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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) is the active form of vitamin B_6 (Figure 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 *E. 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.

1. AAT Reaction Mechanism

The overall reaction catalyzed by AAT is shown in Figure 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 (Figure 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 Figure 3, while the active site structure of AAT is shown in Figure 4. This 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 Figure 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 nitrogen (bonded to C4′) leads to the second carbinolamine intermediate. Finally, deprotonation and collapse of the second carbinolamine leads to the E-PMP/oxalacetate Michaelis complex which dissociates to free enzyme and the first product. The reverse of this same sequence of steps with α-ketoglutarate as the α-keto acid substrate leads to L-Glu as the amino acid product. Remarkably, AAT facilitates the chemically difficult Cα-H deprotonation step so much that it is only partially rate-limiting for the half-reaction, with the chemically easier ketimine hydrolysis being partially rate-limiting.[46]

2. PLP Protonation State and Reaction Specificity

Schiff bases of pyridoxal and its derivatives can exist in several different protonation states that differ in their intrinsic reactivity toward carbanion formation.[39, 42, 47] Limbach and coworkers, in collaboration with our group, have used N-15 NMR of pyridine nitrogen-labeled PLP to understand the factors that control protonation states of free and AAT-bound PLP.[48–55] Initially, model studies were performed with 3,5-dibromosalicylaldehyde imines in solvents of varying polarity.[55] It was found that more polar solvents promoted the proton transfer from the phenolic oxygen to the imine nitrogen, as expected for formation of a dipolar ionic species from a neutral one. This is consistent with previous work in this area.[7, 56–60] Further experiments using N-15 NMR showed that protonation of the pyridine nitrogen promotes proton transfer from O3′ to the imine nitrogen when aldimines are formed from aliphatic amines but not from aromatic ones, and detailed the natures of the inter- and intramolecular O-H-N hydrogen bonds.[53, 54]

Using model studies as a guide, it was possible to determine the protonation state of the pyridine nitrogen of PLP bound to AAT.[52] In crystalline samples of both the unliganded and maleate-liganded enzyme forms at low pH, the pyridine nitrogen is fully protonated. The Asp222 oxygen - pyridine nitrogen distance is 2.63 Å in the unliganded crystals and somewhat shorter at 2.60 Å in the closed, maleate-liganded conformation, in agreement with X-ray structures.[61] From the absorption spectra of these enzyme forms it is also known that the imine nitrogen is protonated under these conditions. AAT dissolved in water is also fully protonated at the pyridine nitrogen, with an N-H distance of 1.07 Å and an Asp222 oxygen - pyridine nitrogen distance of 2.71 Å.

Thus, the coenzyme protonation states of the various intermediates shown in Figure 3 are correct, unless proton transfer between Asp222 and the pyridine nitrogen occurs in intermediates not probed by the NMR experiments. The stepwise 1,3-proton transfer interconverting the external aldimine and the ketimine requires pyridine nitrogen protonation to stabilize the carbanionic intermediate.[39, 42, 62–65] We expect that pyridine nitrogen protonation is a prerequisite for catalysis by all PLP enzymes that *require a stabilized carbanionic intermediate*. This includes PLP dependent aminotransferases, decarboxylases, enzymes catalyzing (retro)aldol and Claisen condensations, eliminases with poor leaving groups, etc.

An important corollary to consider is whether or not PLP enzymes that do not necessarily require a stabilized carbanionic intermediate with a significant lifetime form one or not. This was discussed in detail in a recent review.[66] In summary, it appears that enzymes that do not require a well stabilized carbanionic intermediate do not form one, since this intermediate is a branchpoint for unwanted side reactions.

The requirement for pyridine nitrogen protonation was tested experimentally by synthesizing 1-deazaPLP, in which the pyridine nitrogen is changed to carbon.[67] The coenzyme analogue was used to reconstitute apo-AAT. The internal aldimine as well as the external aldimine enzyme forms were crystallized and their X-ray structures determined (Figure 5).[66] Determination of the latter structure was possible because of the extremely low catalytic activity of AAT/deazaPLP with L-Asp (half-reaction $t_{1/2} > 20$ days). The binding interactions of deazaPLP in the AAT active site are extraordinarily similar to those of PLP itself, and the external aldimine structure shows Lys258 positioned directly over Cα, poised to act as a base for deprotonation.

The activity of AAT/deazaPLP was shown to be at least 10^9-fold less than the activity of AAT/PLP. Compared to the ~1000-fold decrease for alanine racemase and the 260-fold decrease for O-acetylserine sulfhydrylase, 10^9-fold is very large.[68] The fact that AAT suffers such a large decrease in activity from changing the pyridine nitrogen to carbon, while that for alanine racemase is only ~10^3-fold and that for O-acetylserine sulfhydrylase even less, strongly supports the proposal that not all PLP enzymes need to, or necessarily should, strongly stabilize the carbanionic intermediate by protonation of the pyridine nitrogen. These results correlate well with the nature of the interactions of the pyridine nitrogen with active site residues (Figure 6) and mechanistic evidence in the literature. For example, changing Asp222 in AAT to Ala leads to a 10^5-fold decrease in activity.[69] The difference between this result from mutagenesis and that with deazaPLP is presumably due to the fact that the D222A simply leads to deprotonation of the pyridine nitrogen, whereas deazaPLP fully exchanges the nitrogen for the less electronegative carbon atom. The results with the D222A mutant contrast with those of the analogous mutant in O-acetylserine sulfhydrylase where S272, which hydrogen bonds to the pyridine nitrogen, is mutated to alanine; this mutant reduces catalytic activity by only 3-fold.[70, 71] Results with deazaPLP also corroborate the conclusions on AAT from N-15 NMR.

