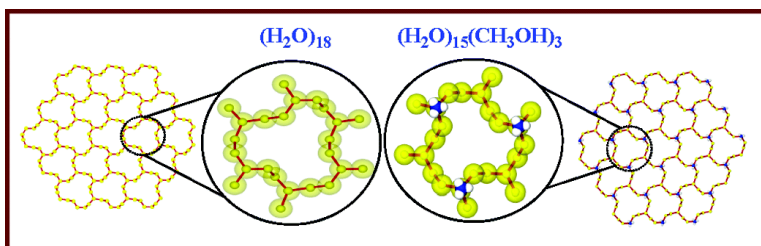**Article**

Characterization of Supramolecular (HO) Water Morphology and Water-Methanol (HO)(CHOH) Clusters in a Novel Phosphorus Functionalized Trimeric Amino Acid Host

Kannan Raghuraman, Kavita K. Katti, Leonard J. Barbour,
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$(H_2O)_{18}$ $(H_2O)_{15}(CH_3OH)_3$

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Characterization of Supramolecular (H₂O)₁₈ Water Morphology and Water-Methanol (H₂O)₁₅(CH₃OH)₃ Clusters in a Novel Phosphorus Functionalized Trimeric Amino Acid Host

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Abstract: Phosphorus functionalized trimeric alanine compounds (L)- and (D)-P(CH₂NHCH(CH₃)COOH)₃ **2** are prepared in 90% yields by the Mannich reaction of Tris(hydroxymethyl)phosphine **1** with (L)- or (D)- Alanine in aqueous media. The hydration properties of (L)-**2** and (D)-**2** in water and water−methanol mixtures are described. The crystal structure analysis of (L)-**2**·4H₂O, reveals that the alanine molecules pack to form two-dimensional bilayers running parallel to (001). The layered structural motif depicts two closely packed monolayers of **2** each oriented with its phosphorus atoms projected at the center of the bilayer and adjacent monolayers are held together by hydrogen bonds between amine and carboxylate groups. The water bilayers are juxtaposed with the H-bonded alanine trimers leading to 18-membered (H₂O)₁₈ water rings. Exposure of aqueous solution of (L)-**2** and (D)-**2** to methanol vapors resulted in closely packed (L)-**2** and (D)-**2** solvated with mixed water−methanol (H₂O)₁₅(CH₃OH)₃ clusters. The O−O distances in the mixed methanol−water clusters of (L)-**2**·3H₂O·CH₃OH and (D)-**2**·3H₂O·CH₃OH (O−O(average) = 2.857 Å) are nearly identical to the O−O distance observed in the supramolecular (H₂O)₁₈ water structure (O−O(average) = 2.859 Å) implying the retention of the hydrogen bonded structure in water despite the accommodation of hydrophobic methanol groups within the supramolecular (H₂O)₁₅(CH₃OH)₃ framework. The O−O distances in (L)-**2**·3H₂O·CH₃OH and (D)-**2**·3H₂O·CH₃OH and in (H₂O)₁₈ are very close to the O−O distance reported for liquid water (2.85 Å).

Introduction

The chemistry, physics, and biological aspects of hydrogen bonded liquids have attracted considerable theoretical and experimental interests.[1−3] Water and methanol constitute important prototypes of hydrogen bonded liquids and investigations toward understanding hydrogen bonding patterns that exist within the individual liquids, and also more complex phenomenon that ensue upon mixing H₂O and MeOH, has been at the focus of attention for a number of decades.[1−3] Because water plays an indispensable role in life-sustaining processes, investigations on its structure, properties and functions have received more scientific attention than any other substance.[1−3] Several investigations to date, have provided evidence that the water trimer,[4] tetramer,[5] and pentamers[6] have hydrogen bonded 2D cyclic minimum energy structures whereas pioneering efforts by Saykally and others suggest 3D geometries for the larger water clusters with the hexameric forms representing the transition from 2D cyclic to the 3D geometries.[7−11] Recent studies are focused on unraveling structural morphologies of water cocrystallized with various chemical entities because the lattice of a crystal host offers an attractive environment for stabilizing various topologies of water clusters and thereby provide quantitative characterization of the hydrogen bonded networks that exist in liquid water.[3,12−15] Studies toward

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understanding the growth and properties of water clusters that are larger than hexamers are highly relevant in understanding solvation processes although the development of large size water clusters has been a challenging scientific endeavor.[8,16−18]

Hydrogen bonding liquid methanol (MeOH) is the simplest amphiphile functionalized with hydrophobic (−CH₃) and hydrophilic (−OH) groups. Understanding of the solvation properties of MeOH as it relates to the disposition of hydrophobic/hydrophilic groups in polar or nonpolar solvents is of fundamental importance in biological and chemical sciences because amphiphiles are essential constituents making up cell membranes and are also extensively used in chemical industries as micelles (e.g., detergent action). In fact, the eccentric properties associated with water-alcohol mixtures have been the focus of numerous scientific investigations and significant technological applications.[16,18−21] Frank and Evans interpreted the unusual entropic changes that ensue upon mixing methanol and water by proposing that the hydrogen bonds of water act like an "ice berg" and encapsulate the hydrophobic headgroups of methanol in a clatharate-like fashion,[22] although this model has never been confirmed by concrete structural evidence. In contrary to this widely accepted model, Crain and co-workers have recently provided experimental evidence inferring that the hydrogen bonded network structure of water is not lost upon mixing methanol with water and that the polar hydroxyl group of methanol dictates the thermodynamic properties of methanol−water mixtures.[23,24]

