Abstract

Carbohydrates are vital to life. In their naive form, they serve as a primary energy source for supporting life. However, in most cases carbohydrates do not exist as simple sugars in nature. Instead they occur as more complex molecular conjugates known as the glycans and Glycobiology is the study of the roles of these glycans in various biological events. The study of sugars is gaining importance in all strata of today's scientific world, be it cell biology, immunology or even neurobiology. These glycans do not exist at the cell surface or in the extracellular matrix as free-standing polymers. Rather, they are organized onto specific proteins to form protein-glycan conjugates. One such specific class of proteins is the lectins.

Lectins are ubiquitously distributed in nature and are recognized universally by their property of carbohydrate recognition. Much work has been done in the field of lectins to delineate the roles of different lectins in their recognition events and proper propagation of the protein folding machinery. However, these studies mainly concentrate on the animal lectins. Nonetheless, a good deal of structural work has been done on plant lectins and these plant lectins serve as excellent paradigms to work on the ever-exciting problem of protein folding.

This thesis primarily concentrates on a glycoprotein plant lectin, Soybean agglutinin. I have mainly addressed the issues of glycosylation and oligomerization on the stability of the protein.

Chapter 1 is a prelude to glycosylation in proteins and their functions in vivo. Glycosylation, particularly the N-linked kind, profoundly affects protein folding, oligomerization, and stability. The increased efficiency of folding of glycosylated proteins could be due to the chaperone like activity of glycans, which is observed even when the glycan is not attached to the protein. Covalently linked glycans could also facilitate oligomerization by mediating inter-subunit interactions in the protein or stabilizing the oligomer in other ways. Glycosylation is also seen to affect the rate of fibril formation in prion proteins, with N-glycans reducing the rate of fibril formation, and O-glycans affecting the rate either way based on factors such as position and orientation. While it is still not apparent if there is a common theme in the significance of glycosylation sites in multiply glycosylated proteins or in the conservation of glycans in a related family of glycoproteins, it is evident that glycosylation is a multifaceted post translational modification that incredibly affects proteins.

This is followed by a brief description on lectins, their structure, and functions with the emphasis on the various kinds of quaternary associations that they are involved in. Further I have also included a very concise discussion on the major forces involved in protein folding and stability.

Chapter 2 deals with the stability studies of two structurally similar legume lectins, Soybean agglutinin and Concanavalin A. The unfolding pathway of these two legume lectins are determined using GdnCl induced denaturation. Both displayed a reversible two-state unfolding mechanism. The analysis of isothermal denaturation data provided values for conformational stability of the two proteins. It was found that the DG of unfolding of SBA was much higher than Con A at all the

temperatures at which the experiments were done. Con A had a Tg 18°C lower than SBA. The higher conformational stability of SBA in comparison to Con A is largely due to substantial differences in their degrees of subunit interactions. Ionic interactions at the interface of the two proteins especially at the non-canonical interface seem to play a significant role in the observed stability differences between these two proteins. Further, SBA is a glycoprotein with a GlcNac2Man9 chain attached to Asn-75 of each subunit. The sugar chain in SBA spans the entire non-canonical interface of the protein. However, the entire glycan chain in SBA is not seen in its crystal structure. Only the two proximal GlcNac residues are visible in the crystal structure of the protein. I probed the interactions of these two sugar residues with the amino acid residues of the protein and determined that there were a number of inter-subunit interactions. These interactions further stabilize SBA with respect to Con A, which is not glycosylated.

In **Chapter 3** I have presented my investigations on the role of glycosylation in the stability of the glycoprotein SBA. I have used the non-glycosylated recombinant form of the protein rSBA expressed in E. coli cells for the stability studies and compared it with the native glycosylated form. The non-glycosylated form features a lower stability when compared to the glycosylated form. Further the unfolding pathways in the two are widely different. While the glycosylated form undergoes a simple two state unfolding, the non-glycosylated species unfolds via a compact monomeric intermediate which is not a molten globule. Representative isothermal and thermal denaturation profiles show that glycosylation accounts for a stabilization of nearly 9 kcal/mol of the tetramer, while the difference in Tm in the two forms is 26oC. To my knowledge this is the first report which shows that glycosylation imparts huge conformational stability to the macromolecule to which it is attached. As already mentioned, the density for the entire oligomannosidic chain is not observed in the crystal structure of the protein. Hence, I attempted to obtain a three-dimensional structure of the glycoprotein by combining the structure of the protein (from the protein data bank) and the glycan (from the www.glycosciences.de database). The glycoprotein thus obtained portrayed substantial inter-subunit glycan protein interactions (hydrogen bonding and hydrophobic) which is perhaps the major cause for the stability of the glycoprotein tetramer.

In **Chapter 4** I present the thermodynamic analysis on the monomer of SBA and estimate the stability of SBA monomer. Almost all legume lectins show oligomerization-deoligomerization with change in pH. However, pH dependent association/dissociation was not reported for SBA. As evident from size exclusion chromatographic and dynamic light scattering studies, I show that the monomeric form of the protein is existent at pH 1.9. The analyses of CD and fluorescence spectroscopy suggest that the monomer is well folded, and it has a very different characteristic feature when compared to the tetramer. The conformational stabilities of the tetramer and the monomer at the temperature of their maximum stabilities were widely different indicating that oligomerization contributes huge stability to the native molecule. Also, the Tg difference in the two forms of the protein is~40K, while the difference in DCp is only 1.6kcal/mol/K. This suggests

that the major hydrophobic core is present in the monomer and oligomerization involves mainly ionic interactions.

The thesis ends with a summary of all the findings in **Chapter 5.** I have tried to review the vital factors that contribute to the stability and quaternary integrity of the protein. Brief discussions involving the roles of oligomerization and glycosylation have been discussed. Further I have also tried to focus on the various unfolding thermodynamic parameters obtained by solution denaturation studies of glycosylated SBA, non-glycosylated SBA and the monomer of SBA and give a comparative account of all with the possible rationale for the variations.

Appendix A deals with one of the much-discussed issues of the recent times: the use of quantum dots in biology as imaging and targeting agents. The quantum dots surpass the already accessible existing organic fluorophores by virtue of fluorescence, resistance to photo bleaching and optical properties that facilitate the simultaneous imaging of multiple fluorophores.

One-pot synthesized neoglycoconjugates with reactive thiol group are introduced here for functionalization with carbohydrates for solubilization and stabilization of CdSe-ZnS quantum dots (QDs) in aqueous solution. Three different sizes of QDs with lactose, melibiose and maltotriose on them surface have been utilized, for the first time, for lectin detection through agglutination assay. Agglutination of Sugar-QDs (S-QDs) by three different lectins occurred through specific carbohydrate-lectin interactions and was studied extensively by monitoring the scattered light at 600 nm. This assay is very selective, which has been demonstrated by a more selective binding of Soybean agglutinin (SBA) with melibiose-QD as compared to lactose- QD, and specific de-agglutination caused by a-D-galactose while a-Dmannose did not show any effect. The detection sensitivity of the maltotriose-QD was tested with Concanavalin A (ConA), and as low as 100 nM of the lectin was detected using light scattering. Furthermore, the visual detection sensitivity of S-QDs has been enhanced by the emission properties of semiconductor QDs.