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Water and water clusters in biological systems

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Abstract. Water is inherently a simple substance, but from Aristotle's time until today it raises a lot of questions. Living cells are about eighty per cent water. Organisms consist essentially of liquid water, which fulfils a lot of functions and should never be considered just an inert diluent. The unique properties of water are of fundamental relevance for human life and play a substantial role in many biochemical and biological systems. In the second half of the previous century, researchers came to an understanding about the differences between biological water and ordinary water. This article reviews previous studies on water function and its significance in biological systems. Present knowledge about water clusters, the understanding of water cluster role in biological systems and common methods used in the analysis of determining water clusters are examined in this paper.

Key words: biological water; water structure; water biology; clustered water; cell water; hydrogen bonding.

INTRODUCTION

About 600 BC, the Greek philosopher Thales asserted that water is the primal substance from which all things arose and of which they consist (Morra, 2001). Two hundred years later, the philosopher Aristotle deemed that water is one of the four fundamental elements, in addition to fire, air and earth (Sokolowski, 1970). Nowadays it is known that water is the most opulent molecule on earth. Almost 70% of the planet is covered by water (Robinson et al., 1996). The properties of liquid water are unique for a liquid: it has huge heat capacity and uncharacteristically high melting and boiling temperatures. Water also has one of the most highly dielectric constants amongst non-metallic liquids and many anomalies in its specific volume as e.g. ice floats on water (Cabane et al., 2005). At first sight, water looks like an ordinary molecule consisting of two hydrogen atoms connected to one oxygen atom, and only a few molecules are known to be smaller in size. Its size misleads the complexity of its characteristics, which seem to fit ideally into the conditions of carbon-based life (Chaplin, 2001). This substance is the most extraordinary and indispensable constituent in every living organism. Water makes up about 80% of the chemical compounds in a living cell; it is the matrix and medium for the origin and operation of life (Szent-Györgyi, 1971). However, water is not just a tasteless, neutral transporter system for biochemical processes, but also an active participator in biology, which simply could not act the way it does without the specific mediating properties of water. Moreover, it is widely believed that water is the first molecule to contact biomaterials in any clinical application (Vogler, 2004).

This paper gives an overview of the current knowledge on water function and its significance in biological systems. The present concept of water clusters, understanding water cluster role in biological systems and common methods used in the analysis of determining water clusters are studied in this paper.

Formation of understandings about water in a biological system

Cells, organs and all living organisms are in constant water demand. Without the presence of water, several chemical reactions would not take place. Also, biological systems would not function, and life, as we know it, might not even have come to be without water (Ellabaan et al., 2012). The task of water in chemical, biochemical and cellular occasions has been recognized as a universal solvent. The common belief was that biological water is not markedly different from normal liquid water. However, the relevance of biophysical and biochemical characteristics of water have been pointed out since the second half of the last century. These researches focused on the fundamental question: what are the differences between biological water and ordinary water (Lo et al., 2000). Water molecules that surround solute molecules form with them frozen patches or microscopic icebergs (Frank & Evans, 1945). The concept of cell as a membranous bag of solutions has been seriously challenged. For the first time, it was claimed that the partitioning of solutes between the cell and extracellular solution is not determined only by the throughput of the membrane, but that protoplasm itself preferentially accumulates some solutes and excludes others. The understanding that water in the protoplasmic gel is different from water in a simple aqueous solution was at the centre of this concept (Troschin, 1966). Many extensive studies on water in biological systems have been carried out. It has been concluded that the water density of *Artemia cyst* was smaller than of normal water at the same temperature (Clegg, 1984). Some authors claim there is both high and low-density water inside the cell. Others claim that the water inside the cell is different from free water in bulk (Wiggins, 1990). One of the main problems in biochemistry is to understand how the structure is built up and what are the functions of proteins at the molecular rate. Water molecules also act as catalysts for many enzymes in biochemical reactions. The particular inclusion of water is indispensable for detecting many problems in biochemistry. Thus, it could be implied that without principal and detailed knowledge of water, many phenomena in molecular biology would be impossible to understand (Robinson et al., 1996). Bulk water is only one of the many various ways that liquid water can be organized. ‘Dense’ water (low degree of hydrogen bonding) and ‘less dense’ (‘expanded’) water (high degree of hydrogen bonding—extreme in ice with straight hydrogen bonds) were proposed to exist under specific conditions (Wiggins, 2001). Water ionizes and permits simple proton exchange between molecules to facilitate the affluence of ionic synergies in biology. The structuring of water around molecules makes it possible to sense them and helps them sense each other from a distance. The unique hydration properties of water towards biological macromolecules (especially proteins and nucleic acids) designate their three-dimensional structures to a large range, and hence their functions in a solution (Chaplin, 2001). A surprising characteristic of water is its preferential orientation in the hydration cell of nonpolar solutes and nonpolar side groups appended to biopolymers. The structure obtained by liquid water that is in close proximity to nonpolar solutes is a primary characteristic of modern theories about hydrophobic hydration and effects, which are of high importance to our knowledge of many important chemical and

