

Human serum albumin (HSA) is the most abundant protein in the systemic circulation, with comprising 60% in plasma. This protein can play a dominant role on the drug disposition and the efficiency. The pharmacokinetics and pharmacodynamics of any drug depend, largely, on the interaction with HSA. Hence, in present study, the interaction between new designed Pd(II)-complexes, 2,2'-bipyridin ethylglycinato Pd(II) nitrate, 2,2'-bipyridin butylglycinato Pd(II) nitrate and 2,2'-bipyridin octylglycinato Pd(II) nitrate, anti-tumor components, with human serum albumin as a carrier protein, were studied at different temperatures of 27 and 37° C by fluorescence spectroscopy, circular dichroism (CD) spectrophotometry and differential scanning calorimetry (DSC) techniques. Results showed Pd(II)-complexes have strong ability to quench the intrinsic fluorescence of HSA through static quenching procedure. The binding parameters were evaluated by fluorescence quenching method. From enthalpy and entropy of binding, it can be concluded that hydrophobic interactions may play an important role in the binding of Pd(II)-complexes to HSA. Far-UV-CD results represented that Pd(II)-complexes induced decreasing in content of α helical structure of protein.

From above results, it can be concluded that the blood carrier protein of HSA could bind and transfer of these new anti-cancer drugs, but the stability of the protein decreased upon the interaction with this complexes.

Keywords: Pd(II) complex, DSC, HAS, Quenching, Thermodynamic parameters.

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Molecular Aspects of Calcium Binding in NCAD12 Nagamani Vunnam.

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Cadherins are calcium dependent, transmembrane proteins, which mediate cell-cell adhesion through homophilic interactions. They play a fundamental role in embryogenesis and tissue morphogenesis. The primary structure of cadherins comprises five extracellular domains, a transmembrane segment, and a conserved C-terminal cytoplasmic region, which interacts with the cytoskeleton through catenins. It has been known that cadherins require calcium binding at the interfaces of the extracellular domains for cell adhesion and for protection from proteases. Recent literature has proposed that binding of three calcium ions (Ca1, Ca2 and Ca3) at the domain interfaces causes a conformational change, a "closed" to "open" transition, in the extracellular domain 1, which leads to cell-cell adhesion. However, to date, no solution studies have demonstrated the conformational change upon calcium binding. Despite the important function of calcium binding in cadherin mediated cell adhesion, the characteristics of calcium binding are still unclear. In order to determine the molecular aspects of binding of Ca1, Ca2 and Ca3 in cell adhesion, a comprehensive study of the calcium binding properties is required. Our work focuses on the first two extracellular domains of neural cadherin (NCAD12). We mutated an essential residue D134 for the binding of Ca3 in NCAD12 wild type. We used Circular dichroism and Fluorescence spectroscopy in order to obtain global and local information of conformational change on calcium binding to NCAD12 wild type and D134A. These studies were repeated with a mutant, D136N, designed to probe the cooperativity between calcium binding sites. Spectroscopic and proteolytic footprinting studies of NCAD12 wild type, D134A and D136N show that these proteins behave differently in presence of calcium. The mutations significantly lowered the stability and increased protease sensitivity in the presence of calcium. These preliminary results imply that binding of Ca3 is a critical step in conformational change.

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Effect of Osmolytes on Protein Stability and Folding

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Cells exhibit certain cellular coping mechanisms when faced with osmotic stresses by importing or producing organic compounds called osmolytes which aid in osmotic regulation. Proline is an example of such a compound. The primary function of these compounds is to combat the effects of dehydration in the cell. Stabilization of proteins, which are particularly susceptible to osmotic stresses, is of key importance to the cell's health. Osmolytes have been shown to directly impact the stability and solubility of proteins, and certain organic osmolytes also exhibit the function of aiding in protein folding and refolding and in preventing protein aggregation. The mode by which osmolytes aid in assuring protein stability is believed to be a solvent-oriented process by which protein folding is facilitated by the preferential ordering of solvent molecules, but the exact mechanism of stabilization remains elusive. In this research project, we characterized the supramolecular structure of proline at high concentration in solution using multi-dimensional NMR spectroscopy and dynamic light scattering. The molecular mechanism underlying the stabilizing effect of proline on a protein is studied using thermal denaturation monitored by