Indeed, structural elucidations and understanding hydrogen-bonding properties of supramolecular networks of water and water-methanol at nanometer scales have been a topic of significant challenge.[3] Well characterized mixed water−methanol clusters are rare,[25−28] although structural elucidation of such clusters is imperative to gaining insight into the anomalous properties and to understanding the hydrogen bonding and dynamics of water−methanol binary mixtures. We, herein, report (a) the experimental evidence for the existence of a 2D cyclic supramolecular (H₂O)₁₈ morphology within the layers of (HOOCCH(CH₃)NHCH₂)₃P, **2**, with structural features (average O−O bond distance in (H₂O)₁₈ = 2.858 Å) similar to those of liquid water (O−O bond distance in liquid water = 2.85 Å);[17] (b) Our results on the application of single-crystal X-ray diffraction methods to probe the molecular scale hydrogen bonded structure of mixed supramolecular water-methanol (H₂O)₁₅(CH₃OH)₃ clusters and the evidence for the accom-

Scheme 1. Synthesis of P(CH₂NHCH(CH₃)COOH)₃ (**2**)
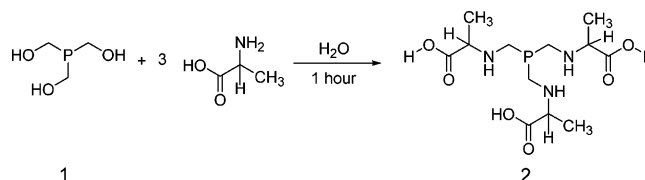
modation of methanol groups within the hydrogen bonded (H₂O)₁₈ framework.

Results and Discussion

Synthesis and Characterization of (L)-2 and (D)-2. As part of our continued efforts in the design and development of cancer diagnostic and therapeutic agents,[29,30] we have recently developed new strategies for the preparation of peptide-mimetic dimeric and trimeric amino acid conjugates.[31] Phosphorus functionalized trimeric amino acid (L)-(HOOCCH(CH₃)NHCH₂)₃P (**2**) and (D)-(HOOCCH(CH₃)NHCH₂)₃P (**2**) were prepared by the addition of tris(hydroxymethyl phosphine) **1** to 3-fold excess of (L)- or (D)-alanine, via a Mannich-reaction pathway, in water in 90% yields (Scheme 1). Compounds (L)-**2** and (D)-**2** are white, air-stable, crystalline compounds and are readily soluble in water. The ³¹P{¹H} NMR spectra of (L)-**2** and (D)-**2** in D₂O showed sharp singlets at δ −39.9 ppm. The ¹H and ¹³C NMR spectra of (L)-**2** and (D)-**2** in D₂O are consistent with the structure. The mass spectrum of (L)-**2** showed a molecular ion [M+H⁺] peak with m/z = 338.0 consistent with the formula weight (337.31).

Hydration Studies of (L)-2 and Characterization of (H₂O)₁₈. The high solubility and a balanced hydrophobic/hydrophilic functionalities in the trimeric alanine compound **2** prompted us to utilize this molecule as a host template with the hope of encapsulating water clusters within its crystal lattice. Indeed, this trimeric alanine molecule **2** crystallizes with four molecules of water as revealed by X-ray crystallography. The crystallographic data are summarized in Table 1.

The crystal structure analysis of (L)-**2**·4H₂O, reveals that the alanine molecules pack to form two-dimensional bilayers running parallel to (001). Figure 1 shows perspective view of the structurally rigid (H₂O)₁₈ supramolecular ice-like hydrogen bonding arrangement of water molecules. The layered structural motif depicts two closely packed monolayers of **2** each oriented with its phosphorus atoms projected at the center of the bilayer and adjacent monolayers are held together by hydrogen bonds between amine and carboxylate groups. Space filling model as shown in Figure 1 depicts the alanine amino acid clefts interfacing the supramolecular ice-like (H₂O)₁₈ bilayers. The ball-and-stick perspective view shows how the water bilayers are juxtaposed with the H-bonded alanine trimers leading to 18-membered water rings. This motif consists of two closely packed monolayers of (L)-**2**, each oriented with its phosphorus atoms projected toward the center of the bilayer. Although no direct intermolecular interactions are apparent between the molecules of each monolayer, adjacent monolayers are held together by hydrogen bonds formed between their amine and

