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Vibhor Mishra
PREFACE

To be biologically active, proteins must adopt specific folded three-dimensional tertiary structures. Yet the genetic information for the protein specifies only the primary structure, the linear sequence of amino acids in the polypeptide backbone. How this occurs has come to be known as ‘the protein folding problem’. 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 is still cannot be determined. It can only be possible by complex experimental analyses. 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 for a complete understanding of the folding/unfolding mechanism of larger proteins.

The research work presented in the thesis reports structural, functional and stability characteristics of the first two enzymes phosphoserine aminotransferase (EhPSAT) and D-phosphoglycerate dehydrogenase (EhPGDH) of the phosphorylated serine biosynthesis pathway from enteric human parasite Entamoeba histolytica. The thesis primarily has been divided into following 7 chapters. Chapter 1 gives an overview of the protein folding phenomenon. Chapter 2 is an account of structural and functional features of aminotransferases and dehydrogenases. The chapter also provides a glimpse of earlier works on phosphoserine aminotransferase and D-phosphoglycerate dehydrogenase. Chapter 3 contains Insights into the structure-function relationship of EhPSAT. Chapter 4 deals with the effect of cofactor and domains on stability and subunit assembly of EhPSAT. Chapter 5 defines the role conserved active site Trp-101 in activity and stability of EhPSAT. Chapter 6 describes the role of Glu-108 in subunit assembly and dimer stability of EhPGDH. Finally, Chapter 7 is an account of novel protein-protein interactions between EhPGDH and EhPSAT.