

Amidohydrolase Superfamily

Liu Aimin, *University of Mississippi Medical Center, Jackson, Mississippi, USA*

Li Tingfeng, *University of Mississippi Medical Center, Jackson, Mississippi, USA*

Fu Rong, *University of Mississippi Medical Center, Jackson, Mississippi, USA*

The amidohydrolase superfamily is a structure-based cluster of enzymes that contain a sturdy and versatile triosephosphate isomerase (TIM)-like β -barrel embracing a mono- or dinuclear *d*-block metal cofactor essential for catalysis. Up to date, it has had several thousand members catalysing a wide range of hydrolytic and nonhydrolytic metabolic reactions important in amino acid and nucleotide metabolism as well as biodegradation of agricultural and industrial compounds.

Introduction

The concept of the 'amidohydrolase superfamily' was introduced by Holm and Sander. The striking similarities of the three-dimensional structures of adenosine deaminase (ADA), phosphotriesterase (PTE) and urease (URE) inspired the unification of a broad set of *d*-block metal-dependent hydrolase enzymes into a unique enzyme superfamily (Holm and Sander, 1997). From its inception, the term 'amidohydrolase superfamily' has referred to a structural relationship among the metal-dependent proteins that contain a triosephosphate isomerase (TIM)-like barrel fold in the catalytic domain.

The amidohydrolase superfamily is more than just a group of hydrolase enzymes. It is a functionally diverse enzyme group. Members of this enzyme superfamily catalyse the cleavage of not only C–N but also C–C, C–O, C–Cl, C–S and O–P bonds of organic compounds. Most of the characterized members of the amidohydrolase superfamily are important enzymes for histidine and tryptophan metabolism, *de novo* biosynthesis of purine and pyrimidine nucleotides, and biodegradation of agricultural and industrial materials including rubber chemicals, herbicides, leather, paper and others. Some members of this enzyme superfamily are medically relevant. For instance, ADA catalyses ammonia elimination from the heterocyclic nitrogenous base of the substrate. The deficiency of this enzyme is linked to a common form of severe combined immunodeficiency (SCID). Since the superfamily is not function-designated, an enzyme with amidohydrolase in its name is not necessarily a member of the amidohydrolase superfamily. For instance, penicillin amidohydrolase (also known as penicillin acylase, EC 3.5.1.11) contains neither a catalytic metal ion nor a TIM-barrel domain; thus, it is not a member of the amidohydrolase superfamily. **See also:** Biodegradation of Organic Pollutants

In structure-based protein databases such as SCOP (<http://scop.mrc-lmb.cam.ac.uk/scop>), the amidohydrolase superfamily is a large 'metal-dependent hydrolase' protein group under the 'alpha and beta proteins (α/β)' clan. The

amidohydrolase superfamily contains three related subfamilies in the Pfam database annotation (Bateman *et al.*, 2004). The Amidohydrolase_1 subset (accession number: PF01979) contains a large group of 2812 protein sequences (<http://pfam.janelia.org>). Its members catalyse the hydrolysis of a wide range of substrates bearing amide, ester, halogen or other functional groups at carbon and phosphorus centres. This family includes well-characterized enzymes such as ADA, PTE, URE, cytosine deaminase (CDA), *D*-amino acid deacetylase (AAD), dihydroorotase (DHO), hydantoinase (HYD), *N*-acetylglucosamine-6-phosphate deacetylase (AGD) and renal dipeptidase (RDP). Among them, DHO and URE also belong to MEROPS peptidase family M38 (β -aspartyl dipeptidase, clan MJ), where they are classified as nonpeptidase homologues.

The Amidohydrolase_2 subset (accession number: PF04909) contains 771 aligned protein sequences. These proteins are related to the metal-dependent hydrolases but they appear to be either structurally or functionally divergent from the Amidohydrolase_1 group. Members of this branch are known to catalyse nonhydrolytic reactions including decarboxylation and hydration (Liu and Zhang, 2006). The prototypic member of the Amidohydrolase_2 is α -amino- β -carboxymuconate- ϵ -semialdehyde decarboxylase (ACMSD, EC 4.1.1.45), which converts α -amino- β -carboxymuconate- ϵ -semialdehyde to α -aminomuconate semialdehyde in a nonhydrolytic C–C bond cleavage process. Despite the similar tertiary structures, it is not clear if members of the Amidohydrolase_2 subset have arisen from a single evolutionary origin with those in the Amidohydrolase_1 because of their very low overall sequence similarities.

