MOLECULAR PRESENTATIONS

SURFACE HYDRATION OF POLYPEPTIDES AND PROTEINS

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Dedicated to the late Professors Carl Djerassi and William S. Johnson of Stanford University

Based on the concept that the folding and assembly of natural polypeptides into proteins is driven by the spontaneous movement of water from order in covalent transient linear elements of surface hydration toward disorder in surrounding water, hypothetical forms of hydrated polypeptides are presented as they emerge from ribosomes and move into coils, beta sheets and beta turns. The same principles of transient linear hydration are used to interpret the folding of the 42-peptide terminal segment of the Carboxypeptidase-A enzyme into its natural protein structure.

In spite of the fact that proteins form and function only in aqueous media, water is never displayed around them and little attention is directed to the roles it may play in their motions and interactions.[1] In the renowned polypeptide-folding experiment performed by Anfinsen in 1961, a ribonuclease protein was denatured by the addition of urea to the media and then found to be restored to normal function when urea was removed.[2] At the time, urea was considered to disrupt hydrogen-bonding within proteins so it was concluded that all the information required for correct folding was within the polypeptide; that the driving force for assembly was a decrease in internal free energy and that water was simply the solvent. Although the concept still prevails,[3] numerous studies have documented that water produces structuring on surfaces[4] and that urea disrupts that structuring.[5] Although intensive efforts have been devoted to examining water around proteins, there seems to be no consensus regarding its structuring properties.[6] The fact is that there are two different forms of hydrogen-bonding between water molecules in the liquid state and on surfaces and that each performs unique functions in the folding and assembly of polypeptides. The purpose of this presentation is to describe those two forms and illustrate how they most likely perform their functions.

Historically, each water molecule in the liquid state has been viewed as hydrogen-bonded in tetrahedral configurations to three or four other water molecules by point charges on their surfaces.[7] In proteins, water molecules bridge between polar and ionic atoms at a variety of distances and angles to stabilize the molecules in their thermodynamically-stable states.[6] However, in 2004, neutron bombardment at the Stanford Synchrotron Radiation Laboratory provided evidence that, at any instant, the largest structural unit in liquid water is a “trimer” with two water molecules hydrogen-bonded to a central molecule.[8] Molecular orbital calculations in the 60’s and 70’s forecast that such a trimer (with 2.76 Angstroms between the molecules) would be the most stable structural unit in liquid water[9] and, in 1972, X-ray scattering from the surface of liquid water at 25^{o}C revealed the presence of both trimers and tetramers, again with 2.76Å between the molecules.[10] The trimer and tetramer form spontaneously but are unstable and last only about 10^{-12} seconds, a million millionth of a second.[11]

When the trimer was first reported, most scientific attention was directed to crystalline proteins and there was a debate regarding the nature of the hydrogen bond.[7,9] However, in 1999,

Dr. Isaacs at Bell Laboratories concluded, based on detailed X-ray analysis, that water-to-water hydrogen bonding in ice is not the same as single water molecule bonding in proteins: in ice, hydrogen-bonding is "covalent"- similar to bonding between carbon atoms with electron orbital clouds of adjacent water molecules encompassing a central proton.[12] Further evidence for this second form of hydrogen-bonding between water molecules was provided in 2003 by Professor Stanley at Boston University who, as a consequence of a detailed study of the properties of liquid water, concluded that liquid water and water on surfaces are composed of two density-forms of hydrogen-bonded water molecules.[13]

If liquid water is carefully cooled to -30°C, it turns into a glassy fluid containing linear elements and clusters but no ice. However, if cooled down to -40°C, crystallization occurs immediately to produce a form of ice called "cubic" in which all of the molecules are in linear elements, 2.75 Angstroms between the molecules.[14] Cubic ice is formed kinetically by the linear overlap of orbitals[15] but is unstable and immediately isomerizes at 0°C into the more stable "hexagonal" form of ice in which some of the elements are not linear and the molecules are 2.75 to 2.84 Angstroms apart.[14] It is important to realize that water molecules have extremely high kinetic energy with a low probability of spontaneously adopting ordered forms, even below 0°C.[7] However, if liquid water is placed in contact with a surface in which the atoms are in the same hexagonal arrangements as they are on the surface of ice, like those of oil molecules or iodine crystals, crystallization occurs immediately at 0°C.[7] If even a trace of oil is on the surface, super-cooling is impossible because the ends of oil molecules in contact with water assemble in the same hexagonal arrangements as water molecules in ice.[16]

