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Mechanistic Insights into Water−Protein Interactions of Filamentous Bacteriophage

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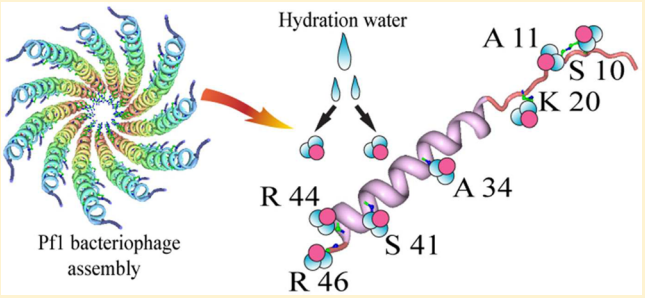
Supporting Information

ABSTRACT: Water plays a major structural and functional role around proteins. In an attempt to explore this mechanistic structural aspect of proteins, we present site-specific interaction of hydration water with the major coat protein subunit of filamentous virus Pf1 by magic angle spinning (MAS) solid-state NMR. The interaction of surrounding water with 36 MDa Pf1 virion is investigated in uniformly ^{13}C, ^{15}N isotopically labeled; polyethylene glycol precipitated fully hydrated samples by solid-state nuclear magnetic resonance spectroscopy. Dipolar edited two-dimensional (2D) $^{1}H-^{15}N$ heteronuclear correlation (HETCOR) experiments lead to unambiguous assignments of cross-peaks originating exclusively from ^{1}H resonances of water molecules correlating to the protein amide nitrogen. An enhanced resolved ^{1}H chemical shift dimension in these experiments also precludes the need of perdeuteration. We report seven residues spanning the 40-residue continuous α-helical conformation assembly of Pf1 interacting with surrounding water. It shows a highly hydrated inner core inside this viral filamentous assembly. The results obtained also suggest the first evidence of a water-mediated interface cluster formed at the site of Arg44 with the single-stranded DNA genome of the filamentous phage supramolecular assembly.



1. INTRODUCTION

Water is an active participant in different biological processes. The structural and functional aspects of water molecules around biomolecules[1] and their hydration patterns are important and have been investigated employing various biophysical techniques.[2,3] Protein molecules are lubricated by water to facilitate different biological functions.[4] Interactions at the binding interface of proteins are often determined by coupled interplay with surrounding water.[5] Solid-state NMR spectroscopy (SSNMR) has recently contributed insights into the structure and dynamics of water in and around microcrystalline[6−9] and antifreeze proteins,[10,11] ion channels,[12] amyloid fibrils,[13] pharmaceuticals,[14] etc. High-resolution SSNMR allows the site-resolved observation of water−protein interactions and, as such, represents a potentially powerful tool to obtain a more detailed picture of hydration in biomolecular solids. Another important class of biological systems amenable to SSNMR methods is filamentous bacteriophage. With a genetically engineered filamentous bacteriophage being utilized for ordering of quantum dots[15] and showing interesting properties like piezoelectric effect generation,[16] these viruses are becoming more promising for investigation. These systems are an important class of macromolecular assemblies with DNA surrounded by coat proteins. Various structural and dynamic studies have been carried out by SSNMR methods on these supramolecular assemblies.[17,18] Class II filamentous phage Pf1, which infects *Pseudomonas aeruginosa*, has served as a paradigm for structure elucidation as well as grasping the significance of complex macromolecular interactions. This bacteriophage can exist in two forms with slightly different symmetries undergoing a reversible temperature and salt concentration dependent macromolecular transition.[19] Pf1 phage has two unusual features—a 1:1 nucleotide:coat protein ratio packaging pattern and a highly distorted DNA structure.[20,21]

Hitherto all studies on this phage coat protein have seldom probed any water−protein interactions or water molecules interacting at the protein−DNA interface. This study sought to gain insight into the contribution of water in its high temperature form ($Pf1_H$) and its interaction with the coat protein. In this Article, we report the first experimental evidence of specific water−protein interactions in fully hydrated samples of $Pf1_H$. We performed 2D $^{1}H-^{15}N$ dipolar edited medium- and long-distance (MELODI) HETCOR experiments[22,23] on $U-^{15}N$, ^{13}C labeled $Pf1_H$ to probe interaction with the surrounding hydration water. A detailed knowledge of these interactions will engender insights into the role of water mediated specificity and affinity of capsid protein−DNA interactions.[24−26]

