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A novel proteolytic mechanism in membrane proteins

Nami Tajima[1], Kaoru Nishimura[1], Sam-Yong Park[1], Jeremy R.H. Tame*[1]
[1]Yokohama City University, Tsurumi, Yokohama 230-0045, Japan

Introduction

Autotransporters are a family of virulence factors secreted from Gram-negative bacteria. They have many different roles in diseases, but share a common secretion mechanism which remains poorly understood. A C terminal domain of the protein inserts into the outer membrane of the bacterium and to make a beta-barrel. The N terminal domain (called the passenger) is moved across the outer membrane, and it is controversial how this is achieved. The role of the barrel is not clear. In a sub-family of autotransporters, the polypeptide chain is cleaved within the barrel domain by an unknown mechanism to release the passenger into the medium. We have studied an autotransproter called Hbp (hemoglobin protease) which is produced by pathogenic *E. coli*. This protease has an unknown role in peritonitis, but has been isolated from bacteria causing disease in both humans and birds. Hbp shows the self-cleaving property and has been used by several groups as a model system. Our group has solved the structure fo the passenger domain, and we are also interested in the membrane domain, and understanding its role in the secretion process.

Crystallisation

In order to examine the nature of the secretion intermediate just prior to self-cleavage we created a truncated form of Hbp in which most of the passenger domain is missing. The cleavage site occurs between two highly conserved asparagines residues, and it is known that mutating the first of these to aspartate can stop the reaction. We therefore expressed this mutant, and purified the barrel domain carrying approximately 25 residues of the passenger attached to its N terminus. This integral membrane domain was extracted from the membrane in detergent and purified using a C terminal histidine tag. Crystallisation was carried out under various conditions, the best of which produced crystals diffracting to 1.9Å resolution at beam line 5A of the Photon Factory. The cell parameters were a=73Å, b=69Å, c=78Å and β=117º. The space group is $P2_1$. Molecular replacement gave a unique solution, using a related barrel structure as a search model.

Mechanism

The refined model gives a very clear picture of the barrel, and in particular the residues at the catalytic site. The aspartic acid residue replacing the asparagine is found in a position suitable for nucleophilic attack on its own main-chain.

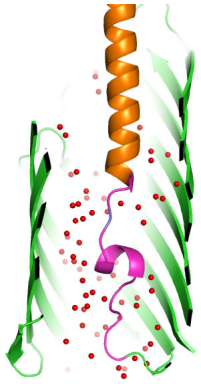

Figure 1.

In Figure 1, the overall structure is shown. The passenger residues are shown in orange, and for a helix. The cleavage site is found between the passenger helix and another short helix within the barrel. Residues coloured pink are highly conserved among the autotransporter proteins which show self-cleavage.

A closer look at the active site reveals that the mechanism relies on a novel intein-like mechanism, shown in Figure 2.

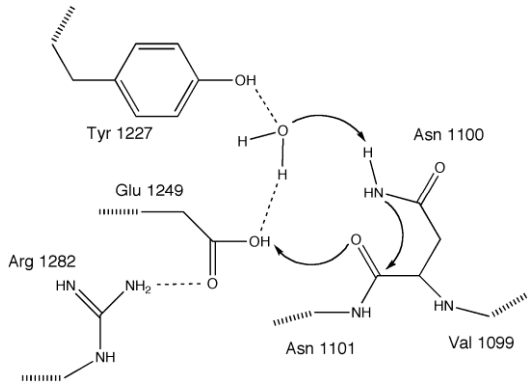

Figure 2.

Self-attack by the asparagine is promoted by a nearby glutamate residue which is required for the reaction. Unlike inteins, the mechanism requires no other residues such as histidines, and is robust to many mutations within the barrel itself. It is hoped the barrel domain will be useful for biotechnological purposes, allowing different proteins to be secreted from bacteria and automatically released from the cell surface.

References

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* jtame@tsurumi.yokohama-cu.ac.jp