References

Dideberg, O., Charlier, P., Dive, G., Joris, B., Frère, J.M. & Ghuysen, J.M. (1982) Structure of a Zn^{2+}-containing D-alanyl-D-alanine-cleaving carboxypeptidase at 2.5 Å resolution. *Nature* **299**, 469–470.

Hall, T.M.T., Porter, J.A., Beachy, P.A. & Leahy, D.J. (1995) A potential catalytic site revealed by the 1.7-A crystal structure of the amino-terminal signalling domain of Sonic hedgehog. *Nature* **378**, 212–216.

Lee, J.J., Ekker, S.C., von Kessler, D.P., Porter, J.A., Sun, B.I. & Beachy, P.A. (1994) Autoproteolysis in hedgehog protein biogenesis. *Science* **266**, 1528–1537.

Porter, J.A., von Kessler, D.P., Ekker, S.C., Young, K.E., Lee, J.J., Moses, K. & Beachy, P.A. (1995) The product of hedgehog autoproteolytic cleavage active in local and long-range signalling. *Nature* **374**, 363–366.

Vallee, B.L. & Auld, D.S. (1990) Zinc coordination, function, and structure of zinc enzymes and other proteins. *Biochemistry* **29**, 5647–5659.

Vanderslice, P., Copeland, W.C. & Robertus, J.D. (1988) Site-directed alteration of serine 82 causes nonproductive chain cleavage in prohistidine decarboxylase. *J. Biol. Chem.* **263**, 10583–10586.

463. Zinc D-Ala-D-Ala carboxypeptidase

Databanks

Peptidase classification: clan MD, family M15, MEROPS ID: M15.001
NC-IUBMB enzyme classification: EC 3.4.17.14
Databank codes:

Species	SwissProt	PIR	EMBL (cDNA)	EMBL (genomic)
Streptomyces albus	P00733	A00913	X55794	–

Brookhaven Protein Data Bank three-dimensional structures:

Species	ID	Resolution	Notes
Streptomyces albus	1LBU	1.8	

Note: There used to be a sequence from *Streptomyces griseus* in SwissProt, which has disappeared.

Name and History

Bacteriolytic enzymes isolated from culture supernatants of *Streptomyces albus* G and selected for their specificity on critical linkages were useful in establishing the structure of the bacterial cell wall peptidoglycan (Ghuysen, 1968). The ***KM endopeptidase*** has the specificity of a carboxypeptidase (Ghuysen *et al.*, 1969, 1970). It selectively hydrolyzes bacterial walls in which the peptidoglycan interpeptide bonds extend between a D-alanine residue and another D-amino acid residue in α-position to a free carboxylate; it cleaves the bis-disaccharide peptide dimer (obtained by muramidase treatment of the peptidoglycan of *Escherichia coli*) into monomers by hydrolyzing the C-terminal D-alanyl-D-*meso*-diaminopimelic acid interpeptide bond; and it hydrolyzes the C-terminal D-alanyl-D-alanine linkage of the precursor UDP-*N*-acetylmuramoyl-L-alanyl-γ-D-glutamyl-(L)-*meso*-diaminopimelyl-(L)-D-alanyl-D-alanine. Subsequently, the KM endopeptidase was identified as a zinc enzyme (Dideberg *et al.*, 1980) and has been given the name ***zinc D-Ala-D-Ala carboxypeptidase***. It contains 1 mol of zinc per 18 000 Da protein molecule, the apoprotein binds Zn^{2+} with a K_a of $2 \times 10^{14}\ M^{-1}$ and the Zn^{2+} cofactor is required for activity.

Specificity and Activity

The specificity of the zinc D-Ala-D-Ala carboxypeptidase was studied on peptides of the form R3-R2-R1-COOH (Leyh-Bouille *et al.*, 1970a). There is a strict requirement that the C-terminal amino acid R1 should have a free carboxylate.

R1 may be glycine, leucine, alanine, ornithine, lysine or diaminopimelic acid, but if the amino acid is not glycine, then the asymmetric carbon must be in the D configuration. The side chain of R1 may be very bulky. High substrate activity is compatible with the presence of polypeptide substituents on the ε-amino group of D-lysine, the δ-amino group of D-ornithine, and the amino group on the L-carbon of *meso*-diaminopimelic acid.

There is a strict requirement that the R2 side chain be either a hydrogen atom or, much better, a methyl group, in which case the asymmetric carbon must be D. Replacement of the penultimate D-Ala in N^{α},N^{ε}-diacetyl-L-Lys-D-Ala-D-Ala by a Gly does not modify the V value but drastically decreases the K_m value. The length, structure and polarity of the R3 side chain is of great importance. The presence at this position of Gly, L-Ala, L-Tyr, L-homoserine, N^{γ}-acetyl-L-diaminobutyric acid, N^{δ}-acetyl-L-ornithine and N^{ε}-acetyl-L-Lys is paralleled by a progressive and large increase of the rate of hydrolysis of the terminal D-Ala-D-Ala linkage. The presence of an acyl substituent on the ω-amino group of the R3 side chain is another requirement. The N^{α},N^{ε}-diacetyl-L-Lys-D-Ala-D-Ala is a much better substrate than the N^{α}-monoacetyl derivative. Also, the transformation of the ε-amino group of L-Lys to an α-amino group by introduction of a carboxyl group in α-position (as occurs when L-Lys is replaced by diaminopimelic acid) and its substitution by a pentaglycine sequence result in a large increased substrate activity of the corresponding peptides.

