Effects of *Staphylococcus aureus* Foldase PrsA on Protein A Secretion

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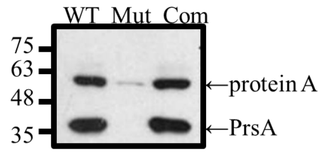
ABSTRACT

PrsA is a membrane-anchored lipoprotein, which is found in many Gram-positive bacteria including *S. aureus*. This protein functions as a foldase to assist post-translocational folding and enhance the stability of exported proteins in the microenvironment of the membrane–cell wall interface. However, the role of PrsA in *S. aureus* remains unclear. In a mice infection model, we found that PrsA is involved in virulence and pathogenesis of *S. aureus* infections. Additionally, proteomic analysis revealed that PrsA is required for secretion of protein A, which is a major virulent factor and involved in immune evasion. The objective of this study is to investigate how PrsA influences protein A secretion. We found that in comparison with wild-type strain HG001, the *prsA*-defective mutant HG001Δ*prsA* secreted much less protein A during different growth phases. PrsA does not influence protein A expression at the level of transcription. We also found that deletion in *prsA* decreases the stability of exported protein A. Furthermore, pull down assay demonstrated that PrsA interacts with protein A *in vitro* and *in vivo*. The domain mapping assay showed that both N- and C-terminal domain of PrsA are required for binding of protein A. This study reveals that secretion of protein A is PrsA-dependent. Protein A is also the first identified folding substrate of PrsA in *S. aureus*. In conclusion, the information derived from this study provides new insights into the protein export pathways that are crucial to pathogenesis of *S. aureus*.

INTRODUCTION

PrsA is a member of Parvulin peptidyl-prolyl cis/trans-isomerases family of membrane-associated lipoproteins involved in protein folding and stability. Our previous studies found that deletion in *prsA* decreases the amount of protein A in cell wall. When the PrsA-defective mutant was complemented with a plasmid that carries *prsA*, the expression of PrsA and protein A are restored. The results suggested that PrsA is required for secretion of protein A, which binds to human immunoglobulin binding protein and contributed to immune evasion.



By Lee *et. al.*, unpublished data

Fig. 1 Expression of PrsA and Protein A in *S. aureus*. Cell wall proteins were extracted from *S. aureus* wild type strain HG001 (WT), *prsA*-deletion mutant HG001Δ*prsA* (Mut) and complementary strain HG001(pHY-PrsA) (Com). The amount of protein A and PrsA were analyzed by immunoblotting using anti-Protein A and anti-PrsA antibodies.

RESULTS

PrsA affects the amount of protein A on different fraction of *S. aureus*

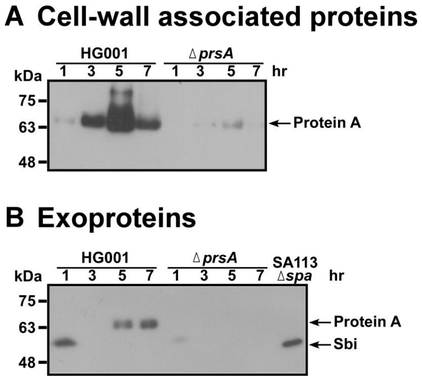


Fig.2. The amount of protein A in cell wall and culture medium in different growth phases of *S. aureus*. Proteins were extracted from the cell wall (A) and culture medium (exoprotein) (B) of *S. aureus* HG001 (wild-type) and HG001Δ*prsA* (mutant), which had been cultured for 1, 3, 5, 7 hrs. The amount of protein A was analyzed by immunoblotting using anti-Protein A antibodies. *S. aureus* SA113Δ*spa* was used as a control to demonstrate the presence of Sbi in exoprotein (B).

