communication with macromolecules on the surface of electrode. In this project, the structures of nafion nanocomposite polymer and toluidine blue- mediator (which causes catalyzing oxide reaction and restoring protein) are investigated. Then by modifying the surface of electrode we make it possible the observation of redox enzyme metalo-proteins reaction through electrochemistry which can be useful in bioelectrochemistry applications.In the first phase of this project, the surface of electrode is modified with nanocomposite polymer nafion-toluidine blue. Then we examine the surface of obtained nanocomposite using Cyclic Voltametry, Cronoamperometry, DPV and electron microscopy data. Our data confirms the formation of nanoparticles of nafion-toluidine blue in the size (dimensions) of 70 nm. We observe that toluidine blue can move the peak of cathode enzyme catalas to 100 mv. Then, by stabilizing the enzyme catalase we measure the hydrogen proxide (as a substrate of this enzyme) and introduce this biosensor with the detection limit of 0/28 µM.

Keywords: Nanobiosensor, Hydrogen Peroxide, Catalase Polymer Nafiyn, Toluidine blue.

Abstract No.225

Purification of Amine Oxidase from Pea Seedlings and Effects of Sucrose on its Stability

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The copper amine oxidase (AO, E.C 1,4,3,6) is found in all known organisms including planrts, mammals and microorganism. There is an increasing interest in amine oxidases due to their involvement in polyamine metabolism correlated in physiopathological processes. Osmolytes as solvent additives favorably affect protein stability extreme environmental stress without any enzymatic activity.Amine oxidase was purified from pea seedlings. Residual activity of pea seedling amine oxidase (PSAO) was investigated as a function of various sucrose concentrations and incubation time in different temperature. Then for more elucidation of the effect of sucrose the studies were extended using by far-UV CD, fluorescence spectroscopy. PSAO loses its activity by incubation in 63 °C for 60 min. Presentation of sucrose increases the stability of PSAO in terms of activity losing temperature and incubation time. More detailed studies using fluorescence and Far-UV CD showed that sucrose increases the PSAO stability as a concentration dependent manner.

Keywords: Osmolyte, Stabilization, CU-amine Onidase, Thermal unfolding.

Abstract No.226

Exploring Serpin Inhibitory Mechanism by Molecular Dynamics Simulation

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Serine protease inhibitor (serpin) is a family of structurally related proteins that control many physiological reactions by inhibition the protease. This inhibitor is found in many organisms, from nematode to human. However, a detailed mechanism of inhibition, how a protein could inhibit a protease, is yet to be understood. In a well known mechanism, serpin binds to the active site like a substrate, leading to small conformational change required to lock the protease. On the other hand, hydrogen bond network around the active site of serine protease family plays an important role in stabilization of the transition state. Herein, to explore mechanism of Serpin's inhibition, we studied the effect of binding of ecotin (a natural protein in the periplasmic space of Escherichia coli with a broad range of inhibition) on changing of hydrogen bond network near the active site of the thrombin (a key enzyme in the processes of thrombosis and haemostasis). Three all atomic molecular dynamic simulations of 10 nanosecond were performed on three forms of thrombin enzyme (free enzyme, substrate-enzyme and ecotin-enzyme complex). More than 20 hydrogen bonds were selected around the active site. Distance changes between the donor and acceptor in the length of trajectory was extracted and distance-distance correlation (DDC) was computed. Affected hydrogen bond by ecotin binding was detected by comparison of the DDC profile amongst the three structures. Results of this investigation revealed that change in the hydrogen bond network has the primary role in enzyme catalysis.

Keywords: Molecular Dynamic Simulation, Serpin, Ecotin, Thrombin.