steady-state fluorescence. Results from the thermal denaturation studies indicate that the T_m (the temperature at which 50% of the molecules are in the native state) of the protein increases in the presence of increasing concentrations of proline by about 20 °C, suggesting that thermodynamic stability of the protein is enhanced upon binding to proline. Stability studies using several other osmolytes like TMAO, glycerol, 4-hydroxy proline, and betaine show that proline is the osmolyte which stabilizes the protein to the largest extent. Two-dimensional HSQC NMR experiments were used to reveal the binding sites of proline on FGF-1. The results of this study provide useful insights on the molecular mechanism of proline.

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Strategy And Biophysical Tools For Developing Modern Diagnostic Assays

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Neutrophil Gelatinase-Associated Lipocalin (NGAL) is a 20 kDa monomeric protein secreted by activated human neutrophils. NGAL is believed to bind small lipophilic substances such as bacteria-derived lipopolysaccharides, siderophores, formylpeptides, and may function as a modulator of inflammation. Clinical studies have shown NGAL can serve as an early diagnostic marker for acute kidney injury (AKI). We have developed a sensitive immunoassay to measure NGAL level in patient urine. During the course of assay development, we employed a variety of biophysical methods to characterize the physical and binding properties of several anti-NGAL antibody candidates and a recombinant NGAL protein. CD spectroscopy was used to study the structure and stability of NGAL; Förster Resonance Energy Transfer (FRET) was used to determine the binding affinity of NGAL toward anti-NGAL mAbs; Dual-Color Fluorescence Cross-Correlation Spectroscopy (DC-FCCS) was used to compare the capability of two antibodies forming a sandwich with NGAL; NMR was used to identify epitopic regions of NGAL for each antibody candidate. Two antibodies, which have the highest binding affinity toward NGAL and recognize distinct discontinuous epitope regions on NGAL, were chosen as the reagents for a sandwich type microparticle based immunoassay. This work demonstrates a modern strategy and biophysical tools, which are necessary to build a sensitive and robust diagnostic assay.

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Recognition and Discrimination of Gases by the Signal Transducers HemAT

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The recognition and discrimination of diatomic gases that affect biological processes through the perturbation of protein function is a formidable challenge towards our understanding of the molecular mechanisms involved in signal transduction by heme-based sensor proteins (1). There is a general consensus now that internal cavities in proteins are involved in controlling the dynamics and reactivity of the protein reactions with small ligands, such as O₂, CO, and NO usually through ligand accommodation. These cavities serve as a local storehouse for ligands near the active site, thereby increasing the effective concentration of the ligand many times. In addition to having functional role in ligand binding they are also important for determining relative affinities. In the heme-based oxygen sensors such as HemAT the recognition and discrimination of the specific gas leads to either activation or inhibition of a regulated domain. The dynamic coupling between two distinct binding sites as the underlying allosteric mechanism for gas-recognition/ discrimination that triggers a conformational switch for signaling by the oxygen sensor protein HemAT will be presented (2-3).

References

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Fluorescent-tagged Kinases: A New Assay System For Detecting And Screening For Allosteric Kinase Inhibitors

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Targeting the ATP binding pocket has been the most common strategy for inhibiting kinases. Recently, a less-conserved allosteric site was identified in some kinases (i.e. Abl, EGFR and p38 α), opening up patent space for novel drug scaffolds which are more kinase specific. Kinases are typically in the active conformation (DFG-in) with the activation loop open and extended, allowing ATP and substrates to bind. Alternatively, the adjacent allosteric site is available only in the inactive conformation (DFG-out) in which the activation