PREFACE

Proteins are biological workhorses that carry out vital functions inside the cell. To carry out their tasks, proteins must fold into complex three-dimensional structures, but what tells a protein which shape it should be or how it achieves and what forces stabilize the native conformation is still a puzzle for scientists. One of the most important results in understanding the process of protein folding was a thought-provoking experiment that was carried out by Christian Anfinsen and colleagues with RNase in 1960. On the basis of this result, Anfinsen concluded that the amino acid sequence determines the shape of protein, a finding for which Anfinsen received Nobel Prize in chemistry in 1972. Although, now it is possible to deduce the primary structure of a protein from genes sequence, but its native structure cannot be determined. It can only be possible by complex experimental analyses and, at present, this information is only known for few proteins. Additionally, the folding of a protein is not a chemical reaction, with a bond breaking here and a new one forming there. It is more like the weaving of an intertwined molecular pattern, the stability of which is defined by astonishing number of interactions. Mutual shuffling of these interactions involved in the regulation of functions and structural dynamics of the proteins.

Recent studies of protein folding and stability have been focused on small proteins and domains of larger multidomain proteins. This is not only because of simplicity of the mechanism and reversibility of folding reaction, but also because of probability that these reactions reflect earlier events in the folding process of larger proteins. On the other hand, direct characterization of folding and stability behavior with the entire molecule is necessary to complete understanding of the unfolding mechanism of larger proteins.

The work presented in this thesis is mainly concerned with the characterization of structural, folding, and stability properties of a two-domain protein, Ferredoxin NADP+ reductase from Toxoplasma gondii. We have divided the entire study into various chapters. Chapter 1 gives a brief introduction of various types of noncovalent forces involved in maintenance of structure and stability of proteins along with an overview of the process of protein folding. Chapter 2 contains the details of FNR enzyme, have chosen as a model in the study. This chapter also introduces the structural and functional properties of FNR from various sources and their importance in physiological processes. Chapter 3 contains materials used in the study and details of the experimental procedures, in the manner as they were carried out in lab. Chapter 4 deals with the effect of modulation of ionic interactions by alterations in environmental pH on the domain transitions of TgFNR. Chapter 5 deals with the unusual behavior of Hofmeister series salts on folding and stability of TgFNR and folding/unfolding studies on TgFNR is detailed in Chapter 6. pH dependent ANS binding studies shows that ANS itself can able to unfold the TgFNR at low pH is described in chapter 7. Bibliography in alphabetical order has been put at the end of thesis.