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^{17}O NMR of water in ordered environments

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Abstract

Two NMR experiments are designed for selective excitation of spin $I = 5/2$ nuclei that exhibit residual quadrupolar splittings. The $I = 5/2$ Jeener–Broekaert experiment is preferred to the four-quantum filtration experiment as it is shown to be a more sensitive technique in experimental practice. Both techniques are applied to ^{17}O-enriched water in biological systems. The occurrence of water which displays a residual ^{17}O quadrupolar splitting is demonstrated for the first time in a model biological system and an excised tissue sample. The resulting ^{17}O NMR spectra are shown to have the characteristics predicted in computer-simulated $I = 5/2$ NMR spectra. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: Jeener–Broekaert experiment; Four-quantum filtration experiment; ^{17}O NMR; Quadrupolar splittings

1. Introduction

With a spin quantum number of $I = 5/2$, ^{17}O is the only magnetic nuclide of oxygen. The combination of its low natural abundance (0.037%) and low gyromagnetic ratio [$\gamma(^{17}\mathrm{O}) \approx 0.135\gamma(^{1}\mathrm{H})$] result in an absolute NMR sensitivity (the product of its sensitivity relative to ^{1}H and its natural abundance) of only 1.08×10^{-5}. However, the association of water with biological macromolecules, such as proteins, membrane lipids and DNA helices, has led to interest in the possibility of using ^{17}O NMR spectroscopy as a probe of the environment of water in biological systems [1–5]. Such a probe might, for example, prove valuable in comparing the macromolecular construction of biological tissues or in assessing changes to macromolecules induced by disease or pharmaceuticals. In biological systems, ^{17}O nuclei in molecular water do not exhibit any relative chemical shifts and, despite the relatively large value of the $^{1}J_{OH}$ scalar coupling constant in water (~ 80 Hz), proton exchange is of a sufficient rate for no couplings to be observable in the ^{17}O NMR spectrum. In contrast, one ^{17}O NMR

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parameter that is anticipated to be sensitive to the motional behaviour and environment of water molecules is the quadrupolar interaction.

The occurrence of ^{17}O nuclei outside the extreme-narrowing limit in aqueous solutions of proteins has been demonstrated using three- and five-quantum filtered NMR experiments [1–4] and this work is currently being extended to suspensions of cells and organelles [5]. In systems of this type, however, it is necessary to investigate whether multiple-quantum filtered ^{17}O NMR signals are the result of multiexponential transverse quadrupolar relaxation or of residual quadrupolar splittings. Experimental techniques that discriminate between nuclei in isotropic, liquid-like environments and those in anisotropic, solid-like environments, are now routine in NMR of $I=3/2$ nuclei [6–11], particularly ^{23}Na, but have so far not been used for $I=5/2$ nuclei. In this article, two NMR pulse sequences are designed for *selective* excitation of $I=5/2$ nuclei in anisotropic environments and used to search for evidence of ordered ^{17}O nuclei in water in biological samples.

2. Theory

In the $I=5/2$ multiple-quantum filtered experiments in Refs. [1–4] it was assumed (almost certainly correctly) that the ^{17}O nuclei existed in a purely liquid environment, hence excitation of multiple-quantum coherences was the result of triexponential quadrupolar relaxation alone. Chung and Wimperis have presented a description of spin $I=5/2$ quadrupolar relaxation in terms of irreducible spherical tensor operators [12,13]. Transverse relaxation of in-phase single-quantum coherences, $T_{1,\pm1}$, can be described by

$$T_{1,\pm 1} \overset{R^{(\pm 1)}}{\rightarrow} f_{11}^{(\pm 1)}(t)T_{1,\pm 1} + f_{31}^{(\pm 1)}(t)T_{3,\pm 1} + f_{51}^{(\pm 1)}(t)T_{5,\pm 1} \qquad (1)$$

