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MINI-REVIEW

Bacterial β-peptidyl aminopeptidases: on the hydrolytic degradation of β-peptides

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Abstract The special chemical and biological features of β-peptides have been investigated intensively during recent years. Many studies emphasize the restricted biodegradability and the high metabolic stability of this class of compounds. β-Peptidyl aminopeptidases form the first family of enzymes that hydrolyze a variety of short β-peptides and β-amino-acid-containing peptides. All representatives of this family were isolated from Gram-negative bacteria. The substrate specificities of the peptidases vary greatly, but the enzymes have common structural properties, and a similar reaction mechanism can be expected. This review gives an overview on the β-peptidyl aminopeptidases with emphasis on their biochemical and structural properties. Their possible physiological function is discussed. Functionally and structurally related enzymes are compared to the β-peptidyl aminopeptidases.

Keywords Beta-peptide · Beta-amino acid · Aminopeptidase · Proteobacteria

Introduction

A wide variety of β-amino acids are part of bioactive molecules and complex peptidic as well as non-peptidic natural products. There are, by far, more natural β-amino acids than proteinogenic α-amino acids, and this number is even exceeded by the various γ-amino acids. One reason for this structural diversity is the increased number of substitution sites found in β- and γ-amino acids (Scheme 1). β-Peptides and β-amino acids are designated according to the rules outlined by Seebach et al. (2004) throughout this manuscript.

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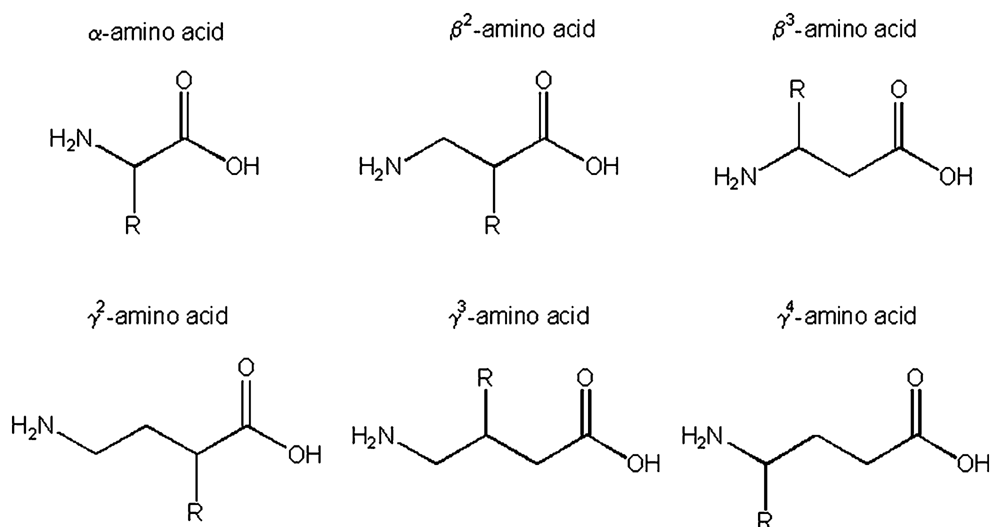
The most prevalent and simple β-amino acid is β-homoglycine (commonly called β-alanine), a building block of pantothenic acid, coenzyme A, and carnosine.

Many natural products, such as microcystin, paclitaxel (Taxol), carnosine, and bestatin, contain β-amino acids, but natural peptides that are solely composed of β-amino acids have not been described so far. Nevertheless, different unusual amide linkages, in which γ-carboxylic groups and ε-amino groups are involved, can be found in natural polymers such as poly(ε-L-lysine) and poly(γ-glutamic acid) (Obst and Steinbüchel 2004).

The chemical synthesis of β-peptides with homologated proteinogenic side chains was published in 1996 for the first time (Seebach et al. 1996), and since then, the special features of these compounds have been investigated intensively (Koyack and Cheng 2006; Seebach et al. 2004). β-Peptides are mainly synthesized by classical chemical methods that were originally developed from ligation methods for α-peptides (Kimmerlin and Seebach 2005). A wide variety of chemical and biocatalytical methods for the synthesis of enantiomerically pure β-amino acids were reviewed recently (Juaristi and Lopez-Ruiz 1999; Liljeblad and Kanerva 2006; Liu and Sibi 2002). These articles mainly focus on the synthesis of β^3-amino acids because the synthesis of β^2-amino acids is far more complicated (Lelais and Seebach 2004) and not yet as advanced as the one of β^3-amino acids.