biological processes. Placing a solute molecule in liquid water brings about a rearrangement of the random H-bond network. Also, in order to create some space for the guest molecule, the water tries to strengthen its network around the non-polar solute. This can best be done by placing its tetrahedral bonding directions in a straddling mode (Ludwig, 2001). In a closed system, these kinds of water moieties are balanced: deviation of water density from that of bulk water in one partial volume of the system is presumably compensated by an inverse deviation of the density of the water moiety in some neighbouring partial volume to maintain a balance. There seem to be no strictly defined borderlines between the different water densities, and it should be kept in mind that the partitioning of cell water into water moieties of different density cannot be static, but rather a procession of transient states of high dynamic activity (Mayer et al., 2006). Self-association of water through hydrogen bonding is the essential mechanism behind water solvent properties and understanding self-association effects near surfaces is a key to understanding water properties in contact with biomaterials (Vogler, 2004). Specifically for proteins, the dynamics of water–protein interactions govern various activities, including facilitating protein folding, maintaining structural integrity, mediating molecular recognition and accelerating enzymatic catalysis. Thus, it is important to characterize the dynamic behaviour of biomolecule-associated water—biological water—at a molecular level. Every water molecule in the hydration shell is dynamic (not in an iceberg), and their ultrafast motion is established experimentally to be significantly slower than the one in bulk water. The reorientation motion of water in the shell occurs in a few picoseconds in the inner layers and becomes faster in the outer layers (Zhong et al., 2011). Water transport through biological membranes can occur in three ways: 1) over diffusion through the lipid matrix; 2) through transport proteins, such as channels, and some occlusion transport proteins, such as glucose transporters, and 3) through water channel proteins specifically expressed by cells for such purpose (Disalvo et al., 2015).

Water clusters

The simplest water system, the water cluster, is an accurate assembling of water molecules synergized together by hydrogen bonds. In the biological system, water molecules form an unlimited hydrogen-bonded net with structured clustering. It has been hypothesized that small clusters composed of four water molecules already have the ability to form comparatively stable water octamers. These clusters may further form much larger water clusters that can interlink and tessellate throughout space (Chaplin, 2001). The fundamental knowledge of water clusters can be investigated computationally or experimentally (Ludwig, 2001). Water clusters influence a large number of aspects of biological functions. The water-protein interaction has been accepted as a major decider of chain folding, internal dynamics, conformational stability, binding specificity and catalysis for a long time (Pocker, 2000). A different allocation of water clusters in natural water (rain and river) and in the biological matrix of fruits (apples, bananas) and vegetables (potatoes, carrots, tomatoes, onions, red beets) has been determined. It has been found that the structure of natural water clusters is different from the water of the biological matrix of fruits and vegetables and it is dependent on dryness and the biological matrix. It has been identified that water clusters in natural water are not as stable and reproducible as water clusters in plants (Zubov et al., 2006). Lo *et al*. advance the hypothesis that the water clusters are active partners in any biochemical

reactions occurring inside a living organism. These water clusters are created by the interaction of minute inorganic or organic substance with water (Lo et al., 2000). During plant growth, water clusters and their distribution (downfallen or expanded) play an important role. The behaviour of water clusters in plants can possibly be exploited in the future as the indicator for biological matrix development during the time of plant growth. Oscillation of water clusters inside plants makes communication between plants and surroundings possible through a resonance field. The right choice of natural water concerning the long-range order of water clusters could advance plant growth and decrease costs (Zubow et al., 2010). Wang *et al*. explored the effects of small water clusters in *Escherichia coli* bacterial culture and found that the *E. coli* grew faster in small water clusters than in normal water (Wang et al., 2013).

Calculated water clusters

There have been numerous semi-empirical and *ab initio* quantum mechanical studies on water clusters (Ludwig, 2001). Discussions have shown that water clusters, depending on their size and connectivity, can play an important part in experimental and theoretical investigations.