2. MATERIALS AND METHODS

2.1. Sample Preparation. Uniformly ^{15}N, ^{13}C-labeled $Pf1_H$ bacteriophage was isolated and purified from infected

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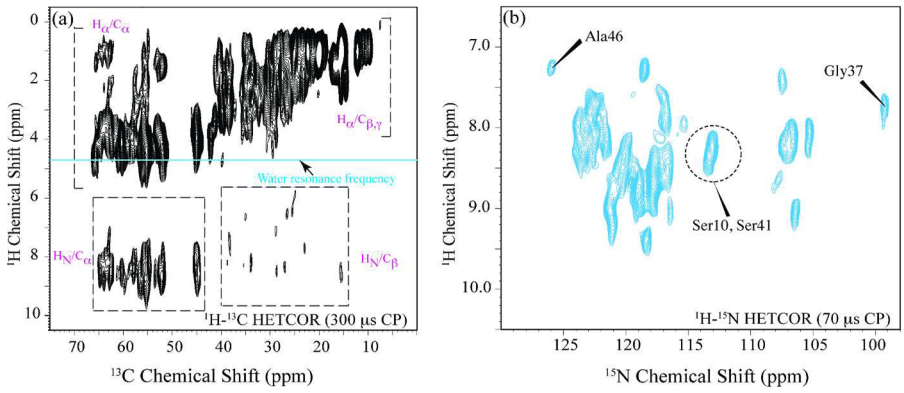

Figure 1. 2D conventional HETCOR spectra of U−^{13}C, ^{15}N-labeled $Pf1_H$: (a) 2D LG-CP ^{1}H−^{13}C HETCOR and (b) 2D ^{1}H−^{15}N HETCOR spectra recorded with 300 and 70 μs contact times, respectively.

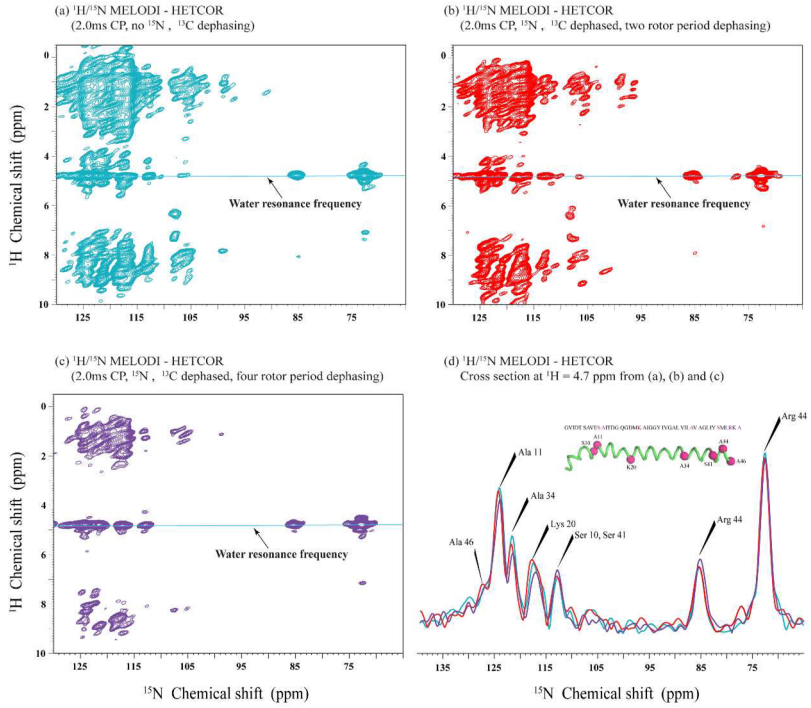

Figure 2. 2D MELODI-HETCOR spectra of U−^{13}C, ^{15}N-labeled $Pf1_H$ with 2 ms spin diffusion. (a) 2D ^{1}H−^{15}N MELODI-HETCOR with no dephasing, (b) 2D ^{1}H−^{15}N MELODI-HETCOR with simultaneous ^{13}C, ^{15}N dephasing for two rotor period, (c) 2D ^{1}H−^{15}N MELODI-HETCOR with simultaneous ^{13}C, ^{15}N dephasing for four rotor period. (d) Rows extracted at the water frequency from a to c of 2D ^{1}H−^{15}N MELODI-HETCOR with both ^{13}C, ^{15}N dephasing with different rotor periods. The assignments of various resonances and residues interacting with water in the Pf1 major capsid protein (PDB ID: 1PJF) are also shown. The amino acid sequence of the major capsid protein is GVIDT SAVES AITDG QGDMK AIGGY IVGAL VILAV AGLIY SMLRK A.

