[M189]. Mirabilysin

Databanks

[M189]P001 *MEROPS name*: Mirabilysin

[M189]P002 *MEROPS classification*: clan MA(M), family M10B, peptidase M10.057

[M189]P003 *Species distribution*: *Proteus mirabilis*

Name and History

[M189]P004 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 (Wassif *et al*., 1995). Many strains of *P. mirabilis* produce this protease, also referred to as an IgA protease because of its activity on both serum and secretory forms of IgA1 (immunoglobulin A1) and IgA2, as well as IgG (Loomes *et al*., 1990, 1992, 1993; Senior *et al*., 1991).

Activity and Specificity

[M189]P005 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 (Loomes *et al*., 1992). Enzyme activity is detected from pH 6.0 to 10.0, with an optimum activity at pH 8.0 (Loomes *et al*., 1992). 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 (ca. 65% of maximal activity) suggests that the purified protein has divalent metal ions already bound to it, in agreement with the observations of Senior *et al*. (1987, 1988) and Loomes *et al*. (1992).

[M189]P006 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 (Loomes *et al*., 1992), a result that is typical of peptidases in family M10.

[M189]P007 The *zapA* gene that encodes mirabilysin has been cloned and its gene product overexpressed (Wassif *et al*., 1995). 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 IgA from human and related primates (Kornfeld & Plaut, 1981 (cf. Chapter [M047]). Recombinant mirabilysin also digested human serum IgA2 and secretory IgA, as well as casein and azocasein, in agreement with the observations of others (Senior *et al*., 1991; Loomes *et al*., 1993). The enzyme does not have activity against serum albumin, cytochrome *c*, *P. mirabilis* flagellin, ovalbumin, or phosphorylase *b* (Senior *et al*., 1991; Loomes *et al*., 1993; Wassif *et al*., 1995), yet, recent evidence suggests that the specificity of the enzyme is not limited solely to IgA. Fernandes *et al*. (2000) 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{XAFR#SAAQ} was ★1 cleaved at the Arg-Ser bond with a K_m of 4.6 μM, a k_{cat} of 1.73 s^{-1}, and a catalytic efficiency of 376 mM s^{-1}, while a second peptide, Abz-Arg-Pro-Pro-Gly-Phe┼Ser-Pro-Phe- ★2 Arg-Gln-EDDnp{PPGF#SPFR}, 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} (Fernandes *et al*., 2000). Indeed, unpublished work from my laboratory has provided convincing evidence that mirabilysin can cleave many proteins found associated with the human urinary tract, including complement proteins (C1q and C3) and structural components of urinary epithelial cells (actin, tubulin, fibronectin, collagen and laminin). Thus, it may not be appropriate to refer to mirabilysin as an 'IgA protease', rather its spectrum of activity suggests that has a more generalized activity.

Structural Chemistry

[M189]P008 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. The enzyme is rich in glycine (11.2 mol %).

[M189]P009 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 subgroup of family M10, which includes the proteases of *Serratia marcescens* (serralysin: Chapter [M604]), *Erwinia chrysanthemi*, and *Pseudomonas aeruginosa* (aeruginolysin: Chapter [M188]). 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 (Baumann, 1994). 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 subgroup of proteases is the Met-turn of metzincins (Baumann, 1994), which in mirabilysin is located at Thr222 to Tyr226, with Met224 being the conserved residue in this motif.

[M189]P010 The proteases of the serralysin subgroup are secreted by the ATP-binding cassette (ABC) superfamily of prokaryotic and eukaryotic transporters (Higgins, 1992; Pugsley, 1992; Wandersman, 1992; see also Chapters [M604] and [M188]). 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 (Delepelaire & Wandersman, 1991; Holland *et al*., 1990). 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 (Delepelaire & Wandersman, 1990). This sequence ends with the conserved four amino acid motif DXXX (Ghigo & Wandersman, 1994). In the case of mirabilysin and serralysin this sequence is DFIV (Nakahama *et al*., 1986). 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 (Baumann, 1994). 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

[M189]P011 Mirabilysin is purified by hydrophobic chromatography on phenyl-Sepharose using the procedure described by Wassif *et al*. (1995). This purification scheme typically provides between 200 and 500 μg of protein per liter of culture supernatant. The relative protease activity of the recombinant enzyme is ca. 15–17 units per μg of protein as measured by azocasein digestion.

