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Chapter 182

c0182

Mirabilysin

s0010 ## Databanks

p0030 *MEROPS name*: mirabilysin

p0035 *MEROPS classification*: clan MA, subclan MA(M), family M10, subfamily M10B, peptidase M10.057

p0040 *Species distribution*: known only from *Proteus mirabilis*

p0045 *Reference sequence from*: *Proteus mirabilis* (UniProt: Q11137)

s0015 ## Name and History

p0050 Among the virulence components known to be expressed by the urinary tract pathogen *Proteus mirabilis* is a metalloprotease referred to as ***mirabilysin***. This is an extracellular protease that can be isolated when *P. mirabilis* is grown on media containing a suitable substrate such as skim milk agar [1]. Many strains of *P. mirabilis* produce this protease which has broad substrate specificity but has been referred to as an IgA protease because of its activity on both serum and secretory forms of IgA1 (immunoglobulin A1) and IgA2 [2], as well as IgG [3–6]. The enzyme is not related structurally or functionally to the IgA proteases produced by a number of important pathogens (*e.g. Streptococcus, Neisseria*; Chapters 279 and 683) which have exquisite specificity for human IgA1 and a limited number of proteins with sequence similarity to the IgA1 hinge region [2,7].

Activity and Specificity s0020

p0055 The purified enzyme is stable for long periods at 4°C at pH 8.0 and is unaffected by heating at 60°C for 5 min [4]. Enzyme activity is detected from pH 6.0 to 10.0, with an optimum activity at pH 8.0 [4]. The addition of either Mg^{2+} or Ca^{2+} increases activity, while EDTA is inhibitory. The finding of activity in the absence of added divalent cations (*circa* 65% of maximal activity) suggests that the purified protein has divalent metal ions already bound to it [4,8,9].

p0060 In addition to EDTA, 1,10-phenanthroline and α,α'-dipyridyl both inhibit the activity of mirabilysin, while incubation in the presence of either DFP or iodoacetamide has no effect. Phosphoramidon, an inhibitor of some metalloproteases, has no effect on mirabilysin [4], a result that is typical of peptidases in family M10.

p0065 The *zapA* gene that encodes mirabilysin has been cloned and its gene product overexpressed [1]. SDS-PAGE was used to examine the substrate specificity of the recombinant mirabilysin. Both human serum IgA1 and human IgG were digested by the recombinant metalloprotease. Recombinant mirabilysin degrades human IgA1 in a time-dependent manner resulting in complete digestion of the IgA1 substrate into numerous smaller fragments. Mouse IgA is also a substrate for mirabilysin, an unusual finding because the action of IgA proteases is

normally limited to IgA1 from human and related primates [19] (*cf.* Chapter 683). Recombinant mirabilysin also digested human serum IgA2 and secretory IgA, as well as casein and azocasein, in agreement with the observations of others [5,6]. The enzyme does not have activity against serum albumin, cytochrome c, *P. mirabilis* flagellin, ovalbumin, or phosphorylase b [1,5,6]. The relevance of proteolytic activity to pathogenicity of *Proteus* may not be limited solely to cleavage of IgA and IgG, many proteins found in the urinary tract are substrates of ZapA proteolysis, including complement (C1q and C3), cell matrix (collagen, fibronectin, and laminin), cytoskeletal proteins (actin and tubulin) and the antimicrobial peptides human beta-defensin 1 (hBD1) and LL-37, components of the human renal tubule innate immune response [11].

