The PlySs9 endolysin and its amidase subdomain reveal potential roles in the treatment of Gram-positive bovine mastitis

Niels Vander Elst1,2,3, Sara B. Linden2, Rob Lavigne3, Eylene Meyer1 and Daniel C. Nelson2

1Laboratory of Biochemistry, Department of Pharmacology, Toxicology and Biochemistry, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium; 2Laboratory of Institute for Bioscience and Biotechnology Research, University of Maryland, Rockville, United States of America; 3Laboratory of Gene Technology, Department of Biosystems, KULeuven, Leuven, Belgium. Contact: niels.vanderelst@ugent.be

Introduction & Aim

Bovine mastitis, an infection of the cow’s udder, causes major economic losses in the global dairy industry and remains one of the main reasons for antibiotic use in dairy cattle. The most prevalent Gram-positive causative agents of this disease are Streptococcus uberis (S. uberis) and Staphylococcus aureus (S. aureus). Bacterioype-derived endolysins, such as LysK, B30 and AS2A [1-2], are each known to lyse at least one of these pathogens both in vitro and in preclinical models. These endolysins typically consist of a cell wall-binding domain (CBD) and one or more enzymatically active domains (EADs), which allow them to either bind or cleave the bacterial peptidoglycan, respectively. Their known working spectrum can nevertheless be improved and/or expanded by engineering chimeric endolysins using platforms such as Versatile Shuffling [3] or non-covalent B barnase-Bstar fusion [4]. From this perspective, the in-depth functional characterization of different CBDs and EADs is mandatory before creating novel chimeric endolysins. Therefore, here we characterized the LysM-based CBD and N-terminal amidase EAD of PlySs9, an endolysin derived from a Streptococcus suis (S. suis) bacterioype that also displays lysis activity against bovine S. uberis and S. aureus.

Methods

Gene Cloning: The amidase EAD and CBD subdomains were amplified from the PlySs9 genome (the latter artificially synthesized and codon-optimised) through polymerase chain reactions (PCR) and Gibson assembled in a pBAD24 vector. After transformation in competent E. coli cells, colonies were picked from selective plates and PCR-screened for presence of the gene. Positive clones were subsequently sequenced. Protein expression: Transformed E. coli BL21 were grown in 1 L of selective Luria-Bertani broth until the OD600 reached 0.8 - 1.0. These cultures were subsequently induced with 0.25% L-arabinose and shaken overnight (18°C, 150 rotations per minute (RPM)). Protein purification: E. coli BL21 were pelleted at 4000 RPM and resuspended in phosphate buffered saline (PBS) with 10 mM imidazole and 1 mM PMSF. Thereafter, cells were sonicated on ice for 15 minutes. Insoluble cellular debris was removed through centrifugation for 45 minutes at 13000 RPM and 4°C. His-tagged chromatography further purified the protein from the centrifuged supernatant. Eluted fractions that contained the envisaged protein were combined and dialyzed for 4 hours at 4°C in 5.0L distilled water and embedded in an SDS-PAGE gel. Proteins were boiled for 2 minutes in an SDS buffer, ran on the gel, refolded with 5% Triton X-100, incubated in phosphate buffer at 37°C and stained with 0.1% crystal violet. Viable counts: Overnight cultures were washed, resuspended in PBS and incubated for 2 hours with an equal volume of purified protein. Serial dilutions were made and 10µL was subsequently spotted on nutrient agar. AlexoFluor 555 labeling: Performed according to the manufacturer’s protocol.

Results

1. Lytic activity of the PlySs9 parental endolysin

Figure 1. Turbidity reduction assays with the PlySs9 parental endolysin.

Optical density measured at 600nm during 20 minutes at 37°C with 0 and 20 µg/ml concentrations against (A) S. aureus 730982, (B) S. uberis 0140J and (C) S. suis NRS-14.

2. Lytic activity of the PlySs9 amidase EAD subdomain

Figure 2. Lysis observed in a spot-on-plate assay with 3.0 mg/ml PlySs9 EAD amidase versus phosphate buffered saline (PBS) as a negative control after overnight incubation at 37°C.

(A) S. suis 730982, (B) S. uberis 0140J and (C) S. suis NRS-14.

Conclusions

- The parental PlySs9 endolysin and its amidase EAD subdomain expose lytic activity against Streptococcus suis and two major Gram-positive bovine mastitis pathogens: Streptococcus uberis and Staphylococcus aureus.
- The PlySs9 LysM-based CBD subdomain is able to bind Streptococcus suis. Binding to relevant mastitis-causing pathogens requires further in-depth analysis.

References


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