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Structural and mechanistic insights into the kynurenine aminotransferase-mediated excretion of kynurenic acid

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ABSTRACT

Kynurenine aminotransferase (KAT) is a homodimeric pyridoxal protein that mediates the catalytic conversion of kynurenine (KYN) to kynurenic acid (KYA), an endogenous N-methyl-D-aspartate (NMDA) receptor antagonist. KAT is involved in the biosynthesis of glutamic and aspartic acid, functions as a neurotransmitter for the NMDA receptor in mammals, and is regulated by allosteric mechanisms. Its importance in various diseases such as schizophrenia makes KAT a highly attractive drug target. Here, we present the crystal structure of the *Pyrococcus horikoshii* KAT (PhKAT) in complex with pyridoxamine phosphates (PMP), KYN, and KYA. Surprisingly, the PMP was bound to the LYS-269 of phKAT by forming a covalent hydrazine bond. This crystal structure clearly shows that an amino group of KYN was transaminated to PLP, which forms a Schiff's base with the LYS-269 of the KYN. Thus, our structure confirms that the PMPs represent an intermediate state during the KAT reaction. Thus, PhKAT catalyzes the sequential conversion of KYN to KYA via the formation of an intermediate 4-(2-aminophenyl)-2,4-dioxobutanoate (4AD), which is spontaneously converted to KYA in the absence of an amino group acceptor. Furthermore, we identified the two entry and exit sites of the PhKAT homodimer for KYN and KYA, respectively. The structural data on PhKAT presented in this manuscript contributes to further the understanding of transaminase enzyme reaction mechanisms.

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1. Introduction

Kynurenine–oxoglutarate transaminase (EC 2.6.1.7), which is also known as kynurenine (KYN) aminotransferase (KAT) (Tobes and Mason, 1977; Tomita et al., 2009), is a pyridoxal enzyme containing two covalently bound (Schiff base linkages) pyridoxal-5-phosphate (PLP) cofactor moieties and is an essential enzyme involved in kynurenic acid (KYA) biosynthesis. PLP, which is covalently bound to a lysine residue in KAT, accepts an amino acid from KYN resulting in the formation of pyridoxamine phosphate (PMP). Subsequently, PMP donates an amino group to the α-ketoglutaric acid (2OG) (Fig.S1), thus enabling the catalytic conversion of KYN to KYA in the mitochondria of mammals (Fig. 1 and B). This leads to the generation of L-glutamic acid (GLU) from the 2OG that originated from the Krebs cycle (Fig. 1B and S1). Recently, we reported that oxaloacetic acid (OXA) acts as an amino group acceptor in the *Pyrococcus horikoshii* KAT (PhKAT)-mediated synthesis of L-aspartic acid (ASP) (Fig.S1) (Okada et al., 2012). In humans, GLU and ASP function as neurotransmitters for the N-methyl-D-aspartate receptor (NMDAR) (Chen et al., 2005; Meldrum, 2000).

In the human brain, KAT is involved in the metabolism of L-tryptophan to KYA via a KYN catabolic intermediate (Han et al., 2010; Rossi et al., 2008; Stone and Darlington, 2002; Stone et al., 2012), and is therefore a key enzymatic target for the treatment of psychiatric diseases such as schizophrenia (Chen and Guillemin, 2009). KAT catalyzes the sequential biosynthesis of KYA via the formation of a 4-(2-aminophenyl)-2,4-dioxobutanoate (4AD) intermediate (Fig.S1). In the human brain, KYA acts as a natural antagonist for the glycine site of NMDAR (Schwarcz et al., 1992; Stone, 2001) and plays key roles in the glutamatergic neurotransmission system (Wu et al., 2010). Recently, Schwarcz et al. reported that the levels of KYA are elevated in the prefrontal cortex of individuals with schizophrenia (Sathyasaikumar et al., 2011). Thus, it is hypothesized to play a role in the pathogenesis of Alzheimer's disease (Baran et al., 1999; Gong et al., 2011; Hartai et al., 2007) and

