A subfamily of PLP-dependent enzymes specialized in handling terminal amines

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Abbreviations: PLP, pyridoxal 5'-phosphate; PMP, pyridoxamine 5'-phosphate; α-KG, α-ketoglutarate; GABA, γ-aminobutyrate; AT, aminotransferase; AT-I, Subgroup-I aminotransferases; AT-II, Subgroup-II aminotransferases (also denominated class-III aminotransferases, e.g. in the Pfam database); AspAT, Aspartate aminotransferase; AroAT, Aromatic aminotransferase ; ω-AT, ω-aminotransferase. Other abbreviations for specific aminotransferases are given in Table 1.

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ABSTRACT

The present review focuses on a subfamily of pyridoxal phosphate (PLP)-dependent enzymes, belonging to the broader fold-type I structural group and whose archetypes can be considered ornithine δ-transaminase and γ-aminobutyrate transaminase. These proteins were originally christened “subgroup-II aminotransferases” (AT-II) but are very often referred to as “class-III aminotransferases”. As names suggest, the subgroup includes mainly transaminases, with just a few interesting exceptions. However, at variance with most other PLP-dependent enzymes, catalysts in this subfamily seem specialized at utilizing substrates whose amino function is not adjacent to a carboxylate group.

AT-II enzymes are widespread in biology and play mostly catabolic roles. Furthermore, today several transaminases in this group are being used as bioorganic tools for the asymmetric synthesis of chiral amines. We present an overview of the biochemical and structural features of these enzymes, illustrating how they are distinctive and how they compare with those of the other fold-type I enzymes.

1. *Introduction*

Pyridoxal phosphate (PLP) arguably represents the most versatile organic cofactor in biology, being used by a variety of enzymes in all organisms [1-5]. PLP-dependent enzymes almost invariably catalyze reactions that involve amino compounds, since PLP can bind covalently to the amino group of the substrate and then act as an electrophile to stabilize different carbanionic intermediates [1, 4, 5]. Building on this shared mechanistic feature, PLP-dependent enzymes comprise, among others, transaminases, decarboxylases, racemases, aldolases, lyases, and enzymes that catalyze β- or γ-replacement reactions [1-5]. In spite of this functional diversity, all PLP-dependent enzymes structurally characterized to date can be grouped in just seven distinct structural families, or 'fold-types' [6-9], presumably corresponding to different evolutionary lineages. Of these, the so-called fold-type I is the most populated, functionally and structurally diverse and arguably the most evolutionarily ancient.

We focus herein on a subgroup of PLP-dependent enzymes belonging to the broader fold-type I family. Mehta, Christen and co-workers reported for the first time in 1993 a classification of aminotransferases (ATs) in different subgroups, based on an algorithm that took into account sequence comparison, hydropathy patterns and secondary structure features of 51 ATs [2, 10, 11]. One of four identified subgroups included as prototypical enzymes ornithine δ-transaminase and γ-aminobutyrate (GABA) transaminase. Mehta, Christen and co-workers termed these enzymes "subgroup-II aminotransferases" (AT-II; [2, 10-12]).

An updated list of AT-II enzymes is provided in Table 1. As suggested by the name, most enzymes in this subfamily are indeed aminotransferases, using either α-ketoglutarate (α-KG) or pyruvate as the preferred amino group acceptor. The amino group donors instead are generally compounds where the amino group is located distal to a carboxylate and usually at the end of an alkyl chain. These compounds are conventionally called ω-

amines [13, 14], and their transamination leads to production of an aldehyde (or less frequently a ketone) as shown in Eq. 1.

$$\omega\text{-amine} + \alpha\text{-ketoacid} \longleftrightarrow \text{aldehyde (ketone)} + \alpha\text{–amino acid}$$

Eq. 1

A great amount of biochemical, physiological, structural and applicative information has been accumulating on these enzymes, especially in recent years [15], due to several reasons. First, some enzymes in this subgroup are being explored as potential drug targets [16]. Second, it has been shown that, in spite of the name, AT-II also includes enzymes that are not aminotransferases (Table 1). Last but most important, today several transaminases belonging to AT-II are being used as bioorganic tools in the industrial synthesis of chiral amines [15, 17, 18].

In relation to this, we examine the literature and analyze sequences and three-dimensional structures trying to address some general points: What are the peculiar features of the reactions catalyzed by AT-II enzymes? What differentiates them structurally from the other enzymes of the fold type I? Are there structural properties that make the enzymes in this subfamily particularly apt at handling terminal amines? What features contribute to their substrate and reaction specificity? Our analysis also offers an overview of the evolution of these proteins, integrating it with the broader issue of the evolution of fold-type I enzymes.

1.1 - Nomenclature issues

To describe the group of enzymes in table 1, we will follow here the original designation of AT-II given by Mehta, Christen and co-workers [2, 10, 11]. However it is essential to note that just a couple of years after the first study by Mehta et al. [10], Grishin and coworkers renamed the very same subfamily of enzymes ‘class III aminotransferases’

[6]. The change arose due to those authors splitting subgroup I (identified by Mehta et al.) into two smaller subfamilies. While this operation was based on reasonable structural considerations, Grishin and coworkers quite arbitrarily proceeded to rename the new subfamilies “Class I” and “Class II” (rather than, for example, Ia and Ib), shifting up the roman numbering for all other subgroups [6].

The Grishin et al. classification has been adopted by some databases such as PROSITE [19]. Pfam [20] reunites the classes I and II (as defined by Grishin et al.) into a single family, but instead of reverting to the original Mehta et al nomenclature calls this family “Aminotransferases Class I and II”, while it retains the definition “Class III aminotransferases” for the enzymes in Table 1 (http://pfam.sanger.ac.uk/family?entry=PF00202) [13].

Often the aminotransferases of the AT-II group are also referred to as ω-transaminases (ω-ATs; [17, 18, 21-23]). Indeed the physiological reactions of these enzymes almost invariably involve a terminal amino group or at least (e.g., in the cases of ABS or FUMAT) an amino group not located in α-position with respect to a carboxylate (Table 1); compounds containing such groups can be broadly described as ω-amines [13] [1] [14, 24, 25]. However the reader should be aware that while the AT-II (or “Class III AT”

[1] The expression “amine transaminases” is also used when describing ATs employed for bio-organic synthetic applications [14] F. Steffen-Munsberg, C. Vickers, H. Kohls, H. Land, H. Mallin, A. Nobili, L. Skalden, T. van den Bergh, H.J. Joosten, P. Berglund, M. Hohne, U.T. Bornscheuer, Bioinformatic analysis of a PLP-dependent enzyme superfamily suitable for biocatalytic applications, Biotechnol Adv (2015). , [24] F. Steffen-Munsberg, C. Vickers, A. Thontowi, S. Schätzle, T. Meinhardt, M. Svedendahl Humble, H. Land, P. Berglund, U.T. Bornscheuer, M. Höhne, Revealing the structural basis of promiscuous amine transaminase activity, ChemCatChem 5 (2013) 154-157. , [25] H. Mallin, M. Höhne, U.T. Bornscheuer, Immobilization of (R)- and (S)-amine transaminases on chitosan support and their application for amine synthesis using isopropylamine as donor, J. Biotechnol. 191 (2014) 32-37. . In the field, this tautological expression is used in a restricted sense, to indicate enzymes acting on amines that are neither terminal nor adjacent to a carboxylate. These amines are normally chiral and ATs can produce specifically the *(S)*- or *(R)*- stereoisomer. AT-II enzymes employed for bioorganic applications are usually *(S)*-amine transaminases.

according to Grishin and coworkers) classification is based on sequence and structure criteria, the definition ω-ATs is functional, so the two denominations do not really overlap.

In particular, as noted, there are some AT-II members that are not aminotransferases at all (Table 1). On the other hand, at variance with the statements found in some authors (e.g., [18]), AT-II is not the only subgroup of PLP-dependent enzymes containing ATs that act on ω-amino-compounds. For example 2-aminoethylphosphonate-pyruvate AT (belonging to fold-type I, but not to AT-II; [26]) acts on a substrate that definitely qualifies as an ω-amine. Several ω-transaminases have also been identified belonging to fold-type IV; these enzymes are being used (much like some belonging to the AT-II subgroup; see section below) for both the asymmetric synthesis and resolution of chiral amines [18, 27].

2- Chemical peculiarities of the reactions catalyzed by AT-II enzymes

2.1- Equilibria in ω--ATs reactions

Aminotransferase reactions involving standard amino acids (as donors of their α-amino group) and α-keto acids are generally reversible and their equilibrium constant is often not far from unity [28]. AT-II enzymes are quite peculiar not just because one of the substrates is an amine, but also because one of the two amino group acceptors involved in the equilibrium is not an α-keto acid but an aldehyde. Given the high reactivity of aldehydes, one might expect the reaction in Eq. 1 to proceed more favorably in the direction of the amine, but under physiological conditions this is not the case.

In fact, while most of the transaminase reactions catalyzed by the AT-II enzymes are reversible *in vitro,* in cells they are nearly always driven towards consumption of the amine. The factors contributing to this behavior do not seem attributable to the transaminases themselves, but rather to the occurrence of spontaneous or tightly coupled

enzymatic reactions that follow formation of the aldehyde and determine an almost unidirectional metabolic flux.

For example, the products of lysine AT (α-aminoadipate-γ-semialdehyde) and of putrescine AT (4-aminobutanal), spontaneously convert to cyclic compounds (Δ1-piperidine-6-carboxylate and 1-pyrroline, respectively) through formation of an intramolecular Schiff base [29], and this is expected to drive the reaction in Eq. 1 to the right. *In vivo,* the reactions catalyzed by these enzymes are strongly biased in favor of degradation of the ω-amino substrate [30, 31].

Likewise, the reaction of taurine-pyruvate aminotransferase is reversible *in vitro* but in bacteria it only proceeds towards the consumption of taurine and the production of sulfoacetaldehyde [32]. In this case the phenomenon can be attributed to the presence of an acetyltransferase, which metabolizes sulfoacetaldehyde to acetyl phosphate and sulfite [33, 34].

An analogous discrepancy between the *in vitro* and *in vivo* situation is observed in the case of the reaction catalyzed by mammalian GABA-AT. The equilibrium of this reaction apparently favors the formation of GABA *in vitro* [35], but *in vivo* GABA-AT serves primarily to the degradation of the amine (e.g., [36]), presumably because the product of GABA transamination, the reactive succinic semialdehyde, is detoxified by succinic semialdehyde dehydrogenase in a tightly coupled reaction [37].

In plants, GABA-PAT transaminates GABA using pyruvate or glyoxylate instead of α-KG as an amino group acceptor. The reaction seems to proceed unidirectionally towards GABA degradation [38]. In addition to the presence of succinic semialdehyde dehydrogenase in plants [39], this is probably due to the fact that the reaction catalyzed by GABA-PAT with glyoxylate is completely irreversible [38]. An analogous near-irreversibility is observed also in other PLP-dependent transaminations where glyoxylate serves as the amino group acceptor [40-44] as well as in the transamination between free pyridoxamine

and glyoxylate [45]. Such a strong bias presumably reflects the high reactivity and relative thermodynamic instability of glyoxylate, which not only is an aldehyde, but is also subject to the electron-withdrawing effect of the nearby carboxylic group.

A rare case of ω-AT reaction that appears to be effectively reversible both *in vitro* and *in vivo* is represented by the ornithine aminotransferases (OAT) reaction. In this instance the spontaneous and reversible cyclization of the product glutamate-γ-semialdehyde, to Δ1-pyrroline-5-carboxylate, does not seem to prevent the reverse process, at least in mammals [46, 47]. In plants, however, OAT appears to play only a catabolic role [48].

The striking predominance of metabolic reactions in which the amine is consumed and the aldehyde is formed may have a biological, as well as chemical, significance. Due to their substantial reactivity, it is important that aldehydes do not accumulate during metabolism [49]. Aminotransferase reactions, on the other hand, are intrinsically rather slow processes, owing to their complex ping-pong mechanism. Accordingly it may be speculated that, if the main task of ω-ATs was to consume (rather than to produce) aldehydes, buildup of these compounds might be relatively facile and represent a liability for the organism.

In any instance, understanding the factors that modulate the equilibria and fluxes of ω-AT reactions has a very concrete and practical interest, as these transaminases are being used as bioorganic tools for the enantiopure synthesis of chiral amines. In such syntheses, the amines are produced from the corresponding ketones, exploiting the reaction of eq. 1 in the reverse direction. The enantiopure synthesis of chiral amines is highly desirable for the preparation of many compounds of pharmaceutical interest [15, 17, 18] [50].

In the asymmetric synthesis of chiral amino compounds, in order to obtain a good yield of the desired product, it is necessary to define and, often, to change the chemical

equilibrium of the reaction. As noted above, the amino acceptor in this case is not an aldehyde but a ketone and it is commonly accepted that transaminations of ketones are thermodynamically unfavourable [51-55]. Ketones have in fact a relatively low electrophilicity of the carbonyl carbon, as compared with α-keto acids and aldehydes, and are more stable than the corresponding amines. To overcome these problems, in the bioorganic applications of ω-ATs, the equilibrium is usually shifted from the side of substrate ketone to the desired product amine by removing the co-product through chemical or enzymatic means. In particular, if the co-product is pyruvate, it can be reduced to lactate by lactate dehydrogenase. [2] [25, 52, 56]

2.2 - Specificity and dual-specificity issues.

