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Not as simple as just punching a hole

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Abstract

Like a variety of other pathogenic bacteria, *Aeromonas hydrophila* secretes a pore-forming toxin that contribute to its virulence. The last decade has not only increased our knowledge about the structure of this toxin, called aerolysin, but has also shed light on how it interacts with its target cell and how the cell reacts to this stress. Whereas pore-forming toxins are generally thought to lead to brutal death by osmotic lysis of the cell, based on what is observed for erythrocytes, recent studies have started to reveal far more complicated pathways leading to death of nucleated mammalian cells.

Keywords: Aerolysin; *A. hydrophila*; Pore-forming; GPI anchored protein; Membrane insertion; Oligomerization

1. Introduction

Among the great variety of virulence factors produced by bacterial pathogens, a sub-family is composed by the pore-forming toxins (van der Goot, 2001). The purpose of producing such a membranolytic factor varies form one type of bacterium to another. Some invasive bacteria such as *Listeria monocytogenes* (Cossart and Lecuit, 1998) produce pore-forming toxins (listeriolysin O) to break the phagocytic vacuole after the microorganism has been taken up by the host cell. Interestingly it has recently been shown, for the first time, that production of a pore-forming toxin can also allow the escape of a bacterium from the host cell once it has multiplied in the cytoplasm (Alli et al., 2000). The expression of a pore-forming toxin upon termination of bacterial replication to allow cytolysis of the host cell and egress of the intracellular bacteria was shown for *Legionella pneumophila* but will most probably apply to other bacteria. Pore-forming toxins are also produced by extracellular bacteria such as *Aeromonas hydrophila* (aerolysin), *Staphylococcus aureus* (alpha-toxin, Gouaux, 1998) and *Clostridium septicum* (alpha-toxin, Ballard et al., 1995). Although these toxins clearly contribute to the virulence and the spreading of the bacteria, their exact role during infection has not been established. Surprisingly however, they constitute by far the best-characterized pore-forming toxins.

We will here summarize our current knowledge about the structure and function of aerolysin, emphasizing the findings of the last 5 years. Aerolysin is produced by a variety of *Aeromonas* sp. These Gram negative bacteria are responsible for food-born infections in humans and may also lead to deep wound infection (Altwegg and Geiss, 1989). Recently *A. hydrophila* has also emerged as a potential pathogen in the immunocompromised host (Chang et al., 1997). Aerolysin has been shown to contribute to the spreading of the pathogen and immunization against aerolysin will protect animals against the bacterium. Also disruption of the aerolysin gene will attenuate virulence (Chakraborty et al., 1987) although it has recently been proposed that a two-hemolytic toxin model provides a more accurate prediction of *Aeromonas* virulence (Heuzenroeder et al., 1999).

The birds eye view of the mechanism of action of aerolysin is the same as for all extracellular acting pore-forming toxins. The toxin is secreted by the bacterium as a soluble protein that can readily diffuse. Upon interaction with the target cell, the toxin oligomerizes into a ring-like structure that inserts into the membrane and forms a pore. Pore formation leads to permeabilization of the host plasma membrane. We will describe in detail what is known at the molecular level about these different steps and how the host cell reacts toward this membrane damage. Finally we will summarize our current knowledge of how the structure of aerolysin relates to its function.

