Experimental evaluation of hydrogen-bonding energy of water at protein surfaces using a photo-prototropic hydration probe

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A repertoire of researches have been performed on hydration dynamics in biological systems, revealing the relation of water and proteins interactions to the dynamics. On the other hand, the energetics of H-bonds of biological water has been overlooked, the scrutiny of which is fundamental to the understanding the foundation of hydration dynamics at biological surfaces.

To evaluate the H-bonding energy of biological water, the measurement of hydration rates at a series of temperatures is requisite with applying Arrhenius equation. However, when not only the available energy to populate a reactive configuration along a reaction coordinate but other factors such as conformation of a protein is a function of temperature, the interpretation of obtained activation energies from such analysis is not straightforward.

Here, we propose a new, experimental approach to elucidate the H-bonding energy of biological water. To this end, the fluorescent, non-canonical amino acid, 7-azatryptophan (AW) was used in this study as a hydration probe. AW has a similar molecular structure and size as tryptophan does, but exhibits unique H-bonding interactions with water molecules. The chromophoric moiety of AW, 7-azaindole, has been extensively investigated and reported to undergo excited-state proton transfer (ESPT) catalyzed by a water molecules via forming a transient 1:1 cyclically H-bonded complex. The efficiency of ESPT depends on the free energy difference between the well configured cyclic complex requisite for ESPT and prevalent, noncyclically H-bonded AW-water complexes. To achieve the cyclically H-bonded configuration in water, the complexing water molecule should replace one H-bond to other adjacent water molecule with a new H-bond to AW, which is inserted in a model protein and highly exposed to water environment. The H-bonding energy of biological water is evaluated by comparing the ESPT rates of AW at the surface of the protein and in bulk water.