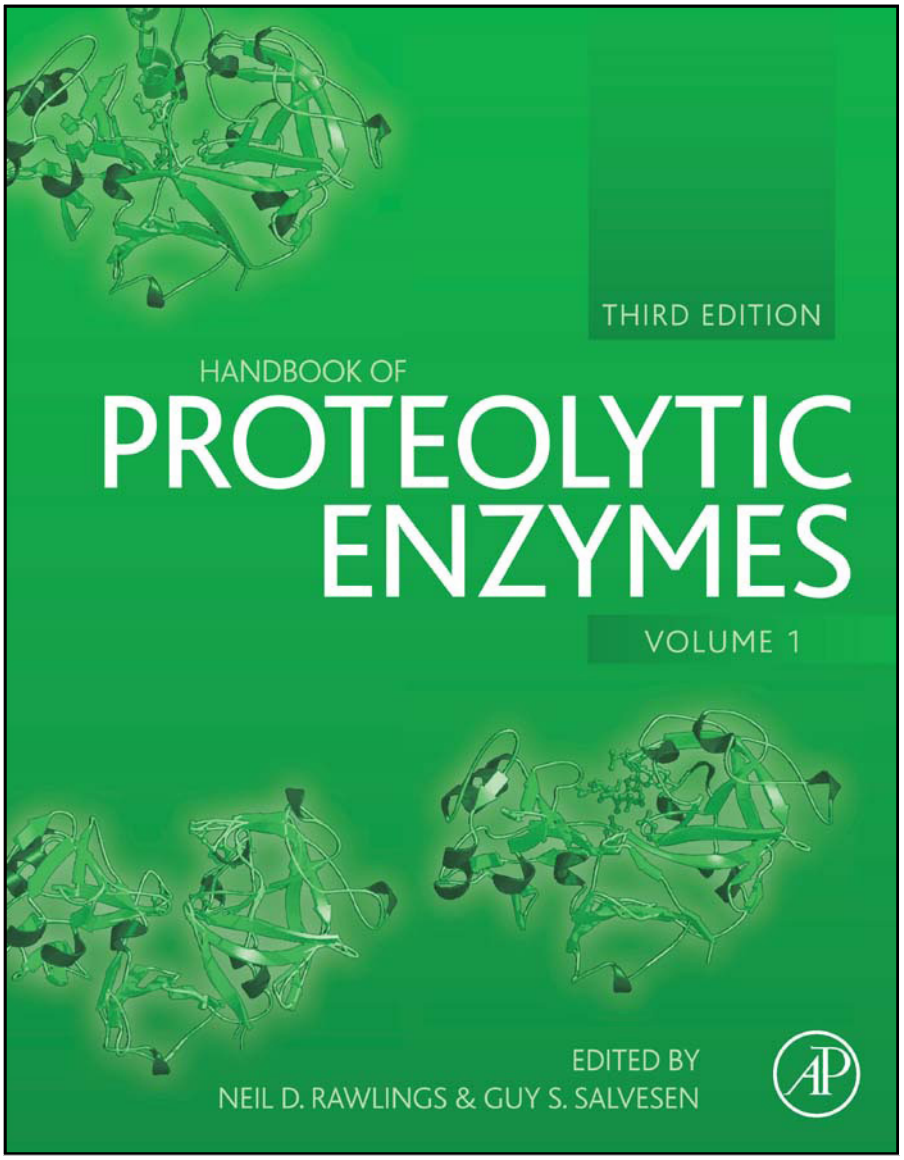

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Chapter 374

Isoaspartyl Dipeptidase (IadA)

Databanks

MEROPS name: isoaspartyl dipeptidase (metallo-type)
MEROPS classification: clan MJ, family M38, peptidase M38.001
Tertiary structure: Available
Species distribution: superkingdom Bacteria
Reference sequence from: *Escherichia coli* (UniProt: P39377)

Name and History

Isoaspartyl or β-aspartyl residues in proteins arise from spontaneous non-oxidative degradation reactions at aspartyl and asparaginyl sites [1,2]. These two amino acids are particular hot spots for protein damage due to the favorable nucleophilic attack by the peptide-bond nitrogen on the side chain carbonyl groups [3]. The resulting succinimidyl intermediate can non-enzymatically epimerize

and/or hydrolyze to yield a mixture of approximately 60–85% L-isoaspartyl and 40–15% L-aspartyl residues, in addition to small amounts of D-isoaspartyl and D-aspartyl residues [4–7]. Isoaspartyl peptides can also be formed enzymatically from asparagine or other small molecule substrates [8,9]. In either case, the isoaspartyl residue, which introduces a kink in the polypeptide chain by rerouting the backbone through the β-carboxyl rather than the α-carboxyl group, can lead to protein aggregation and loss of function [10–13]. Proteins containing altered aspartyl residues can be partially repaired by the L-isoaspartyl/D-aspartyl methyltransferase, leading to the major formation of L-aspartyl residues and a minor formation of D-isoaspartyl residues [14–16].

Isoaspartyl-containing proteins not repaired by the methyltransferase can be subject to enzymatic proteolysis. Several early studies using isoaspartyl-containing peptides showed that the isoaspartyl β-carboxyl bond is generally resistant to proteolysis. For example, in rat liver and kidney extracts, no hydrolytic activity was found directed to an isoaspartyl-alanine dipeptide [17]. Furthermore, leucyl aminopeptidase (Chapter 329) was shown to hydrolyze angiotensin II (Asp-Arg-Val-Tyr-Val-Ala-His-Pro-Phe) but not the corresponding isoaspartyl form [18]. More recently it was shown that five different caspases were incapable of cleaving the isoaspartyl peptide bond in a variety of synthetic peptides [19]. In the same study the authors also showed that the presence of an isoaspartyl residue near the cleavage site could eliminate or reduce the exo- and endoproteolytic activities for prolyl oligopeptidases, amino peptidase N, matrix metalloproteinase 3, and enteropeptidase [19]. However, enzymatic activities have been found that can cleave isoaspartyl linkages. The first evidence for hydrolysis of this type of linkage was the cleavage of an isoaspartyl-histidine dipeptide by a partially purified preparation of pig kidney carnosine (β-alanyl-histidine hydrolase; Chapters 358 and 359) [20], although it is not clear whether this activity was due to the carnosine dipeptidase or a contaminating activity.