planktonic batch cultures of *P. aeruginosa* strain K (PAK) grown in M9 minimal media with $^{15}NH_4Cl$ and U−^{13}C glucose as the sole nitrogen and carbon sources. The phage was prepared by already established protocols.[18] All the sample preparation steps were performed at room temperature for $Pf1_H$. The PEG-8000 (Sigma-Aldrich, USA) precipitated pellet was then transferred into a 3.2 mm magic angle spinning NMR rotor. Extraneous bulk water was removed physically to enhance the quality of the spectra. The empty space after physical removal was refilled further with more protein sample after rotation in the NMR probe. Approximately 6.5 mg of the protein sample was used for NMR experiments.

2.2. Solid State NMR (SSNMR) Spectroscopy. SSNMR experiments were carried out on a Bruker AVANCE-III 600 MHz (14.1 T) spectrometer using a 3.2 mm triple-resonance E^{free} MAS probe. All conventional 2D ^{1}H−^{15}N and ^{1}H−^{13}C Lee−Goldberg (LG)[27] HETCOR experiments were carried out at 298 K under 7.5 kHz MAS. PMLG-9 ^{1}H homonuclear decoupling[28] with a 99 kHz transverse radio-frequency field was applied during the t_1 period for optimal resolution in proton dimension. SPINAL-6 heteronuclear decoupling was applied during acquisition. For ^{1}H−^{15}N LG-HETCOR experiments, a LG cross-polarization (CP) contact time of 800 μs and a maximum t_1 evolution time of 4.5 ms were used. For ^{1}H−^{13}C LG-HETCOR experiments, the LG-CP contact time was 2.0 ms and the maximum t_1 evolution time was 3 ms. MELODI-HETCOR experiments with two rotor periods of dipolar dephasing were carried out at 298 K under 7442 Hz MAS. The ^{13}C and ^{15}N 180° pulse lengths were 10 and 10 μs, respectively. Hartmann−Hahn (HH)[29] CP with a contact time of 2 ms was

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used to allow ^{1}H spin diffusion, and a maximum t_1 evolution time of 3.2 ms was used. Chemical shift calibration in ^{1}H, ^{15}N, and ^{13}C dimensions was carried out from a standard spectrum.

3. RESULTS AND DISCUSSION

In order to investigate plausible direct dipolar contacts between water protons and the coat protein, a set of ^{13}C, ^{15}N dipolar-edited 2D $^{1}H-^{15}N$ MELODI HETCOR experiments was performed. Figure 1a displays conventional 2D $^{1}H-^{13}C$ HETCOR[22,30] spectra recorded with 300 μs contact time allowing spin diffusion to neighboring protons, and Figure 1b shows the 2D $^{1}H-^{15}N$ HETCOR spectra of the amino proton region recorded with 70 μs contact time to restrict the correlation to one-bond only. Optimal proton line widths of 0.7 ppm were achieved in the 2D $^{1}H-^{15}N$ HETCOR spectrum. It shows a good resolution with resolved cross-peaks. This spectrum confirms that protein is well folded under the present experimental conditions. We observed strong correlation of H_N/C_ω, H_α/C_α and $H_{\alpha,\beta}/C_{\beta,\gamma}$ and weak correlation of H_N/C_β. Besides these, several correlation peaks are seen at the water ^{1}H chemical shift of 4.78 ppm (indicated by a blue line). At this frequency, the resonances can be from water protons or overlap from Hα resonances from protein backbone and amino protons.