3. Ground State Destabilization in Catalysis

There are two mechanistically distinct ways to reduce the activation energy for a chemical reaction. The first is the more familiar concept of transition state stabilization, while the second is that of ground state destabilization. In 1948, Linus Pauling first proposed that enzymes employ transition state stabilization.[72] The concept of ground state destabilization, on the other hand, has only recently been embraced. [73–77] In elegant work on AAT, Hayashi and coworkers have shown that ground state destabilization occurs in the free enzyme through strain imposed on the internal aldimine, which increases the value of

k_{cat}/K_M.[24] Additional strain is present in the Michaelis complex with amino acids induced by domain closure.

Our recent work on ground state destabilization is motivated by Dunathan's original hypothesis on PLP enzyme reaction specificity.[78] He proposed that PLP enzymes determine the bond to be broken in the external aldimine intermediate, leading to the carbanionic intermediate, simply by aligning that bond parallel to the *p* orbitals of the conjugated π system of the imine and pyridine ring. This allows greatest stabilization of developing negative charge at Cα as bond cleavage occurs, and additionally allows hyperconjugation of the aligned bond in the ground state. The latter effect would manifest itself as a weakening of the σ bond that is oriented parallel to the π system. This is illustrated in Figure 7a.

The effect of hyperconjugation on weakening the σ bond in the ground state can be observed, in principle, by binding isotope effects that report on external aldimine formation from free enzyme and free substrate. If hyperconjugation, and thereby weakening of the Cα-H bond, occurs in the external aldimine, then Cα-protiatated amino acid will preferentially bind to the enzyme over Cα-deuterated amino acid. In our work on another PLP enzyme, alanine racemase, we determined the free energy profiles for protiated and deuterated alanine.[79] External aldimine formation in alanine racemase indeed shows a deuterium binding isotope effect of 1.26 ± 0.07 and 1.27 ± 0.07 for the L→D and D→L directions, respectively, which are relatively large. This was interpreted as direct evidence for ground state weakening of the Cα-H bond from hyperconjugation with the imine and pyridine ring.

The results with alanine racemase led us to measure binding isotope effects with AAT. We expected larger binding isotope effects with AAT than alanine racemase since AAT fully protonates the pyridine nitrogen, whereas in alanine racemase the interaction of the pyridine nitrogen with Arg219 precludes its protonation.[80] Instead of extracting binding isotope effects from a larger analysis of the enzymatic reaction as was done with alanine racemase, we sought to isolate the binding step to enable direct measurement. This was possible with the K258A mutant of AAT, which has no general base to deprotonate Cα and therefore forms a very stable external aldimine intermediate.[43]

The procedure used for binding isotope effect measurements is outlined in Figure 8. K258A/PLP was reacted with a 50:50 mixture of protiated and deuterated L-Asp at varying stoichiometries with the enzyme. The equilibrated solutions were next concentrated in centrifugal ultrafiltration devices and the eluate containing free L-Asp was collected. The free L- Asp was analyzed by mass spectrometry to determine the change in the ratio of protiated to deuterated substrate, being repeated for each of the different L-Asp:K258A stoichiometries. The varying ratio of L-Asp:K258A corresponds to different fraction reactions for binding L-Asp and allowed us to plot the data to determine the binding isotope effect (Figure 8). Binding isotope effects were measured in a similar manner for K258A/deazaPLP.

The results presented in Figure 7b show that the binding isotope effect with K258A/PLP and L-Asp is 1.34 ± 0.05, which, as predicted, is significantly larger than that for alanine racemase (unpaired t-test P value = 0.0012 for n =15 data points). The hypothesis for K258A/deazaPLP was that the $>10^9$-fold reduction in catalytic activity of AAT with this coenzyme analogue would be reflected in the binding isotope effect. That is, the weaker electron withdrawing capacity of the deazaPLP imine compared to the PLP imine would be manifested as a smaller binding isotope effect with deazaPLP. The binding isotope effect of 1.15 ± 0.04 for K258A-deazaPLP fulfills this prediction, being smaller than that for either K258A-PLP, which has a protonated pyridine nitrogen, or alanine racemase (unpaired t-test

P value < 0.0001 for n =15 data points), which has an unprotonated pyridine nitrogen. The smaller binding isotope effect again reflects the lower electronegativity of carbon compared to nitrogen.

