Prsa lipoprotein promotes efficient extracytoplasmic protein

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“ PrsA Lipoprotein Promotes Efficient Extracytoplasmic Protein Secretion with Possible Beneficial Applications for Medicine and Industry”

Abstract

This paper explores the essential function of the PrsA-mediated system of influencing the amount and rate of protein secretion from Gram positive cells. During secretion, bacterial proteins face an obstacle course of extremes of charge, pH, unfolding and re-folding into native conformations in order to be of service in the adaptation of the bacterial cell to its environment. Recent evidence suggests that PrsA enhances the yield and efficiency of this secretion process through its influences on protein shape and stability (as a chaperone) as well as its effects on the bacterial cell wall itself. PrsA up- or down-regulation also affects cellular levels of many other factors that exert effects on the ubiquitous Sec secretory pathway. This represents an additional avenue for manipulating Gram positive bacteria to secrete the type and amount of heterologous proteins required, whether it is large or small. The PrsA system offers an attractive target for many beneficial clinical as well as industrial applications. More work should be done to fine-tune our understanding of its mechanisms of action.

Keywords: PsrA, Gram positive, protein secretion, protein folding, antibiotic resistance

Introduction and Significance

To ensure survival, bacterial cells must adapt to the challenging conditions of diverse environments in order to resist stress, acquire nutrients, replicate, and evade destruction by the immune response. Secreted proteins are the

structural and enzymatic functional tools that facilitate their environmental adaptation to survive these threats. Following synthesis, these proteins must be translocated across the bacterial cell membrane and properly folded to be functional [Cahoon & Freitag, 2014].

Proteins containing a signal peptide are secreted from bacterial cells by a complex system of multiple interconnected components. This apparatus directs protein targeting, translocation, signal peptide processing, and posttranslational folding [Bronn, Bolhuis & Tjalsma et al. and Danese & Silhavey, 1998]. The individual components of this pathway, which ultimately translocate proteins across the Gram positive bacterial cytoplasmic membrane, have been identified and are well characterized [Vitikainen, Pummi, & Airakinsen, et al., 2001].

The stages of protein secretion which take place outside the cytoplasmic membrane are less well characterized. The key components of the folding pathway and their mechanisms are conserved and are highly similar between Gram positive and Gram negative bacteria [Manting & Driessen, 2000]. An essential feature of bacterial protein secretion is posttranslocational folding, assisted by foldase enzymes and molecular chaperones. The correct folding of many secreted proteins does not occur spontaneously, but requires the assistance of additional folding factors. [Jones, Danese, & Pinkeret al. (1997)] After location and cleavage of the signal peptide, secreted proteins fold into their native, active conformations. Although details of this pathway are still being elucidated, some important components that assist posttreanslocationsl folding are chaperones, peptidylprolyl *cis-trans* isomerases (PPIases), and thio-disulphide oxido-

reductases. These factors assist in post-translocational folding [Tjalsma, Bolhios, & Jongbloedet al., 2000)]. Without them, bacteria would be unable to adapt effectively to their environments and changing conditions like stress, infection, or cell growth.

PrsA Enables Protein Folding in Hostile Posttranslational Environments

PrsA is one of the few known proteins that assists posttranslocational protein folding in Gram positive bacteria. PrsA has been best characterized in *B. subtilis* , a lipoprotein of 270 amino acid residues which is essential for growth [Kontinen& Sarvas, 1993]. Depletion of PrsA lead to gross morphological alterations (bacteria look like spheres instead of rods) and eventually cell death. Another effect of PrsA depletion in *B. subtilis* is reduction in the production of AmyQ, or *B. amyloliquefasciens* ï¡-amylase [Harwood & Cranenbergh, 2007]. The opposite effect occurs during overproduction of PrsA, which causes overproduction of AmyQ. However, most secretory proteins in Gram positive bacteria are PsrA independent [Vitikainen et al., 2004].