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Table 1. Crystallographic Data for L-**2**·4H$_2$O, L-**2**·3H$_2$O(CH$_3$OH) (193 K and 293 K), D-**2**·3H$_2$O(CH$_3$OH)

compd	L-**2** containing (H$_2$O)$_{18}$	L-**2** containing (H$_2$O)$_{15}$(CH$_3$OH)$_3$ (T = 193 K)	D-**2** containing (H$_2$O)$_{15}$(CH$_3$OH)$_3$ (T = 193 K)	L-**2** containing (H$_2$O)$_{15}$(CH$_3$OH)$_3$ (T = 293 K)
formula	C$_{12}$H$_{32}$N$_3$O$_{10}$P	C$_{12.50}$H$_{33}$N$_3$O$_{10}$P	C$_{12.50}$H$_{33}$N$_3$O$_{10}$P	C$_{12.50}$H$_{33}$N$_3$O$_{10}$P
formula weight	409.37	416.39	416.39	416.39
T	193(2) K	193(2) K	193(2) K	293(2) K
λ (Å)	0.71073	0.71073	0.71073	0.71073
crystal system, space group	hexagonal, P6$_3$	hexagonal, P6$_3$	hexagonal, P6$_3$	hexagonal, P6$_3$
a (Å)	12.2838(7)	12.2122(4)	12.2272(7)	12.3133(3)
c (Å)	15.7572(14)	15.8761(11)	15.8762(17)	16.0239(9)
volume	2059.1(2) Å^3	2050.51(17) Å^3	2055.6(3) Å^3	2104.01(14) Å^3
Z,^{a} D_{Calc}	4, 1.321 Mg/m^3	4, 1.349 Mg/m^3	4, 1.345 Mg/m^3	4, 1.315 Mg/m^3
$F(000)$	880	896	896	896
crystal size (mm)	0.50 × 0.25 × 0.25	0.45 × 0.25 × 0.05	0.50 × 0.35 × 0.05	0.50 × 0.35 × 0.10
θ range (°)	1.91 to 27.12	1.93 to 27.15	1.92 to 27.09	1.91 to 27.11
reflections collected/unique	12424/2992 [R(int) = 0.0468]	12976/2865 [R(int) = 0.0616]	12936/3039 [R(int) = 0.0661]	13419/3043 [R(int) = 0.0385]
GOF	1.066	1.080	1.041	1.089
R	0.0485	0.0519	0.0529	0.0415
R_w	0.1274	0.1138	0.1191	0.1007

^{a} Z = 4 can be rationalized as follows: There are 6-symmetry related positions in the unit cell, and the asymmetric unit consists of two separate fragment each consisting of a third of a molecules. For example, two independent trimeric alanine molecules are included in an asymmetric unit (2/3 × 6 = 4) and two water oxygens are located on the 3-fold axis 1/4 ((2 + 2/3) × 6) = 4).
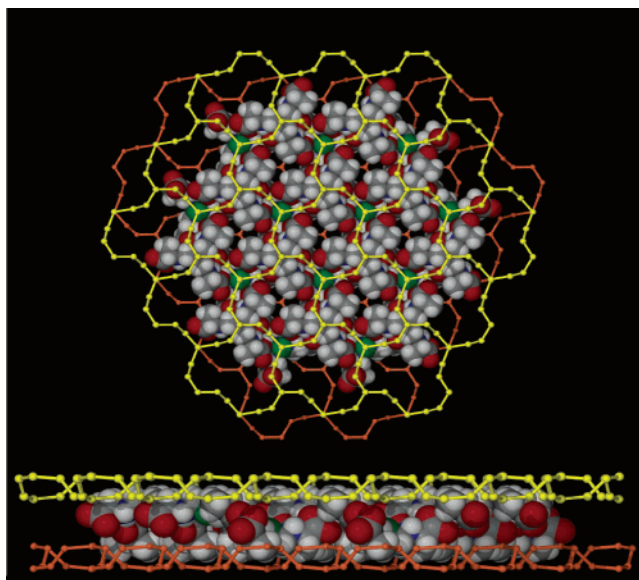

Figure 1. Perspective view of supramolecular (H$_2$O)$_{18}$ hydrogen bonding arrangement of water molecules. Colors are as follows: gray, carbon; dark blue, nitrogen; green, phosphorus; red, oxygen (carboxyl); yellow and orange, oxygen (water); white, hydrogen. Hydrogen atoms in water are omitted for clarity.

Figure 2. Perspective view showing triangular amino acid clefts accommodating four water molecules. Colors are as follows: gray, carbon; red, oxygen (carboxyl); green, phosphorus; yellow, oxygen (water); dark blue, nitrogen; white, hydrogen.