PrsA does not influence the expression of protein A

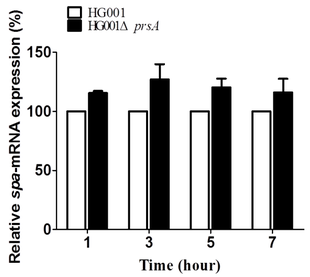


Fig.3. Effect of PrsA on transcription of protein A. RNA was extract from HG001 and HG001Δ*prsA*. Expression of protein A was determined by RT-qPCR. The amount of *spa* transcript was set as 100%.

Deletion in *prsA* decrease the stability of exported protein A

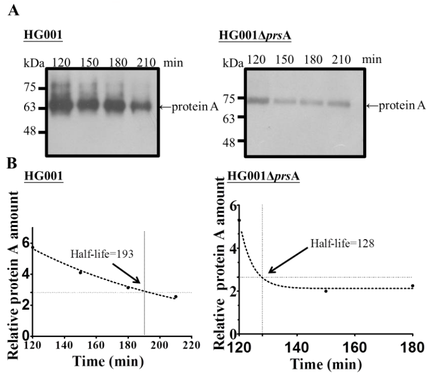


Fig.4 Stability of exported protein A. (A) Bacteria strains were subcultured for 5 hr and treated with erythromycin to stop the protein synthesis. The cell wall proteins were then extracted at the indicated time after adding erythromycin. The amount of Protein A was determined by immunoblotting. (B) The relative intensity of each band was quantified using densitometer. The half-life of protein A was calculated by exponential regression analysis.

Protein A interacts with PrsA *in vivo*

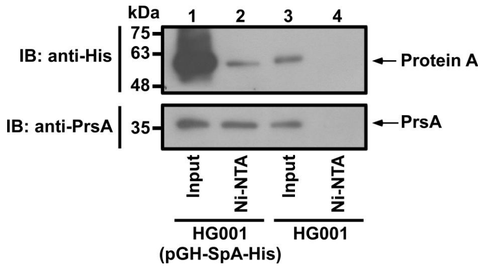


Fig.5 Interaction between PrsA and protein A. Ni-NTA beads were added to the cell wall proteins extracted from HG001 and HG001(pGH-spa-His) which expressed His-tag protein A. Proteins that were pulled down by the beads were analyzed by immunoblotting using anti-His and anti-PrsA antibodies. Input lanes were loaded with 8% of cell wall proteins.

Protein A interacts with the N- and C-terminal region of PrsA

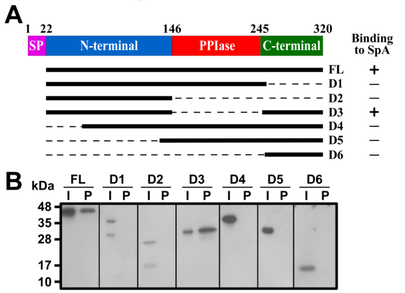


Fig.6 Mapping the interaction domains on PrsA. (A) Schematic representations of the full-length His-tag PrsA (FL) and its derivatives (D1-D6) used in this study. The recombinant proteins were extracted from *E. coli* BL21(DE3) and purified using Ni-NTA beads. (B) Protein A beads were added with purified His-tag PrsA and its derivatives. Proteins that bound to the beads were detected by immunoblotting using anti-PrsA antibody (P). 10% of purified proteins were loaded as input control (I).

CONCLUSION

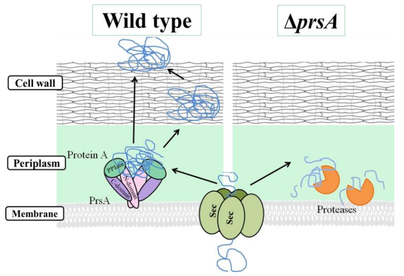


Fig. 7. Interactions between PrsA and protein A. Protein A is exported through Sec complex. After secreted out of cell membrane, protein A interacts with NC domain of PrsA in the membrane-cell wall interface. Protein A is folded by PrsA and then anchored to cell wall or released to external environment. If PrsA is absent, unfolded protein A will be degraded by quality control proteases in periplasm.