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Of the several macromolecules that constitute biological systems, proteins are known to perform an awesome repertoire of functions that mediate the numerous biological processes governing life. Therefore, an understanding of the structure, function and interactions of these cellular moieties is crucial towards gaining insights into life processes. However, the often scarce levels of subject protein molecules in the cellular milieu hinder such investigations. Although formation of the three-dimensional structure of protein molecules from their primary sequences appear to be facilitated with great ease by the cellular machinery, accomplishment of the same in vitro appears to be a task of titanic magnitude. Thereby bringing forth the interest of understanding the fundamental rules underlying the generation of the folded structure from the natively unfolded polypeptide, or the "protein folding problem". Furthermore, the need to generate copious amounts of protein molecules for academic investigations as well as industrial applications and the attribution of several diseases to abberations of protein molecules further augment the importance of understanding the mechanistics of protein folding.

Fragment complementarity, in addition to other spectroscopic techniques such as stopped flow kinetics employing circular dichroism and fluorescence, hydrogen-deuterium exchange studies and genetic manipulation of proteins have been used to probe the folding problem. Given the fleeting disposition of the early events in the protein folding reaction and the existence of sub-structures such as domains and sub-domains; it is suggested that an understanding of the folding intermediates can be gained from studies pertaining to protein fragments. Investigations involving fragments provide information related to the local events in the folding reaction. Furthermore, possible initiation sites of the folding intermediates as well as structures stabilized as a consequence of local interactions can be detected (Dyson and Wright, 1993; Dobson, 1993; Fersht and Dill, 1994).

Ever since the pioneering studies of Richards and Vithyathil (1959) showing fragment complementation of RNaseA, similar studies have been conducted with numerous systems to decipher the fundamental principles of folding and binding events.
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(Taniuchi et al., 1967; Williams and Shoelson, 1993; de Prat Gay et al., 1994). Amongst other techniques such as genetic engineering and solid phase peptide synthesis, limited proteolysis has been extensively used to generate fragments of the protein under investigation. Importantly, most of the fragment complementary systems studied thus far involve only two fragments that bind together to form the native-like complex. Furthermore, limited proteolysis has been used as a tool to understand the structural aspects of protein molecules (Hubbard, 1998). Structural analysis of several proteolyzed molecules with spectroscopic methods have been employed to show the native-like conformation of the proteolyzed species as well as the non-covalent complexes generated as a result of fragment complementation (Williams and Shoelson, 1993, Musi et al., 2004). The importance of tertiary interactions is highlighted in proteolyzed structures wherein despite the severing of peptide bonds the molecule maintains a native-like conformation (Taniuchi et al., 1967).

Present understanding of the mechanism of protein folding is mainly derived from studies involving small domains or molecules due to the relatively lower complexity of such structures as well as the understanding that investigations related to these independently folding units can be applied to larger systems. However, the principles guiding protein folding appear to vary from one protein to the other, therefore augmenting the need to study different protein systems in order to construe the mechanism of their folding.

The present work is an enterprise towards further probing the mechanistics of protein folding using Glutathione-S-transferase, derived from pGEX-KG vector as the model system with fragment complementarity as the tool. Glutathione-S-transferase (GST) from Schistosoma japonicum has been noted to be a member of the important superfamily of enzymes involved in metabolism of electrophilic compounds (Armstrong, 1991). Considering the physiological role played by GST in cellular detoxification as well as the extensive usage of this molecule in expression of fusion proteins, it was of interest to understand the basis of folding and assembly of this molecule using fragment complementarity. Biochemical and biophysical investigations as well as stability studies performed on the proteolyzed molecule of GST with an objective towards understanding
the contribution of tertiary interactions in the formation of the folded molecule are delineated in Chapter 3. Interesting results pertaining to peptide bond religation, gained as an off-shoot of the earlier endeavours with complementarity studies of GST fragments are outlined in Chapter 4. Furthermore, Chapter 5 presents the crystal structure of the non-covalent complex of lysozyme. Additionally, biophysical characterization of the non-covalent complex of this molecule is demonstrated. Since proteases had been extensively employed in the present study, remarkable observations pertaining to the usage of protease inhibitors in proteolysis reactions with a wide variety of substrates has been detailed in Chapter 6. Chapter 1 presents a review of the earlier work performed using fragment complementarity as a tool to understand protein folding and explores the efforts put forth towards the \textit{in vitro} synthesis of the peptide bond. Furthermore, it recapitulates the forces, concepts and tools pertaining to protein folding. Chapter 2 details the materials and methods employed in the study. Finally, the summary and conclusions section towards the end brings out the essence of the dissertation.