To interpret the binding isotope effects quantitatively, we performed *ab initio* calculations that were then used to calculate binding isotope effects.[75, 81] The Cα-H bond of a model 5′-deoxypyridoxal-Ala imine was incrementally lengthened and held fixed while the geometry of the remaining atoms was optimized. This series of calculated structures and their corresponding calculated binding isotope effects was used to construct the plot shown in Figure 7b. For this plot, the Cα-H bond lengths were converted to changes in Pauling bond order, which allows one to find by inspection the extent to which the Cα-H bond is broken in the ground state for a given observed binding isotope effect. For example, with K258A/PLP the observed binding isotope effect of 1.34 corresponds to ~20% loss of the original Cα-H bond order in the ground state. That is, the Cα-H bond is ~20% broken simply by it binding to the enzyme and forming the external aldimine intermediate. This extraordinary result shows that ground state destabilization through hyperconjugation in the external aldimine intermediate is a major factor in determining reaction specificity and catalytic power in AAT by moving the substrate very substantially from its structure in solution toward the transition state. We expect that similar conclusions hold for PLP enzymes as a group.

Binding isotope effects for PLP and amino acids in water have not been measured experimentally. It was calculated for a model of the external aldimine structure shown in Figure 3 (N1-protonated 5′-deoxypyridoxal Schiff base with alanine) in water solvent. The calculated binding isotope effect is 1.06 when the Cα-H bond is fixed perpendicular to the plane of the imine/pyridine ring. This value is likely to be lower when measured experimentally because of the freedom of rotation about the N-Cα bond and the angular dependence of hyperconjugation and thereby the binding isotope effect.

4. Light activation

Few enzymes actually require light for catalytic activity, but many enzymes require cofactors that absorb light in the UV and visible regions of the spectrum. Observations from the 1980s showing that light increases the rate of nonenzymatic PLP catalyzed decarboxylation, possibly increases enzymatic decarboxylation of DOPA, and promotes quinonoid formation in AAT sparked our interest in probing the enhancement of AAT activity by light.[82–84] Our first publication in this area examined changes in spectroscopic properties on the femtosecond to minute time scale of PLP in aqueous solution as well as Schiff bases with valine and aminoisobutyrate.[85] It was found that free PLP and the Schiff bases convert from an excited singlet state to a triplet state on the μs time scale and that the charge structure of the triplet state leads to rapid loss of a proton to form the carbanionic intermediate with valine.

AAT was next examined for light dependence of its kinetics.[86] When AAT is irradiated with 250 mW of 440 nm light, the value of k_{cat} is increased 2.3-fold (Figure 9). A photochemical mechanism similar to that for Schiff bases in water was found for AAT, wherein the excited singlet state undergoes intersystem crossing to a triplet state that is responsible for accelerated loss of the proton from Cα to give the carbanionic intermediate. The small enhancement of AAT catalytic activity by light (up to ~4-fold increase in k_{cat} at high power) is primarily due to the fact that deprotonation of the external aldimine intermediate is only partially rate limiting for k_{cat}.[46] Subsequent light-insenstive steps in the L-Asp half-reaction (ketimine hydrolysis and product dissociation) are partially rate-limiting as is the second half-reaction. In the K258A mutant where the catalytic base is

removed, deprotonation is fully rate-limiting, and 250 mW of 440 nm light increases the rate of L-Asp deprotonation 530-fold. Maximal rate enhancement of ~2000-fold occurs with K258A at high power. Finally, 250 mW of 440 nm light enhances the transamination reaction of the PLP-Asp imine in solution ~60,000-fold. This wide variation in enhancement by 440 nm light with imine reactivity is expected; the less reactive (lower energy) Schiff bases in water are enhanced to a greater extent by photoexcitation than the more reactive (higher energy) enzymatic species. Similar results are commonly found in organic chemistry (*e.g.*, in correlations of β values for base catalysis with carbon acid pK_a). It was estimated that the Cα-H bond in the triplet state is ~14 orders of magnitude more acidic than the ground state singlet in water.

The selective nature of the perturbation of AAT by light (*i.e.*, only the deprotonation step is affected) allowed us to extract the rate constant for the thermally activated deprotonation step from the power dependence of the value of k_{cat}. Thermal deprotonation was calculated to occur with a rate constant of 230 s^{-1}, while the sum of all subsequent steps (*i.e.*, ketimine hydrolysis, oxalacetate dissociation, the second half-reaction) have a net rate constant of 425 s^{-1}. These values agree with stopped-flow studies of the L-Asp half-reaction and other experiments.[87]

The power dependence of AAT catalytic enhancement allowed an additional important conclusion to be drawn, namely that the quinonoid intermediate is indeed on the productive reaction pathway for AAT. The existence of the quinonoid intermediate on AAT is clear from both equilibrium and transient absorption spectra, but it had been debated in the literature whether or not the interconversion of the external aldimine and ketimine intermediates occurred by a concerted proton transfer between Cα and C4′ or by stepwise one with an intervening carbanionic intermediate.[46] Simulations of the power dependence of the light enhancement of AAT allowed us to show definitively that the carbanionic quinonoid intermediate is on the thermal reaction pathway at a low steady-state concentration.