Various isoaspartyl-containing peptides, with the most abundant being β-aspartylglycine and β-aspartylserine, were identified in normal human urine [21]. Comparing fasted patients to non-dietary restricted individuals, it was concluded that total urinary β-aspartyl peptides is the sum of a constant base level release from endogenous metabolism and a larger, more various contribution by dietary sources [22,23]. The finding that excreted isoaspartyl peptides did not increase in similar proportion to the consumption of protein foods [23] implied the existence of a proteolytic mechanism that minimizes the accumulation of certain isoaspartyl peptides. In rats given radiolabeled isoaspartyl di- and tripeptides by stomach tube, only a small percentage of the radioactive peptide was rapidly excreted, suggesting peptide metabolism by one or more enzymes that can cleave the isoaspartyl linkage [24].

An enzyme (or enzymes) with maximal activity toward isoaspartylglycine was partially purified (15-fold over crude cytosol) from rat liver and classified as EC 3.4.19.5 [24]. Although various di- and tripeptides were hydrolyzed, the best activity was observed with L-isoaspartylglycine [24]. No hydrolytic activity was present for α-aspartyl- and γ-glutamyl-containing substrates, nor for asparagine or carnosine [24]. To our knowledge, the enzyme or enzymes responsible for this activity have not been further characterized.

An enzyme from *Escherichia coli* with β-aspartyl-directed activity towards dipeptides but not tripeptides was partially purified by Haley [25], and later purified to homogeneity by Gary & Clarke and designated ***IadA*** for ***isoaspartyl dipeptidase*** [26]. This enzyme is a member of the amidohydrolase superfamily that includes dihydroorotases [27,28], and will be discussed further in this chapter.

Finally, a third type of enzyme active on the isoaspartyl linkage was initially found in *Salmonella enterica* serovar Typhimurium and named IaaA for **I**so**A**spartyl **A**minopeptidase [29]. The catalytic mechanism of this enzyme is similar to members of the N-terminal nucleophile group, and is capable of hydrolyzing an array of both isoaspartyl-containing di- and tripeptides. Family members have now been found widely in nature including other bacteria [30,31], cyanobacteria and plants [30,32,33], in addition to mammals [9,34]. This enzyme is discussed in Chapter 819.

Activity and Specificity

In *Escherichia coli*, the IadA dipeptidase was initially characterized with preparations enriched 110-fold over crude cytosol [25], and later purified to homogeneity [26]. This enzyme is distinguished from the mammalian activity characterized by Dorer *et al.* [24] in having essentially no activity towards isoaspartylglycine dipeptides and no ability to hydrolyze tripeptides [25,26]. Although IaaA also differs from IadA in that it has tripeptide activity, both are maximally active with isoaspartylleucine dipeptides, with IadA having an apparent K_m of 0.81 mM to 1.02 mM [25,28,35]. Finally, as with the rat liver and IaaA enzymes, α-aspartyl and γ-glutamyl dipeptides are not significant substrates for IadA [25,26,29]. Table 374.1 summarizes the substrate activities of IadA, IaaA, the rat liver enzyme, in addition to human glycosylasparaginase [9] and asparaginase-like protein 1 [34], two enzymes that exhibit isoaspartyl-directed activities similar to IaaA.

Interestingly, the *E. coli* IadA enzyme activity is unaffected by iodoacetamide (5 mM), *o*-iodosobenzoate

TABLE 374.1 Activities of select enzymes on β-aspartyl peptides and related compounds

Substrate	Isoaspartyl dipeptidase [25,26] (IadA, *E. coli*)	Isoaspartyl aminopeptidase [29] (IaaA, *S. enterica*)	β-Aspartyl peptidase [24] (Rat liver)	Glycosyl-asparaginase [9] (GA, human)	Asparaginase-like protein 1 [34] (hASRGL1, human)
Asn	0	1.6	0	41	90
Asp-Ala	0	0.01	10		
Asp-Asn	0				
Asp-Gly			3		
Asp-Ile	0				
Asp-Leu	0	0.2	9		
Asp-Phe	0				
Asp-Ser	0		10		
Asn-Leu	0	<0.001			
Glu-Leu	0	<0.001	5		
Gly-Ala			40		
Gly-Gly			40		
Gly-Leu	0		Trace		
Leu-Gly	0		50		
Asp-GlcNac					
Asp-Gly-Ala	0		3		
Asp-Gly-Gly		<0.001	3		
Asp-Leu-Gly	0				
Gly-Gly-Gly			30		
Ala-β-Asp-Leu-Ala		<0.001			
β-Asp-methyl ester					100
β-Asp-Ala	33	83	51		78
β-Asp-Asn	10				
β-Asp-Gly	0	5	100	3	
β-Asp-Gln	48				
β-Asp-His	0	69			
β-Asp-Ile	19		37		
β-Asp-Leu	100	100	65		65
β-Asp-Lys		39			69
β-Asp-Met	68		82		
β-Asp-Phe	38	24			39
β-Asp-Ser	82		56		
β-Asp-Thr	18		29		
β-Asp-Val	56		28		
γ-Glu-Cys	<10				
γ-Glu-Gly	<10				

(*Continued*)

TABLE 374.1 (Continued)

Substrate	Isoaspartyl dipeptidase [25,26] (IadA, *E. coli*)	Isoaspartyl aminopeptidase [29] (IaaA, *S. enterica*)	β-Aspartyl peptidase [24] (Rat liver)	Glycosyl-asparaginase [9] (GA, human)	Asparaginase-like protein 1 [34] (hASRGL1, human)
γ-Glu-His	<10				
γ-Glu-Leu	<10	<0.001	0		
β-Asp-Gly-Ala	0		55		
β-Asp-Gly-Gln				27	
β-Asp-Gly-Gly	0		95		
β-Asp-Gly-Gly-Gly-Gly				8	
β-Asp-Gly-Val			13	49	
β-Asp-Leu-Ala		35			
β-Asp-Leu-Gly	0				
β-Asp-Ala-Amide				35	
β-Asp-Gly-Amide				15	
β-Asp-Phe-methyl ester		0.3		23	45
β-Asp-Ser-Amide				71	
Asn-GlcNac		<0.001		100	0
Carnosine	0		0		

(1 mM), and ammonium persulfate (0.4 mM), but is sensitive to *p*-hydroxymercuribenzoate (2 mM), Mn^{2+}, Co^{2+}, and Zn^{2+}, which inhibit the enzyme 17, 42, 90, and 100%, respectively [25]. EDTA also inhibits this enzyme, in contrast to IaaA [29]. Finally, Ca^{2+} and Mg^{2+} slightly stimulated hydrolytic activity 125 and 135%, respectively, over control values [25]. A comparison between the enzyme characteristics of IadA and rat liver β-aspartyl peptidase are summarized in Table 374.2.