β-Peptides adopt secondary structures, are extremely stable against proteolytic and peptidolytic hydrolysis (Frackenpohl et al. 2001; Gopi et al. 2003; Hintermann and Seebach 1997; Hook et al. 2004; Lelais and Seebach 2003; Seebach et al. 1996, 1998), and are not degraded in vivo by rats (Wiegand et al. 2002, 2004) or by Mexican



Scheme 1 Structures of single substituted α-, β- and γ-amino acids

sweet maize (Lind et al. 2004). The high biological stability of β-peptides may overcome the major disadvantages in the application of physiologically active α-peptides as pharmaceuticals and peptidomimetics, e.g., their quick degradation by luminal, pancreatic, cytosolic, or lysosomal proteases, which results in low bioavailability and relatively short half-lifes in vivo. However, complete inertness inevitably will create unwanted pharmacological as well as environmental problems. Fortunately, slow degradation of β-peptides is observed when they are incubated with microbial consortia that were enriched with β-peptides as sole carbon and energy sources. Such experiments indicated for the first time that β-peptides are biodegradable (Schreiber et al. 2002).

The metabolism of specific β-amino acid-containing peptides such as the dipeptide carnosine (Teufel et al. 2003; Vongerichten et al. 1994; Wood and Johnson 1981) and the cyclic heptapeptide microcystin (Bourne et al. 1996, 2001; Tillett et al. 2000) is known in detail, but for other peptides containing β-amino acids, no information about their metabolism is available.

β-Peptidyl aminopeptidases are hydrolases that specifically catalyze the N-terminal cleavage of β-amino acids from oligopeptides and hydrolyze β-peptides completely to their constituting amino acids. These enzymes might play a crucial role in the degradation of small peptides carrying N-terminal β-amino acids. Until now, four β-peptidyl aminopeptidases from *Proteobacteria* have been isolated and characterized (Table 1). They are classified into the peptidase family S58 according to the MerPOS database (http://merops.sanger.ac.uk/index.htm; Rawlings et al. 2004).

The focus of this minireview is to provide an overview on the recently discovered β-peptidyl aminopeptidases to illustrate their unusual reaction and biochemical characteristics.

β-Peptidyl aminopeptidases

The L-aminopeptidase D-Ala-esterase/amidase from *Ochrobactrum anthropi* (DmpA) was the first enzyme of this class to be purified (Fanuel et al. 1999a,b). It was detected due to its ability to hydrolyze D-alanyl-, L-alanyl-, and glycyl-*p*-nitroanilides, -amides, and -esters with preference for the D-configuration of alanine, but it also acts as an L-stereoselective aminopeptidase on different α-peptides. Just recently, Heck et al. (2006) showed that DmpA was able to cleave peptides and amides containing small N-terminal β-homoamino acids such as βhGly and β^3hAla with high activities.

Recently, two further enzymes were described that exhibit sequence similarities to DmpA (Table 2). They originate from environmental isolates, *Sphingosinicella xenopeptidilytica* 3-2W4 and *Sphingosinicella microcystinivorans* Y2, that utilize short synthetic β-peptides as sole source of carbon, nitrogen, and energy (Geueke et al. 2005–2007). Because β-peptides exhibit a high pharmaceutical potential and an astonishing metabolic inertness, the aim of these studies was to investigate the biodegradability and potential environmental fate of β-peptides. It was shown that the initial steps of the catabolic pathway are catalyzed by β-peptidyl aminopeptidases with fairly broad substrate specificities. The enzymes were named 3-2W4 BapA and Y2 BapA, respectively.

