Review Article

Functional Hydration Behavior: Interrelation between Hydration and Molecular Properties at Lipid Membrane Interfaces

Nozomi Watanabe, Keishi Suga, and Hiroshi Umakoshi

Graduate School of Engineering Science, Osaka University, Toyonaka, Osaka, Japan

Correspondence should be addressed to Hiroshi Umakoshi; umakoshi@cheng.es.osaka-u.ac.jp

Received 31 August 2018; Revised 30 November 2018; Accepted 16 December 2018; Published 13 January 2019

Academic Editor: Ester Chiessi

Copyright © 2019 Nozomi Watanabe et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Water is an abundant commodity and has various important functions. It stabilizes the structure of biological macromolecules, controls biochemical activities, and regulates interfacial/intermolecular interactions. Common aspects of interfacial water can be obtained by overviewing fundamental functions and properties at different temporal and spatial scales. It is important to understand the hydrogen bonding and structural properties of water and to evaluate the individual molecular species having different hydration properties. Water molecules form hydrogen bonds with biomolecules and contribute to the adjustment of their properties, such as surface charge, hydrophilicity, and structural flexibility. In this review, the fundamental properties of water molecules and the methods used for the analyses of water dynamics are summarized. In particular, the interrelation between the hydration properties, determined by molecules, and the properties of molecules, determined by their hydration properties, are discussed using the lipid membrane as an example. Accordingly, interesting water functions are introduced that provide beneficial information in the fields of biochemistry, medicine, and food chemistry.

1. Introduction

Water is an abundant and interesting molecule that has various functions in biological systems. In this context, it has attracted much interest in a variety of research fields. Water is the only solvent that can be drunk without serious risks to human health, and is therefore indispensable in the food and medical fields. Specifically, the hydration state of food is one of the important and determining factors of its quality. The water (moisture) content of food controls its subtle taste and texture. Accordingly, controlling the amount and state of water is critical for the preservation of the quality of food because the elimination of water drastically reduces the growth of living species (bacteria and fungi). Drying and freezing foods prevent the growth of microorganisms, and they thus constitute useful techniques for food preservation. Conversely, drying and freezing may diminish the taste of food because the characteristics of water (or ice) play very important roles in the organization of the constituent structures of materials in food. Salting and sugaring also improve food stabilities, while higher concentrations of salt lead to the denaturation of proteins. The colloidal properties of solution are also sensitive to the amount of water. In systems which only consist of oily components, the phase separation behaviors can be dependent on the composition, temperature, pH, salt concentration, and the presence of surfactants. In the case of agar hydrogels often used as edible polymers, their stiffness can contribute as a determinant of taste, wherein the hydration state is a considerable factor that determines the sponge structure of the formed hydrogel. Considering the above, the quality of food is relevant to the hydration state and to the structure of the component.

Regarding the stability and freshness of food, an important concept relates to the method/approach required to maintain the structure of cells, proteins (or enzymes), and self-assembled compounds in food materials. The capacity of water molecules to functionalize biological molecules and their assemblies has been investigated for many years [1]. Water molecules are essential for the maintenance of the physiological cell function and structure. Therefore, it is
required to understand how it behaves as a modulator from various viewpoints, including chemical, physical, and biological. The biological interfaces, e.g., the surfaces of proteins, nucleic acids, and lipid membranes, are usually in well-hydrated states, and the properties of interfacial water have been attracting the interest of researchers for years [2–5]. The functions of proteins are in many cases strongly related to their structure, including the hydration water layer, wherein the hydration state of the protein surface affects the protein conformation [6]. Additionally, the water molecules associated with proteins can work to modulate the conformational properties of protein motifs [7, 8]. In cell systems, the behaviors of water at the lipid membrane surface are directly related to the maintenance of the cell structure and to their interactions with biomolecules [9]. Information on the hydration properties of the lipid membrane is also useful in the medical and biological fields. Furthermore, the polarity of the lipid membrane (hydrophilicity) is an important indicator in the targeting of nutrients and drugs to cells, both in terms of the estimation of biocompatibility and modulation of bioavailability [10, 11]. Therefore, a precise understanding of the fundamental hydration properties of the lipid membrane is required.

