

Abstract No.222

pKa Calculation of the Positively Charged Amino Acids in a Protein Chain, An Assessment of Theoretical Procedure

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In this study, the aqueous pKa for two cationic amino acids including lysine and arginine in a peptide chain which composed of eight glycine molecule have been calculated. HF and B3LYP methods using the 6-31+G(d) [1-3] combined with solvation energies that were computed by the SCRF-(CPCM/UFF) continuum models as implemented in Gaussian 09 computational package [4]. pKas were further calculated using two thermodynamic schemes (scheme1 and 2), namely the direct method and the proton exchange method with the inclusion of an explicit solvent water molecule. The results of this research verify that the direct method is not suitable for computing pKa of the amino acids in a peptide chain, while the other scheme in the presence of water molecule significantly improved the pka in comparison to the experimental data. The combination of the proton exchange scheme and CPCM-UFF model performed well with mean absolute deviations (MADs) of ~0.9 pKa unit. Because of the convergence problems, the inclusion of large numbers of water molecule in scheme 2, the computation procedure in the solvated model produces weak results. Comparison between the pKa α , β and random (R) structures of the studied proteins, reveals that the strongest acidic character belongs to β , α and R, respectively at the HF and B3LYP levels of the theory. The reason for this new result returns to this fact that structural differences from the hydrogen bond point of view and natural orbital populations affect the proton affinity of the studied amino acids. Atom in molecule (AIM) and Natural population analysis (NBO) on all structures show that the β structure possess the maximum hydrogen bonds with high electron density relative to other second structures which induces a more positive charge on the acidic hydrogen of the amino acid.

Keywords: pKa, SCRF, Second Structure, Amino acid, Lysine, Arginine.

Abstract No.223

Effects of some Flavonoids on the Mushroom

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The inhibitory effects of some flavonoids on the diphenolase activity of mushroom tyrosinase have been investigated with the spectrophotometric technique. The results of kinetic assays showed that the flavonoids induced a reversible inhibition on the enzyme activity. Furthermore, gallic acid and quercetin show non competitive-type of inhibition. The chrysin and naringin induced a competitive manner of inhibitor. The inhibition constants have been determined for these flavonoids and the inhibition strength follows the order of: quercetin < chrysin < naringin < gallic acid. The values of the inhibitor binding constant (K_i) obtained 16.0, 7.9, 3.0, 1.5 mM, respectively. Thus the flavonoids played an important role in the inhibition of the mushroom tyrosinase enzyme.

Keywords: Inhibition, Flavonoids, Kinetic, Mushroom Tyrosinase.

Abstract No.224

Nanobiosensors Designed to Detect Hydrogen Peroxide by Using Catalase Polymer Nafiyon

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The bare surfaces of electrode are not suitable in electrochemical survey of proteins. This, not only leads to decreasing the speed for electron transfer between electrode and protein but also can lead to irreversible absorption of protein on the surface of the electrode, accompanying the conformation changes and loss of protein activity. Hence, it is necessary to provide required groups for the active

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 metallo-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 catalase to 100 mv. Then, by stabilizing the enzyme catalase we measure the hydrogen peroxide (as a substrate of this enzyme) and introduce this biosensor with the detection limit of 0/28 μ M.

Keywords: Nanobiosensor, Hydrogen Peroxide, Catalase Polymer Nafion, 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 plants, 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 Oxidase, 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.