The gene sequence *bapA* of the β-alanyl-Xaa dipeptidase from *Pseudomonas* sp. MCI3434 (Ps BapA) was isolated due to its relative position to *ramA*, a gene coding for an *R*-amidase with biotechnological potential for producing an important building block for an HIV protease inhibitor (Komeda and Asano 2005; Komeda et al. 2004). Cloning and expression of the *bapA* gene from *Pseudomonas* sp. revealed that it codes for an aminopeptidase with

Table 1 Structural, genetic, and biochemical characteristics of β-peptidyl aminopeptidases from *Proteobacteria*

Enzyme (accession number), microorganism	Structural and genetic analysis						Enzyme characteristics			References
	Number of amino acids	Signal sequence (aa)	Subunits (aa)	Guanine-cytosine content of the gene (%)	Flanking regions coding for putative genes: Upstream	Flanking regions coding for putative genes: Downstream	pH Optimum	Temperature stability ($t_{1/2}$ in min)	Cofactors	
DmpA (CAA66259), *O. anthropi* LMG7991	375	none	α: 1–249 β: 250–375	59.8	Spermidine/putrescine transporter	n. d.[a]	7.5–8.5	30 at 55°C	Not required	Fanuel et al. 1999a; Frère and van Beeumen 2004; Heck et al. 2006
Ps BapA (BAE02664), *Pseudomonas* sp. MCI3434	366	none	α: 1–238 β: 239–366	63.1	Periplasmic polyamine binding protein	Dipeptidase	9–10	10 at 55°C	Not required	Komeda and Asano 2005
3-2W4 BapA (AAX93858), *S. xenopeptidilytica* 3-2W4	402	29	α: 30–278 β: 279–402	66.6	Sugar transporter	Threonine dehydratase	8–9	26 at 70°C	Not required	Geueke et al. 2005; Geueke et al. 2006
Y2 BapA (ABC59253), *S. microcystinivorans* Y2	399	26	α: 27–275 β: 276–399	64.1	Sugar transporter	Threonine dehydratase	10	60 at 60°C 5 at 70°C	Not required	Geueke et al. 2006

[a] *n. d.* Not determined

high specificities for β-alanyl dipeptides (Table 3) and that the enzyme has sequence similarities with DmpA, 3-2W4 BapA, and Y2 BapA (Table 2). An alignment of the four β-peptidyl aminopeptidases was published by Geueke et al. (2006).

Structural properties and molecular biology

DmpA from *O. anthropi* and the BapA enzymes from *Pseudomonas* sp., *S. xenopeptidilytica* 3-2W4, and *S. microcystinivorans* Y2 are composed of two polypeptides (α- and β-subunit) that form the active enzymes. N-terminal sequencing of the subunits after protein purification combined with cloning and sequencing of the corresponding genes clearly proved that both subunits are encoded by the same gene and originate from the respective precursor protein (Fanuel et al. 1999a; Geueke et al. 2005; Komeda and Asano 2005). All four enzymes are processed by a cleavage in front of a conserved serine residue, but the cleavage sites are different for DmpA and Ps BapA (Gly↓Ser) when compared to 3-2W4 BapA and Y2 BapA (Asn↓Ser).

It was shown for all enzymes that the processing also takes place when the genes are expressed in *Escherichia coli* strains. This observation supports the hypothesis of an autocatalyzed cleavage of the precursor proteins. Whether the reaction is catalyzed by an inter- or intramolecular mechanism might be an interesting topic for future investigations. Western-blot analyses with *O. anthropi* and a recombinant *E. coli* strain expressing *dmpA* reveal that the precursor is not detectable in *O. anthropi* cell extracts, but it is present as inclusion bodies in *E. coli* (Fanuel et al. 1999a). Mutagenesis studies at the cleavage site Gly249↓Ser250 of DmpA show that all tested mutants produce a soluble, inactive precursor protein that is not cleaved even in the presence of wild-type DmpA after prolonged incubation time (Fanuel et al. 1999a).