The Amidohydrolase_3 subset (accession number: PF07969) consists of 868 protein sequences, most of which are hypothetical proteins. Some, including formyltransferase/hydrolase complex Fhc subunit A, are amidohydrolase-like enzymes. This branch of the amidohydrolase superfamily contains the most diverse set of sequences, including a high proportion of outlier sequences that have only low levels of sequence identity to their closest superfamily relatives.

Advanced article

Article Contents

- Introduction
- Structure
- Structural Diversity: Imperfect Barrel
- One-fold, Numerous Reactions
- Enzyme Mechanisms
- Concluding Remarks

doi: 10.1002/9780470015902.a0020546

Structure

Members of the amidohydrolase superfamily consist of a central $(\beta/\alpha)_8$ barrel in which eight parallel β strands flanked on the outer face by eight α helices (Figure 1). This gives rise to a prominent architectural feature: a sturdy pocket with an internal cavity adjacent to the metal cofactor. The pocket has a propeller-like shape made by the eight β strands with a depth of around 15–18 Å and a diameter of about 14–16 Å. The pocket is a parallel β -barrel that forms a compact thermostable core. The $(\beta/\alpha)_8$ -barrel fold is a common structural platform found in about 10% of all proteins with known three-dimensional structures and in about 30 superfamilies in the SCOP database (<http://scop.mrc-lmb.cam.ac.uk/scop>). It is also known as the TIM-barrel fold because it was first defined from the three-dimensional structure of TIM. Unlike most of the other enzyme superfamilies that contain a TIM-barrel fold, members of the amidohydrolase superfamily utilize a *d*-block metal cofactor with either one or two metal ions in conjunction with the TIM-barrel for catalysis. Figure 1 shows an example of the TIM-barrel along with a mononuclear metal cofactor. There are currently 18 functionally annotated unique members of the amidohydrolase

superfamily for which high-resolution X-ray crystal structures are available (Table 1).

Biochemical studies on the amidohydrolase superfamily have revealed that the metal cofactor is catalytically essential (Seibert and Raushel, 2005). The metal cofactor is located at the opening of the barrel near the C-terminal ends of several β strands, coordinated by several histidine, aspartic acid and in some occasions cysteine residues of the β strands and $\beta\alpha$ loops. The metal-containing site is referred to as the catalytic face of the barrel, in contrast to the stabilizing face of the opposite end of the barrel (Sermer and Höcker, 2005). To accommodate the metal centre and substrates, the catalytic face often has a wider width than the stabilizing face, consequently making the other side of the barrel appear more compressed. Thus, the barrel sometimes looks like a conoid.

The most common metal in this enzyme superfamily is a divalent zinc ion or a dinuclear Zn_2 pair. However, transition metals, such as divalent iron, nickel and manganese ions, are also observed in amidohydrolase enzymes. Cobalt ion can often be substituted for zinc *in vitro*, producing a comparable or even higher level of enzyme activity. Non *d*-block metal ions, such as Mg^{2+} and Ca^{2+} , are usually unable to perform the biochemical functions at the enzyme

