Abstract No.269

Understanding the Role of Conserved Residues in Folding and Stability of Cytochromes-C

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There are more than 287sequences of cytochromes-c (cyts-c) known (http://pir.georgetown.edu/). 37 of them are of mammalian origin. All the mammalian mitochondrial cyts-c are ~104-residue long single polypeptide chain in which the heme is covalently linked through Cys14 and Cys17. A sequence alignment of cyts-c from all kingdoms with that of the horse cyt-c led to the conclusion that there are only 5 positions (Cys17, Gly29, Gly41, Leu68, Pro71) which are conserved throughout the kingdoms. Interestingly, all mammalian cyts-c have Leu at position 94 that, baring 13 species which have either Ile or Val or Phe at this position, is conserved throughout the kingdom. Such conserved residues are sometimes also called as 'key residues'. We have been interested in understanding the role of this key residue (Leu94) of the mammalian cyts-c in protein folding and stability. We present here the results of mutation of Leu by Gly at the position 94 of the horse cyt-c whose 3-D structure is known. In silico study shows that this mutation (Leu94Gly) will lead to removal of 10 van der Waals interactions. This study therefore suggests that the mutant Leu94Gly will be less stable than the wild-type protein. We prepared the mutant Leu94Gly by expressing it in E. collie, and we carried out in vitro studies of structural and thermodynamic characterizations. Our main findings from the in vitro studies are: (a) the mutant protein exists as molten globule under the native condition of the wild-type protein, (b) a weak salt denaturant induces a biphasic transition in which the equilibrium intermediate has structural characteristics of the pre-molten globule, (c) the mutant leu94Gly is unfolded at pH 2, and titration of the this unfolded protein with NaCl also induces a pre-molten globule state, and (d) Tm (thermodynamic stability) and ΔGDº (thermodynamic stability) of the mutant are respectively, 29 ºC and 5 kcal mole-1 less than those of the wild-type protein. A comparison these results with those of the wild-type protein led us to conclude that the conserved residue Leu94 in the wild-type protein is required for proper protein folding and stability. The mechanism of folding of cyts-c may be described by the process, Unfolded state ↔ Pre-molten globule state ↔ Molten globule state ↔ Native folded state under physiological condition.

Keywords: Conserved Residue in Protein, Folding, Protein, Cytochromes-C.

Abstract No.270

A Hydrogen Peroxide Biosensor Using Functionalized-Carbon Nanotubes and Clay

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Among the different analytical devices, biosensors play an important role due to some generally claimed advantages: intrinsic specificity, low costs and fast analyses. . In this study, we demonstrate the biosensor based on deposition of carbon nanotubes (CNTs) on clay minerals, and the development of biosensors based on COOH–MWCNT/Clay/horse radish peroxidase (HRP) for the detection of hydrogen peroxide (H_2O_2). The mixed hybrid film of CNT/Clay/HRP was coated on the glassy carbon (GC) electrode. This film exhibited a detection limit of 5.0×10^{-5} M towards H_2O_2 with a sensitivity of 280 μA mM– 1. A higher sensitivity and more stability of enzyme are observed with increasing H2O2 content in the composite matrix film. Consequently, the CNT/Clay medium can probably be a useful electrode for the development of sensors due to its high sensitivity and applicability.

Keywords: Carbon Annotates, Clay, Biosensor, Horse Radish Peroxidase, COOH Functionalized -Carbon Nanotubes, Hydrogen Peroxide.

