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Comparative Evaluation of Sildenafil Effects on the Structure and Activity of Native and Modified States of Human Carbonic Anhydrase II

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Human carbonic anhydrase (hCA, EC 4. 2. 1. 1), is a zinc – containing enzyme which catalyes the reversible hydration of carbon dioxide to bicarbonate and hydrogen ions. Many of CA isozymes have been discovered as important targets for activators and inhibitors with clinical applications. It has recently been demonstrated that sildenafil, which widely used for the treatment of erectile dysfunction, acts as activator of hCA II. We observed that histidine residues on the rim of hCA II active site are critical for its activity and suggestive of their role as a proton transfer group. The treatment of histidine-modified hCA II with sildenafil revealed a moderate hCA II activation profile. Furthermore, the effects of sildenafil on kinetic and structural properties of native and modified hCA II were investigated employing different spectroscopic techniques such as UV-Vis, circular dichroism (CD) and fluorescence spectroscopy. Fluorescence data proposed that sildenafil acts as quencher of native and modified hCA II fluorescence. Both the Stern-Volmer analysis and molecular docking revealed the existence of one binding site in the both forms of enzymes for sildenafil. The thermodynamic parameters indicated that the driving force of this processes are not same. Calculation of the protein surface hydrophobicity (PSH), using 1- anilinonaphthalene-8-sulfonic acid (ANS), indicate the increment of PSH of native and modified hCA II in the presence of sildenafil. The near-UV CD results as well as the determination of PSH results reiterate that, in the presence of sildenafil, flexibility of the native and modified hCA II tertiary structures has been increased. We will discuss the importance of our observations.

Keywords: Human Cabonic AnhydraseII, Sildenafil, Fluorescence Quenching, Activator, Diethyl Pyrocarbonate, Histidine Residue.

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Differential Binding and Effect of Leucovorin on Stability and Aggregation of IgG

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The interaction of leucovorin (LV), an adjuvant used in cancer chemotherapy, with a non-carrier protein IgG was performed by different spectroscopic and computational approaches in absence and presence of physiological blood solutes (NaCl, urea and glucose) as well as in those conditions where their concentrations increases abruptly (in diabetes and uremia). A continuous decrease in Stern-Volmer fluorescence quenching constants with the increase in temperature (from 25 to 37°C) and a higher value of bimolecular rate constant showed static mode of fluorescence quenching. The specific nature of LV binding with a non-carrier protein is confirmed by SPR. For this interaction, the enthalpy (ΔH) and the entropy (ΔS) changes were found to be 6.46 kcal mol^{-1} and 45 cal K^{-1} mol^{-1} respectively along with negative signs of free energy change (ΔG) which suggest that hydrophobic interaction is the predominant intermolecular forces stabilizing the complex. The binding results suggested that the main responsible driving forces are H-bonds, hydrophobic and electrostatic interactions which are consistent with docking results. We have postulated that both the uremic and diabetic patients are at higher risk regarding the bioavailability of required amount of drug. We have also investigated the effect of LV on IgG conformation accompanying the change in protein stability as well as the amyloidogenic propensity of IgG in the presence and absence of normal and increased level of the blood solutes. The plausible effect of LV on IgG was to enhance the stability and somehow to diminish the aggregation of IgG molecules.

Keywords: Disease Mimetic Conditions, Bioinformatics, Non-Carrier Protein, Protein Aggregation, Protein-Ligand Interaction, Protein Stability, Thermodynamics.