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The impact of kosmotropes and chaotropes on bulk and hydration shell water dynamics in a model peptide solution

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Abstract

Kosmotropic (order-making) and chaotropic (order-breaking) co-solvents influence stability and biochemical equilibrium in aqueous solutions of proteins, acting indirectly through the structure and dynamics of the hydration water that surrounds the protein molecules. We have investigated the influence of kosmotropic and chaotropic co-solvents on the hydrogen bonding network dynamics of both bulk water and hydration water. To this end the evolution of bulk water and hydration water dynamics of a prototypical hydrophobic amino acid with polar backbone, *N*-acetyl-leucine-methylamide (NALMA), has been studied by quasielastic neutron scattering as a function of solvent composition. The results show that bulk water and hydration water dynamics, apart from a dynamical suppression that depends on the NALMA solute, exhibit the same dependence on addition of co-solvent for all of the co-solvents studied (urea, glycerol, $MgSO_4$, and dimethyl sulfoxide). The hydrophobic solute and the high concentration water-structuring additive have the same effect on the water hydrogen bonding network. Water remains the preferential hydration of the hydrophobic side chain and backbone. We also find that the reorganization of the bulk water hydrogen bond network, upon addition of kosmotrope and chaotrope additives, is not dynamically perturbed, and that the hydrogen bond lifetime is maintained at 1 ps as in pure bulk water. On the other hand the addition of NALMA to the water/co-solvent binary system causes reorganization of the hydrogen bonds, resulting in an increased hydrogen bond lifetime. Furthermore, the solute's side chain dynamics is not affected by high concentrations of co-solvent. We shall discuss the hydration dynamics results in the context of protein folding and protein–solvent interactions.

Keywords: Hydrophobic interaction; Water dynamics; Hydration water dynamics; Neutron scattering; Kosmotrope; Chaotrope; Proteins

1. Introduction

Protein unfolding occurs when the balance of forces between the protein–protein interactions and the protein–solvent interaction is disrupted. The disruption may be the result of a perturbation of water structure and dynamics around the protein. Water molecules in the vicinity of a biomolecule may be classified in three categories: internal water, hydration water and free water. Internal water, or structural water, is a relatively immobile water within the solute, and its dynamics is often restricted to slow rotation [1–3]. Hydration water consists of structured water shells that depend on the protein surface interaction; its diffusion dynamics is slow, and it can exchange with outer shells [4–11]. Free water is water of and beyond the third hydration shell, which in principle is not strongly influenced by the protein surface other than due to the excluded volume effect of the protein molecule [4]. In principle the internal and hydration water are more important for protein stability and function. Water structure and dynamics can be perturbed either through temperature or pressure, or through kosmotropic (order-making) and/or chaotropic (order-breaking) co-solvents [12]. Kosmotropic co-solvents are very soluble compounds that are well hydrated with strong hydrogen bonds to water molecules. Kosmotropic substances decreases the solubility of hydrophobic particles and stabilize their aggregates and are excluded from the

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immediate surrounding of non-polar solute particle. Chaotropes are poorly hydrated co-solvents that break down hydrogen bonds and are thought to interact directly with protein. They increase the solubility of hydrophobic particles and encourage protein extension and denaturation [13–15]. Several hypotheses have been offered to explain the effects of solutes on protein stability. The principal hypothesis states that stabilisers and destabilisers of globular protein act indirectly by altering water structure and dynamics, but other hypotheses suggest that this is not the determining factor and that other effects such as excluded volume, affinity for protein surface, and ability to attenuate or accentuate the hydrophobic effect, should be considered [16].

In this paper we address the question of how the hydrogen bonding network dynamics of *bulk* and *hydration water,* together with protein side chain dynamics, may be influenced at the molecular level by water structure stabilizing (kosmotropic) and destabilizing (chaotropic) co-solvents. Considering the complexity of the whole system and experimental limitations for studying molecular events in the dynamics of a protein's first hydration shell we use a simplified model system.

