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Crystal structure analysis of staphylococcal α-hemolysin H35A monomer

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1 Introduction

Staphylococcal α-hemolysin is a β-barrel pore-forming toxin (PFT) expressed by *Staphylococcus aureus*. α-Hemolysin is secreted as a water-soluble monomeric protein with a molecular mass of 34 kDa, which binds to target membranes and forms membrane-inserted heptameric pores. With the appearance of the pore on the membrane, the cells are killed through leakage. The structure of the heptameric pore has been determined, and each protomer was shown to assemble along a noncrystallographic seven-fold axis. Moreover, crystal structure of α-hemolysin monomer in complex with antibody was reported recently. Although the α-hemolysin has a strong propensity to spontaneously form heptameric pores, the bound antibody inhibited pore formation. The overall structure of α-hemolysin is divided into three domains, i.e. the cap, rim, and stem domains. The stem region folded beside the cap domain in monomeric form protrudes out from the cap domain to form transmembrane β-barrel in the pore form. Although the monomeric structure was determined in antibody-bound form, that without binder has not yet been determined.

Since the gene of the toxin was identified, a number of mutational studies have been carried out to clarify the mechanism of pore formation. In the present study, we focused on the substitution of His35 among the reported mutants. Substitution of His35 caused marked decreases in oligomerization and lysis activities instead of sufficient cell binding activity. His35 is located at the interface between protomers in the heptameric pore structure. It is likely that His35 mutant retains the monomeric structure but is unable to assemble heptamers due to inability to form the correct interprotomer interactions due to the substitution. Therefore, this mutant is probably the most suitable for obtaining crystals of monomeric α-hemolysin. In the present study, we expressed, purified and crystallized α-hemolysin H35A mutants [1]. The diffraction data were collected at a resolution of 2.91 Å. The results of molecular replacement showed that the obtained crystal consisted of monomeric α-hemolysin.

2 Experiment

Crystals of α-hemolysin most suitable for further diffraction experiments were grown from a buffer containing 0.05 M MES (pH 6.5), 0.01 M magnesium chloride, 2.1 M lithium sulfate and 5% (w/v) ethylene glycol. The X-ray diffraction dataset of α-hemolysin was collected at beamline BL5A with a Quantum 315r detector under cryogenic conditions at 100 K. Crystals were soaked in mother liquor containing 10% (w/v) ethylene glycol and flash-cooled under a stream of liquid nitrogen. The distance between the crystal and detector was set to 452.6 mm. A total range of 180° was covered with 0.5° oscillation per frame. The diffraction data were indexed, integrated and scaled using the program XDS. Data collection statistics are summarized in Table 1. Molecular replacement was carried out with the program Phaser using the structure of the α-hemolysin pore protomer (PDB ID 3ANZ).

3 Results and Discussion

The diffraction dataset was collected at a resolution of 2.90 Å. The crystal belonged to space group $P6_1$ or $P6_5$, with unit cell parameters a = b = 151.3 Å, c = 145.0 Å (Table 1). Molecular replacement was carried out using a protomer of heptameric α-hemolysin pore as a search probe. Four molecules were found by the program Phaser on the assumption of space group $P6_1$. Positive electron density corresponding to the stem region was observed beside the cap domain for all molecules. Further model building and structure refinement are currently underway. Intermolecular interaction similar to that observed in the pore form was not observed among molecules, showing that the crystal is composed of monomeric α-hemolysin H35A mutant. To our knowledge, this is the first report of a crystal of monomeric α-hemolysin without binder. The structure will provide insight into the pore-forming mechanism of α-hemolysin.

Table 1: X-ray data collection statistics

Beamline	BL5A
Space group	$P6_1$
Unit cell parameters (Å)	a = b = 151.3, c = 145.0
Resolution	48.58 – 2.90 (3.09 – 2.90)
R_{merge} (%)	13.5 (70.0)
Completeness (%)	99.7 (99.4)
Multiplicity	10.72 (8.70)
Average I / σ	13.48 (2.38)

References

[1] Sugawara et al. ***Acta Cryst. F*** in press

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