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Probing membrane protein structure using water polarization transfer solid-state NMR

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ABSTRACT

Water plays an essential role in the structure and function of proteins, lipid membranes and other biological macromolecules. Solid-state NMR heteronuclear-detected ^{1}H polarization transfer from water to biomolecules is a versatile approach for studying water–protein, water–membrane, and water–carbohydrate interactions in biology. We review radiofrequency pulse sequences for measuring water polarization transfer to biomolecules, the mechanisms of polarization transfer, and the application of this method to various biological systems. Three polarization transfer mechanisms, chemical exchange, spin diffusion and NOE, manifest themselves at different temperatures, magic-angle-spinning frequencies, and pulse irradiations. Chemical exchange is ubiquitous in all systems examined so far, and spin diffusion plays the key role in polarization transfer within the macromolecule. Tightly bound water molecules with long residence times are rare in proteins at ambient temperature. The water polarization-transfer technique has been used to study the hydration of microcrystalline proteins, lipid membranes, and plant cell wall polysaccharides, and to derive atomic-resolution details of the kinetics and mechanism of ion conduction in channels and pumps. Using this approach, we have measured the water polarization transfer to the transmembrane domain of the influenza M2 protein to obtain information on the structure of this tetrameric proton channel. At short mixing times, the polarization transfer rates are site-specific and depend on the pH, labile protons, sidechain conformation, as well as the radial position of the residues in this four-helix bundle. Despite the multiple dependences, the initial transfer rates reflect the periodic nature of the residue positions from the water-filled pore, thus this technique provides a way of gleaning secondary structure information, helix tilt angle, and the oligomeric structure of membrane proteins.

1. Introduction

Water is one of the most essential molecules for the proper structure and function of proteins and other biological macromolecules. Protein hydration, protein folding, ion channel function, and lipid self-assembly, all rely on water interactions with these molecules [1–6]. Solid-state NMR (SSNMR) spectroscopy is an excellent method to study water interactions with non-crystalline and insoluble biological macromolecules. We are particularly interested in understanding how water interacts with proteins in phospholipid membranes, and to what extent water can serve as a probe of membrane protein structure. In this perspective article, we review SSNMR pulse sequences for measuring water–biomolecule polarization transfer and recent findings of the mechanism of water interactions with proteins, lipids and other biological solids. We focus on studies that detect protein signals to obtain site-specific information about water dynamics and binding, and do not attempt to cover the large literature of direct observation of water dynamics using relaxation NMR. We then present our new study of the water interaction with the four-helix bundle formed by the influenza M2 transmembrane peptide (M2TM). The goal is to explore the extent to which secondary structure and oligomeric structure of membrane proteins can be extracted from site-specific water–protein polarization transfer rates.

2. Solid-State NMR techniques for studying water–protein interactions

The main SSNMR approach for probing water–biomolecule interactions is to transfer the water ^{1}H polarization to biomolecules and detect the result of the transfer via ^{13}C, ^{15}N or other heteronuclear signals of the biomolecule. This polarization transfer technique has many pulse sequence variations. When implemented in a 1D fashion, a ^{1}H polarization gradient needs to be

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established first, which is usually accomplished by a ^{1}H T_2 filter. The Hahn echo retains the polarization of the dynamic water, which has long T_2, while destroying the polarization of the rigid molecules, which have much shorter T_2's. A subsequent longitudinal mixing period (t_{mix}) allows the water polarization to transfer to the biomolecule, followed by ^{1}H–X (X = ^{13}C, ^{15}N, etc.) cross-polarization (CP) for detection (Fig. 1a). The CP step can be implemented in a spin-diffusion-free fashion by locking the ^{1}H magnetization along the magic-angle direction (LG-CP), or can include spin diffusion using the conventional Hartman–Hahn (HH) CP. In the latter case, the longitudinal mixing period may be skipped to shorten the total polarization transfer time, if one wishes to selectively examine only the nearest water protons [7,8].

This polarization transfer framework is modified from the Goldman–Shen experiment [9,10]. The heteronuclear signals reveal which sites are close to water, and the distance information can be made quantitative by measuring the heteronuclear signals as a function of t_{mix}.

When water is not the only species that survives the ^{1}H T_2 filter and other dynamic species also exist, then the 1D experiment needs to be modified either by selective excitation of the water resonance [11] or by extending the experiment to 2D and encoding the ^{1}H chemical shift before the mixing period [12,13] (Fig. 1b). In the resulting 2D spin-diffusion heteronuclear correlation (HETCOR) experiment, ^{1}H homonuclear decoupling is optional during the evolution period. Without ^{1}H–^{1}H decoupling, only highly mobile molecules such as water and liquid–crystalline lipids will be detected in the indirect dimension. When homonuclear decoupling is applied, the ^{1}H signals of the rigid solid also become detectable, thus providing higher information content to the spectra. For the undecoupled 2D experiment, the ^{1}H T_2 filter is necessary for suppressing the rigid proton signals in the first t_1 slice, while for the homonuclear-decoupled experiment, the T_2 filter can be removed.