5. Interconversion of AAT and TAT

The rapid increase in the availability of amino acid sequences for enzymes in the last ~15 years has spurred an interest in being able to predict, from comparisons of amino acid conservation patterns, the positions in structurally homologous enzymes that determine reaction and substrate specificity differences.[88] Our interests in this area originated in attempts to convert GABA aminotransferase into a decarboxylase.[89, 90] Our frustrating failures based on manual analysis of multiple sequence alignments and chemist's intuition led us to devise a statistical method for extracting this information.[91] Over the course of developing the program (named JANUS) several other groups developed programs with similar goals, but ours has the following unique features: it employs a direct comparison of two enzymes (the starting point and target of conversion), it employs an amino acid distance (substitution) matrix based on a principle component analysis of 237 amino acid physicochemical properties, it accounts for both structural conservation and covariation, and it was experimentally validated using AAT-to-TAT as an example. The AAT-to-TAT conversion was chosen because of the excellent work from Kirsch's laboratory on the same conversion, which provides an important benchmark for comparison.[92–97]

The JANUS program is illustrated schematically in Figure 10. It uses sum-of-pairs statistics within and between families to calculate a priority list of mutations to make to convert the "start" enzyme into the 'target" enzymes. One must then make a subjective choice for the number of mutations to introduce into the start enzyme to initiate the conversion. With AAT, we chose to include the top 35 mutations to include in gene synthesis of the initial

hybrid. This enzyme, called J0, showed the expected substrate specificity with a K_M for phenylalanine of ~1 mM (wild type AAT does not saturate with L-Phe), but the k_{cat} value was low (0.3 s^{-1} compared to 160 s^{-1} for wild type AAT).

Careful consideration led us to conclude that most likely too many mutations from the JANUS priority list were included initially. Given that there was no way of estimating the correct number of mutations to include, we opted to perform gene shuffling with wild type AAT to remove a variable number of mutations and create a diverse library of mutants. The mutant library was used in a complementation selection in an *E. coli* strain that is missing TAT, and a large number of clones were found that allowed rapid growth under selection conditions. The six most active of these were expressed, purified, and characterized kinetically and structurally.

Back-crossing with wild type AAT removed approximately half of the original mutations and led to a diverse set of enzymes that had increased k_{cat} values (compared to J0) in common. The structural changes observed were small, with the greatest active site changes in the neighborhood of Trp140. This can be rationalized as opening up the substrate side-chain binding pocket to allow the larger aromatic side chain to bind in a relaxed manner, which at first blush would be a K_M effect. The origins of the increases in k_{cat} are less clear. Although they may be due to Trp140 relocation, it is enticing to speculate that some of the mutations outside the active site might play a role through protein dynamics.

JANUS has now been applied to two other enzymes in the author's laboratory successfully. We hope to improve its predictive abilities even further and explore whether different catalytic properties such as reaction and substrate specificity are orthogonal and can be "mixed and matched" more or less at will. The implications for such a tool in engineering humanized enzymes and industrial catalysts are broad.

Acknowledgments

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Highlights

- AAT is a source of important fundamental mechanistic information on PLP catalysis
- Protonation state of the external aldimine plays a major role in controlling reaction specificity
- Ground state destabilization due to hyperconjugation is important in AAT
- Transamination is enhanced by light absorption both in solution and on AAT

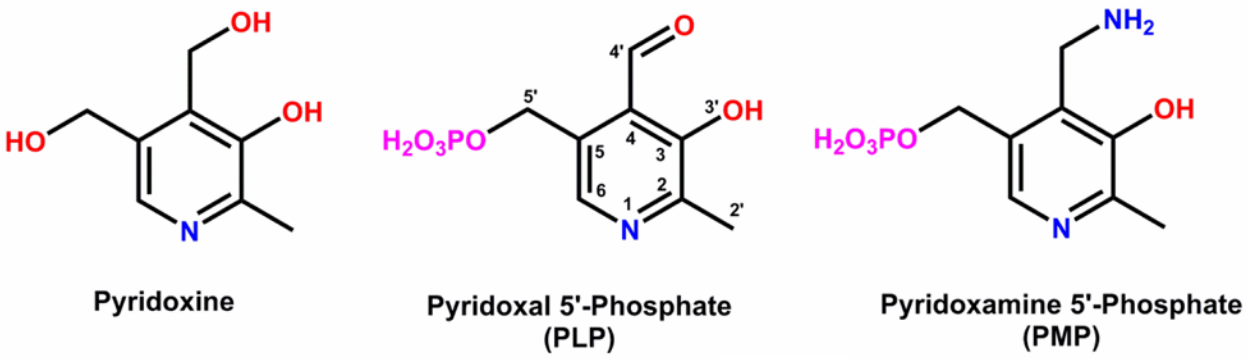

Figure 1.

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.

