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 P3121 space group, with unit-cell parameters of a = b = 81.48, c = 82.53 Å and α = β = 90.0, γ = 120.0°. Three monomers of the protein are present in each asymmetric unit, with a calculated crystal volume per protein weight (Vm) of 2.04 Å³ 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.

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