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2Q NMR of ^2H_2O ordering at solid interfaces

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ABSTRACT

Solvent ordering at an interface can be studied by multiple-quantum NMR. Quantitative studies of ^2H_2O ordering require clean double-quantum (2Q) filtration and an analysis of 2Q buildup curves that accounts for relaxation and, if randomly oriented samples are used, the distribution of residual couplings. A pulse sequence with absorption mode detection is extended for separating coherences by order and measuring relaxation times such as the 2Q filtered T_2. Coherence separation is used to verify 2Q filtration and the 2Q filtered T_2 is required to extract the coupling from the 2Q buildup curve when it is unresolved. With our analysis, the coupling extracted from the buildup curve in ^2H_2O hydrated collagen was equivalent to the resolved coupling measured in the usual 1D experiment and the 2Q to 1Q signal ratio was in accord with theory. Application to buildup curves from ^2H_2O hydrated elastin, which has an unresolved coupling, revealed a large increase in the 2Q signal upon mechanical stretch that is due to an increase in the ordered water fraction while changes in the residual coupling and T_2 are small.

1. Introduction

Solvent molecules interacting with a surface can be differentiated from the bulk solvent and studied using multiple-quantum (MQ) spectroscopy. The first examples were ^{131}Xe interacting with a glass surface [1] and strain induced ordering of ^2H_2O in blood vessels [2]. The key feature of this approach is that quadrupolar or dipolar couplings in the bulk, isotropic phase are averaged to zero while molecules at the surface are weakly ordered and have a non-zero coupling. The residual coupling is used to generate MQ coherence and the characteristic response of the MQ coherence to shifts in the transmitter offset or phase is used to separate or filter NMR signals from molecules at the surface. Herein we address practical aspects of this approach when applied to an important class of interfacial phenomena; water at the surface of a matrix protein. From an NMR perspective, water is an attractive probe since the anisotropic spin coupling can be dipolar (between protons) or quadrupolar (if the solvent is labeled with ^2H_2O or $H_2^{17}O$). In this paper, we discuss the simplest case, ^2H NMR, which has a single double-quantum (2Q) transition. Applications have included the observation of ordered water in elastin [3], purple membrane [4] and tendon [5–7] and likely involve water interacting at the nonpolar surface of a hydrophobic protein or at the polar surface of a hydrophilic protein.

Important properties to be determined are the degree of solvent ordering and the solvent accessible surface area. These are closely related to two NMR parameters, the residual quadrupole coupling and the intensity of the indirectly detected 2Q signal. In this paper we address practical difficulties that arise when these parameters are measured. The goals are to confirm adequate 2Q filtration and to develop a quantitative analysis of 2Q buildup data that incorporates relaxation and the distribution of residual couplings inherent to randomly oriented samples. In many cases, the coupling is not resolved [1,3,8] so detection of an antiphase doublet using the standard sequence can lead to signal cancellation. Also, overlap of a potentially large signal from the bulk solvent occurs if 2Q filtration is insufficient. To eliminate cancellation of antiphase signals, absorption mode signals are detected [7,9] and, to confirm that 2Q filtration is adequate, we have added a constant time indirect dimension for separating coherences by order. The sequence with absorption mode detection is also extended so that three relaxation times specific to the ordered solvent, T_1 and T_2 with 2Q filtration and $T_{2,2Q}$, can be measured. With randomly oriented samples, there is a "powder" distribution of quadrupole coupling frequencies and this is incorporated into our analysis. The pulse sequence and analysis are tested against collagen and applied to elastin. Collagen has a small but resolved quadrupolar coupling and we show that this coupling can be determined from the buildup curve using our analysis albeit with less accuracy than measured in the 1Q experiment. Also, the observed 2Q signal strength is in accord with theory if relaxation and the powder distribution of couplings are accounted for. With elastin, the residual coupling is not

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resolved and the detected NMR signal has a contribution from bulk solvent if 2Q filtration is not complete. With a purge period and a 64-step phase cycle, adequate filtration is confirmed by 2D separation of the coherences and we find that the residual quadrupole coupling and the mole fraction of ordered solvent can be determined from the buildup curve if T_2 of the ordered solvent is also measured. When elastin is subjected to mechanical strain, large changes in the 2Q spectra are observed and these are parsed into changes in the mole fraction of ordered solvent and the residual coupling.