Water in contact with hydrophobic surfaces like oil and lipid molecules, displays the nuclear magnetic resonance doublet peaks of ice, not the singlets of liquid water[17] and molecular orbital calculations indicate that transient linear elements of five and six covalently hydrogen-bonded water molecules form in particular orientations on such surfaces.[18] Although half-lives of these linear elements on hydrophobic surfaces of polypeptides, as they are released from ribosomes, are too brief to permit visualization, ultra-high-speed crystallographic analysis of water on the solid planar surface of graphite, by Professor Zewail and his group at Caltech, revealed that it is present as layers of hexagonally-bonded linear elements with cubic patterning between the layers - similar to cubic ice.[19]

Although the covalent linear elements which form on hydrophobic surfaces last only about 10^{-11}seconds;[20] they must fill voids and bind between polar and charged atoms as polypeptide chains transition from one thermodynamically-stable state to another. As linear elements between hydrophobic surfaces and charged atoms increase and decrease in length by admitting and releasing single water molecules;[27] polypeptides must move in quantized steps to form complexes and perform vital functions.[23] In fact, on broad planar hydrophobic surfaces, short covalent linear elements of hydration might well propagate the formation of time-dependent planar sheets with cubic layering[4] - similar to cubic ice.[19]

However, the formation of relatively-ridged covalent linear elements on surfaces may not only be responsible for the orderly assembly of proteins but for spontaneity as well. As a water molecule approaches a hydrophobic surface and forms a covalent hydrogen bond, it loses 5 to 6 kcal/mole of energy to adjacent water molecules.[21] However, as the bond breaks and the water molecule returns to the more random state, similar units of quantized energy are absorbed from surface molecules and they are driven toward lower energy and higher order.[22] For example, oil molecules, which can twist and turn and bend in the liquid state, lose energy and entropy and are forced to align as linear segments in layers in contact with water - motions are limited to lateral movements and rotations around axes.[16]

Water molecules are unique in that their small size and highly-revesible hydrogen-bonding into short covalently-bonded units permits them to move rapidly back and forth between order and disorder. By rapidly forming and degrading on ordering surfaces, covalent linear elements of hydration not only transfer units of energy from more massive slower-moving molecules to surface water,[22] they move in quantized steps.[23] It is this unidirectional transfer of quantized units of energy from biomolecules to water which drives (and possibly directs) the spontaneous folding and assembly of natural polypeptides into functional proteins. By obeying the Second Law of Thermodynamics and moving spontaneously from order toward disorder, transient linear elements of hydration move natural molecules in the opposite, non-spontaneous, direction. In 1937, it was Erwin Schrödinger, in his little book "What is Life?" who concluded that it was this unidirectional energy-transfer property of water which drove molecular evolution from disorder to toward order - it was water which produced the order for life.[24]

Actually, the spontaneous movement of polypeptides into functional proteins involves surface dehydration.[6] As polypeptides emerge from ribosomes, hydrophobic surfaces must immediately become coated with transient linear elements of hydration which, by continually and repetitively forming in preferred orientations, must influence the directions and dimensions of folding and, by moving from order to disorder, drive them toward internal order.[22] When final proteins are produced, central regions are dehydrated - peptides which provided hydration order are on the inside while peptides with amides and ions in their side chains are on the outside, hydrogen-bonding with surface water in multiple orientations to increase mobility, stability and solubility.[6,7] In fact, recent studies suggest that exit tunnels in ribosomes, through which newly-synthesized polypeptides must pass, may have regions which function like chaperone proteins to assist hydrophobic water-ordering regions of polypeptides to fold into coils before they are released into ordering surface water.[25] Although outer surfaces of most proteins do not reflect cubic geometry, they often display external structures which permit them to assemble into complexes with each other and with other proteins in a variety symmetries.

