

Structural Studies of α -Hemolysin Assembly Intermediates

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Beamline(s): X25

α -Hemolysin (α HL) is a water soluble toxin produced by most pathogenic strains of *Staphylococcus aureus*. It is secreted as a monomeric species and assembles to form heptameric transmembrane pores on susceptible cell membranes. Pore formation on target cell membranes leads to cell death due to cell lysis and the flow of ions, small molecular weight molecules (< 1 kD) and water through the toxin pore.

Our work is focused on answering specific structural questions related to the mechanism of membrane binding and assembly of α HL. After secretion from *S. aureus* as a water soluble monomer (α 1), α HL binds to the membrane of a susceptible cell. This membrane bound monomer (α 1*) assembles to a membrane-embedded heptamer (α 7) via a nonlytic heptameric intermediate (α 7*). The membrane-embedded heptamer creates a water-filled channel in the membrane bilayer. We have solved the structures of the heptameric pore form (α 7)¹ and the water soluble monomer (α 1) of LukF², a closely related *S. aureus* toxin.

The H35W mutant of α HL is arrested in an intermediate stage of heptamer assembly (α 1* or α 7*), i.e. the mutant is folded like the wild-type protein and can bind to cell membranes, but it does not assemble to form a heptameric pore. We have crystallized the H35W mutant in the presence of phospholipids that catalyze assembly of wild-type α HL and have collected a native data set to 3.4 Å at Beamline X25. We will attempt to solve the structure of H35W using molecular replacement (MR) with a protomer from the assembled heptamer as a search probe. If an MR solution is not attainable, we have prepared seleno-methionine derivitized H35W to be used in a MAD phasing experiment.

It should be noted that data of medium resolution (3.5 Å) for H35W crystals could only be obtained at the synchrotron. Crystals of equal or superior quality diffracted very poorly or not at all at our home source.

References:

1. L. Song, M.R. Hobaugh, C. Shustak, S. Cheley, H. Bayley, J.E. Gouaux, "Structure of Staphylococcal α -Hemolysin, a Heptameric Transmembrane Pore," *Science*, **274**, 1859-1866, 1996.
2. R. Olson, H. Nariya, K. Yokota, Y. Kamio and E. Gouaux, "Crystal Structure of Staphylococcal LukF Delineates Conformational Changes Accompanying Formation of a Transmembrane Channel," *Nat. Struct. Biol.*, **6**, 134-140, 1999.