Soutenance

THESE

Jeudi 21 Décembre 2017 à 14h30

Salle des séminaires

Institut de biologie structurale - 71 avenue des Martyrs CS 10090 38044 Grenoble Cedex 9 - T.+33 (0)4 57 42 85 00

www.ibs.fr

par **Marko Nedeljkovic**

Institut de Biologie Structurale

Groupe Pathogénie bactérienne

Structural characterization of a bacterial defense complex

Thèse de Doctorat de l'Université de Grenoble

The widespread resistance to antibiotics developed by bacterial pathogens calls for the characterization of original, yet unexplored potential targets in bacteria. Alpha2- macroglobulins (α2Ms) are broad-spectrum protease inhibitors that play key roles in eukaryotic immunity. They are multi-domain molecules that carry approximately 1,800 residues and harbor a central amino acid sequence, the 'bait site', which is recognized and cleaved by a large number of proteases. Upon cleavage, the resulting conformational change exposes a buried thioester bond between a cysteine and a glutamine, which is readily hydrolyzed, allowing the resulting glutamate to associate covalently to the target protease, trapping it within the α2M cage-like structure. Recently, α2M homologs from pathogenic and colonizing bacteria have also started to be characterized. These findings suggest that bacteria possess a rudimentary immune system that mimics initial key steps of the eukaryotic immune pathway and that could represent a yet unexplored target in pathogen biology.

The genes for two types of α2M are present in bacterial genomes: type 1, which contains the thioester bond, and type 2 that does not harbor it. Type 1 bacterial α2Ma persistently co-occur within the same operon with a gene that encodes a cell wall biosynthesis enzyme, Penicillin-Binding Protein 1c (PBP1c). This suggests that the association between the two proteins could be highly advantageous for the cell during infection/colonization, when the outer cell wall is targeted by host defenses. In this situation, α2M and PBP1c could exert the role of 'guardians of the periplasm', with PBP1c repairing damaged peptidoglycan, and α2M trapping invading proteases.

The aim of this work was to demonstrate the existence of such complex and characterize this interaction structurally and functionally. For this purpose, α2M and PBP1c from E. coli were studied. The proteins were expressed and purified separately. α2M (also called ECAM in E. coli) is a highly soluble, monomeric protein with a mass of 182 kDa, monodisperse and stable during the course of time. PBP1c is a membrane-bound, 87 kDa protein, predominantly present as a dimer. The complex, reconstituted in vitro by mixing and incubating the proteins for 2 hours, resulted in formation of a complex, demonstrated by appearance of a new peak in size exclusion chromatography. This result was further confirmed by SDS-PAGE, analytical centrifugation and small-angle x-ray scattering (SAXS) experiments. ECAM and PBP1c associated with 2:2 and 4:4 stoichiometries. The activity test confirmed that PBP1c performs polymerization of glycan chains and that its activity is enhanced in the presence of ECAM.

L'accès au campus EPN nécessite un avis de rendez-vous

Contactez O. Cavoret (au moins 48h à l'avance) - T. 04 57 42 87 04 - email : ibs.seminaires@ibs.fr