The lipid bilayer membrane is organized based on the hydrophobic interactions between amphiphilic molecules that constitute the hydrophobic-hydrophilic interface in an aqueous system. The lipid bilayers, usually found in cell membranes and a part of the outer membranes of intracellular compartments, act as physical barriers to prevent the free permeation of water and water-soluble compounds, thus playing an essential role in the maintenance of cellular homeostasis. In the various types of biological interfaces (including lipid membranes), the key aspects pertaining to the roles of water molecules are as follows: (1) hydration properties that can be influenced by the formation of hydrogen bonds between the hydration water molecules and the substance of interest and (2) classification of water molecules according to their hydration characteristics, such as the strong hydration properties/characteristics exhibited at the interface or in the bulk. Although the hydration properties of food are complicated, the hydration water layer of each component (proteins, nucleic acids, membranes, and other small molecules) is still relevant from the viewpoints of chemistry, biology, medicinal chemistry, and so on (Figure 1).

In systems, such as proteins, lipid membranes, and polymer surfaces, the interfacial water molecules exist as hydration water layers and exhibit different properties compared to those in bulk water. In the case of the hydration water in a phospholipid bilayer membrane system, the binding state of the hydration water varies, depending on the binding position [12]. The water group that binds directly at the interface can be considered as a first-order hydration water group. Additionally, the associating water molecules around the first group can be more flexible, and these groups can be regarded as second-order hydration water groups. Recently, an increased focus has been documented on the classification of the interfacial hydration water on such groups because each hydration water group can play important roles in (a) modulating protein structure and function and (b) regulating adhesion among (bio)materials [13]. It is also important to evaluate the thermodynamic properties of hydration instead of observing the binding properties. The classification of interfacial water molecules into several groups allows us to understand their contributions. The understanding of the hydration properties is essential for food materials from the viewpoint of the structural stability and function of each component, including the lipid membranes. In this review, the recent approaches adopted for the investigation of water properties, and the fundamental interrelation between hydration and molecular properties, are summarized with a special focus on lipid membrane properties.

2. Basic Properties of Water Molecules/Observation of Water

Water molecules thermally diffuse in liquid to perform certain functions by assuming certain structures: Eisenberg and Kauzmann assumed that the structure of water molecules is classified according to the observation time (Figure 2) [14].

(a) I, structure at each instant \( t < \tau_v \)
(b) \( \tau_v \), structure averaged with respect to vibration \( (\tau_v < t < \tau_D) \)
(c) \( \tau_D \), structure averaged with respect to the orientation and movement of molecules \( (\tau_D < t) \)

The orders of \( \tau_v \) and \( \tau_D \) are \( 10^{-13} \) s and \( 10^{-12} \) s, respectively. Most of the water properties are investigated to relate to the D-structure, whereas there are few measurement methods relevant to the \( \tau_v \)-structure. The evaluation of the hydration water behaviors can be dependent on the measurement method in accordance with the characteristics of the target water.

2.1. Experimental Approach: Observations of Direct Motion of Water Molecules. X-ray and neutron diffraction spectroscopy are representative experimental methods that have been used to evaluate water molecules on nucleic acids, proteins, lipid membranes, reverse micelles, etc. The orientations of the water molecules within the hydration layer have also been discussed [15–19]. The properties of the hydration layer are not simple. Accordingly, related studies on hydration layers are discussed in Section 3. Given that X-ray diffraction and neutron diffraction measurements require crystallized samples, the molecular structure and patterns might be different compared to those in aqueous solution systems. Specifically, these approaches are effective in identifying potential properties, such as the stereoscopic characteristics at the interface (including the hydration water). Small-angle X-ray scattering (SAXS) and small-angle neutron scattering (SANS) have been adopted to evaluate molecular structures at the nanometer scale, and they are often used for the investigation of the hydration layer [20–23]. A molecular distribution analysis suggests that the density of water in the hydration layer on the protein is
approximately 15% higher than the water density in the bulk [21, 23]. Additionally, in lipid bilayer systems, the excess number of water molecules can be distributed at the membrane-bulk interface region [24]. By employing SAXS, the structural properties of food can be evaluated. From a scientific viewpoint, the self-organized structure of mayonnaise can be categorized in emulsion gels (oil-in-water emulsions) or in bicontinuous cubic phases [25].

