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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 Proteoletic 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 other 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 length of times. In this regards, 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 caueses increased interactions and consequently decreased accessibility of digest sites of trypsin. This results along with fluorescence and CD data suggest that these mutations exert an important role in decreasing structural felexibility 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 (Aβ) peptide. Aβ, 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 Aβ 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 role in thia 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

presumably through interactions with Aβ. In this study we have investigated about this promotive effect of AChE and BChE on amyloid aggregation in vitro. Our preliminary results showed that both enzymes could significantly increase Aβ42 thioflavine T (ThT) fluorescence intensity, considered as a quantitive index of amyloid aggregation, when they were incubated with Aβ. In the continuation of these studies we will examine the synergistic effects of these two enzymes (if any) and the effects of their inhibitors of Aβ aggregation, with the use of techniques such as circular dichroism (CD) and atomic force microscopy (AFM) in addition to thioflavine T fluorescence spectroscopy.

Keywords: Beta Amyloid (Aβ), Fibril Formation, Acetylcholinesterase (AChE), Butyrylcholinesterase (BChE).

Abstract No.91

The Effects of Addictive Drugs on Zebrafish Behavior and its Correlation with Brain Metabolites Changes

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Drug addiction is a worldwide problem and is considered as a chronic brain disease and government spend huge resource to eradicate this problem. However, eradication could only be possible if we understand the behavior and molecular mechanism of addiction. Currently, numbers of animal models including zebrafish were used to understand the behavior and molecular mechanism of drugs addiction and withdrawal symptoms. We use zebrafish as model organism to understand how alcohol and nicotine separately or in combination affect known zebrafish behavior and brain metabolite. Our findings suggest that addictive drugs disrupt shoaling behavior and reduce frightening behavior in adult zebrafish but induce in larvae zebrafish. The effect, however, was less prone to highly active young adult fish but more prone to energy starved adult fish (such as hungry and old age or full-term egg bearing female fish). Drugs also produced progressively decreased feeding pattern with time compared to control except in case of nicotine where no significant feeding was observed. Feeding pattern however, recovered during drug withdrawal period except the fish group co-treated with alcohol and nicotine (co-abuse). This finding clearly suggests that addictive drugs can manipulate appetite. Drugs also found to influence learning and memory example, nicotine produced improve understanding of stimulating environment whereas alcohol causes decreases in this activity. In contrast, fish group co-abuse with drugs, the memory seemed to be largely compromise by the anxiolytic as well as anxiogenic effect. Further, sleeping pattern and duration during night was significantly disrupted in the fish co-abuse with drugs. Our zebrafish brain metabolites analysis also indicates that the addictive drugs manipulate the concentration of the metabolite critical in memory formation such as N-acetyl L-aspartate (excitatory activator) and taurine (excitatory inhibitor).

Keywords: Behavioral Study, Addictive Drugs, Addiction, Alcohol, Nicotine, Brain Metabolite.

Abstract No.92

Employment of Phage Display Technology To Construct GFP-Bearing Phage Nanoparticles with Peptide-Ligands Targeting Into Intestinal Epithelial Cells

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Gene and drug targeting is a promising strategy to treat various diseases. Over the recent years, phages thanks to attractive features such as lack of intrinsic tropism for mammalian cells, the presence of a capside structure surrounding DNA and the background of safe use have fostered various attempts to develop novel gene and drug carriers as attractive alternatives to existing viral and non-viral vehicles used for gene and drug delivery. To circumvent the problem of low efficiency of phage vehicles, one great technological achievement called phage display is exploited in order to express targeting ligands on the surface of phage thereby developing a platform for specific and targeted gene and drug delivery into cells. Here, our aim was to construct bacteriophage nanoparticles with the capability of targeted delivery into intestinal cells. To this end, XL1-Blue MRF′ bacterial cells were infected with M13 phages thereby amplifying phage particles. Following titering of phage particles by preparing serial dilutions of phage suspension, M13 plaques were obtained on solid medium. These plaques were employed for extracting double-stranded DNA of M13. GFP gene cassette was cloned in M13 bacteriophage genome as a reporter gene. Making use of phage display, two oligonucleotide