NW12A/2011G237

Overexpression, crystallization and preliminary X-ray crystallographic analysis of hypothetical protein SAV0479 from *Staphylococcus aureus* Mu50

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1 Introduction

Staphylococcus aureus bacteria have developed extensive antibiotic resistance. An example of this phenomenon is the methicillin-resistance *S. aureus* (MRSA), which was isolated in 1961. *S. aureus* has become resistant to new antibiotics through mutations and by the acquisition of exogenous genes. To protect patients from the methicillin-resistant strain, glycopeptides, such as vancomycin, are being used as the therapeutic drug of choice for MRSA infections. As a result, the vancomycin-resistant strains (VRSA) have emerged rapidly. One of the vancomycin-resistant MRSA strains was reported in Japan in early 1990s. Since then many laboratories have reported the vancomycin-resistance strain. Several studies have been done to understand the mechanism of vancomycin-resistance in methicillin-resistant *S. aureus*. Many genes and protein databases of different strains of *S. aureus* are available (GeneBank accession numbers BA000017, BA000018, BA000033 for *S. aureus* Mu50, *S. aureus* N315 and *S. aureus* MW2 respectively). Even though genome sequencing spreads some light on many proteins, a considerable number of protein sequences remain designated as “hypothetical proteins” with a very little or no functional studies associated with them. Using the GeneBank and Comprehensive Microbial Resource, CMR (http://cmr.jcvi.org, currently offline), we chose hypothetical proteins which might provide new information about antibiotic resistant pathogens and possible targets for drug development. In the search of functional proteins from methicillin-and vancomycin-resistant strain *S. aureus* Mu50, which is resistant to methicillin and vancomycin and almost all antibiotics including the potent β-lactams, we selected SAV0479, a hypothetical protein from *S. aureus* Mu50 strain.

2 Experiment

The expression plasmid for native SAV0479 was constructed using vector pET21a(+) having C-terminus hexahistidine tag to facilitate purification. The crystallization conditions for SAV0479 was obtained by the hanging-drop vapour-diffusion method. 1 μl of protein solution was mixed with an equal volume of buffer on a siliconized cover slip and the mixture was equilibrated over 500 μl of reservoir solution at 20 °C. The crystallization condition for SAV0479 was 1.8 M NaCl, 0.1 M sodium acetate at pH 4.2 and 1 mM taurine. The needle-shaped crystals appeared in 1 day to the largest dimension of 1.0 x 0.2 x 0.1 mm (Fig. 1). Crystals were soaked in 4.5 M NaCl containing the crystallization solution for 10 s and were flash-frozen in liquid stream before data collection. X-ray diffraction data from a single crystal of SAV0479 was collected to a resolution of 2.8 Å at 100 K using an ADSC Quantum210 CCD detector, scanning a total of 360° rotations in ω on synchrotron beamline NW12A at the Photon Factory, Japan. 360 images were collected for the full data set. Each image was recorded with an exposure of 1.5 s per 1° oscillation range. The data set was indexed and integrated, and images from 31 to 300 were used for scaling with the HKL-2000 program suite. Molecular replacement was performed using MOLREP from the CCP4 program suite.

3 Results and Discussion

The recombinant SAV0479 from *S. aureus* (MRSA Mu50 strain) was overexpressed in E. coli in soluble form with an approximate yield of 10 mg/L LB medium. The molecular weight of the protein was estimated to be 11.5 kDa by ProtParam (http://www.expasy.org/tools). X-ray diffraction data were collected to 2.8 Å resolution. A total of 123 166 measured reflections were merged into 8154 unique reflections, resulting in an Rmerge of 7.1%. The crystals belong to the $P3_121$ space group, with unit-cell parameters of $a = b = 81.48$, $c = 82.53$ A ˚ and $\alpha = \beta = 90.0$, $\gamma = 120.0°$. Three monomers of the protein are present in each asymmetric unit, with a calculated crystal volume per protein weight (V_M) of 2.04 $Å^3$ Da^{-1} and a solvent content of 39.89%. Model building and further refinement are in progress.

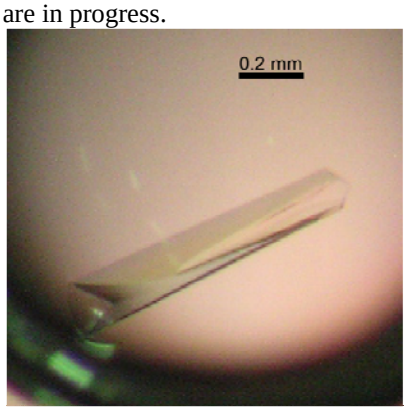

Figure 1 : Single crystal of SAV0479 having dimension of approximately 1.0 x 0.2 x 0.1 mm.

Acknowledgement

The authors thank the staff at beamline NW12A of the Photon Factory, Japan for assistance during the X-ray data collection experiments. The authors would also like

to thank the National Institute for International Education (NIIED), Ministry of Education, Science and Technology (MEST), Republic of Korea for funding a graduate scholarship to Chinar Pathak.

References

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