FOLDING OF PROTEINS : EXPERIMENTAL STUDIES ON THE EFFECT OF SURFACTANTS AND THEORETICAL STUDIES ON SOME WILD AND MUTANT PROTEINS

by

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CERTIFICATE

This is to certify that the thesis entitled **"FOLDING OF PROTEINS: EXPERIMENTAL STUDIES ON THE EFFECT OF SURFACTANTS AND THEORETICAL STUDIES ON SOME WILD AND MUTANT PROTEINS"** being submitted by Mr. Shashank Deep to the Indian Institute of Technology, Delhi for the award of degree of Doctor of Philosophy in Chemistry is a record of bonafide research work carried out by him. Mr. Shashank Deep has worked under my guidance and supervision, and has fulfilled the requirements for the submission of this thesis, which to my knowledge, has reached requisite standard.

The results contained in this dissertation have not been submitted in part or in full, to any other University or Institute for the award of any degree or diploma.

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ABSTRACT

The surfactants have found widespread applications in biochemical research. In the biological sciences, there are several major areas of research, which make use of interactions between surfactants and biological systems. In the present work, Micro-differential scanning calorimeter is used to determine the nature of interactions of various anionic surfactants: sodium dodecyl sulfate (SDS), sodium octyl sulfate (SOS) and cationic surfactants: dodecyl trimethyl ammonium bromide (DTAB), tetradecyl trimethyl ammonium bromide (TTAB) and cetyl trimethyl ammonium bromide (CTAB) with proteins: Bovine Serum Albumin (BSA), Lysozyme and the β-lactoglobulin. The thermodynamic parameters of protein folding: denaturation temperature (T_d), enthalpy of denaturation (ΔH_d) and cooperativity (η) (ratio of calorimetric enthalpy and van't Hoff enthalpy) were determined. Investigations were also carried out on very dilute solutions of BSA by spectropolarimeter, fluorescence spectrophotometer and UV spectrophotometer for greater understanding of the interaction of surfactants with proteins.

At the lower molar ratio of SDS/BSA (less than 20), thermograms for the denaturation of BSA are biphasic as reflected by two endotherms. The biphasic endotherm are associated with the unfolding of the ligand - poorer and ligand - enriched protein forms respectively. The both endotherms shift to higher temperature with increase in SDS concentration. Also, calorimetric enthalpy of first endotherm decreases while that of second endotherm increases. With increasing level of SDS, the endotherms coalesce to give a single asymmetric peak skewed to the lower temperature side. However, at the higher molar ratio of SDS/BSA (more than 20), the

results are opposite to the ones obtained for low molar ratio of SDS/BSA. Thus SDS plays two opposite functions for the folding and the stability of bovine serum albumin. At low concentration, it acts as a structure-stabilizing additive and increases the stability toward thermal denaturation. This is attributed to the binding of SDS to sites with fairly high affinity on BSA. Additionally, weak interactions and/or indirect solvent mediated effect lead to a continuing but smaller increase in stability at intermediate surfactant concentration. At higher concentration, the strongly destabilizing character of sodium dodecyl sulfate predominates and it behaves as a classical denaturant. This is due to the binding of SDS to denatured protein. With increasing sodium octyl sulfate (SOS) concentration, the denaturation temperature increases with concomitant decrease in half height width. However, the increase in denaturation temperature is smaller than for SDS showing strong binding in case of SDS emphasizing the importance of hydrophobic part of surfactant in binding with BSA. Further, the unfolding of bovine serum albumin in presence of urea was studied using fluorescence spectrophotometer and spectropolarimeter. CD and fluorescence results are consistent with the calorimetric results that SDS stabilizes at low concentration and destabilizes at high concentration.

DSC investigations on the effect of cationic surfactants, dodecyl trimethyl ammoniumbromide (DTAB), tetradecyl trimethyl ammoniumbromide (TTAB) and cetyl trimethyl ammoniumbromide (CTAB) with lysozyme and bovine serum albumin show that the cationic surfactants act as destabilizers of proteins. With increasing surfactant concentration, the denaturation temperature decreases with concomitant increase in half height width. The effectiveness of cationic surfactants in decreasing T_d increases with its alkyl chain length. The decrease in the denaturation temperature and enthalpy of denaturation are observed to be in the following order: DTAB <

TTAB< CTAB. These results can be explained on the basis of hydration cosphere overlap model. With an increase in alkyl chain length and consequently the hydrophobic hydration propensity of amino acid, there is enhancement of the hydrophobic hydration of the nonpolar residues of the unfolded protein which favours the denatured state, resulting in reduced Td of the protein. UV-difference spectra results of the interaction of the BSA with SDS, SOS and DTAB are consistent with the DSC results.

The heat capacity measurement is important in specifying the denatured state of proteins since this parameter is a sensitive index of the completeness of the protein unfolding. The heat capacities (ΔC_p) for the temperature induced unfolding of proteins: paraalbumin carp, lysozyme T4, papain, pancreatic trypsin inhibitor, trypsin, ribonuclease T1, stap. nuclease, chymotrypsinogen A, chymotrypsin, lysozyme, myoglobin, ribonuclease A and metmyoglobin were calculated from the change in solvent accessible surface area between the native proteins and extended polypeptide chain. To visualize the effect of disulfide crosslinks on molar heat capacity, loops of varying number of alanine residue and extended alanine chains with terminal cystein were modeled. The difference in ΔC_p values between the extended state and the loop conformation is found to be linearly related to the number of residues in the loop. Corrections are applied for proteins with crosslinks based on above observation. Corrected values of ΔC_p are found to be close to experimental values.

Atomic solvation parameters are widely used to estimate the solvation contribution to the thermodynamic stability of proteins as well as the free energy of association for protein-ligand complexes. In view of discrepancies in the results of free energies of solvation of folding for various proteins obtained using different atomic solvation parameter sets, systematic studies were carried out for calculation of

accessible surface area and the changes in free energy of solvation of folding of a large number of proteins: paraalbumin carp, lysozyme T4, papain, pancreatic trypsin inhibitor, trypsin, ribonuclease T1, stap. nuclease, chymotrypsinogen A, chymotrypsin, lysozyme, myoglobin, ribonuclease A and metmyoglobin. The calculated free energy of solvation (ΔG_s) of folding values for various ASP sets differ in absolute value and sign in such a way that certain ASP sets predict the unfolded state to be more stable than the folded whereas other yield precisely the opposite. This behaviour has been explained in terms of weightage to hydrophobic and hydrophilic atoms.

A detailed calculation of accessible surface area and free energy of solvation for mutants of lysozyme T4 where threonine157 is replaced by aminoacids alanine, cysteine, aspartate, glutamate, phenylalanine, glycine, histidine, isoleucine, leucine, asparagine, arginine, serine and valine, for which thermodynamic data of free energy of denaturation and crystal structures are reported in literature were carried out using various atomic solvation parameter sets. The change in free energy of solvation between wild and mutant lysozyme T4 were calculated. The deviation of calculated results with experimental results are discussed to highlight the discrepencies in the atomic solvation parameter sets and possible reasons for the same. The octanol to water based ASP sets perform better in comparison to the vacuum to water based ASP sets. This can be attributed to more weightage to hydrophilic atoms in the vacuum based sets. However, the vacuum to water based ASP "Oons" performs well when combined with chain free energy values. The results are also discussed to throw light on the effect of hydrogen bonding on the stability of mutants.

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