PLP-dependent enzymes acting on α-amino acids can bind their substrates by interacting with two parts of the substrate molecule, in addition to the amino group -namely the α-carboxylate and the side chain. As a first approximation, since all amino acids contain an α-carboxylic group, interactions with this group will only contribute to binding affinity, while interactions with the side chain will confer specificity. In contrast, enzymes acting on terminal amines must seek both binding affinity and specificity *via* interactions with the side chain of the substrate.

[2] This system has the disadvantage of requiring a continuous regeneration of NADH, which is usually accomplished by adding to the system a NADH-recycling enzyme such as glucose dehydrogenase. Nevertheless it is difficult to obtain the best activity and stability for all the three elements of this enzymatic system. To overcome this hurdle, a few alternative approaches are reported in literature, such as the use isopropylamine as the amino-donor [25] H. Mallin, M. Höhne, U.T. Bornscheuer, Immobilization of (R)- and (S)-amine transaminases on chitosan support and their application for amine synthesis using isopropylamine as donor, J. Biotechnol. 191 (2014) 32-37. , [56] C.K. Savile, J.M. Janey, E.C. Mundorff, J.C. Moore, S. Tam, W.R. Jarvis, J.C. Colbeck, A. Krebber, F.J. Fleitz, J. Brands, P.N. Devine, G.W. Huisman, G.J. Hughes, Biocatalytic asymmetric synthesis of chiral amines from ketones applied to sitagliptin manufacture, Science 329 (2010) 305-309. or an alternative coupled reaction with pyruvate decarboxylase, which does not need NADH [52] M. Hohne, S. Kuhl, K. Robins, U.T. Bornscheuer, Efficient asymmetric synthesis of chiral amines by combining transaminase and pyruvate decarboxylase, Chembiochem 9 (2008) 363-365.).

The implementation of specificity is particularly delicate in aminotransferases, whose reactions typically require two different substrates to bind in succession to the same cofactor in the same active site. Accordingly, these enzymes must be able to accommodate both substrates while discriminating against all others [4, 57]. Consider for example the case of aromatic amino acid aminotransferase (AroAT) , which uses α-KG as the amino group acceptor: for this enzymes, the problem of dual specificity entails accommodating the negatively charged γ-carboxylate of α-ketoglutarate in a site that must also accept the large hydrophobic side chain of an aromatic amino acid [4].

The problems associated to dual specificity are more pronounced for transaminases where the donor substrate is a terminal amine, since their substrates, besides differing in their 'side chain' properties, also differ due to the presence/absence of an α-carboxylate. For example, the active site of putrescine AT (PUAT, Table 1) must accommodate both α-KG (which possesses a negatively charged side chain and an α-carboxylate) and putrescine (positively charged side chain, no α-carboxylate) while discriminating against L-ornithine (positively charged side chain plus an α-carboxylate). Despite the complexity of this exercise, the enzyme from *E. coli* fares relatively well, reacting with putrescine some 50-fold better than with L-ornithine [58]

3- Structural peculiarities of AT-II enzymes

3.1 - AT-II vs. AT-I enzymes. Comparing the overall structures

To try to assess what features help AT-II enzymes achieving their peculiar functional specialization, it seems useful to compare the structure of the most studied AT-II transaminases with that of the prototypical aminotransferase, the aspartate aminotransferase (AspAT, assigned by Mehta and Christen to the structural subgroup AT-I). Both AT-I and AT-II enzymes are generally homodimers or homotetramers

(Supplementary Table 1); a first inspection of the subunit architecture reveals however some important differences between the two types of proteins (Figure 1 A-B).

It is possible to subdivide a fold-type I subunit into two domains: a larger one, whose central feature is a seven-stranded mixed β sheet, and a smaller discontinuous domain. This small domain is formed by the C-terminus of the protein chain, which contributes an antiparallel β-sheet (e.g., four strands in GABA-AT) plus some α-helices that lean against it, and by the N-terminus, contributing a few α-helices (usually one or two) and some antiparallel β strands (three or four).

Analyzing Figure 1A-B**,** it is evident that the N-terminal part is not conserved between AT-II and AT-I, as confirmed by the literature [6, 10], where this is described as the major determinant of diversity between the subgroups of the fold type I [8, 59]. For example in AspAT and AroAT (both AT-I) the N-terminal part starts on the surface of the second subunit and then crosses over the active site cleft before joining the small domain. Moreover an α-helix of the N-terminus is part of the active site entrance and interacts with the β-sheet in the C-terminal part of the small domain. These elements are not conserved in AT-II enzymes, where the N-terminal β-sheet and an α-helix simply form a meander in the active site, wedged between the large and the small domains [59].

The C-terminal part of the small domain and the large domain are instead quite conserved between AT-I and AT-II. The large domain has some structural elements that are retained in all the enzymes of fold type I [8]. It is constituted by an helix-loop-helix segment followed by a central seven-stranded β-sheet with complex topology: six parallel and one antiparallel β-strands. The β-strands are surrounded by various α-helices (e.g., 10 in GABA-AT [60]) in a typical α/β fold [61].

3.2 - AT-II vs. AT-I. Comparing the PLP-binding sites

The description above points to some macroscopic structural differences between AT-I and AT-II enzymes, but does not indicate whether such differences are important to explain the peculiar specialization of AT-II enzymes towards terminal amines. Much clearer insights can be gained by an analysis of active site structures.

The active site of these enzymes can be schematically divided in two parts, one that interacts with the substrate and another one with the cofactor. This latter part is relatively conserved and in the case of AT-II enzymes it is located in the large domain at the interface with the small domain. The phosphate group of PLP is nestled in a specific subsite, sometimes called ‘phosphate binding cup’, forming a network of several hydrogen bonds to side chains, backbone amide groups and water molecules. All these interactions recur similarly in the structure of AT-II enzymes as well as in that of AspAT [62, 63]. Also, in both the AT-I and AT-II enzymes, the PLP ring is held in place by a hydrogen bond between the pyridine nitrogen and the side chain carboxylate of an Asp residue. This is probably the most conserved residue among the entire fold type I and the mechanistic importance of its interaction with the pyridine ring has been analyzed in several works [5, 59, 64] (Figure 2).

In contrast to the above, other features of the active site and of the interactions between the enzyme and the cofactor are quite distinctive of AT-II enzymes as compared to AT-I [8, 59]:

First of all, the catalytic Lys residue, that covalently binds to PLP and that plays a crucial role in all the PLP-dependent reactions, is not always in the same position in the two subfamilies of enzymes [8, 59]. Even if it is always placed in the loop that connects the last two strands of the large domain, the Lys residue is located earlier in the polypeptide chain of AT-II enzymes (Supplementary Fig. 1) [8]. This fact is in all likeliness due to a deletion occurred during the evolution of AT-II.

Furthermore, a residue providing a hydrogen bond to the phenolic oxygen of the cofactor is present in the majority of AT-II enzymes; exceptions include DAPA-AT [8, 59] and other enzymes (e.g. [65]), where the hydrogen bond is water-mediated. Hydrogen bonding the phenolic oxygen may be important for modulating the pK_a of the internal aldimine (the Schiff base of the PLP with the catalytic lysine) [66] and also the reactivity of PLP [67].

Finally, in both AT-I and AT-II enzymes there is an aromatic residue stacking on the PLP pyridine ring. This residue is part of a loop that faces in the active site and it has an important role in the formation of external aldimine and quinonoid [8, 59]. In AT-II enzymes, however, the aromatic side chain is perpendicular to the cofactor while in other fold type I enzymes it is close to parallel [59]. The parallel position is made possible by the fact that the aromatic residue is located at the end of the loop, just before the beginning of an α-helix. In AT-II instead the aromatic residue is not at the end of the loop, but it constitutes the active site entrance (which is much more open than in other fold type I ATs) and it cannot be parallel to PLP because it would clash with the chain surrounding the loop in which it is included.

3.3 – The substrate binding site: a gateway system in α-KG-specific AT-II transaminases

The part of the active site of AT-II transaminases that interacts with the substrate also shows some important differences, when compared to AT-I.

The first one regards the changes in molecular conformation that accompany substrate binding. In AspAT and AroAT (both AT-I), the small domain rotates towards the large domain to close the active site upon binding of ligands (Figure 1C). In contrast, if we compare the ligand-bound with the unligated forms of GABA-AT , OAT or AcOAT (all AT-II) they exhibit no large-scale changes in the overall conformation (Figure 1-D). This is also true for other AT-II transaminases (such as DAPA-AT [59] and PUAT [68]) suggesting that

that these enzymes do not generally undergo closure of the active site as the AT-I enzymes do [57, 69].

One notable exception to this observation is found in glutamate 1-semialdehyde aminomutase (GSA). In this enzyme there is an active site gating loop, which is open when the enzyme contains bound PMP and closed when it contains PLP [70, 71]. This differential accessibility agrees with the fact that the natural substrate of GSA must initially enter the active site and bind to the PMP (not PLP) form of the cofactor (see the legend of Table 1) [71].

A second difference between AT-II and AT-I transaminases is closely related to the dual-specificity issue described above. The AT-II enzymes must be able to bind a terminal amine and to position their substrates in a way that permits to the ω-amino group to be transferred, while preventing transamination of α-amino acids. To accomplish this, AT-II enzymes that use α-KG as the amino acceptor and amines with long side chain as main donor (GABA, ornithine, lysine…), have evolved a so-called gateway system (Figure 3) [4]. The key elements of this gateway are two juxtaposed residues, an Arg and a Glu (Arg398 and Glu211 in *E. coli* GABA-AT; Arg413 and Glu235 in human OAT). Of these, only the Arg residue is conserved in AspAT (Arg374 in the *E. coli* enzyme), where it serves to bind and position the α-carboxylate of the aspartate substrate, making the transamination of the α-amino group possible. The Glu residue instead is missing in AspAT and it is conserved only in α-KG specific ATII transaminases (Supplementary figure 1).

Through site-directed mutagenesis experiment with both OAT and GABA-AT, it was concluded that this conserved Glu residue forms a salt bridge with the Arg residue during the first half of the transamination reaction, when the ω–amino substrate enters the active site. The presence of this salt bridge helps restrict the binding and reaction of undesired α-amino acids (e.g., glutamate) or α-keto acids, that are required only for the second half-reaction [16, 21, 69].

On the other hand, in the presence of PMP during the second half-reaction, the salt bridge opens up to allow α-KG to interact with the Arg residue. In other words, in the transition between the first and second half-reactions of these α-KG specific AT-II transaminases, the Glu residue acts as functional switch, triggered by the formation of PMP [21]. Such a switch allows these enzymes to be specific for amino-group donors lacking an α–carboxylate while retaining the ability to bind the α-ketoacid acceptor[3].

The active sites of α-KG-specific AT-II transaminases also contain typically a second Arg residue (Arg141 in GABA-AT; Figure 3) that forms a salt bridge with the γ-carboxylic group of α-KG [69, 72]. This interaction is a crucial determinant of the selectivity for α-KG as the specific acceptor ketoacid. Furthermore, the same residue also helps recognizing and properly positioning the amino group donor. In fact the α-KG -specific ATs often bind substrates (L-ornithine, GABA, L-lysine…) that contain a carboxylate at the opposite end with respect to the ω-amino group; this carboxylate seems able to interact with the Arg residue as efficiently as the γ-carboxylate of α-KG (Figure 3) [21].

What about the putrescine aminotransferase discussed in section 2? In contrast to L-ornithine, GABA and the like, putrescine does not contain any carboxylate, but just two amino groups, located at the opposite ends of an aliphatic (linear) chain of carbon atoms. A very recent structural study shows that, in the active site of PUAT from *E. coli*, a Lys residue (Lys183) replaces the Arg, which in OAT and GABA-AT salt-bridges with the carboxylate of the amino-group donor [68]. Compared to Arg, the Lys side chain is smaller and less basic (meaning that it can more easily exist in neutral form), and these differences may help explain the low activity of PUAT towards ornithine and other amino donors containing an α-carboxylate. Another difference noted by the authors of the structural paper relates to the active site entrance, which appears narrower and more

[3] In contrast AspAT (like other transaminases of subfamily AT-I) lacks the gateway system and retains only the Arg residue, rather rigidly positioned within the active site; this biases the enzyme towards binding both donors and acceptors containing an α-carboxylate.

restricted in PUAT, possibly favoring access of the 'leaner' poliamines (putrescine and cadaverine) with respect to the bulkier amino acids [68].

3.4 – The substrate binding site: P and O pockets in pyruvate-specific AT-II transaminases

The gateway system, as described in section 3.3, is not operational in AT-II transaminases that employ pyruvate as the preferred amino group acceptor: in fact, in these enzymes the Glu residue of the gateway is not conserved (Supplementary Figure 1). An Arg residue is still involved in recognition of the α-carboxylate of substrates, as indicated among other data by the available structures of pyruvate-specific ATs (a list of the three-dimensional structures of AT-II enzymes currently deposited in the Protein Data Bank is provided in Supplementary table 1) but its position is different (Supplementary Figure 1). Furthermore one is left to wonder why in these enzymes, the Arg does not need to form a strong interaction during the first part of the reaction, to mask its positive charge when the ω-amine binds. More generally, one may wonder why the substrate specificity of several pyruvate-specific ATIs tends to be relatively relaxed.

