Name of the Scholar	**Shah Ubaid Ullah**
Name of the Supervisor	**Professor Faizan Ahmad, Ph.D., F.N.A.Sc., F.N.A**
Name of the Co-Supervisor	**Dr. Janendra K Batra, Ph.D., F.N.A.Sc., F.A. Sc. (Deputy Director/Staff Scientist VIII, National Institute of Immunology(NII), New Delhi - 67)**
Centre	**Centre for Interdisciplinary Research In Basic Sciences (CIRBSc.)**
Title of the Thesis	**Role of Extra N-Terminal Residues in the Folding and Stability of the Yeast Cytochrome *c***

ABSTRACT

A direct comparison of sequences of yeast iso-1-cyt *c* and horse cyt *c* shows that the two sequences are almost 60% identical and their tertiary structures are remarkably similar. However, the horse cytochrome *c* is one of the most stable proteins, while the yeast cytochrome is the least stable one. This large difference in stability is difficult to explain as the yeast protein contains additional five residues at N-terminal as compared to its higher eukaryotic relatives. Here we have made an attempt to understand the effect of deletion of these extra five N-terminal residues on stability and folding of the yeast cytochrome *c*.

In silico studies suggested that a number of interactions are lost upon deletion of the extra N-terminal residues, thus affecting the stability of the protein. We successfully cloned, expressed and purified WT and Δ(-5-1) yeast iso-1-cyt *c* lacking extra five N-terminal extension. Conformational studies carried out by CD, absorption spectroscopy, fluorescence and DLS confirmed that the deletion of extra five N-terminal residues does not affect the conformation of the protein. To establish the role of the deletion on the stability of the protein, we carried out thermal denaturation of the native proteins by monitoring changes in spectral properties ($[\theta]_{222}$ and $\Delta\varepsilon_{405}$ vs temperature). Thermal denaturation studies showed that the T_m (midpoint of thermal denaturation) of Δ(-5/-1) iso-1- cyt *c* is ~4.5 ^oC less than WT protein, and ΔG_D^o is 0.90 kcal mol^{-1} less than WT. DSC studies also confirmed the results

obtained from spectroscopic studies and provided direct evidence that heat-induced denaturation of both the proteins follows a two-state mechanism.

The equilibrium denaturation of WT and Δ(-5/-1) iso-1-cyt *c* by GdmCl at pH 6.0 and 25 ^{o}C was monitored by following changes in $\Delta\varepsilon_{405}$, $[\theta]_{405}$, $[\theta]_{222}$ and $[\theta]_{416}$. Δ(-5/-1) iso-1-cyt *c* was found to be less stable than WT. Further, the normalized transition curves from different optical probes are not coincident in both the proteins, suggesting GdmCl-induced denaturation is not a two-state process. On other hand, the normalized urea-induced denaturation curves of different physical properties were coincident in both WT and Δ(-5-/1) yeast iso-1-cyt *c*, suggesting that urea-induced denaturation is a two-state process.

The denaturation of WT and Δ(-5/-1) iso-1-cyt *c* induced by LiCl at pH 6.0 and 25 ^{o}C monitored by $\Delta\varepsilon_{409}$, $\Delta[\theta]_{405}$ and $[\theta]_{222}$ shows a biphasic transition (N ↔ X ↔ D). Thus, there exists a thermodynamically stable intermediate state, X on the folding/unfolding pathway of the protein. Characterization of this X state by far and near-UV CD, intrinsic and ANS binding fluorescence, and DLS led us to conclude that X state has all the common characteristics of a premolten globule (PMG) at pH 6.0 and 25 ^{o}C. A-state of WT and Δ(-5/-1) iso-1-cyt *c* was also formed by addition of 0.33 M Na_2SO_4 to acid denatured proteins (pH 2.1). This state on characterization by far-UV and near-UV CD, ANS binding and DLS measurements showed resemblance to the molten globule state (MG). On the basis of the results obtained in this study it is possible to conclude that; the thermodynamically stable intermediate state on the reversible folding/unfolding pathway of yeast iso-1- cyt *c* and its deletant Δ(-5/-1) at pH 6.0 and 25 °C has all the common characteristics of PMG state, and the extra five N-terminal residues have no effect on the folding/unfolding pathway as both proteins follow N ↔ X ↔ D transition.