Infrared (IR) and Raman spectroscopy can be utilized to investigate the behaviors of hydration water at interfaces [26–29]. Nonhydrogen bonding water molecules have been reported by many researchers based on Raman spectroscopic analyses [29–31]. Typically, H₂O or D₂O is employed as solvents for biological species, and the contribution of the O-H or O-D vibration stretching modes from target molecules is analyzed. Walrafen et al. observed the isosbestic point in the O-D vibrational modes measured at different temperatures. In overlays of these spectra, each spectrum yielded a cross point at a specific frequency. By varying the temperature, the frequencies derived from pure α² and pure

**Figure 1:** Possible role of water in various fields.

**Figure 2:** Classification of water molecules. Illustration was drawn according to Eisenberg and Kauzmann [14]. (a) I-structure. (b) V-structure. (c) D-structure.
β² (whereby α and β correspond to polarizability and anisotropy, respectively) yielded the isosbestic point. This suggests that the hydrogen-bonded and nonhydrogen-bonded water are in a thermodynamically equilibrium state [32, 33]. The ΔΗ value of the OH–O bond was estimated to be –2.6 kcal/mol [34]. In Raman and IR measurements, several types of vibrational peaks are summed in one spectrum, and peak deconvolution is thus an effective tool in assigning the contribution of each molecule or bond [35–37]. These methods are very useful for evaluating the dynamic behaviors of water molecules.

Flexible molecules, such as solutes in aqueous systems, or molecules in self-assembly systems, exhibit dynamic behaviors in response to external stimuli. This is derived from the reorientation of the associated water molecules. Spin relaxation of hydrogen or oxygen isotopes can be measured as a function of time [38]. In general, the molecular dynamics can be evaluated at the order of nanoseconds to milliseconds. By contrast, the MRD analysis has been extensively employed (e.g., MD simulations, etc.) [42–44]. Furthermore, NMR measurements reflect the information of all the molecules from the entire system. However, it could be difficult to identify the signals of the hydration water and solvent water in solution NMR measurements. By contrast, the MRD analysis has been extensively studied to monitor the dynamic behavior of the molecules and has been shown to elicit results that are highly correlated with the outcomes of simulation studies [45–47].

The sum-frequency generation (SFG) vibrational spectroscopy can observe an asymmetric molecular fraction in the vicinity of the interface [48]. It is mainly used for analyzing the hydration state to the lipid membrane at an air-water interface [43], and it is applied to monitor the direction at which the water molecules orient themselves around the lipid head group (H-up, H-down) [49, 50]. Although there are limitations associated with the directionality at the interface and with the experimental conditions, SFG is a powerful tool used to evaluate the orientation of water molecules at the molecular surface.

2.2. Experimental Approach: Observations of Water Molecules as a Group. In dielectric relaxation spectroscopy, it is possible to observe the behavior of the dipole moments of water molecules [51–54]. If the system is swept by an alternating electric field, the dipole of the water molecule exhibits a Debye relaxation. Given that the relaxation time of water is relevant to the hydration characteristics, its value depends on the reorientation time of individual molecules [55]. The precise definition of the scale of each water group is still under discussion. In addition, the bulk water molecules affect the hydrated water molecules in the vicinity of the target molecule [41, 56, 57]. Therefore, it is necessary to consider the fact that the observed dynamics of water are cooperatively restricted compared to its original characteristics. As an improvement of the experimental method, the number of hydration water molecules can be estimated based on the deconvolution of the multiple relaxation peaks [44].

Dynamic light scattering (DLS) and the optical Kerr effect are sensitive to the refractive index of the solvent. Similar to the dielectric relaxation analysis, the polar tensor relaxation can be measured using these methods. Given that the molecular polar tensor of water is almost isotropic, these techniques essentially probe the collective translational rearrangements of the water molecules [56, 57]. Terahertz spectroscopy can measure the physical properties corresponding to the distortion of the hydrogen bond network in the far IR range. By comparing the bands near the bulk and the molecule, the hydrogen bond in the vicinity of the molecule can be detected. Utilizing this technique, the existence of a dynamic hydration shell with a thickness of 20 Å has been observed around proteins [58–61]. There are some assumptions in this definition pertaining to the restriction of this shell, including its uniform distribution, for example.

Dielectric properties relate to the water activity (w), that constitutes a representative index for the water affinity on food material. There are some reports on the application of the dielectric analysis mentioned above for the purpose of the investigation and monitoring of the quality of the food products [62, 63].