To address this sort of questions, as well as to interpret the stereoselectivity of amine:pyruvate transaminases (ω-PATs), Shin and Kim put forward a schematic model of the active site, based on both structure and reactivity analysis [73]. This scheme has been adopted in somewhat modified forms by other researchers in the field [18, 74, 75]. In essence the model is based on a bipartite substrate binding site, consisting of two subsites or 'pockets'. One of the subsites, which accommodates the α-carboxylate of the keto acid, is generally located in the vicinity of the O3' atom of PLP, and it can be indicated as the O-pocket. The other pocket is near the phosphate moiety of PLP and hence is called P-pocket [75]. The two pockets occur both in AT-I and AT-II transaminases, although their relative sizes are different (Figure 4).

In the ω-PAT from *Vibrio fluvialis* studied by Shin and Kim, the O pocket could accept both a carboxylate group and hydrophobic substituents. A similar situation can be proposed also for other AT-II transaminases reacting with pyruvate, where the O-pocket appears to be large and the presence of aromatic residues provides a hydrophobic environment and π–π interactions to stabilize aromatic and alkyl amino substrates [15, 74]. In sum, the O-pocket of ω-PATs seems able to bind both the α-carboxylate of the amino group acceptor and the hydrophobic side chains of the amino group donor substrates (Figure 4B).

This dual specificity can presumably be achieved without substantial structural rearrangement. Since in a large O-pocket the internal arginine can move relatively freely, it does not need to mask its positive charge, as it happens in the gateway system, to avoid clashes with the hydrophobic side chain of the amino substrate. Recently Steffen-Munsberg et al., based on the analysis of some ω–ATs, contrasted the mobility of the Arg residue in AT-I and AT-II enzymes [24]. According to this analysis, in AT-I the conserved Arg residue is quite packed within the active site, and it can only contact the α-carboxylic group of the substrate, while in AT-II enzymes the Arg is very flexible. Its position in fact is rather variable in the structures of similar enzymes and also in different monomers of the same structure (see also [14, 76]).

The flexibility of this Arg residue allows the enzyme to bind both pyruvate and much larger aldehydes and even a bulky or aromatic substrate. In this last case the Arg side chain is oriented away from the active site, leaving a large cavity behind. The wide size of the O-pocket may also explain the substantial substrate promiscuity not just of ω-PATs [18] but also of other pyruvate-specific transaminases such as AGXT2 [77].

What about the P pocket? In the study by Shin and Kim, this subsite was small and unable to accommodate substituents larger than an ethyl group. It also showed a strong aversion towards carboxylate groups [18, 73]. Therefore, this site contributes by steric

constrains and electrostatic repulsion to impart the enzyme specificity for pyruvate *versus* α-KG as the amino group acceptor (Figure 4B). The P-pocket is the only part of the enzyme that can distinguish between the two keto acids.

The O-pocket of α-KG-specific transaminases is smaller and more constrained than that of pyruvate-specific enzymes [24] (Figure 4C). This fact, together with the occurrence of the gateway system and the presence, for most of these enzymes, of an Arg residue in the P-pocket, as discussed in section 3.3 appears to implement a tendency of α-KG specific ATs to show lower substrate promiscuity. Exceptions exist however, e.g. *Pseudomonas aeruginosa* GABA-AT also accepts diamino acids (ornithine) and diamines (putrescine) [78].

3.5 – An overview of substrate specificity in AT-II transaminases

To summarize very schematically the data discussed in sections 3.3 and 3.4, α-KG- and pyruvate-specific ATs appear to have adopted two very distinct strategies to select donors bearing an ω-amino group while still permitting binding of the α-ketoacid acceptors. In pyruvate-specific enzymes, binding of an α-carboxylate and binding of a (large) side chain are mutually exclusive because they both should occur in the same pocket (the O pocket). Thus, these enzymes can only accommodate substrates containing no α-carboxylate (ω-amines) or containing an α -carboxylate and a very minimal side chain (pyruvate, glyoxylate). In α-KG-specific ATs, dual specificity is achieved through a more sophisticate and more rigid mechanism – namely, when the active site contains PLP, the O-pocket is not accessible to α-carboxylate groups because the Arg-Glu gateway is operational. However, upon formation of PMP the Arg residue in the O-pocket is released and can interact with α-carboxylate groups, whereas another positively charged residue in the P-pocket interacts with the γ-carboxylate of α-KG, imparting specificity for the ketoacid.

There are however exceptions and intermediate situations. One interesting example is a bacterial β-AT, called MesAT, whose physiological function is unknown. The enzyme was selected for applicative purposes (the preparation of enantiopure β-phenylalanine) and shown to react with a broad range of substrates, using α-KG as the amino acceptor [79]. The structure of MesAT however shows elements in common with both those observed in pyruvate-specific and in α-KG-specific AT-II transaminases, as well as in AT-I enzymes.

The P-pocket for γ- and β- carboxylate is relatively small and resembles the O-pocket of AspAT. On the other hand the O-pocket is quite large, it can accept both α-carboxylate and bulky hydrophobic groups and it is hence similar to the O-pocket of AT-II enzymes that use pyruvate as the acceptor (Figure 4D). Furthermore, even if interacting with α-KG, this enzyme does not possess a gateway system but a mobile arginine that acts as a switch (when a β-amino acid binds, the Arg side chain is oriented away from the active site, producing an hydrogen bond with another residue. This re-orientation enlarges the O-pocket permitting the accommodation of the hydrophobic side chain of substrates. Similar 'arginine switch' mechanisms occur in other PLP-dependent enzymes [4]). This arrangement is different from (and somewhat hybrid between) the models previously presented for the active sites of the other pyruvate and α-KG acceptors, as shown in Figure 4D.

4 – AT-II enzymes that are not aminotransferases

Despite the fact that the initial classification of Metha and collaborators dealt only with ATs, some enzymes belonging to the subgroup II show other activities. Not considering DGDA (which catalyzes both decarboxylation and transamination reactions) and GSA (whose reaction is in effect an internal transamination between two positions of

the same substrate; see Table 1), AT-II has been shown to encompass enzymes with *bona fide* racemase and lyase functions (Table 1 and Figure 5).

The first AT-II enzyme reported in the literature to catalyze a reaction different from transamination, was 2-aminohexano-6-lactam racemase (AH6L-R, table 1). In fact, AH6L-R was characterized before the classification work of Christen, Metha and co-workers [80, 81] and its three dimensional structure is currently known [82].

A comparison of the structure of this enzyme with those of GABA–AT (29% identity with AH6L-R) and DGDA (30% identity) confirmed that both the overall architecture and the active site structure surrounding the cofactor are comparable to those of the other AT-II [82]. AH6L-R however, unique among fold-type I enzymes, possesses an additional C-terminal 'tail' (about 12 residues, forming an α–helix) that is essential for function. Some of the residues in this C-terminal part contribute to shaping the active site cavity, determine substrate specificity, recognize the nitrogen of an amide of lactam in the substrate and control the access to the active site [82].

On the other hand, the racemase lacks an Arg residue to interact with the carboxylic group of the substrate. AH6L-R does not need to bind a substrate bearing an α carboxylate, thus the absence of the Arg residue prevents the interaction of the enzyme with α–keto acids and ultimately the undesired occurrence of transamination. This seems to be a key determinant of reaction specificity. Another key determinant may be a Tyr residue (Tyr137 in the enzyme from *Achromobacter obae*) which has been proposed to be involved acid/base catalysis [82]. This Tyr residue is conserved in several AT-II transaminases, but it can be occasionally replaced by Phe (in OAT, for example) or by Trp (in DGDA; Supplementary Fig. 1), making it unlikely that it may play an acid-base role during transamination. Nevertheless, since racemase reactions require different proton transfers as compared to transamination reactions, Tyr137 may have acquired a new function during evolution of AH6L-R.

Recently, a second racemase belonging to AT-II was identified, namely isoleucine 2-epimerase [83]. This enzyme is quite peculiar not only because it is a racemase, but also because it operates on a standard α-amino acid substrate (together with D-FGAT and DGDA, it is one of the few AT-II enzymes whose preferred substrate is an α–amino acid rather than an ω–amine).

There is no three-dimensional structure available for ILE-R, but inspection of the the sequence alignments shows that the Arg residue involved in the interaction with the α-carboxylate is conserved, whereas the Glu that in α-KG-specific AT-IIs is involved in the gateway system, although not conserved, is replaced by the chemically similar Asp (Supplementary Figure 1). It is possible that the same residue that confers the dual specificity to some transaminases of the AT-II subgroup in this case is involved in the catalysis of the racemization with isoleucine. Further biochemical and structural data are necessary to better define the interaction of this enzyme with its substrate and the catalytic mechanism.

The only other known AT-II enzymes not catalyzing transaminations are two closely related lyases, ethanolamine phospho-lyase (PEA-PL) and 5-phosphohydroxy-lysine phospho-lyase (PHK-PL), that catalyze the elimination of phosphate from phosphoethanolamine and 5-phosphohydroxy-lysine, respectively. The molecular identification of both lyases was accomplished in a single remarkable study by Veiga-da-Cunha and coworkers [84].

The structures of these two phospho-lyases are not known, but some hypotheses can be made about the structural changes that, during evolution, led to their emergence from ancestors that was almost certainly a transaminase [85].

Several similarities can be observed with the sequence of the DGDA (30% identity). For example, as observed in DGDA, the Arg that in most ATs interacts with the substrate α-carboxylic group is not conserved. This is in accordance with the low affinity of PEA-PL

towards α-amino acids and with its ineffectiveness as a transaminase (for this enzyme, β-elimination is at least 500-fold more efficient than transamination) [85]. Again similar to DGD (and more generally to pyruvate specific ATs) the lyases also lack the Arg residues that in α-KG-specific ATs interacts with the γ-carboxylate of the substrate (see Figures 3 and 4C). This residue is replaced by Met in DGD and by His in both PEA-PL and PHK-PL. This histidine could perhaps perform acid/base catalysis, permitting the elimination of the phosphate leaving group (Supplementary Figure 1).

5 - Inferences on the evolution of AT-II enzymes

It is believed that fold type I enzymes evolved divergently for 1500 millions years and, despite their diversification and specialization to cover a wide range different catalytic functions, they have maintained their structure conserved during evolution. The presence of the cofactor and some conserved hydrophobic residues are probably the driving forces that have kept the original structure conserved during evolution [86]. Analyzing sequences, structures and function of this class of PLP-dependent enzyme is useful for better understanding what are the evolutionary steps that have driven the acquisition of new enzymatic functions or substrate specificities [2, 11].

Figure 6 shows a phylogenetic tree illustrating the relationships between AT-II enzymes. The tree was constructed starting from a set 89 sequences that included all AT-II proteins with a validated physiological function, obtained from the B6 Database [9] (Supplementary Table 2). Among the sequences in the set, only a few were ω-PATs and β-ATs (for comparison, see [15]). AspATs were used for adding an out-group, belonging to the AT-I. An inspection of the tree, combined with the information provided in the previous sections, allow drawing some inferences and putting forward some hypotheses about evolution of AT-IIs.

The long branch that connects AT-I, here represented by the AspATs, and AT-II is indicative of the evolutionary differences among these groups of enzymes. As described in section 3, there are many parts of the protein sequence that are not conserved between the two subfamilies, and that justify the branch length. In particular, the divergence between AT-I and AT-II seems to have been accompanied by changes in the structure of the small domain and by some significant rearrangements of the active site, such as a repositioning, along the sequence, of the catalytic Lys and changes in the organization of the substrate-binding pockets.

It seems reasonable to speculate that the ancestral AT-II enzyme was an aminotransferase, or at least that its main catalytic function was to carry out transaminase reactions. This hypothesis is in agreement with the current overabundance of AT enzymes within the subfamily (as well as within subgroup AT-I) and with the observation that non-AT enzymes are found far from the tree root. The hypothetical ancestral enzyme might also have had some preference towards the amino group acceptor; in fact, in Fig. 6 the branches of the tree most close to the root represent α-KG specific ATs (DHNEAT, NEAT, LAT and D-FGAT: for the last enzyme, a PDB-deposited crystal structure exists (2CY8; [87]), which however does not contain bound PLP and lacks other structural details).

In any case, a neat divide separates the majority of extant α-KG specific ATs from the pyruvate-specific ATs, even though a unique branchpoint leading to this separation is difficult to individuate. Ultimately, the loss of the Glu residue of the gateway system and other rearrangements of the two pockets of the active site seem the major determinants of difference among the two AT groups. However, specialization for pyruvate may have been acquired progressively and/or separately in different lineages. For example DABA-AT, which occupies a position close to the emergence of pyruvate-specific ATs, is able to catalyze its reaction also with pyruvate, but the K_M is severalfold higher than that with α-KG [88]. Over time, the different groups of enzymes may have optimized their specificity

towards one or the other amino group acceptor, owing to the availability and role of a given keto acid in one organism or to specific physiological contexts.

Whatever the evolutionary mechanisms that led to the current situation, there are hints that the absence of a gateway system (as found in pyruvate-specific ATs) correlates with a greater enzyme versatility and/or promiscuity. For example DAPA-AT enzymes, that display a very peculiar substrate specificity, shows a relatedness with pyruvate-specific ATs. Non-AT enzymes (lyases, racemases) cluster together and seem most closely related to some pyruvate-specific ATs showing relaxed substrate specificity (DGDA, AGXT2). Eliot and Kirsch argued that one preferential route towards the evolution of a new PLP-dependent catalytic activity is the acquisition of an altered substrate specificity [4]. Elimination for example can be a very facile reaction if an enzyme binds a substrate with a good leaving group. The presence of the phospho-lyase and DGDA in the same tree branch, the fact that phosphate is a good leaving group and that they possess some active site residues in common is in accordance with the hypothesis described.