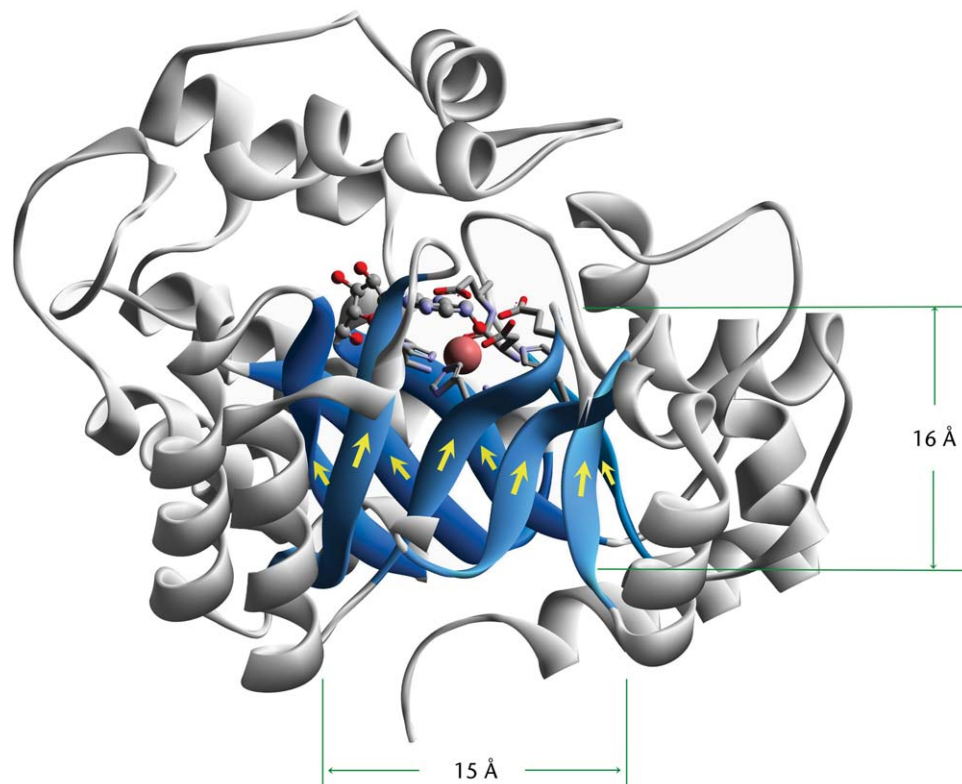


Figure 1 Metal cofactor and the TIM-like parallel barrel core in adenosine deaminase (ADA, from PDB file 1a4m). The metal ion is depicted as CPK sphere, Metal ligands are shown in sticks and the propeller structural fold is highlighted in blue colour in the representation. Substrate analogue 6-hydroxy-1,6-dihydro purine nucleoside is represented in scaled ball and stick. The fifth α helix covering the segment of residues 223–228 is represented transparent for uncovering the β strands. The ADA is a homotetramer in the structure; only the subunit A is shown.

Table 1 Functionally annotated and structurally characterized members of the amidohydrolase superfamily

Enzyme	Abbreviation	E.C. number	PDB code
α -Amino- β -carboxymuconate- ϵ -semialdehyde decarboxylase	ACMSD	4.1.1.45	2hbv
Adenosine deaminase	ADA	3.5.4.4	1a4m
Adenine deaminase	ADase	3.5.4.2	2ics
<i>N</i> -acetylglucosamine-6-phosphate deacetylase	AGD	3.5.1.25	1o12
Allantoate amidohydrolase	ATase	3.5.2.5	1z2l
Adenosine 5'-monophosphate deaminase	AMPD	3.5.4.6	2a3l
Cytosine deaminase	CDA	3.5.4.1	1k6w
D-Aminoacylase	DAA	3.5.1.81	1m7j
Dihydroorotase	DHO	3.5.2.3	1j79
Dihydropyrimidinase (L-hydantoinase) ^a	DHPase	3.5.2.2	1gkr
Guanine deaminase	GAH	3.5.4.3	1wkq
D-Hydantoinase	HYD	3.5.2.2	1nfg
Isoaspartyl dipeptidase	IAD	3.4.19.5	1onw
Imidazolonepropionase	IPase	3.5.2.7	2bb0
Phosphotriesterase	PTE	3.1.8.1	1hzy
Renal dipeptidase	RDP	3.4.13.11	1itq
γ -Resorcyate decarboxylase	RSD	4.1.1.44	2dvt
Urease	URE	3.5.1.5	2ubp

^aAlthough DHPase and HYD have the same E.C. number, they are different enzymes and have different substrate specificities. DHPase enzymes (including L-hydantoinase) catalyse the reversible hydrolytic ring opening of six- or five-membered cyclic diamides such as dihydropyrimidines and 5'-monosubstituted hydantoins to the corresponding 3-ureido acids and carbamoyl amino acids, respectively. HYD is an industrial enzyme that is widely used in the production of D-amino acids which are precursors for synthesis of antibiotics, peptides and pesticides.

active site, suggesting that the role of the *d*-block metal cofactor is beyond simply providing a cationic charge for substrate binding.

The metal cofactor is tethered to the protein through a few protein residues contributed by the β strands. There are several types of active site structural architecture known in this enzyme superfamily, and the origin of the metal ligands is a valuable tool to divide the enzyme superfamily into seven subgroups (Seibert and Raushel, 2005). A mononuclear cofactor requires four or five ligands from the β strands, while a dinuclear cofactor demands five or six protein ligands. One notable structural feature is that at least one water molecule or water-derived hydroxide is coordinated to the metal ion(s) in the enzyme structures. The solvent-derived ligand generally remains bound to the metal in the enzyme–substrate complex, but it is replaced by substrate on some occasions such as 2,6-dihydroxybenzoate (γ resorcyate) decarboxylase (RSD) (Goto *et al.*, 2006).

For a mononuclear metal cofactor of this enzyme superfamily, the fifth β strand invariably provides a histidine ligand for the metal coordination. The sixth β strand denotes a histidine residue either as a metal ligand or a non-ligand but an important catalytic component at the enzyme active site. These two histidine residues are the signature amino acids conserved across the amidohydrolase superfamily, regardless it is a mononuclear or a dinuclear metal cofactor bound at the active site of the enzyme. It is common for the first and/or eighth β strands to add more protein ligands for metal coordination. An aspartic acid residue from the eighth β strand in many amidohydrolase

enzymes plays a dual role, i.e. acid/base catalyst and metal ligand.