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2. Synthesis and secretion

Aerolysin is synthesized by Gram-negative bacteria of the *Aeromonas* sp. as a pre-protoxin containing an N-terminal signal sequence and a C-terminal activation peptide (Howard and Buckley, 1985a, b) (see below). The signal sequence targets the protein to the *sec* machinery that translocates the precursor toxin into the periplasmic space. Probably with the help of chaperones such as dsbA (Hardie et al., 1995), proaerolysin then acquires its tertiary structure within the periplasm. Quaternary assembly into dimers can also occur (Barry et al., 2001; van der Goot et al., 1993a). It is however not clear whether dimerization is required for secretion or whether it only occurs when the periplasmic toxin concentration reaches a certain threshold (Fivaz et al., 1999). The fully folded toxin is then transported across the outer bacterial membrane via a type II secretion apparatus (Pugsley et al., 1997). The first evidence that folding precedes transport across the outer membrane came from a study by Buckley and colleagues (Hardie et al., 1995), where they engineered a mutant aerolysin that had a disulfide bridge linking the two monomers in the dimer together. They could show that disulfide bridge formation, and therefore dimer assembly, could occur prior to secretion into the medium. Similar conclusion has recently been reached by Nomura et al. (2000). The property of folding and assembling in the periplasm is shared by other virulence factors that are released by type II secretion such as cholera toxin which form a hetero hexamer (Dalbey and Robinson, 1999). Searches for consensus sequences that would target proteins to the type II secretion pathway have failed and it is therefore now believed that the information lies in the three-dimensional structure (Lu and Lory, 1996). The fold that is required has however not yet been identified. In the case of proaerolysin, the translocation targeting motif appears to reside in the large lobe of the protein (domains 2–4, see below and Fig. 2) since domain 1 on its own could not be secreted (Diep et al., 1998a). It is important to note however that the large lobe was secreted far less efficiently than the wild type toxin suggesting that domain 1 does somehow contribute to efficient secretion.

How passage across the outer membrane occurs is not fully understood. At least 12 gene products appear to be involved (Howard et al., 1993; Jahagirdar and Howard, 1994) one of which probably forms an outer-membrane protein pore complex (Guilvout et al., 1999; Howard et al., 1996). Transport also requires ATP and the electromotive force (Letellier et al., 1997; Wong and Buckley, 1989).

3. Interaction with the target cells

3.1. Binding to the host cell

Once secreted into the extra bacterial milieu, proaerolysin can diffuse towards target cells. Binding to the mammalian host occurs via a high affinity interaction (Abrami et al., 1998b; MacKenzie et al., 1999) between the toxin and host glycosylphosphatidyl inositol (GPI) anchored proteins (Abrami et al., 1998b; Diep et al., 1998b; Nelson et al., 1997; Wang et al., 1999). The toxin indeed opportunistically uses these ubiquitous cell surface molecules as receptors. Aerolysin however does not bind to the polypeptide moiety of these lipid anchored proteins but to the conserved glycan core (Diep et al., 1998b) they all share (Fig. 1) (for review on GPI-anchored proteins see Ferguson, 1999). With some exceptions such as the folate receptor (Diep et al., 1999), aerolysin does not appear to discriminate between different GPI anchored proteins within a given cell type (Fivaz et al., submitted). Binding to the glycan core also ensures that the toxin is located in the direct vicinity of the plasma membrane. The importance of this will become apparent below. It must be noted that on erythrocytes, aerolysin in addition binds to the major surface glycoprotein glycophorin (Diep et al., 1999). Whether other receptors, in addition to GPI-anchored proteins, exist on other cell types has not been address. The observation that GPI deficient cells are resistant to aerolysin up to concentrations of 1 μg/ml (Gordon et al., 1999; Abrami, submitted), however suggests that interaction with putative additional receptors would be of lower affinity.

A puzzling question that remains is how aerolysin reaches its high-affinity binding site on the glycan core of the GPI-anchor. Indeed this part of the receptor is very little exposed at the surface of the cell. The interesting possibility has been forwarded that, in order to cross the thick layer of glycocalyx covering epithelial cells, aerolysin initially binds to surface oligosaccharides with low affinity, possibly through its lectin-like domain 1 (see below) or other tryptophan rich regions of the molecule, before being progressively shuttled to the membrane vicinity where it would bind to the glycan core of the GPI-anchor (MacKenzie et al., 1999). This attractive relay mechanism, involving low- and high-affinity binding sites, remains however to be proven.