Structural Chemistry

The *E. coli* isoaspartyl dipeptidase is encoded by the gene *iadA*, located at the 98-min chromosome region [26]. With no localization signal or membrane-spanning regions, the deduced 390 amino acid sequence most closely resembles dihydroorotases and imidases in terms of amino acid sequence (Figure 374.1) and the amide bond cleavage reaction (Figure 374.2) [26–28]. While gel filtration analyses suggested complexes of about 120 kDa for the *E. coli* enzyme [25] and about 200 kDa for the *Salmonella enterica* serovar Typhimurium enzyme [29], two X-ray crystal structures demonstrated IadA is a 330 kDa 'tetramer of dimers' octomeric structure of 422 symmetry, with each subunit composed of an N-terminal domain of eight mixed β-strands and a C-terminal $(\alpha\beta)_8$ triosphosphate isomerase barrel domain [27,36]. Although it is unclear why lower molecular weights were indicated previously, it is possible the enzyme can dissociate under certain conditions into smaller complexes that retain activity.

Because IadA can accommodate an assortment of dipeptides with hydrophobic, aromatic and hydrophilic residues at the C-terminus (Table 374.1), the active site cannot be too specific for the side chain of this part of the substrate. For example, for a β-Asp-His dipeptide, only Arg233, Ile257, and Pro291 are within 4.0 Å of the substrate imidazole side chain [28]. However, evidence suggests the backbone amides of Gly75, Thr106, and Ser289 can hydrogen bond with the α-carboxylate of the substrate [28]. Furthermore, to orient the substrate for proper hydrolysis, Glu77 ion pairs with the N-terminal α-amine, while Arg169 and Arg233 interact with the C-terminal carboxylate [28,35]. Two zinc atoms, termed 'α' and 'β', bridged by a hydrolytic water or hydroxide ion and a

TABLE 374.2 Characteristics of IadA and rat liver β-aspartyl peptidase

	Isoaspartyl dipeptidase [25,27,35] (IadA, *E. coli*)	β-aspartyl peptidase [24] (Rat liver)
pH optimum	7.4–8.0	7.5–8.0
Optimal buffer	Tris-maleate, Tris-HCl	Sodium phosphate
Preferred substrate	β-Asp-Leu	β-Asp-Gly
Additives not affecting activity	Iodoacetamide, *o*-Iodosobenzoate, Ammonium persulfate	EDTA, Iodoacetamide, 2-mercaptoethanol, Mg^{2+}, Mn^{2+}, Ca^{2+}, Co^{2+}
Additives decreasing activity	EDTA, Sodium phosphate, *p*-Hydroxymercuribenzoate, Co^{2+}, Mn^{2+}, Zn^{2+}	Tris-HCl, *p*-Hydroxymercuribenzoate, Zn^{2+}
Additives increasing activity	Ca^{2+}, Mg^{2+}	Na^{+}, K^{+}
Alkaline or transition metal required for activity	Zn^{2+}	None