The two divalent metals are separated by ~ 3.6 Å in the dinuclear metal cofactor. The more buried metal cation is coordinated to two histidine residues from β strand 1 and an aspartate from β strand 8. The more solvent-exposed metal ion is ligated to protein through two imidazole side chains of histidine from β strands 5 and 6 (Seibert and Raushel, 2005). The two divalent metal ions are bridged by a hydroxide in addition to a carbamate functional group originating from the posttranslational modification of a lysine residue from β strand 4 in the presence of bicarbonate. In some cases, an unmodified glutamic acid from β strand 4 or a cysteine from β strand 2 is employed as a bridge instead of the modified lysine.

Until now, the characterized dinuclear amidohydrolase enzymes have a homogeneous metal preference. However, some mixed metal cofactors may exist. Enamidase, for instance, is a bifunctional enzyme belonging to the amidohydrolase family that mediates hydrolysis of 1,4,5,6-tetrahydro-6-oxonicotinate to ammonia and (*S*)-2-formylglutarate; it reportedly contains a Fe–Zn cofactor (Alhapel *et al.*, 2006) although the three-dimensional structure of this enzyme remains to be determined.

Other than the histidine residues from the fifth and sixth β strands, which are conserved throughout the entire family, the remaining metal ligands exhibit some variations. For example, a HxH metal-binding motif is commonly seen in strand 1 of the β -barrel, but HxD and ExH have been observed from the ACMSD protein subfamily of

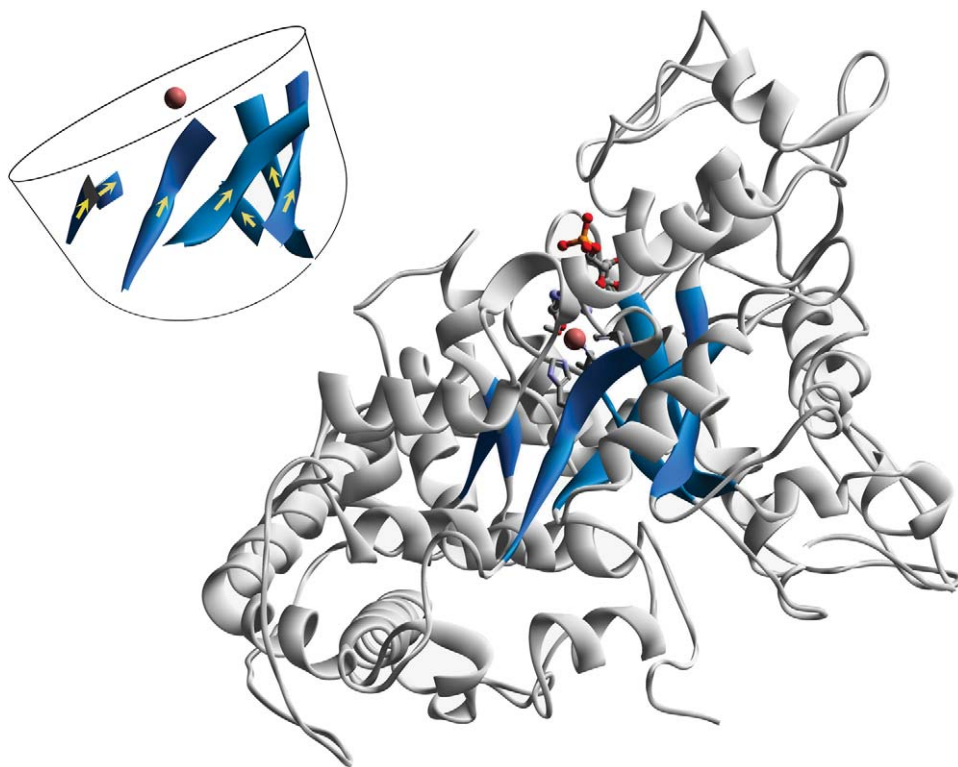


Figure 2 An imperfect TIM-barrel exhibited in the structure of adenosine 5'-monophosphate deaminase (AMPD) in complex with coformycin 5'-phosphate (ball and stick) (from PDB file 2a3 l). The inset highlights the distorted barrel and the zinc ion (CPK sphere).

Amidohydrolase_2 (Liu and Zhang, 2006). Moreover, the metal-binding motif from strand 1 does not always serve as metal ligands. For instance, in the structures of AGD, the HxH motif is present in the active site but it is not ligated directly to the divalent metal.

