CONCLUSIONS

Taken together, our results suggest that

- The denaturation of SSA induced by urea and GdmCl at pH 7.4 and 25 °C monitored by $\Delta\varepsilon_{287}$, $[\theta]_{222}$ and F_{347} shows a biphasic transition (N↔X↔D).Thus there exists a thermodynamically stable intermediate state, X on the folding/unfolding pathway of the protein. Characterization of this X-state by far and near-UV CD, intrinsic and ANS binding flouresence, and DLS led us to conclude that X state has all the common characteristics of a molten globule (MG) at pH 7.4 and 25 °C.
- On the basis of urea- and GdmCl-induced denaturation studies of SSA protein, we may hypothesis that domain III may be responsible for formation of intermediate state during protein folding because of its lower stability and fewer interactions with other parts of the molecule (PDB ID 4LUF). It is possible that domain III unfold before the other two domains (I and II) of SSA protein. If we assume that N state ↔ X state transition in the absence of osmolytes corresponds to unfolding of domain III, it then means that osmolytes stabilize this domain, and all domains of SSA unfold cooperatively in their presence. It is interesting to note that osmolytes strengthen hydrophobic interactions due to preferential exclusion, and a more compact structured member of ensembles is favoured thermodynamically over less structured ensembles [446]. We thus conclude that osmolytes modulate the folding mechanism of SSA
- Since sorbitol is not used by kidney cells as the counteractant against the deleterious effect of urea, however, it may be used to maintain osmotic balance of the kidney cells under high osmotic stress conditions.
- The order of stabilization of kidney proteins by kidney osmolytes follows the order: glycine betaine > myo-inositol > sorbitol. There are strong evidence from literature that osmolytes stabilize the protein towards its native conformation by the preferential

exclusion of osmolytes from the protein surface. Methylamines such as glycine betaine are more osmophobic (i.e. unfavorable interaction of osmolyte with peptide back bone) as compared to myo-inositol and sorbitol and thus greater is the stabilization effect.

- Although still there are many unsolved questions pertaining to the mechanism of functioning of kidney osmolytes, however, the interesting outcome of this work is that addition of the co solutes stabilizes an intermediate state of an intact protein, which is of novel significance. The intermediate state is prone to aggregation due to sticky surfaces and exposed hydrophobic patches. The kidney osmolyte stabilzes the intermediate state into native like conformations. The practical outcome of this study is that kidney osmolytes provides a favorable environment for refolding of proteins from their aggregated states.

- A similar study was also planned for other kidney proteins, namely, CRT, H2AX and CRT-DNA which we had isolated and purified to homogeneity. Since urea-induced and GdmCl-induced denaturation of each protein in the absence and presence of each kidney osmolyte were irreversible, thermodynamic stability parameters of these proteins could not be measured.