STRUCTURAL CHARACTERIZATION OF *STAPHYLOCOCCUS AUREUS* α-HEMOLYSIN – A model for understanding the molecular mechanism of bacterial pore-forming β-toxins

Author: Pablo Gracia González

Supervisor: Ester Boix, (Department of Biochemistry and Molecular Biology, Faculty of Biosciences, Universidad Autónoma de Barcelona).

ABSTRACT

How can a water-soluble protein insert into hydrophobic lipid bilayers? Staphylococcus Aureus alpha-hemolysin is a well studied model to explain the molecular mechanism underlaying this event. Its water-soluble monomer binds to glycerophospholipid-rich membranes and, after a 4-stage stepwise mechanism it forms an heptameric, partially-hydrophobic transmembrane pore. The key structural determinant responsible for this membrane-spanning action are the unfolding of the stem domains to form a beta-barrel, the protomer-protomer interactions and the protein-membrane (both polar head and acyl chains) interactions. Furthermore, this bacterial toxin potent capacity to form pores offers useful biotechnological tools: alpha-hemolysin and similar nanopores are used as biosensors to identify molecules travelling through them and also for DNA sequencing. This purposes are based in the current changes inside the channel at the moment a molecule enters and blocks the nanopore.

SECONDARY AND TERTIARY STRUCTURE

α-hemolysin shows a 100% β folding for both monomeric and heptameric conformations. It comprises three structural domains: N-terminal β-sandwich domain formed of two six-stranded anti-parallel β-sheets and called “cap” domain, a C-terminal “rim” domain that is rich in β-strands, and a central domain called a “stem, which is folded against the core of the molecule but unfolds upon oligomerization, penetrating the membrane and lining the transmembrane pore.

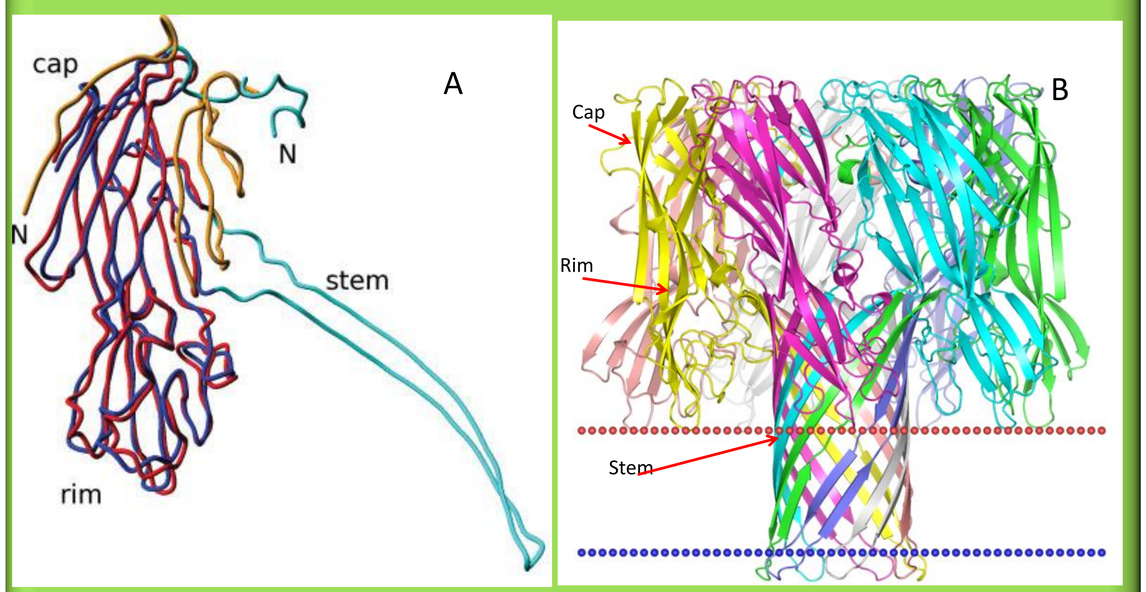

Fig.1. a) [1]Conformational rearrangements upon α-hemolysin oligomerization. Protomer (blue and cyan) and monomer (red and orange) structures are shown. Sequence stretches which seem to exhibit the greatest movements upon membrane insertion are displayed in cyan and orange. Domain identifiers refer to the pore structure. b) [2] Ribbon representations of the α-hemolysin heptamer with each protomer in a different color View perpendicular to the sevenfold axis and approximately parallel to the putative membrane plane.

α-HEMOLYSIN – LIPID BILAYER INTERACTIONS

α-Hemolysin establishes two kinds of interactions with the lipid bilayer: polar interactions between residues Met-197, Lys-198, Thr-199, and Arg-200 and stem residue Trp-179 have been unequivocally observed by means of high-resolution X-ray crystallography . This residues form the so called “rim-stem crevice”. On the other hand, apolar residues in the stem domain are very likely to make hydrophobic contacts with the acyl chains of the membrane glycerophospholipids. This protein-lipid interactions trigger conformational rearrangements which, in turn, drive the pre-pore to pore transition.

