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Review

Aspartate aminotransferase: An old dog teaches new tricks

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ABSTRACT

Aspartate aminotransferase (AAT) is a prototypical pyridoxal 5′-phosphate (PLP) dependent enzyme that catalyzes the reversible interconversion of L-aspartate and α-ketoglutarate with oxalacetate and L-glutamate via a ping-pong catalytic cycle in which the pyridoxamine 5′-phosphate enzyme form is an intermediate. There is a bountiful literature on AAT that spans approximately 60 years, and much fundamental mechanistic information on PLP dependent reactions has been gained from its study. Here, we review our recent work on AAT, where we again used it as a test bed for fundamental concepts in PLP chemistry. First, we discuss the role that coenzyme protonation state plays in controlling reaction specificity, then ground state destabilization via hyperconjugation in the external aldimine intermediate is examined. The third topic is light enhancement of catalysis of Cα–H deprotonation by PLP in solution and in AAT, which occurs through a triplet state of the external aldimine intermediate. Lastly, we consider recent advances in our analyses of enzyme multiple sequence alignments for the purpose of predicting mutations that are required to interconvert structurally similar but catalytically distinct enzymes, and the application of our program JANUS to the conversion of AAT into tyrosine aminotransferase.

Introduction

Pyridoxal 5′-phosphate (PLP)[1] is the active form of vitamin B_6 (Fig. 1). It is required for a wide variety of reactions in amine and amino acid metabolism, and has been the subject of continuous mechanistic research since the 1930's [1–7]. The diversity of PLP dependent enzymes is well documented, and classification into evolutionary subfamilies has been well studied [8–17]. PLP dependent enzymes also present excellent drug targets [18].

For decades, aspartate aminotransferase (AAT) was the workhorse for understanding the mechanism of PLP dependent enzymatic catalysis, largely due to the ease of purifying it in large quantities from readily available sources such as chicken hearts and its stability. The molecular biology revolution over the past three decades has rapidly expanded the repertoire of PLP enzymes available in quantity and mechanistic work on AAT has slowed in recent years. Nevertheless, the rich literature on AAT makes it an ideal test bed for fundamental questions regarding PLP catalysis. It was the first PLP dependent enzyme to have its X-ray structure determined [19]. The structure allowed an insightful and detailed proposal for the catalytic mechanism that still holds today [7,20]. Currently, there are 150 structures of different AATs and mutants of (mainly) the *Escherichia coli* isozyme in the RCSB PDB, including structures of true reaction intermediates [21,22]. A majority of the mechanistic insight gained from studying AAT has been applicable to other PLP dependent enzymes, especially aminotransferases [12,17,23–35].

In recent years, we have again turned to AAT to test basic ideas in PLP and protein chemistry. This review summarizes our recent work on AAT focusing on: (1) the protonation state of PLP in the active site and how it influences reaction specificity and catalytic power, (2) the magnitude of ground state destabilization in the external aldimine intermediate, (3) the enhancement of the catalytic activity of free and AAT bound PLP by light, and (4) the interconversion of the substrate specificity of AAT and tyrosine aminotransferase (TAT) by bioinformatics methods we have developed.

AAT reaction mechanism

The overall reaction catalyzed by AAT is shown in Fig. 2A: L-Asp and α-ketoglutarate are reversibly interconverted to L-Glu and oxalacetate. The reversible transformation is accomplished via two half-reactions in a ping-pong kinetic mechanism (Fig. 2B). In the first, the PLP enzyme reacts with L-Asp to generate the pyridoxamine 5′-phosphate (PMP) enzyme and oxalacetate. The reverse of this half-reaction with α-ketoglutarate regenerates the PLP enzyme and gives the amino acid product, L-Glu, which is the common nitrogen currency for metabolism.

The accepted half-reaction mechanism of AAT is shown in Fig. 3, while the active site structure of AAT is shown in Fig. 4. This

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[1] *Abbreviations used:* AAT, aspartate aminotransferase; PLP, pyridoxal 5′-phosphate; TAT; tyrosine aminotransferase; PMP, pyridoxamine 5′-phosphate.

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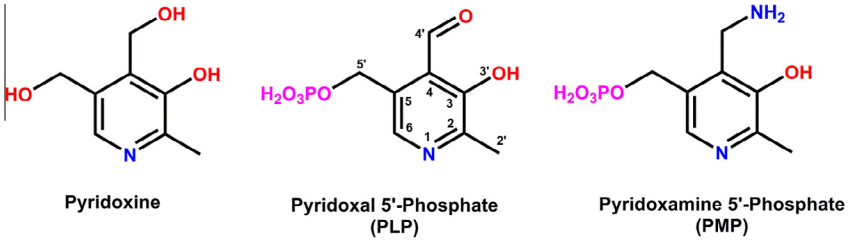

Fig. 1. Vitamin B_6 group. Pyridoxine is the most common form ingested as a nutritional supplement. PLP is the most common form of B_6 found in metabolism. PMP is an obligatory intermediate in the ping-pong mechanism of aminotransferases.