carboxylate groups. Thus, within the bilayer, each molecule of one monolayer interacts with three molecules of the adjacent monolayer. This intricate hydrogen bonded arrangement ensures that the bilayer is structurally rigid. Both surfaces of each bilayer are hydrophobic, being composed primarily of hydrogen atoms on aliphatic carbon atoms. Adjacent bilayers are in van der Waals contact with one another by virtue of these hydrogen atom surfaces. However, the packing arrangement of the molecules of each bilayer results in the formation of relatively large fissures in its surface. These fissures expose carboxylate oxygen atoms just below the plane of the surface, thus forming triangular hydrophilic clefts in the bilayer (Figure 2). Indeed, the clefts of adjacent bilayers overlap to form a two-dimensional network of hydrophilic channels running through the structure. These channels are occupied by water molecules which hydrogen bond to one another to form fused 18-membered water rings
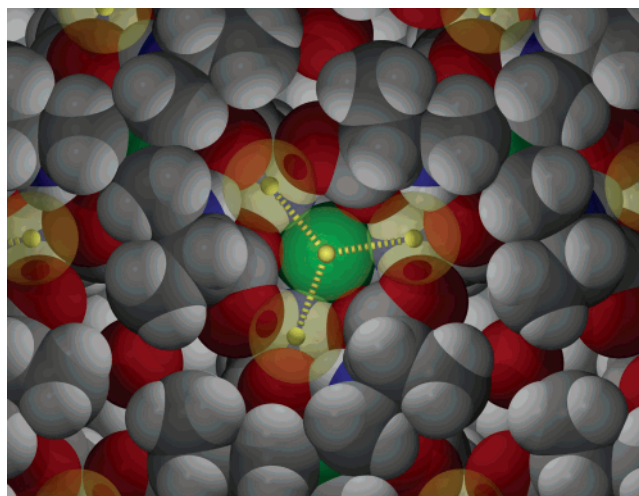
(Figure 1). The water molecules in the ice layer also hydrogen bond to the exposed oxygen atoms of the carboxylate groups of **2**. Each triangular cleft on the surface of the bilayer accommodates four water molecules (Figure 2). Three of these molecules are arranged as a regular equilateral triangle (side length = 4.951 Å). The fourth water molecule is situated 0.25 Å above the centroid of the triangle and hydrogen bonds to those at the vertexes (O···O = 2.869 Å). A slight overlap of the triangle vertexes of adjacent bilayers exists such that each triangle links to three triangles of the neighboring bilayer via an O···O hydrogen bond (2.848 Å) between the water molecules. The interplanar spacing between the best-fit planes defined by the triangles of adjacent bilayers is approximately 2 Å.

The nature of interaction of (H$_2$O)$_{18}$ with the host template (L)-**2** was probed by thermogravimetric analysis (TGA). The TGA data on (L)-**2**·4H$_2$O showed an 17.352% weight loss between room temperature and 150 °C which corresponds to 3.94 molecules of water (Figure 3). The facile removal of four
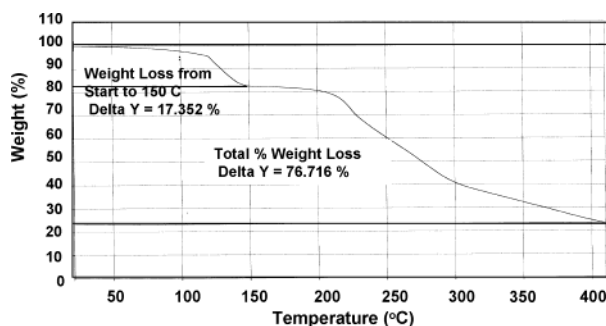
Figure 3. Thermogravimetric Curve of (L)-**2**·4H$_2$O at a heating rate of 10 °C.

molecules of water from (L)-**2**·4H$_2$O reconfirms that the (H$_2$O)$_{18}$ polygons have weak interactions with the host framework in **2** and that the supramolecular (H$_2$O)$_{18}$ cyclic water manifests liquid water properties. Indeed, such a large supramolecular 2D cyclic structure for water has not been observed before either in liquid water or in other low dimensional ice. Hitherto, pentagons/hexagons have been characterized to be the basic hydrogen bonding subunits in water clusters [(H$_2$O)$_n$ n = 8, 10, and 12] in supramolecular compounds.[3,12−15] The unique structural morphology of water in (H$_2$O)$_{18}$ is in stark contrast to the ordered pentagonal and hexagonal water morphologies found in a majority of organic/inorganic supramolecular compounds.[12−15] This supramolecular association of 2D water molecules in layers of (L)-**2** is presumably enforced by the shape of trimeric alanine host channels, whose relatively narrow openings inhibit the formation of more stable three-dimensional water clusters. The average O−O distance of 2.858 Å in the supramolecular (H$_2$O)$_{18}$ morphology of (L)-**2** is strikingly similar to the O−O distance of 2.85 Å found in liquid water suggesting close structural similarity of (H$_2$O)$_{18}$ in (L)-**2** to liquid water structure.[17]

This experimentally observed (H$_2$O)$_{18}$ water cyclic morphology in **2** may be rationalized as follows: As the hydrophilicity of the solute molecules increase, water clusters tend to grow in size to the hexameric and higher membered water rings.[8,17] Indeed, the high hydrophilicity of the trimeric alanine **2** may presumably result in a spatial arrangement of water such that it encloses hydrophobic sites within the polyhedral (H$_2$O)$_{18}$ water structure as a means of effecting minimal disruption from the preferred tetrahedral hydrogen bond arrangement. The O−O−O angle of 125.7° (average) observed in the supramolecular (H$_2$O)$_{18}$ is a significant deviation from the tetrahedral geometry and implies that this opening up of the O−O−O angle allows efficient perpetuation of hydrogen bonds.