β-ATs, like the one shown to have an intermediate active site organization between α-KG and pyruvate-specific ATs [79], forms a separate cluster together with two other peculiar ATs: FUMAT and GSA. Not only all three enzymes lack the Glu residue of the gateway system: in FUMAT and GSA it is also absent the Arg (strongly conserved among AT-II ATs) interacting with the α-carboxylate of the amino group acceptor. In the case of GSA this residue is substituted by a Glu, which interacts with the amino group of the diaminovalerate (produced during the catalysis) and excludes glutamate or other α-amino acids from the active site, since this is the only AT-II transaminase not interacting with any α-carboxylate [89]. The absence of the Arg residue in FUMAT is more difficult to rationalize, but it might be due to the long and bulky side chain of the substrate. In the absence of structural information, it can be hypothesized that the enzyme has evolved a different system to bind this substrate and consequently also the amino group acceptor

(FUMAT was reported to use pyruvate [90], despite being located outside the main cluster of pyruvate-specific ATs).

6 - Conclusions

Over twenty years after the first clear definition of AT-II as a structural subfamily [10], we have provided an up-to-date overview of these enzymes, based on the most recent biochemical and structural literature, illustrating how their properties are distinctive and how they compare with those of the other fold-type I enzymes, as well as sketching a picture of their evolution. As more enzyme functions and protein structures emerge within this structural subgroup, there will be possibilities for elucidating in greater detail their specific properties and for incorporating this knowledge into the current picture. As mentioned in section 2, the practical interest in AT-II enzymes has increased over time, due to their use for the enantiopure synthesis or resolution of chiral amines, as reviewed in detail in a number of recent publications [15, 17, 18]. Hence, new structural and enzymological studies on AT-II enzymes are expected to not only benefit our general knowledge but to also improve our ability of selecting and tailoring these catalysts for applicative purposes.

FIGURE LEGENDS

Figure 1: Comparison of the structure of a prototypical AT-I enzyme (mitochondrial AspaAT from *Gallus gallus*) with a prototypical AT-II enzyme (GABA-AT from *Escherichia coli*)**.** (A) Structure of holo-AspAT (PDB ID: 7AAT). The large domain is shown in green and magenta while the small domain is in dark blue-dark gray (N-terminal part) or light blue-light gray (C-terminal part). PLP is shown in yellow. (B) Structure of holo-GABA-AT (PDB ID: 1SF2). GABA-AT and AspAT share similar large domains, while the small domain, and in particular the N-terminal part, is not conserved. (C) Structure of AspAT in closed conformation, bound to the inhibitor aminooxyacetate (PDB ID: 1OXP) [91]. The transition between the open and closed conformation is determined by some structural rearrangements: one involves the movement of three α-helices and few β-strands, indicated by a continuous curved arrow. Other minor rearrangements are indicated by with dotted arrows. (D) Structure of GABA-AT complexed with aminooxyacetate (PDB ID: 1SFF) [69]. In this case, no large structural rearrangements are observed upon ligand binding.

Figure 2: Schematic active site model of *E. coli* GABA-AT and structural comparison with other enzymes belonging to the AT-II subgroup. Some key residues (or group of residues) are indicated by numbers from (1) to (4): (1) Side-chain involved in the interaction with the α-carboxylic group of the substrate (the corresponding residue in human OAT is Arg413) (2) Residues interacting with the phosphate of PLP (Gly142, Val143 and Thr322 fulfill this role in human OAT). (3) Aspartate interacting with the pyridine nitrogen of the PLP ring (Asp263 in human OAT) (4) Residue interacting with the 5’ hydroxyl group of PLP (Gln266 in human OAT). Residues in (1) and (3) are conserved among all the ATs in AT-II. The tabular part of the figure resumes the actual amino acids identities in some known 3D structures. These residues often are not aligned

(supplementary figure 1) and they can have different positions inside the structure (Arg residues, column 1) but they have the same role in contacting the cofactor or substrate. The symbol ***** indicates residues that, despite not being alignable with the Gln found in the majority of the enzymes, appear to play the same role in the three-dimensional structure. The symbol **-** Indicates that a residues in the active site is not directly interacting with the PLP O3' group, but through hydrogen bond with a molecule of water.

Figure 3: Scheme of the gateway gystem in GABA aminotransferase (residues numbering refers to the *E. coli* enzyme; [69]). At the beginning of the physiological reaction, the enzyme forms a Schiff base with GABA and during this process the crucial Arg398 residue remains salt-bridged with Glu211. The first half of the reaction ends with release of succinic semialdehyde and conversion of PLP to PMP. In the second half of the transamination, α-KG reacts with PMP to yield the external aldimine of glutamate, which will be released as a second product. Triggered by the formation of PMP, the Arg398 switches its position and becomes available for interacting with the α-carboxylic group of α-KG (or glutamate). A second Arg residue (Arg141) interacts instead with the γ-carboxylic group of both GABA and α-KG and serves for productive binding and correct positioning of the substrates. This residue is conserved also in other α-KG specific AT-II transaminases [4, 16, 60, 69].

Figure 4: Schematic features of the O and P pockets in different types of ATs. The circle and the triangle indicate the different chemical groups that can be found on the substrates. (A) Model referring to AroAT and other AT-I transaminases [74]. (B) Model for AT-II transaminases that use pyruvate as the amino group acceptor. Based on the work of Shin and Kim on an ω-PAT [24, 73]. (C) Model referring to α-KG-specific ATs [57]. (D) Scheme of the atypical active site of a β–AT described by Wybenga and collaborators [79].

Figure 5: Reactions catalyzed by the currently-known AT-II enzymes that are not ATs. Despite the apparently drastic differences, these reactions share among them (and with the transaminase reactions) some mechanistic foundations. In fact, PLP-dependent transaminases, racemases and lyases are all expected to begin their reaction by forming a Schiff base between PLP and the substrate amino group, followed by deprotonation of the amino carbon. Only at that point mechanisms diverge, to yield specific reactions and products [1, 5]

Figure 6: A phylogenetic tree of the functionally validated AT-II enzymes. The sequences of enzymes whose function had been experimentally validated were taken from the B6 database (http://bioinformatics.unipr.it/cgi-bin/bioinformatics/B6db/home.pl) and grouped using the neighbor joining method. A multiple sequence alignment was performed using ClustalX2 (http://www.clustal.org/); the number of sequences was subsequently reduced from 89 to 48, in order to facilitate reading of the alignment (see Supplementary Table 2 and Supplementary Fig. 1) and of the resulting tree. The radial tree was obtained using aspartate aminotransferases form AT-I as an out-group and displayed through the phylogenetic tree generator FigTree (http://tree.bio.ed.ac.uk/software/figtree/). Branches are colored in: blue for α-KG-specific ATs and for AspATs, red for pyruvate-specific ATs, orange for DAPA-AT and GSA, black for lyases and racemases. Symbols are used to indicate, respectively, the change in the position of the catalytic lysine and reorganization of the small domain (✶) and the loss/acquisition of the Glu involved in the gateway system (○).

Table 1: PLP-dependent enzyme belonging to the AT-II subgroup.

Enzyme name	Acronym[1]	Sequence[2]	E.C.	Substrates[3] First	Substrates[3] Second
Aminotransferases					
γ-aminobutyrate AT	GABA-AT	GABT_HUMAN[4] GABT_ECOLI[4]	2.6.1.19	GABA	α-KG
Ornithine AT	OAT	OAT_HUMAN	2.6.1.13	L-ornithine	α-KG
Acetylornithine AT	ACOAT	ARGD_ECOLI	2.6.1.11	N_2-Acetyl-L-ornithine	α-KG
Succinylornithine AT	SOAT	ASTC_ECOLI	2.6.1.81	N_2-succinyl-L-ornithine	α-KG
L-lysine ε-aminotransferase	LAT	LAT_STRC[5] Q9EVJ7_FLALU[5]	2.6.1.36	L-lysine	α-KG
Putrescine AT	PUAT	PAT_ECOLI	2.6.1.29/ 2.6.1.82[6]	cadaverine, putrescine	α-KG
Diaminobutyrate AT	DABA AT	DAT_ACIBA	2.6.1.76	L-2,4-diaminobutyrate	α-KG
D-4-hydroxyphenylglycine AT	D-FGAT	Q6VY99_PSEST	2.6.1.72	D-4-hydroxyphenylglycine	α-KG
Neamine transaminase	NEAT	NEON_STRFR	2.6.1.93	Neomycin C, Neamine	α-KG
2'-deamino-2'-hydroxyneamine AT	DHNEAT	KANB_STRKN	2.6.1.94	2'-deamino-2'-hydroxy-neamine, Neamine	α-KG
acyl-CoA β-transaminase[7]	COA-β-AT	KAT_CLOAI	-	3-aminobutyryl-CoA	α-KG
3-aminobenzoate synthase[8]	ABS	A8R0K5_9ACTO	-	L-glutamate[8]	3-DHS[8]
3-acetyloctanal transaminase[9]	AcOCT-AT	L7ZI44_SERMA	--	(L-glutamate?)[9]	3-acetyloctanal
L-glutamate 1-semialdehyde (GSA) aminomutase[10]	GSA	GSA_ECOLI	5.4.3.8	glutamate 1-semialdehyde	-
β-alanine-pyruvate AT	βA-PAT	OAPT_PSEPU	2.6.1.18	β-Alanine	Pyruvate
γ-aminobutyrate-pyruvate AT	GABA-PAT	J9XGZ5_MALDO	2.6.1.96	GABA	Pyruvate, Glyoxylate
Putrescine-pyruvate AT	PU-PAT	Q9I6J2_PSEAE	--	Putrescine	Pyruvate
*D-3-a*minoisobutyrate pyruvate AT	AGXT2[8]	AGT2_RAT	2.6.1.40 /43/44[11]	(D)-3-amino-isobutyrate	Pyruvate, Glyoxylate
Taurine-pyruvate AT	TAU-PAT	TPA_BILWA	2.6.1.77	Taurine	Pyruvate
Fumonisin-pyruvate AT[12]	FUMAT	FUMI_SPHMC	-	fumonisin B_1	Pyruvate
Dialkylglycine decarboxylase[13]	DGDA	DGDA_BURCE	4.1.1.64	(Isopropylamine) [13]	Pyruvate
7,8-diamino-pelargonate AT[14]	DAPA AT	BIOA_ECOLI	2.6.1.62	S-adenosyl-L-methionine	KAPA [14]
Lysine-7,8-diamino-pelargonate AT[14]	K-DAPA AT	Q8KZN0_BACIU	-	Lysine	KAPA [14]
Amine-pyruvate AT[15]	ω-PAT	F2XBU9_VIBFL	_	Various ω-amines	Pyruvate
β-amino acid AT[15]	β-AT	A3EYF7_9RHIZ	-	Various β-amino acids	Pyruvate, α-KG
Polyketide synthase AT domain[16]	PKS-ATd	MYCA_BACIU	--	Glutamine	β-ketothioester
Isomerases					
2-aminohexano-6-lactam racemase	AH6L-R	ACLR_ACHOB	5.1.1.15	L-2-aminohexano-6-lactam	-
Isoleucine 2-epimerase	ILE-R	M1GRN3_LACBU	--	L-Isoleucine	_

Lyases					
O-phosphoethanolamine phospho-lyase	PEA-PL	AT2L1_HUMAN	4.2.3.2	*O*-phospho-ethanolamine	-
5-phosphohydroxy-L-lysine phospho-lyase	PHK-PL	AT2L2_HUMAN	4.2.3.134	5-phospho-hydroxy-L-lysine	_

[1] Abbreviation used in this review to indicate to the specific enzyme.

[2] Accession number in UniProtKB of a representative enzyme with validated activity.

[3]For ATs (which normally use two substrates) the first entry represents the amino group donor, the second entry is the amino group acceptor under physiological conditions.

[4] Two phylogenetically distinct groups of GABA-AT enzymes exist, one bacterial [69] and one eukaryotic [92], thus one representative sequence per group is given.

[5] Two phylogenetically distinct groups of LAT enzymes have been described, one from Gram+ [29] and the other from Gram- bacteria [31]; one representative sequence per group is given.

[6] The listed PUAT enzyme (from *Escherichia coli*) can transaminate efficiently both putrescine and cadaverine [58], and can be legitimately assigned to two different EC numbers.

[7] COA-β-AT is an AT recently discovered based on a bacterial metagenome analysis [93]. It serves to form acetoacetyl-CoA from 3-aminobutyryl-CoA.

[8] 3-DHS = 3-dehydroshikimate. The enzyme ABS catalyzes the transamination between L-glutamate and 3-DHS, yielding α-KG and 3-amino 4,5-dihydroxy cyclohex-1-ene-1-carboxylate; the latter product rapidly and irreversibly undergoes the elimination of two water molecules, becoming converted to 3-aminobenzoate [94]. Throughout the review we include ABS among the α-KG-specific ATs, even though physiologically α-KG represents a product, rather than a substrate.

[9] The enzyme AcOCT-AT catalyzes a transamination reaction in the biosynthesis of the antibiotic prodigiosin [95, 96]. Even though the amino group donor has not been reported,

we tentatively assume it is L-glutamate, since the enzyme is phylogenetically related to α-KG-specific transaminases.

