

Abstract No.269

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

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There are more than 287 sequences 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, barring 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. coli*, 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) T_m (thermodynamic stability) and ΔG_D^0 (thermodynamic stability) of the mutant are respectively, 29 °C 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 \leftrightarrow Pre-molten globule state \leftrightarrow Molten globule state \leftrightarrow 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 (H₂O₂). 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 H₂O₂ with a sensitivity of 280 μ A 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.

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Spectrophotometry 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)Cl₃-Au(III) new complex in physiological buffer (pH=7.2) was investigated using UV-Vis