Abstract No.253

Molecular Dynamics Simulation Study of Lipase B from Candida Antarctica

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Candida antarctica lipase B (CalB) belongs to the α/β hydrolase family which is act on the ester bonds of tri-acyl glycerol. CalB is a psychrophilic lipase that is able to perform lypolytic activity at low temperatures. Cold-active lipases have several applications in industry, production of pharmaceuticals, food, and fine chemical. To understand the mechanisms involving in the psychrophily of CalB, molecular dynamics (MD) simulation has been undertaken to explain its dynamics at atomic level. Crystal structure of CalB was obtained from Protein Data Bank (1TCA) as the initial structure for MD simulations. MD simulations were performed by AMBER 10.0 with all-atom force filed ff99SB. The structure was hydrated in a 10 Å layer of TIP3P water model. In this study, several MD simulations have been performed for 30 ns at three different temperatures (5, 35 and 60 °C). A highly flexible alpha helix (α5 helix) was identified in which act as a lid for the active site clef of CalB. According to the results obtained from RMSF graphs, the extent of flexibility is much higher at low temperature rather than higher temperatures. Further investigation of the active site revealed that the starting open conformation of the enzyme become close immediately at 35 and 60 °C while it remains open for a considerable time. Radial distribution function of water molecule in the activeite also verifies the movement of the lid. The presence of water molecules at open conformation is higher than that of closed state. It is suggested that the cold-activity of Calb is tightly related to the movement of the lid at low temperatures. As it observed in the current study, open state is more stable at 5 °C rather that 35 and 60 °C.

Keywords: Candida Antarctica Lipase B, Psychrophilic Enzymes, Molecular Dynamics Simulation, Open-Closed Conformations.

Abstract No.254

Molecular Dynamics Study of two Mechanistic Mutations in Mnemiopsin from Mnemiopsis leidy

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Mnemiopsin from Mnemiopsis leidy emits light in the presence of Ca^{2+} decomposing into apomnemiopsin, coelenteramide and CO_2. To understand mechanism of the reaction, a comparative structure-function study was undertaken with respect to two single mutants, Leu36His and Phe186His located in the substrate binding cavity. Our experimental results showed that these mutations stop bioluminescent reaction. Molecular dynamic simulation studies indicated an increase in overall flexibility of Ca^{2+}-binding loops and substrate binding cavity residues in mutants compared to the wild type. Further analyses on Phe186His mutant revealed that mobility of coelenterazine in some regions and substrate-protein interaction energy is decreased, while these structural properties are not changed in Leu36His mutant. Finally these findings revealed that these two substitutions prevent disruption of peroxide group of coelentrazine and consequently bioluminescent reaction by altering the dynamic and energetic properties in this region.

Keywords: Mnemiopsin, Structure-function, Molecular Dynamics Study.

Abstract No.255

Artificial Superoxide Dismutase Activity of Copper-Cysteine to Electrochemical Detection of Superoxide

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Superoxide dismutase (SOD) represents an essential defense system against oxygen-derived free radicals, specifically superoxide radical anion. Superoxide can initiate a series of free radical reactions, which causes a vast number of diseases. So, quantitative analysis of in vivo is very important. In most of the analysis methods, SOD was immobilized through cysteine self assembled monolayer on gold (Cys/Au) electrode. Here, we design and compare three approaches of superoxide

detection by using SOD/Cys/Au, Cu^{2+}/Cys/Au and Cys/Au electrodes. The Cu/Cys/Au electrode shows quasi reversible peaks with formal potential of 29 mV versus Ag/AgCl at scan rate 50 mVs^{-1} as same as SOD/Cys/Au electrode. The ampromrtic response for was monitored at an electrode potential 250 mV at pH 7.4 phosphate buffer and 500 rpm. In addition, the linear detection range and detection limit of superoxide anion radical at Cu^{2+}/Cys/Au electrode were 3.4-254.2 and 2.3 μM respectively. Comparison between voltammograms of different electrodes revealed that current intensity was increasing by the order of Cu^{2+}/Cys/Au > SOD/Cys/Au > Cys/Au electrodes. This increasing order was also seen for the amprometric response. The experimental results revealed that Cu^{2+}, either as coordinated with Cys or as SOD redox center, plays a critical role in electrochemical response on the Cys/Au electrode. It seems that in Cu^{2+}/Cys/Au electrode, Cu^{2+} coordinate with amine and carboxyl groups of Cys and form a complex. Thus, Cu^{2+}/Cys/Au electrode shows better superoxide dismutase activity than SOD/Cys/Au electrode, since Cu^{2+} in the metal active-site of SOD is structurally located deep in a channel and direct electron transfer between enzyme and the electrode is difficult.

Keywords: Superoxide Dismutase, Superoxide Detection, Cysteine, Electrochemistry.

Abstract No.256

Evaluation of the Smac Based Peptides and Protein in Antagonizing XIAP as Anticancer Agents

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XIAP prevents apoptosis through inhibition of caspase-9 by the BIR-3 and caspases -3 and -7 through BIR2 domain. SMAC which is released from the mitochondria competes with caspases in binding to XIAP unleashing caspase activity and causing cell death. SMAC peptides and protein were used to investigate their ability in relieving the executioner caspase activities inhibited by both the BIR1-2 domains and the BIR1-2-3 domains of XIAP. Furthermore, the potency of these peptides was compared to the Smac protein in antagonizing XIAP. AKPD, ANPR, SGVD, AVPI peptides and the SMAC protein were preincubated with the IAP domains and the activity of caspases was studied in the presence of these mixtures. Moreover, the ability of these peptides in preventing the interaction of BIR1-2 domain with the large and the small subunits of executioner caspases was studied. It was observed that the Smac protein by far is the most potent agent in reversing caspase inhibition. In addition, Caspase-3 inhibition by XIAP domains was more sensitive to SMAC peptides than that of caspase-7. Finally, while, BIR1-2 inhibited caspase-3 was very sensitive to SMAC interference, BIR1-2 inhibited caspase-7 responded to SMAC antagonism very weakly. These results indicate that under conditions of extensive XIAP cleavage and involvement of caspase-7 as the driving force for execution of apoptosis, Smac, and by extension Smac based anticancer agents, cannot be effective in inducing cell death.

Keywords: Apoptosis, XIAP, Executioner Caspases, Smac Peptides and Protein.

Abstract No.257

Comparison of Wild Type and Double Mutated Aequorin Variants from Luminescence and Kinetic Aspects

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The photoprotein aequorin is a small calcium-dependent bioluminescent protein which emits blue light by an intramolecular reaction. The emission properties, stability and decay kinetics of this reporter protein can be changed by directed mutagenesis of key residues. In the present work, three double mutants including variants of Y82F/W86F, Y82F/D153G, and W86F/D153G are prepared. With respect to our results, it seems that presence of W86F mutation shifts the emission to shorter wavelengths, while the Y82F mutation results in shift of emission to longer wavelengths. Furthermore, analysis of the variants for light half-life showed decreased t1/2 for the two mutants of Y82F/D153G and W86F/D153G. Conversely, the Y82F/W86F variant displayed a 2-fold increase of light half-life compared to wild type aequorin. Finally, comparative thermostability analyses of double mutants showed higher stability only for Y82F/D153G variant while the single W86F mutant reached the highest stability against thermal treatment. Our results suggest that replacement of few residues in the active site or binding pocket of aequorin affects its luminescence and kinetic properties and promises the feasibility of new reporter production with limited substitutions.