Mixed Water−Methanol Clusters. Despite numerous investigations on water-alcohol binary mixtures, there is still considerable uncertainty about the nature of the hydration structure in aqueous alcohol.[16,18−21,32−40] To gain insight into the reasons for the anomalous thermodynamic properties of alcohol−water mixtures, it is imperative to perform detailed structural analysis of hydrogen-bonded networks of water in the vicinity of alcohols. The structural integrity of hydrogen bonded (H$_2$O)$_{18}$ in (L)-**2** and the interlayer spacing of ∼2 Å as found within the layers of (L)-**2** prompted us to investigate the feasibility of incorporation of other hydrogen bonding liquids within its layered lattice. In particular, we were interested in examining if the layers of (L)-**2** serve as a fertile hydrogen
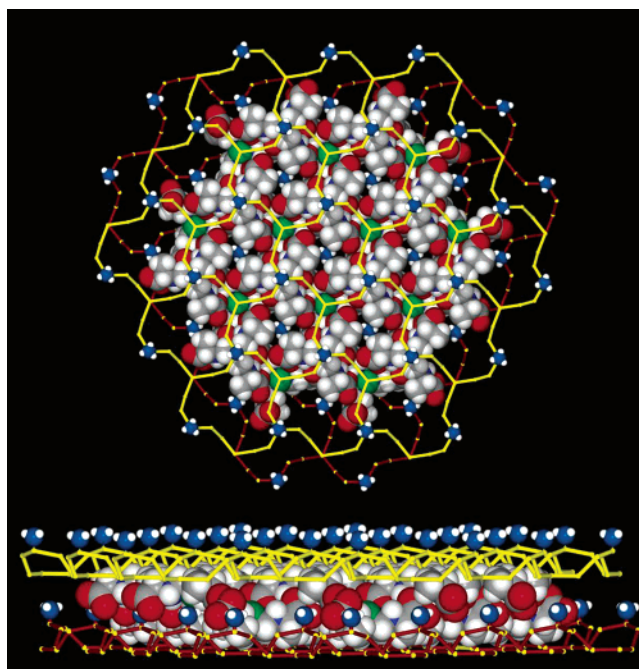

Figure 4. Perspective view of supramolecular hydrogen bonding network of water and methanol molecules in (H$_2$O)$_{15}$(CH$_3$OH)$_3$. Colors are as follows: gray, carbon; dark blue, carbon (methyl from methanol); green, phosphorus; red, oxygen (carboxyl); yellow and orange, oxygen (water); white, hydrogen.

bonding terrain to stabilize mixed water-methanol clusters. Our approach to the development of mixed methanol−water clusters involved exposure of methanol vapors to the aqueous solutions of (L)- or (D)-trimeric alanine compound **2**. Indeed, these studies resulted in the formation of mixed methanol−water clusters within the (L)- and (D)-trimeric amino acid **2** hosts. Single crystals suitable for X-ray diffraction studies were grown from exposing aqueous solution of (L)-**2** or (D)-**2** (0.05 g/mL of water) to the vapors of methanol (20 mL). The X-ray crystallographic data for (L)-**2**·3H$_2$O·CH$_3$OH and (D)-**2**·3H$_2$O·CH$_3$OH are shown in Table 1. The crystal structure of (D)-**2**·3H$_2$O·CH$_3$OH is isostructural to that of (L)-**2**·3H$_2$O·CH$_3$OH. We will, therefore, restrict our structural descriptions to (L)-**2**·3H$_2$O·CH$_3$OH. The crystal structure of (L)-**2**·3H$_2$O·CH$_3$OH constituted closely packed monolayers of the mixed methanol−water (H$_2$O)$_{15}$(CH$_3$-OH)$_3$ cluster. Perspective view of supramolecular hydrogen bonding network of water and methanol molecules in (H$_2$O)$_{15}$-(CH$_3$OH)$_3$ is given in Figure 4. Figure 4 shows space filling model of the alanine amino acid clefts interfacing the supramolecular mixed water−methanol (H$_2$O)$_{15}$(CH$_3$OH)$_3$ bilayers. The cross-sectional view (Figure 4) shows how the water-methanol bilayers are juxtaposed with the H-bonded alanine trimers leading to 18-membered water-methanol rings. The presence of the supramolecular hydrogen bonded mixed water-methanol (H$_2$O)$_{15}$(CH$_3$OH)$_3$ clusters encompass the amino acid layers in a two-dimensional network. Although no direct intermolecular interactions are apparent between the molecules of each monolayer, adjacent amino acid monolayers are held together by hydrogen bonds formed between their amine and carboxylate groups. Thus, within the bilayer, each molecule of one monolayer interacts with three molecules of the adjacent monolayer. This intricate hydrogen bonded arrangement ensures that the bilayer is structurally rigid. Both surfaces of each bilayer are hydrophobic, being composed primarily of hydrogen atoms on
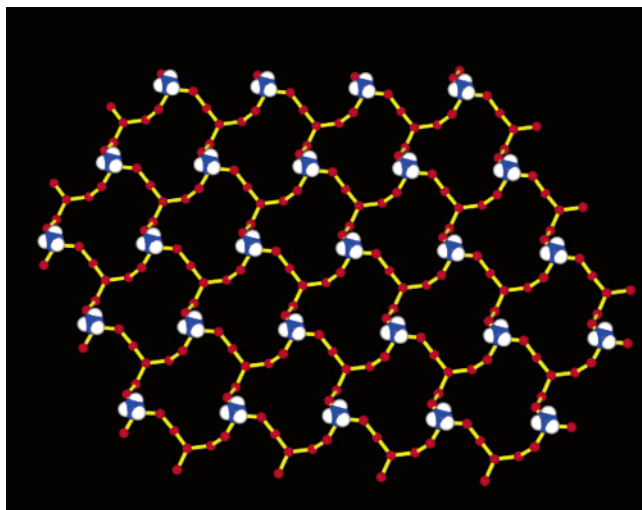
Figure 5. Perspective view showing the accommodation of hydrophobic headgroups of methanol within the water structure. Colors are as follows: dark blue, carbon (methyl from methanol); red, oxygen; white, hydrogen.

