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Helicobacter pylori VacA, a pore-forming toxin causing several alterations in human cells, plays an important role in the development of peptic and duodenal ulcerations as well as gastric adenocarcinoma. The N- and C-terminal cleavage of VacA during the secretion process yields a mature 88-kDa toxin which its partial proteolysis produces p33 and p55 domains. The latter is encoded by the middle (m) region of the vacA gene which includes m1/m2 polymorphisms. The m1 and m2 VacA proteins bind different cell-surface receptors. This is relevant clinically because the strains harboring m1 vacA genotype are associated with gastric cancer and VacA is a candidate antigen for vaccine development. The crystallographic structure of the m1 VacA p55 domain has been released and docked into the 19-Å cryo-EM map of the wild-type dodecamer in order to discern the p55 structure in the context of VacA oligomers. The purpose of this study was to produce the theoretical three-dimensional structure of m2 VacA p55 domain in order to perform a comparative structural analysis of two forms of the domain. The models were constructed using comparative modeling method and the stereochemical quality of protein structures and molecular dynamics simulation of the refined model was performed. The structural analysis showed the key features of m2 relative to m1 domain that may illustrate their different potentiality for interaction with host receptors and formation of oligomeric structures.

Keywords: *Helicobacter pylori*, VacA, Gastric Cancer, Comparative Modeling.

Abstract No.89

The Proteolytic Analysis of Firefly Luciferase with Disulfide Bridge and Hydrophilic Residue Substitution

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Luciferase from the North American firefly *Photinus pyralis* is a well-characterized enzyme that catalyzes the emission of yellow-green light. It is a useful protein that has been extensively used for sensitive in vitro and in vivo applications. Limited proteolysis has become a powerful tool for probing the higher order structure of proteins which determine the location of particular peptide bonds within the overall

fold of the protein. In order to determine the effect of disulfide bridge and substitution of hydrophilic residue with surface hydrophobic residue on stability against protease, limited proteolysis of native, A296C/A326C (to create disulfide bridge), I232R (hydrophilic residue substitution) and A296C/A326C/I232R mutant luciferases was carried out using trypsin at 23°C in different lengths of time. In this regard, first of all mutant luciferases were created using site direct mutagenesis. Afterward, they were expressed and purified. At last, proteolysis was done. Based on the analysis of SDS-PAGE and the percent of full-length remaining enzyme (using UN-SCAN-IT software (Silk Scientific, Inc)), all mutants shown different sensitivity against trypsin hydrolysis over a period of time, and apparently they were more stable than native one (especially the enzymes containing I232R mutation). In the case of luciferases with disulfide bridge, some fragments were found to be more resistant to further degradation, whereas in native and I232R mutant, fragments released from the intact protein were subdigested by trypsin, giving rise to smaller peptides. Therefore disulfide bridge causes increased interactions and consequently decreased accessibility of digest sites of trypsin. This result along with fluorescence and CD data suggest that these mutations exert an important role in decreasing structural flexibility and consequently proteolysis.

Keywords: Firefly luciferase, *Photinus pyralis*, Proteolysis.

Abstract No.90

A Comparative Study of Non-hydrolytic Activities of Acetyl and Butyrylcholinesterase Enzymes and their Impact on the Formation of Beta-amyloid Aggregation

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Alzheimer disease (AD) is one of the most common neurodegenerative dementias, caused by silent plaques that are created by fibril formation of beta amyloid (A β) peptide. A β is a portion of transmembrane receptor-like amyloid precursor protein (APP) in neuron cells. Recent investigations have shown that acetylcholinesterase (AChE) plays a crucial role in the promotion of A β aggregation beside its role in the rapid hydrolysis of the neurotransmitter acetylcholine (ACh). Butyrylcholinesterase (BChE), because of similar structure and function to AChE may also have a role in this phenomena. Both AChE and BChE have a peripheral anionic site, beside their active site, and it is proposed that it is involved in the promotion of amyloid fibrillation