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Molecules in focus
IgA1 protease

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Abstract

IgA1 proteases are proteolytic enzymes that cleave specific peptide bonds in the human immunoglobulin A1 (IgA1) hinge region sequence. Several species of pathogenic bacteria secrete IgA1 proteases at mucosal sites of infection to destroy the structure and function of human IgA1 thereby eliminating an important aspect of host defence.

IgA1 proteases are known as autotransporter proteins as their gene structure encodes the information to direct their own secretion out of the bacterial cell. The *iga* gene structure is also thought to contribute to the antigenic heterogeneity demonstrated by the IgA1 proteases during infections and the cleavage specificity of the IgA1 proteases for human IgA1.

The IgA1 proteases have therefore been implicated as important virulence factors that contribute to bacterial infection and colonisation. The development of strategies to inactivate these IgA1 proteases has become the subject of recent research, as this has the potential to reduce bacterial colonisation at mucosal surfaces.

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1. Introduction

The bacterial IgA1 proteases are a group of proteolytic enzymes that cleave human immunoglobulin A, subclass 1 (IgA1). The first IgA1 protease was discovered in 1973 (Mehta, Plaut, Calvanico, & Tomasi, 1973), and subsequently several species of pathogenic bacteria were found to produce IgA1 protease including *Haemophilus influenzae*, *Streptococcus pneumoniae*, *Neisseria gonorrhoeae* and *Neisseria meningitidis* (Plaut, Gilbert, Artenstein, & Capra, 1975; Mulks, Kornfeld, & Plaut, 1980). This group of proteolytic enzymes was found to cleave specific proline-serine and proline-threonine peptide bonds within the sequence TPPTPSPSTPPTSPSPS (T, P and S are threonine, pro-

line and serine residues, respectively) found in the hinge region of human IgA1 (Frangione & Wolfenstein-Todel, 1972). The IgA1 proteases were therefore thought to be an important virulence factor, which influenced colonisation and infection of bacteria at the mucosal surfaces.

2. Structure

The various IgA1 proteases have been characterised as serine, metallo or cysteine type proteases (Bachovchin, Plaut, Flentke, Lynch, & Kettner, 1990; Plaut, Genco, & Tomasi, 1974; Frandsen, Kjeldsen, & Kilian, 1997). The IgA1 protease genes (*iga*) generally code for two protein domains, known as the protease and beta domains (Fig. 1a).

The serine-type protease domain sequence is composed of approximately 1000 residues and contains the active site sequence VLGDSGSPLF (Fig. 1a), which is also found in the trypsin/chymotrypsin family of

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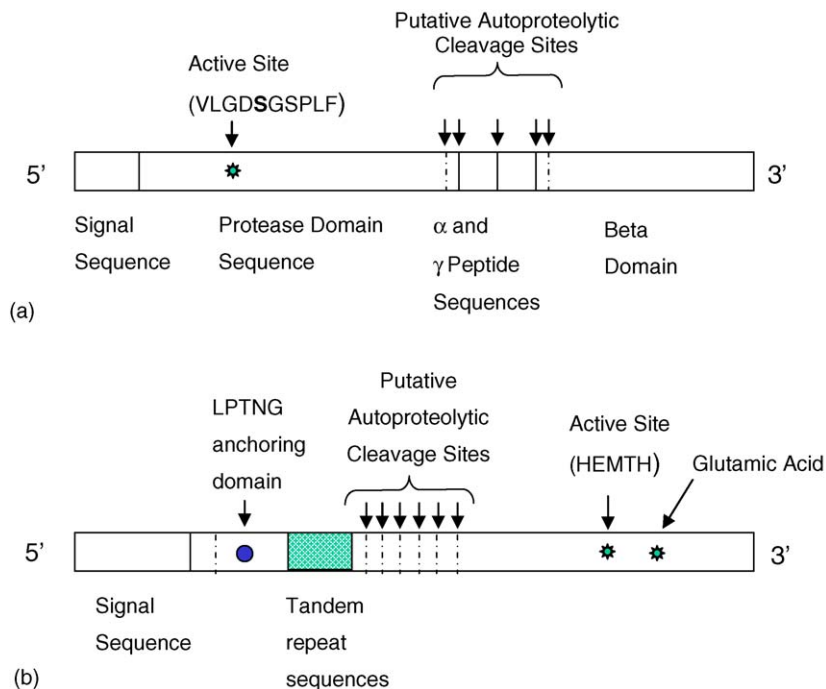