where a triexponential function $f_{l'l}^{(p)}(t)$ is the $I=5/2$ equivalent of the biexponential functions described for $I=3/2$ in Jaccard et al. [6]. The relationship $f_{nm}^{(-1)}(t)=(-1)^{n-m}f_{nm}^{(+1)}(t)$ applies. It can be shown in the extreme-narrowing limit ($\omega_0\tau_c \ll 1$) that $f_{31}^{(\pm 1)}(t) \approx f_{51}^{(\pm 1)}(t) \approx 0$. This feature can be exploited in multiple-quantum experiments, equivalent to the $I=3/2$ three-quantum filtration experiment [6], designed to detect selectively nuclei outside the extreme-narrowing limit ($\omega_0\tau_c \geq 1$). In the five-quantum filtration experiment [13], magnetization is filtered through a state of five-quantum coherence which can only be supported by the fifth-rank tensors formed for nuclei outside the extreme-narrowing limit. In the $I=5/2$ three-quantum filtration experiment [13], tensors with both $l=3$ and 5 are selected via a state of three-quantum coherence. The fifth-rank tensor contribution is then suppressed by a flip-angle effect on the two filtration pulses, with $\beta=\beta'=70.5°$.

Before experiments can be designed for selective excitation of ordered water, it is necessary to have a description of the time evolution of the $I=5/2$ density matrix, in this case in the irreducible spherical tensor operator basis set, under the influence of a first-order quadrupolar Hamiltonian. Consider a system of isolated spin $I=5/2$ nuclei evolving under quadrupolar splitting alone. Expressed in the eigenbasis of the Zeeman Hamiltonian, the evolution of the $p=+1$ single-quantum elements of the density matrix, $\sigma(t)$, can be described by the equation

$$\frac{\mathrm{d}}{\mathrm{d}t}\boldsymbol{\sigma}^{(+1)}(t) = -i\mathbf{H}^{(+1)}\boldsymbol{\sigma}^{(+1)}(t) \qquad (2)$$

where $\mathbf{H}^{(+1)}$ is the 5×5 Hamiltonian supermatrix describing the effect of the quadrupolar splitting on the $p=+1$ coherences and $\sigma^{(+1)}(t)$ is a 5×1 column vector containing the $p=+1$ elements of the density matrix. The solution to Eq. (2) is

$$\boldsymbol{\sigma}^{(+1)}(t) = \exp\{-i\mathbf{H}^{(+1)}t\}\boldsymbol{\sigma}^{(+1)}(0) \qquad (3)$$

Since $\mathbf{H}^{(+1)}$ is diagonal, with elements consisting merely of the rotating frame frequencies of the five $I=5/2$ resonances $(0, \pm 2\omega_Q, \pm 4\omega_Q)$, the exponential term in Eq. (3) can be written easily. This description of the time evolution of the single $p=+1$ elements is converted into the irreducible tensor basis by means of the transformation

$$\begin{aligned}\mathbf{b}^{(+1)}(t) &= \mathbf{U}\boldsymbol{\sigma}^{(+1)}(t)\\ &= \mathbf{U}\exp\{-i\mathbf{H}^{(+1)}t\}\boldsymbol{\sigma}^{(+1)}(0)\\ &= \mathbf{U}\exp\{-i\mathbf{H}^{(+1)}t\}\mathbf{U}^{-1}\mathbf{U}\boldsymbol{\sigma}^{(+1)}(0)\\ &= \mathbf{U}\exp\{-i\mathbf{H}^{(+1)}t\}\mathbf{U}^{-1}\mathbf{b}^{(+1)}(0)\\ &= \mathbf{g}^{(+1)}(t)\mathbf{b}^{(+1)}(0) \qquad (4)\end{aligned}$$

where **U** is the unitary matrix [13]

$$\mathbf{U} = \begin{pmatrix} -\sqrt{\frac{1}{7}} & -\sqrt{\frac{8}{35}} & -\sqrt{\frac{9}{35}} & -\sqrt{\frac{8}{35}} & -\sqrt{\frac{1}{7}} \\ -\sqrt{\frac{5}{14}} & -\sqrt{\frac{1}{7}} & 0 & \sqrt{\frac{1}{7}} & \sqrt{\frac{5}{14}} \\ -\sqrt{\frac{1}{3}} & \sqrt{\frac{1}{30}} & \sqrt{\frac{4}{15}} & \sqrt{\frac{1}{30}} & -\sqrt{\frac{1}{3}} \\ -\sqrt{\frac{1}{7}} & \sqrt{\frac{5}{14}} & 0 & -\sqrt{\frac{5}{14}} & \sqrt{\frac{1}{7}} \\ -\sqrt{\frac{1}{42}} & \sqrt{\frac{5}{21}} & -\sqrt{\frac{10}{21}} & \sqrt{\frac{5}{21}} & -\sqrt{\frac{1}{42}} \end{pmatrix} \qquad (5)$$