Table 2. O—O Distance (Å) in $(H_2O)_{18}$ and $(H_2O)_{15}(CH_3OH)_3$

bond	$(H_2O)_{18}$[a] (Å)	$(H_2O)_{15}(CH_3OH)_3$[b] (Å)	$(H_2O)_{15}(CH_3OH)_3$[c] (Å)	$(H_2O)_{15}(CH_3OH)_3$[d] (Å)
O1W—O2W	2.869	2.852	2.861	2.904
O2W—O3W	2.848	2.859	2.851	2.859
O3W—O1M	2.860	2.861	2.864	2.885

[a] Crystallized with (L)-2 crystal structure determined at 193 K. [b] Crystallized with (L)-2 crystal structure determined at 193 K. [c] Crystallized with (D)-2 crystal structure determined at 193 K. [d] Crystallized with (L)-2 crystal structure determined at 293 K.

aliphatic carbon atoms. Adjacent bilayers are in van der Waals contact with one another by virtue of these hydrogen atom surfaces. However, the packing arrangement of the molecules of each bilayer results in the formation of relatively large fissures in its surface. These fissures expose carboxylate oxygen atoms just below the plane of the surface, thus forming triangular hydrophilic clefts in the bilayer. Indeed, the clefts of adjacent bilayers overlap to form a two-dimensional network of hydrophilic channels running through the structure. These channels are occupied by water and methanol molecules which hydrogen bond to one another "cooperatively" to form a supramolecular two-dimensional lattice composed of fused 18-membered rings in $(H_2O)_{15}(CH_3OH)_3$ (Figure 4). The extended structure is stabilized by an extensive array of intermolecular hydrogen bonds between layers of molecules with three molecules of the hydrophobic methanol encompassing the supramolecular $(H_2O)_{15}(CH_3OH)_3$ structure (Figure 5).

The X-ray structural details obtained for (L)-**2**·3H$_2$O·CH$_3$-OH and (D)-**2**·3H$_2$O·CH$_3$OH (Figures 4 and 5) clearly suggests that the solute **2** in water upon exposure to the vapors of methanol, produces a mixed water-methanol cluster $(H_2O)_{15}(CH_3OH)_3$ constitution within **2**. The molecular constitution of the mixed water-methanol clusters in (L)-**2**·3H$_2$O·CH$_3$OH further confirms that the hydrophobic methanol molecules are readily accommodated within the hydrogen bonded water structure (Figure 5). The O—O distances (Table 2) in the mixed methanol—water clusters of (D)-**2**·3H$_2$O·CH$_3$OH and (L)-**2**·3H$_2$O·CH$_3$OH (O—O(average) = 2.857 Å) are nearly identical to the O—O distance observed in the supramolecular $(H_2O)_{18}$ water structure (O—O(average) = 2.859 Å) implying the

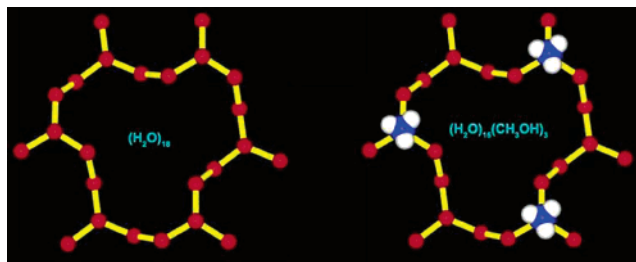**Figure 6.** Perspective view for the comparison of structurally rigid supramolecular $(H_2O)_{18}$ and mixed methanol—water $(H_2O)_{15}(CH_3OH)_3$ clusters. Hydrogen atoms are omitted for clarity. Colors are as follows: dark blue, carbon; red, oxygen; white, hydrogen.