Clearly, quantized transient linearization in surface water must play a dominant role in filling voids and stabilizing spaces in intermediate states in the formation of functional proteins. However, they must also play a critical role in the transfer of charges between polar and ionic atoms on surfaces of proteins as polypeptide chains move from one position to another.[26] By forming as covalent dielectric linear elements between ions, they permit proton tunnelling to generate counter ions and lower charge potentials.[27] By transiently forming in enzyme and receptor binding sites in their open forms, they provide quantized spaces for substrate and regulator molecules to bind and perform functions. NMR analyses indicate that water is in particular orientations on smooth muscle and collagen fibers[29] and molecular analyses indicate that the phosphate head-groups of lecithin/cholesterol complexes, which compose the inner surfaces of the large axons of nerve fibers, are the same distances apart as covalently-hydrogen-bonded linear elements of water molecules. During depolarizations, when potentials between nerve endings and nodes are high, proton pulses most likely tunnel through extended linear elements of hydration at extremely high speeds with almost no loss in energy.[30] If our nerves were filled with metal rather than water, we would be combusted by the resistance. Nanotechnology today is searching for superconductivity in electrons - nature has already found it in the protons of linear elements of water molecules in nerve cells.

Indeed, it is unfortunate that water is never displayed in biomolecular structures. For example, the classical structure of DNA is never viewed as hydrated but the crystallographic pattern which was used by Watson and Crick to develop their helical model was obtained by Rosalind Franklin by spraying the sample with water.[31] Only when DNA is hydrated with at least 13 water molecules per base pair, does it exist in the uniform helical coil that has become the symbol of modern molecular biology.[32] Only when spaces between phosphate oxygens on opposite sides of the wide groove are transiently-bridged by linear elements of six-to-seven covalently hydrogen-bonded water molecules to delocalize the charge and by three-to-four across the narrow groove, are filaments of double helix DNA stabilized as they oscillate and move through their various functional states. In fact, transient linear hydration is so dominant around DNA to stabilize its high negative charge that the water is referred to as "Ice-like" and spherically-hydrated sodium ions are held out away from it by several layers of water molecules.[32,33]

Indeed, it is unfortunate that chemists, biologists and the public at large are denied the truth that it is surface water which plays a critical role in the structures and functions of natural molecules. The fundamental problem is that the kinetic formation of transient quantized covalent linear elements of surface hydration must be accepted as one of the primary mechanisms of energy and spatial control within living cells.[13,15,23] For the past century, chemists have used the concept of kinetic control and quantized units of covalent structure to devise synthetic pathways to complex natural molecules;[34] there is no reason why the same fundamental principles of preferred transient linear hydrogen bonding cannot be used to interpret the role of surface water in transitions of natural molecules from one stable conformation to another.

The purpose of this brief article is to provide an introduction to the use of theoretical principles of covalent hydrogen bonding in the standard linear 4.5-Angstrom trimer and related transient linear elements of hydration to derive hypothetical conformational interpretations of hydration-stabilized forms of polypeptides as they transition from linear states to coils, beta-sheets and beta-turns.[35] Since surface water is dynamic and continually shifting from one state to another, there is no way to calculate precise energy changes.[36] However, as you will see from the proposed intermediates, there is a continual decrease in linear ordering in surface hydration as folding and assembly proceed. Peptide side chains are displayed in specific low-energy conformations realizing that most of them are dynamic and move relatively freely from one low-energy to another in aqueous solution but are confined to more specific conformations in finished proteins.[3] Finally, the folding of the terminal 42 peptides at the acid end of the Carboxypeptidase-A polypeptide is presented as an example of how hydration principles derived above can be used to interpret its folding into a natural intermediate protein conformation.[37]

Within the past few years, an increasing number of studies have provided evidence that surface water and water at sub-zero temperatures display the properties of a quantized media[23] and, recently, the quantum mechanical property of entanglement has been revealed as a property of protons within water molecules.[38] Undoubtedly, these properties permit the development of entirely new concepts regarding the role of water in life processes. The question is: When is the field of molecular biology going to accept the importance of surface water in life processes?

For more information on the author, the quantization of surface hydration, the assembly of proteins, the hydration of receptor sites and the possible role of water in molecular evolution, check out: www.linearwater.com and www.molecularcreation.com.