and where $\mathbf{b}^{(+1)}(t)$ is the 5×1 column vector containing the coefficients $b_{l,p=+1}(t)$ in the tensor operator expansion of the density operator:

$$\sigma(t) = \sum_{l=0}^{2I}\sum_{p=-l}^{l} b_{l,p}(t)T_{l,p} \qquad (6)$$

Hence the matrix

$$\mathbf{g}^{(+1)}(t) = \mathbf{U}\exp\{-i\mathbf{H}^{(+1)}t\}\mathbf{U}^{-1} \qquad (7)$$

describes the evolution of $p = +1$ tensors under quadrupolar splitting and its elements, $g_{l'l}^{(+1)}(t)$, are the quadrupolar splitting equivalent of the triexponential relaxation functions, $f_{l'l}^{(+1)}(t)$, used in Eq. (1). Of particular importance to this article are the $g_{l1}^{(+1)}(t)$ functions and these are given by

$$g_{11}^{(+1)}(t) = \frac{1}{35}(9 + 16\cos 2\omega_Q t + 10\cos 4\omega_Q t) \qquad (8a)$$

$$g_{21}^{(+1)}(t) = \frac{i\sqrt{2}}{7\sqrt{5}}(4\sin 2\omega_Q t + 5\sin 4\omega_Q t) \qquad (8b)$$

$$g_{31}^{(+1)}(t) = \frac{2}{5\sqrt{21}}(-3 - 2\cos 2\omega_Q t + 5\cos 4\omega_Q t) \qquad (8c)$$

$$g_{41}^{(+1)}(t) = \frac{2i}{7}(-2\sin 2\omega_Q t + \sin 4\omega_Q t) \qquad (8d)$$

$$g_{51}^{(+1)}(t) = \frac{\sqrt{2}}{7\sqrt{3}}(3 - 4\cos 2\omega_Q t + \cos 4\omega_Q t) \qquad (8e)$$

The following relationships apply:

$$g_{nm}^{(\pm 1)}(t) = g_{mn}^{(\pm 1)}(t) \qquad (9a)$$

$$g_{nm}^{(-1)}(t) = (-1)^{n-m} g_{nm}^{(+1)}(t) \qquad (9b)$$

The spin $I = 5/2$ $f_{l'l}^{(\pm 1)}(t)$ and $g_{l'l}^{(\pm 1)}(t)$ functions show explicitly, as they do for the $I = 3/2$ case [6], that even-rank tensor operators can arise through the action of a quadrupolar splitting but cannot be formed by transverse quadrupolar relaxation alone. In principle, the presence of certain additional relaxation mechanisms can lead to the appearance of even-rank tensors in the absence of quadrupolar splittings but practical experience with $I = 3/2$ nuclei in biological systems has failed to reveal a single example of this. Therefore if magnetisation can be filtered purely through even-rank tensor operators alone then it may be assumed that only ordered nuclei will contribute to the final signal [6].

3. Experiment design

One approach to selective excitation of ordered $I = 5/2$ nuclei, which can be thought of as analogous to the two-quantum filtration experiment widely used for $I = 3/2$ [6,7,10], is to filter magnetisation through a state of four-quantum coherence, which can only be supported by tensor operators with $l = 4$ or 5, and then use a flip-angle effect to suppress the undesirable fifth-rank tensors. The pulse sequence and coherence level diagram for such a four-quantum filtration experiment is shown in Fig. 1a. The pulse sequence begins with a $90°_0$ pulse to create first-rank sin-