Preparation

The wild-type strain produces about 100–200 ng enzyme per liter of culture. The enzyme can be purified using classical chromatographic procedures (Duez *et al.*, 1978). Gene overexpression in *Streptomyces lividans* (Duez *et al.*, 1990) results in the export of the synthesized enzyme (yield: 10 mg liter^{-1}).

Structural Chemistry

The sequence of the mature 213 amino acid residue zinc-containing D-Ala-D-Ala carboxypeptidase was determined by chemical degradation (Joris *et al.*, 1983) and firmly established by gene sequencing (Duez *et al.*, 1990). The enzyme is synthesized as a 255 residue precursor that contains a cleavable signal peptide of 42 amino acids. The three-dimensional structure of the enzyme was established by X-ray diffraction at high resolution (Dideberg *et al.*, 1982; Wéry, 1987) (see Fig. 462.1). The molecular structure in solution was studied by small-angle X-ray scattering (Labischinski *et al.*, 1984). The model compares well with that of the crystal structure. Radius of gyration: 182 ± 0.05 nm; largest diameter: 5.9 ± 0.2 mm; relative molecular mass: $17\,000 \pm 200$; volume: 35 ± 2 mm^3; degree of hydration: 0.25 ± 0.02 g water g^{-1} protein.

Bimodular Design

The fold adopted by the zinc D-Ala-D-Ala carboxypeptidase, consisting of two globular modules connected by a single link, is unprecedented. The catalytic 132 amino acid residue C-terminal module possesses three α helices and a five-stranded β sheet that defines a cleft in which the Zn^{2+} ion is ligated by one water molecule, two His and one Asp residues (Wéry, 1987). The open cleft can accommodate extended structures, a feature which is related to the specificity profile of the enzyme. Arg138 is probably concerned with the binding, by charge pairing, of the carboxylate substrate, and His and Asp residues probably play the required functions of proton abstraction-donation.

Fused to the catalytic module, the noncatalytic, 81 amino acid residue, N-terminal module possesses three α helices and an elongated crevice defined by a loop-helix-loop-helix motif consisting of two repeats, each 16 amino acid residues long, connected by a heptapeptide (Ghuysen *et al.*, 1994). Similar motifs are borne by the C-terminal regions of the *N*-acetylmuramoyl-peptide amidases of *Bacillus subtilis* and *Bacillus licheniformis*, and the lysozyme of *Clostridium acetobutylicum*. As a common feature of these exocellular enzymes is their substrate, it is likely that their noncatalytic modules are involved in the binding to the insoluble wall peptidoglycan.

Active-site-directed Inactivators

The zinc D-Ala-D-Ala carboxypeptidase can be inactivated by acyclic compounds possessing both a C-terminal carboxylate and, at the other end of the molecule, a thiol, hydroxamate or carboxylate (Charlier *et al.*, 1984). 3-Mercaptopropionate (racemic) and 3-mercaptoisobutyrate (L-isomer) are competitive inhibitors ($K_i \approx 5$–10×10^{-9} M). 6β-Iodopenicillinate binds to the active site in front of the Zn^{2+} cofactor, close to His190 and causes permanent inactivation of the enzyme (Charlier *et al.*, 1984). Classical β-lactam compounds are very weak inhibitors (Leyh-Bouille *et al.*, 1970b). Enzyme inhibition may be competitive or noncompetitive, in which case (as observed with cephalothin and cephalosporin C) binding causes disruption of the protein crystal lattice (Charlier *et al.*, 1984) and drastically alters the scattering behavior of the protein in solution (Labischinski *et al.*, 1984).

In summary, in spite of differences in stereospecificity, polypeptide folding and function, the zinc D-Ala-D-Ala carboxypeptidase is mechanistically similar to carboxypeptidase A (Chapter 451) and thermolysin (Chapter 351). A similar mechanism, dictated by common catalytic requirements, has evolved from different starting tertiary structures by converging evolution. The zinc D-Ala-D-Ala carboxypeptidase and other peptidoglycan hydrolases have similar binding-site-shaped repeated sequences. The acquisition of a noncatalytic substrate-binding module is an evolutionary advantage for exocellular enzymes that hydrolyze bonds in an insoluble polymer.

References

Charlier, P., Dideberg, O., Jamoulle, J.C., Frère, J.-M., Ghuysen, J.-M., Dive, G. & Lamotte-Brasseur, J. (1984) Active-site directed inactivators of the Zn^{++} D-alanyl-D-alanine-cleaving carboxypeptidase of *Streptomyces albus* G. *Biochem. J.* **219**, 763–772.