p0070 aThe molecular specificity of mirabilysin was defined using small peptides (β-chain insulin) to be similar to many other bacterial metalloproteinases, *i.e.* cleavage after hydrophobic amino acids. Fernandes *et al.* [12] examined the enzyme specificity using fluorogenic substrates and found two that were effectively cleaved. In their study, the synthetic peptide Abz-Ala-Phe-Arg↓Ser-Ala-Ala-Gln-EDDnp was cleaved at the Arg-Ser bond with a K_M of 4.6 μM, a k_{cat} of 1.73 mM s^{-1}, and a catalytic efficiency of 376 mM s^{-1}, while a second peptide, Abz-Arg-Pro-Pro-Gly-Phe↓Ser-Pro-Phe-Arg-Gln-EDDnp, was cleaved at the Phe-Ser bond with a K_M of 13.6 μM, a k_{cat} of 3.96 s^{-1}, and a catalytic efficiency of 291 mM s^{-1} [12].

s0025 Structural Chemistry

p0075 The amino acid sequence of mirabilysin has been deduced from the nucleotide sequence of the *zapA* gene. A 1473 bp open reading frame provides convincing evidence that this is the structural gene of the secreted metalloprotease. The deduced amino acid sequence of mirabilysin predicts an acidic protein (pI 4.30) composed of 491 residues with a total molecular mass of 54 000 Da. Based on gel electrophoresis analysis, recombinant mirabilysin is composed of a single protein of 55 kDa. Analysis of the metalloproteases of *P. mirabilis* and other *Proteus spp*. on polyacrylamide-gelatin gels under various constraints of pH and other factors showed the presence of cell-associated proteinase before the other forms were secreted. The secreted protease was modified to two isoforms whose mass (53–46 kDa) varied with the *Proteus spp*. and the strain [13].

p0080 Computerized protein homology searches comparing mirabilysin to other protein sequences from both prokaryotes and eukaryotes revealed that mirabilysin is homologous to members of the serralysin subfamily of family M10, which includes the proteases of *Serratia marcescens* (serralysin: Chapter 180), *Erwinia chrysanthemi*, and *Pseudomonas aeruginosa* (aeruginolysin: Chapter 181). Four distinctive protein signature motifs are found in the mirabilysin sequence that support this idea. The first signature motif, encompassing Asn178 to Gly203, is homologous to the zinc-binding region of this protein family. The location of Glu187 is appropriate for this residue to act as the catalytic base [14]. Additionally, the three histidines in this region, His186, His190 and His196, are correctly located to function as a putative zinc-binding site. The second motif found in the serralysin subfamily of proteases is the Met-turn of metzincins [14], which in mirabilysin is located at Thr222 to Tyr226, with Met224 being the conserved residue in this motif.

p0085 The proteases of the serralysin subfamily are secreted by the ATP-binding cassette (ABC) superfamily of prokaryotic and eukaryotic transporters [15–17]. In these systems, three transport proteins probably combine to form zones of adhesion between the inner and outer membranes, through which the proteins are secreted [18,19]. The proteins secreted by this system do not possess an N-terminal signal sequence, but they do contain a C-terminal targeting signal that is essential for secretion [20]. This sequence ends with the conserved four amino acid motif DXXX [21]. In the case of mirabilysin and serralysin this sequence is DFIV [22]. A further characteristic of this group of secreted proteins is that the member proteins contain 4–13 repeats of the consensus sequence GGXGXD near the C-terminal secretion signal. This motif produces a β-roll conformation that serves as a Ca^{2+}-binding site [14]. Mirabilysin has three of these sites located at Gly343 to Asp348, Gly361 to Asp366, and Gly379 to Asp384. A fourth Ca^{2+}-binding motif may also exist (Gly388 to Asn393), although it lacks consensus at the last residue with asparagine substituted for aspartate.

Preparation s0030

p0090 Mirabilysin can be purified by hydrophobic chromatography on phenyl-Sepharose [1,4]. This purification schemes typically provide between 200 and 500 μg of protein per liter of culture supernatant. The relative protease activity of the recombinant enzyme is *circa* 15–17 units per μg of protein as measured by azocasein digestion.