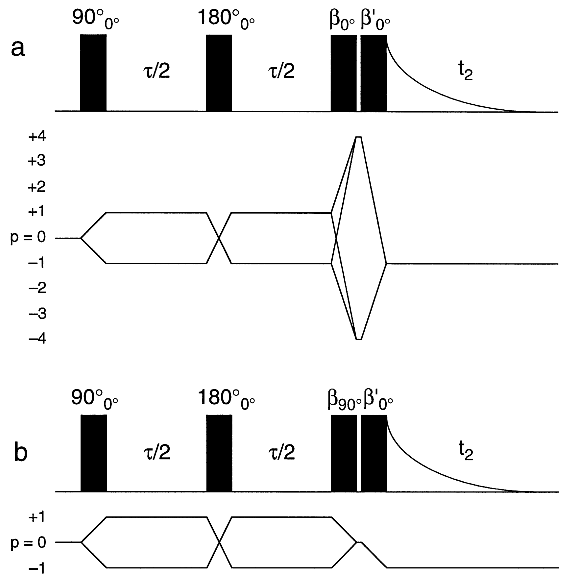

Fig. 1. Pulse sequences and coherence transfer pathway diagrams for: (a) the four-quantum filtration experiment; and (b) the Jeener–Broekaert experiment. In (a), the pulse flip-angles are calculated in the text to be $\beta = \beta' = 63.4°$ for optimum suppression of fifth-rank four-quantum coherences, $T_{5,\pm 4}$, while in (b), they are calculated to be $\beta = \beta' = 49.1°$ for optimum suppression of hexadecapolar order, $T_{4,0}$.

gle-quantum coherences:

$$T_{1,0} \xrightarrow{90°_0} \frac{1}{\sqrt{2}}(T_{1,-1} - T_{1,+1}) \tag{10}$$

Free precession under the influence of a residual quadrupolar splitting for an interval τ, punctuated by a $180°_0$ pulse at $\tau/2$, results in a state

$$\begin{aligned}\sigma(\tau) = -\frac{1}{\sqrt{2}}\{&g_{11}(\tau)(T_{1,-1} - T_{1,+1})\\ &+g_{21}(\tau)(T_{2,-1} + T_{2,+1})\\ &+g_{31}(\tau)(T_{3,-1} - T_{3,+1})\\ &+g_{41}(\tau)(T_{4,-1} + T_{4,+1})\\ &+g_{51}(\tau)(T_{5,-1} - T_{5,+1})\},\end{aligned} \tag{11}$$

where the superscripts (± 1) have been omitted from the evolution functions for clarity and use has been made of the simplification afforded by Eq. (9a). Excitation of four-quantum coherences leads to retention of only fourth- and fifth-rank tensors:

$$\begin{aligned}&\sigma(\tau,\beta_0) =\\ &\frac{1}{\sqrt{2}}\{g_{41}(\tau)[d^4_{4,1}(\beta) + d^4_{4,-1}(\beta)](T_{4,-4} - T_{4,+4})\\ &+g_{51}(\tau)[d^5_{4,1}(\beta) - d^5_{4,-1}(\beta)](T_{5,-4} + T_{5,+4})\}\end{aligned} \tag{12}$$

The reduced rotation matrix elements in Eq. (12) are given by

$$d^4_{4,\pm 1}(\beta) = -\frac{\sqrt{7}}{4\sqrt{2}}\sin^3\beta(1 \pm \cos\beta) \tag{13a}$$

$$d^5_{4,\pm 1}(\beta) = -\frac{\sqrt{21}}{16}\sin^3\beta(1 \pm \cos\beta)(5\cos\beta \mp 1). \tag{13b}$$

Inclusion of these into Eq. (12) yields

$$\begin{aligned}\sigma(\tau,\beta_0) = -\frac{1}{\sqrt{2}}\Bigg\{&\frac{\sqrt{7}}{2\sqrt{2}}g_{41}(\tau)\sin^3\beta(T_{4,-4} - T_{4,+4})\\ &+\frac{\sqrt{21}}{8}g_{51}(\tau)\sin^3\beta(5\cos^2\beta - 1)(T_{5,-4} + T_{5,+4})\Bigg\}\end{aligned} \tag{14}$$