2.3. Experimental Approach: Indirect Observation of Water Behavior. Highly sensitive and specific information on the biological interface can be obtained from molecular probe methods. To evaluate the hydration environment at biological interfaces, numerous fluorescent molecules have been developed in which the probes are sensitive to their local environment [64]. Various probe molecules have been designed and optimized: herein the probe preferentially binds to the target molecule and elicits strong fluorescence signals reflecting their microscopic surroundings. Usually, the internal part of biomolecules (core of proteins, strand regions of nucleic base-pairs) is hydrophobic compared to water, and the inserted fluorescent probe can thus emit a stronger fluorescence signal. For example, the exposed hydrophobic site in the denatured protein or the self-assembly surface, such as the lipid membrane, can be detected by polarity-sensitive probes, such as the 8-anilino-1-naphthalenesulfonic acid (ANS) and 6-(p-toluidino)naphthalene-2-sulfonate (TNS) [65–69]. Solvent-sensitive fluorescent probes, such as 6-propionyl-2-dimethyl-aminonaphthalene (prodan), 6-dodecanoyl-2-dimethylaminonaphthalene (laurdan), ANS, and TNS, are extensively used for the characterization of the lipid membrane [70–73]. These molecules have a dipole moment following their excitation and induce the relaxation of the surrounding water molecules. Hence, they exhibit various fluorescence characteristics.
according to the degree of solvent relaxation [74, 75]. The specificity of the fluorescent probe’s location in the lipid membranes has advantages and disadvantages. An advantage is the fact that the choice of a specific probe enables us to analyze more localized information toward the lipid bilayer, such as the membrane surface region monitored by 1-(4-trimethylammoniumphenyl)-6-phenyl-1,3,5-hexatriene (TMA-DPH), for example. The membrane inner region can be monitored by 1,6-diphenyl-1,3,5-hexatriene (DPH) [76]. A disadvantage is the fact that the fluorescence property (quantum yield) can be varied depending on the polarity and viscosity of the solvent. Thus, the information obtained from a single fluorescence probe could include multiple factors. Accordingly, the alteration of the membrane property can be induced by an external molecule (e.g., protein binding) and vice versa. The use of multiple fluorescence probes can solve these problems. Therefore, it is recommended that several types of probes be used such that the interfacial polar environment can be systematically investigated [77–79].

The type and quantity of water molecules can be identified by using differential scanning calorimetry (DSC) based on the heat balance of the water freezing/thawing processes. In the polymer systems, the water molecules can be classified into the intermediate and free water types and can be observed with (a) endothermal peak temperatures > 0°C and (b) < 0°C, respectively [80, 81]. Because the water in polymers does not freeze at temperatures less than 0°C, it can be distinguished from free water (bulk water). Therefore, the amount of nonfreezing water can be evaluated by identifying the difference between the amount of intermediate water and free water obtained from the entire system. This is a very effective method because the heat capacity can be evaluated from the dilution/melting peak. Regarding the evaluation of water in the biomolecules, the structure of the molecule largely affects the hydration characteristics. Thus, the differentiation of the type of water constitutes a significant problem. Each experimental approach has limitations regarding the observable time allotted to the dynamics of the target molecule. According to previous reports, the experimental approaches used for the investigation of water dynamics are summarized in Figure 3.

2.4. Simulation Approach of Water Behavior. For the computational simulation of water dynamics, it is essential to set the force fields of water and coexisting molecules. Until now, many potential functions have been proposed to produce the molecular behaviors in simulation approaches [83]. Given that the most extensively used approaches are based on the theoretical and experimental results, various models of water molecules have been developed, such as TIP3P, TIP4P, SPC, etc. [84–87]. The average number of nearest neighboring molecules was increased to almost five in liquid water. Additionally, the average number of hydrogen bonds per molecule decreased to three in comparison to the state of ice. Recent studies revealed the possible structures of water clusters composed of four or eight molecules, and the mechanism responsible for the density fluctuations is well supported by simulation results [88]. Some representative water clusters, such as the cyclic pentamer, bicyclo-octamer, and tricyclo-decamer, are relatively stable, and the dynamic behavior exchanging continuous formation of hydrogen bonding networks has also been studied [89].

Considering the behavior of water as solvent, simulation calculations at the hydration layer must be conducted. The hydration shell can be defined as a group of water molecules in which the orientation of the dipole moment is in a good arrangement in the first layer (with a thickness which is approximately equal to 3.5 Å). However, the definition of the hydration shell (or layer) is controversial because the water molecules in these layers exhibit different properties depending on their interaction states with other molecules [13, 42, 59, 60]. The distance to the first minimum value in the radial distribution function can be defined as hydration shell including whole contributions of the motional properties of water molecules such as rotation, translation, and hydrogen bonds [82, 87, 90, 91]. Finally, the mechanical properties of the water molecules present in the hydrated shell can be investigated [92].