As mentioned above, proaerolysin as well as aerolysin can exist as dimers or monomers depending on the toxin concentration (Barry et al., 2001; Fivaz et al., 1999; van der Goot et al., 1993a). There is however evidence, that the monomer is the binding competent form of the toxin (Fivaz et al., 1999). It is not clear how this 52 kDa protein, once it has managed to reach the membrane vicinity, is able to bind to the comparatively small glycan core that is situated between the lipid bilayer and the polypeptide moiety of the GPI anchored protein (Abrami et al., 2000).

3.2. Activation

Proaerolysin is not toxic because it is unable to polymerize into a ring-like structure. An activation step is absolutely required which consists in removing a C-terminal peptide of

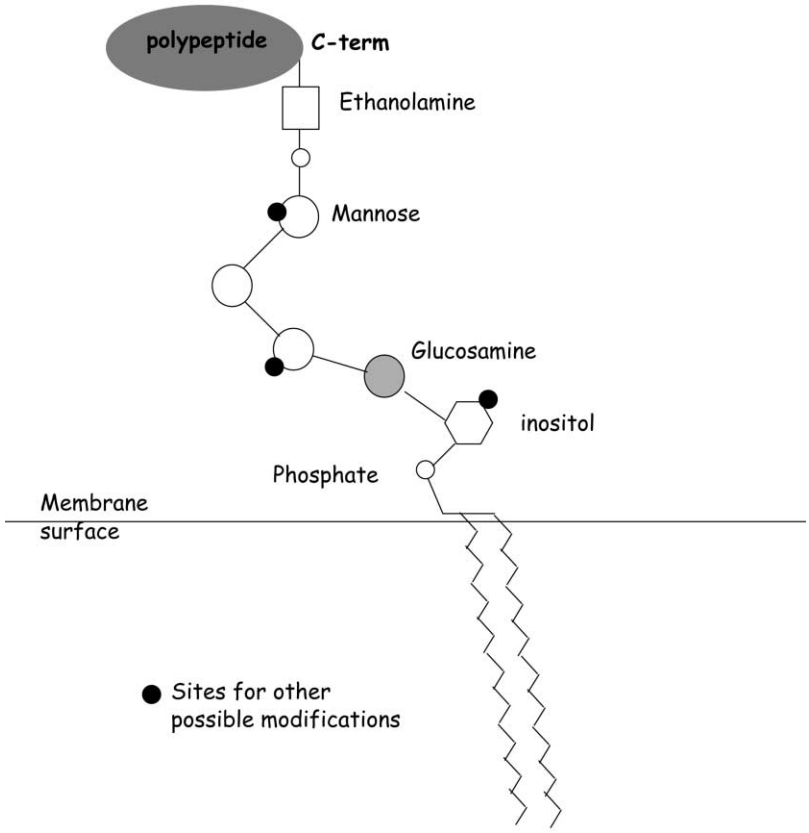

Fig. 1. Schematic diagram of a mammalian GPI anchor (Ferguson, 1999; Kinoshita et al., 1997). The GPI anchor is added to the C-terminus of the protein of interest.

approximately 40 amino acids (Howard and Buckley, 1985a). Soluble proteases such as trypsin and chymotrypsin (van der Goot et al., 1992) or proteases produced by *Aeromonas* itself (Howard and Buckley, 1985a), will activate proaerolysin into mature aerolysin. Membrane anchored proteases of the host cells belonging to the proprotein convertase family are also able to process the precursor toxin (Abrami et al., 1998a). More specifically we have shown that processing at the surface of Chinese hamster ovary (CHO) and baby hamster kidney (BHK) cells is due mainly to furin. The fact that both soluble and membrane anchored proteases are able to activate the precursor is in agreement with the fact that both proaerolysin and aerolysin are able to bind to the receptors (Abrami et al., 1998a; MacKenzie et al., 1999). It is important to note that whereas furin has been localized mainly intracellularly it is also present at the cell surface where processing of proaerolysin occurs.

Whether processing occurs via soluble or membrane anchored proteases might depend on the site of infection. For example trypsin is likely to activate proaerolysin in the digestive tract, whereas furin might activate the precursor during deep found infection.