The final pulse, β'_0, creates $p = -1$ coherences:

$$\begin{aligned}&\sigma(\tau,\beta_0,\beta'_0) =\\ &\frac{1}{\sqrt{2}}\Bigg\{\frac{7}{8}g_{41}(\tau)\sin^3\beta\sin^3\beta' T_{4,-1} + \frac{21}{64}g_{51}(\tau)\sin^3\beta\\ &\quad\times(5\cos^2\beta - 1)\sin^3\beta'(5\cos^2\beta' - 1)T_{5,-1}\Bigg\}\end{aligned} \tag{15}$$

Eq. (15) shows that the use of a flip-angle effect on either filtration pulse with β or $\beta' = \cos^{-1}(1/\sqrt{5}) = 63.4°$ will suppress the fifth-rank tensor operators. In Eq. (15), these arise through the action of a quadrupolar splitting, but Eq. (1) shows that, unlike fourth-rank tensor operators, they can also arise as a result of triexponential relaxation. Thus, the four-quantum filtration experiment with 63.4° flip angles yields $I = 5/2$ signal which is due exclusively to ordered nuclei. Maximum sensitivity is produced by having the

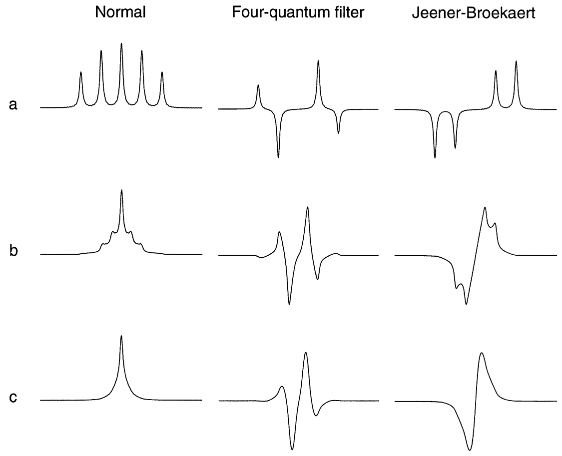

Fig. 2. Computer simulations of normal, four-quantum filtered ($\beta = \beta' = 63.4°$), and Jeener–Broekaert ($\beta = \beta' = 49.1°$) $I = 5/2$ NMR spectra. The program calculates numerically the evolution of the density matrix during pulses and free precession intervals using the time-independent solution to the Liouville–von Neumann equation. Transverse relaxation is not included within the density matrix calculation and the simulations are thus only valid for the case where the residual quadrupolar splitting is larger than the transverse relaxation rates. Spectra are shown with (a) a single value of the quadrupolar splitting parameter ($\omega_Q/2\pi = 2.5$ kHz); (b) a powder distribution of ω_Q($\omega_Q^{\max}/2\pi = 2.5$ kHz, $\eta = 0$); and (c) a sum of powder patterns with a Gaussian distribution of the maximum quadrupolar splitting parameter in each powder pattern (mean, standard deviation, minimum and maximum values of $\omega_Q^{\max}/2\pi = 1.25$, 0.625, 0, and 2.5 kHz, respectively). The spectral width is 40 kHz in each case. Intense pulses are used in all spectra, while in (b) and (c) the free precession intervals used are $\tau = 133$ μs (four-quantum filter) and $\tau = 66.7$ μs (Jeener–Broekaert).

other filter pulse set to 90°, but maximum suppression, achieved with $\beta = \beta' = 63.4°$, is the usual goal.

Computer simulations of the $I = 5/2$ NMR spectra resulting from the application of the four-quantum filtration pulse sequence are given in Fig. 2. Spectra are shown with: (a) a single value of the quadrupolar splitting parameter ω_Q; (b) a powder distribution of ω_Q; and (c) a sum of powder patterns with a Gaussian distribution of the maximum quadrupolar splitting parameter in each powder pattern. All three four-quantum filtered spectra have the characteristic features of the $T_{4,-1}$ tensor; triply antiphase and no central transition. (It should be noted that these simulations are based on a density matrix calculation that excludes the effects of transverse relaxation and these are mimicked in Fig. 2 by inclusion of a simple homogeneous line broadening function. The simulations are, therefore, only valid for the case where the residual quadrupolar splitting is larger than the transverse relaxation rates.)

