Towards the Structure Determination of the HtrA1 Protein from *Staphylococcus aureus*

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The HtrA1 protein of *Staphylococcus aureus* has a high degree of sequence homology to members of the HtrA/DegP family from Gram-negative bacteria. Expression of this protein is induced in response to heat shock or secretion stress signals in *Bacillus subtilis* [1]. HtrA/DegP shows a temperature dependent "switch" from chaperone to (serine)protease activity [2], a function that has also been proposed for the corresponding protein in Gram-positive bacteria [3]. We are expressing HtrA1 with a view to crystallization and structure determination. A His-tagged wildtype HtrA1 protein was expressed, containing an enterokinase cleavage site in a flexible linker region. Initial purification revealed that the protein probably undergoes self-cleavage during removal of the His-tag. For this reason the serine residue of the proteolytic site was mutated to alanine. This mutant protein was purified and appears stable during the process of enterokinase clevage and subsequent purification. Using size exclusion chromatography the HtrA1(SA) protein shows an elution profile corresponding to a monomeric molecule. Crystallization trials are currently under way, and promising conditions have been determined.

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Keywords: DegP, protease, chaperone