3.3. Oligomerization

The next step, that precedes membrane insertion (van der Goot et al., 1993b), is oligomerization. It is not clear what triggers the assembly process. Clearly activation is required and the removal of the C-terminal peptide triggers subtle conformational changes (Cabiaux et al., 1997) that might promote oligomerization. The surrounding of histidine 132 is moreover thought to act as a nucleation site and deprononation or mutation of this residue blocks the onset of assembly (Buckley et al., 1995). In contrast modification of tryptophans 371 or 373 accelerates the process possibly due to partial destabilization of the monomeric protein.

Oligomerization is a poorly understood but precise event since it always leads to ring-like heptameric structures (Moniatte et al., 1996; Wilmsen et al., 1992). The process can occur either in solution or at the surface of the target cell. In the latter case, the reaction is far more efficient and will occur at 10^6 fold lower concentration than in solution. This difference in efficiency suggests that the cells somehow promote the encounter between aerolysin monomers.

Clearly, since the binding Kd of aerolysin to its receptor is low (20 nM) (Abrami et al., 1998b; MacKenzie et al., 1999),

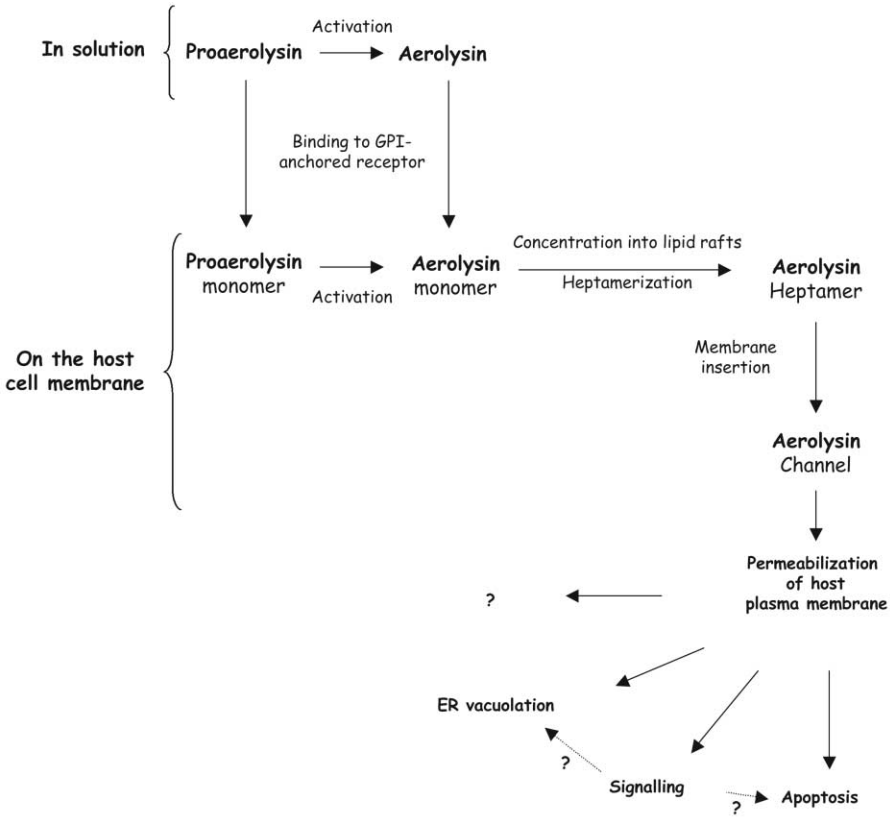

Fig. 2. Schematic representation of the mode of action of aerolysin. The proaerolysin or aerolysin monomer binds to the glycan core of GPI anchored proteins on mammalian cells. In the case of proaerolysin binding, processing occurs via the action of furin. The mature toxin is able to polymerize into a heptameric ring. The transient association of receptor-bound aerolysin with lipid rafts favors this step. This cellular concentration device promotes channel formation. The aerolysin channel selectively permeabilizes the plasma membrane to small ions. As a consequence, several events can occur intracellularly. (i) Calcium can be released from the endoplasmic reticulum through G-protein activation and the IP_3 pathway. (ii) Selective vacuolation of the endoplasmic reticulum can be observed and (iii) apoptosis is triggered in certain cell types such as T-lymphocytes.

it can be expected that binding concentrates the toxin. Indeed, as discussed by McLaughlin and Aderem (1995), membrane binding reduces the dimensionality from three to two. We estimated that toxin binding to its receptor leads to an increase in concentration by a factor of ≈1500. Although this estimate may be somewhat approximate, it appears that binding could not solely account for the increased efficiency of oligomerization observed at the cell surface as compared to in solution.