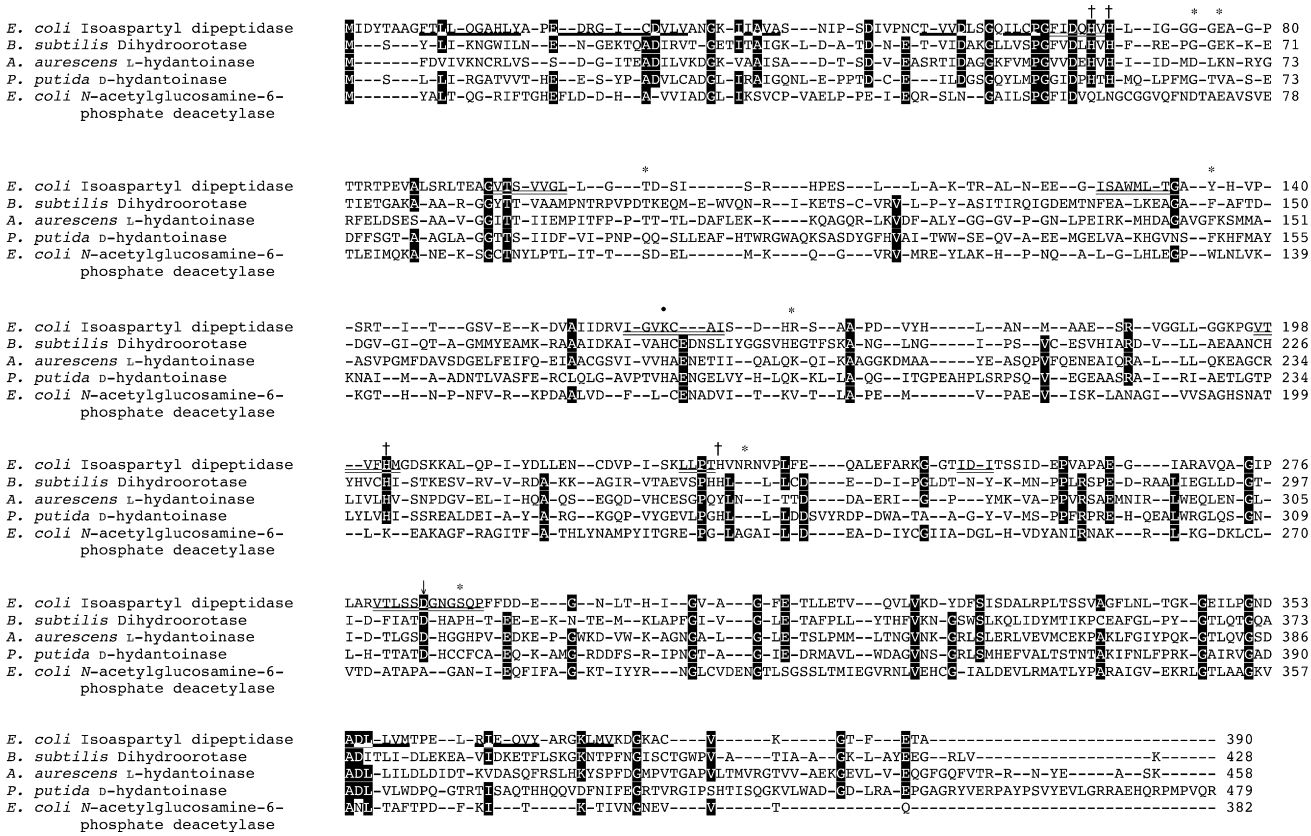

FIGURE 374.1 Multiple sequence alignment of the amidohydrolase superfamily, including the IadA isoaspartyl dipeptidase. Shaded residues indicate an identity shared between at least four of the five enzymes. Asp285, which is critical for IadA enzymatic activity, is marked with an arrow. Histidine residues associated with coordinating zinc ions are labeled with crosses, and the bridging carboxylated Lys162 is indicated with a black dot. The mixed β-sheets domain of IadA (strand residues in bold underline) ranges from residues Met1 to Gly63 and from Gly346 to Thr389 [36]. Amino acids Phe64 to Lys345 comprise the eight β-strands of the TIM-barrel motif (strand residues in double underline) [36]. IadA recognizes isoaspartyl substrates with the specific residues marked with asterisks [27,28,35,36]. Although the phenylalanine in dihydroorotases and hydantoinases may be functionally equivalent to the tyrosine at position 137 in IadA, the other interacting residues are completely different and confer the specific function of IadA.

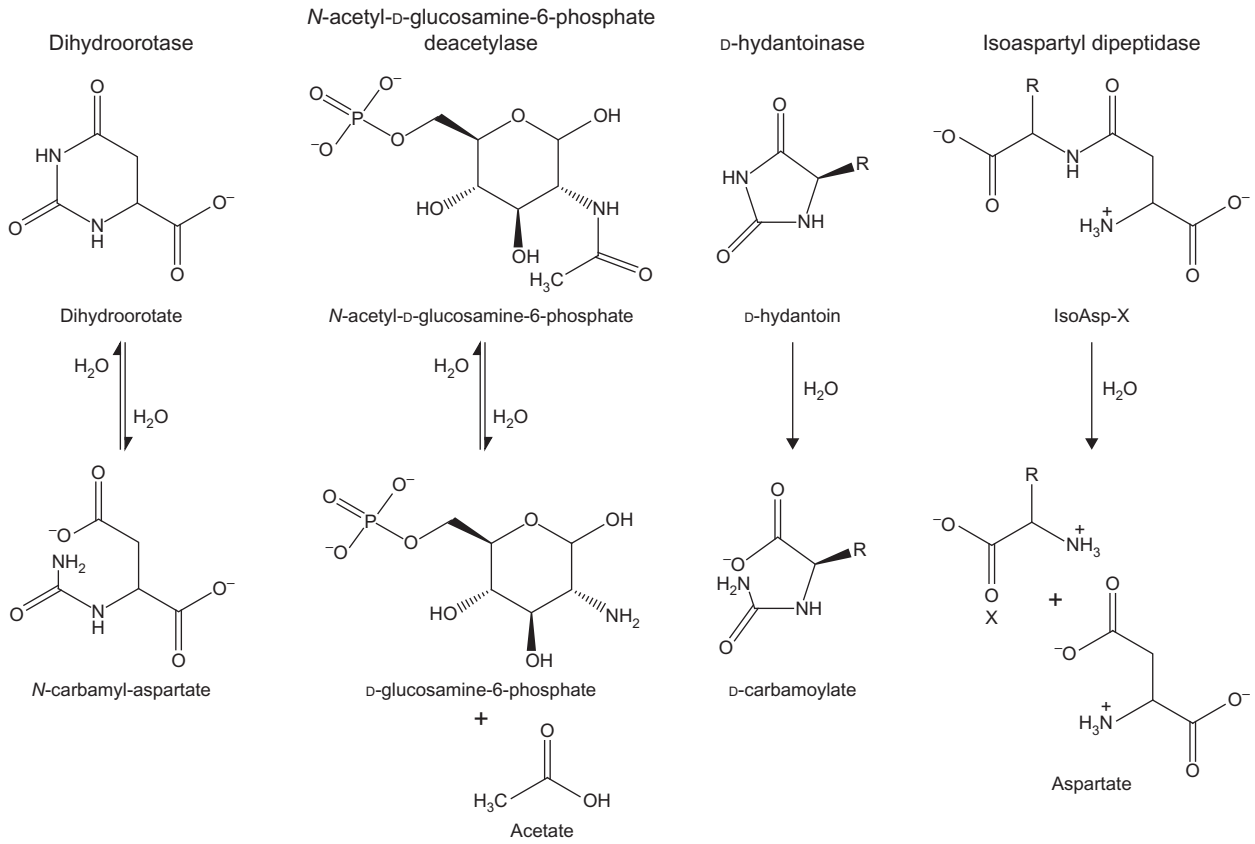

FIGURE 374.2 Comparison of reactions catalyzed by IadA, dihydroorotases, D-hydantoinases, and *N*-acetylglucosamine-6-phosphate deacetylase showing the similarity of the reactions and substrate structures.

carboxylated Lys-162, comprise the binuclear metal reaction center [27,36]. Using a mechanism similar to the amidohydrolase superfamily and M38 family of the MJ clan of metalloproteases, it has been proposed that the carbonyl group of the substrate is polarized by the β-metal ion [27,28]. After transfer of a water proton to Asp285, the hydrolytic hydroxide ion initiates the reaction by a nucleophilic attack on the C-terminal amide bond, forming a tetrahedral intermediate stabilized by Tyr137 [28,35]. Upon transferring the Asp-285 proton to the α-amino group of the leaving residue, the C-N bond is cleaved and the products are released [28] (Figure 374.3).

Although Zn^{2+} provides maximal activity with a K_m and k_{cat} of 1.02 ± 0.09 mM and $104 \pm 3\ s^{-1}$, respectively, Co^{2+} and Cd^{2+} allowed for enzymatic activity, but to a much lower extent [28]. Loss of activity at lower pH corresponds to the protonation of the bridging hydroxide, an effect correlated to the bound metal in the active site [35]. It is proposed that activity losses at higher pH are a result of deprotonation of the aspartyl α-amino group, a moiety essential for enzymatic activity [28].

