotinamide adenine dinucleotide, *TDO* tryptophan 2,3-dioxygenase

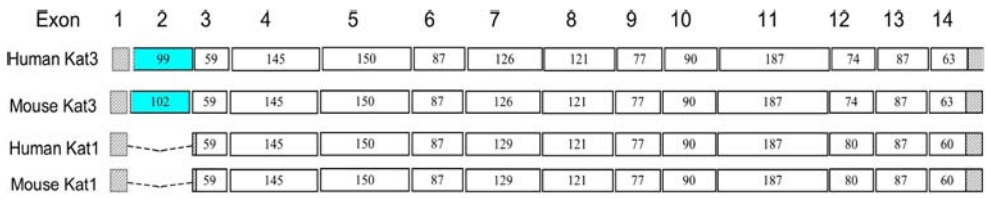

Fig. 2.

Genomic structure of human KAT I/CCBL1 and KAT III/CCBL2. Schematic representation of the organization of KAT I and KAT III genes. Each *box* represents an exon and the number of nucleotides with the exon. The *shaded regions* at N- and C-termini are UTR. The exon 2 is skipped in KAT I isoform a

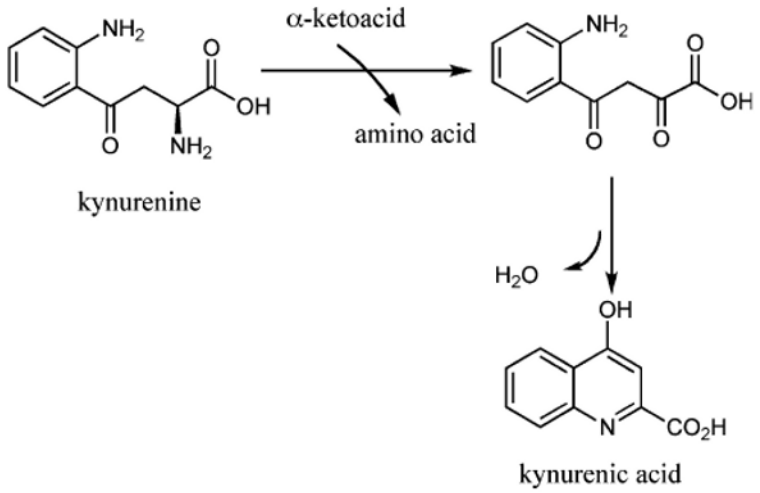

Fig. 3.
KAT catalyzed transamination reactions

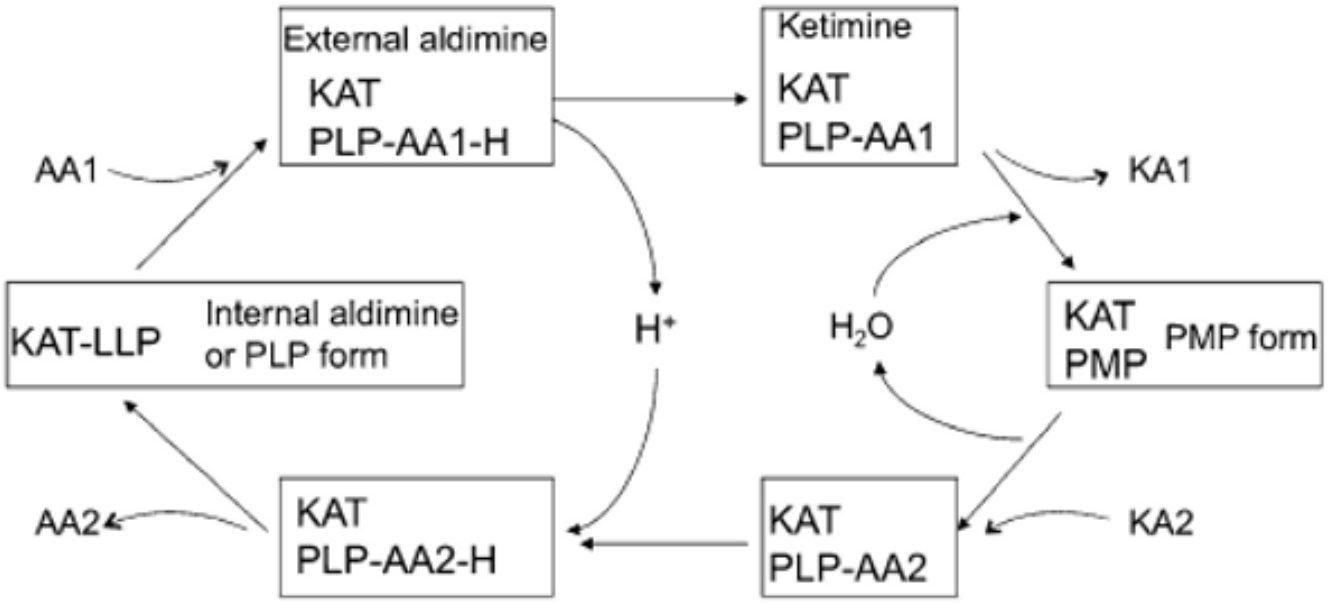

Fig. 4.
A schematic presentation of KAT catalyzed transamination reactions. *AA* Amino acid, *KA* α-ketoacid

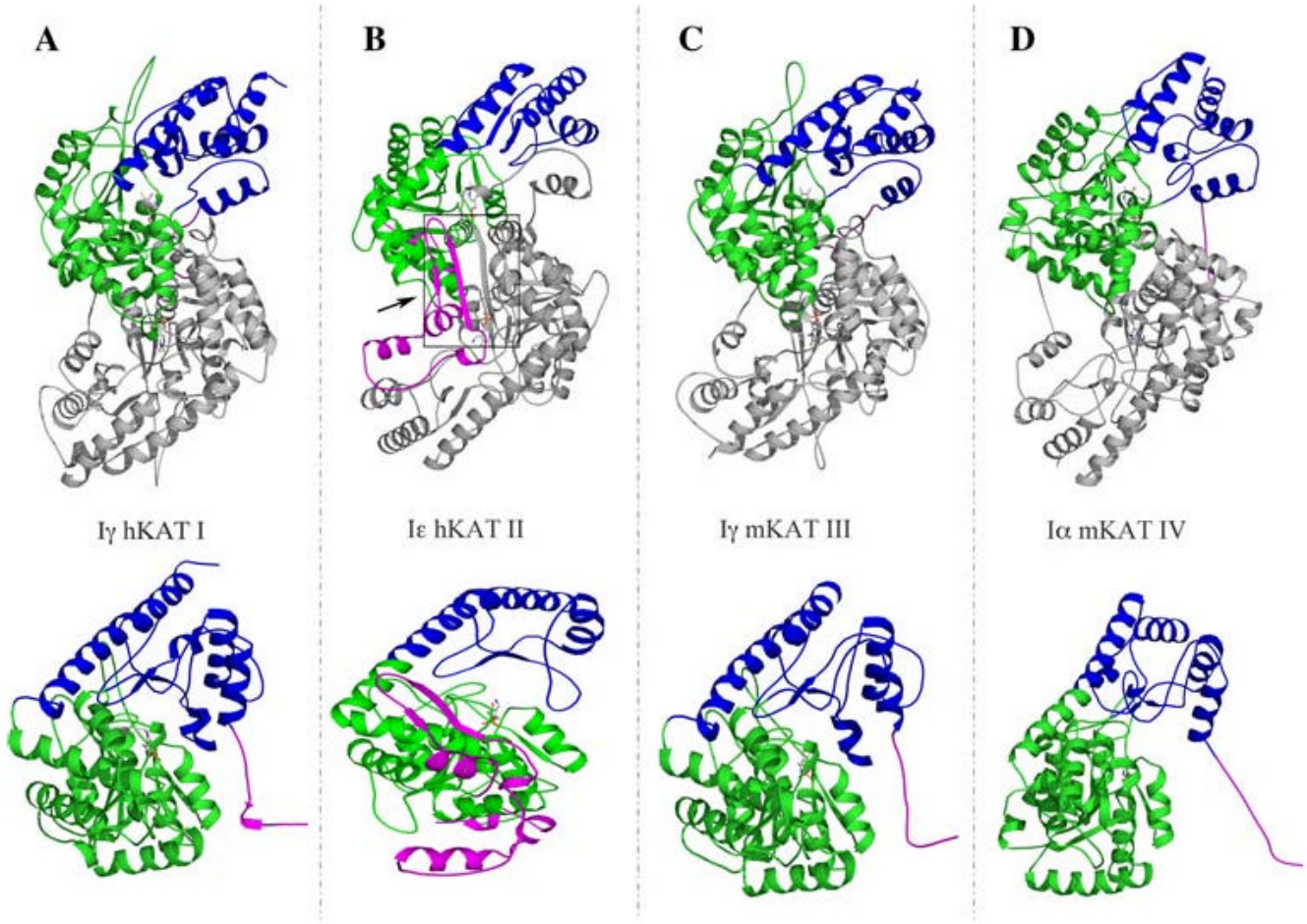

Fig. 5.