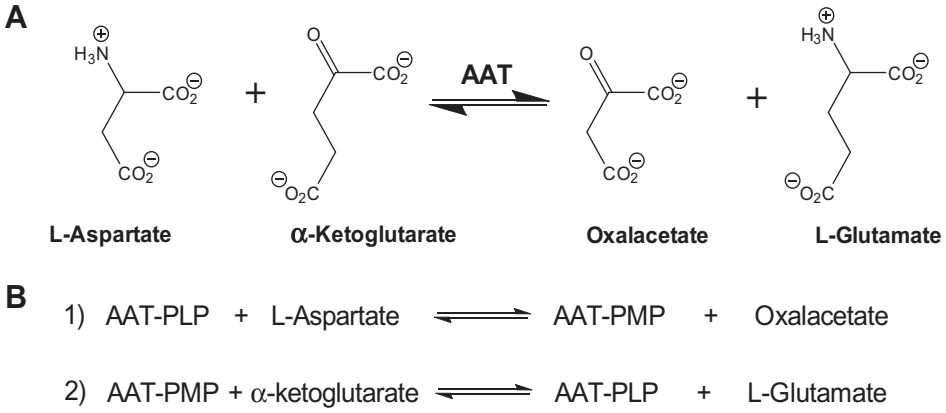

Fig. 2. (A) The overall reaction catalyzed by AAT. L-Aspartate and α-ketoglutarate are reversibly converted to oxalacetate and L-Glutamate. (B) The AAT catalyzed reaction is achieved through a ping-pong catalytic cycle in which L-Asparate first reacts with the PLP enzyme to form free oxalacetate and free PMP enzyme. The PMP enzyme then reacts with α-ketoglutarate to regenerate the PLP enzyme and produce L-glutamate.

detailed mechanism shows all required steps including those that are not kinetically significant. It allows one to appreciate the true complexity of a single half-reaction of the ping-pong catalytic cycle. Hayashi et al. have shown that there are two routes to the formation of a productive Michaelis complex [32]. In one, the protonated internal aldimine can bind unprotonated amino acid, and, in the other, the unprotonated internal aldimine can bind protonated amino acid. In either case, there is a single "extra" proton that can readily be transferred between the substrate α-amino group and the imine nitrogen of the internal aldimine. The reactive Michaelis complex has the proton on the imine nitrogen and the substrate amino group as the free base. This combination leads to rapid attack of the substrate amino group on the electrophilic C4′ of PLP to give the first geminal diamine intermediate. This step is facilitated by the fact that *transimination* (i.e., the conversion of one imine into another) is more facile than imine formation from an aldehyde and an amine, and strain that is imposed on the internal aldimine by the enzyme [24,36,37]. Forward progress requires the first geminal diamine to be converted to the second, which occurs via a proton transfer between the two geminal nitrogens. This step is probably facilitated by the 3′-oxygen of the coenzyme given its proximity to both nitrogens and the bidentate ionic interaction of the substrate α-carboxylate with Arg386. The second geminal diamine collapses into the external aldimine intermediate by displacing the free base of Lys258 as the leaving group. These steps leading to the external aldimine intermediate are all rapid and do not contribute to rate limitation under physiological conditions; kinetically, they are generally modeled as a single step because of this.

Forward reaction of the external aldimine intermediate occurs by deprotonation of the substrate Cα–H bond, which is considered to be chemically the most difficult step in the reaction when referenced to model reactions in solution [38–42]. The free base form of Lys258 acts as a general base catalyst for deprotonation, which has been shown experimentally by mutant enzymes and their rescue with exogenously added amine catalysts [43–45]. Although the central 1,3-proton transfer between Cα of the substrate and C4′ of the coenzyme could, in principle, occur via a concerted mechanism with Lys258 both deprotonating Cα and protonating C4′ in the same transition state, the evidence weighs in favor of a stepwise mechanism with an intervening carbanionic intermediate. This carbanionic intermediate has three major resonance contributors, which are shown in Fig. 3. The first two, with negative charge at Cα and C4′ are required for the obligatory protonation/deprotonation steps intrinsic to the reaction mechanism. The third resonance contributor has the electron pair from the Cα–H bond delocalized onto the pyridine nitrogen, and is called the quinonoid intermediate due to its structural similarity to *p*-quinones. This charge-neutralized resonance form has traditionally been accepted as the most important source of the catalytic power of PLP, and is certainly important to the mechanisms of PLP enzymes that *require a stabilized carbanionic intermediate* as a component of the reaction mechanism: AAT requires a stabilized carbanionic intermediate to achieve the 1,3-proton transfer. This concept is expanded to other mechanisms below. In AAT and many other enzymes, the pyridine nitrogen interacts with a side chain carboxylate, which presumably maintains the pyridine nitrogen protonated so the carbanionic intermediate is stabilized.

The carbanionic intermediate is readily protonated at both Cα and C4′, leading either to the original external aldimine or the ketimine intermediate; productive reaction occurs with C4′ protonation to give the ketimine intermediate. The ketimine reacts forward by Lys258 catalyzed addition of water to Cα to give the first carbinolamine intermediate. Proton transfer to the PMP