The overall sequence conservation among the amidohydrolase superfamily is rather low, indicating that the TIM-like barrel fold is not dictated by details of sequences but rather by overall distribution of polar or charged and nonpolar or noncharged residues. Also, it is not unusual that enzymes in this superfamily contain noncatalytic domains in addition to the catalytic domain. Thus, conventional sequence alignments occasionally fail to reveal the general characteristics of a potential new member of the amidohydrolase superfamily. When either the metal-binding motif or the TIM-like barrel is not obvious, an advanced sequence alignment may be required. If this is the case, a secondary structure pattern can be calculated by the PSIPRED protein structure prediction server at <http://bioinf.cs.ucl.ac.uk/psipred> (Bryson *et al.*, 2005). Then a 'secondary structure-based' sequence alignment with those members with known three-dimensional structures may reveal clusters of similar residues at topologically equivalent positions. An example using this strategy was described in a recent study of ACMSD, which successfully predicted that this is a new member of the amidohydrolase superfamily. A site-directed mutagenesis analysis was followed and the results revealed important information concerning the enzyme such

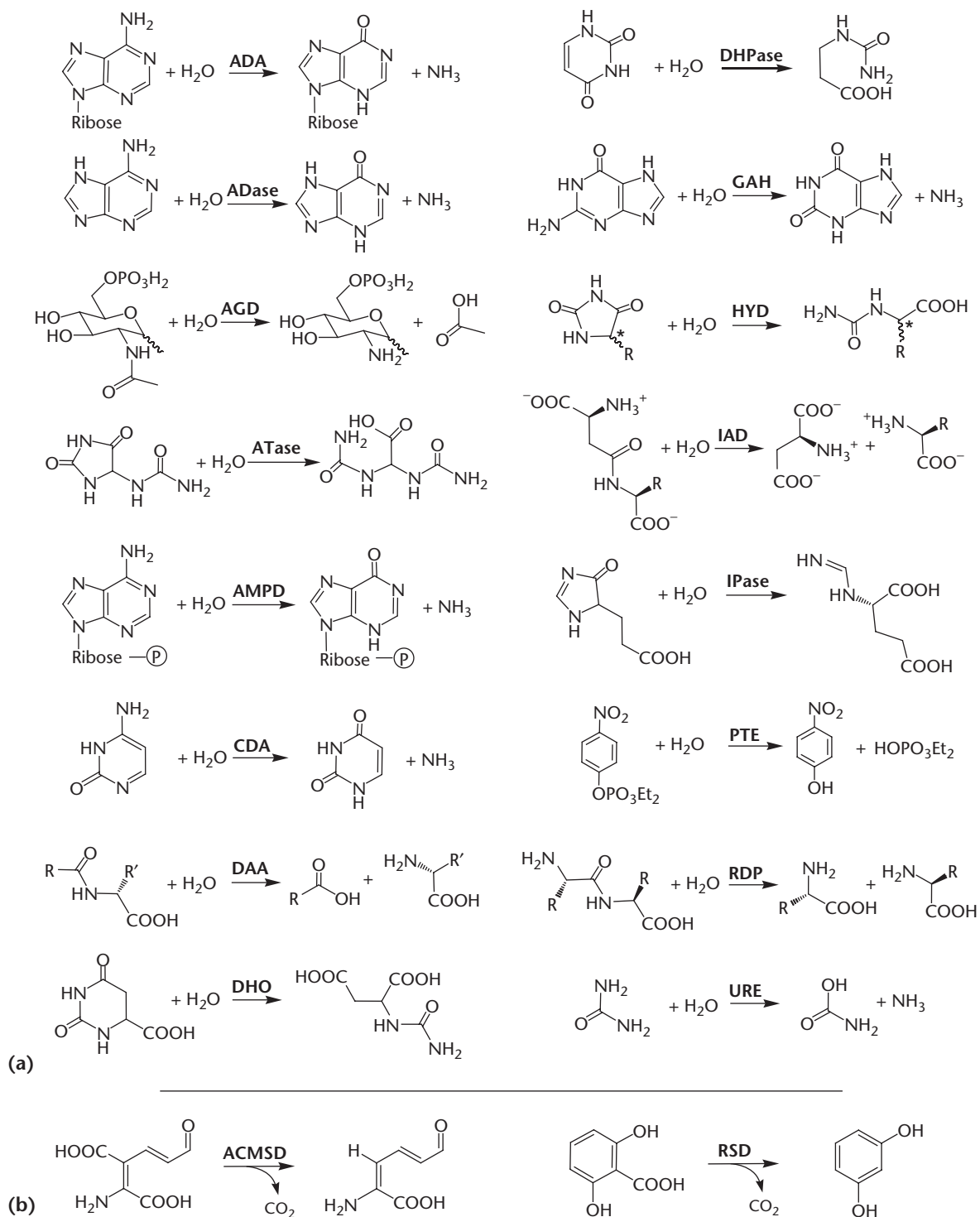
as metal ligand identities and active site residues as well as their possible roles in the catalytic process (Li *et al.*, 2006).