The 2D $^{1}H-^{15}N$ HETCOR spectra (Figure S1a, Supporting Information) exhibit spin diffusion Hartmann–Hahn cross-polarization (HH–CP) and cross-peaks from directly bonded (NH) as well as non-directly bonded (aliphatic) protons and ^{15}N spins. If dephasing pulses are turned on the ^{15}N channel, this will lead to dephasing of amino ^{1}H signals (5–9 ppm in the ^{1}H chemical shift range), whereas the cross-peak intensities originating from aliphatic protons and ^{15}N spins are almost unaffected. Dephasing on the ^{15}N channel will eventually rule out the possibility of any ^{1}H cross polarizing to nitrogen at the water frequency from the amino proton region. Dephasing on the ^{13}C channel will lead to complete suppression of cross-peaks originating due to cross-polarization from aliphatic ^{1}H signals (1–5 ppm), whereas the cross-peak intensities from amino ^{1}H signals remain. If simultaneous dephasing is done on both ^{13}C and ^{15}N channels (Figure S1b, Supporting Information), almost all resonances should get suppressed but some cross-peaks with long-range correlation are retained. In this experiment, dephasing was done for two rotor periods. The 2D MELODI-HETCOR spectral editing technique unambiguously discriminates between the ^{1}H NMR signals from water and protein (mainly backbone α-protons and amino protons), which could create obscurity with measurements at the water frequency in non-deuterated proteins. In our case, 2D $^{1}H-^{15}N$ HETCOR was preferred over $^{1}H-^{13}C$ HETCOR due to less spectral overlap at the water resonance frequency. Due to strong $^{13}C-^{13}C$ homonuclear dipolar coupling, the dephasing of attached ^{1}H magnetization will not be complete in U–^{13}C, ^{15}N labeled samples. However, the signal intensity will reduce as a function of increasing rotor period (Figure 2a–c). In the case of ^{1}H of water molecules, the signal intensity will not change due to increase in rotor period. This is what has been observed by comparing ω_2 rows extracted at the water frequency from the various 2D $^{1}H-^{15}N$ MELODI HETCOR experiments with different rotor periods (Figure 2d). We observed clear cross-peaks between water and seven residues of the coat protein: ser10, ala11, lys20, ala34, ser41, arg44, and ala46 (Figure 2d). The assignments (Figure S3, Supporting Information) were confirmed from 2D $^{13}C-^{13}C$ correlation with a phase-alternate recoupling irradiation scheme (PARIS) mixing,[31] NCA[32] experiments, and previously reported assignments of the coat protein.[18]

The amino acids that showed interactions with water in the $Pf1_H$ virion are serine, alanine, lysine, and arginine. Serine O–H groups are generally responsible for hydrogen bond (H-bond) formation and in recognition of nucleotides through direct/water-mediated H-bonds. The capacity of proteins to form H-bonds is an important determinant of protein stability. Out of 7 alanine residues of the 46-residue-long coat protein, 3 residues were involved in interaction with water. The hydration properties of alanine are slightly kosmotropic, and alanine rich amino acids are known to have a predominantly right α-helical structure.[33] Lysine residues by evolutionary design are found on the surface of proteins to minimize self-aggregation and avoid crystal formation. This shows the fascinating design principle of the minute marvels of complex architecture.

The surface hydration of proteins/DNA is the manifestation of their local stereochemistry. The interaction of arg44 with water explains the perplexing highly dynamic nature of the side chain at the C-terminus in spite of its spatial location inside the deep inner core of the virus.[34] This residue in particular has also been reported to interact with the DNA bases in $Pf1_H$.[35] Interfacial water serves as glue at protein surfaces, providing the amino acids a certain degree of flexibility eventually contributing to hydrogen bonding networks.[36,37] This structural adaptability of water in some instance plays a pivotal role by mediating sequence-specific recognition between amino acid residues and DNA base pairs, which are out of reach, nevertheless are known to interact. The unusual 1:1 ratio of nucleotides to major capsid protein in Pf1 suggests specific protein–nucleic acid interactions. Thus, it is quite plausible that water mediates arg44–DNA interaction in $Pf1_H$ at this biological interface. We also presume that hydration water also enhances the affinity of capsid protein for the twisted viral DNA. To investigate this aspect, mutagenesis studies perturbing the arg44 residue are under progress.

4. CONCLUSION

In conclusion, we present the first experimental study of water–protein interaction in uniformly ^{13}C, ^{15}N labeled fully hydrated $Pf1_H$ bacteriophage by SSNMR experiments. The present finding reveals a hydrated inner core in $Pf1_H$ and explains the highly dynamic nature of arg44 and its proposed interaction with the DNA.[35] In addition, we exemplify the potential of MELODI-HETCOR spectral editing experiments for probing site-specific water–protein interactions in non-deuterated large supramolecular proteins which earlier were only limited to small antimicrobial peptides[12,19] and ion channels.[12] The present experimental results may have profound implications in unraveling the role of hydration water in supramolecular assemblies and biological interfaces.

■ ASSOCIATED CONTENT

Supporting Information

Additional experimental details and figures, as mentioned in the manuscript, are given. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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