One structural difference between the four proteins concerns signal sequences. Whereas the *bapA* genes from *S. xenopeptidilytica* 3-2W4 and *S. microcystinivorans* Y2 encode signal sequences of 29 and 26 amino acids, respectively, *dmpA* and *bapA* from *Pseudomonas* sp. do not. This observation indicates that the enzymes are located in different compartments of the cell; DmpA and Ps BapA are cytosolic, but 3-2W4 BapA and Y2 BapA are probably transported via the Sec-pathway into the periplasm. In the case of DmpA and Ps BapA, the reducing conditions in the cytoplasm do not allow the formation of disulfide bridges; 3-2W4 BapA and Y2 BapA do not contain any cysteines and therefore cannot form such structures anyway. Nevertheless, expression of the latter two *bapA* genes without their signal sequences in *E. coli* showed that the autocata-

Table 2 Identities between the amino acid sequences of the β-peptidyl aminopeptidases from *S. xenopeptidilytica* (3-2W4 BapA), *S. microcystinivorans* (Y2 BapA), *Pseudomonas* sp. (Ps BapA), and *O. anthropi* (DmpA) and two putative aminopeptidases from *S. xenopeptidilytica* (3-2W4 ABK40072) and *S. microcystinivorans* (Y2 ABK40073)

Protein	Hits/alignment length (percent)				
	3-2W4 BapA	Y2 BapA	3-2W4 ABK40072	Y2 ABK40073	Ps BapA
Y2 BapA	335/373 (89%)				
3-2W4 ABK40072	174/374 (46%)	177/373 (47%)			
Y2 ABK40073	175/371 (47%)	177/372 (47%)	352/378 (93%)		
Ps BapA	146/371 (39%)	141/358 (39%)	170/377 (45%)	169/372 (45%)	
Oa DmpA	157/372 (42%)	162/375 (43%)	186/384 (48%)	188/381 (49%)	162/373 (43%)

lytical cleavage into the two subunits also takes place in the cytoplasm of the recombinant strains (Geueke et al. 2006).

The mature β-peptidyl aminopeptidases are composed of four α- and four β-polypeptide chains as determined by gel permeation chromatography or mass spectrometry (Frère and van Beeumen 2004; Geueke et al. 2005, 2006; Komeda and Asano 2005). The elucidation of the crystal structure of DmpA confirmed this observation (Bompard-Gilles et al. 2000b). None of the four enzymes contained any cofactors such as divalent metal ions. Isoelectric focusing of DmpA revealed a slightly acidic isoelectric point (pI) of 6.0 (Frère and van Beeumen 2004).

The *bapA* gene is present as single copy in the genome of *S. xenopeptidilytica* 3-2W4 (Geueke et al. 2006), but a similar gene with yet unknown function (EF043282) was also isolated from this strain. *S. microcystinivorans* Y2 also contains a second gene (EF043283) that is similar to its *bapA* gene. The degrees of similarity of these two putative aminopeptidases (ABK40072, ABK40073) to the four characterized β-peptidyl aminopeptidases are shown in Table 2.

Enzymatic properties and substrate specificities

The general reaction of the β-peptidyl aminopeptidases is the N-terminal cleavage of β^3-homoamino acids from oligopeptides, amides, and esters (Table 3). The N-terminus of the substrate molecule needs to be unprotected because none of the four enzymes cleaves substrates that have a protected amino group. 3-2W4 BapA and Y2 BapA exclusively hydrolyze N-terminal β^3-homoamino acids and accept a variety of amino acid side chains, whereas they do not cleave α-amino acids (Geueke et al. 2006). The activity of both enzymes is only slightly influenced by the second position of the substrate molecule; β^3-homoamino acids bound to an α-amino or β^3-homoamino acid as well as β^3-homoamino acid amides serve as substrates.

Ps BapA cleaves peptides, amides, and esters carrying an N-terminal βhGly, but no peptide with an N-terminal α-amino acid is accepted as substrate (Komeda and Asano 2005). The chromogenic substrates L-Ala-*p*NA and D-Ala-*p*NA are both hydrolyzed. A comprehensive study on the

Table 3 Substrate specificity of β-peptidyl aminopeptidases for peptides and amides with varying N-terminal amino acids

