Post-doctoral activities of Salahaddin University-Erbil academic staff

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Summary

During my post doctorate in Faculty of Biochemistry, Biophysics and Biotechnology at Jagiellonian University in Krakow, Poland I was working on a project carried out in collaboration with prof. F.Xavier Gomis-Ruth from the Department of Structural Biology of the Molecular Biology Institute of Barcelona (CSIC, Barcelona, Spain), and the following three publications are the effect of my postdoctorate:

1. The structure of the catalytic domain of *Tannerella forsythia* karilysin reveals it is a bacterial xenologue of animal matrix metalloproteinases. Núria Cerdà-Costa, Tibisay Guevara, **Abdulkarim Y. Karim**, Miroslaw Ksiazek, Ky-Anh Nguyen, Joan L. Arolas, Jan Potempa and F. Xavier Gomis-Rüth. Molecular Microbiology 2011;**79**:119-132.

   Summary

   Metallopeptidases (MPs) are among virulence factors secreted by pathogenic bacteria at the site of infection. One such pathogen is *Tannerella forsythia*, a member of the microbial consortium that causes peridontitis, arguably the most prevalent infective chronic inflammatory disease known to mankind. The only reported MP secreted by *T. forsythia* is karilysin, a 52 kDa multidomain protein comprising a central 18 kDa catalytic domain (CD), termed Kly18, flanked by domains unrelated to any known protein. We analysed the 3D structure of Kly18 in the absence and presence of Mg2+ or Ca2+, which are required for function and stability, and found that it evidences most of the structural features characteristic of the CDs of mammalian matrix metalloproteinases (MMPs). Unexpectedly, a peptide was bound to the active-site cleft of Kly18 mimicking a left-behind cleavage product, which revealed that the specificity pocket accommodates bulky hydrophobic side-chains of substrates as in mammalian MMPs. In addition, Kly18 displayed a unique Mg2+ or Ca2+ binding site and two flexible segments that could play a role in substrate binding. Phylogenetic and sequence similarity studies revealed that Kly18 is evolutionarily much closer to winged-insect and mammalian MMPs than to potential bacterial counterparts found by genomic sequencing projects. Therefore, we conclude that this first structurally characterized non-mammalian MMP is a xenologue co-opted through horizontal gene transfer during the intimate coexistence between *T. forsythia* and humans or other animals, in a very rare case of gene shuffling from eukaryotes to prokaryotes. Subsequently, this protein would have evolved in a bacterial environment to give rise to full-length karilysin that is furnished with unique flanking domains that do not conform to the general multidomain architecture of animal MMPs.
2. A metalloproteinase karilysin present in the majority of *Tannerella forsythia* isolates inhibits all pathways of the complement system. Monika Jusko, Jan Potempa, **Abdulkarim Y.Karim**, Miroslaw Ksiazek , Kristian Riesbeck, Peter Garred, Sigrun Eick and Anna M. Blom. Journal of Immunology, 2012;**188**:2338-2349.

   Summary

   Tannerella forsythia is a poorly studied pathogen despite being one of the main causes of periodontitis, which is an inflammatory disease of the supporting structures of the teeth. We found that despite being recognized by all complement pathways, T. forsythia is resistant to killing by human complement, which is present at up to 70% of serum concentration in gingival crevicular fluid. Incubation of human serum with karilysin, a metalloproteinase of T. forsythia, resulted in a decrease in bactericidal activity of the serum. T. forsythia strains expressing

karilysin at higher levels were more resistant than low-expressing strains. Furthermore, the low-expressing strain was significantly more opsonized with activated complement factor 3 and membrane attack complex from serum compared with the other strains. The high-expressing strain was more resistant to killing in human blood. The protective effect of karilysin against serum bactericidal activity was attributable to its ability to inhibit complement at several stages. The classical and lectin complement pathways were inhibited because of the efficient degradation of mannose-binding lectin, ficolin-2, ficolin-3, and C4 by karilysin, whereas inhibition of the terminal pathway was caused by degradation of C5. Interestingly, karilysin was able to release biologically active C5a peptide in human plasma and induce migration of neutrophils. Importantly, we detected the karilysin gene in >90% of gingival crevicular fluid samples containing T. forsythia obtained from patients with periodontitis. Taken together, the newly characterized karilysin appears to be an important virulence factor of T. forsythia and might have several important implications for immune evasion.

3. A novel mechanism of latency in matrix metalloproteinases. López-Pelegrín, M., Ksiazek, M., **Karim, AY**., Guevara, T., Arolas, J.L., Potempa, J. & Gomis-Rüth, F.X. J Biol Chem 2015;**290**:4728-4740.

   Background: Animal and plant matrix metalloproteinases (MMPs) are kept zymogenic through large prodomains and a cysteine-switch mechanism.

   Results: Bacterial MMP karilysin has only a short N-terminal peptide upstream of the catalytic domain, which lacks cysteines.

   Conclusion: This peptide inhibits through an aspartate-switch mechanism and also exerts other functions of authentic prodomains.

   Significance: Karilysin is kept latent by a novel mechanism for MMPs.

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