doi 10.18699/bgrs2024-3.1-47

Structural studies of the point mutant S25C of the gram-negative targeting endolysin LysSi3 with broad bactericidal activity

Matyuta I.O.[1]*, Sluchanko N.N.,[1] Vasina D.V.[2], Boyko K.M.[1]

[1] *Institute of Biochemistry named after A.N. Bach, Research Center of Biotechnology RAS, Moscow, Russia*

[2] *N.F. Gamaleya National Research Centre for Epidemiology and Microbiology, Ministry of Health of the Russian Federation, Moscow, Russia*

** i.matyuta@fbras.ru*

Key words: X-ray crystallography; endolysin; point mutant; bacteriophage

Motivation and Aim: Antimicrobial resistance (AMR) is alarmingly increasing in medicine and is one of the major concerns of healthcare today. Due to this fact there is a growing interest in bacteriolytic enzymes that act to lyse bacterial cell wall especially against pathogens with AMR. These types of enzyme include bacteriophage-encoded endolysins which degrade the peptidoglycan (PG) cell wall polymer [1]. Endolysins are synthesized in the cytoplasm of infected bacteria at the final stage of their lytic development for specific degradation the PG polymers of the host bacteria. It leads to abrupt osmotic cell lysis and subsequent release of progeny phages. This phenomenon led to a growing number of studies aimed at using this class of enzymes as antibacterial agents. However, the mechanism of PG binding and degradation by endolysins is still obscure. The LysSi3 is a peptidoglycan hydrolyzing, lysozyme-type enzyme with predicted muramidase activity and broad bactericidal activity. LysSi3 was shown to be a monomer in solution. However, a dimer molecule is observed in the structure of the wild type LysSi3. Examination of the dimer interface revealed that the side chains of S25 of the adjacent subunits are in close proximity. To identify the ability of enzyme to form a dimer it was suggested to obtain and solve the structure of LysSi3 point mutant S25C ($LysSi3^{S25C}$) using X-ray crystallography.

Methods and Algorithms: Initial crystallization screening was performed on a robotic system (Rigaku Americas Corporation, The Woodlands, TX USA) using 96-well VDX plates (Hampton Research, Aliso Viejo, CA USA) and commercial crystallization screens from Hampton Research (Aliso Viejo, CA USA) and Molecular Dimensions Inc (Holland, OH USA) by the “hanging drop” vapor diffusion method. A 15 mg/mL of the $LysSi3^{S25C}$ in 30 mM Tris-HCl buffer pH 8.0 containing 150 mM NaCl was mixed with the crystallization solution in the ratios 1:1, 1:2 (0.1 μL drop volume), and 2:1 (0.2 μL drop volume). The volume of the precipitant solution in the reservoir was 50 μL. The initial crystallization hit was observed under the following conditions: 0.1 M Imidazole pH 7.0 and 20 % v/v Jeffamine ED-2001 pH 7.0 at 1:1 ratio at 288 K.

Crystal of the $LysSi3^{S25C}$ was briefly soaked in a mother liquor containing 20 % glycerol immediately before diffraction data collection and flash-frozen in liquid nitrogen. Dataset was collected at 100K at BL18U beamline (SSRF, China). The dataset was indexed and integrated using the XDS package [2] and scaled using Aimless program of the CCP4 suite [3]. Space groups were suggested by Pointless [4] as $C222_1$.

The structure of LysSi3^{S25C} was solved by the molecular replacement method using the MOLREP program [5] with the atomic coordinates of wild type LysSi3 as a starting model. One copy of the protein was found in an asymmetric unit. The refinement was carried out using the REFMAC5 program of the CCP4 suite [3]. The visual inspection of electron density maps and the manual rebuilding of the model were carried out using the COOT interactive graphics program [6]. The isotropic B-factor and the hydrogen atoms in fixed positions were included during the refinement.
The visual inspection of the modeled structure was carried out using the COOT program and the PyMOL Molecular Graphics System, Version 4.6 (Schrödinger, USA). The contacts were analyzed using the PDBePISA [7].
Results: Structure of LysSi3^{S25C} was solved at 1.8 Å resolution. There is one protein subunit in the asymmetric unit in LysSi3^{S25C} but a dimer similar to LysSi3 was found in the crystal. A double position of the loop with C25 with half occupancy was observed in the electron density. Therefore, there are two possible mutual arrangements of cysteines. In the first case, a disulfide bond is formed. In the second case it is not formed, but then a distance of 2.1 Å is formed between the backbone oxygen atoms of C25 of the adjacent subunits. As a result, the structure shows the formation of a disulfide bond between the C25 of adjacent subunits. Using SEC-MALS and gel electrophoresis it was shown that under oxidized conditions LysSi3^{S25C} is a dimer and in the presence of a reducing agents LysSi3^{S25C} is a monomer.
Conclusion: LysSi3 in solution can form the dimer found in the structures of wild type and point mutant S25C.
Funding: The study is supported by the Ministry of Science and Higher Education of the Russian Federation (No. 075-15-2021-1354).

References

1. Gerstmans H., Rodríguez-Rubio L., Lavigne R., Briers Y. From endolysins to Artilysin®s: novel enzyme-based approaches to kill drug-resistant bacteria. *Biochem Soc Trans*. 2016;44(1):123-128. doi 10.1042/BST20150192
2. Kabsch W. XDS. *Acta Crystallogr D Biol Crystallogr*. 2010;66:125-132. doi 10.1107/S0907444909047337
3. Winn M.D., Ballard C.C., Cowtan K.D. et al. Overview of the CCP4 suite and current developments. *Acta Crystallogr Sect D Biol Crystallogr*. 2011;67:235-242. doi 10.1107/S0907444910045749
4. Evans P. Scaling and assessment of data quality. *Acta Crystallogr Sect D Biol Crystallogr*. 2006;62:72-82. doi 10.1107/S0907444905036693
5. Vagin A.A., Isupov M.N. Spherically averaged phased translation function and its application to the search for molecules and fragments in electron-density maps. *Acta Crystallogr Sect D Biol. Crystallogr*. 2001;57:1451-1456. doi 10.1107/S0907444901012409
6. Emsley P., Lohkamp B., Scott W.G., Cowtan K. Features and development of Coot. *Acta Crystallogr Sect D Biol Crystallogr*. 2010;66:486-501. doi 10.1107/S0907444910007493
7. Krissinel E., Henrick K. Inference of macromolecular assemblies from crystalline state. *J Mol Biol*. 2007;372:774-797. doi 10.1016/j.jmb.2007.05.022