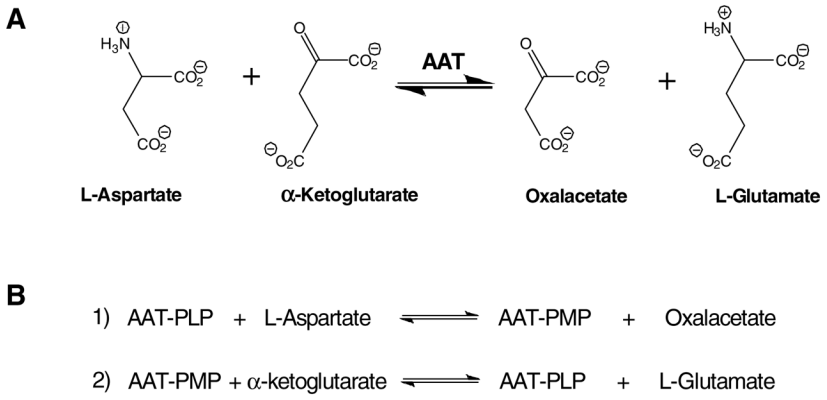

Figure 2.

(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.

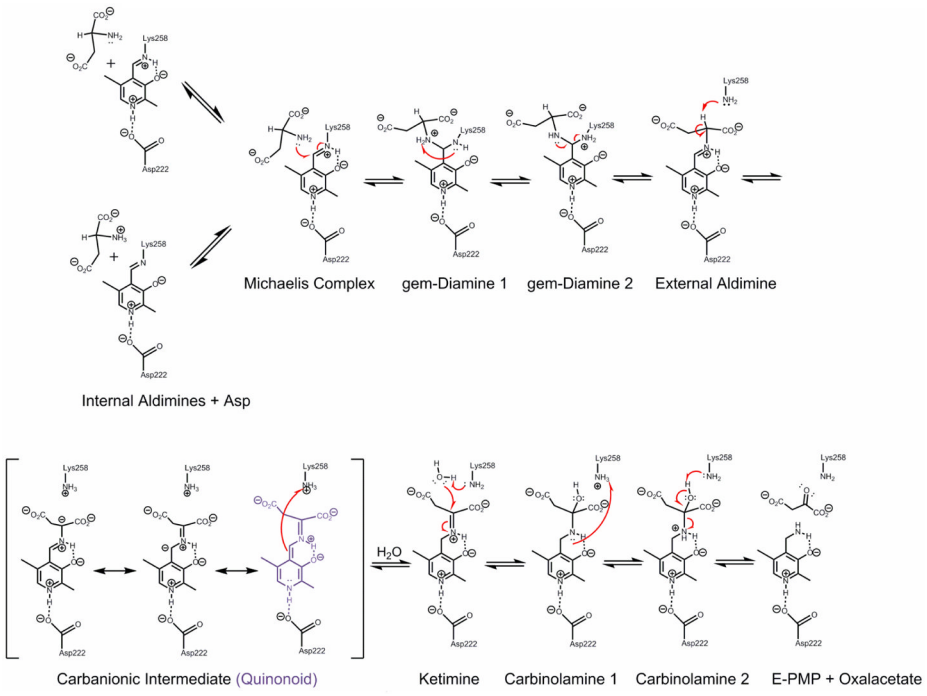

Figure 3.
Detailed mechanism of the L-Aspartate half-reaction of AAT. Electron flow is indicated with red arrows. The quinonoid resonance form of the carbanionic intermediate is shown in purple.

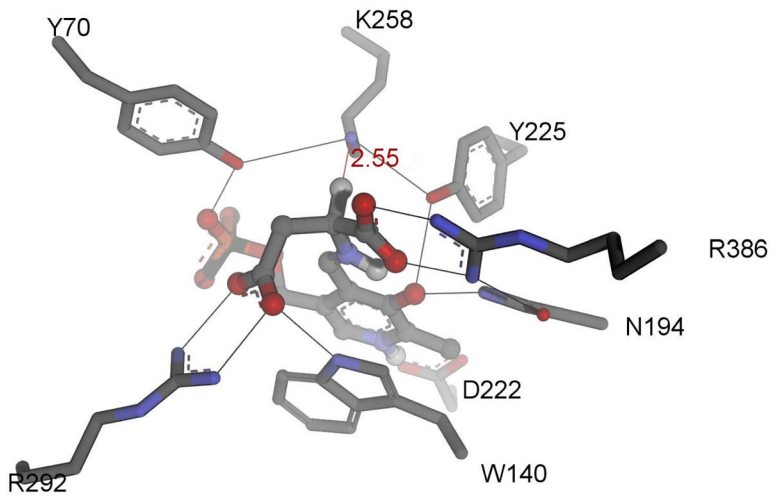

Figure 4.

Active site structure of AAT with hydrogen bonds show as thin solid lines. The external aldimine intermediate with L-Asp was modeled based on the structure of the deazaPLP external aldimine shown in Figure 5 by changing the ring to a protonated pyridine and performing simulated annealing energy minimization using the AMBER03 forcefield in YASARA.

