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Mini Review

Yersinia adhesin A (YadA) – Beauty & beast

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ABSTRACT

The trimeric autotransporter adhesin *Yersinia* adhesin A is the prototype of the type Vc secretion systems. It is expressed by enteropathogenic *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* strains, but not by *Yersinia pestis*. A characteristic trait of YadA is its modular composition and trimeric nature. YadA consists of an N-terminal passenger domain which is exposed on the bacterial cell surface. The translocation of this passenger onto the surface is facilitated by a C-terminal β-barrel domain which concomitantly anchors YadA into the outer membrane with three YadA monomers contributing to the formation of a single β-barrel. In *Y. enterocolitica*, but not *Y. pseudotuberculosis*, YadA is a decisive virulence factor and its deletion renders the bacteria virtually avirulent in mouse models of infection. This striking importance of YadA in infection may derive from its manifold functions in host cell interaction. Presumably the most important function of YadA is that it mediates adhesion to extracellular matrix components of eukaryotic host cells. Only tight adhesion allows for the injection of "anti-host" effector proteins via a type III secretion system into the host cell cytosol. These effector proteins enable Yersinia to subvert the host immune system in order to replicate and establish infection. YadA is also essential for the survival of *Y. enterocolitica* upon contact with serum, an important immune-evasion mechanism called serum resistance. To this end, YadA interacts with several components of the host complement system, the first line of immune defense. This review will summarize recent findings about the structure and biogenesis of YadA and its interactions with the host complement system.

Introduction

YadA is an important virulence factor expressed by the enteropathogenic *Yersinia* species *Yersinia enterocolitica* (Ye) and *Yersinia pseudotuberculosis* (Ypstb). The *yadA* gene is located extrachromosomally on the ~70 kb plasmid of *Yersinia* virulence (pYV). Although present in wild-type *Yersinia pestis* (Yp), there YadA is not expressed due to a silencing frameshift mutation (Rosqvist et al., 1988). Upon host entry and the resulting shift of the temperature to 37 °C, the expression of *yadA* is induced by the temperature sensitive regulator LcrF (VirG) which is known also to activate the expression of the *yop* regulon (Cornelis et al., 1989; Lambert de Rouvroit et al., 1992; Skurnik and Toivanen, 1992). All three pathogenic *Yersinia* species carry the pYV plasmid encoding for YadA (a pseudogene in Yp) and a type three secretion system (T3SS) together with a set of effector proteins, the *Yersinia* outer proteins. Upon tight contact to a host cell the *Yersinia* outer proteins are translocated into the host cell cytoplasm. Their function enables Ye to efficiently colonize the host by undermining the immune response. An imperative prerequisite to effector injection is the adhesion of *Yersinia* to specific host cells. Interestingly, the length of the T3SS injector needle and YadA are functionally coupled. If the distance between the needle tip and host cells is increased by either shortening the needle or by increasing the length of YadA, effector translocation is reduced (Mota et al., 2005).

YadA seems to be the most important single factor contributing to virulence of Ye. This is reflected by the fact that a Ye YadA knock-out strain is avirulent in a mouse infection model (Pepe et al., 1995). However – most likely owing to the differences in infection

Abbreviations: BAM, β-barrel assembly machinery; C3, complement factor 3; C3b, large fragment of the complement factor 3; C4b, large fragment of the complement factor 4; C4BP, C4 binding protein; iC3b, inactivated form of C3b; pYV, plasmid of Yersinia virulence; Skp, seventeen kilodalton protein; SurA, survival factor A; T3SS, type three secretion system; TAA, trimeric autotransporter adhesin; YadA, Yersinia adhesin A; Ye, *Yersinia enterocolitica*; Yp, *Yersinia pestis*; Ypstb, *Yersinia pseudotuberculosis*.

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route – YadA seems to be even detrimental when expressed in Yp (Rosqvist et al., 1988) and dispensable for virulence in Ypstb (Han and Miller, 1997). The reduction of Yp virulence by expression of YadA (from strain Ypstb O:3) is presumably due to the peculiar infection route of Yp. Ye and Ypstb usually enter the host by oral uptake. In the gut, YadA-mediated adhesion is essential in order to invade and disseminate. In contrast, YadA-expressing Yp transferred to the host by a fleabite will immediately bind to extracellular matrix proteins. Thus, YadA$^+$ Yp gets trapped locally and spreading is impossible. YadA may not only act as an adhesin, but also as an invasin, enhancing entry of host cells (Eitel and Dersch, 2002). This function has been assigned to a sequence stretch present exclusively in YadA of Ypstb, which mediates efficient uptake of Ypstb (Heise and Dersch, 2006). Another virulence function of YadA is binding of negative regulators of complement. In Ye, YadA is the most important single factor mediating serum resistance (Biedzka-Sarek et al., 2005). The binding repertoire and even the mode of binding (direct/indirect) may vary between different strains and serotypes of Ye (Biedzka-Sarek et al., 2008; Schindler et al., 2012; Schütz et al., 2010).

Although the role of YadA for *Yersinia* virulence has been investigated for years, we are only at the beginning to understand the interplay of YadA with other *Yersinia* virulence factors, with the host and how subtle differences in YadA sequence may affect function. We know little how YadA is inserted into the outer membrane of *Yersinia*, a process addressed only recently. Central questions which hitherto have only partially been resolved are: What is the structure of full length YadA? Which factors contribute to YadA biogenesis? and What are the mechanics of a trimeric autotransport? Apparently, YadA can display various functions depending on the environment and host. Therefore, to target this virulence factor by anti-infectives such questions have to be addressed. The purpose of this review is to recap the recent findings concerning YadA structure, biogenesis, autotransport mechanics and complement interactions.