[10] Despite being formally classified as an isomerase, GSA catalyzes an internal transamination between carbons 1 and 2 of L-glutamate-1-semialdehyde. The reactive form of the enzyme contains PMP at the active site, and in the first step of the reaction mechanism glutamate semialdehyde acts as the amino group acceptor, receiving the amino group from PMP and forming 4,5-diaminovalerate [70, 97]. This product is not released from the active site, however, and functions as the amino group donor in the second part of the reaction, to yield the final product 5-aminolevulinate.

[11] Mammalian AGXT2 is a promiscuous mitochondrial transaminase that acts on a variety of substrates – in addition to D-3-aminoisobutyrate, these substrates include L-alanine, 5-aminolevulinate, β-alanine [77, 98]. Accordingly, the enzyme can be assigned to different E.C. numbers.

[12] In *Sphingopyxis sp.* MTA144, FUMAT is involved in the degradation of fumonisins (a class of carcinogenic mycotoxins). Pyruvate was found to be the preferred amino group acceptor, although the enzyme could also use other α-keto acids [99, 100].

[13] Dialkylglycine decarboxylase is listed among the transaminases because its catalytic mechanism encompasses an amino transfer [101, 102]. DGD cannot directly transaminate its substrate (a 2,2-dialkylglycine, which lacks an α-proton), but proceeds to decarboxylate it and then catalyzes a transamination with the decarboxylation product (a dialkylamine, such as isopropylamine), to yield the corresponding ketone [101, 102].

[14] KAPA = 7-keto-8-aminopelargonate. Classic DAPA-AT enzymes use S-adenosylmethionine as the amino group donor to produce 7,8-diaminopelargonic acid, an intermediate in biotin biosynthesis [103]. K-DAPA-AT (*B. subtilis*) uses instead L-lysine as the preferred amino group donor. [104, 105].

[11] ω-P AT are enzymes studied and used for their biotechnological potentials. Their

physiological functions are not known. They can react with various substrates and ketoacids. Even if they transaminate an amine (e.g. (S)-α-methylbenzylamine) they are not able to catalyze the same reaction with β-alanine. Similarly also the β-AT enzymes have been studied only for their biotechnological applications [23].

16 PKS-ATd was identified as an AT domain belonging to a multi-domain polyketide synthase. It catalyzes the transfer of an amine to the β-position of the growing acyl chain, using glutamine as the best amino donor [106].

REFERENCES

[1] R.A. John, Pyridoxal phosphate-dependent enzymes, Biochim. Biophys. Acta 1248 (1995) 81-96.

[2] P.K. Mehta, P. Christen, The molecular evolution of pyridoxal-5'-phosphate-dependent enzymes, Adv. Enzymol. 74 (2000) 129-184.

[3] R. Percudani, A. Peracchi, A genomic overview of pyridoxal-phosphate-dependent enzymes, EMBO Rep. 4 (2003) 850-854.

[4] A.C. Eliot, J.F. Kirsch, Pyridoxal phosphate enzymes: mechanistic, structural, and evolutionary considerations, Annu. Rev. Biochem. 73 (2004) 383-415.

[5] M.D. Toney, Controlling reaction specificity in pyridoxal phosphate enzymes, Biochim. Biophys. Acta 1814 (2011) 1407-1418.

[6] N.V. Grishin, M.A. Phillips, E.J. Goldsmith, Modeling of the spatial structure of eukaryotic ornithine decarboxylases, Protein Sci. 4 (1995) 1291-1304.

[7] J.N. Jansonius, Structure, evolution and action of vitamin B6-dependent enzymes, Curr. Opin. Struct. Biol. 8 (1998) 759-769.

[8] G. Schneider, H. Kack, Y. Lindqvist, The manifold of vitamin B6 dependent enzymes, Structure Fold. Des. 8 (2000) R1-6.

[9] R. Percudani, A. Peracchi, The B6 database: a tool for the description and classification of vitamin B6-dependent enzymatic activities and of the corresponding protein families, BMC Bioinformatics 10 (2009) 273.

[10] P.K. Mehta, T.I. Hale, P. Christen, Aminotransferases: demonstration of homology and division into evolutionary subgroups, Eur. J. Biochem. 214 (1993) 549-561.

[11] P. Christen, P.K. Mehta, From cofactor to enzymes. The molecular evolution of pyridoxal-5'-phosphate-dependent enzymes, Chem. Rec. 1 (2001) 436-447.

[12] R.A. Jensen, W. Gu, Evolutionary recruitment of biochemically specialized subdivisions of Family I within the protein superfamily of aminotransferases, J. Bacteriol. 178 (1996) 2161-2171.

[13] P. Berglund, M.S. Humble, C. Branneby, 7.18 C–X bond formation: Transaminases as chiral catalysts: mechanism, engineering, and applications, in: E.M. Carreira, H. Yamamoto (Eds.), Comprehensive Chirality, Elsevier, Amsterdam, 2012, pp. 390-401.

[14] F. Steffen-Munsberg, C. Vickers, H. Kohls, H. Land, H. Mallin, A. Nobili, L. Skalden, T. van den Bergh, H.J. Joosten, P. Berglund, M. Hohne, U.T. Bornscheuer,

Bioinformatic analysis of a PLP-dependent enzyme superfamily suitable for biocatalytic applications, Biotechnol Adv (2015).
[15] C. Rausch, A. Lerchner, A. Schiefner, A. Skerra, Crystal structure of the ω-aminotransferase from *Paracoccus denitrificans* and its phylogenetic relationship with other class III aminotransferases that have biotechnological potential, Proteins 81 (2012) 774-787.
[16] H. Lee, J.I. Juncosa, R.B. Silverman, Ornithine aminotransferase versus GABA aminotransferase: Implications for the design of new anticancer drugs, Med. Res. Rev. (2014).
[17] D. Koszelewski, K. Tauber, K. Faber, W. Kroutil, ω-Transaminases for the synthesis of non-racemic α-chiral primary amines, Trends Biotechnol. 28 (2010) 324-332.
[18] M.S. Malik, E.S. Park, J.S. Shin, Features and technical applications of ω-transaminases, Appl. Microbiol. Biotechnol. 94 (2012) 1163-1171.
[19] C.J. Sigrist, E. de Castro, L. Cerutti, B.A. Cuche, N. Hulo, A. Bridge, L. Bougueleret, I. Xenarios, New and continuing developments at PROSITE, Nucleic Acids Res. 41 (2013) D344-347.
[20] R.D. Finn, A. Bateman, J. Clements, P. Coggill, R.Y. Eberhardt, S.R. Eddy, A. Heger, K. Hetherington, L. Holm, J. Mistry, E.L. Sonnhammer, J. Tate, M. Punta, Pfam: the protein families database, Nucleic Acids Res. 42 (2014) D222-230.
[21] M. Markova, C. Peneff, M.J. Hewlins, T. Schirmer, R.A. John, Determinants of substrate specificity in ω-aminotransferases, J. Biol. Chem. 280 (2005) 36409-36416.
[22] E.M. Valmaseda, S. Campoy, L. Naranjo, J. Casqueiro, J.F. Martin, Lysine is catabolized to 2-aminoadipic acid in *Penicillium chrysogenum* by an ω-aminotransferase and to saccharopine by a lysine 2-ketoglutarate reductase. Characterization of the ω-aminotransferase, Mol. Genet. Genomics 274 (2005) 272-282.
[23] J. Rudat, B.R. Brucher, C. Syldatk, Transaminases for the synthesis of enantiopure beta-amino acids, AMB Express 2 (2012) 11.
[24] F. Steffen-Munsberg, C. Vickers, A. Thontowi, S. Schätzle, T. Meinhardt, M. Svedendahl Humble, H. Land, P. Berglund, U.T. Bornscheuer, M. Höhne, Revealing the structural basis of promiscuous amine transaminase activity, ChemCatChem 5 (2013) 154-157.
[25] H. Mallin, M. Höhne, U.T. Bornscheuer, Immobilization of (R)- and (S)-amine transaminases on chitosan support and their application for amine synthesis using isopropylamine as donor, J. Biotechnol. 191 (2014) 32-37.
[26] C.C. Chen, H. Zhang, A.D. Kim, A. Howard, G.M. Sheldrick, D. Mariano-Dunaway, O. Herzberg, Degradation pathway of the phosphonate ciliatine: crystal structure of 2-aminoethylphosphonate transaminase, Biochemistry 41 (2002) 13162-13169.
[27] C. Sayer, R.J. Martinez-Torres, N. Richter, M.N. Isupov, H.C. Hailes, J.A. Littlechild, J.M. Ward, The substrate specificity, enantioselectivity and structure of the (*R*)-selective amine : pyruvate transaminase from *Nectria haematococca*, FEBS J. 281 (2014) 2240-2253.
[28] Y.B. Tewari, N. Kishore, R.N. Goldberg, T.N. Luong, An equilibrium and calorimetric study of some transamination reactions, J. Chem. Thermodyn. 30 (1998) 777-793.
[29] S. Mani Tripathi, R. Ramachandran, Direct evidence for a glutamate switch necessary for substrate recognition: crystal structures of lysine ε-aminotransferase (Rv3290c) from *Mycobacterium tuberculosis* H37Rv, J. Mol. Biol. 362 (2006) 877-886.
[30] P.J. Large, Enzymes and pathways of polyamine breakdown in microorganisms, FEMS Microbiol. Rev. 8 (1992) 249-262.

[31] T. Fujii, T. Narita, H. Agematu, N. Agata, K. Isshiki, Characterization of L-lysine 6-aminotransferase and its structural gene from *Flavobacterium lutescens* IFO3084, J. Biochem. 128 (2000) 391-397.
[32] H. Laue, A.M. Cook, Biochemical and molecular characterization of taurine:pyruvate aminotransferase from the anaerobe *Bilophila wadsworthia*, Eur. J. Biochem. 267 (2000) 6841-6848.
[33] J. Ruff, K. Denger, A.M. Cook, Sulphoacetaldehyde acetyltransferase yields acetyl phosphate: purification from *Alcaligenes defragrans* and gene clusters in taurine degradation, Biochem. J. 369 (2003) 275-285.
[34] G. Shimamoto, R.S. Berk, Taurine catabolism. II. biochemical and genetic evidence for sulfoacetaldehyde sulfo-lyase involvement, Biochim. Biophys. Acta 632 (1980) 121-130.
[35] F.J. van Bemmelen, M.J. Schouten, D. Fekkes, J. Bruinvels, Succinic semialdehyde as a substrate for the formation of γ-aminobutyric acid, J. Neurochem. 45 (1985) 1471-1474.
[36] K.L. Behar, D. Boehm, Measurement of GABA following GABA-transaminase inhibition by gabaculine: a ^{1}H and ^{31}P NMR spectroscopic study of rat brain *in vivo*, Magn. Reson. Med. 31 (1994) 660-667.
[37] W.G. Hearl, J.E. Churchich, A mitochondrial $NADP^{+}$-dependent reductase related to the 4-aminobutyrate shunt. Purification, characterization, and mechanism, J. Biol. Chem. 260 (1985) 16361-16366.
[38] S.M. Clark, R. Di Leo, P.K. Dhanoa, O.R. Van Cauwenberghe, R.T. Mullen, B.J. Shelp, Biochemical characterization, mitochondrial localization, expression, and potential functions for an *Arabidopsis* γ-aminobutyrate transaminase that utilizes both pyruvate and glyoxylate, J. Exp. Bot. 60 (2009) 1743-1757.
[39] K.B. Busch, H. Fromm, Plant succinic semialdehyde dehydrogenase. Cloning, purification, localization in mitochondria, and regulation by adenine nucleotides, Plant Physiol. 121 (1999) 589-597.
[40] A.H. Liepman, L.J. Olsen, Alanine aminotransferase homologs catalyze the glutamate:glyoxylate aminotransferase reaction in peroxisomes of *Arabidopsis*, Plant Physiol. 131 (2003) 215-227.
[41] Y. Nakamura, N.E. Tolbert, Serine: glyoxylate, alanine:glyoxylate, and glutamate:glyoxylate aminotransferase reactions in peroxisomes from spinach leaves, J. Biol. Chem. 258 (1983) 7631-7638.
[42] M. Kendziorek, A. Paszkowski, Properties of serine:glyoxylate aminotransferase purified from *Arabidopsis thaliana* leaves, Acta Biochim. Biophys. Sin. (Shanghai) 40 (2008) 102-110.
[43] B. Cellini, M. Bertoldi, R. Montioli, A. Paiardini, C. Borri Voltattorni, Human wild-type alanine:glyoxylate aminotransferase and its naturally occurring G82E variant: functional properties and physiological implications, Biochem. J. 408 (2007) 39-50.
[44] S. Donini, M. Ferrari, C. Fedeli, M. Faini, I. Lamberto, A.S. Marletta, L. Mellini, M. Panini, R. Percudani, L. Pollegioni, L. Caldinelli, S. Petrucco, A. Peracchi, Recombinant production of eight human cytosolic aminotransferases and assessment of their potential involvement in glyoxylate metabolism, Biochem. J. 422 (2009) 265-272.
[45] D.E. Metzler, J. Olivard, E.E. Snell, Transamination of pyridoxamine and amino acids with glyoxylic acid, J. Am. Chem. Soc. 76 (1954) 644-648.
[46] A.D. Smith, M. Benziman, H.J. Strecker, The formation of ornithine from proline in animal tissues, Biochem. J. 104 (1967) 557-563.
[47] G. Wu, F.W. Bazer, J. Hu, G.A. Johnson, T.E. Spencer, Polyamine synthesis from proline in the developing porcine placenta, Biol. Reprod. 72 (2005) 842-850.