As described above, the aerolysin receptors are all proteins anchored to the membrane by a GPI-moiety. One of the extensively characterized properties of this family of proteins is that they have a complex and unusual mobility pattern at the cell surface. They can navigate in the phosphoglyceride region of the plasma membrane, and this with a higher mobility than the corresponding transmembrane proteins. They however also have the ability to associate, in a dynamic fashion, with cholesterol and glycosphingolipid rich microdomains of the plasma membrane, called lipid rafts (Brown and London, 1998; Harder and Simons, 1997). Single particle analysis of Thy-1 showed that a GPI-anchored protein can be confined to such a cholesterol rich microdomain for up to 9 s (Sheets et al., 1997). These uncommon characteristics of the aerolysin receptors suggested that lipid rafts might be implicated in promoting cell surface oligomerization of the toxin. This hypothesis is supported by the following observations. Treatment of baby hamster kidney (BHK) cells with the cholesterol binding drug saponin abrogated the capacity of proaerolysin to associate with rafts and concomitantly led to a dramatic inhibition of aerolysin oligomerization suggesting that the concentration threshold required for heptamer formation could no longer be reached. Oligomerization could however be forced when adding higher amounts of toxin to saponin treated BHK cells but remained far less efficient (Abrami and van der Goot, 1999). In cells expressing unusually high levels of GPI-anchored proteins such as T lymphocytes, oligomerization might still occur even upon disruption of

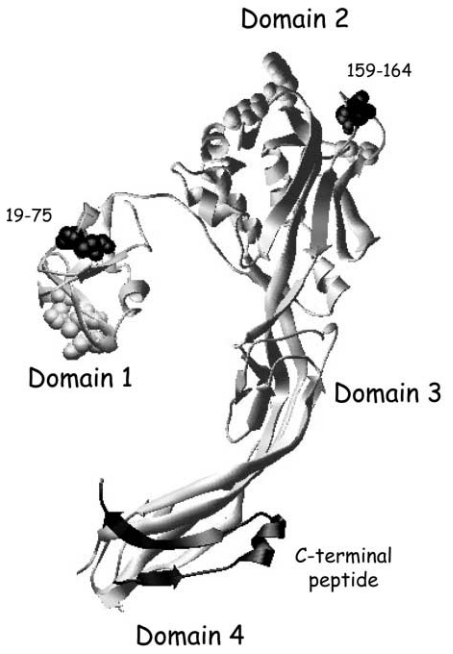

Fig. 3. Ribbon diagram of proaerolysin, based on its X-ray structure (Parker et al., 1994). The activation peptide is shown as a black ribbon, the two disulfide bonds are in black. The amino acids that have been identified as being involved in receptor binding are shown in grey space filled (MacKenzie et al., 1999).

rafts (Nelson and Buckley, 2000). In most cell types however microdomains appears to act as GPI concentration platforms at the cell surface that aerolysin has hijacked to suit its own purpose.

The heptamer that is formed exposes hydrophobic patches at its surface in contrast to the monomer/dimer. Due to this amphipathic nature, the complex is thought to spontaneously insert into the lipid bilayer. It is therefore important that the oligomer only forms in the direct vicinity of the membrane to prevent loss of the complex by hydrophobic interactions (sticking) with molecules in the medium or surrounding the cell. The correct location of the oligomerization reaction is ensured by the receptor. Indeed as mentioned above, aerolysin binds to the glycan core of the GPI-anchor, which lies in the acyl chain-head group boundary region.

