SUMMARY

Comparison of sequences of yeast iso-1-cyt-*c* and horse cyt-*c* shows that the two sequences are almost 60% identical. X-ray diffraction studies of both proteins showed that their tertiary folds are well conserved. However, among the cytochromes-*c*, the horse cytochrome-*c* is one of the most stable proteins, while the yeast cytochrome-*c* 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. Hence we have made an attempt to understand the effect of deletion of these extra N-terminal residues (-1 and -2) on the 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 deletants Δ(-5/-2) and Δ(-5/-3) yeast iso-1-cyt-*c* lacking 4 and 3 residues respectively out of extra five N-terminal extension (deletants, -1 and -2 denoted as Δ(-5/-2) and Δ(-5/-3), respectively). 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 $\Delta\varepsilon_{405}$) as function of temperature. Thermal denaturation studies showed that the T_m (midpoint of thermal denaturation) values of Δ(-5/-2) and Δ(-5/-3) iso-1- cyt *c* are ~3.1 and 1.5 ^oC, respectively, less than that of WT protein, and ΔG_D^0 is 0.91 and 0.49 kcal mol^{-1} less than WT protein. DSC studies also confirmed the results obtained from spectroscopic studies and provided direct

evidence that heat-induced denaturation of WT protein and its deletants follows a two-state mechanism.

The equilibrium denaturation of WT protein and its deletants by GdmCl at pH 6.0 and 25 ^{o}C was monitored by following changes in $\Delta\varepsilon_{405}$, $[\theta]_{405}$, $[\theta]_{222}$ and $[\theta]_{416}$. Both deletants was found to be less stable than WT protein. 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 WT protein and its deletants, suggesting that urea-induced denaturation is a two-state process.

The denaturation of WT and deletants induced by LiCl at pH 6.0 and 25 ^{o}C monitored by $\Delta\varepsilon_{409}$, $[\theta]_{405}$ 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 deletants was also formed by addition of 0.33 M Na2SO4 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 (i) that the thermodynamically stable intermediate state on the reversible folding/unfolding pathway of WT yeast iso-1- cyt c and its deletants Δ(-5/-2), (ii) that Δ(-5/-3) at pH 6.0 and 25 °C has all the common characteristics of PMG state, and (iii) that the extra five N-terminal residues have no effect on the folding/unfolding pathway.