The spin $I = 3/2$ Jeener–Broekaert experiment filters magnetisation through even-rank populations only [8]. This approach can also be applied without modification to spin $I = 5/2$ nuclei to remove all tensors except $T_{2,0}$ and $T_{4,0}$, both of which can only form for nuclei with residual quadrupolar splittings. The pulse sequence and coherence level diagram for this $I = 5/2$ Jeener–Broekaert experiment are the same as that for the $I = 3/2$ case (Fig. 1b), but a tensor calculation shows that different values are preferred for the flip angles of the filtration pulses.

After a $90°_0$ pulse and a period of (spin echo) evolution, the density matrix is given by Eq. (11). The result of a pulse, $\beta_{90°}$, which is phase cycled to give selection of coherence order $p = 0$ is given by

$$\sigma(\tau,\beta_{90°}) = i\sqrt{2}\{g_{21}(\tau)d^2_{1,0}(\beta)T_{2,0} + g_{41}(\tau)d^4_{1,0}(\beta)T_{4,0}\} \tag{16}$$

where the reduced rotation elements are given by

$$d^2_{1,0}(\beta) = -\frac{\sqrt{3}}{\sqrt{8}}\sin 2\beta, \tag{17a}$$

$$d^4_{1,0}(\beta) = -\frac{\sqrt{5}}{16}\sin 2\beta(1 + 7\cos 2\beta). \tag{17b}$$

The final density matrix, produced by a β'_0 pulse, can be expressed as

$$\sigma(\tau,\beta_{90°},\beta'_0) = -i\sqrt{2}\left\{\frac{3}{8}g_{21}(\tau)\sin 2\beta\sin 2\beta' T_{2,-1} + \frac{5}{256}g_{41}(\tau)\sin 2\beta(1 + 7\cos 2\beta)\sin 2\beta'(1 + 7\cos 2\beta')T_{4,-1}\right\} \tag{18}$$

Both $T_{2,-1}$ and $T_{4,-1}$ can arise only from nuclei in ordered environments. However, it is usually advantageous to obtain spectra possessing the amplitude characteristics of just one tensor operator. From Eq. (18), suppression of the $T_{4,-1}$ tensor can be achieved using a flip-angle effect, with β or $\beta' = 1/2 \cos^{-1}(-1/7) = 49.1°$, which is also very close to the value at which the $d^2_{1,0}(\beta)$ term is a maximum ($\beta = 45°$). Fig. 2 shows computer simulations of $I = 5/2$ spectra resulting from the application of the Jeener–Broekaert experiment in Fig. 1b with $\beta = \beta' = 49.1°$. All three Jeener–Broekaert spectra have the characteristic features of the $T_{2,-1}$ tensor; singly antiphase and no central transition.

4. Experimental results

A model biological system was prepared to test the predictions of the theory and simulations. The sample consisted of collagen (type I) obtained from bovine achilles tendon (Sigma) wetted with 0.15 M NaCl solution and 100 μl of 20% ^{17}O-enriched H_2O. This system has been used in ^{23}Na NMR studies and selective detection of ordered sodium has been demonstrated [14]. We postulated that water molecules might bind to the collagen molecules in a similar fashion and might thus display residual quadrupolar splittings.

Fig. 3a shows normal, four-quantum filtered, and $I = 5/2$ Jeener–Broekaert ^{17}O NMR spectra of this collagen sample. In the normal spectrum, a broad component is barely discernible beneath a much sharper signal. In both the four-quantum filtered and Jeener–Broekaert spectra, the general characteristics of the ^{17}O lineshapes can be seen to match those generated by simulations for systems with macroscopic disorder and a Gaussian distribution of $\omega_Q^{\max}$ values (Fig. 2c), as would be expected for a heterogeneous biological sample.