2. Theory

The pulse sequence begins with the density operator at thermal equilibrium, i.e., aside from an invariant identity term and the Curie proportionality constant, $\rho_{eq} = I_z$. Standard density matrix theory with equation of motion $\dot{\rho} = -i[H, \rho]$ is used and implemented with scripts listed in the Appendix A.3. With rotating frame Hamiltonian, H, the density operator evolves according to

$$\rho' = U^{-1}\rho U \text{ with } U = e^{iHt}. \tag{1}$$

Quadrupole coupling, frequency offset and relaxation are reasonably neglected during pulses and the relevant Hamiltonian is

$$H_{RF} = \omega_1(\cos\phi I_x + \sin\phi I_y). \tag{2}$$

In the above, ω_1 is the resonant RF field strength and ϕ is the RF phase.

Pulse sequence design is facilitated by expanding ρ in operators related to coherences and populations of the spin system transitions. Here, we use the spin-1 single transition coherences of Wokaun and Ernst [10] combined with I_z and $Q = (3I_z^2 - 2)$ to represent the two independent populations (0Q coherences) [11]. This set is complete, trace orthogonal and expectation values are directly related to matrix elements of ρ by,

$$I_x^{(rs)} = \rho_{rs} + \rho_{sr}, \quad I_y^{(rs)} = i(\rho_{sr} - \rho_{rs}), \quad I_z = \rho_{11} - \rho_{-1-1} \text{ and}$$
$$Q = (\rho_{11} - 2\rho_{00} + \rho_{-1-1}). \tag{3}$$

Between pulses, coherent evolution is due to the quadrupole, H_Q, and offset, H_{OS}, Hamiltonians,

$$H = H_Q + H_{OS} = \omega_Q\left(I_z^2 - 2/3\right) - \omega_{OS}I_z, \tag{4}$$

where ω_Q and ω_{OS} are the effective quadrupole coupling and the offset frequencies. Relaxation and potentially spin exchange must also be accounted for when ω_Q is comparable to $1/T_2$. In the context of spin-3/2 ^{131}Xe NMR, Deschamps et al. [12] used an effective Liouvillian approach. With ^{2}H, the exchange narrowed limit can likely be assumed since the quadrupole coupling is small and narrow lines or well-resolved couplings are typically observed. In this limit, coherences relax with a single exponential time constant [13] and the effective quadrupole coupling is a population weighted average [14]. This assumption greatly simplifies subsequent calculations and a straightforward calculation shows that between pulses,

$$\rho_{rs}(t) = \rho_{rs}(0)\exp[-(i\bar{\omega}_{rs} + R^{(rs)})t] \text{ and}$$
$$I_z(t) = I_z + (I_z(0) - I_z)e^{-R_1 t} \approx I_z(0), \tag{5a}$$

with

$$\omega_{-10} = (\omega_{OS} + \omega_Q), \quad \omega_{01} = (\omega_{OS} - \omega_Q),$$
$$\omega_{-11} = 2\omega_{OS} \text{ and } \omega_{rr} = 0. \tag{5b}$$

In the above, $R^{(rs)}$ are reciprocal relaxation times and we have noted that longitudinal relaxation can be neglected when T_1 is long compared to the pulse sequence duration.