However, one should be cautious in drawing conclusions solely based upon a sequence study. Sequence, and even structural, similarities do not always translate into functional similarities. One extreme example in the functionally diverse amidohydrolase superfamily is the difference between melamine deaminase and atrazine chlorohydrolase. These two enzymes share 98% sequence identity, but catalyse completely different reactions (Seffernick *et al.*, 2001).

Structural Diversity: Imperfect Barrel

About 200 amino acids are required to assemble a TIM-like parallel barrel. To form the barrel, a β strand/ α helix pair will have to repeat eight times and linked by $\beta\alpha$ loops. Occasionally, there is a deletion or an insertion in a strand that causes a single residue interruption of the secondary structure in the amidohydrolase enzymes. It is not unusual for a member of the superfamily to exhibit a distorted propeller barrel or additional insertion domains and loops for specific structural or functional requirements, such as substrate specificity.

Owing to high variability in the lengths of barrel strands and helices, evolution has produced highly distorted (β/α)₈-propeller folds for some special biochemical needs. A small



Scheme 1 Hydrolytic (a) and nonhydrolytic (b) reactions catalysed by the structurally characterized members of the amidohydrolase superfamily.

subset of the amidohydrolase superfamily with imperfect folds has been reported (Han *et al.*, 2006). In the structure of adenosine 5'-monophosphate deaminase (AMPD), six or seven of the eight β strands can be recognized, but the

fifth and/or sixth are replaced by somewhat irregular loop structures, resulting in an apparently imperfect β -barrel (Figure 2). Although the barrel is not composed of eight strands, the catalytic zinc ion is coordinated to an aspartic

acid and three histidine residues, as seen in the active site of other amidohydrolase enzymes (Han *et al.*, 2006).

One-fold, Numerous Reactions

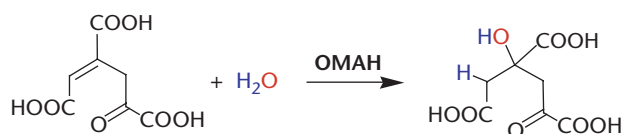
Hydrolytic reactions

Members of the Amidohydrolase_1 subfamily described thus far catalyse only hydrolytic reactions (Scheme 1). There are two classes of hydrolytic reactions that differ in their substrate structural properties. Accordingly, members of the Amidohydrolase-I subfamily may be divided into cyclic amidohydrolase enzymes and noncyclic amidohydrolase enzymes. Cyclic amidohydrolase enzymes catalyse the hydrolysis of cyclic C–N bonds. They are commonly found in nucleotide metabolites of purines and pyrimidines. These enzymes share similar catalytic mechanisms and show considerable sequence and structural homogeneity, suggesting that they might have evolved from a common ancestral protein. Enzymes in the noncyclic group catalyse hydrolysis of C–O, P–O, C–Cl and C–S bonds in addition to the common C–N bond cleavage.

It seems that there is an exception. The enzyme uronate isomerase (URI) is believed to be a member of the amidohydrolase superfamily but it catalyses a nonhydrolytic aldose/ketose isomerization reaction between D-glucuronate and D-fructuronate. However, this enzyme's catalytic function is independent of a metal ion (Williams *et al.*, 2006). URI contains no *d*-block metal in its structure (PDB code, 1j5s), nor does its structure contain the characteristic histidine from the sixth β strand. Thus, the enzyme URI deserves reconsideration for its protein superfamily classification. It is not an amidohydrolase enzyme but rather an amidohydrolase-like neighbour.