In Gram positive bacteria (like *B. subtilis*), a matrix of cell wall polymers, peptidoglycan, and teichoic acids surrounds the cell membrane, forming a porous structure [Archibald, Hancock & Harwood, 1993]. Macromulecules as large as 50kD may pass through the wall [Demchick & Koch, 1996]. Bacterial proteins are unfolded and secreted secreted through the pores by complex machinery, and re-folding after translocation is critical for the proteins to retain their proper structure and therefore function [Vitikainen 2001]. PrsA is the only protein outside the cytoplasmic membrane known to be involved in

protein secretion. It is a lipoprotein that consists of a 33-kDa lysine-rich protein moiety and an N-terminal cysteine residue with a thiol-linked diacylglycerol (DAG) anchoring the protein to the outer surface of the cytoplasmic membrane [Jacobs, Anderssen & Kontinen 1993; Kontinen & Sarvas 1993; Leskela, Wahlstrom & Kontinen et al. 1999]. The PrsA protein is crucial for the efficient secretion of many exoproteins. In *psrA* mutants, for example, the stability and secretion of certain model proteins is decreased, while PsrA enhances secretion levels of proteins designed for high expression levels [Jacobs et al. 1993; Kontinen et al. 1993; Leskela et al. 1999]. Many specific aspects of this mechanism remain to be elucidated. Further, despite its numerous functions to increase exoprotein secretion efficiency, many barriers late in the secretion process may interfere with its functions. These issues will need to be solved to make the PrsA system maximally effective as a target for the development of new medical and industrial applications.

Barriers to Efficient Protein Secretion: Posttranslational Folding to Native Conformation

At the cytoplasmic membrane / cell wall interface of *B. subtilis* , PrsA lipoprotein acts as a folding factor (“ foldase”) for exoproteins [Rahfeld, J. U., Ragel, K. P., Schelber, B., et al., 1994] In addition to its role in promoting correct posttranslational protein folding, experimental evidence indicates that PsrA may help Gram positive bacteria overcome two additional barriers to efficient protein secretion. PsrA influences neither the expression nor the translocation of secretory proteins. However, it *is* required for protein stability in the posttranslational phase of secretion [Vitikainen, M.,

Hyyrylainen, H. L., Kivimaki, A., et al., 2005; Jacobs, M., Andersen, J. B., Kontinen, V. P. et al. 1993; Hyyrylainen, H. L., Bolhuis, A., Darmon, E. et al. 2001; Vitikainen et al. 2001]. Several model proteins are secreted at increased levels when PsrA is expressed [Kontinen and Sarvas 1993; Vitikainen 2001]. This indicates that posttranslational folding is a rate-limiting step in protein secretion. PrSA is also required for Gram positive bacterial cell viability [Kontinen and Sarvas 1993; Vitikainen 2001]. This indicates that it probably catalyzes the folding of some of the essential components of the cell wall and/or cell membrane.

Barriers to Efficient Protein Secretion: Gram Positive Cell Walls Block Protein Passage

The bacterial cell wall matrix is another factor located outside the cell membrane that affects protein secretion efficiency in Gram-positive bacteria. This matrix consists of a complex peptidoglycan heteropolymer to which are covalently linked anionic polymers of teichoic acid [Archibald, Hancock & Harwood, 1996]. These anionic polymers interact with the membrane-bound lipoteichoic acids, resulting in an area of high-density negative charge at the cell wall and within the microenvironment found at the cytoplasmic membrane / cell wall surface [Vitikainen, Hyyrylainen & Kivimaki, 2005]. The net negative charge of the cell wall is determined by the degree of *D* -alanine esterification of the teichoic and lipoteichoic acids bound there. The *D* -anylation is carried out by enzymes encoded by the *dlt* operon [Perego, Glaser & Minutello et. al, 1995].

In *B* . *subtilis* , inactivating the *dlt* operon leads to increased negative charge density which stabilizes and increases the secretion rates of some mutant

proteins that are usually very susceptible to proteolysis after the posttranslational secretion phase. This observation may result from the protein's slow innate protein folding kinetics or conformations that make them sensitive to attack by proteases [Hyyrylainen, Vitikainen & Thwaite, et al., 2000]. The *dlt* mutation does not affect transcription of the *prsA* gene or genes of secreted proteins Hyyrylainen et al., 2001]. One of the major barriers, therefore, in effective protein secretion is moving secreted proteins through the single cell wall of Gram positive bacteria, then ensuring that it can be re-folded properly (and therefore be functional) on the other side of the wall. Another barrier prevents proteins from traveling through the wall efficiently.