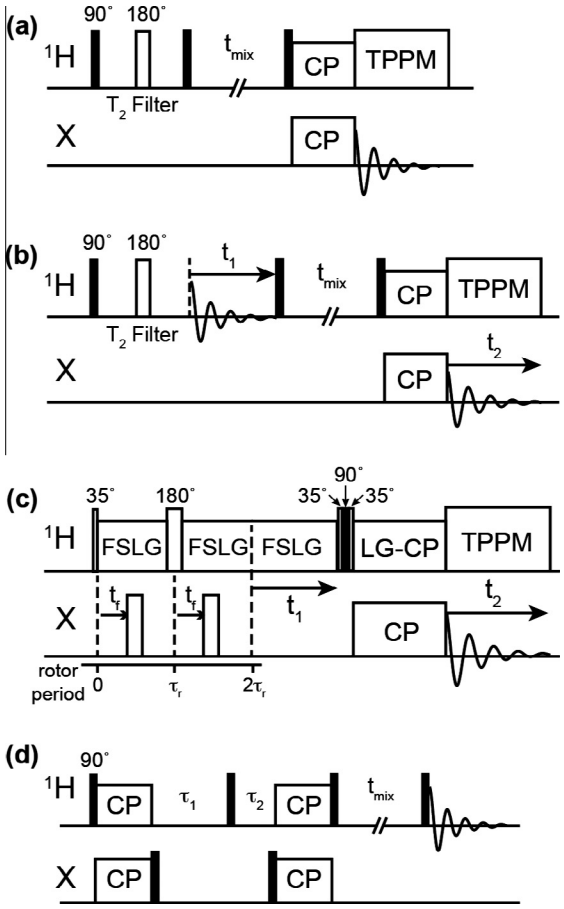

Fig. 1. Representative pulse sequences used to measure water polarization transfer to biomolecules. (a) 1D T_2-filtered experiment. (b) 2D ^{1}H-undecoupled HETCOR experiment with spin diffusion mixing. (c) 2D dipolar-dephased MELODI-HETCOR experiment. (d) ^{1}H-detected 1D XHH experiment for sensitivity-enhanced detection of biomolecule–water polarization transfer.

In practice, even with ^{1}H chemical shift encoding, some solid protons such as protein Hα can resonate near the bulk-water chemical shift of 4.8 ppm to cause ^{1}H resonance overlap. To address this problem, and to simplify the 2D HETCOR spectra, we developed a dipolar-filtered HETCOR experiment called MELODI (medium- and long-distance heteronuclear correlation) HETCOR, which detects only cross peaks resulting from non-bonded spin pairs [14,15] (Fig. 1c). Before the ^{1}H evolution period, a ^{1}H–X REDOR dephasing period is inserted in which an 180° X-pulse is applied every half a rotor period to recouple the ^{1}H–X dipolar interaction. A single ^{1}H 180° pulse in the center of the dephasing period refocuses the ^{1}H chemical shift. This REDOR filter destroys the magnetization of protons that are directly bonded to an X spin. The protons that survive this dipolar filter include water, protein protons without directly bonded ^{13}C or ^{15}N nuclei, and highly dynamic protons with weaker ^{1}H–X dipolar couplings. Demonstration on natural-abundance amino acids confirmed that only two-bond cross peaks such as Hα–Cβ and Hβ–Cα remained in the ^{13}C-dephased ^{13}C–^{1}H MELODI-HETCOR spectra [14]. For a selectively ^{13}C-labeled model protein, ubiquitin, the ^{13}C-dephased ^{13}C–^{1}H MELODI-HETCOR spectra contain cross peaks between labeled ^{13}C and protons without directly bonded ^{13}C, and cross peaks between labeled ^{13}C and ^{15}N-bonded protons such as backbone amides, Lys NH_3 and Arg guanidinium Hη and Hε. For a uniformly ^{13}C, ^{15}N-labeled membrane protein, colicin Ia channel domain, ^{13}C-dephased ^{13}C–^{1}H MELODI-HETCOR spectra exhibit ^{13}C–H^N cross peaks.

The MELODI-HETCOR technique is well suited to the study of water–protein interactions, since water is unaffected by all dephasing pulses (unless ^{17}O pulses are applied). Simultaneous irradiation of ^{13}C and ^{15}N in the dipolar dephasing period will destroy all rigid protein proton signals and leave only water ^{1}H cross peaks. Using this approach, we have investigated water interactions with arginine residues of a cationic antimicrobial peptide [16]. The data allowed the unambiguous assignment of cross peaks at a ^{1}H chemical shift of 4.9 ppm to water–Arg correlations, rather than Arg Hα-sidechain correlations. This means that water solvates membrane-inserted guanidinium ions, which provides direct evidence of the long-suspected but rarely proven phenomenon that water penetrates into the lipid membrane to lower the free energy of insertion of these cationic membrane peptides.

To enhance the sensitivity of water–protein polarization transfer experiments, we developed a ^{1}H-detected experiment called XHH, in which the ^{1}H mixing period is preceded by dual CP steps: forward ^{1}H–X CP and reverse X–^{1}H CP. In this way, only protons directly attached to the X spins are selected to undergo polarization transfer [17] (Fig. 1d). Under moderate MAS frequencies and without ^{1}H dilution by deuteration, such a CHH experiment detects only the ^{1}H magnetization of mobile water and lipids that is transferred from the ^{13}C-bonded protein protons. Between the two CP steps, the X-spin magnetization is stored along the *z*-axis while the initial ^{1}H magnetization is destroyed in the transverse plane. 90° ^{1}H "purge" pulses during the dephasing period can be applied to ensure clean suppression of all initial ^{1}H magnetization. The sensitivity enhancement factor of the CHH experiment depends on the ^{13}C labeling level and the number of protein protons relative to the number of mobile water and lipid protons. For ^{13}C-labeled colicin Ia channel domain, we showed that the 1D CHH experiment