Substrates	Enzyme			
	DmpA	Ps BapA	3-2W4 BapA	Y2 BapA
Peptides with N-terminal				
L-α-amino acids	Gly, Ala, Leu, Phe, Ser, Lys, Arg	n.d.	n.d.	n.d.
D-α-amino acids	Ala	n.d.	n.i.	n.i.
L-β-amino acids	*βhGly*, *β³hAla*, β³hSer, β³hVal	*βhGly*	βhGly, *β³hAla*, *β³hVal*, *β³hIle*, *β³hLeu*, *β³hPhe*, *β³hTyr*, β³hTrp, β³hSer, β³hThr, β³hGln, β³hHis, β³hLys, β³hArg	βhGly, *β³hAla*, *β³hVal*, *β³hIle*, *β³hLeu*, *β³hPhe*, *β³hTyr*, β³hTrp, β³hSer, β³hThr, β³hGln, β³hHis, β³hLys, β³hArg
D-β-amino acids	n.d.	n.i.	β³hVal	β³hVal
Amides with N-terminal				
L-α-amino acids	Ala, Lys, Arg	Ala	n.d.	n.d.
D-α-amino acids	Gly, Ala	Ala	n.d.	n.d.
L-β-amino acids	*βhGly*, *β³hAla*	*βhGly*	βhGly, *β³hAla*, *β³hPhe*	βhGly, *β³hAla*, β³hPhe
D-β-amino acids	n.d.	n.i.	β³hPhe	β³hPhe

Amino acids that are cleaved with high activities are in italics. For nomenclature of β-peptides and β-amino acids, see Seebach et al. 2004.
n.d. No activity detected, *n.i.* not investigated

specificity of this enzyme with respect to β-homoamino-acid-containing substrates has not been published yet.

DmpA is the only enzyme of this group that is able to cleave α-peptides, but it exhibits much higher specific activities towards peptides and amides carrying βhGly and β^3hAla at the N terminus (Heck et al. 2006). Although DmpA cleaves D-Ala-*p*NA and L-Ala-*p*NA with fairly high activities (Fanuel et al. 1999a), the hydrolysis of βhGly-*p*NA is much faster. This is illustrated clearly by the catalytic efficiencies k_{cat}/K_m of DmpA for Gly-*p*NA, L-Ala-*p*NA, D-Ala-*p*NA, and βhGly-*p*NA reaching values of 23,000, 1,550, 7,500 (Fanuel et al. 1999a), and 7,800,000 M^{-1} s^{-1} (Geueke et al. 2006), respectively.

The largest peptide known to be degraded by β-peptidyl aminopeptidases is the hexapeptide (β^3hAla-β^3hLys-β^3hPhe)$_2$. In accordance with the substrate specificities, 3-2W4 BapA and Y2 BapA hydrolyze the hexapeptide completely, whereas DmpA only cleaves the first amino acid and forms the corresponding pentapeptide (Heck et al. 2006).

With regard to peptidic substrates, 3-2W4 BapA, Y2 BapA, and DmpA hydrolyze N-terminal L-amino acids faster than N-terminal D-amino acids (Fanuel et al. 1999a; Geueke et al. 2006; Heck et al. 2006), but the opposite effect occurs when Ps BapA and DmpA are incubated with L-Ala-*p*NA and D-Ala-*p*NA. This modification of the stereoselectivity is a very unusual feature and might be explained when further experiments aimed at elucidating the structure of the active sites will have been performed.

All four β-peptidyl aminopeptidases have basic pH optima. Data on the temperature stability show that the enzymes are mesophilic. Their temperature stability increases in the following order: Ps BapA < DmpA < Y2 BapA < 3-2W4 BapA (Table 1).

The activity of DmpA is not reduced in the presence of several specific peptidase inhibitors such as antipain, aprotinin, bestatin, chymostatin, E64, ethylenediamine tetra-acetic acid (EDTA), leupeptid, Pefabloc SC, and 1,10-phenanthroline (Fanuel et al. 1999a; Frère and van Beeumen 2004), whereas Ps BapA is inhibited to different extents by *p*-chloromercuribenzoate, *N*-ethylmaleimide, dithiothreitol, $HgCl_2$, $ZnSO_4$, $ZnCl_2$, $AgNO_3$, and $CdCl_2$ (Komeda and Asano 2005). 3-2W4 BapA and Y2 BapA are completely inactivated in the presence of the specific serine protease inhibitor Pefabloc SC but not by EDTA, leupeptin, phenyl-methanesulfonyl fluoride, bestatin, or 1,10-phenanthroline (Geueke et al. 2006).