retention of the hydrogen bonded structure in water despite the accommodation of the hydrophobic methanol groups within the supramolecular $(H_2O)_{15}(CH_3OH)_3$ framework. It is even more striking that the O—O distances in (D)-**2**·3H$_2$O·CH$_3$OH and (L)-**2**·3H$_2$O·CH$_3$OH and in $(H_2O)_{18}$ (found in D-**1**) are very close to the O—O distance reported for liquid water (2.85 Å).[17]

Global Picture of H$_2$O—MeOH Interactions. Dielectric spectroscopy, thermodynamic measurements, neutron scattering and simulation studies on dilute aqueous methanol solution suggest that water molecules solvate the polar moiety in methanol before solvating the non polar moiety.[23,24,33-41] However, structural evidence of such solvation models are still rare. Our results on the structures of $(H_2O)_{15}(CH_3OH)_3$ provide experimental evidence for the local structural environment of hydrophobic solutes. The three methyl groups within the $(H_2O)_{15}(CH_3OH)_3$ rings are situated within the hydrophobic pockets created by the methyl groups of the trialanine solute **2** (Figures 4 and 5). This spatial arrangement of hydrophobic groups not only maintains the structural integrity of the hydrogen-bonding network within the cyclic water structure but also aids in maximizing the cooperative hydrogen bonding with methanol molecules. The accommodation of hydrophobic headgroups of methanol within the water structure observed in $(H_2O)_{15}(CH_3OH)_3$ may be rationalized in terms of the cooperativity in hydrogen bonding networks of water and methanol with consequent structural arrangements leading to an optimized hydrogen bonded water structure embedded with methanol subunits (Figure 6). The cooperative phenomenon in hydrogen bonded chains in $(H_2O)_{15}(CH_3OH)_3$ is driven by the dual property of methanol to accept and donate protons. Each molecule of methanol in $(H_2O)_{15}(CH_3OH)_3$ interacts with three water molecules to form the 18-membered cyclic structure (Figure 6). Methanol molecules in $(H_2O)_{15}(CH_3OH)_3$ are hydrogen-bonded as a single proton donor and double acceptor to water molecules present in the water cluster (Figure 6).

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It is a well-accepted view that aqueous solution of alcohols at ambient temperatures must enhance water structure, as compared to the pure liquid water, if the observed heat of mixing and negative excess entropy are to be rationalized.[16,18−24,34,37] It is also hypothesized that enhancement of water structure leads to a significant ordering of hydrogen bonds near the hydrophobic headgroups of alcohols. To put our own results in the perspective of currently held beliefs and much speculation on the nature of hydrophobic interaction with water at ambient temperatures, we have performed the X-ray crystallographic analysis of L-**2**·3H₂O·CH₃OH at ambient temperatures (see Table 1). Surprisingly, the H-bonded network in $(H_2O)_{15}(CH_3OH)_3$ cluster did not show any significant changes on going from 193 K to 293 K (Table 2). The uniformity in the ordered structure of water within $(H_2O)_{15}(CH_3OH)_3$ suggests that the hydrophilic group of methanol is capable of involving in hydrogen bonding interaction with water without causing orientation changes in the tetrahedral arrangement of bonding in water. This experimental finding provides a new mechanism for the loss of entropy via the generation of a highly ordered hydrogen bonded network incorporating hydrophobic entities within the water structure.

Several theoretical models have been used to examine the anomalous thermodynamic properties of H_2O/MeOH solutions and also to gain insight into the intrinsic static hydration structure of water around methanol in dilute solutions.[16,18−21,38−40] Conventional models on hydrophobic hydration proposed by Frank and Evans attribute undue importance to the enhancement of water structure in the proximity of hydrophobic solutes.[22] Water being unable to form hydrogen bonds with hydrophobic solutes, aggregation of the solute is considered a rationale to expel the structured water to the bulk solvent and thereby causing favorable entropy increase of the system. Although experimental demonstration for testing these models are scarce, the molecular structure studies of water−methanol solution, using neutron diffraction with H/D isotope substitution, recently reported by Crain and co-workers[23,24] underscores the importance of experimental evidence in unraveling new information on the structure and dynamics of hydrogen bonds in mixed water-methanol systems. Molecular solids that can embed water or water−alcohol mixtures causing minimum or no alteration in the nature of hydrogen bonds are of paramount importance in gaining important information causing the hydrogen bonding patterns that exist in hydrogen bonding liquids. The results presented in this paper show how new chemical host templates, such as the trimeric alanine **2**, will allow the development and consequent structural elucidation resulting in an unprecedented understanding into the global picture of MeOH hydration.