Structural organization of the functional dimer and overall structures of KATs. Functional dimers (*top*) and different domains of a monomer (*bottom*) of human KAT I (**a**), human KAT II (**b**), mouse KAT III (**c**), and mouse KAT IV (**d**). The two subunits are differently *colored* in each image. The small and large domains are depicted in *blue* and *green*, respectively; the N-terminal arms or N-terminal parts are in *pink*. The PLP cofactor is depicted with *sticks*. The *box* in B indicates the two two-stranded *β*-sheets that provide a major contribution to dimer stability in human KAT II. Figures were generated with PyMOL [137]

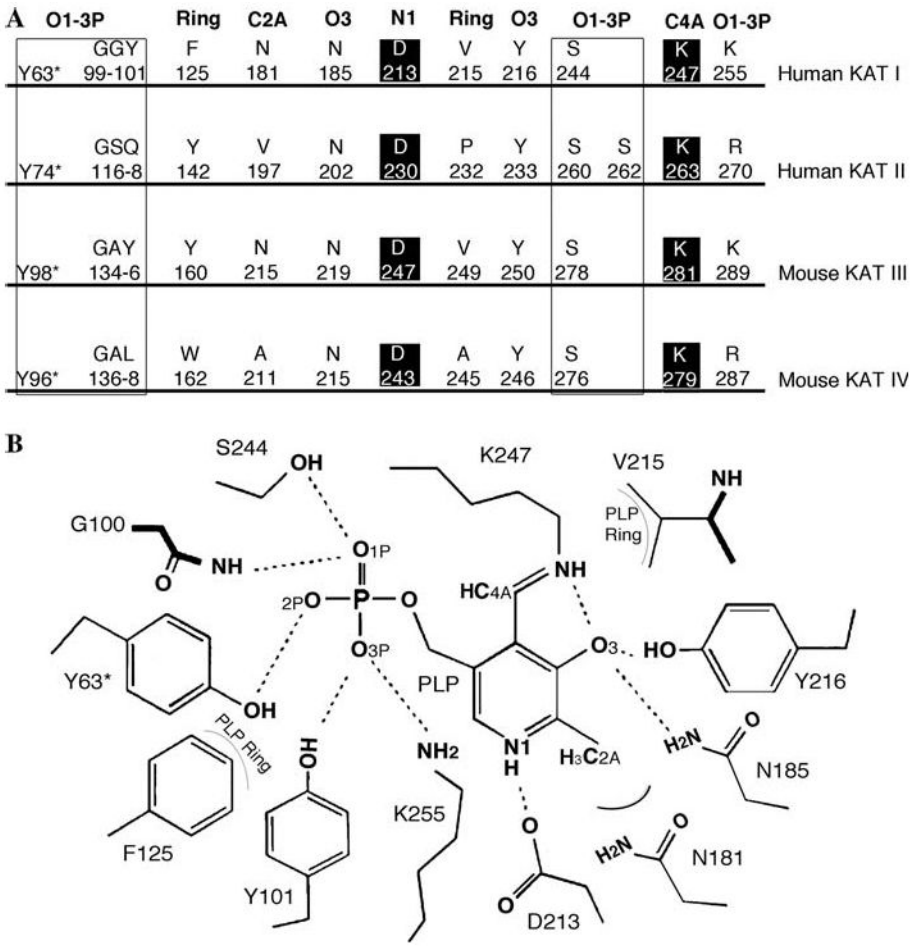

Fig. 6.