The final membrane insertion step is very poorly understood. It has been suggested that unfavorable energetic effects exist at the junctures between lipid rafts and the fluid-phase phosphoglyceride region of the plasma membrane (Brown, 1998). These unstable boundaries might facilitate membrane insertion of the aerolysin heptamer. Also other characteristics of lipid rafts might promote membrane penetration. In vitro studies have however recently revealed that lipids that favor the lamellar to inverted hexagonal phase transition can enhance channel formation by aerolysin in artificial liposomes (Alonso et al., 2000).

4. Cellular effects of aerolysin

Insertion of the heptameric complex into artificial bilayers leads to the formation of a well-defined transmembrane channel (Chakraborty et al., 1990; Wilmsen et al., 1990) that remains open between −80 and +80 mV (Wilmsen et al., 1990). In vitro studies suggest that the pore has a diameter of 17–40 Å (Howard and Buckley, 1982; Tschödrich-Rotter et al., 1996; Wilmsen et al., 1992) and allows passage of molecules as large as glucagon (3550 Da). In vivo experiments however suggest smaller size pores. Aerolysin channels in living cells indeed seem to discriminate between an ethidium monomer and an ethidium dimer (Krause et al., 1998) and do not allow entry of trypan blue into cells (960 Da) (Abrami et al., 1998b). These observations raise the possibility that the channels formed in vitro are somewhat different from those formed in vivo.

In living cell, aerolysin channels selectively permeabilize the plasma membrane to small ions such as potassium or calcium but not proteins (Abrami et al., 1998b; Krause et al., 1998). At present we have been unable to observe any repair of plasma membrane lesions in contrast to what has been described for other toxins (Valeva et al., 2000). This could be a result of the extraordinary stability of the aerolysin heptamer, its resistance to proteolysis and degradation (Lesieur et al., 1999). Alternatively aerolysin might not be internalized by cells and therefore not be removed from the surface.

In the presence of aerolysin, cells remain viable for several hours depending on the toxin concentration (Abrami et al., 1998b). Channel formation however induces a number of cellular responses. In human granulocytes, aerolysin was shown to trigger release of calcium from the ER (Krause et al., 1998). This process could be inhibited by pre-treating cells with Pertussis toxin or by treating cells with a phospholipase C-β inhibitor indicating that aerolysin had triggered the activation of G proteins and the production of inositol(1, 4, 5)-triphosphate (Krause et al., 1998). The mechanism by which channel formation by aerolysin induces activation of G-protein is unclear. Using inactive aerolysin mutants, we could rule out that signaling was trigger through binding of aerolysin to its GPI-anchored receptors (Krause et al., 1998). One possibility is that the aerolysin channels affect the integrity of lipid rafts, which have been implicated in modulating and integrating signaling events at the plasma membrane (Simons and Toomre, 2000). In agreement with the activation of G-proteins, certain cellular function such as chemotaxis where found to be stimulated (Jin et al., 1992; Krause et al., 1998). Aerolysin was also found to stimulate production of cyclic AMP (Fujii et al., 1999) in cultured cells, to activate arachidonic acid metabolism in macrophages and to upregulate the production of tumor necrosis factor alpha as well as IL-1 and IL-6 (Chopra et al., 2000).

In T cells, channel formation by aerolysin was shown to trigger apoptosis, as witnessed by degradation of genomic DNA, possibly due to massive calcium entry (Nelson et al., 1999). This process could be overcome by overexpression of

the anti-apoptotic protein Bcl-2. It is important to note however that apoptosis, addressed by DNA degradation, was not observed in all cell types (see below) indicating that the reactions to selective plasma membrane permeabilization by aerolysin may be cell type specific.