Preparation

There have been several strategies for bacterial IadA isoaspartyl dipeptidase purification. Haley homogenized *E. coli* strain B grown to mid-logarithmic phase with a Waring blender [25]. Using ammonium sulfate fractionation, Sephadex G-200 size-exclusion and DEAE-cellulose anion exchange chromatography, IadA was partially purified (110-fold), achieving a 34% yield and L-isoaspartyl-L-leucine hydrolysis with a specific activity of 770 $nmol^{-1}\ mg^{-1}$ [25]. Subsequent to French press homogenization of late-logarithmic phase cells, Gary & Clarke [26] included an additional phenyl Sepharose hydrophobic-interaction chromatography step to achieve enzyme homogeneity (>3000-fold purification) from the wild-type *E. coli* strain MC1000, as well as from strain JDG100, which overexpresses IadA 40-fold over MC1000 via a pUC19 vector. Both preparations have a 15% yield and L-isoaspartyl-leucine specific activity of 1900 $nmol\ min^{-1}\ mg^{-1}$. More recently, *iadA* has been cloned into pET30 [28,35,36] and expressed in Bl21 (DE3)star cells and then purified in a similar manner to Gary & Clarke [26]. Alternatively, *iadA* has been cloned

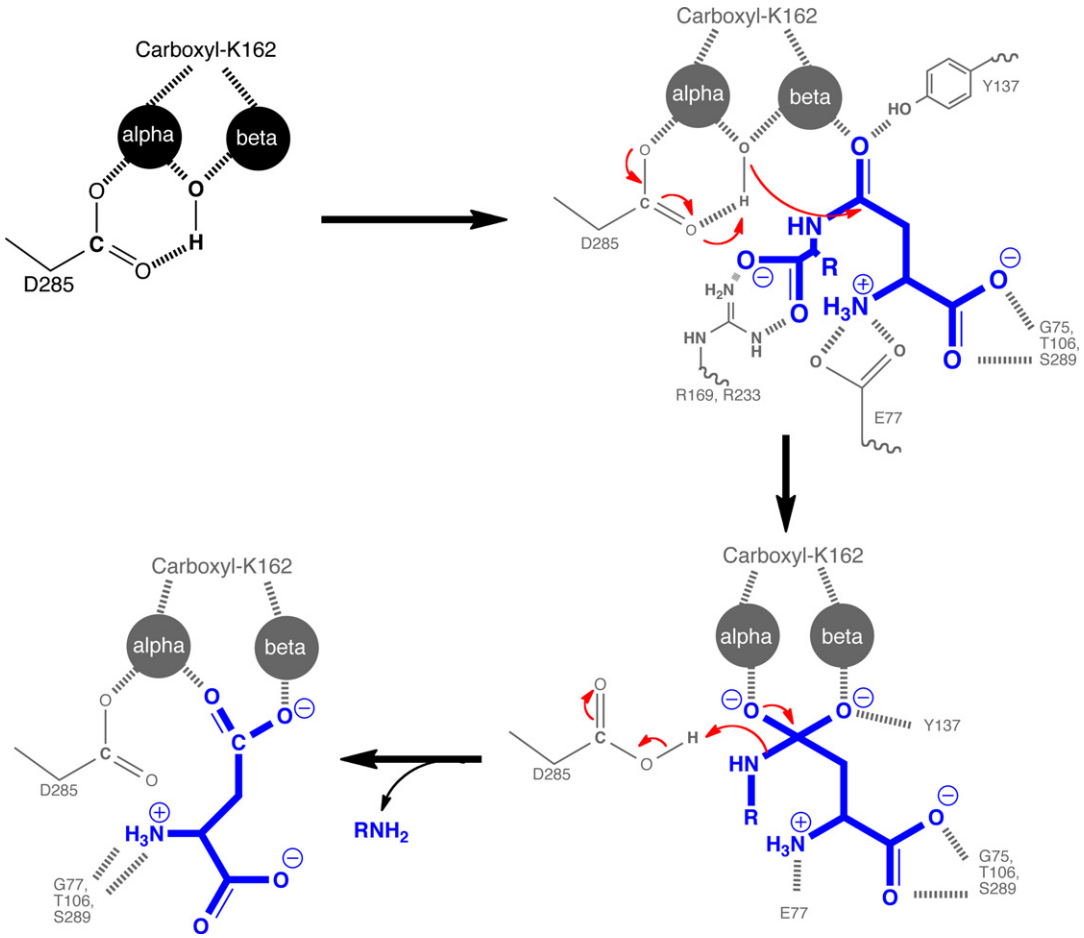

FIGURE 374.3 Proposed reaction mechanism for the hydrolysis of isoaspartyl-containing dipeptides by IadA. The illustrated mechanism for this binuclear metalloenzyme is derived from Thoden *et al.* [36], Jozic *et al.* [27], and Martí-Arbona *et al.* [28,35]. The two active site zinc cations are labeled as 'alpha' and 'beta', and the isoaspartyl dipeptide substrate is highlighted in blue. Hashed bonds indicate chemical interactions.

into pTrc99a and expressed in JM109/pIadA [27]. For the latter purification, the authors used sonication followed by DE-52 anion exchange chromatography, ammonium sulfate precipitation, phenyl Sepharose high performance chromatography, dialysis, and finally Sephadex 75 gel filtration chromatography [27].

Biological Aspects

Cellular proteins and peptides are prone to chemical modifications that can detrimentally alter their stability and function. One of the most common modifications is the isomerization and deamidation of aspartyl and asparaginyl residues, respectively. *In vivo* studies have shown that isoaspartyl damage to calmodulin [37], Bcl-x_L [38], type-I collagen [39] and protein kinase A [40] can be detrimental, impacting intracellular localization, biological function or biochemical activity (for a review see Shimizu *et al.* [41]). Furthermore, the existence of isoaspartyls in amyloid β-peptides [10,19], in addition to long-lived proteins such as the human brain myelin basic protein [42] and αB-crystallin [43], illustrates the importance of this modification on aging organisms. Finally, isoaspartyl formation has been shown to make immunologically ignored proteins, such as melanoma antigen tyrosine-related protein (TRP)-2 [44], immunogenic in comparison to the normal aspartyl form, and has been associated with altered T cell proliferation in MRL mice [45].

In *E. coli*, the accumulation of isoaspartyl residues during exponential growth has been found to be minimal due to the short 20 min doubling time. However, isoaspartyl residues and their associated dipeptide degradation products may overcome necessary biomolecule turnover rates and build up during prolonged time periods at stationary phase, resulting in cell toxicity or the depletion of amino acid pools [46–49]. To determine if the isoaspartyl

dipeptidase prevents the accumulation of these dipeptides, experiments were performed using JDG11000, an *E. coli* strain lacking the *iadA* gene [26]. In this null mutant, growth in both exponential and stationary phase was unaffected and no obvious phenotypes were detected [26]. Interestingly, radiolabeling studies did not reveal any accumulation of isoaspartyl-leucine dipeptides, suggestive of a compensatory dipeptidase [26]. This was confirmed by the analysis of null mutant cytosol, which indicated the presence of a secondary activity accounting for approximately 31% of the total isoaspartyl dipeptidase activity [26]. Studies in *S. enterica* serovar Typhimurium later showed this secondary activity was from the isoaspartyl aminopeptidase IaaA, which also preferentially hydrolyzes isoaspartyl-leucine over aspartyl-leucine and isoaspartyl-glycine, but is also capable of cleaving tripeptides [29]. Although *S. enterica* deficient in both *iadA* and *iaaA* showed no further isoaspartyl peptidase activity, preliminary results surprisingly indicate this strain grows normally [29].

