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Title of Thesis - Role of Extra N-terminal Residues (-4 & -3) in the Folding and Stability of the Yeast Cytochrome- c

Abstract

Cyt-*c* is a small (104 ±10 amino acid residues), globular, electron transport protein which is present in almost all living forms. More than 280 sequences have been reported but its tertiary structure and function is highly conserved throughout the evolution. Though cyt-*c* is highly conserved in terms of function and structure, the difference in amino acid sequence is noteworthy. When amino acid sequences of two most well studied cytochromes i.e., horse cyt-*c* (h-cyt-*c*) and yeast iso-1-cytochrome-*c* (y-cyt-*c*) are aligned, it is observed that they share 61% sequence identity. A significant difference in h-cyt-*c* and y-cyt-*c* is the presence of an N-terminal extension of five residues in y-cyt-*c*. These amino acid residues, Thr-Glu-Phe-Lys-Ala (TEFKA), are generally referred to as residues -5 to -1, when using the eukaryotic numbering system for cyts-*c*. The opinion about the contribution of these residues in structure, function and stability varies. While the X-ray structure shows this N-terminal extension to be in extended conformation, NMR studies show an inherent structure for this extension in solution. Our research work here, aims to investigate the role of these extra N-terminal residues, in particular those at position -5 and -4, in folding and stability of the WT y-cyt-*c*. We sequentially deleted the residues at position -5 and -4 to respectively form deletants Δ(-5/-5) and Δ(-5/-4) and studied the effects of these deletions on the folding and stability of the protein. We started with *in silico* analysis of the wild-type (WT) cyt-*c* and deletants to predict the effect of deletion on the structure and stability of the protein. We carried out structural characterization of each protein using circular dichroism (CD), absorption and fluorescence spectroscopy, and dynamic light scattering (DLS) to monitor if deletion of extra N-terminal residues caused any alteration in the

native and denatured state of the protein. We also estimated the thermodynamic stability of each protein using different denaturants (heat, urea and GdmCl). We carried out LiCl-induced denaturation of each protein to monitor the effect of deletion on the folding mechanism of the protein. Important findings of the thesis

1. Deletion of extra N-terminal residues do not cause any alteration in either secondary or tertiary structure of the native states of WT cyt-c and its deletants, Δ(-5/-5) and Δ(-5/-4). Denatured states induced by different denaturants of each protein also remained unperturbed due to deletion. Thus, suggesting that these extra N-terminal residues at position -5 and -4 do not contribute to the structure of the protein.
2. ΔG^{o} obtained for each protein from their respective transition curves induced by heat, GdmCl and urea showed the order of stability of proteins to be Δ(-5/-4) > WT > Δ(-5/-5). In silico studies showed that the removal of Thr(-5) from the WT protein is accompanied with the removal of two H bonds associated with Thr(-5) and that leads to destabilization of Δ(-5/-5). On the other hand, extensive interaction of new N-terminal, Phe(-3), with the rest of the molecule, after the removal of Glu(-4), causes stabilization of the deletant Δ(-5/-4). Thus, it can be concluded that Thr(-5) and Glu(-4) contribute to the stability of the protein.
3. LiCl-induced denaturation of all three proteins showed a biphasic transition, N state ↔ X (intermediate) state ↔ D state. Structural characterization of X state of each protein suggested that X state have structural characteristics of PMG (pre-molten globule) state. The deletion of extra N-terminal residues have no effect on the stability of PMG state. So, it can be concluded that extra N-terminal residues do not have any role in the folding of the protein.