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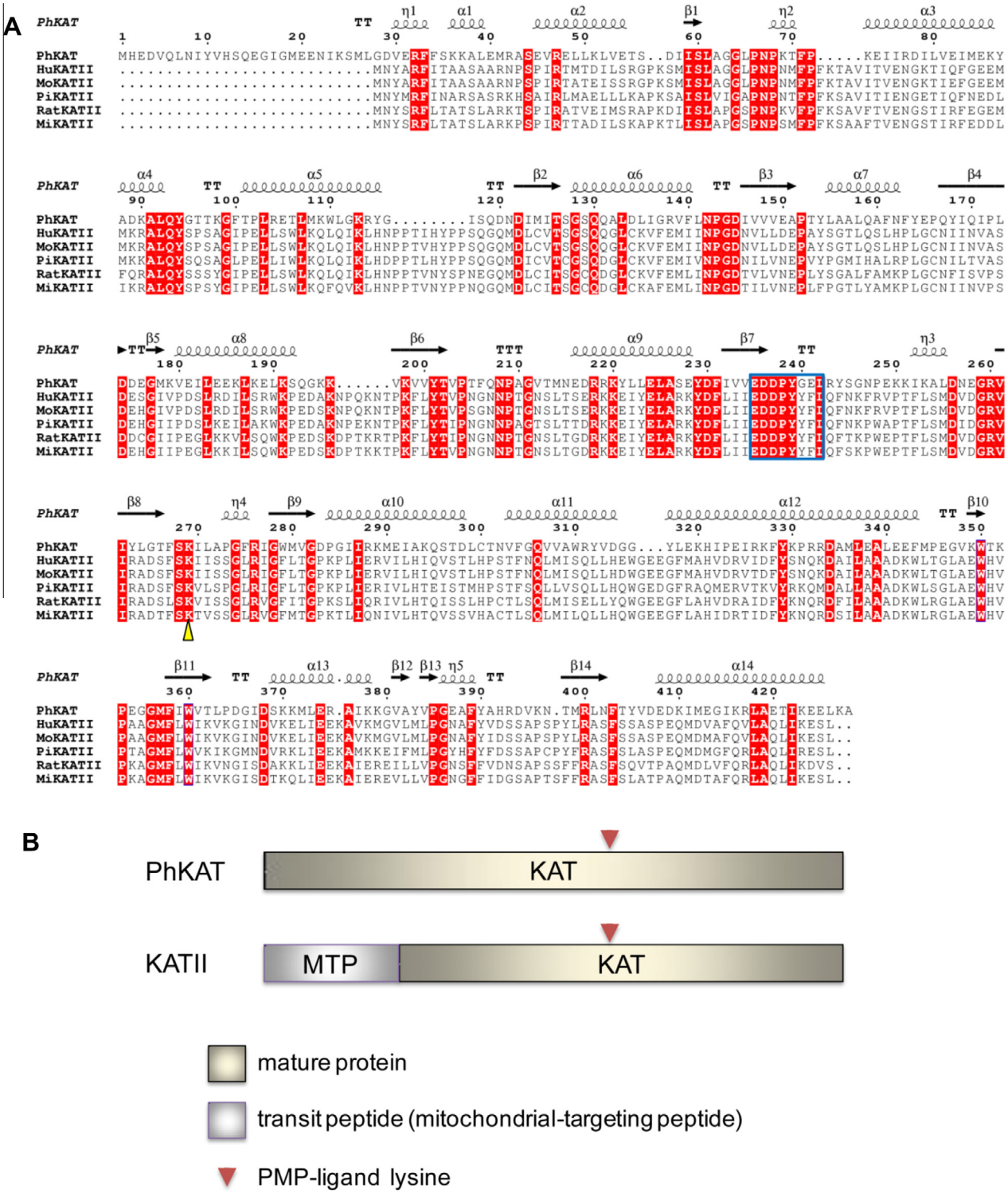

Fig.1. Comparative analysis of protein sequences of PhKAT and mammalian KAT IIs. (A) Protein sequence alignment of mammalian KAT IIs and *P. horikoshii* KAT. Ph, *P. horikoshii* OT3 (GenBank™ accession number NP_142204); Hu, *Homo sapiens* (GenBank™ accession number NP_057312); Mo, *Macaca mulatta* (GenBank™ accession number AFE66661); Pi, *Sus scrofa* (GenBank™ accession number XP_001924647); Rat, *Rattus norvegicus* (GenBank™ accession number NP_058889); and Mi, *Mus musculus* (GenBank™ accession number NP_035964). The alignment was performed usingClustalW2 and ESPript. Red highlights the identical amino acid residues. The blue box indicates the binding site of an allosteric effector of PhKAT. A yellow arrowhead indicates the lysine that binds the PMP ligand. (B) Comparison of *P. horikoshii* KAT and mammalian KAT IIs. The mammalian KAT II precursors have heterogeneous N-terminal extensions that resemble a mitochondrial targeting transit peptide. These two regions (transit peptide and mature KAT) are represented with differently colored boxes. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

schizophrenia (Erhardt et al., 2003). The above findings suggest a protective role for KYA in neurodegenerative disorders (Schwarcz et al., 2012).

The enzyme, phKAT isolated from the hyperthermophilic archaeon shares sequence homology with the mammalian KAT II enzymes (Han et al., 2008), including those from *Homo sapiens*

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