A second possible physiological function for IadA may be to utilize environmental β-aspartyl peptides as nutrition sources. It has been shown that both wild type and peptidase-deficient *E. coli* strains are unable to grow on minimal media supplemented with isoaspartyl-leucine [29]. However, *E. coli* overexpressing IadA were able to grow on this medium [29].

Distinguishing Features

A database search performed in March 2011 for protein sequences similar to *E. coli* IadA using the NCBI BLAST program [50] revealed that similar species (with expect E-values less than 1×10^{-10} and 30% or more amino acid identity) were strictly limited to bacteria. These bacteria included enterobacteria (*i.e. Shigella* sp., *Salmonella* sp., *Citrobacter* sp., *Photobacterium* sp., *Vibrio* sp., and *Marinomonas* sp.), ε-proteobacteria (*e.g. Campylobacter* sp.), firmicutes (*e.g. Thermoanaerobacter* sp., *Clostridium* sp., *Bacillus* sp., *Desulfitobacterium* sp., *Carboxydibrachium* sp., *Mitsuokella* sp., and *Ruminococcus* sp.) and fusobacteria (*Fusobacterium* sp.). Other enzymes, such as dihydroorotases, amidohydrolases and N-acetylglucosamine-6-phosphate deacetylases, are present at E-values and identities below this standard. To date, there has been no evidence for IadA in organisms other than this small number of prokaryotes.

Despite the limited identity in the full sequence, IadA is most similar to the first 70 amino-terminal residues of dihydroorotases and imidases [26] including the sequence PGFIDQHVHI that contains two of the four histidine ligands for the zinc ions (Figure 374.1) [51–53]. More significant, however, is the topological similarities not only between IadA and dihydroorotases, but also other enzymes with binuclear metal centers, such as L- and D-hydantoinases, adenosine and cysteine deaminases, phosphotriesterases and ureases [27,54]. For example, in *Thermus* sp. (D-hydantoinase), *Arthrobacter aurescens* (L-hydantoinase), as well as *Klebsiella aerogenes* and *Bacillus pasteurii* (ureases), 70% of the α-carbon atoms in the β-sandwich domains are in equivalent positions as in IadA [27]. Furthermore, the α-carbon atoms in the catalytic domains of the *E. coli* dihydroorotase and *Pseudomonas diminuta* phosphotriesterase are 67 and 69% equivalent as IadA, respectively [27]. Crystal structures have confirmed this close relationship to dihydroorotases and the existence of two zinc ions in the catalytic site of *E. coli* IadA [27,36]. Isoaspartyl dipeptides and dihydroorotate have similar molecular geometries and functional groups, and hydrolysis occurs at a similar position on each molecule. The *E. coli* isoaspartyl dipeptidase, however, has no dihydroorotase activity and, unlike IaaA, does not cleave asparagine [26].

Interestingly, another enzyme has recently been found that cleaves a different product of aspartyl modification. This lactacystin-sensitive endopeptidase, termed the **D-a**spartyl **e**ndo**p**eptidase (DAEP), was discovered to have activity toward D-aspartyl-containing proteins [55]. Found in organisms such as mice [55], rabbit [55], and *Paenibacillus* sp. [56], DAEP is localized to the inner mitochondrial membrane rather than the cytosol [55] and has a topology similar to the AAA^+ protease family [57], unlike the structures of IadA and IaaA.

Isoaspartyl damage is the most common type of non-oxidative protein damage due to the natural propensity of aspartyl and asparaginyl amino acids to isomerize and deamidate at physiological conditions. Because these residues are vital for protein function in various biological processes, cells require mechanisms to deal with this detrimental modification. Rather than defining a unique class of proteolytic enzymes, IadA uses a similar catalytic mechanism and overall structure to dihydroorotases to work alongside the L-isoaspartyl/D-aspartyl methyltransferase in ensuring the removal of isoaspartyl residues from cells.

Further Reading

Aswad *et al.* [4] and Clarke [14] review the formation, metabolism, and significance of isoaspartyl residues in proteins. Details of the properties of the bacterial IadA enzyme are summarized in Haley [25], Gary & Clarke [26], Jozic *et al.* [27], Marti-Arbona *et al.* [28,35], and Thoden *et al.* [36]. Finally, mammalian and plant pathways for the hydrolysis of isoaspartyl residues are reviewed in Noronkoski *et al.* [9], Hejazi *et al.* [32], and Cantor *et al.* [34].

References

[1] Stephenson, R.C., Clarke, S. (1989). Succinimide formation from aspartyl and asparaginyl peptides as a model for the spontaneous degradation of proteins. *J. Biol. Chem.* 264(11), 6164−6170.

[2] Lindner, H., Helliger, W. (2001). Age-dependent deamidation of asparagine residues in proteins. *Exp. Gerontol.* 36(9), 1551−1553.

[3] Radkiewicz, J.L., Zipse, H., Clarke, S., Houk, K.N. (2001). Neighboring side chain effects in asparaginyl and aspartyl degradation: An *ab initio* study of the relationship between peptide conformation and backbone NH acidity. *J. Amer. Chem. Soc.* 123(15), 3499−3506.

[4] Aswad, D.W., Paranandi, M.V., Schurter, B.T. (2000). Isoaspartate in peptides and proteins: formation, significance, and analysis. *J. Pharm. Biomed. Anal.* 21(6), 1129−1136.

[5] Dehart, M.P., Anderson, B.D. (2007). The role of the cyclic imide in alternate degradation pathways for asparagine-containing peptides and proteins. *J. Pharm. Sci.* 96(10), 2667−2685.

[6] Vigneswara, V., Lowenson, J.D., Powell, C.D., Thakur, M., Bailey, K., Clarke, S., Ray, D.E., Carter, W.G. (2006). Proteomic identification of novel substrates of a protein isoaspartyl methyltransferase repair enzyme. *J. Biol. Chem.* 281(43), 32619−32629.