3. Hydration Layer

The water molecules randomly distribute depending on their dipole-dipole interactions and form a hydrogen bond network among water molecules via electrostatic interactions. Ultimately, a layer of water molecules is formed from a hydrophobic interface with a thickness that spans several nanometers [93, 94]. Although the definition of the hydration layer varies depending on the interface to be targeted, a similar tendency can be observed within biological interfaces. Ueda et al. suggested a concept of structured hydration layers based on studies using the 17O NMR spin-lattice relaxation [95]. The authors defined the dynamic hydration number nDHN that represents the dynamic characteristics of hydration. The thickness of the structured water is at least several nm irrespective of the type of material constituting the interface, that is, the hydration layers comprising approximately 10 water molecules are structured [95].

Israelachvili et al. measured the intermolecular forces between mica plates in dilute KCl solutions [96]. The interaction of the two interfaces was well explained by the DLVO theory using distances of 10 nm or less. When the distance of the interfaces was less than 1.5 nm, the oscillations appeared at every 0.25 ± 0.03 nm [97]. In this case, 6–7 oriented water molecules existed between the mica plates. The short-range hydration force between the smooth rigid surfaces was always oscillatory as water molecules attached to the hydrated surface groups and formed an ordered layer [97]. This repulsive force is a synergistic hydrogen bond (polarized) interaction that attenuates as a function of distance from the surface [98, 99]. Interestingly, in flexible surfaces, such as lipid membranes, these vibrations are averaged into a monotonous repulsive force because of the roughness of the head group of lipid molecule and the repulsive thermal
fluctuation force that arises from the dynamic nature of the lipid membrane surface [100–102].

The hydration layer on the MgO solid surface is consistent of approximately 10 molecules (with single-molecule layers and layers with thicknesses spanning two or three molecules) [103]. The first water molecules orient in such a way that (a) one hydrogen atom faces the oxygen atom of MgO and (b) the OH groups are aligned parallel to the MgO plane. The thickness of the first layer is in the range of 0.2–0.3 nm, while the second layer is a more distributed alignment of the water molecules with thickness values in the range of 0.4–0.6 nm. Thicknesses equal to three or more layers are equivalent to bulk water, and the orientation anisotropy disappears. According to Zhang et al., the self-diffusion coefficient of water in the wall of the capillary ($\varepsilon = 5$) ranges from $4.5$ to $4.9 \times 10^{-9}$ m$^2$·s$^{-1}$ and that of bulk water is two times smaller and equal to $2.7 \times 10^{-9}$ m$^2$·s$^{-1}$ [104]. A group of water molecules in the form of a layer on such an interface has been extensively studied as functional water [13, 61].

In the polymer system, the water layer, commonly referred to as an intermediate layer, is assumed to adjust the accumulation of proteins [59, 82]. In a separation system using a polymer, such as in artificial dialysis, grasping the activity of water molecules in this intermediate layer leads to the design of a high-performance separation membrane. The water molecules at these intermediate layers are measured by using DSC, dielectric spectroscopy, NMR, and so on. In the case of biopolymers, it is argued that a layer comprising a single molecule is defined as the first hydration layer, while a second hydration layer has a thickness that spans 2-10 molecules [13]. In biomolecules, some polar or charged sites appear on the surface that can strongly interact with the first layer of water molecules via hydrogen bonds or electrostatic interactions. These heterogeneous states in the hydration properties can result in the generation of electric fields. However, these properties have not been well clarified. The concepts related to the hydration layer according to the reviewed studies are summarized in Figure 4. The food materials derived from animals or plants can be considered as the assembled cell tissue, wherein the front part of the food material surface can consist of cell membranes. In the following sections, the correlation between the hydration behavior and molecular properties is discussed, especially focusing on the lipid membranes as representatives of the potential functions of biological interfaces.

4. Hydration Properties in Lipid Membranes

The lipid bilayer membrane provides different polar environments, whereby the surface regions from the lipid head group to the glycerol group are hydrophilic. In contrast, the inner membrane region is hydrophobic owing to the accumulated hydrocarbon chain [105]. For the experimental study of lipid bilayer membranes, the “liposome” can be used as the artificial self-assembled entity with a phospholipid membrane (Figure 5). The hydration layer is formed between bulk and hydrophobic core regions. Herein, the word “membrane surface” indicates the hydrophilic region of the lipid bilayer, which acts as the interface of interaction with the surrounding solvent water. The “membrane interface” indicates the border between the hydrophilic and the