Small Cyclic Water Clusters (n = 3–6), including ring structures, are the optimal structures with harmonic vibrational frequencies (Xantheas et al., 1993).

Isoenergetic Water Hexamer Clusters (n = 6) or cyclic hexamers, as discussed above, are the building blocks of numerous ice forms and they appear to be relevant to liquid water as well (Kim et al., 1998).

A variation of Water Heptamers (n = 7), although, to date, only a few theoretical investigations on the water heptamer are available and knowledge is still scarce (Mir & Vittal, 2007).

Water Octamers (n = 8): Cubic or Cyclic. The cyclic topology has fewer H-bonds than the cubic octamers (8 versus 12) and is therefore energetically not favoured. Its powerful thermodynamic balance is induced by entropic factors (Weinhold, 1998).

Ice-Like and Clathrate-Like Structures (n = 12–26) are well known structural components of crystallographic ice forms (Ludwig & Weinhold, 1999).

Larger Clusters: Icosahedral Networks (n = 280), which are built in a way that each water molecule is connected with four H-bonds. Two of them act as acceptors and two as donors (Chaplin, 2001).

Experimental determination of water clusters

The physical characteristics of water clusters are principally investigated through size-dependent multitude researches in the mass spectra of protonated water clusters of $(H_2O)_nH^+$ and deprotonated water clusters $(H_2O)_nH^-$ in a size ranging up to a hundred molecules (Hansen et al., 2009), through infrared (IR) spectroscopy (Mizuse et al., 2010) and by using photoelectron spectroscopy (Ma et al., 2009). Electron diffraction tests were performed during the years 1975–2000 and it was the preferred method for experiments with crystalline water where the size range of clusters was n = 200–1,000 molecules (Torchet et al., 1989). Vibrational IR spectroscopy experiments validate these results in a study on pure water and Na-doped clusters (Mizuse et al., 2010). With IR excitation-modulated photoionization spectroscopy, the beginning of crystallization in a range of n = 275 water molecules was registered (Pradzynski et al., 2012). In the computer simulations it is assumed that the amorphous-crystalline transition takes place

at n = 200 water molecules (Kazimirski & Buch, 2003). Turning to amorphous structures is expected to occur between n = 21–275 molecules.

All up-to-date published studies which examine water in biological systems have reached the conclusion that water is not a passive spectator in the molecular biological processes. Research on water has given the result that water performs a variety of important roles in cells and tissues and in the functioning of the biological organisms overall (Lo et al., 2000). Almost all the activity of proteins and nucleic acids depends on the presence of water. Theories that describe the role of water in proteins and nucleic acids activity are under ongoing scientific debate. Long-standing 'iceberg' theory has replaced the theory of water molecules in the hydration shell being all dynamic (Frank & Evans, 1945; Zhong et al, 2011). Water in biological systems is surrounded by intervening cellular components and also affected by structural effect, as well as having different structure and characteristics than bulk water. The biological system generates stabilized clustered networks from water molecules (Wiggins, 1990). Researchers have shown that water clusters have internal and external influence on biological processes. Many studies describe the influence of biological processes in which water clusters help to complete biological systems, for example, in conformational stability, internal dynamics, catalysis, etc. The results which have been obtained by the external contact with clustered water and biological systems are also remarkable. However, the mechanisms of action in those processes need more investigation. There is still a lot of incomplete knowledge about water clusters or biological water and theories of cell biochemistry are not explicit. Some studies have shown that clustered water also has an effect on the yield of crops (Zubow et al., 2010). Until now there have been a lot of theoretical researches carried out on structures of water clusters in biological systems, which help to contribute to interpreting biological processes of nature. Different physical experimental methods are used for researching biological water and determining water clusters. However, there is no unique method for defining biological objects in water clusters—the existing methods need continuous development to be in an agreement with specific biological system characteristics.

CONCLUSIONS

Water clusters in biological systems have an active role in all biochemical reactions that exist inside living organisms. Understanding the mechanism of the self-association of water molecules through hydrogen bonding is essential for understanding water properties in contact with biomaterials. It can be derived that clustered water can have a similar essential effect in the human organism as clustered water has in plants. Considering that water clusters in plants may have an influence on the human organism, there is a need for further research of water clusters in plant cells. It is very likely that further research on water clusters in plant cells will reveal new insights into fresh herbal food and plant juice benefits for human health.

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