Different assays are applied to follow the reaction rates of β-peptidyl aminopeptidases. A simple standard assay is the spectrophotometrical determination of the hydrolysis of amide bonds between amino acids and *p*-nitroaniline at 405 nm (Fanuel et al. 1999a; Geueke et al. 2006; Komeda and Asano 2005). The degradation of peptides can be determined by detecting disappearance or appearance of substrates or products, respectively, after separation with reversed-phase high-performance liquid chromatography (HPLC) at 205 nm (Geueke et al. 2005). The release of N-terminal amino acids from peptides can also be quantified by HPLC using 2 mM $CuSO_4$ as solvent, whereby the complexes of amino acids and copper are detected at 254 nm (Komeda and Asano 2005; Miyazawa et al. 2004; Oi et al. 1992). Separation by thin-layer chromatography followed by detection with ninhydrin and quantification by densitometry is a further method suitable for the simultaneous quantification of substrates and products (Fanuel et al. 1999a).

3-D Structure and reaction mechanism

Soon after the first description of DmpA from *O. anthropi*, the crystal structure of this protein was reported (PDB 1b65; Bompard-Gilles et al. 1999, 2000b). The structure does not show any similarity to known aminopeptidases such as leucine aminopeptidase (Burley et al. 1990), methionine aminopeptidase (Roderick and Matthews 1993), microbial aminopeptidases from *Aeromonas proteolytica* (Chévrier et al. 1994) and *Streptomyces griseus* (Greenblatt et al. 1997), D-specific aminopeptidases from *O. anthropi* (Bompard-Gilles et al. 2000a), and proline iminopeptidase from *Xanthomonas campestris* pv. *citri* (Medrano et al. 1998). These enzymes are either metal-dependent (Zn^{2+}, Co^{2+}) or have a catalytic triad (Ser/His/Asp) or tetrad (Ser/Lys/Ser/Glu), whereas in DmpA, the autocatalytically uncovered N-terminal Ser250 functions as both nucleophile and catalytic base.

DmpA was assigned to the N-terminal nucleophile (Ntn) hydrolase superfamily that was described by Brannigan et al. (1995). Ntn-hydrolases are characterized by a typical four-layered αββα-core structure, are activated by a post-translational, autocatalytic cleavage step, and hydrolyze amide bonds. The DmpA fold shows structural similarities to that of other Ntn-hydrolases, and functionally equivalent amino acids were found in the catalytic centers (Bompard-Gilles et al. 2000b; Frère and van Beeumen 2004). On the other hand, the direction and connectivity of the secondary structure elements differ significantly from the consensus Ntn-hydrolase family (Bompard-Gilles et al. 2000b). Because of these differences, DmpA was not included in a detailed structural comparison of Ntn hydrolases (Oinonen and Rouvinen 2000) but assigned to a novel consensus structure, the DOM-fold (Cheng and Grishin 2005), that is defined as a four-stranded β-sheet packed against two parallel α-helices with the sequential order βββαβα and believed to be a result of convergent evolution.

Nevertheless, the catalytic mechanism that was postulated for DmpA is similar to the mechanism of the other Ntn-hydrolases (Bompard-Gilles et al. 2000b). The free α-

amino group of the catalytic active nucleophile Ser250 acts as a base and enhances the nucleophilic character of the hydroxyl group. The carbonyl carbon of the substrate is attacked by the Ser250 hydroxyl group and a covalent enzyme-substrate complex is formed. This oxyanion intermediate is stabilized by the backbone-NH-group of Tyr146 and the side chain-NH_2 of Asn218. Ser288 and Gly289 are interacting with the Ser250 and could be indirectly involved in the catalytic mechanism. Glu144 is the only acidic amino acid close to the active site; it seems to bind the N-terminal α-amino group of the substrate molecule.

All the above-mentioned amino acids are conserved in the primary structure of Ps BapA (Komeda and Asano 2005), but in 3-2W4 BapA and Y2 BapA, Tyr146 is replaced by Leu164 and Leu161, respectively (Geueke et al. 2006). However, the high homologies indicate that the four β-peptidyl aminopeptidases have a conserved reaction mechanism and similar folding.