Figure 3: Experimental approach for the investigation of water dynamics. The molecular properties of water are summarized according to Fogarty and Laage [82].
hydrophobic regions of the membrane, that is, the region around the carbonyl group of the lipid molecules. The hydration state of the lipid membrane is complicated (cannot be simply defined) owing to the various contributing factors, such as for example, the chemical structure of the lipid head group, acyl chain packing state, lateral interaction between lipids, etc. A typical zwitterionic phospholipid, e.g., diacylglycerophosphocholine (PC), known as lecithin, possesses both a negatively charged phosphate group and a positively charged choline group. These zwitterionic head
groups are strongly hydrated via hydrogen bonds with solvent water. In a phosphate group, the bound water molecules are retained in a tetrahedral structure around the oxygen atoms [106–108]. The water molecules associated with the positively charged choline group are weakly connected to each other in a clathrate hydration state [106, 107]. As shown in the 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) membrane, the water molecules around the CH₂ moieties in the choline group are distributed according to the Gaussian distribution, regardless of the membrane phase state. Hence, their existence cannot be ignored [82].

In general, the hydration layer formed on the surface of the PC membrane prevents the access of water-soluble molecules, despite the existence of the thin hydration layer which has a thickness of approximately 1 nm [24]. It is still unclear how such a hydration layer can inhibit the access to external molecules. A thicker hydration layer can stabilize the self-assembled structure of the membrane, which is one of the important topics related to the design of drug carriers [110]. The hydration property of the membrane can be varied depending on the lipid head group. In addition, the lipids modified with polyethylene glycol (PEG) and glycolipids allow the existence of large numbers of water molecules in the form of a hydrated sponge [111]. In consideration of other factors that influence the hydration behaviors, the simulation study on the associated dynamics has investigated the fact that the hydrogen bonds between the water molecules are strengthened on the lipid membrane that is composed of phosphoethanolamine (PE) lipids [107]. The PE has a small head group which creates a flat membrane surface, thus resulting in the enclosure of lipid molecules. From this point-of-view, it is considered that the uniformity of the charge characteristics of the surface layer also contributes to the stability of the bonds between the water molecules [112].

In research studies using SFG, the direction and hydration characteristics of the water molecules at the hydrophilic/hydrophobic interface were evaluated. One of the advantages of SFG measurements is the observation of the orientation of molecules localized at the bulk-membrane interface [48, 106]. In the lipid monolayer system formed at the air-water interface, the water molecules in anionic lipid membranes, such as 1,2-dipalmitoyl-sn-glycero-3-phospho(1’-rac-glycerol) (DPPG), are oriented to direct the hydrogen atoms toward the lipid head, while the water molecules exceeding the phosphate group are oriented so that the hydrogen atoms are directed in the opposite direction [48]. For cationic lipid membranes, such as 1,2-dipalmitoyl-3-trimethylammonium-propane (DPTAP), the orientation of the hydration water layer is opposite to that in the case of DPPG, wherein the water on the bulk side and inside the membrane directs oxygen atoms toward the head group [48]. Such orientations of water are very important for the understanding of the complex hydration environment at the lipid membrane surface.

From the structural point-of-view, it has also been suggested that the water molecules existing among acyl chains contribute to the stability of the structure of the molecule, based on simulation and FTIR studies [113, 114]. Alarcón et al. analyzed the hydration state of DPPC using simulations, and confirmed that the water molecules between the acyl chains formed a chain-like configuration which was stabilized in the acyl chain pocket. Additionally, these results were consistent with the experimental results [19]. In the case of lipid molecules which possess hydrogen bond donor or acceptor groups, such as sphingomyelin, the water molecules can align with respect to the NH group of the backbone structure as well as with respect to the phosphate and choline groups because of the strong hydrogen bond acceptors of oxygen atoms in water. Comparison of PC and sphingomyelin with the same acyl chain lengths indicates that the sphingomyelin membranes have multiple hydration layers owing to the hydrogen bond with solvent waters, as confirmed by NMR measurements [115]. Regarding the unsaturated sphingomyelin, the possibility of intermolecular hydrogen bond interactions can be replaced by the hydrogen bonds formed with water molecules that increase molecular flexibility according to quantum chemistry approaches [116]. It is suggested that the subtle configuration differences, such as saturated and unsaturated configurations, could affect the hydration behavior.

Generally, the lipid membranes composed of unsaturated acyl chain lipids exhibit a hydrophilic property owing to their loosely packed lipid orientation. By contrast, the lipid membrane—composed of saturated lipids—elicits rather hydrophobic properties owing to the high-packing-lipid density at the temperature below the phase transition temperature. The lipid rafts, mainly composed of sphingomyelin and cholesterol, are hydrophobic because of their highly packed membrane states [117]. The configuration of the “umbrella model,” whereby the head group of sphingomyelin covers cholesterol and the shielded inner membrane region, may contribute to the dehydrated inner membrane environment [118]. It is considered that this umbrella structure moves the hydration water layers around the head group out. Finally, the expelled water molecules can be accumulated like a shell, which might be observed as high-density water molecule layers at the membrane surface.