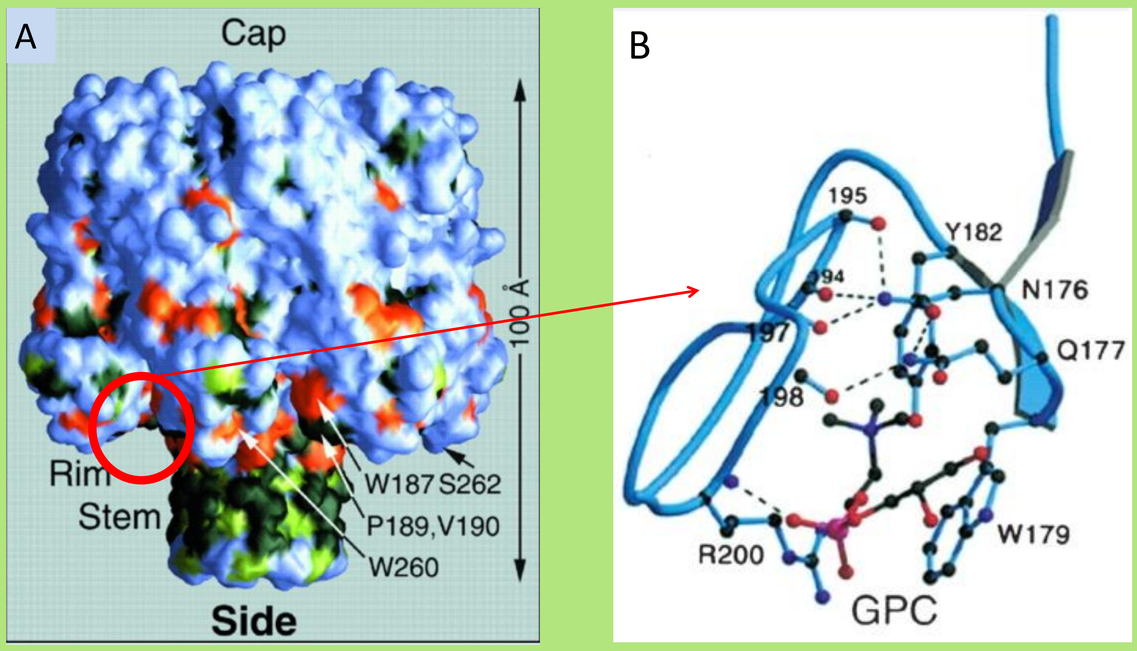

Fig.2. a) [3]Crystal structure with predominantly polar mushroom-shaped cap domain, rim-stem crevice and stem domain hydrophobic-belt are shown. Residues are color coded: Ala, Val, Pro, Leu, lie, Met, Phe: dark green; Gly: light green; Asp, Glu, Lys, Arg, His, Tyr, Trp, Ser, Thr, Asn, Gln: light purple. b) [4]Rim-stem crevice zoom. Interactions between GPC polar head and key residues R200 and W179 are shown. Hydrogen bonds are marked as dashed lines.

BIOTECHNOLOGICAL APPLICATIONS: NANOPORES AND DNA SEQUENCING

α-hemolysin and α-hemolysin-like synthetic nanopores allow passage for molecules up to 2kDa or even large lineal molecules such as ssDNA. When a molecule pass through the nanopore it blocks the channel therefore altering the ion current inside. These current variations can be measured, and characteristic of each molecule. Taking benefit of this biosensor property, several applications have been developed during last years: DNA sequencing and DNA methylations can be determined by nanopore-through passing. Also, some other useful applications have been developed: it’s ability to let Ca2+ enter the cell while leaving intact the cytoplasmic enzyme machinery, has been used to investigate exocytosis requirements. On the cancer therapy field, a tumour proteases specifically activated pore was created by insertion of a cleavable segment in the stem domain.

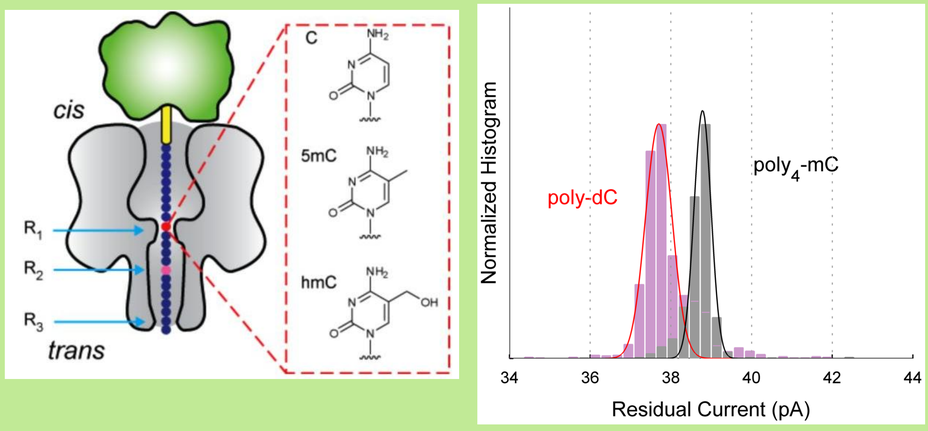

Fig. 4. a) [5]Biotin (yellow)-streptavidin (green) -assisted DNA translocation trhough a nanopore. Three DNA recognition sites are marked as R1, R2 and R3. Methylaed, Hyrdromethylated and non-methylated forms of an hypothetic ssDNA strand are shown. B) [6] Residual current measurements for both poly-deoxicytosine and poly-methylcytosine oligonucleotides: discrimitation of methylated ssDNA strands.