Fig. 1. Diagrammatic representation of the *iga* genes structures of the serine and metallo type IgA1 proteases: (a) the *iga* structures of the serine protease; (b) the *iga* structure of the metallo enzyme from *Streptococcal* species.

serine proteases. The beta domain is composed of heterogeneous sequences in the N terminus and a highly conserved C terminus sequence of approximately 300 residues that is thought to be required for protein translocation (Lomholt, Van Alphen, & Kilian, 1993). A linking sequence, which can be of variable lengths, connects the protease domain to the beta domain.

The metallo-type IgA1 protease contains the sequence LPNTG, which is characteristic of an anchoring domain, resulting in the IgA1 protease being mainly associated with the cell membrane of the bacteria (Fig. 1b). This is followed by a heterogeneous region of sequence composed of varying numbers of tandem repeats, each approximately 20 amino acids in length. The subsequent sequence is highly hydrophobic and is thought to encode the transmembrane domain. The metallo-type *iga* sequence contains the zinc-binding motif HEMTH and a glutamic acid residue E located 20 amino acids downstream from the critical histidine of the active site (Poulsen et al., 1998).

The *iga* gene structure for the cysteine-type IgA1 protease has yet to be determined (Arzese & Botta, 1995).

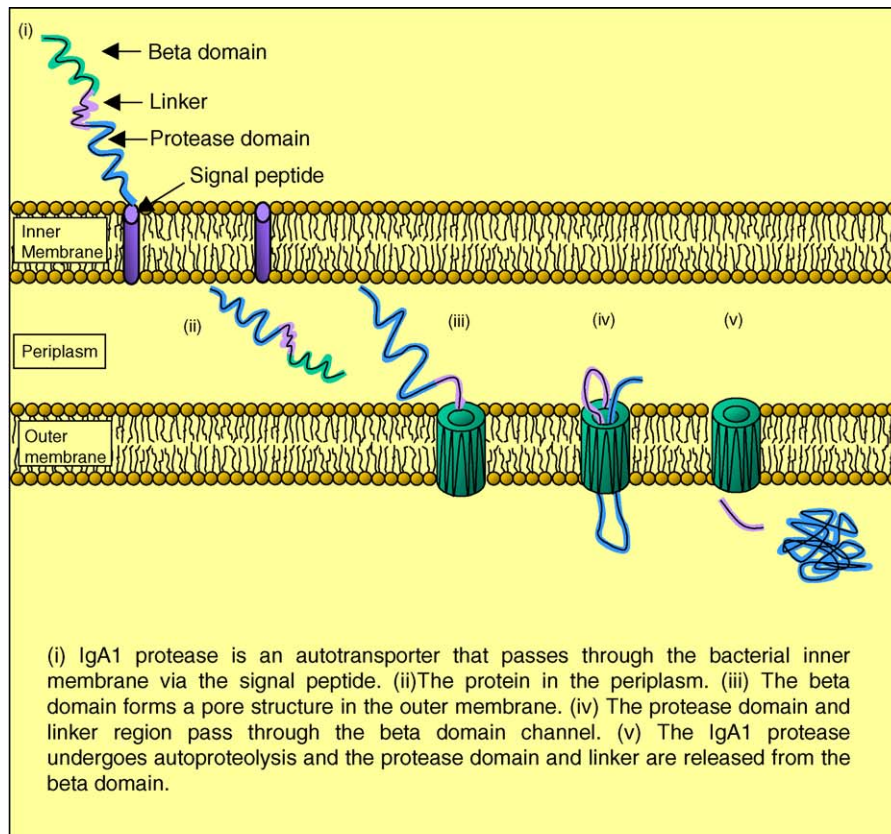
3. Expression, activation and turnover

The *iga* genes are constitutively expressed and secreted during the bacterial exponential growth phase