Conclusions

Our results provide new insight and an improved understanding of the two-dimensional structural aspects of water with important implications in protein biology. Water molecules at the surfaces of proteins, which dictate their conformation, stability, function, and dynamics are implicated in important biological processes such as molecular recognition, catalysis and antigen−antibody association. Determination of the precise structure of water present at the protein surface is challenging because of the complex macromolecular domain of proteins. In this context, the results discussed in this paper are of particular significance because of the new supramolecular $(H_2O)_{18}$ morphology at the amino acid interface in **2** may serve as an

important model to understanding the nature of water packing at the protein water interface. The supramolecular ice-like $(H_2O)_{18}$ cyclic structure of water, complementary to that of bulk water, can be a favorable conformation for water at the amino acid interface. The packing diagram (Figure 1) show how the $(H_2O)_{18}$ structure provides high mobility and flexibility resulting in the hydrophilic amino acid water interface. Move across the interface, from one amino acid layer to the other, the $(H_2O)_{18}$ water cluster arrangement interlink amino acid surfaces via hydrogen bonds to polar groups on amino acids while making van der Waals contacts with non polar atoms. This arrangement of water facilitates a complementary relationship resulting in inter and intramolecular hydrogen bonds within and between the amino acid interfaces and provides a new macroscopic model for water−amino acid interactions in terms of packing efficiency and maximization of macromolecular interactions.

Our direct crystallographic measurements of mixed water-methanol clusters in (L)-**2**·3H₂O·CH₃OH and (D)-**2**·3H₂O·CH₃OH confirm the existence of a cooperative hydrogen bonding between polar groups in methanol and water. The disposition of the hydrophobic headgroups within the supramolecular domain of the mixed water-methanol $(H_2O)_{15}(CH_3OH)_3$ cluster, as shown in Figure 4, confirms that the hydrophobic methyl groups of methanol orient in such a fashion as to minimize disruption of hydrogen bonds in the water structure. The larger size of the water rings in $(H_2O)_{15}(CH_3OH)_3$ (and also in $(H_2O)_{18}$) clusters contrast the compact pentameric and hexameric water clusters found in a majority of structures discussed to date. The larger water rings presumably provide higher degrees of freedom and consequently favor larger distances between the hydrophobic headgroups within the $(H_2O)_{15}(CH_3OH)_3$ cluster. This spatial arrangement suggests that the cooperative hydrogen bonding of polar groups in methanol with the hydrogen bonds within the water structure is the dominant phenomenon within the larger water rings and that the well-known Frank and Evans hypothesis[22] on the generation of clatharate-like cage water structure around hydrophobic headgroups may be limited to smaller pentameric or hexameric water structures.

Experimental Section

General Considerations. Synthesis of **2** was performed under dinitrogen atmosphere. The starting materials (L)-Alanine and Tris-(hydroxymethyl)phosphine were commercially available and used without any purification. ¹H, ¹³C, and ³¹P NMR spectra were recorded at 300, 75 and 121 MHz, respectively, on a Bruker ARX300 spectrometer. Thermal gravimetry analysis (TGA) was performed on a Perkin-Elmer Pyris 1 TGA system using 17.135 mg and heating from 30 °C to 600 °C at a heating rate of 10 °C min⁻¹.

Conjugation of (L)-Alanine/(D)-Alanine with Tris(hydroxymethyl)phosphine. Tris(hydroxymethyl)phosphine (**1**) (0.500 g, 4.033 mmol) in 5 mL of distilled water was added dropwise to (L)-alanine/(D)-alanine (1.077 g, 12.00 mmol) in 10 mL of distilled water at 25 °C. The reaction mixture was stirred under dry nitrogen for 1 h. The solvent was removed in vacuo to obtain a white solid. The white solid was washed with methanol, and dried in vacuo to give the pure product (L)-**2** or (D)-**2** in 90% yield. ESI−MS calcd for $C_{12}H_{24}N_3O_6P$ 337.3. Found 337.6. ¹H NMR (300 MHz, D₂O, δ): 1.38 (d, 9H, −NCH(C*H₃*)COOH, J = 6.0 Hz), 3.47 (d, 6H, PC*H₂*), 3.65 (m, 3H, −NC*H*(CH₃)COOH). ¹³C NMR (75 MHz, D₂O, δ): 14.84 (s, NCH(*C*H₃)COOH), 42.65 (d, P*C*H₂, $J_{P−C}$ = 12.82 Hz), 59.35 (d, −N*C*H(CH₃)COOH, $J_{P−C}$ = 5.77 Hz), 174.10 (s, NCH(CH₃)*C*OOH). ³¹P NMR (121.5 MHz, D₂O, δ): −39.9 (s).

Single Crystals of (L)-2 in Water. Single crystals of X-ray quality of **2** were obtained by dissolving 0.05 g of **2** in 1 mL of water and left undisturbed for 3 d.

Single Crystals of (L)-2/(D)-2 in Water−Methanol. Single crystals of X-ray quality of **2** containing water-methanol cluster were obtained by exposing methanol (20 mL) vapors to solution of **2** (0.05 g) in water (1 mL). Single crystals were obtained after 12 h.

Crystallographic Data Collection and Refinement of Structure. A suitable crystal was chosen and mounted on a glass fiber with epoxy resin. The Crystal data and refinement results were given in Table 1. See the Supporting Information for CIF files. Data reduction and processing followed routine procedures. Structures were solved by direct methods and refined on F_o^2. Absorption correction were done by semiempirical equivalents.

Supporting Information Available: Crystallographic information files (CIF) for structures of **2** with $(H_2O)_{18}$ and $(H_2O)_{15}(CH_3OH)_3$. This material is available free of charge via the Internet at http://pubs.acs.org.

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