Nonhydrolytic reactions

The more diverse catalytic reactions of the Amidohydro_2 subfamily are not restricted to hydrolytic reactions (Li *et al.*, 2006). ACMSD is the first structurally characterized member that catalyses a nonhydrolytic reaction in this subfamily (PDB code, 2hbv). This enzyme catalyses a C–C bond cleavage of a nonoxidative decarboxylation reaction. The second described member of this subfamily is RSD, which also catalyses a C–C bond cleavage reaction (Goto *et al.*, 2006). A recent bioinformatics study has led to the suggestion that at least 60 nonredundant protein sequences constitute a new ACMSD protein family as a subgroup of the amidohydrolase superfamily (Liu and Zhang, 2006). The members of the ACMSD protein family include several functionally annotated members such as isoorotate decarboxylase, 5-carboxyvanillic acid decarboxylase, 4-oxalomesaconate hydratase (OMAH) and RSD in addition to ACMSD. Unlike ACMSD and RSD, the enzyme 4-oxalomesaconate hydratase catalyses a hydration



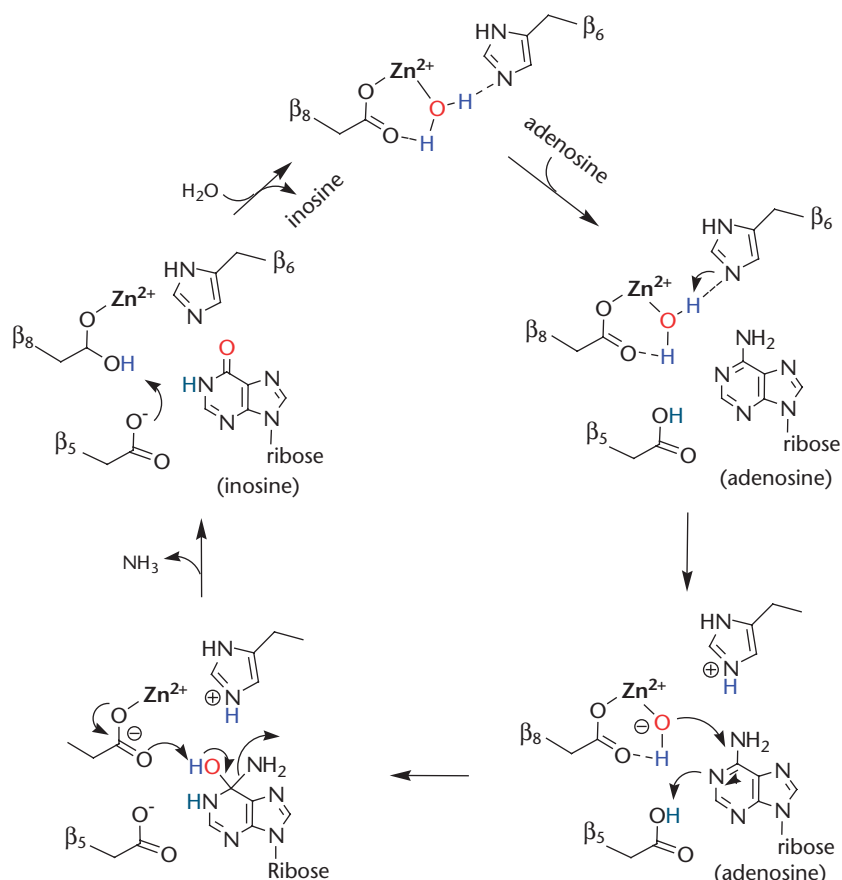
Scheme 2 Chemical reaction catalysed by 4-oxalomesaconate hydratase (OMAH).

reaction that opens up a C=C double bond and adds a proton and an OH[−] across the original C=C double bond of the substrate (Scheme 2).

Enzyme Mechanisms

The best-characterized members of the amidohydrolase superfamily share a common catalytic mechanism. Scheme 3 illustrates the proposed hydrolytic mechanism for the mononuclear Zn-dependent enzyme adenosine deaminase. The common feature of the mechanism is that a metal-bound water becomes a hydroxide with the assistance of an active site catalyst. It then attacks the substrate-bearing amide or other functional groups at carbon and phosphorus centres and forms a tetrahedral carbon intermediate on the substrate. Subsequent collapse of the substrate-based intermediate leads to the hydrolytic products (Wang and Quioco, 1998). The dinuclear enzymes proceed similarly, with a second divalent metal ion providing additional activation power towards the substrate. DHO is one of the best characterized dinuclear amidohydrolase enzymes. It is believed that the original *keto* oxygen of the substrate interacts with the more solvent exposure metal ion in DHO, while the more buried metal ion activates the nucleophilic attack during catalysis.