Comparison of the residues implicated in cofactor binding of four selected KATs. **a** The Lys residue which covalently binds PLP and Asp residue which binds PLP N1 atom are indicated with *white lettering on black background.* The PLP atoms interacting with the residues are given on the *top*. **b** Schematic diagram shows interactions of active site residues and PLP molecule in human KAT I structure, where the interacting atoms from PLP are *labeled*

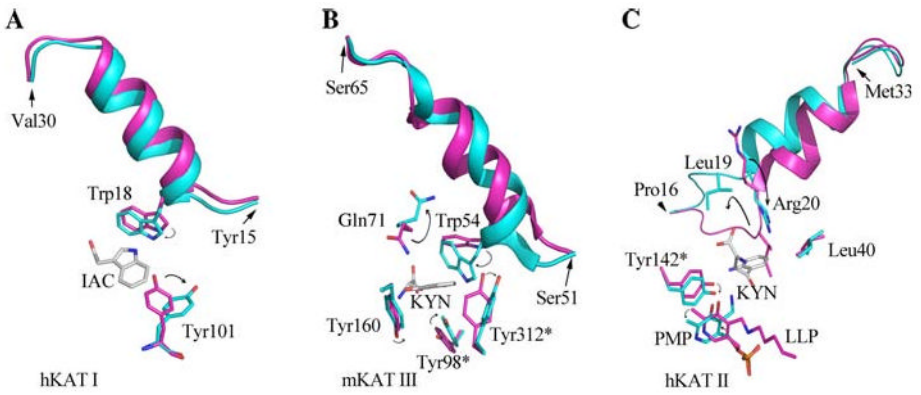

Fig. 7.

Conformational changes of KATs during catalysis. The structures of free (*magenta*) and ligand-bound (*cyan*) forms of each KAT structure are superposed upon each other. **a** Human KAT I, **b** human KAT II, **c** mouse KAT III. The N-terminal α-helices are shown as *ribbons* and the other residues that show significant side chain repositioning are depicted as *sticks*. Residues with an *asterisk* are from the secondary subunit in each functional dimer. The *arrows* show the direction of the movement the N-terminal α-helices and some crucial residues upon ligand binding

Table 1

Altered CNS (cerebrospinal fluid/brain tissue) KYNA levels in selected disease states

Disease state	KYNA	Reference
Alzheimer's	↓	[8,9]
Parkinson's	↓	[10]
Multiple sclerosis	↓	[11]
Huntington's	↓	[8,12–14]
Neonatal asphyxia	↓	[15,16]
Cerebral malaria	↑	[17]
HIV infection	↑	[8,18]
Down's syndrome	↑	[19]
Amyotrophic lateral sclerosis	↑	[20]
Schizophrenia	↑	[21–23]
Epilepsy	↑	[24]

Table 2

Gene information for encoded four human KATs

Gene name	Protein name	Amino acids	Chromosome locus	GenBank acc. no.	Cellular targeting	Reference
CCBL1	CCBL 1/GTK/ KAT I	422[a]	9q34.11	NP_004050[a]	Cyt[a]	[62]
		516		AK297995[c]	Mit[d]	
CCBL2	CCBL 2/KAT III	454[a]	1p22.2	NP_001008661[a]	Mit [a]	[34]
		420[b]		NP_001008662	Cyt [b]	
AADAT	AADAT/KAT II	425	4q33	NP_872603	Mit[d]	[63]
GOT2	GOT2/Mitochondrial ASAT/KAT IV	430	16q21	NP_002071	Mit	[64]

Mit Mitochondrion, *Cyt* cytoplasm

[a] Isoform a

[b] Isoform b

[c] Further confirmation of the sequence is needed

[d] Further confirmation is needed

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Table 3

Major biochemical differences of four KATs

	Optimal pH range	Recommended assay pH	Recommended co-substrate	Specific inhibitors of KAT activity	Unique substrates
KAT I	7.5–10	9–10	Glyoxylate	Tryptophan	
				3-indolepropionic acid	
				DL-indole-3-lactic acid	
KAT II	7–9	7–8	α-ketoglutarate	(*S*)-4-ethylsulfonylbenzoylalanine (rat KAT II)	Aminoadipate
KAT III	9–10 (mouse)	9–10 (mouse)	Glyoxylate	Methionine (mouse)	
KAT IV	7–9 (mouse)	7–8 (mouse)	α-ketoglutarate	Aspartate (mouse)	Aspartate (mouse)

Unless specified, the listed parameters are for human enzymes

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Table 4

Differences in active center residues and substrate specificity

	Substrate	Human KAT I	Mosquito KAT	Mouse KAT III
Different residues in active centers		Phe 125	Phe 135	Tyr 160
		Phe 278	Tyr 286	Tyr 312
Catalytic efficiency k_{cat}/K_m (min^{-1} mM^{-1})	Leucine	44.7	22	No activity
	Histidine	26.6	112	171.2