[48] D. Funck, B. Stadelhofer, W. Koch, Ornithine-δ-aminotransferase is essential for arginine catabolism but not for proline biosynthesis, BMC Plant Biol. 8 (2008) 40.
[49] P.J. O'Brien, A.G. Siraki, N. Shangari, Aldehyde sources, metabolism, molecular toxicity mechanisms, and possible effects on human health, Crit Rev Toxicol 35 (2005) 609-662.
[50] H. Kohls, F. Steffen-Munsberg, M. Höhne, Recent achievements in developing the biocatalytic toolbox for chiral amine synthesis, Curr. Op. Chem. Biol. 19 (2014) 180-192.
[51] P. Tufvesson, J.S. Jensen, W. Kroutil, J.M. Woodley, Experimental determination of thermodynamic equilibrium in biocatalytic transamination, Biotechnol. Bioeng. 109 (2012) 2159-2162.
[52] M. Hohne, S. Kuhl, K. Robins, U.T. Bornscheuer, Efficient asymmetric synthesis of chiral amines by combining transaminase and pyruvate decarboxylase, Chembiochem 9 (2008) 363-365.
[53] J.-S. Shin, B.-G. Kim, Asymmetric synthesis of chiral amines with ω-transaminase, Biotechnol Bioeng 65 (1999) 206-211.
[54] J.H. Seo, D. Kyung, K. Joo, J. Lee, B.G. Kim, Necessary and sufficient conditions for the asymmetric synthesis of chiral amines using ω-aminotransferases, Biotechnol. Bioeng. 108 (2011) 253-263.
[55] J.-S. Shin, B.-G. Kim, Kinetic modeling of ω-transamination for enzymatic kinetic resolution of α-methylbenzylamine, Biotechnol. Bioeng. 60 (1998) 534-540.
[56] C.K. Savile, J.M. Janey, E.C. Mundorff, J.C. Moore, S. Tam, W.R. Jarvis, J.C. Colbeck, A. Krebber, F.J. Fleitz, J. Brands, P.N. Devine, G.W. Huisman, G.J. Hughes, Biocatalytic asymmetric synthesis of chiral amines from ketones applied to sitagliptin manufacture, Science 329 (2010) 305-309.
[57] K. Hirotsu, M. Goto, A. Okamoto, I. Miyahara, Dual substrate recognition of aminotransferases, Chem. Rec. 5 (2005) 160-172.
[58] N.N. Samsonova, S.V. Smirnov, I.B. Altman, L.R. Ptitsyn, Molecular cloning and characterization of *Escherichia coli* K12 ygjG gene, BMC Microbiol. 3 (2003) 2.
[59] H. Kack, J. Sandmark, K. Gibson, G. Schneider, Y. Lindqvist, Crystal structure of diaminopelargonic acid synthase: evolutionary relationships between pyridoxal-5'-phosphate-dependent enzymes, J. Mol. Biol. 291 (1999) 857-876.
[60] P. Storici, D. De Biase, F. Bossa, S. Bruno, A. Mozzarelli, C. Peneff, R.B. Silverman, T. Schirmer, Structures of γ-aminobutyric acid (GABA) aminotransferase, a pyridoxal 5'-phosphate, and [2Fe-2S] cluster-containing enzyme, complexed with γ-ethynyl-GABA and with the antiepilepsy drug vigabatrin, J. Biol. Chem. 279 (2004) 363-373.
[61] F.W. Alexander, E. Sandmeier, P.K. Mehta, P. Christen, Evolutionary relationships among pyridoxal-5′ -phosphate-dependent enzymes, Eur. J. Biochem. 219 (1994) 953-960.
[62] A.I. Denesyuk, K.A. Denessiouk, T. Korpela, M.S. Johnson, Functional attributes of the phosphate group binding cup of pyridoxal phosphate-dependent enzymes, J. Mol. Biol. 316 (2002) 155-172.
[63] A.I. Denesyuk, K.A. Denessiouk, T. Korpela, M.S. Johnson, Phosphate group binding "cup" of PLP-dependent and non-PLP-dependent enzymes: leitmotif and variations, Biochim. Biophys. Acta 1647 (2003) 234-238.
[64] T. Yano, S. Kuramitsu, S. Tanase, Y. Morino, H. Kagamiyama, Role of Asp222 in the catalytic mechanism of *Escherichia coli* aspartate aminotransferase: the amino acid residue which enhances the function of the enzyme-bound coenzyme pyridoxal 5'-phosphate, Biochemistry 31 (1992) 5878-5887.

[65] K.S. Midelfort, R. Kumar, S. Han, M.J. Karmilowicz, K. McConnell, D.K. Gehlhaar, A. Mistry, J.S. Chang, M. Anderson, A. Villalobos, J. Minshull, S. Govindarajan, J.W. Wong, Redesigning and characterizing the substrate specificity and activity of *Vibrio fluvialis* aminotransferase for the synthesis of imagabalin, Protein Eng. Des. Sel. 26 (2013) 25-33.
[66] T. Yano, T. Mizuno, H. Kagamiyama, A hydrogen-bonding network modulating enzyme function: asparagine-194 and tyrosine-225 of *Escherichia coli* aspartate aminotransferase, Biochemistry 32 (1993) 1810-1815.
[67] M. Chan-Huot, A. Dos, R. Zander, S. Sharif, P.M. Tolstoy, S. Compton, E. Fogle, M.D. Toney, I. Shenderovich, G.S. Denisov, H.H. Limbach, NMR studies of protonation and hydrogen bond states of internal aldimines of pyridoxal 5'-phosphate acid-base in alanine racemase, aspartate aminotransferase, and poly-L-lysine, J Am Chem Soc 135 (2013) 18160-18175.
[68] H.J. Cha, J.H. Jeong, C. Rojviriya, Y.G. Kim, Structure of putrescine aminotransferase from *Escherichia coli* provides Insights into the substrate specificity among class-III aminotransferases, PLoS One 9 (2014) e113212.
[69] W. Liu, P.E. Peterson, R.J. Carter, X. Zhou, J.A. Langston, A.J. Fisher, M.D. Toney, Crystal structures of unbound and aminooxyacetate-bound *Escherichia coli* γ-aminobutyrate aminotransferase, Biochemistry 43 (2004) 10896-10905.
[70] R. Contestabile, S. Angelaccio, R. Maytum, F. Bossa, R.A. John, The contribution of a conformationally mobile, active site loop to the reaction catalyzed by glutamate semialdehyde aminomutase, J. Biol. Chem. 275 (2000) 3879-3886.
[71] J. Stetefeld, M. Jenny, P. Burkhard, Intersubunit signaling in glutamate-1-semialdehyde-aminomutase, Proc. Natl. Acad. Sci. U.S.A. 103 (2006) 13688-13693.
[72] P. Storici, G. Capitani, R. Muller, T. Schirmer, J.N. Jansonius, Crystal structure of human ornithine aminotransferase complexed with the highly specific and potent inhibitor 5-fluoromethylornithine, J. Mol. Biol. 285 (1999) 297-309.
[73] J.S. Shin, B.G. Kim, Exploring the active site of amine:pyruvate aminotransferase on the basis of the substrate structure-reactivity relationship: how the enzyme controls substrate specificity and stereoselectivity, J. Org. Chem. 67 (2002) 2848-2853.
[74] B.-Y. Hwang, B.-K. Cho, H. Yun, K. Koteshwar, B.-G. Kim, Revisit of aminotransferase in the genomic era and its application to biocatalysis, J. Mol. Catal. B: Enzym. 37 (2005) 47-55.
[75] A. Lyskowski, C. Gruber, G. Steinkellner, M. Schurmann, H. Schwab, K. Gruber, K. Steiner, Crystal structure of an (*R*)-selective ω-transaminase from *Aspergillus terreus*, PLoS One 9 (2014) e87350.
[76] C. Sayer, M.N. Isupov, A. Westlake, J.A. Littlechild, Structural studies of *Pseudomonas* and *Chromobacterium* ω-aminotransferases provide insights into their differing substrate specificity, Acta Crystallogr. D Biol. Crystallogr. 69 (2013) 564-576.
[77] R.N. Rodionov, N. Jarzebska, N. Weiss, S.R. Lentz, AGXT2: a promiscuous aminotransferase, Trends Pharm. Sci. 35 (2014) 575–582.
[78] R. Voellym, T. Leisinger, Role of 4-aminobutyrate aminotransferase in the arginine metabolism of *Pseudomonas aeruginosa*, J. Bacteriol. 128 (1976) 722-729.
[79] G.G. Wybenga, C.G. Crismaru, D.B. Janssen, B.W. Dijkstra, Structural determinants of the β-selectivity of a bacterial aminotransferase, J. Biol. Chem. 287 (2012) 28495-28502.
[80] S.A. Ahmed, N. Esaki, H. Tanaka, K. Soda, L-α-amino-β-thio-ϵ-caprolactam, a new sulfur-containing substrate for α-amino-ϵ-caprolactam racemase, FEBS Lett. 174 (1984) 76-79.

[81] S.A. Ahmed, N. Esaki, H. Tanaka, K. Soda, Mechanism of α-amino-ϵ-caprolactam racemase reaction, Biochemistry 25 (1986) 385-388.
[82] S. Okazaki, A. Suzuki, T. Mizushima, T. Kawano, H. Komeda, Y. Asano, T. Yamane, The novel structure of a pyridoxal 5'-phosphate-dependent fold-type I racemase, α-amino-ϵ-caprolactam racemase from *Achromobacter obae*, Biochemistry 48 (2009) 941-950.
[83] Y. Mutaguchi, T. Ohmori, T. Wakamatsu, K. Doi, T. Ohshima, Identification, purification, and characterization of a novel amino acid racemase, isoleucine 2-epimerase, from *Lactobacillus* species, J. Bacteriol. 195 (2013) 5207-5215.
[84] M. Veiga-da-Cunha, F. Hadi, T. Balligand, V. Stroobant, E. Van Schaftingen, Molecular identification of hydroxylysine kinase and of ammoniophospholyases acting on 5-phosphohydroxy-L-lysine and phosphoethanolamine, J. Biol. Chem. 287 (2012) 7246-7255.
[85] D. Schiroli, S. Cirrincione, S. Donini, A. Peracchi, Strict reaction and substrate specificity of AGXT2L1, the human *O*-phosphoethanolamine phospho-lyase, IUBMB Life 65 (2013) 645-650.
[86] A. Paiardini, F. Bossa, S. Pascarella, Evolutionarily conserved regions and hydrophobic contacts at the superfamily level: The case of the fold-type I, pyridoxal-5'-phosphate-dependent enzymes, Protein Sci. 13 (2004) 2992-3005.
[87] P. Kongsaeree, C. Samanchart, P. Laowanapiban, S. Wiyakrutta, V. Meevootisom, Crystallization and preliminary X-ray crystallographic analysis of D-phenylglycine aminotransferase from *Pseudomonas stutzeri* ST201, Acta Crystallogr. D Biol. Crystallogr. 59 (2003) 953-954.
[88] C.S. Vandenende, M. Vlasschaert, S.Y. Seah, Functional characterization of an aminotransferase required for pyoverdine siderophore biosynthesis in *Pseudomonas aeruginosa* PAO1, J. Bacteriol. 186 (2004) 5596-5602.
[89] M. Hennig, B. Grimm, R. Contestabile, R.A. John, J.N. Jansonius, Crystal structure of glutamate-1-semialdehyde aminomutase: an α_2-dimeric vitamin B6-dependent enzyme with asymmetry in structure and active site reactivity, Proc. Natl. Acad. Sci. U.S.A. 94 (1997) 4866-4871.
[90] S. Heinl, D. Hartinger, M. Thamhesl, G. Schatzmayr, W.D. Moll, R. Grabherr, An aminotransferase from bacterium ATCC 55552 deaminates hydrolyzed fumonisin B(1), Biodegradation 22 (2010) 25-30.
[91] Z. Marković-Housley, T. Schirmer, E. Hohenester, A.R. Khomutov, R.M. Khomutov, M.Y. Karpeisky, E. Sandmeier, P. Christen, J.N. Jansonius, Crystal structures and solution studies of oxime adducts of mitochondrial aspartate aminotransferase, Eur. J. Biochem. 236 (1996) 1025-1032.
[92] P. Storici, G. Capitani, D. De Biase, M. Moser, R.A. John, J.N. Jansonius, T. Schirmer, Crystal structure of GABA-aminotransferase, a target for antiepileptic drug therapy, Biochemistry 38 (1999) 8628-8634.
[93] A. Perret, C. Lechaplais, S. Tricot, N. Perchat, C. Vergne, C. Pellé, K. Bastard, A. Kreimeyer, D. Vallenet, A. Zaparucha, A novel acyl-CoA beta-transaminase characterized from a metagenome, PLoS One 6 (2011) e22918.
[94] A. Hirayama, T. Eguchi, F. Kudo, A single PLP-dependent enzyme PctV catalyzes the transformation of 3-dehydroshikimate into 3-aminobenzoate in the biosynthesis of pactamycin, Chembiochem 14 (2013) 1198-1203.
[95] N.R. Williamson, H.T. Simonsen, R.A. Ahmed, G. Goldet, H. Slater, L. Woodley, F.J. Leeper, G.P. Salmond, Biosynthesis of the red antibiotic, prodigiosin, in *Serratia*: identification of a novel 2-methyl-3-n-amyl-pyrrole (MAP) assembly pathway, definition of the terminal condensing enzyme, and implications for undecylprodigiosin biosynthesis in *Streptomyces*, Mol. Microbiol.. 56 (2005) 971-989.