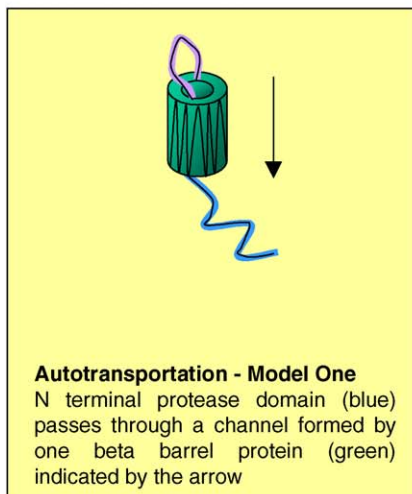
(Plaut et al., 1974). Fig. 2A shows the initial gene product is a preproprotein, which undergoes several post-translocational modifications as the protein is secreted into the extracellular space. The IgA1 protease is described as an autotransporter, as the protein encodes all the information required for the protein to transport itself out of the cell. The beta domain has been shown to insert into the outer membrane and one model of secretion suggests the beta domain forms a pore in the membrane (Halter, Pohlner, & Meyer, 1984) (Fig. 2B). However recent studies suggest that several beta domain proteins are required to group together to form a pore structure for the protease domains to traverse the outer membrane (Veiga, Sugawara, Nikaido, de Lorenzo, & Fernandez, 2002) (Fig. 2C).

4. Biological function

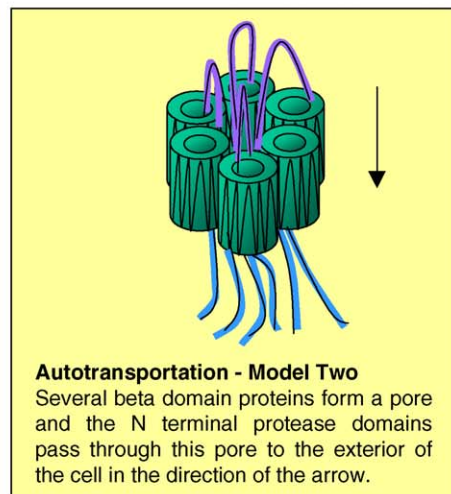
The precise biological function of the IgA1 proteases is unclear, as the development of an animal model has been hampered due to the specificity of IgA1 proteases for human IgA1. Furthermore, several in vitro organ culture models have been unsuccessful in defining the exact role of IgA1 proteases in bacterial infection (Farley et al., 1986; Cooper, McGee, Mulks, Koomey, & Hindman, 1984).



(A)



(B)



(C)

Fig. 2. IgA1 protease – an autotransporter protein that directs its own translocation through the bacterial cell membranes.

However, it is known that bacterial pathogens secrete IgA1 proteases that cleave the human IgA1 hinge region, thereby producing intact Fab α and Fc α fragments. The Fab α fragments still retain their capacity to bind to bacterial surface antigens, a term called *fabulation* (Mansa

& Kilian, 1986). Bacterial surface epitopes are therefore masked from intact antibodies and the lack of Fc α fragments required for agglutination and opsonophagocytosis facilitates bacterial survival on the mucosal surface.

There is indirect evidence to support the importance of IgA1 proteases based upon the host response to IgA1 proteases. They invoke a strong immunogenic response in vivo and antibodies to IgA1 proteases have been detected in patients during acute disease, colonisation and recovery (Brooks, Lammel, Blake, Kusecek, & Achtman, 1992). However, the IgA1 proteases are highly heterogeneous and inhibiting antibodies produced during previous infections do not neutralise the IgA1 proteases of new strains of bacteria. This is thought to be one mechanism of evasion from host defences. In addition, higher concentrations of IgA1 proteases have been detected in patients with acute infection compared to asymptomatic carriers (Vitovski, Dunkin, Howard, & Sayers, 2002), suggesting increased immunogenicity during such episodes, perhaps as a result of bacterial invasion.

