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Phage-Related Ribosomal Protease (Prp): A Novel Antibiotic Target in *Staphylococcus*

Julia A. Hotinger*, Heather A. Pendergrass, Adam Johnson and Aaron E. May

Department of Medicinal Chemistry and Institute for Structural Biology, Drug Discovery and Development, Virginia Commonwealth University, Richmond, Virginia, USA.

*Corresponding author: hotingerja@vcu.edu

Phage-Related Ribosomal Protease (Prp)

- L27 is necessary for normal ribosomal function
- Some strains of bacteria (*Staphylococcus aureus*) code for an additional N-terminal tag
- Prp (a cysteine protease) cleaves this additional tag, allowing for normal protein production
- S. aureus* cannot survive with uncleavable L27 or pre-cleaved L27.
- Possible way to combat methicillin resistant *S. aureus* (MRSA)

L27 N-Terminus Extends into Peptidyl Transferase Center

PDB: 4V5C

N-terminal tail of L27 (pictured in blue) is involved in stabilizing the 3' ends of the ribosomal substrates (tRNA pictured in green and pink). When N-terminal tag is present stabilization does not occur.

S. aureus Prp structure

Image A – Crystal structure of *S. aureus* Prp dimer bound to our custom-synthesized peptidyl chloromethyl ketone substrate analog (seen below).

The refined structure of our Prp inhibition complex (2.35Å resolution) reveals 6 – 7 residues of the peptidyl group covalently bound as a thioether to Cys34 of Prp.

Image B - L27 cleavable sequence (KLNQLQFF) in the Prp binding site showing key interactions

Protease Activity Assay Design

Prp Inhibition Assay

Normal protease activity
Control = inhibition

Mersalyl acid is used as the positive control. In the past it has been used clinically as a mercurial diuretic.

Assay Miniaturization

96-well plate

200 microliters total volume
Hand pipetting
CV ~2%

384-well plate

60 microliters total volume
Echo 550 acoustic liquid handler
CV ~2%

1536-well plate

~6 microliters total volume
Echo 550 acoustic liquid handler
CV ~2%

Structure of Known Protease Inhibitors

Protease inhibitor library was screened, none were identified as hits meaning that Prp is highly selective. Below: 5 of the screened compounds displaying the variety in structure and mechanism.

- vr23** – a potent proteasome inhibitor with IC50 of 1 nM, 50-100 nM, and 3 μM for trypsin-like proteasomes, chymotrypsin-like proteasomes, and caspase-like proteasomes, respectively.
- Aebsf** – a broad spectrum, irreversible serine protease inhibitor.
- Aloxistatin** – an irreversible and membrane-permeable cysteine protease inhibitor with blood platelet aggregation inhibiting activity.
- Darunavir** – a nonpeptidic HIV protease inhibitor, used to treat HIV infection.
- Paritaprevir** – a nonstructural (NS) protein 3/4A protease inhibitor.

Conclusions

- Structural and computational studies need to be done in order to better understand the Prp binding site
- More screenings to find hits in order to do drug design and structure-activity relationship studies

References

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