MOLECULAR MECHANISM

α-Hemolysin follows a stepwise mechanism, as represented below: the water-soluble monomer binds to a glycerophospholipid (GPI)-rich membrane, and this triggers oligomerization due to protomer-protomer interactions and, finally, stem domain unfolding and pore formation. Protein-lipid interactions are the key determinant that drives the innocuous monomer to lytic pore transtion, and are responsible for amino-latch closing and stem unfolding. Note that only the fully assembled pore, and not the pre-pore, is the cytolytic form of α-Hemolysin.

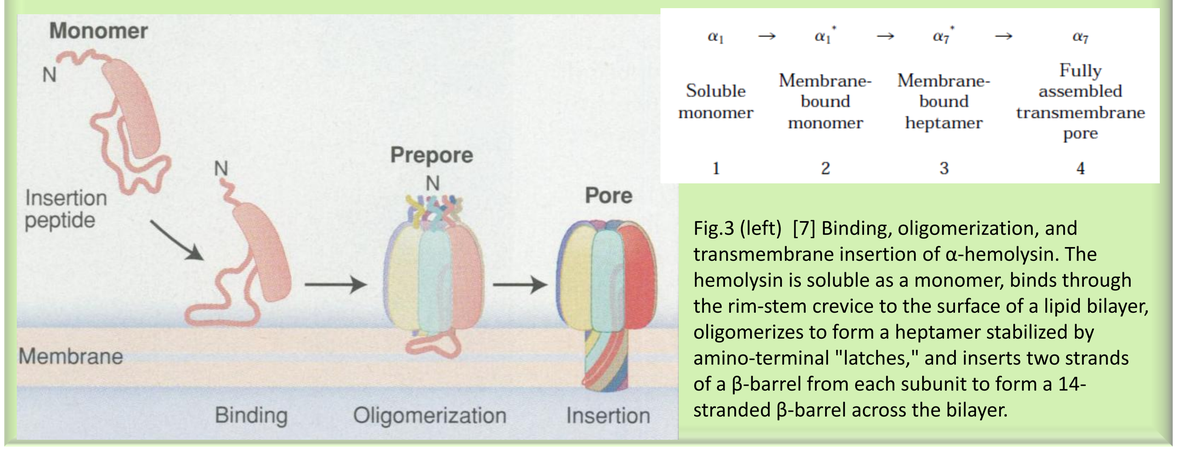

Fig.3 (left) [7] Binding, oligomerization, and transmembrane insertion of α-hemolysin. The hemolysin is soluble as a monomer, binds through the rim-stem crevice to the surface of a lipid bilayer, oligomerizes to form a heptamer stabilized by amino-terminal "latches," and inserts two strands of a β-barrel from each subunit to form a 14-stranded β-barrel across the bilayer.

CONCLUDING REMARKS

1.- β-barrel pore forming toxins follow a lipid membrane-assisted stepwise mechanism in which they transform a water soluble monomer into a transmembrane heptamer, therefore, protein-lipid interactions are a rquirement for this event.

2.- α-Hemolysin architecture provides insight into membrane-damaging toxins molecular mechanism, thus contributing to possible biomedical research and rational drug design.

3.- New structural approaches to the molecular features of the lipid bilayer during α-hemolysin four stages of pore formation could be the next logical step to enlarge the insight in this field.

4.- Nanopore sequencing has the potential to become a fast and low-cost DNA sequencing platform.

REFERENCES

1.- *Structural characterization of the α-hemolysin monomer from Staphylococcus aureus. Christian Meesters, Antje Brack, Nadja Hellmann, and Heinz Decker*

2.- *http://opm.phar.umich.edu/protein.php?pdbid=7ahl*

3.-*Structure of Staphylococcal α-Hemolysin, a Heptameric Transmembrane Pore*. Langzhou Song, Michael R. Hobaugh,* Christopher Shustak, Stephen Cheley, Hagan Bayley, J. Eric Gouaux .

4.- *High resolution crystallographic studies of alpha-hemolysin–phospholipid complexes define heptamer–lipid head group interactions: Implication for understanding protein–lipid interactions*

5.- *Identification of epigenetic DNA modifications with a protein nanopore*†
Emma V. B. Wallace , David Stoddart , Andrew J. Heron , Ellina Mikhailova , Giovanni Maglia , Timothy J. Donohoe and Hagan Bayley

6.- *Nucleotide Discrimination with DNA Immobilized in the MspA Nanopore* Elizabeth A. Manrao, Ian M. Derrington, Mikhail Pavlenok, Michael Niederweis, Jens H. Gundlach

7.- *Crossing the Hydrophobic Barrier: Insertion of Membrane Proteins*. Donald M. Engelman