Preface

The properties of water near the surface of a protein are different than that of bulk water. These water molecules, commonly referred to as 'biological water', are dynamically coupled with the protein and play crucial roles in controlling the protein's structure, dynamics, and function. In this thesis we have studied in detail the correlated properties of water present in the hydration layer of a small 36-residue globular protein villin headpiece subdomain or HP-36 (PDB ID: 1VII) using atomistic molecular dynamics (MD) simulations. HP-36 is present at the extreme C-terminus of the villin protein and contains one of the two binding sites of villin. It contains three short α-helices connected by few turns and loops. The activity of the protein is centered around helix-3, which contains ten residues. Effect of partial unfolding of the protein on the properties of surrounding water has also been investigated.

The thesis consists of seven chapters. The primary focus of each of the chapters and the important findings obtained from those are highlighted below.

Chapter 1 presents a brief overview of the current status of knowledge on the hydration properties of proteins and protein folding/unfolding phenomena. Some important issues in these areas and the scope of the present work are also discussed.

In Chapter 2, the structure and dynamics of an aqueous solution of HP-36 have been studied from a constant volume and temperature (300 K) MD simulation. It is observed that within the time scale of the simulation, the residues in helix-3 exhibit large-amplitude oscillatory motions. Such large-amplitude oscillations of helix-3 residues have been found to influence the structure and dynamics of the surrounding water molecules. A reduced structuring of water molecules around helix-3 has been noticed, as against that around the other two helices. Interestingly, both translational and rotational motions of the water molecules within the hydration layer of helix-3 have been found to be faster compared to those in the hydration layers of the other two helices. In agreement to these

findings, it is further noticed that the density of water around helix-3 fluctuates faster than those around the other two helices. A blue shift of 25 cm^{-1} in the low-frequency vibrational band corresponding to the O···O···O bending or the oscillatory caging motion of water molecules present in the hydration layers of the helices confirms the rigidity of the layers. We believe that the low structuring of water molecules coupled with their fast translational and rotational motions around helix-3 may have important consequences on the biological activity of HP-36, as helix-3 contains the active site of the protein.

In Chapter 3, the dynamics of protein–water (PW) and water–water (WW) hydrogen bonds around the three helical segments of the protein HP-36 have been investigated. The calculations reveal that the hydration layer water molecules form strong PW hydrogen bonds with average time constants 2–3 times longer than the WW hydrogen bonds in pure bulk water. Interestingly, significant differences in the dynamical behavior of PW hydrogen bonds have been noticed among the three helices. It is found that the structural relaxation of the hydrogen bonds formed between the helix-3 residues and water is faster than that for the other two helices. The results indicate an excellent correlation between the differential relaxation behavior of the intermittent PW hydrogen bond time correlation functions among the helices and the translational and rotational motions of the corresponding hydration layer water molecules as discussed in Chapter 2. It is further noticed that faster relaxation of PW hydrogen bonds and diffusion of water molecules make the hydration layer of helix-3 less rigid than the hydration layers of the other two helices. We find that the reformation of broken PW hydrogen bonds is more significant for helix-1 and helix-2, as compared to helix-3. Such rapid reformation of hydrogen bonds is also responsible for the higher rigidity of the hydration layers of helix-1 and helix-2.

Polar solvation dynamics of the three α-helical segments of the protein HP-36 has been investigated in Chapter 4. The calculations are carried out using the polar residues as intrinsic probes. It is found that within the time scale of our simulation, the most dominant contribution arises from the interactions between the polar amino acid residues and the water molecules. The solvation dynamics of helix-2 has been found to be faster

than that of the other two helices. This is surprising, as the water molecules around helix-2 exhibit slower motions as compared to those around the other two helices (as discussed in Chapter 2). A careful analysis revealed that the origin of such a counterintuitive behavior lies in the dependence of the solvation time correlation function on the degree of exposure of the probe residues to solvent. It is found that the polar residues of helix-2 are more exposed to water and hence exhibit significantly faster solvation dynamics, even though the interfacial water molecules are slow near helix-2.

In Chapter 5, atomistic MD simulations have been carried out to study the temperature-induced unfolding of the protein HP-36 in aqueous solution. The protein molecule has been found to undergo an unfolding transition at a high temperature of 600 K. It is noticed that a partially unfolded molten globule (MG) structure has been formed with complete unfolding of helix-2 present in the middle of the protein. The other segments of the protein remained mostly intact during the transition. Further examination revealed that the amino acid residue Phe-18 acted as the nucleation center to initiate the unfolding transition. During the transition process, the unfolding of helix-2 has been found to be correlated with higher relative displacement of the surrounding water molecules and a consequent relative lowering of rigidity of the corresponding hydration layer as compared to that for the other two helical segments.

In Chapter 6, we have compared the dynamical properties of water around the three α-helical segments of the folded native structure and a partially unfolded molten globule (MG) structure (as obtained from simulations described in Chapter 5) of the protein HP-36 by means of atomistic MD simulations at room temperature (300 K). The calculations reveal that unfolding of helix-2 of the protein has a strong influence on the dynamics of water surrounding it. As a result, inhomogeneities in the relative translational and rotational motions of water in the hydration layers of the three helical segments have been observed for the folded native and the partially unfolded structures. It is noticed that the water molecules around helix-3 exhibit faster motions than that around the other two helices for both the structures. However, compared to the native structure, a reverse trend in the relative mobility of water around the segments

corresponding to helices 1 and 2 has been observed in the unfolded structure. This is primarily due to faster motions of water around helix-2 in the unfolded structure as against that in the folded native structure. It is also found that the unfolding of the segment corresponding to helix-2 is strongly correlated with the dynamics of hydrogen bonds formed by its residues with water.

Chapter 7 presents a comparative study of the solvation dynamics of the folded native structure and a partially unfolded molten globule (MG) structure (as obtained from simulations described in Chapter 5) of the protein HP-36 by means of atomistic MD simulations at room temperature (300 K). The calculations reveal that the unfolding of helix-2 of the protein influences the polar solvation dynamics of all the three helical segments, but in a nonuniform heterogeneous manner. It is noticed that while the solvation time correlation function for helix-3 relaxes faster in the unfolded form as compared to the native structure, the corresponding function for helix-1 and helix-2 segments relax slowly in the unfolded structure. Contrary to the normal expectation, it is observed that the exposure of the side chains of some of the polar residues of helices 1 and 2 to bulk solvent have been reduced due to unfolding. This leads to slower solvation dynamics of these two helical segments in the unfolded structure. By using the polar residues as intrinsic probes, we have been able to microscopically identify such differences in the exposure of the residue side chains due to unfolding. Our findings indicate that the sensitivity of the solvation response of a protein to its immediate environment can in general be used to discriminate between the folded native and the molten globule states of the protein.