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.