[7] Young, G.W., Hoofring, S.A., Mamula, M.J., Doyle, H.A., Bunick, G.J., Hu, Y., Aswad, D.W. (2005). Protein L-isoaspartyl methyltransferase catalyzes *in vivo* racemization of Aspartate-25 in mammalian histone H2B. *J. Biol. Chem.* 280(28), 26094−26098.

[8] Burton, E.G., Schoenhard, G.L., Hill, J.A., Schmidt, R.E., Hribar, J.D., Kotsonis, F.N., Oppermann, J.A. (1989). Identification of N-beta-L-aspartyl-L-phenylalanine as a normal constituent of human plasma and urine. *J. Nutr.* 119(5), 713−721.

[9] Noronkoski, T., Stoineva, I.B., Ivanov, I.P., Petkov, D.D., Mononen, I. (1998). Glycosylasparaginase-catalyzed synthesis and hydrolysis of beta-aspartyl peptides. *J. Biol. Chem.* 273(41), 26295−26297.

[10] Bohme, L., Hoffmann, T., Manhart, S., Wolf, R., Demuth, H.U. (2008). Isoaspartate-containing amyloid precursor protein-derived peptides alter efficacy and specificity of potential beta-secretases. *Biol. Chem.* 389(8), 1055−1066.

[11] Noguchi, S. (2010). Structural changes induced by the deamidation and isomerization of asparagine revealed by the crystal structure of *Ustilago sphaerogena* ribonuclease U2B. *Biopolymers* 93(11), 1003−1010.

[12] Visick, J.E., Clarke, S. (1995). Repair, refold, recycle: how bacteria can deal with spontaneous and environmental damage to proteins. *Mol. Microbiol.* 16(5), 835−845.

[13] Wakankar, A.A., Borchardt, R.T., Eigenbrot, C., Shia, S., Wang, Y.J., Shire, S.J., Liu, J.L. (2007). Aspartate isomerization in the complementarity-determining regions of two closely related monoclonal antibodies. *Biochemistry* 46(6), 1534−1544.

[14] Clarke, S. (2003). Aging as war between chemical and biochemical processes: Protein methylation and the recognition of age-damaged proteins for repair. *Aging Res. Rev.* 2(3), 263−265.

[15] Lowenson, J.D., Clarke, S. (1992). Recognition of D-aspartyl residues in polypeptides by the erythrocyte L-isoaspartyl/D-aspartyl protein methyltransferase. Implications for the repair hypothesis. *J. Biol. Chem.* 267(9), 5985−5995.

[16] Murray, E.D. Jr., Clarke, S. (1984). Synthetic peptide substrates for the erythrocyte protein carboxyl methyltransferase. Detection of a new site of methylation at isomerized L-aspartyl residues. *J. Biol. Chem.* 259(17), 10722−10732.

[17] Greenstein, J.P., Price, V.E. (1949). alpha-Keto acid-activated glutaminase and asparaginase. *J. Biol. Chem.* 178(2), 695−705.

[18] Riniker, B., Schwyzer, R. (1964). Synthetische Analoge des Hypertensins. α-L-, β-L-, α-D- und β-D-Asp-Val-Hypertensin II; desamino-Val-Hypertesin II (Synthetic analogs of the hypertensins. α-L, β-L-, α-D- und β-D-Asp-Val-Hypertensn II; desamio-Val-Hypertensin II). *Helv. Chim. Acta* 47, 2357−2375.

[19] Bohme, L., Bar, J.W., Hoffmann, T., Manhart, S., Ludwig, H.H., Rosche, F., Demuth, H.U. (2008). Isoaspartate residues dramatically influence substrate recognition and turnover by proteases. *Biol. Chem.* 389(8), 1043−1053.

[20] Hanson, H.T., Smith, E.L. (1949). Carnosinase; an enzyme of swine kidney. *J. Biol. Chem.* 179(2), 789−801.

[21] Buchanan, D.L., Haley, E.E., Markiw, R.T. (1962). Occurrence of beta-aspartyl and gamma-glutamyl oligopeptides in human urine. *Biochemistry* 1, 612−620.

[22] Buchanan, D.L., Haley, E.E., Markiw, R.T., Peterson, A.A. (1962). Studies on the *in vivo* metabolism of alpha- and beta-aspartyl-glycine-1-C14. *Biochemistry* 1, 620−623.

[23] Dorer, F.E., Haley, E.E., Buchanan, D.L. (1966). Quantitative studies of urinary beta-aspartyl oligopeptides. *Biochemistry* 5(10), 3236−3240.

[24] Dorer, F.E., Haley, E.E., Buchanan, D.L. (1968). The hydrolysis of beta-aspartyl peptides by rat tissue. *Arch. Biochem. Biophys.* 127(1), 490−495.

[25] Haley, E.E. (1968). Purification and properties of a beta-aspartyl peptidase from *Escherichia coli*. *J. Biol. Chem.* 243(21), 5748−5752.

[26] Gary, J.D., Clarke, S. (1995). Purification and characterization of an isoaspartyl dipeptidase from *Escherichia coli*. *J. Biol. Chem.* 270(8), 4076−4087.

[27] Jozic, D., Kaiser, J.T., Huber, R., Bode, W., Maskos, K. (2003). X-ray structure of isoaspartyl dipeptidase from *E. coli*: a dinuclear zinc peptidase evolved from amidohydrolases. *J. Mol. Biol.* 332(1), 243−256.

[28] Marti-Arbona, R., Fresquet, V., Thoden, J.B., Davis, M.L., Holden, H.M., Raushel, F.M. (2005). Mechanism of the reaction catalyzed by isoaspartyl dipeptidase from *Escherichia coli*. *Biochemistry* 44(19), 7115−7124.

[29] Larsen, R.A., Knox, T.M., Miller, C.G. (2001). Aspartic peptide hydrolases in *Salmonella enterica* serovar Typhimurium. *J. Bacteriol.* 183(10), 3089−3097.

[30] Borek, D., Michalska, K., Brzezinski, K., Kisiel, A., Podkowinski, J., Bonthron, D.T., Krowarsch, D., Otlewski, J., Jaskolski, M. (2004). Expression, purification and catalytic activity of *Lupinus luteus* asparagine beta-amidohydrolase and its *Escherichia coli* homolog. *Eur. J. Biochem.* 271(15), 3215−3226.

[31] Michalska, K., Brzezinski, K., Jaskolski, M. (2005). Crystal structure of isoaspartyl aminopeptidase in complex with L-aspartate. *J. Biol. Chem.* 280(31), 28484−28491.