Barriers to Efficient Protein Secretion: Misfolded Proteins Accumulate at Cell Wall

When Gram positive cells experience stress from environmental conditions or from the secretion process itself, misfolded proteins may accumulate at the membrane / cell wall interface. This condition induces expression of genes encoding Htr-A type membrane-bound proteases [Pallen, M. J. & Wren, B. W., 1997; Clausen, Southan, & Ehrmann, 2002). The proteases clean the interface by degrading any misfolded or aberrant (nonfunctional) proteins that they find within the cell envelope [Vitikainen et al., 2005].

As scientists apply their increasing understanding of how to overcome these barriers through PrsA-related interventions, both medicine and industry may benefit from new applications.

PrsA Applications: Clinical And Industrial Uses

Manipulating the Electrical Charge of the Cell Wall

It has been shown that increasing the negative charge density of the cell wall (by inactivating genes of the *dlt* operon) improves experimental mutant protein production as well as secretion in *B. subtilis* [Hyyrylainen et al 2000; Thwaite & Baillie 2002]. In order to test whether this method could be applied generally to enhance heterologous exoprotein secretion, Vitikainen et. al. [2005] determined the effect of a *dlt* mutant (*dltD*) on protein secretion. They grew *dltD* mutant as well as wild-type strains of *B. subtilis* . Compared to wild-type, the mutated strain conferred a negative effect on production of three proteins: pertussis toxin subunit S1, TEM-1 ï)¢-lactamase, and PehA endopolygalatouronase. Both PrsA over-production and inactivation of the D-alanylation of the teichoic acids increased secretion of a protein called pneumolysin. All 4 of these affected bacterially secreted proteins are medically important.

Pneumolysin (Ply) is a cholesterol-dependent cytolysin produced by all clinical isolates of S. pneumoniae. Pneumolysin travels through the bloodstream of pneumonia patients and interferes with the normal immune response against the pneumonia bacteria, prolonging the infection and making symptoms more severe [Lu, Sun & Xu, et al. 2014]. The enzymatically active A unit of pertussis toxin forms around the S1 subunit [Locht & Antoine, 1995]. TEM-1 is responsible for 90% of ampicillin resistance in E. coli [Cooksey, Swenson, & Clark, et al., 1990]. ï),-lactamases are enzymes that produce resistance toï€ ï)¢-lactam antibiotics like penicillins, cephamycins and carbapenems [Neu, 1969]. With an enhanced

understanding of how PrsA contributes to bacterial secretion of these medically essential proteins, it may be possible to attenuate the effects of infections and antibiotic resistance by decreasing the ability of different types of bacteria to secrete large amounts of these proteins. The result might be improved symptoms for pertussis and pneumonia patients, as well as decreased bacterial resistance to antibiotics like penicillin.

A deeper understanding of how to manipulate the PsrA pathway will also have unlimited practical value for industry, where there is high demand for chemical “ manufacture” of heterologous proteins secreted by bacteria and then collected from culture media. PrsA has been best characterized in B. subtilis [Vitikainen et al., 2005]. When heterologous proteins from B. subtilis emerge from the translocase and enter the compartment between the cell wall and the cytoplasmic membrane, they face a challenging environment. In that compartment, there is a high negative charge, high concentration of cations, as well as low pH immediately outside the membrane. This environment encourages delicate proteins to become denatured, lowering the yield, utility, and profitability of this process to harvest industrially vital proteins for industry. Further, these heterologous proteins usually have slow folding kinetics and are especially vulnerable to protolytic degradation [Stephenson, Carter, & Harwood, et al., 1998]. It is known that the major extracytoplasmic folding factor in B. subtilis is PsrA. Determining and manipulating the PrsA mechanism to ensure proper folding of these valuable proteins would increase yield, profit, and availability of these products to both patients and researchers. Clearly, PsrA research has potentially

valuable applications and needs to continue until it becomes practical to apply in the clinic as well as industrial applications.

Conclusion

PrsA is essential for both Gram positive bacterial cell viability as well as efficient secretion of proteins out of bacterial cells. It has wide-ranging effects on entire networks of factors, just a few of which have been mentioned in this brief review. As knowledge of the scope of PsrA's involvement in resolving late-translocational barriers to protein secretion, extremely targeted interventions may result, either to enhance or retard the rate and amount of production of various protein classes. The ability to tailor-make mutant strains of bacteria which produce custom blends of heterologous proteins " to order" has the capacity to revolutionize both medicine and industry.

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