As shown in the examples above, the hydration state in the lipid membrane is very complicated, and mechanisms of stabilization of each hydration can be varied by different molecules. However, it is a very interesting task to ascertain whether the membrane properties determine the hydration characteristics, or whether the hydration characteristics determine the membrane properties, and how this works for the interaction with biomolecules. In the next section, the membrane characteristics determined by the hydration properties are introduced.

5. Membrane Properties Determined by Hydration

Considering the interfacial interaction, electrostatic interactions operate at longer distances. By contrast, the hydration waters provide a strong repulsion (referred to as a hydration force, as already mentioned in Section 3), which applies at very short distances [119]. Based on the
measurements of surface forces developed between bilayer membranes, it was confirmed that there was an additional repulsive force derived from the hydration force for hydration thicknesses of approximately 2 nm or less [120]. Additional energy is required to induce the dehydration of the head group region. Additional approaches increase the repulsion force at distances within the range of 0.2-0.3 nm [121]. This repulsive force prevents the adhesion of each lipid membrane. Therefore, it is interesting to know how lipid membranes interact with other biomolecules, such as enzymes, beyond this hydration wall.

In the zwitterionic lipid bilayer, the lipid molecules have a net charge that is equal to zero. Surface charge properties are determined by the hydration shell. The carbonyl and phosphate groups possess lone-pair electrons, and the water molecules bound to them can be polarized. The charge properties of the fixed layer (lipid) are shielded by anions (van der Waals interactions rather than electronegativity) and cations [122]. The width of this layer is defined by the slip plane which determines the ζ potential [123]. Given that PC groups have phosphate groups oriented outward, it can be assumed that slip planes from phosphate groups can be considered. However, attention should be paid to interfaces where unevenness occurs on the surface layers, such as negatively charged phosphatidylserine (PS) and hydrogen donor phosphatidylinositol (PI) moieties. Thus, the heterogeneity in the lateral lipid distribution can be an important factor to generate a potential field for the interaction.

The degree of water saturation also affects the orientation of the dipole moment of the lipid head group. In the highly hydrated interfacial region, the orientation of hydration water molecules around the carbonyl and phosphate moieties could modulate the orientation of the head group [114, 124]. For low degrees of hydrations, the direction of the dipole potential in the head group is reversed, and the surface charge potential of the membrane thus exhibits negatively charged properties [125, 126]. The increase in the mean head area can be induced by hydration swelling. For example, the mean head group area of a lecithin molecule and the distance between neighboring lipids molecules on its membrane surface are 0.7 nm² and 2.7 nm, whereas 0.45–0.55 nm² and 1.3 nm for PE lipids, respectively. Thus, the repulsive force between the PE head groups is quite small [122]. Water molecules in the hydration shell of the membrane strongly bind to polyhydroxyl compounds, such as trehalose, sucrose, and arbutin, thus affecting the dipole potential of the lipid [127–132]. The direct correlation between the polarized water among the polar head group and the dipole potential can be explained by water displacement by trehalose and phloretin [127]. The hydration may support the alignment of the dipole moment of the lipid head group and results in the adjustment of the electrochemical property of the lipid membrane.

The permeability of water molecules affects the packing of the membrane plane and domain formation [125, 133–135]. According to a prior review that summarized the membrane structure and its repulsion against permeability, the number of water molecules that stabilize the inner membrane plane is considered to be 10 per PC [136]. Water molecules directly hydrating the carbonyl and phosphate groups are impermeable, and the indirectly hydrated water molecules are regarded as permeable. When the population of water molecules exceeds the referred number, the lipid-lipid interactions can be affected by the existence of water. As the packing density in the hydrophilic part of the membrane decreases, the water penetration increases. It also relates to the ability of water molecules to be exchanged because the water molecules could strongly interact with the polar groups of other molecules that could prevent the water penetration via hydrogen bonds. For example, in the presence of the dextran or PEG, water molecules in the hydration shell of the lipid membrane can be extruded owing to the strong affinity with the polar molecules [137, 138]. Dehydration based on the outward penetration compresses the membrane and reduces its volume [139]. Structural changes would occur in an energetically favorable manner by adjusting some parameters, such as the membrane packing density, or the orientation of the lipid head group. These behaviors are elicited in the membranes and in the interactions between individual molecules. Therefore, a configuration defect caused by water extrusion can be a target to replace other molecules, such as the lipid head group or the penetration of other molecules [107, 140].

