Abstract No.239

Analysis of the Binding Interaction of Curcumin with Lysozyme

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Curcumin [1,7-bis-(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione] is a polyphenol compound extracted from the herb Curcuma longa Linn. Modern scientific community discovers that curcumin exhibits an enormous variety of pharmacological and biological activities including anti-oxidant, anti-tumor, anti-inflammatory, anti-bacterial, and anti-protozoal activity. Further, curcumin has the possibility to slow down the progress of Alzheimer's diseases by reducing b-amyloid formation. Lysozyme is an antimicrobial proteinase that has ability to lyse the cell walls of bacteria by hydrolyzing the bond between N-acetylglucasamine and N-acetylmuramic acid of the peptidoglycan. Lysozyme consists of a single chain polypeptide containing 129 amino acid residues which is crosslinked with 4 disulfide bridges. The investigation of interactions between small molecules and lysozyme has an important meaning on realizing the transport and metabolism process of the small molecules and the relation of structure and function of lysozyme. With respect to the different physiological and pharmaceutical functions of lysozyme and its widely distribution in various biological fluids and tissues including avian egg, animal secretions, human milk, and tears, we decided to study the interaction of curcumin with chicken egg white lysozyme. This study was carried out using different techniques including steady sate fluorescence, synchronous fluorescence, three-dimensional fluorescence, UV-vis absorption, fluorescence resonance energy transfer, and molecular docking. The fluorescence experiments revealed that addition of curcumin effectively quenched the intrinsic fluorescence of lysozyme by formation of a non-fluorescent complex (static quenching). The number of substantive binding sites and the binding constant were calculated by relevant fluorescence quenching data. Based on the Förster's theory of non-radiative energy transfer, distance between the donor (lysozyme) and acceptor (curcumin) as well as the critical energy transfer distance has also been calculated. The molecular docking studies revealed that specific interactions were observed with the Trp-62 and Trp-63 residues. The calculated thermodynamic parameters showed that H-bonds and van der Waals interactions played a major role in stabilizing the curcumin–lysozyme complex.

Keywords: Lysozyme, Curcumin, Fluorescence Spectroscopy, Molecular Docking.

Abstract No.240

Structural and Functional Study of Lysozyme from Rutilus Frisii Kutum

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Lysozyme is an antibacterial protein, has been implicated in innate immunity in invertebrates, but its activity in shrimp and some other marine animals, remained to be determined. We are going to clone the white Rutiluslysozyme cDNA using a PCR strategy following to designing sutable primers according to the nearest spesiec to Rutilus, and detected its activity in haemocytes using a lytic-zone assay against Micrococcus luteus, The deduced amino acid sequence resulted in 150 amino acid with 46% identity to hen egg white lysozyme. RT-PCR was used to detect lysozyme mRNA in haemocytes. Analysis of the amino acid sequence of the lysozyme may be showed that it belongs to the C-type family of lysozymes. Furthermore, the lysozyme amino acid sequence contained extra residues at its C-terminus, which are characteristic of marine invertebrates. This information will be useful in future studies on the molecular mechanisms of immunity in marine invertebrates.

Keywords: Lysozyme, Shrimp, Prawn, Purification, Haemocyte, PCR-cloning, EST.

Abstract No.241

The Effect of di- and Polyamines on 2ʹ,3ʹ-cyclic Cytidine Monophosphate: a Spectroscopic Study

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The nucleotide 2ʹ,3ʹ-cyclic cytidine monophosphate is produced during hydrolysis process of RNA by RNAse A, although this compound is an intermediate and hydrolyzed in the next step. A large number of

studies (e.g. effect of different ligands and their interactions) have been carried out on it. On the other hand, polyamines are produced naturally in living cells. They are implicated in a wide range of cell processes including cell growth, cell division, differentiation, gene regulation, enzyme activity and signal transduction.In this study effect of various concentrations of di and poly-amines has been investigated on the structure of 2˙,3˙-cyclic cytidine monophosphate by following ΔA284. 2˙,3˙-cyclic cytidine monophosphate and all amines were dissolved in Tris-EDTA buffer (Tris 100mM, EDTA 2mM, pH 7.5). As our results showed, none of the diamines, including 1,3-diaminopropan, 1,4-diaminobutane (putrescine) and 1,5-diaminopentane (cadaverine), had significant effect on the nucleotide spectrum, while both of polyamines (spermidine and spermine) caused significant and meaningful change on 2˙,3˙-cCMP absorbance. Overall, it seems that polyamines interaction with phosphate group and/or cytosine base of the nucleotide resulting in a change in its absorbance while diamines may interact only with phosphate group of the nucleotide which has no effect on its absorbance. This difference may be related to size of polyamines that are larger than the diamines.

Keywords: 2˙,3˙-cyclic Cytidine Monophosphate, Diamine, Polyamine, Interaction.

Abstract No.242

In Silico Analysis of Conformational and Immunological Differences of Linked StxB and CtxB

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CtxB and StxB are of the most impacting factors in initiation of cholera and shigella infections. These two proteins are working as carriers of catalytic domains of shigella and cholera toxins. All such proteins have 5 same subunits along with a catalytic domain among. These two proteins are considered as novel targets for immunological studies. It is assumed that accompaniment of both of proteins can boost the immunological effects. Linking both proteins with a linker is a possible solution, but structural integrity and proper folding of proteins must remain stable. In order to discover suitable number of linker molecules for proper separation of proteins, CtxB-linker(n)-StxB and StxB-Linker(n)-CtxB manner of proteins are designed and structures modeled with Modeller 9 program based on protein data bank structures of proteins. A Furin cleavable linker is used for evaluation of structures. Best scored models are studied for their stability and original structures by over fitting of models and initial structures, solvent accessible surface and ramachandran plot. Results demonstrate that presence of StxB at the N-Terminal of structure stabilizes the total fusion because of C-Terminal structure of StxB. Number of linker repeats must be at least one but more than 3 repeats those not change the structure stability.

Keywords: CtxB, StxB, Modeling, Furin linker, Shigella, Cholera.

Abstract No.243

Spectroscopic Investigation on the Interaction of c-MYC quadruplex DNA with Water-Soluble Tetrapyridinoporphyrazinatozinc(II)

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Nucleic acid sequences which are rich in guanine are capable of forming four-stranded structures called G-quadruplexes. Oncogenes are especialy rich in quadruplex. It is known that ~90% of c-MYC transcription is controlled by a 27-nt purine rich strand is composed of consecutive five guanine stretches. Recent experimental data suggest that even a brief inhibition of c-MYC expression may be sufficient to permanently stop tumor growth and induce regression of tumors. The ligands that bind to hypersensitivity element III1 (NHE III1) of the c-MYC promoter can control the transcriptional activity of the c-MYC oncogene. Here, interaction between water-soluble N,N′,N″,N′″-tetramethyltetra-3,4-pyridinoporphyrazinatozinc(II) {$[Zn(3,4\text{-}tmtppa)]^{4+}$} and c- MYC G-rich oligonucleotide was investigated. The absorption spectrum of {$[Zn(3,4\text{-}tmtppa)]^{4+}$ displays a Q band at ~670 nm. It was found that at low concentrations of DNA, a hypochromicity in the Q band of the complex is shown but, at higher concentrations, the intensity of spectra increases and the maximum absorption shifts to higher wavelengths considerably. It seems two types of complexes form due to interaction of the porphyrazine with c-MYC G-quadruplex DNA. The quenching of [Zn(3,4-tmtppa)]4+ by G4 DNA and the G4–thiazole orange by the complex were measured by fluoresnce spectroscopy. Stern–Volmer