Finally, in a variety of polarized and non-polarized epithelial cells, aerolysin was shown to trigger vacuolation of the ER (Abrami et al., 1998a, b). It has not been determined whether intracellular calcium is released in cells that undergo ER vaculoation. Although ER vacuolation can be observed for some forms of apoptosis, we could not detect any degradation of genomic DNA and moreover vacuolation could not be prevented by Bcl-2 overexpression. Vacuolation was restricted to the first compartment of the biosynthetic pathway as neither the morphology of the Golgi complex, nor that of endocytic compartments was altered by aerolysin. Vacuolation led to an arrest in transport of newly synthesized proteins out of the ER. It is however not known whether this is due to an effect on protein folding/quality control or on vesicular transport out of the ER, or on both. Vacuolation was inhibited by ATP depletion of cells or depolymerization of the microtubule network indicating that the process is dependent on the dynamic properties of ER membranes, perhaps because aerolysin interferes with normally occurring ER fission events. It is not clear how this inhibition is achieved, especially as there is no evidence that the toxin enters the target cell.

The fact that aerolysin triggers ER vacuolation leaving the morphology of endocytic compartments unchanged underlines that its mode of action is very different from that of the *Helicobacter pylori* toxin VacA which leads to vacuolation of late endocytic compartments (Reyrat et al., 1999). Two other toxins have however recently been described to induce massive intracellular vacuole formation, the *Serratia marcescens* hemolysin (Hertle et al., 1999) and the *Vibrio cholera* El tor hemolysin (Coelho et al., 2000; Mitra et al., 2000). For both these toxins, the cytoplasmic vacuoles were found to have, to a large extend, a neutral pH, in contrast to the VacA induced vacuoles which are acidic as expected for compartments of the endocytic pathway. It is therefore likely that both *S. marcescens* hemolysin and the *V. cholera* El tor hemolysin, as aerolysin, affect the ER.

As mentioned above, aerolysin induces different downstream effects depending on the cell type. These various events might however be initially triggered by a common mechanism, i.e. pore formation followed by membrane depolarization or calcium efflux or both. It is important to note however that neither streptolysin O (which also leads to membrane depolarization and calcium influx), nor calcium, or potassium, ionophores, led to ER vacuolation as does aerolysin.

5. Structure-function

The various steps that allow aerolysin to form a pore in the plasma membrane of a target eucaryotic cell have now been identified: secretion, activation, receptor binding, heptamerization and membrane insertion but the mechanisms are not fully understood. Since the solving of the proaerolysin structure in 1994 by Parker and colleagues (Parker et al., 1994), our knowledge of the structure-function relationships has however considerably increased.

Proaerolysin is composed of 470 amino acids. Homology searches with other proteins in the databases reveal only two other proteins, alpha;-toxin from *C. septicum* (Ballard et al., 1995) and a plant protein enterolobin (Sousa et al., 1994).

The crystal structure of proaerolysin reveals an L-shaped molecule (Fig. 3) which can be divided into a small N-terminal globular domain (domain 1) and a long elongated domain (the large lobe). The toxin is rich in beta-sheet (42%) but contains a significant amount of helical structure (21%). The shape and the secondary structure are reminiscent of those of the cholesterol binding toxin perfringolysin O (Rossjohn et al., 1997b). The threading through the domains is however quite different and the two proteins do not have any significant sequence homology.

Domain 1 of aerolysin shares a strong structural homology with a fold found in the S2 and S3 subunits of *Bordetella pertussis* toxin (Rossjohn et al., 1997a). A similar fold is also found in C-type lectins suggesting that it is involved in carbohydrate binding (see below). The large lobe can be divided into three structural domains (domains 2–4). Except for domain 1, none of the domains are continuous in sequence (domain 2, residues 83–178 and 311–398; domain 3, residues 179–195, 224–274, 299–310 and 399–409; domain 4, residues 196–223, 275–298 and 410–470).

Proaerolysin contains four cysteine residues that form two disulfide bridges (C19–C75 in domain 1 and C159–C164 in domain 2) (Lesieur et al., 1999). Both bridges significantly contribute to the overall stability of the protein. In addition the C159–C164 bridge protects the protein from proteolytic attack within a loop at the top of domain 2.