[96] X. Lou, T. Ran, N. Han, Y. Gao, J. He, L. Tang, D. Xu, W. Wang, Crystal structure of the catalytic domain of PigE: A transaminase involved in the biosynthesis of 2-methyl-3-n-amyl-pyrrole (MAP) from *Serratia sp*. FS14, Biochem. Biophys. Res. Commun. 447 (2014) 178-183.
[97] C.E. Pugh, J.L. Harwood, R.A. John, Mechanism of glutamate semialdehyde aminotransferase. Roles of diamino- and dioxo-intermediates in the synthesis of aminolevulinate, J. Biol. Chem. 267 (1992) 1584-1588.
[98] Y. Kontani, M. Kaneko, M. Kikugawa, S. Fujimoto, N. Tamaki, Identity of D-3-aminoisobutyrate-pyruvate aminotransferase with alanine-glyoxylate aminotransferase 2, Biochim. Biophys. Acta 1156 (1993) 161-166.
[99] D. Hartinger, H. Schwartz, C. Hametner, G. Schatzmayr, D. Haltrich, W.-D. Moll, Enzyme characteristics of aminotransferase FumI of *Sphingopyxis sp. MTA144* for deamination of hydrolyzed fumonisin B1, Appl. Microbiol. Biotechnol. 91 (2011) 757-768.
[100] H. Leslie, W.D. Paul, Z. Lishan, Transaminases, deaminases and aminomutases and compositions and methods for enzymatic detoxification, Google Patents, 2004.
[101] G. Bailey, W. Dempsey Purification and properties of an α-dialkyl amino acid transaminase, Biochemistry 6 (1967) 1526–1533.
[102] S. Sun, R.F. Zabinski, M.D. Toney, Reactions of alternate substrates demonstrate stereoelectronic control of reactivity in dialkylglycine decarboxylase, Biochemistry 37 (1998) 3865-3875.
[103] G.L. Stoner, M.A. Eisenberg, Purification and properties of 7, 8-diaminopelargonic acid aminotransferase, J. Biol. Chem. 250 (1975) 4029-4036.
[104] S.W. Van Arsdell, J.B. Perkins, R.R. Yocum, L. Luan, C.L. Howitt, N. Prasad Chatterjee, J.G. Pero, Removing a bottleneck in the Bacillus subtilis biotin pathway: BioA utilizes lysine rather than S-adenosylmethionine as the amino donor in the KAPA-to-DAPA reaction, Biotechnol Bioeng. 91 (2005) 75-83.
[105] S. Dey, J.M. Lane, R.E. Lee, E.J. Rubin, J.C. Sacchettini, Structural characterization of the Mycobacterium tuberculosis biotin biosynthesis enzymes 7, 8-diaminopelargonic acid synthase and dethiobiotin synthetase, Biochemistry 49 (2010) 6746-6760.
[106] Z.D. Aron, P.C. Dorrestein, J.R. Blackhall, N.L. Kelleher, C.T. Walsh, Characterization of a New tailoring domain in polyketide biogenesis: The amine transferase domain of MycA in the mycosubtilin gene cluster, J. Am. Chem. Soc. 127 (2005) 14986-14987.

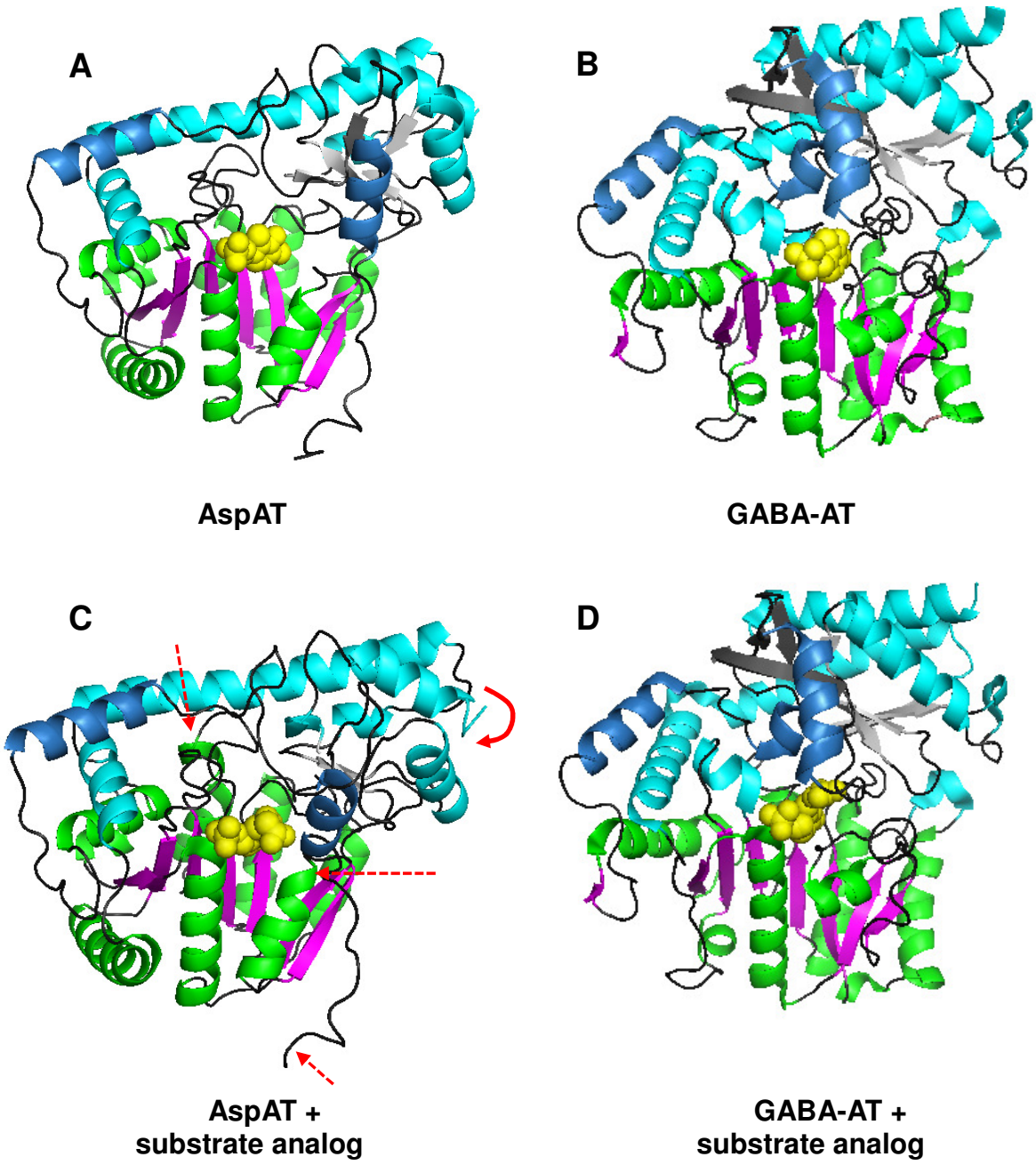
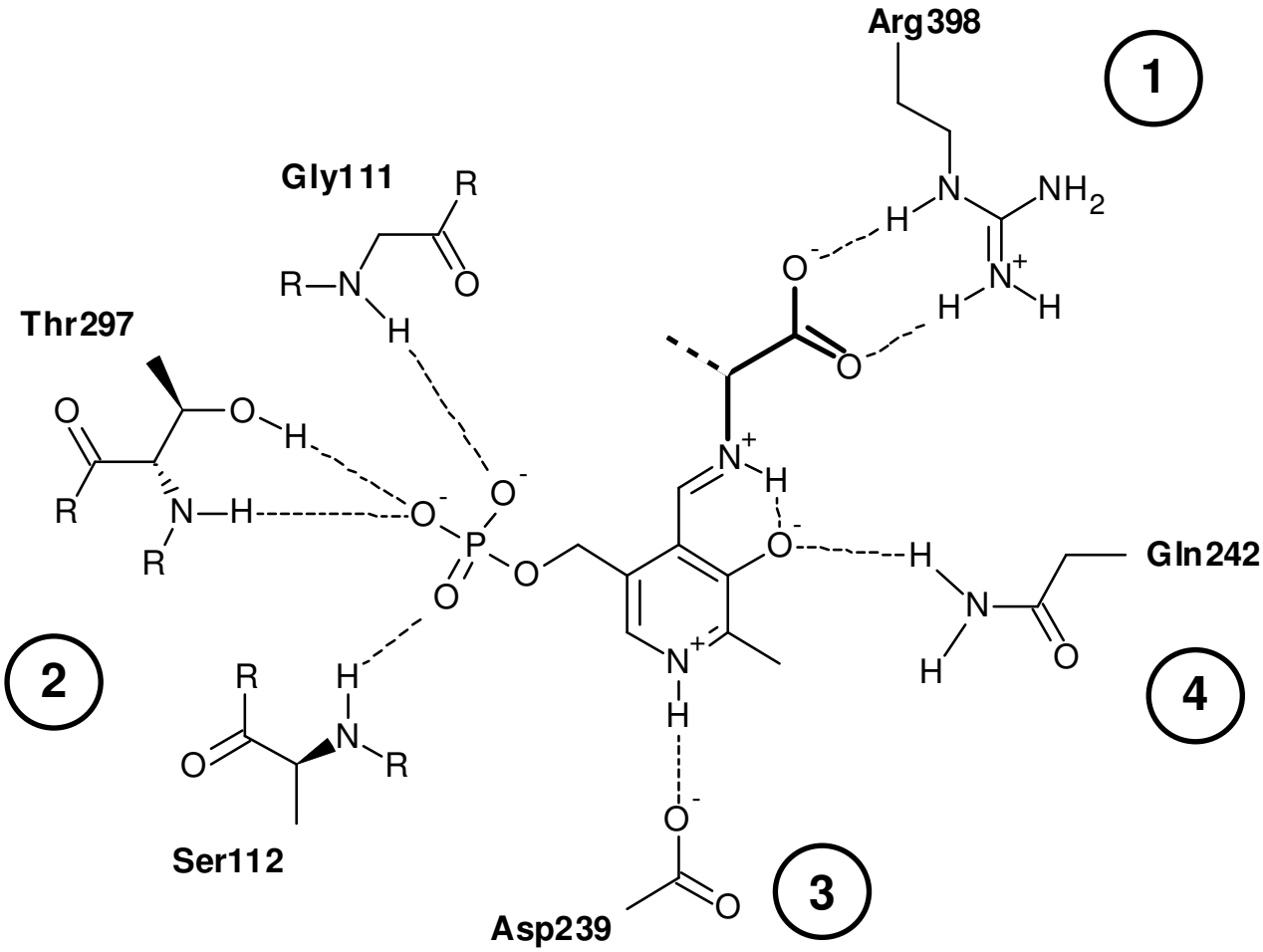

A
B
AspAT
GABA-AT
C
D
AspAT +
substrate analog
GABA-AT +
substrate analog

Figure 1



Enzyme	PDB ID	Position			
		1	2	3	4
GABA-AT	1SFF	R	GST	D	Q
OAT	2OAT	R	GVT	D	Q
ACOAT	1WKG	R	GST	D	Q
SOAT	4ADD	R	GST	D	Q
LAT	2CJD	R	GAT	D	Q
GSA	2GSA	S	GST	D	N*
DGDA	1D7V	R	GTT	D	Q
DAPA-AT	1QJ3	R	GST	D	-
β-AT	2YKU	R	GTT	D	-
ω-PAT	4B98	R	GST	D	-
AH6L-R	3DXV	E°	GST	D	-