Experimental limitations arise from several factors. Water–monomer interactions fluctuate on a short time scale and involve many complex factors such as hydrogen bonds and hydrophobic interactions, and different types of water confinement. One of the principal limitations of these studies is that the most important techniques to investigate the water dynamics give information averaged over all kinds of interactions. Thus it is impossible to distinguish between contributions from hydrophobic and hydrophilic sites or between regions that are more or less exposed to the solvent, and therefore difficult to characterize with confidence events at the molecular level [17]. Another real limitation has to do with the molecular environment. Highly concentrated solutions, adapted to have only one or two hydration water layers around the biomolecule, are difficult to obtain *in vitro* due to aggregation and precipitation. On the other hand dilute solutions are difficult to study because contributions from bulk water dominate and overwhelm the interesting effects due to hydration water.

In our recent work the approach has been to separate out different types of water dynamics, involving hydrophilic/hydrophobic interactions, and backbone/local side chain interfaces, through the study of model peptide systems whose dimensions are of the order of several water diameters. Short simple peptides are highly mobile, and the central side chain is forced to interact with solvating water. In addition, the possibility of end-capping the short sequence helps to reduce the charge effect and the choice of simple blocks is important in allowing the side chain to visit many possible solvation states. The side chain's effects on the structure of water become more pronounced as the surface of the residue accessible to the solvent increases [18]. Infrared spectroscopy studies show that the size of water clusters around the side chains of hydrophobic amino acids increases in the following order: Gly < Ala < -Val < Ile, Leu [19]. Russo et al. have controlled protein surface inhomogeneity by characterizing the dynamics of the first interacting water molecule near a completely homogeneous hydrophobic oligo-peptide, penta-Alanine, which adopts a beta-sheet conformation [1]. Then, in order to study the hydrophobic effect on the first hydration layer in solution, we considered the dynamics of hydration water near *N*-acetyl-leucine-methylamide (NALMA), a hydrophobic amino acid side chain attached to a blocked polypeptide backbone (equivalent to a Gly–Leu–Gly peptide, Fig. 1). Previous work, using X-ray scattering experiments and molecular dynamics simulations [20], on the structural organization of these peptides in solution, through the full concentration range from 0.5 M (1 mole NALMA to 110 moles H_2O) to 2.0 M (1 mole NALMA to 27 moles H_2O), revealed that water stabilizes either mono-dispersed or small clusters of amino acids, rather than causing complete segregation of the hydrophobic solute molecules into one large cluster. (Note that a 1.0 M solution has a concentration of 1 mol/L.) In addition small angle scattering experiments performed on NALMA in water over the same range of concentrations exclude the formation of NALMA aggregates [4].

Given this structural hypothesis a number of quasielastic neutron scattering experiments have been performed in order to study hydrophobic effects on water dynamics and solute dynamical relaxation as a function of hydration layer [21,22]. Because of its high solubility the NALMA–water system is ideally suited to studies of the dynamics of different hydration layers near a hydrophobic amino acid. High solute concentrations, such as 2M, simulate a biological interface with a shared water layer whereas more dilute concentrations, such as 0.5 M, have in principle enough water for 2–3 complete hydration layers surrounding each NALMA molecule. This solute configuration permits the separation of inner and outer hydration shells around a purely hydrophobic amino acid hydration site, enabling characterization of the first hydration layer dynamics and its influence on the outer layer dynamics [4,20]. Another interesting aspect of the high concentration solute with the corresponding single hydration layer is that it simulates a protein core, permitting investigation of the hydrophobic core dynamics, or the dynamics of the trapped water. These simple models are also important to study the influence of water molecule translational diffusion on protein dynamics and the role of protein–water

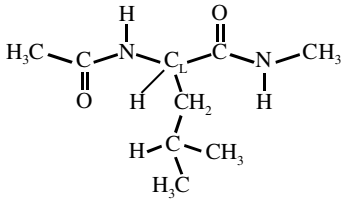

Fig. 1. The NALMA molecule.