Biological Aspects

[M189]P012 Mirabilysin is similar to the proteases of *P. aeruginosa* (Chapter [M188]) and *S. marcescens* (Chapter [M604]), both of which degrade IgA and IgG (Döring *et al*., 1981; Molla *et al*., 1988), and to the proteases of *E. chrysanthemi* (Létoffé *et al*., 1990). *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 (Senior *et al*., 1991). The requirement of mirabilysin in virulence was demonstrated by comparing the survival of a mirabilysin-defective mutant to wild-type *P. mirabilis* in a murine model of ascending urinary tract infection (Walker *et al*., 1999). The 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 (1.90×10^2 colony forming units (CFU) ml^{-1} for the mirabilysin-defective mutant compared with 1.67×10^7 CFU ml^{-1} for wild type) (Walker *et al*., 1999). The same study also found that *zapA* was expressed coordinately with the development of the swarmer cell, another component of virulence. 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*.

Further Reading

For reviews, see Hooper (1994) and Maeda & Morihara (1995). [M189]P013

References

Baumann, U. (1994) Crystal structure of the 50 kDa metallo protease from *Serratia marcescens*. *J. Mol. Biol.* **242**, 244–251. PMID:8089845 [M189]P014

Delepelaire, P. & Wandersman, C. (1990) Protein secretion in gram-negative bacteria. The extracellular metalloprotease B from *Erwinia chrysanthemi* contains a C-terminal secretion signal analogous to that of *Escherichia coli* α-hemolysin. *J. Biol. Chem.* **265**, 17118–17125. PMID:2211614 [M189]P015

Delepelaire, P. & Wandersman, C. (1991) Characterization, localization and transmembrane organization of the three proteins PrtD, PrtE and PrtF necessary for protease secretion by the gram-negative bacterium *Erwinia chrysanthemi*. *Mol. Microbiol.* **5**, 2427–2434. PMID:1791757 [M189]P016

Döring, G., Obernesser, H.-J. & Botzenhart, K. (1981) Extracellular toxins of *Pseudomonas aeruginosa*. II. Effect of two proteases on human immunoglobulins IgG, IgA and secretory IgA. *Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. Reihe A* **249**, 89–98. PMID:NONE [M189]P017

Fernandes, B.L., Aneas, M.A., Juliano, L., Palma, M.S., Lebrun, I. & Portaro, F.C. (2000) Development of an operational substrate for ZapA, a metalloprotease secreted by the bacterium *Proteus mirabilis*. *Braz. J. Med. Biol. Res.* **33**, 765–770. PMID:10881051 [M189]P018

Ghigo, J.M. & Wandersman, C. (1994) A carboxyl-terminal four-amino acid motif is required for secretion of the metalloprotease PrtG through the *Erwinia chrysanthemi* protease secretion pathway. *J. Biol. Chem.* **269**, 8979–8985. PMID:8132636 [M189]P019

Higgins, C. (1992) ABC-transporters: from microorganisms to man. *Annu. Rev. Cell Biol.* **8**, 67–113. PMID:1282354 [M189]P020

Holland, I., Kenny, B. & Blight, M. (1990) Haemolysin secretion from *E. coli*. *Biochimie* **72**, 131–141. PMID:2116181 [M189]P021

Hooper, N.M. (1994) Families of zinc metalloproteases. *FEBS Lett.* **354**, 1–6. PMID:7957888 [M189]P022

Kornfeld, S.J. & Plaut, A.G. (1981) Secretory immunity and the bacterial IgA proteases. *Rev. Infect. Dis.* **3**, 521–534. PMID:6792682 [M189]P023

