Structural studies of LysSi3, Gram-negative bacteria targeting endolysin with broad bactericidal activity

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D. Vasina[I,II], N. Antonova[I,III], T. Prudnikova[IV], D. Loginov[IV,V], I.K. Smatanova[IV], F. Dycka[IV], E. Usachev[I], J. Sterba[IV,V], V. Gushchin[I,III]

[I]N.F. Gamaleya National Research Centre for Epidemiology and Microbiology, Ministry of Health of the Russian Federation, Moscow, Russia, [II]A.N. Bach Institute of Biochemistry, Research Center of Biotechnology of the Russian Academy of Sciences, Moscow, Russia, [III]Lomonosov Moscow State University (MSU), Moscow, Russia, [IV]Institute of Chemistry, Faculty of Science, University of South Bohemia, Ceske Budejovice, Czech Republic, [V]Institute of Parasitology, Biology Centre of the Czech Academy of Sciences, Ceske Budejovice, Czech Republic

Phage endolysins comprise a promising alternative capable to replace or supplement conventional antibacterial therapy used in medicine and veterinary in the face of increasing antimicrobial resistance. For endolysins targeting Gram-negative bacteria the specific activity against a broad host range has been shown, however, the molecular mechanisms specifying broad spectrum of action are obscure. The LysSi3 is a peptidoglycan hydrolyzing, lysozyme-like enzyme with predicted muramidase activity (GH24 family) and broad bactericidal activity against ESKAPE pathogens. It showed *in vitro* antibacterial effect for 75 out of the 120 investigated clinical isolates at concentration 100 μg/ml (5.4 μM), reducing the bacterial load up to 5 logs. X-ray crystallography and HDX-MS were applied to elucidate the mechanism of LysSi3 action. Site-directed mutagenesis was performed based on the structural data. Using the 3D structure of muramidase AcLys (PDB ID 6ET6) the LysSi3 catalytic triad (Glu15, Asp24 and Thr30) was predicted and mutated. Modifications of amino acids did not lead to changes in enzymatic activity against the *Acinetobacter baumannii* model strain. Thus, the existence of additional functional domains in the structure of LysSi3 can be proposed. Previously, a significant loss of LysSi3 activity at pH>7, compared to mild acidic conditions has been shown. The addition of permeabilization agent (EDTA) restored its activity, indicating failure of LysSi3 to overcome the bacterial outer membrane at pH>7. Analysis of HDX data revealed that C-terminal peptide of LysSi3, containing two α-helixes and β-strand, becomes less structured with increase of pH. We propose that this charged terminal peptide can play a particular role of membrane-translocating domain, allowing the enzyme to cross outer membrane of bacteria and reach the peptidoglycan substrate. The mechanism of enzymatic action and role of C-terminal peptide in broad bactericidal action of LysSi3 are under investigation.