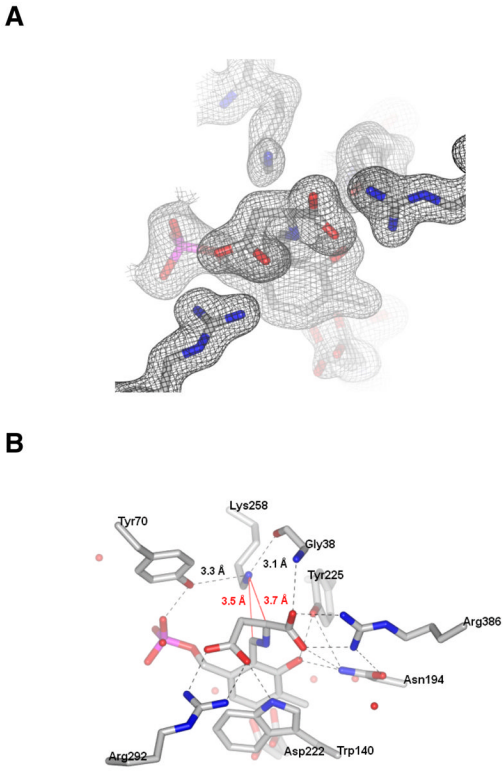

Figure 5.
(A) Active site electron density map for the AAT/deazaPLP/L-Asp structure. (B) Active site structure for AAT/deazaPLP/L-Asp showing hydrogen bonds a dashed lines. The distances from the ε-amino group of Lys258 to Cα and C4′ are marked.

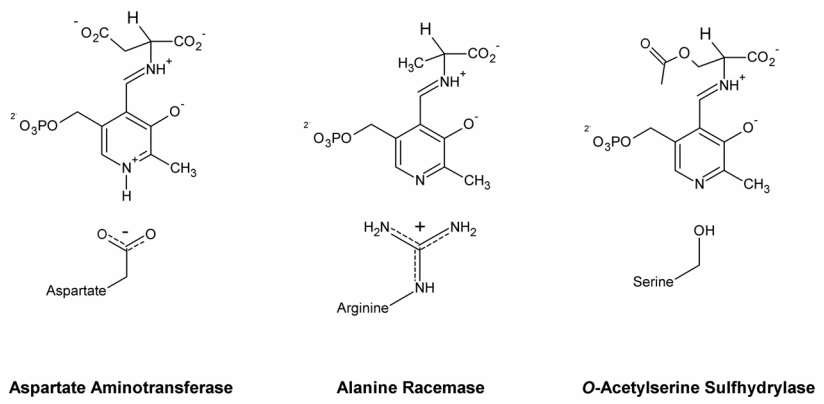

Figure 6.
Interactions between the pyridine nitrogen of PLP and three different mechanistic and structural classes of PLP enzymes.