The sequence used here, Fig. 1, is a modification of sequences used previously for MQ NMR in isotropic solutions [9] and ^{1}H MQ NMR in tendon [6,7,15]. Unlike solution experiments, the coupling is not isotropic and the powder distribution of couplings in unoriented materials must be taken into consideration. The sequence, Fig. 1, has two indirect dimensions, either of which is typically held constant in a 2D experiment. The t_1 (preparation) dimension is used to determine the residual coupling when it is not resolved in the usual 1D experiment and coherences are separated by order in the t_2 (evolution) dimension. Unwanted coherences decay during τ' for absorption mode detection and transmitter phases, α, β and γ, are cycled for 2Q filtration. By varying τ or τ', $R_{2,2Q}$ or R_1 with 2Q filtration are determined and, with an optional refocusing pulse (dotted line), the 2Q filtered R_2 can be measured.

A flowchart summarizing the density operator at the end of each period through conversion is shown in Fig. 2. Expressions for the coherence transfers were obtained by numerical simulations of Eqs. (1)-(5) (see Appendix A.3). During preparation, I_z is partially transformed into 2Q coherence [16,17]. With $\alpha = 0°$, the initial 90° pulse converts I_z into in-phase 1Q coherence $\left(\sqrt{2}\left(I_y^{-10} + I_y^{01}\right)\right)$. Subsequently, the 1Q transitions rotate at frequencies $\omega_{OS} \pm \omega_Q$ and relax with rate R_2. At the end of preparation, net evolution from the frequency offset is eliminated by the central 180° pulse and the 2nd 90° pulse converts in-phase and anti-phase $\left(\sqrt{2}\left(I_y^{-10} - I_y^{01}\right)\right)$ 1Q coherences into 0Q (I_z) and 2Q $\left(I_y^{-11}\right)$ coherences, respectively [17,18]. Bulk solvent ($\omega_Q = 0$) present in the sample adds to the 0Q coherence and the effect of non-ideal pulse widths is to add 1Q coherence.

Coherences created during preparation are separated by order in t_2 and we use this to determine if 2Q filtration is adequate. Ideally, the detected NMR signal is only from the 2Q coherence prepared from the ordered solvent. Losses from relaxation during this period are negligible if the duration, τ, is small compared to $T_{2,2Q}$ and this is achieved by using an offset frequency that is large compared to $R_{2,2Q}$ and sampling a small number of t_2 values at a frequency harmonically related to the offset (see below). An alternative method for separating coherences is to advance the transmitter phase [19]. However, this method and 2Q filtration place similar demands on phase shift accuracy and this is avoided here.

For absorption mode detection, 2Q coherence is converted to and stored as I_z [7,9] and then, after a delay τ', converted to observable 1Q coherence by the final 90° pulse. During τ', unwanted coherences are allowed to decay. In practice, $\tau' \sim 3T_2$ is satisfactory and shorter τ' could be used if gradients are applied. Unwanted signal from I_z terms generated during preparation (the bottom path in Fig. 2) and from bulk solvent are eliminated by the phase cycle described in the Appendix A.1.

With a phase cycle that adds signals coherently along the "2Q path" and cancels signals from other paths, the NMR signal at the beginning of detection is (R_1 relaxation neglected),

$$S(t_1, t_2) = e^{-2R_2t_1 - R_{2,2Q}\tau}\cos(2\omega_{OS}t_2)\int \sin^2(\omega_Q(\Omega)t_1)d\Omega. \tag{6}$$

The exponential factor accounts for relaxation in the course of the pulse sequence and the integral indicates a powder average over orientations of the coupling tensor. With the assumption of axially symmetric coupling, $\omega_Q = \omega_{||}(3\cos^2\theta - 1)/2$, and the substitution, $x = \cos\theta$, the above simplifies as follows for the preparation experiment ($\tau = t_2 = 0$):

$$S(t_1) = S_{\text{prep}}\, e^{-2R_2t_1}\int_0^1 \sin^2\left[\frac{\omega_{||}}{2}(3x^2 - 1)t_1\right]dx. \tag{7}$$

In Eq. (7), $\omega_{||}$ is the quadrupole coupling constant and a constant, S_{prep}, was inserted to account for the gain of the NMR instrument.