SPECTROSCOPIC AND THERMODYNAMIC STUDIES OF THE NON-NATIVE CONFORMATIONS OF HORSE CYTOCHROME C AND THEIR INTERACTION WITH NUCLEOTIDES

BY

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CERTIFICATE

This is to certify that the thesis entitled, **"Spectroscopic and thermodynamic studies of the non-native conformations of horse cytochrome c and their interaction with nucleotides"**, being submitted by **Ms. Unnati Ahluwalia** to the Indian Institute of Technology Delhi for the award of the degree of Doctor of Philosophy in Chemistry is a record of bonafide research work carried out by her. **Ms. Unnati Ahluwalia** has worked under my guidance and supervision and has fulfilled the requirements for the submission of this thesis, which to my knowledge has reached the requisite standard.

The results contained in this dissertation have not been submitted in part or full to any other University or Institute for the award of any degree or diploma.

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If I have seen a little further, it is by standing on the shoulders of Giants -- *Isaac Newton*

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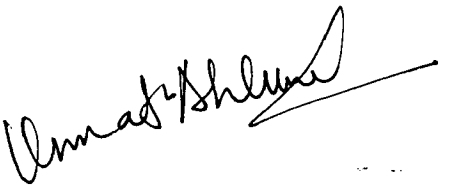

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ABSTRACT

Structural information on the intermediates/non-native conformation in protein folding/unfolding pathway is critical for the understanding of the molecular basis of protein folding. Cytochrome c (cyt c) has long been serving as a model protein for developing new concepts and approaches in protein folding. Cyt c unfolds and refolds by a pathway consisting of same number of intermediates via step-by-step folding of cooperative units. Structural and thermodynamic characterization of these intermediates helps in understanding of the folding mechanism of the proteins. Non-native conformations of proteins are also considered to play an important role in many physiological events. Intermediates/altered forms of cytochrome c (cyt c) are particularly important considering their participation in electron transfer and programmed cell death (apoptosis). Understanding such a complex system requires a thorough investigation of the entire biophysical aspect of these non-native conformations such as their structure, stability and thermodynamics of interaction. The thesis entitled '**SPECTROSCOPIC AND THERMODYNAMIC STUDIES OF THE NON NATIVE CONFORMATIONS OF HORSE CYTOCHROME C AND THEIR INTERACTION WITH NUCLEOTIDES**' is concerned with the characterization of the non-native conformations of cyt c in different environments. Another important aspect of this thesis is the study of interaction of such non-native conformations with nucleotides since these interactions are the basis of many cellular events.

The thesis has been divided into seven chapters. Chapter 1 provides a brief introduction to models of folding and a brief review of different types of non-native conformations described in literature and their significance in metabolic pathways. It also

describes the various tools available in literature to differentiate between different types of non-native conformation of proteins. The chapter also provides an introduction to cytochrome c and a detailed review of progress made in understanding the various non-native conformations of cyt c and their biological significance in processes like electron transfer and apoptosis. Chapter 2 titled "Materials and Methodologies" is about protein and chemical procurement, purification as well as techniques and methodologies used in the investigation. The chapter also describes the procedures for analyzing the data. Chapter 3 (**Sugar and phosphate induced non-native conformations of acid denatured cytochrome c**) discusses the effect of sucrose, a sugar, and phosphate anion on the induction of intermediate conformation in acid denatured cyt c. Based on the data obtained, similarity and subtle differences among the sucrose and phosphate induced conformations were discussed. Chapter 4 (**Interaction of nucleotides with acid denatured cytochrome c and structural and thermal characterization of resulting non-native conformations**) presents the investigation of the effect of the adenosine nucleotides (ATP/ADP/AMP) on the acid denatured cyt c. The chapter also discuss the effect of other nucleotides on the acid-denatured cyt c. Effect of Nucleotides on cyt c was discussed in terms of coupled binding-folding equilibria. Chapter 5 (**The non-native conformations of cytochrome c in sodium dodecyl sulfate and their modulation by ATP**) presents the investigation of the effect of SDS, an anionic detergent, on the conformation of the cyt c. The effect of nucleotides on the conformation of SDS bound cyt c was also investigated. Docking of cyt c with SDS and ATP/GTP was carried out to characterize the putative binding sites of these molecules on cyt c. Information on the docking sites, thus obtained, was used to explain the experimental data obtained from the

binding of cyt c with SDS and the effect of ATP/GTP on the SDS bound cyt c. Chapter 6 **(The non-native conformations of cytochrome c in Cardiolipin/Oleic acid and their modulation by ATP)** deals with the characterization of non-native conformation of cyt c obtained by interaction of native cyt c with lipid cardiolipin/oleic acid and its comparison with other non-native conformations of cyt c. Based on the structural data, the unfolding of the cyt c was explained in terms of various domains. The effect of nucleotides on the cardiolipin/oleic acid induced conformation was also discussed. Information on the docking sites obtained from docking of cyt c and cardiolipin, thus obtained, was used to explain the experimental data obtained from the binding of cyt c with cardiolipin/oleic acid and the effect of ATP on the cardiolipin/oleic acid bound cyt c. **Chapter 7 (Summary and Future Perspectives)** contains salient features of this work. Taken together, the findings/observation of this thesis will help develop a much needed fundamental and molecular-level view of the non-native conformations of the cyt c. In brief, it is concluded that the different non-native conformations of protein may be obtained depending on the protein environment. The effect of nucleotides is different on the different non-native conformation of the protein.

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