Biological Aspects s0035

p0095 Mirabilysin is similar to the proteases of *P. aeruginosa* (Chapter 181) and *S. marcescens* (Chapter 180), both of which degrade IgA and IgG [23,24] and to the proteases of *E. chrysanthemi* [25]. *P. aeruginosa*, *S. marcescens* and *E. chrysanthemi* are pathogenic bacteria, as is *P. mirabilis*, suggesting that mirabilysin may play a role in virulence. Immunoblotting of urine from patients who had

P. mirabilis urinary tract infections showed that 64% of the specimens with IgA contained IgA heavy-chain fragments identical in size to those formed when purified IgA was degraded by pure protease [6]. The importance of mirabilysin as a virulence factor of uropathogenic *Proteus mirabilis* is clearly demonstrated by the dramatic attenuation of virulence in ZapA mutants in a murine model of ascending urinary tract infection [26]. The ZapA mutant failed to produce mirabilysin and did not degrade any immunoglobulin substrates. After a 7-day infection, samples of urine, bladder and kidney were collected and bacterial counts determined. In each sample, the loss of the enzyme resulted in a marked decrease in bacterial survival. For example, a comparison of bacterial counts obtained from urine showed that the survival of the mutant was 100 000 times less than that of the wild type (1.90×102 colony forming units (CFU) ml^{-1} for the mirabilysin-defective mutant compared with 1.67×107 CFU ml^{-1} for wild type) [26]. This might suggest the importance of the degradation of components of the acquired (immunoglobulins) and innate immune systems and possibly other factors [27].

p0100 A number of studies have shown a link between mirabilysin and the characteristic swarming behavior of *Proteus*, another component of virulence [26,28,29]. ZapA was expressed coordinately with the development of the swarmer cell phenotype. Senior *et al.* [9], in a study which showed that almost all clinical isolates of *P. mirabilis* (48 strains), *P. penneri* (25), *P. vulgaris* biogroup 2 (48) and around 70% *P. vulgaris* biogroup 3 (21) isolated from man, produced EDTA sensitive proteolytic enzymes. A strong correlation was found between the ability of a strain to form swarming growth and its ability to secrete proteases. Non-swarming isolates invariably appeared to be non-proteolytic. However, some isolates, particularly of *P. vulgaris* biogroup 3, were non-proteolytic even when they formed swarming growth.

p0105 Walker *et al.* [26] showed that analysis of the nucleotide sequences adjacent to zapA reveals four additional genes, *zapE*, *zapB*, *zapC* and *zapD*, which appear to possess functions required for ZapA activity and IgA proteolysis. Based on homology to other known proteins, these genes encode a second metalloprotease, ZapE, as well as a ZapA-specific ABC transporter system (ZapB, ZapC and ZapD).

p0110 These data, combined with the knowledge that mirabilysin degrades a wide variety of mammalian-host related proteins, strengthen the role of mirabilysin in the uropathogenicity of *P. mirabilis*.

s0040 Uncited reference

p0115 [10]

Further Reading

For reviews of *Proteus* virulence factors including proteases and swarming phenotype, see Morgenstein *et al.* [30] and Rozalski *et al.* [31]. For general review of related proteases, see Gomis-Rüth [32]. p0120

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Chapter 183

c0183

Epralysin

s0010 ## Databanks

p0030 *MEROPS name*: epralysin
p0035 *MEROPS classification*: clan MA, subclan MA(M), family M10, subfamily M10B, peptidase M10.060
p0040 *Species distribution*: known only from *Pseudomonas fluorescens*
p0045 *Reference sequence from*: *Pseudomonas fluorescens*

NON-PRINT ITEM: CHAPTER 182

Abstract
The third edition of the *Handbook of Proteolytic Enzymes* aims to be a comprehensive reference work for the enzymes that cleave proteins and peptides, and contains over 850 chapters. Each chapter is organized into sections describing the name and history, activity and specificity, structural chemistry, preparation, biological aspects, and distinguishing features for a specific peptidase. The subject of Chapter 182 is Mirabilysin.
Keywords:
Aeruginolysin, ATP-binding cassette, immunoglobulin, immunoglobulin A, proteus, serralysin