The precise role of each domain has not been established. Domains 1 and 2 appear to be involved in receptor binding and a number of residues involved directly or indirectly in binding to the GPI anchored receptor Thy-1 have recently been identified (MacKenzie et al., 1999). They include Trp-45, Ile-47, Met 57, Tyr-61 and Lys-66 in domain 1 and Tyr-162, Trp-324 and His-332 in domain 2 (Fig. 3). Strikingly, many of these residues are aromatic as also found for other carbohydrate-binding proteins (Quiocho, 1986). Aromatic residues indeed allow interaction by stacking against the pyranose rings of sugars. This mutational analysis does not determine whether both domains are in direct contact with the receptor. The observation that the large lobe alone can bind to GPI-anchored receptors however argues for direct binding of domain 2 (Diep et al., 1999). It is tempting to speculate that both domains 1 and 2 of aerolysin bind the anchor and that it is the presence of two binding sites that leads to the high overall affinity of proaerolysin for its receptor. It has also been proposed that domain 1 binds to carbohydrates on other surface molecules such as glycophorin (Diep et al., 1999).

In addition to its role in receptor binding, domain 2 most likely plays a role in oligomerization. As mentioned above, mutation of His-132 indeed inhibits oligomerization (Buckley et al., 1995; Green and Buckley, 1990) whereas modification of tryptophans 371 or 373 accelerates heptamer formation (van der Goot et al., 1993b).

Rather than triggering oligomerization, domains 3 and 4 were found to be important in the maintenance of the complex. This was shown by limited proteolysis studies. Since the heptamer is unusually stable, i.e. its tertiary structure is not affected by 24 h incubation in 8 M urea (Lesieur et al., 1999), and very resistant to proteolysis, cleavage required the use of the enzyme boilysin which retains its activity at high temperature (Van den Burg et al., 1998). Proteolysis was performed at 70°C under conditions that would not lead to disassembly of the complex. N-terminal sequencing and MALDI-TOF analysis of the obtained fragments led to the identification of two fragments corresponding to amino acids 180–307 and 401–427 (Lesieur et al., 1999). These experiments indicated that domains 1 and 2 are not required for maintaining the heptamer assembled, and that stable interaction occur in domain 3 and/or domain 4.

Finally domain 4 has been suggested to have an important role both at the beginning and at the end of the life cycle of the toxin. Domain 4 indeed contains the pro-peptide. This peptide has been proposed to stabilize the protein in the bacterial periplasm (Nomura et al., 1999) and to favor dimerization (Nomura et al., 2000). Interestingly this domain does not contain any hydrophobic stretch that would predict membrane insertion, nor does any other part of the protein. Hydrophobicity is therefore thought to be generated through the oligomerization process (Lesieur et al., 1997). It is not clear how this occurs for aerolysin. One possibility is that amphipathic beta hairpins, provided by each protomer, join together to form a β-barrel with an external hydrophobic surface as shown for staphylococcal alpha-toxin (Song et al., 1996).

Whereas a high resolution X-ray structure is available for the proaerolysin dimer, all crystallization attempts of the heptameric complex have failed presumably due to its amphipathic nature and its shape. Low resolution images obtained by negative stained electron microscopy of the heptamer reconstituted with lipids have indeed revealed that the aerolysin channel was a ring-like structure with a mushroom like shape (Wilmsen et al., 1992) as later also observed for the staphylococcal alpha toxin channel (Song et al., 1996). Within this mushroom shaped complex, domains 1 and 2 are thought to form the cap structure and domain 4 the stalk. Experimental evidence to confirm this model is however not yet available

During the last 6 years, the structure of proaerolysin has been solved, the nature of the receptors has been identified, and unsuspected intracellular effects have been described. Many questions however remain open awaiting further investigation. These include the characterization of the various molecular interactions at the atomic level (toxin-receptor, monomer-monomer in the heptamer, …), solving the structure of the membrane imbedded aerolysin channel or the full description of the intracellular effects and the understanding of the underlying mechanisms. Therefore some exciting years are still ahead before the full picture emerges.

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