Abstract No.5

Increasing of Thermostability and Autolysis Resistance in a Neutral Protease by Site-Directed Mutagenesis

Seyedeh Akram Shirdel [1], Hamid Reza Karbalaei Heidari [2], Khosro Khajeh[*1]*

1. Dept. of Biochemistry and Biophysics, Faculty of Biological Science, Tarbiat Modares University, Tehran, IR
2. Dept. of Biology, College of Sciences, Shiraz University, Shiraz, IR
(E-mail: shirdel131@gmail.com)

Neutral proteases (NPs) are sensitive to high temperatures and experience functional and structural alterations upon heating. There are members of a family of homologous metalloproteases which differ considerably in thermostability and autolysis by amino acid differences at the protein surface. The rate-limiting step in thermal inactivation of NPs comprises local unfolding process that cause the enzyme to be susceptible to autolysis as a result of exposing specific regions of its structure, critical for autolysis. Hence, we constructed some representative mutants in order to elucidate the thermal stability differences between wild type and mutant proteins, concerning point mutations. The wild type enzyme was used in this study isneutral protease from Salinivibrio Proteolyticus. The variants were cloned in pQE-80L as expression vector. Maximum expression was reached at 30° C, 1 mM IPTG in LB medium. After purification of the enzyme with Q-Sepharose column chromatography, the variants were characterized for their kinetic and thermodynamic parameters, resistance to temperature and autolysis. Results show that our new mutations are able to slightly increase thermal stability while reduce proteolytic cleavage. It was also concluded that there is no drastic changes in thermodynamic parameters upon mutations. Far-UV CD and intrinsic fluorescence spectroscopyas secondary and tertiary conformational probes were also used to monitor the effect of mutations on the structure of proteins.

Keywords: Autolysis, Local Unfolding, Thermal Stability, Variants And Spectroscopy.

Abstract No.6

Study of Interactions Between Antimicrobial Peptide LAH4 and Model Membranes

Matin Eslami [1], Faramarz Mehrnejad [1], Majid Taghdir[*2], Mahmoud Khadem-Maaref [3]*

1. Dept. of Cellular and Molecular Biology, Faculty of Science, Azarbaijan University of Tarbiat Moallem, Tabriz, IR
2. Dept. of Biology, Faculty of Sciences, University of Guilan, Rasht, IR
3. Dept. of Physics, Faculty of Science, Azarbaijan University of Tarbiat Moallem, Tabriz, IR
(E-mail: mehrnejad@azaruniv.edu)

The designed histidine-rich amphipathic peptide LAH4 exhibits potent antimicrobial and DNA transfection activities. This peptide also was found to mediate intracellular delivery of protein-based vaccines to generate enhanced CD8+ T cell immune responses and antitumor effects, that all of them require interactions with cellular membranes. Bilayer association of peptide has been shown to be strongly pH-dependent, with in-planar alignments under acidic conditions and transmembrane orientations when the histidines are discharged. The mechanism by which these AMPs selectively attack the bacterial membrane is believed to depend on differences in membrane lipid composition. Herein, our results show that the difference in binding affinity of LAH4 for palmitoyl-oleoyl-phosphatidyl-glycerol (POPG) and palmitoyl-oleoyl-phosphatidyl-choline (POPC) by using all-atom molecular dynamics simulations. These results inform us of the detailed location and orientation of the peptide with respect to the bilayer as well as present that, the different binding affinity be related to polar, electrostatic, and hydrophobic protein-lipid interactions that provide an understanding of what occur during membrane insertion. MD Simulations show that the peptide should have stronger interactions with anionic bilayer head group when compared to zwitterionic bilayer head group. The results also indicat changes in the lipid acyl chain order when LAH4 is bound to the different membranes. According to our MD results and previous NMR experimental studies, LAH4 is more effective at the anionic bilayer comparing zwitterionic membrane in acidic condition.This results can be an effective means to improve antimicrobial properties and an approach in against of the growing health problem of antibiotic resistant and serve as an important foundation for future clinical applications to improve protein/peptide-based vaccine potency.

Keywords: Antimicrobial Peptide, Drug Delivery, Molecular Dynamics Simulation, Membrane.