Mechanistic understanding of the nonhydrolytic C–C bond and C=C bond cleavage reactions is still in its infancy. However, recent biochemical and structural studies have yielded evidence supporting the idea that these reactions have mechanisms similar to those proposed for the hydrolytic reactions. The core elements for the required chemistry, a proper Lewis acid metal centre, a water ligand and a nearby histidine, are all in place at the active site of the characterized members of the Amidohydrolase_2 branch, suggesting a common mechanistic paradigm for substrate activation at the early steps of the reaction catalysed by the enzyme of this superfamily. From a chemical perspective, the enzyme 4-oxalomesaconate hydratase of the ACMSD protein family catalyses a mechanistically insightful reaction; it adds a water molecule to the substrate, as if it were the half-reaction in a decarboxylation mechanism proposed for ACMSD (Scheme 3). Although there is no characterized member of the Amidohydrolase_3 subfamily at the present stage, it is almost certain that some structural variations and new functionalities will soon be described for members of this branch.



Scheme 3 Adenosine deaminase catalytic cycle.

Concluding Remarks

The amidohydrolyase superfamily has attracted substantial attention as a large structure-based cluster of enzymes with thousands of members and divergent catalytic functions. In the past few years there have been huge advances in the studies of the amidohydrolyase superfamily. Large volumes of sequence are now becoming functionally annotated, and many members that exhibit distinct biological significances are now structurally defined. It is becoming clear that the metal-cofactor elaborated by the sturdy and versatile TIM-like β barrel is enormously powerful. The substrate specificity appears to be mostly dictated by the loops, insertions and conformational restrictions of the catalytic face of the TIM-like β barrel. This insight has come from site-directed mutagenesis, kinetics, structural and spectroscopic studies of many members of the superfamily.

Major advances have been made, but a structure-based functional annotation is still challenging. Many principals and strategies related to the design of loops, insertions, subunit interactions and the catalytic impact of protein dynamics remain to be elucidated. Nonetheless, the rapid expansion of knowledge concerning the metal-dependent TIM-barrel enzymes is making the amidohydrolyase

superfamily a well-characterized large enzyme group that may enable a reliable evolutionary analysis for the origin of the divergent members. The amidohydrolyase superfamily as a whole is emerging as a popular and valuable asset in the enzymology field for studying structure–function relationships and evolution.

References

- Alhapel A, Darley DJ, Wagener N *et al.* (2006) Molecular and functional analysis of nicotinate catabolism in *Eubacterium barkeri*. *Proceedings of the National Academy of Sciences of the USA* **103**: 12341–12346.
- Bateman A, Coin L, Durbin R *et al.* (2004) The Pfam protein families database. *Nucleic Acids Research* **32**: D138–D141.
- Bryson K, McGuffin LJ, Marsden RL *et al.* (2005) Protein structure prediction servers at University College London. *Nucleic Acids Research* **33**(Web server issue): W36–W38.
- Goto M, Hayashi H, Miyahara I *et al.* (2006) Crystal structures of nonoxidative zinc-dependent 2,6-dihydroxybenzoate (γ -resorcyate) decarboxylase from *Rhizobium* sp. strain MTP-10005. *Journal of Biological Chemistry* **281**: 34365–34373.
- Han BW, Bingman CA, Mahnke DK *et al.* (2006) Membrane association, mechanism of action, and structure of *Arabidopsis*

- embryonic factor 1 (FAC1). *Journal of Biological Chemistry* **281**: 14939–14947.
- Holm L and Sander C (1997) An evolutionary treasure: unification of a broad set of amidohydrolases related to urease. *Proteins* **28**: 72–82.
- Li T, Iwaki H, Fu R *et al.* (2006) α -Amino- β -carboxymuconic- ϵ -semialdehyde decarboxylase (ACMSD) is a new member of the amidohydrolase superfamily. *Biochemistry* **45**: 6628–6634.
- Liu A and Zhang H (2006) Transition metal-catalyzed nonoxidative decarboxylation reactions. *Biochemistry* **45**: 10407–10411.
- Seffernick JL, de Souza ML, Sadowsky MJ and Wackett LP (2001) Melamine deaminase and atrazine chlorohydrolase: 98 percent identical but functionally different. *Journal of Bacteriology* **183**: 2405–2410.
- Seibert CM and Raushel FM (2005) Structural and catalytic diversity within the amidohydrolase superfamily. *Biochemistry* **44**: 6383–6391.
- Serner R and Höcker B (2005) Catalytic versatility, stability, and evolution of the $(\beta\alpha)_8$ -barrel enzyme fold. *Chemistry Review* **105**: 4038–4055.
- Wang Z and Quioco FA (1998) Complexes of adenosine deaminase with two potent inhibitors: X-ray structures in four independent molecules at pH of maximum activity. *Biochemistry* **37**: 8314–8324.
- Williams L, Nguyen T, Li Y, Porter TN and Raushel FM (2006) Uronate isomerase: a nonhydrolytic member of the amidohydrolase superfamily with an ambivalent requirement for a divalent metal ion. *Biochemistry* **45**: 7453–7462.