YadA structure and biogenesis

YadA is a homotrimeric autotransporter adhesin

YadA as a member of the trimeric autotransporter adhesin (TAA) family forms fibrous, often lollipop-like structures on the cell surface (Hoiczyk et al., 2000; Linke et al., 2006). Mature TAA polypeptides possess two functional regions, the N-terminal extracellular or passenger region and the C-terminal membrane-anchored translocation unit. The latter is responsible for extruding the passenger domain through the outer membrane, giving rise to the term 'autotransporter' (Leo et al., 2012).

The passenger regions of TAAs generally contain α-helical coiled coils between globular 'head' domains; these structural modules are joined by various connector elements (Lyskowski et al., 2011; Szczesny and Lupas, 2008). YadA has a single N-terminal head domain, followed by a connector (the 'neck') leading into a uniformly coiled-coil stalk (Fig. 1).

The globular head of YadA from Ye consists of three polypeptides adopting a left-handed parallel β-roll fold that form a compact domain (Nummelin et al., 2004). The left-handed parallel β-roll is composed of a ∼14 residue repeat motif, containing an NSVAIG repeat originally thought to be a collagen-binding motif (Tahir et al., 2000). However, the structure shows that the NSVAIG motifs form hydrophobic cores in the monomer as well as in the trimer thus forming the central structural scaffold of the domain (Fig. 1). Left-handed parallel β-rolls containing NSVAIG motifs are found in numerous other TAAs (Szczesny and Lupas, 2008). As several NSVAIG-containing TAAs do not bind to collagen (Leo et al., 2011; Ray et al., 2002) the misconception of NSVAIG segments as collagen-binding motifs should be dispelled from the literature.

The C-terminal neck of the head domain connects to the coiled-coil stalk. The neck introduces a twist of 120° to the polypeptide chain, such that the stalk α-helix from one monomer starts directly below the left-handed parallel β-roll of the adjacent monomer (Nummelin et al., 2004). The stalk itself is an extended trimeric coiled coil structure. Most of the stalk consists of a right-handed supercoil with repeats that can vary in periodicity (Hoiczyk et al., 2000; Koretke et al., 2006). However, the C-terminus of the stalk leading into the translocation unit is a canonical left-handed coiled coil with heptad periodicity. The transition is mediated by a conserved element, where the degree of supercoiling changes from right-handed to left-handed (Alvarez et al., 2010). The translocation unit of YadA is composed of a transmembrane β-barrel. Recently, the solid-state nuclear magnetic resonance structure of the YadA translocation unit was solved (Shahid et al., 2012a). It shows a trimeric β-barrel, the pore of which accommodates all three linkers (Fig. 1C). Only approximately halfway through the pore do the linkers adopt an α-helical conformation (Shahid et al., 2012b).

Chaperones guide YadA through the periplasm and are important for quality control

Before β-barrel proteins can be inserted into the outer membrane, they have to travel through the inner membrane and across the periplasmic space. To avoid premature folding or aggregation and subsequent degradation by periplasmic proteases, outer membrane proteins are guided through the periplasm by chaperones (Lazar and Kolter, 1996; Sklar et al., 2007). A major periplasmic chaperone is survival factor A (SurA) which is involved in the transit of many outer membrane proteins to the β-barrel assembly machinery which is also termed the BAM complex (Eppens et al., 1997; Ruiz et al., 2006; Volokhina et al., 2011). SurA is an essential factor to maintain monomeric autotransporter proteins unfolded (Bodelon et al., 2009; Oberhettinger et al., 2012; Purdy et al., 2007; Sauri et al., 2009). Is SurA also responsible for the safe passage of YadA across the periplasm? Data dealing with this question are scarce. We observed that overexpression of YadA in a *surA* deletion strain of *E. coli* resulted in only a slight reduction of total YadA protein amount. In a Ye *surA* knockout grown at 37 °C we did not observe any effect on YadA surface exposure levels (unpublished).

Recently, Skp was identified as the most relevant chaperone in the biogenesis of YadA when expressed in a yeast system. Expression of YadA in yeast resulted in the functional assembly of the YadA membrane anchor as well as of full length YadA in mitochondria. Coexpression of Skp leads to an increase of YadA in yeast mitochondria, whereas coexpression of SurA or SecB had no effect (Ulrich et al., 2014). The advantage of yeast compared to the *E. coli* system is that candidate chaperones can be tested individually without interference by other chaperones.

DegP is an important protease in the *E.coli* periplasm where it degrades aggregated or mislocalized outer membrane proteins, but can act also as chaperone (Spiess et al., 1999). It is also a major quality control factor in the biogenesis of YadA. YadA variants that are not properly recognized by the BAM machinery or that have folding defects accumulate in the periplasm and induce an up-regulation of DegP and subsequent degradation of the misfolded proteins (Grosskinsky et al., 2007; Lehr et al., 2010). This is in line with the observation that up-regulation of DegP is also observed under BamA depletion (Lehr et al., 2010) and also with the quality control function of DegP in the biogenesis of monomeric (Jong et al., 2007) and inverse autotransporter proteins (Oberhettinger et al., 2012).

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