Figure 2

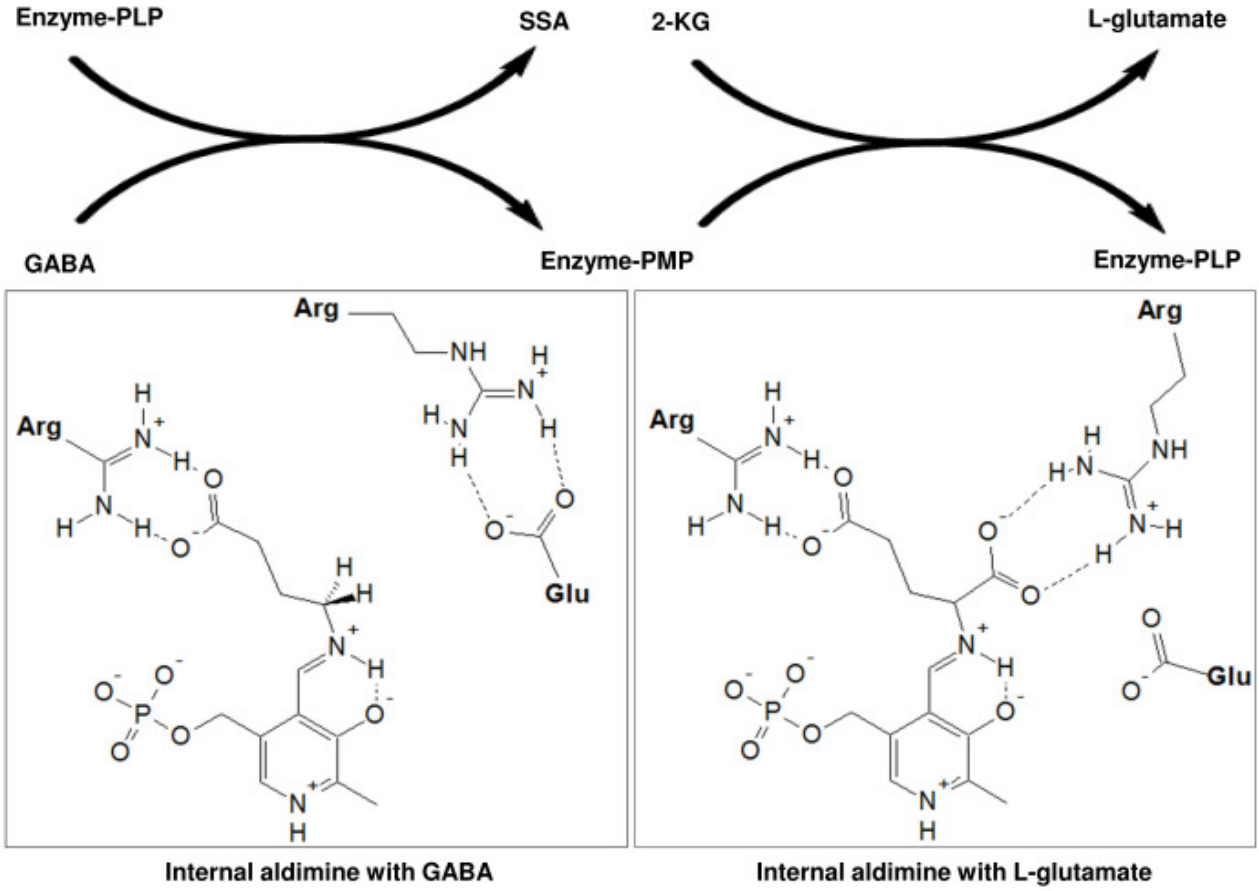

Enzyme-PLP
SSA
2-KG
L-glutamate
GABA
Enzyme-PMP
Enzyme-PLP
Arg
Glu
Internal aldimine with GABA
Internal aldimine with L-glutamate

Figure 3

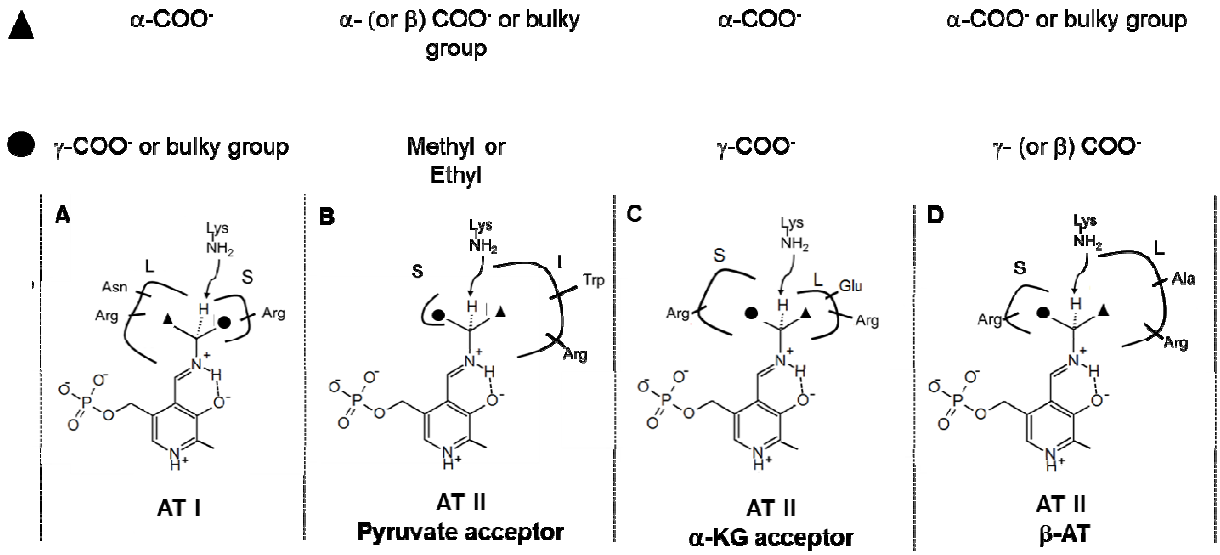

α-COO⁻
α- (or β) COO⁻ or bulky group
α-COO⁻
α-COO⁻ or bulky group
γ-COO⁻ or bulky group
Methyl or Ethyl
γ-COO⁻
γ- (or β) COO⁻
A
B
C
D
Lys NH2
Asn
Arg
Trp
Glu
Ala
AT I
AT II
Pyruvate acceptor
AT II
α-KG acceptor
AT II
β-AT

Figure 4

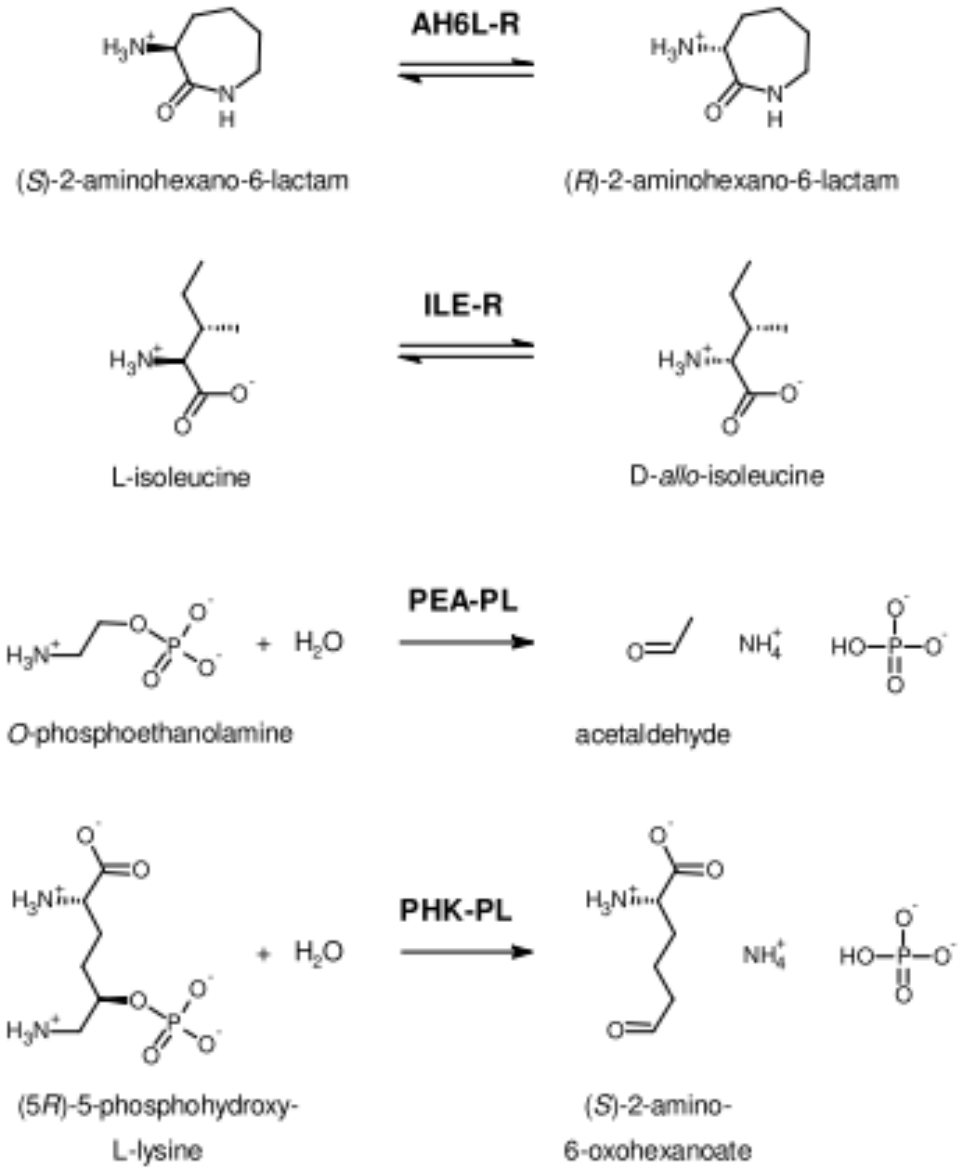

AH6L-R
(S)-2-aminohexano-6-lactam
(R)-2-aminohexano-6-lactam
ILE-R
L-isoleucine
D-allo-isoleucine
PEA-PL
+ H2O
O-phosphoethanolamine
acetaldehyde
PHK-PL
+ H2O
(5R)-5-phosphohydroxy-
L-lysine
(S)-2-amino-
6-oxohexanoate

Figure 5

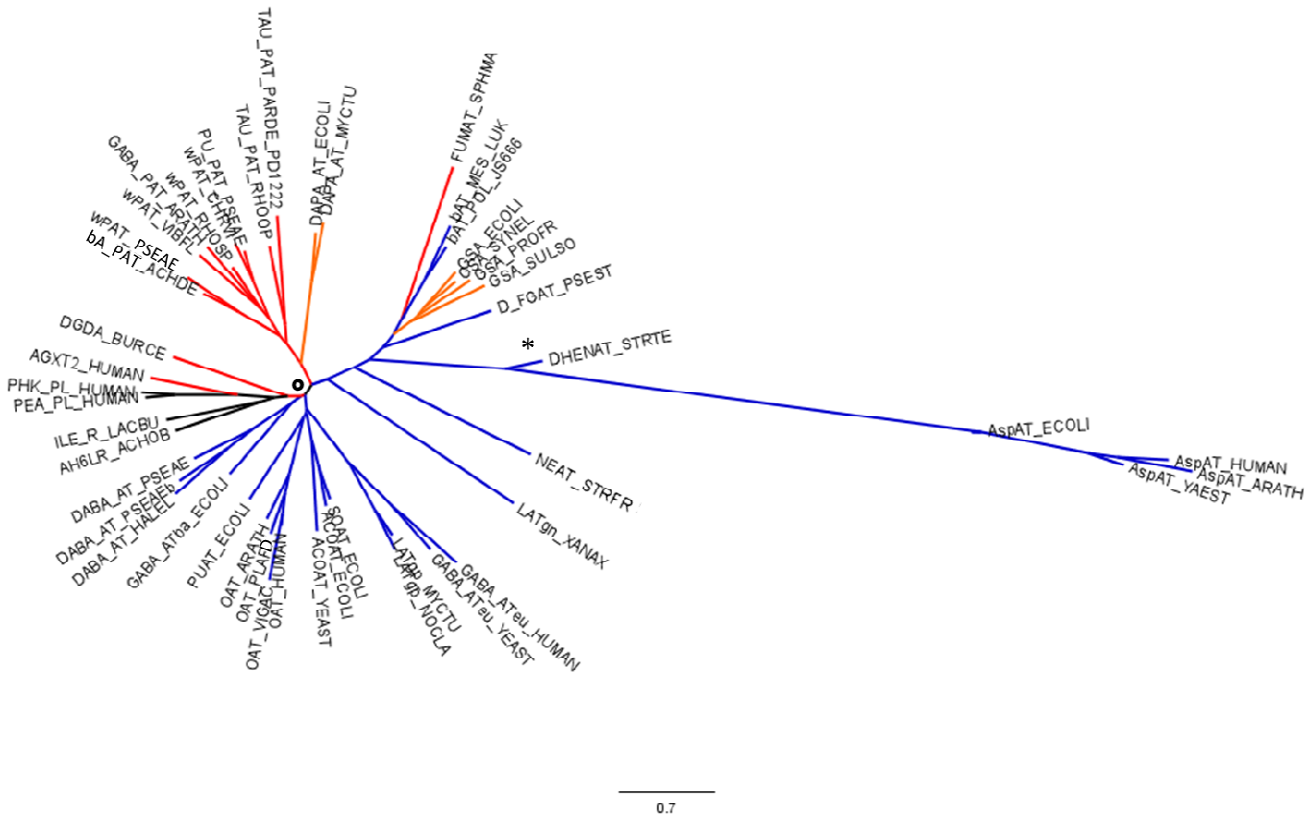

TAU_PAT_PARDE_PD1222
TAU_PAT_RHIOP
PU_PAT_PSEAE
GABA_PAT_AGRTU
WPAT_ECHVR
WPAT_PHOSP
WPAT_VIBFL
WPAT_PSEAE
BA_PAT_ACHDE
DGDA_BURCE
AGXT2_HUMAN
PHK_PL_HUMAN
PEA_PL_HUMAN
ILE_R_LACBU
AH6LR_ACHOB
DABA_AT_PSEAE
DABA_AT_PSEFL
DABA_AT_HALEL
GABA_ATba_ECOLI
PUAT_ECOLI
OAT_ARATH
OAT_PLAFD
OAT_HUMAN
OAT_VIGAC
ACOAT_YEAST
RCOAT_ECOLI
DABA_AT_ECOLI
DABA_AT_MYCTU
FUMAT_SPHMA
BA_PAT_MES_LUK
BA_PAT_OL_IS686
GSA_ECOLI
GSA_SYNEL
GSA_PROFR
GSA_SULSO
D_FOAT_PSEST
DHENAT_STRTE
AspAT_ECOLI
AspAT_HUMAN
AspAT_YAEST
AspAT_ARATH
NEAT_STRFR
LATgn_XANAX
LATgn_MYCTU
LATgn_NOCLA
GABA_ATeu_HUMAN
GABA_ATeu_YEAST
0.7

Figure 6