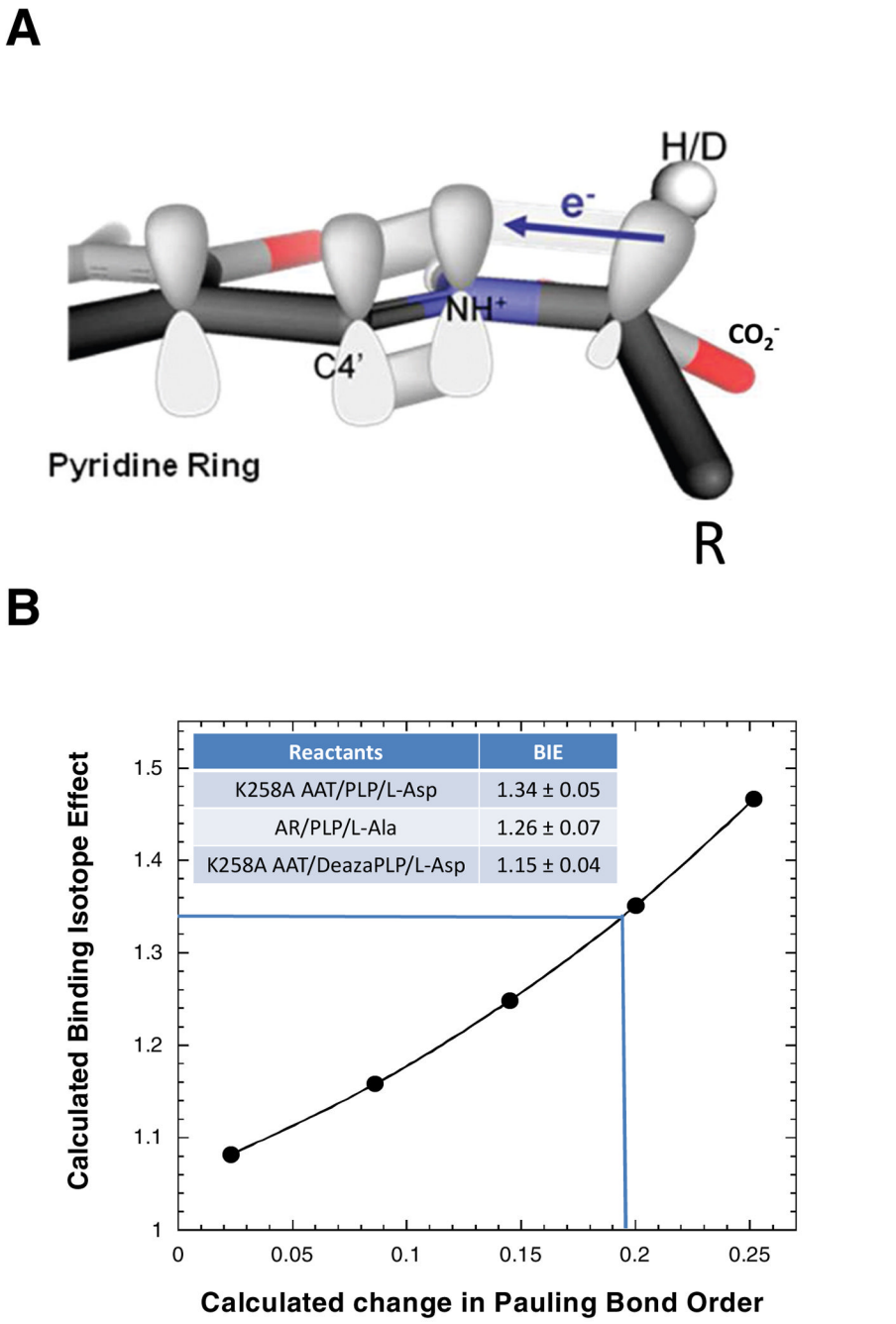

Figure 7.

(A) Illustration of hyperconjugation between the Cα-H bond and the conjugated π system in the external aldimine intermediate, which leads to bond weakening and normal binding isotope effects. (B) Plot of calculated binding isotope effect vs. calculated change in Pauling bond order used to interpret quantitatively the measured binding isotope effects shown in the inset.

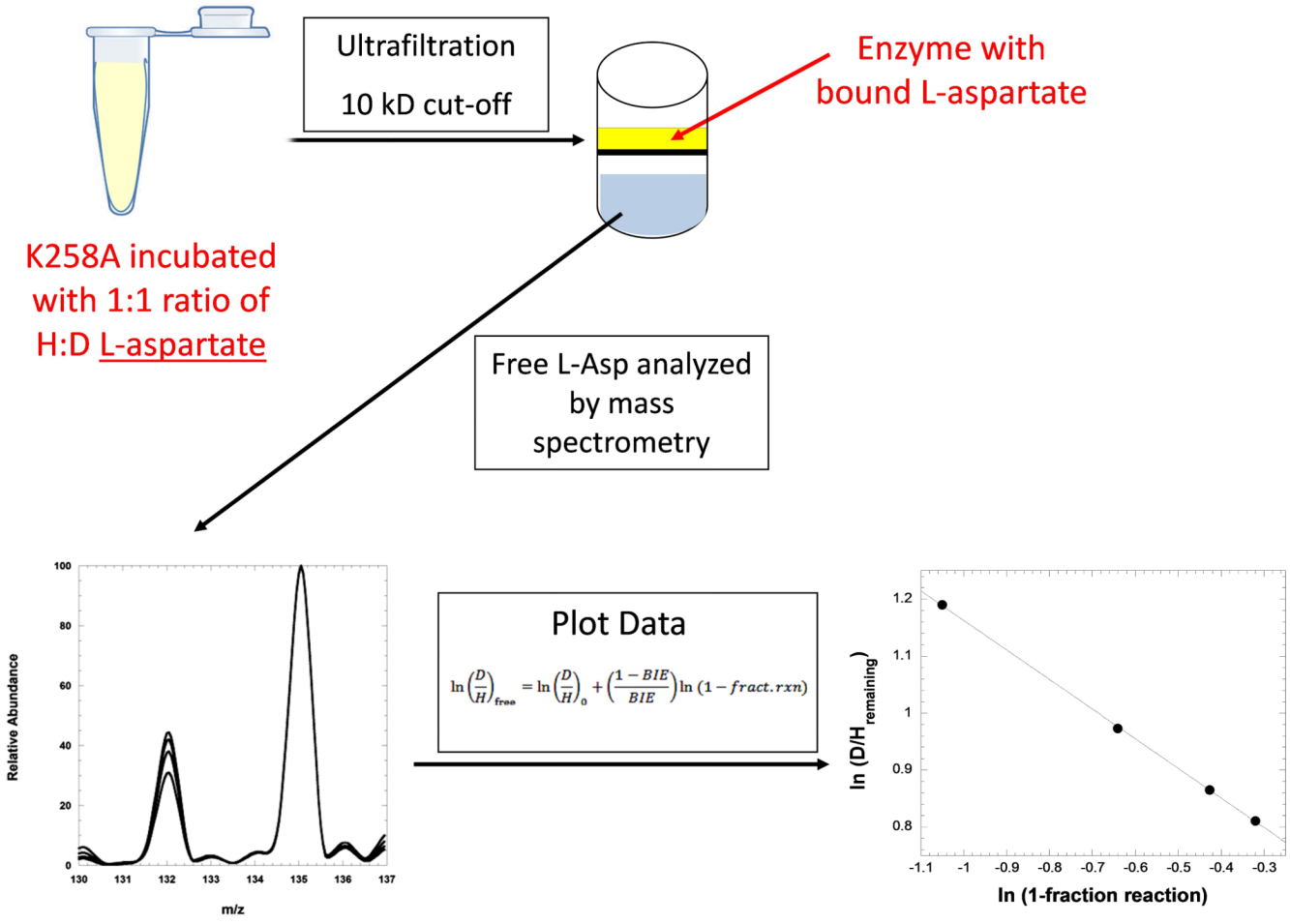

Figure 8.
Illustration of the procedure used to measure binding isotope effects with the K258A mutant of AAT.