Létoffé, S., Delepelaire, P. & Wandersman, C. (1990) Protease secretion by *Erwinia chrysanthemi*: the specific secretion functions are analogous to those of *Escherichia coli* α-haemolysin. *EMBO J.* **9**, 1375–1382. PMID:2184029 [M189]P024

[M189]P025 Loomes, L.M., Senior, B.W. & Kerr, M.A. (1990) A proteolytic enzyme secreted by *Proteus mirabilis* degrades immunoglobulins of the immunoglobulin A1 (IgA1), IgA2, and IgG isotypes. *Infect. Immun.* **58**, 1979–1985. PMID:2111288

[M189]P026 Loomes, L.M., Senior, B.W. & Kerr, M.A. (1992) Proteinases of *Proteus* spp.: purification, properties, and detection in urine of infected patients. *Infect. Immun.* **60**, 2267–2273. PMID:1587593

[M189]P027 Loomes, L.M., Kerr, M.A. & Senior, B.W. (1993) The cleavage of immunoglobulin G *in vitro* and *in vivo* by a proteinase secreted by the urinary tract pathogen *Proteus mirabilis. J. Med. Microbiol.* **39**, 225–232. PMID:8366522

[M189]P028 Maeda, H. & Morihara, K. (1995) Serralysin and related bacterial proteinases. *Methods Enzymol.* **248**, 395–413. PMID:7674934

[M189]P029 Molla, A., Kagimoto, T. & Maeda, H. (1988) Cleavage of immunoglobulin G (IgG) and IgA around the hinge region by proteases from *Serratia marcescens. Infect. Immun.* **56**, 916–920. PMID:3126147

[M189]P030 Nakahama, K., Yoshimura, K., Marumoto, R., Kikuchi, M., Lee, I., Hase, T. & Matsubara, H. (1986) Cloning and sequencing of *Serratia* protease gene. *Nucleic Acids Res.* **14**, 5843–5855. PMID:3016665

[M189]P031 Pugsley, A. (1992) Superfamilies of bacterial transport systems with nucleotide binding components. *Symp. Soc. Gen. Microbiol.* **47**, 223–248. PMID:NONE

[M189]P032 Senior, B.W., Albrechtsen, M. & Kerr, M.A. (1987) *Proteus mirabilis* strains of diverse type have IgA protease activity. *J. Med. Microbiol.* **24**, 175–180. PMID:3309325

[M189]P033 Senior, B.W., Albrechtsen, M. & Kerr, M.A. (1988) A survey of IgA protease production among clinical isolates of Protease. *J. Med. Microbiol.* **25**, 27–31. PMID:3275781

[M189]P034 Senior, B.W, Loomes, L. & Kerr, M. (1991) The production and activity *in vivo* of *Proteus mirabilis* IgA protease in infections of the urinary tract. *J. Med. Microbiol.* **35**, 203–207. PMID:1941989

[M189]P035 Walker, K., Moghaddame-Jafari, S., Lockatell, C., Johnson, D. & Belas, R. (1999) ZapA, the IgA-degrading metalloprotease of *Proteus mirabilis*, is a virulence factor expressed specifically in swarmer cells. *Mol. Microbiol.* **32**, 825–836.

[M189]P036 Wandersman, C. (1992) Secretion across the bacterial outer membrane. *Trends Genet.* **8**, 317–321. PMID:1365398

[M189]P037 Wassif, C., Cheek, D. & Belas, R. (1995) Molecular analysis of a metalloprotease from *Proteus mirabilis. J. Bacteriol.* **177**, 5790–5798. PMID:7592325

Robert Belas
Center of Marine Biotechnology,
The University of Maryland Biotechnology Institute,
701 East Pratt Street,
Baltimore, MD 21202, USA
Email: belas@umbi.umd.edu

Handbook of Proteolytic Enzymes 2nd Edn
ISBN 0-12-[TO BE SUPPLIED]