Functionally and structurally related enzymes

Basic local alignment search tool (BLAST) searches reveal various sequences that show similarities to the β-peptidyl aminopeptidases, for example from *Agrobacterium tumefaciens*, *Chromohalobacter salexigens*, *Photorhabdus luminescens*, *Robiginitalea biformata*, *Mesorhizobium loti*, *Pseudomonas putida*, *Myxococcus xanthus*, *Mycobacterium flavescens*, *Mycobacterium vanbaalenii*, and *Yarrowia lipolytica*. Most of the gene sequences are retrieved from genome sequencing projects, and they originate mainly from Gram-negative bacteria. Despite the high number of possibly related enzymes, only the four enzymes that are described in this paper have been characterized so far. This might be due to the enzymes' unusual substrate specificities that impeded an earlier functional analysis. Nevertheless, the potential in the databases is enormous, and expression and characterization of these genes might help to better understand this rather new family of peptidases.

Unexpectedly, the X-ray structure of the ornithine acetyltransferase (E.C. 2.3.1.35) from *Streptomyces clavuligerus* displays the same fold as DmpA, although the primary sequences do not show a high degree of similarity. Due to the low activity of DmpA on α-peptides, Elkins et al. (2005) assume that DmpA is in fact an acyl-transferase for an as yet unidentified substrate, but this has not been proven so far.

The knowledge about enzymes that cleave peptide bonds between β^3-homoamino acids with proteinogenic side chains is very new (Geueke et al. 2005), but the enzymatic hydrolysis of peptide bonds between βhGly and α-amino acids was reported already in Hanson and Smith (1949). The reaction is catalyzed by bacterial (E.C. 3.4.13.3) and mammalian carnosinases (E.C. 3.4.13.20) that belong to the large family of metallopeptidases and are also called Xaa-His dipeptidases [peptidase subfamily: M20C according to the MEROPS database (Rawlings et al. 2004)]. One well-investigated enzyme of this group is the dinuclear zinc aminopeptidase (PepV) from *Lactobacillus delbrueckii*, which mainly cleaves dipeptides composed of βhGly and α-amino acids as well as pure α-peptides (Jozic et al. 2002; Vongerichten et al. 1994). Recently, the gene sequence of a human carnosinase (CN1) with narrow substrate specificity was described (Teufel et al. 2003). It would be interesting to investigate whether bacterial and mammalian carnosinases also could hydrolyze β^3-amino acids with large side chains or cleave β,β-peptide bonds.

Physiological role

As mentioned previously, natural peptides solely composed of β-amino acids have not been described so far, but mixed α,β-peptides do occur in nature and might serve as physiological substrates for 3-2W4 BapA and Y2 BapA. This suggestion is supported by the fact that the environmental isolates *S. xenopeptidilytica* and *S. microcystinivorans* can utilize different β^3-homoamino acids as carbon and energy source (Geueke et al. 2007).

The high specific activities of DmpA and Ps BapA for carnosine (βhGly-His) give reason to speculate about the function of these enzymes in their natural environment. *O. anthropi* is an emerging nosocomial pathogenic bacterium that causes meningitis and septicemia in immunosuppressed patients (Christenson et al. 1997; Delière et al. 2000; Ezzedine et al. 1994). As carnosine is present in several human tissues such as muscle and brain (Quinn et al. 1992), it might be available as substrate for *O. anthropi* during infection. According to such a scenario, DmpA might function as the key enzyme in a putative degradation of carnosine by *O. anthropi*.

Conclusions

β-Peptidyl aminopeptidases represent a new class of enzymes with remarkable substrate specificities and an unusual protein fold. They cleave peptide bonds between β-amino acids that until now were shown to be resistant towards enzymatic hydrolysis. In contrast to the well-known carnosinases, their substrate specificity is broad, and *N*-terminal β-amino acids with bulky side chains are hydrolyzed. Although only four peptidases of this group are characterized yet, database searches indicate that the group might be much larger. Future studies will need to focus on characterizing related enzymes and more closely elucidating their biological function.

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