Abstract No.271

Spectrophotometery Studies on the Interaction of Au(III)(Phend)Cl3 new Complex with Calf Thymus DNA

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Interaction between calf thymus DNA and $(Phend)Cl_3$-Au(III) new complex in physiological buffer (pH=7.2) was investigated using UV-Vis

spectrophotometery, fluorescence spectrophotometry, cyclic voltammetry and viscosity measurements. The decrease of the absorption spectra of (Phend)Cl_3-Au(III) complex were observed in the presence of DNA, and the fluorescence intensity of (Phend)Cl_3-Au(III) was decreased with the addition of DNA. The relative viscosity of DNA increased with the addition of (Phend)Cl_3-Au(III) complex. The calculated binding constants of (Phend)Cl3-Au(III) complex with DNA at 250 nm and 293 K were 5×10^6 M^{-1}. Cyclic voltamograms due to cyclic voltammetry showed that, cathodic peaks shifted to positive potential that indicates intercalation interaction between (Phend)Cl_3-Au(III) complex and CT-DNA. All these results indicated that (Phend)Cl_3-Au(III) complex can bind to DNA and the major binding mode is intercalative binding.

Keywords: Calf-thymus DNA, (Phend)Cl3-Au(III), Interaction, Spectrophotometry, Intercalation.

Abstract No.272

Hyperthermia Effects on IC-21 Macrophage Like Cell Line To Assay Activity and Expression of Inducible Nitric Oxide Synthase (iNOS) Enzyme

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Nitric oxide (No.) is a uniquely diffusible and reactive molecular messenger in vascular and immune systems, motivated researches for evaluating its biosynthesis by the macrophages. As macrophages are often called to function at times of elevated ambient temperature (e.g. during local inflammation or systemic fever), it is possible that their production of critical effector molecules such as nitric oxide ion or nitric oxide synthase (iNOS) , is sensitive to physiological changes in temperature. To test this possibility, the threshold requirement for production of No. and iNOS in macrophage like cell line of IC-21 under normothermic conditions (37 °C) and following mild hyperthermia (40, 42 and 44 °C) were compared. Temperature gradient showed considerable increases of No. concentration at 40 and 42 °C and decrease at 44 °C (24 h incubation). Further, if IFN-• and lipopolysaccharide (LPS) were given before thermal exposure, a substantial increase in No. and iNOS was observed (highest at 42 °C after 24 h incubation) over that seen using cells kept exposed to hyperthermia alone or that of at normthermic conditions (37 °C). As RT-PCR data have revealed the thermal regulation of iNOS expression is not entirely at the transcriptional level, suggesting possible points of post-transcriptioal thermal sensitivity, so our direct application of hyperthermia on macrophages without any treatment supports this hypothesis. The data in this study reveal the potential of mild hyperthermia (elevated physiological temperature) to increase No. production and iNOS synthesis in using peritoneal type macrophage like cell line. These may support the concept that altering the thermal micro environmental would be an important means by which the host can affect the macrophage responses.

Keywords: Nitric Oxide, Nitric Oxide Synthase (iNOS), Macrophage, IC-21 Cell Line and Hyperthermia.

Abstract No.273

Detection of Fibrillar Aggregates and Inhibition of Amyloid-Mediated Peroxidase Activity Using the Novel Benzothiazole- and Benzofuranone-Derivatives Fluorescence Compounds

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Alzheimer's disease is characterized by the presence of amyloid deposition. Thioflavin T (ThT) has been one of the molecules of choice to attempt the detection of amyloid deposits, however, it has been reported that ThT was unable to cross blood–brain barrier (BBB). Since compounds without a permanent positive charge are mainly capable of crossing the blood–brain barrier, there is a strong motivation to develop suitable compounds for in vitro fibril quantification as well as for in vivo amyloid imaging. Moreover, oxidative stress has frequently been reported to play a critical role in the onset/progression of some neurodegenerative disorders. In this study, we synthesized and employed benzothiazole and benzofuranone derivatives (including neutral ThT analogues), both as fluorescent probes to quantitatively determine the amyloid fibrils made of chymotrypsin (and crystalline) and as potential inhibitors for peroxidase activity. Analyses of the in vitro binding studies indicated that compounds 2 and 4 bind to the amyloid structures successfully while compounds 1 and 3 showed a low affinity in binding to fibrils. Furthermore, compounds 3 and 4 were