The IgA1 proteases have been found to stimulate the release of cytokines such as tumour necrosis factor α (TNF α), interleukin-1 β (IL-1 β), interleukin-6 (IL6), and interleukin-8 (IL8). The IgA1 proteases may therefore be contributing to the propagation of the inflammatory response by stimulating these particular cytokines (Lorenzen et al., 1999). In vivo, IgA1 proteases have also been found to activate CD4(+) and CD8(+) T cells and also CD19(+) B cells and CD56(+) natural killer cells. These findings indicate that IgA1 proteases may also instigate part of the T cell inflammatory response during bacterial infections (Tsirpouchtsidis, Hurwitz, Brinkman, Meyer, & Haas, 2002).

Proteins containing sequences similar to the human IgA1 hinge region sequence have recently been investigated as targets for IgA1 protease. Thus far, in vitro studies have revealed that IgA1 proteases can cleave human chorionic gonadotrophin hormone (Senior, Stewart, Galloway, & Kerr, 2001), granulocyte-macrophage colony stimulating factor, the CD8 surface antigen of cytotoxic T lymphocytes (Kilian, Reinholdt, Lomholt, Poulsen, & Frandsen, 1996) and LAMP 1 a membrane glycoprotein of lysosomes (Hauck & Meyer, 1997). The biological significance of cleaving these proteins requires further investigation.

5. Possible medical and industrial applications

As the IgA1 proteases are thought to be virulence factors in bacterial infection, the inactivation of IgA1 protease proteolytic activity should reduce bacterial colonisation and infection in vivo. Synthetic inhibitors with an analogous structure to the natural substrate (the human IgA1 hinge region) have been found to inactivate the protease by competitive inhibition (Burton, Wood,

Lynch, & Plaut, 1988). Smaller synthetic peptides have also been found to be potent inhibitors of the serine-type IgA1 protease (Bachovchin et al., 1990). The role of these peptides in protection of mucosal surfaces has yet to be explored. Alternatively specific monoclonal antibodies could be produced to block the formation of enzyme-substrate complexes by steric hindrance. Whether such an approach is likely to be effective also requires further study.

In recent studies, the beta domain of the IgA1 protease has been implemented in novel industrial applications as it has been found to be able to transport alternative proteins to the protease domain of IgA1 protease as N terminal passenger proteins (Klauser, Pohlner, & Meyer, 1990). These early investigations have since led to the application of this technology to immobilise heavy metals in soil. A fusion protein of the mouse metallothionein I protein and the beta domain autotransporter has been used to target the mouse metallothionein I protein to the cell surface of *Ralstonia eutropha* (formerly *Alcaligenes eutrophus*). The *R. eutropha* strain was able to reduce CD2(+) toxicity in polluted soils and enabled the growth of *Nicotiana bentamiana* tobacco plants (Valls, Atrian, de Lorenzen, & Fernandez, 2000a). Similarly, the metallothionein-beta domain fusion protein was expressed in *Pseudomonas putida* with a view to utilising this bacteria in heavy metal adsorption of industrial sewage (Valls, de Lorenzen, Gonzalez-Duarte, & Atrian, 2000b).

A fusion protein of the beta domain and a single-chain antibody to transmissible gastroenteritis coronavirus was expressed on the surface of *Escherichia coli*. The single chain antibody was therefore on the *E. coli* cell surface and was able to target and block the virus from infecting epithelial cells (Veiga, de Lorenzo, & Fernandez, 2003).

These studies could be extended to future applications in vivo, by using the beta protein as a transporter domain in replacement therapy for naturally occurring physiological proteins, thereby treating patients with particular protein deficiencies, such as the replacement of mucosal anti-proteases in chronic infections. This approach may reduce the bystander damage occurring as a result of the continued inflammatory process.

6. Concluding remarks

The IgA1 proteases are a group of proteolytic enzymes, which are produced by pathogenic bacteria that infect and colonise mucosal surfaces. They are produced in low concentrations, but are highly potent proteolytic enzymes, which specifically cleave peptide bonds within a restricted sequence in the hinge region

of human IgA1. They also invoke a strong antigenic response although their heterogeneous nature allows the enzymes to evade the human immune system in sequential infections. The cleavage of human IgA1 produces intact Fab fragments, which have been shown to bind antigenic receptors, thereby masking surface epitopes from intact and functional antibodies and the loss of the Fc fragment eliminates the agglutination activity of IgA1. These observations suggest that IgA1 proteases may be important virulence factors central to the colonisation of mucosal surfaces by bacteria.

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