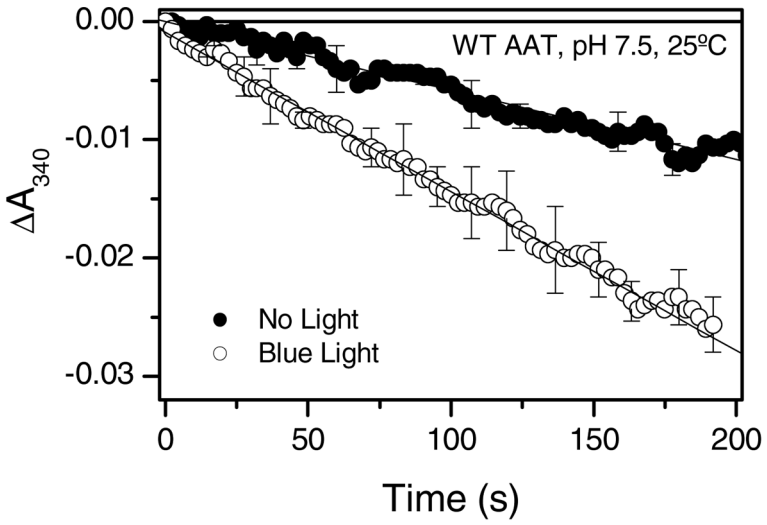

Figure 9.

Enhancement of k_{cat} for AAT by photoexcitation with 250 mW of 440 nm light. In the presence of 250 mW of blue light, the value of k_{cat} is increased 2.3-fold. Saturating concentrations of L-Asp and α-ketoglutarate were used. The figure shows the kinetics of NADH oxidation in the MDH coupled assay.

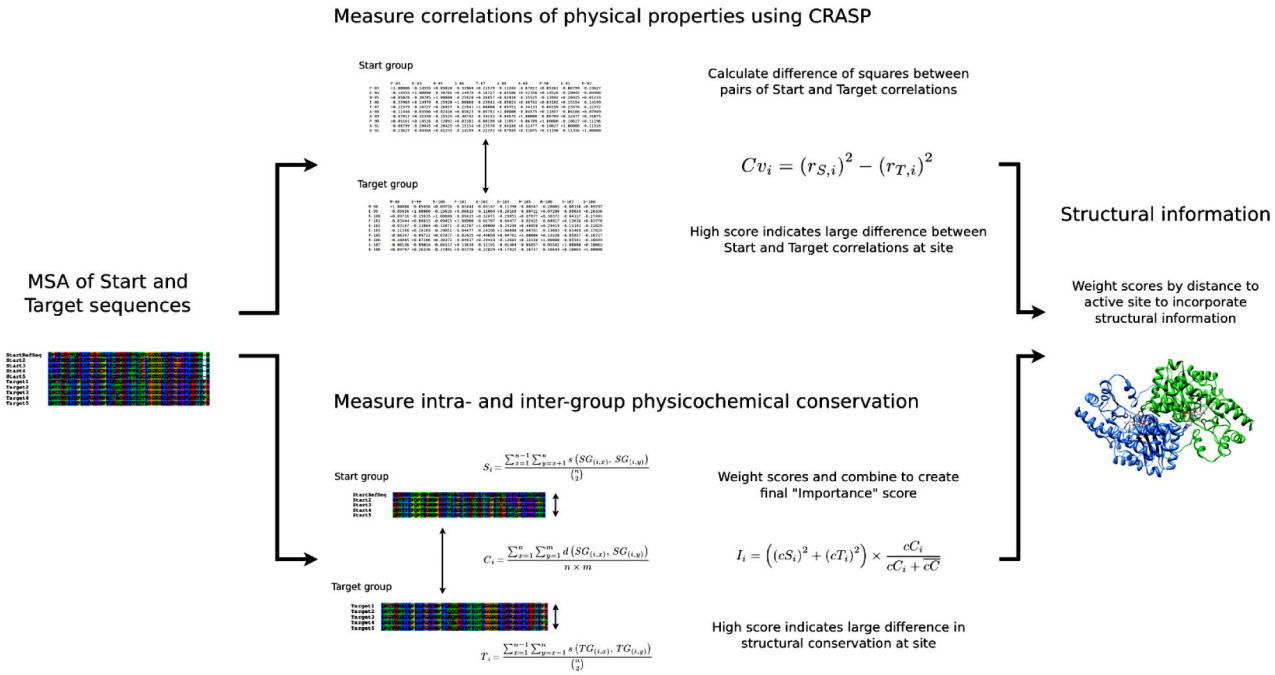

Figure 10.
Schematic illustration of how the JANUS program calculates a priority list of mutations to make to convert one enzyme into another structurally homologous one.