[32] Hejazi, M., Piotukh, K., Mattow, J., Deutzmann, R., Volkmer-Engert, R., Lockau, W. (2002). Isoaspartyl dipeptidase activity of plant-type asparaginases. *Biochem. J.* 364(Pt. 1), 129−136.

[33] Michalska, K., Bujacz, G., Jaskolski, M. (2006). Crystal structure of plant asparaginase. *J. Mol. Biol.* 360(1), 105−116.

[34] Cantor, J.R., Stone, E.M., Chantranupong, L., Georgiou, G. (2009). The human asparaginase-like protein 1 hASRGL1 is an Ntn hydrolase with beta-aspartyl peptidase activity. *Biochemistry* 48(46), 11026–11031.

[35] Marti-Arbona, R., Thoden, J.B., Holden, H.M., Raushel, F.M. (2005). Functional significance of Glu-77 and Tyr-137 within the active site of isoaspartyl dipeptidase. *Bioorg. Chem.* 33(6), 448–458.

[36] Thoden, J.B., Marti-Arbona, R., Raushel, F.M., Holden, H.M. (2003). High-resolution X-ray structure of isoaspartyl dipeptidase from *Escherichia coli*. *Biochemistry* 42(17), 4874–4882.

[37] Johnson, B.A., Langmack, E.L., Aswad, D.W. (1987). Partial repair of deamidation-damaged calmodulin by protein carboxyl methyltransferase. *J. Biol. Chem.* 262(25), 12283–12287.

[38] Deverman, B.E., Cook, B.L., Manson, S.R., Niederhoff, R.A., Langer, E.M., Rosova, I., Kulans, L.A., Fu, X., Weinberg, J.S., Heinecke, J.W., Roth, K.A., Weintraub, S.J. (2002). Bcl-xL deamidation is a critical switch in the regulation of the response to DNA damage. *Cell* 111(1), 51–62.

[39] Lanthier, J., Desrosiers, R.R. (2004). Protein L-isoaspartyl methyltransferase repairs abnormal aspartyl residues accumulated in vivo in type-I collagen and restores cell migration. *Exp. Cell Res.* 293(1), 96–105.

[40] Pepperkok, R., Hotz-Wagenblatt, A., Konig, N., Girod, A., Bossemeyer, D., Kinzel, V. (2000). Intracellular distribution of mammalian protein kinase A catalytic subunit altered by conserved Asn2 deamidation. *J. Cell Biol.* 148(4), 715–726.

[41] Shimizu, T., Matsuoka, Y., Shirasawa, T. (2005). Biological significance of isoaspartate and its repair system. *Biol. Pharm. Bull.* 28(9), 1590–1596.

[42] Shapira, R., Wilkinson, K.D., Shapira, G. (1988). Racemization of individual aspartate residues in human myelin basic protein. *J. Neurochem.* 50(2), 649–654.

[43] Fujii, N., Satoh, K., Harada, K., Ishibashi, Y. (1994). Simultaneous stereoinversion and isomerization at specific aspartic acid residues in alpha A-crystallin from human lens. *J. Biochem.* 116(3), 663–669.

[44] Doyle, H.A., Zhou, J., Wolff, M.J., Harvey, B.P., Roman, R.M., Gee, R.J., Koski, R.A., Mamula, M.J. (2006). Isoaspartyl post-translational modification triggers anti-tumor T and B lymphocyte immunity. *J. Biol. Chem.* 281(43), 32676–32683.

[45] Yang, M.L., Doyle, H.A., Gee, R.J., Lowenson, J.D., Clarke, S., Lawson, B.R., Aswad, D.W., Mamula, M.J. (2006). Intracellular protein modification associated with altered T cell functions in autoimmunity. *J. Immunol.* 177(7), 4541–4549.

[46] Mandelstam, J. (1958). Turnover of protein in growing and non-growing populations of *Escherichia coli*. *Biochem. J.* 69(1), 110–119.

[47] Mandelstam, J. (1960). The intracellular turnover of protein and nucleic acids and its role in biochemical differentiation. *Bacteriol. Rev.* 24(3), 289–308.

[48] Reeve, C.A., Amy, P.S., Matin, A. (1984). Role of protein synthesis in the survival of carbon-starved *Escherichia coli* K-12. *J. Bacteriol.* 160(3), 1041–1046.

[49] Reeve, C.A., Bockman, A.T., Matin, A. (1984). Role of protein degradation in the survival of carbon-starved *Escherichia coli* and *Salmonella typhimurium*. *J. Bacteriol.* 157(3), 758–763.

[50] Altschul, S.F., Gish, W., Miller, W., Myers, E.W., Lipman, D.J. (1990). Basic local alignment search tool. *J. Mol. Biol.* 215(3), 403–410.

[51] Brown, D.C., Collins, K.D. (1991). Dihydroorotase from *Escherichia coli*. Substitution of Co(II) for the active site Zn(II). *J. Biol. Chem.* 266(3), 1597–1604.

[52] Washabaugh, M.W., Collins, K.D. (1984). Dihydroorotase from *Escherichia coli*. Purification and characterization. *J. Biol. Chem.* 259(5), 3293–3298.

[53] Washabaugh, M.W., Collins, K.D. (1986). Dihydroorotase from *Escherichia coli*. Sulfhydryl group-metal ion interactions. *J. Biol. Chem.* 261(13), 5920–5929.

[54] Holm, L., Sander, C. (1997). An evolutionary treasure: unification of a broad set of amidohydrolases related to urease. *Proteins* 28(1), 72–82.

[55] Kinouchi, T., Ishiura, S., Mabuchi, Y., Urakami-Manaka, Y., Nishio, H., Nishiuchi, Y., Tsunemi, M., Takada, K., Watanabe, M., Ikeda, M., Matsui, H., Tomioka, S., Kawahara, H., Hamamoto, T., Suzuki, K., Kagawa, Y. (2004). Mammalian D-aspartyl endopeptidase: a scavenger for noxious racemized proteins in aging. *Biochem. Biophys. Res. Commun.* 314(3), 730–736.

[56] Takahashi, S., Ogasawara, H., Hiwatashi, K., Hori, K., Hata, K., Tachibana, T., Itoh, Y., Sugiyama, T. (2006). Paenidase, a novel D-aspartyl endopeptidase from *Paenibacillus* sp. B38: purification and substrate specificity. *J. Biochem.* 139(2), 197–202.

[57] Kinouchi, T., Fujii, N. (2010). Structural consideration of mammalian D-aspartyl endopeptidase. *Chem. Biodivers.* 7(6), 1403–1407.

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