The water exchange between the inner part of the lipid membrane and the bulk region was observed in NMR experiments [141]. The orientation of the hydrophilic group could influence the water exchange. The amount of hydration water will either tend to decrease as the orientation tilts, or the hydration layer around the head groups will prevent the exchange of water [142–144]. This exchange could be the important concept to consider the activity of hydrated water. When the water enters the membrane from the bulk, the lateral surface tension increases [107]. The increase in the surface pressure would occur in the penetration of the peptide. Specifically, for the analysis of the interaction with peptides in the monolayer system, the “cut off” surface pressure is used in which the surface pressure becomes insensitive to the peptide penetration [122, 145]. The “cut off” surface pressures for PCs and for PEs are approximately 40 mN/m and 30.6 mN/m, respectively. Considering the surface pressure of the saturated PC monolayer, which is approximately in the range of 46.6–48.0 mN/m, the surface pressure of the PC monolayer at which is insensitive to the peptide penetration is relatively lower. From these results, it is suggested that the protein does not penetrate at pressures that are much lower than the pressures at which the head group is filled. This indicates that an extra free energy is required to adsorb the protein, thus suggesting that the thermodynamically active water exceeds that within the hydrated shell. The significantly lower “cut off” surface pressure of the PE monolayer indicates the existence of a smaller amount of thermodynamically active water. In other experiments, lipid membranes composed of PEs interact with protease at remarkably lower rates compared to PC [146]. The difference between the restricted hydrated water molecules around carbonyl or phosphate groups, and water molecules freely dispersing among the head group region, could determine the surface active
energy to interact with other molecules by replacing the hydrogen bonds [146]. The high free energy among lipids implies that these free water molecules could also affect the surface pressure [107]. According to the studies introduced in this review, the correlation of the hydration properties and lipid molecules are summarized in Figure 6.

6. Conclusions

Water is one the most basic and fundamental molecule in nature. It exists in various materials and modulates their unique and interesting properties, yet its actual function and role are still ambiguous. In this review, we introduced the characteristics of molecules hydrated with water by considering the hydration behavior at the lipid membrane interface as an example of a self-assembly system. As mentioned in the Introduction section, it becomes important to understand the science of water to clarify the hydrogen-bonding properties of water (what is and how it is hydrogen bonded) and the classification (how much water of each type is present) around the biomaterial. Specifically, in food chemistry, the structural stability of the cell membrane and the homeostasis of the cell function or protein activity are significant issues in the preservation of the freshness of the material. Given that the number of consumers interested in fine food has been growing in search of healthy, tasty, and antiaging products, the design of food materials with improved performances is required. To deal with these demands, many chemical approaches have been attempted. Some require increased nutrient permeability, while others need to maintain enzyme activity. Therefore, the fundamental behavior of the cell and its activity should be understood properly in order to functionalize and sustain its physiological values.

As shown in the case of lipid membranes, the hydration property of water determines the orientation of the head group of the lipid membrane, the lipid area, the exchangeability with bulk water, and the repulsion forces.
Additionally, water molecules themselves adjust their population depending on the clustering properties in the most energetically favorable ways. The characteristics determined by hydration could lead to the surface charge characteristics of the entire system, interaction with other molecules, and the fusion of lipid membranes. This functional adjustment induced by water is not limited to lipid membranes, but contributes to a basic structure for other molecules, such as proteins, nucleic acids, and others. Various promising prospects can be expected for the health or medicinal efficacy from the synthetic chemistry viewpoint. However, the safety is always challenging. Water is an abundant molecule, and controlling its functional activities aspires to use methods with the lowest energy cost and highest safety responses. Based on the use of simulation calculations and advanced experimental techniques, steady understanding of the function of the water molecule has been accomplished. Future tasks will include the classification based on hydration characteristics that will be further required to comprehend the various systems, to understand the trends, and to grasp the original characteristics of water that could ultimately lead to the engineering of water functions.

**Conflicts of Interest**

The authors have no conflicts of interest to declare.

**Acknowledgments**

This work was primarily supported by the Japan Society for the Promotion of Science (JSPS) KAKENHI Grant-in-Aids for Scientific Research (A) (26249116), Grant-in-Aids for Young Scientists (B) (16K18279), Grant-in-Aids for Challenging Exploratory Research (T15K142040), and Grant-in-Aids for Research Fellow (JP18J11666).

**References**


Submit your manuscripts at
www.hindawi.com