Dideberg, O., Joris, B., Frère, J.-M., Ghuysen, J.-M., Weber, G., Robaye, R., Delbrouck, J.-M. & Roelandts, I. (1980) The

exocellular DD-carboxypeptidase of *Streptomyces albus* G: a metallo (Zn^{++}) enzyme. *FEBS Lett.* **117**, 215–218.

Dideberg, O., Charlier, P., Dive, G., Joris, B., Frère, J.-M. & Ghuysen, J.-M. (1982) Structure at 2.5 Å resolution of a Zn^{++}-containing D-alanyl-D-alanine-cleaving carboxypeptidase. *Nature* **299**, 469–470.

Duez, C., Frère, J.-M., Geurts, F., Ghuysen, J.-M., Dierickx, L. & Delcambe, L. (1978) The exocellular DD-carboxypeptidase-endopeptidase from *Streptomyces albus* strain G. Purification and chemical properties. *Biochem. J.* **175**, 793–800.

Duez, C., Lakaye, B., Houba, S., Dusart, J. & Ghuysen, J.-M. (1990) Cloning, nucleotide sequence and amplified expression of the gene encoding the extracellular metallo (Zn) DD-peptidase of *Streptomyces albus* G. *FEMS Microbiol. Lett.* **71**, 215–220.

Ghuysen, J.-M. (1968) Use of bacteriolytic enzymes in determination of wall structure and their role in cell metabolism. *Bacteriol. Rev.* **32**, 425–464.

Ghuysen, J.-M., Dierickx, L., Coyette, J., Leyh-Bouille, M., Guinand, M. & Campbell, J.N. (1969) An improved technique for the preparation of Streptomyces peptidases and *N*-acetylmuramyl-L-alanine amidase active on bacterial wall peptidoglycans. *Biochemistry* **8**, 213–220.

Ghuysen, J.-M., Leyh-Bouille, M., Bonaly, R., Nieto, M., Perkins, H.R., Schleifer, K.H. & Kandler, O. (1970) Isolation of DD-carboxypeptidase from *Streptomyces albus* G culture filtrates. *Biochemistry* **9**, 2955–2961.

Ghuysen, J.-M., Lamotte-Brasseur, J., Joris, B. & Shockman, G.D. (1994) Binding site-shaped repeated sequences of *Streptomyces albus* G DD-peptidase, *Bacillus subtilis* CwlA amidase, *Corynebacterium acetobutylicum* lysozyme and *Bacillus* sp ORF-L3 gene product. *FEBS Lett.* **342**, 23–28.

Joris, B., Van Beeumen, J., Casagrande, F., Gerday, Ch., Frère, J.-M. & Ghuysen, J.-M. (1983) The complete amino acid sequence of the Zn^{++}-containing D-alanyl-D-alanine-cleaving carboxypeptidase of *Streptomyces albus* G. *Eur. J. Biochem.* **130**, 53–69.

Labischinski, H., Giesbrecht, P., Fischer, E., Barnickel, G., Bradaczek, H., Frère, J.-M., Houssier, Cl., Charlier, P., Dideberg, O. & Ghuysen, J.-M. (1984) Study of the Zn-containing DD-carboxypeptidase of *Streptomyces albus* G by small angle X-ray scattering in solution. *Eur. J. Biochem.* **138**, 83–87.

Leyh-Bouille, M., Ghuysen, J.-M., Bonaly, R., Nieto, M., Perkins, H.R., Schleifer, K.H. & Kandler, O. (1970a) Substrate requirements of the *Streptomyces albus* G DD-carboxypeptidase. *Biochemistry* **9**, 2961–2971.

Leyh-Bouille, M., Ghuysen, J.-M., Nieto, M., Perkins, H.R., Schleifer, K.H. & Kandler, O. (1970b) On the *Streptomyces albus* G DD-carboxypeptidase. Mechanism of action of penicillin, vancomycin and ristocetin. *Biochemistry* **9**, 2971–2975.

Wéry, J.P. (1987) Applications de méthodes de détermination de phases à la résolution de deux structures de protéine [Application of the methods of phase determination to the resolution of two protein structures]. PhD thesis, University of Liège.

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464. N-Acetylmuramoyl-L-alanine amidase

Databanks

Peptidase classification: clan MD, not a peptidase, related to family M15, MEROPS ID: M15.960
NC-IUBMB enzyme classification: EC 3.5.1.28 & EC 3.4.19.10 (discontinued)
Chemical Abstracts Service registry number: 37288-68-9
Databank codes:

Species	Gene	SwissProt	PIR	EMBL (cDNA)	EMBL (genomic)
Homologs of peptidases in family M15					
Bacillus licheniformis	*cwlL*	P36550	S39916	D13377	–
	orfL3	Q99125	D49754	M63942	