Many real biological tissues contain collagen

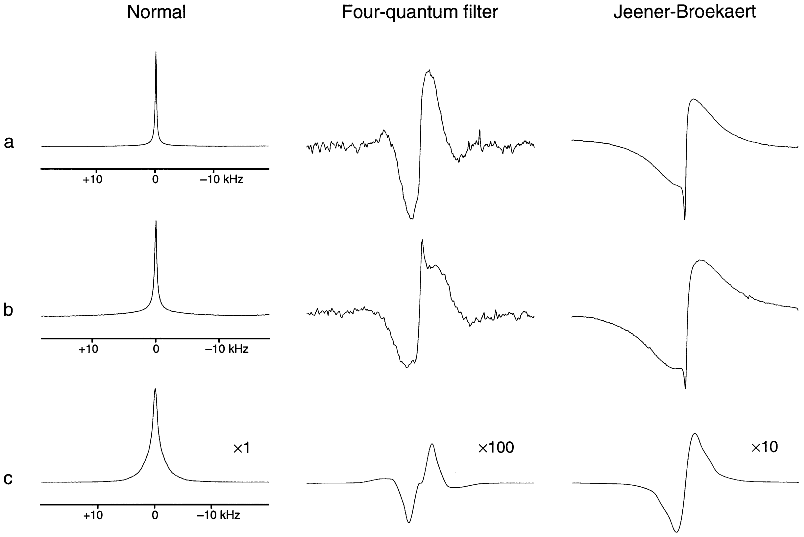

Fig. 3. (a) Normal, four-quantum filtered, and Jeener–Broekaert ^{17}O NMR spectra of a sample of collagen (type I) obtained from bovine achilles tendon (Sigma) wetted with 0.15 M NaCl solution and 100 μl of 20% ^{17}O-enriched H_2O. Acquisition parameters: 54.2 MHz Larmor frequency, 38.5 kHz spectral width; 1024, 49152 and 49152 transients co-added, respectively; 20.3 μs 90° pulse length; $\tau = 64$ μs (four-quantum filter) and 32 μs (Jeener–Broekaert). (b) Normal, four-quantum filtered, and Jeener–Broekaert ^{17}O NMR spectra of a sample of excised bovine tendon, stored in 0.15 M NaCl solution, to which 100 μl of 20% ^{17}O-enriched H_2O was added. Acquisition parameters: 54.2 MHz Larmor frequency, 35.7 kHz spectral width; 64, 12288 and 12288 transients co-added, respectively; 13.5 μs 90° pulse length; $\tau = 48$ μs (four-quantum filter) and 24 μs (Jeener–Broekaert). (c) Computer-simulated normal, four-quantum filtered, and Jeener–Broekaert $I = 5/2$ NMR spectra. Simulation parameters: 40 kHz spectral width; 15 μs 90° pulse length; $\tau = 48$ μs (four-quantum filter) and 24 μs (Jeener-Broekaert). The quadrupolar splitting parameters were the same as those used in Fig. 2c.

macromolecules, notably tendon and cartilage. A sample of bovine tendon from around the front knee joint was excised and stored in 0.15 M NaCl solution (to maintain cell structure). Immediately before use, the tendon was removed from the soaking solution, dried of excess solution and 100 μl of 20% ^{17}O-enriched H_2O added. Fig. 3b presents normal, four-quantum filtered and $I = 5/2$ Jeener–Broekaert ^{17}O NMR spectra taken from this sample. Their resemblance to the model collagen spectra is striking and they clearly show the occurrence of ordered water in the tissue. These experiments on collagen powder and excised tendon are believed to be the first ^{17}O NMR demonstrations of ordered water in biological systems.

5. Discussion

A noticeable feature in Fig. 3a,b is the difference in signal-to-noise ratio between the four-quantum filtration and Jeener–Broekaert experiments. From the $g_{l'l}^{(+1)}(t)$ functions and tensor operator calculations given above, and assuming a single value of ω_Q, it is possible to calculate theoretical maximum values for the $T_{n,-1}$ coefficients occurring at the start of the acquisition period in each experiment. For the four-quantum filtration experiment with $\beta = \beta' = 63.4°$ we find that

$$\sigma^{\max}(\tau = 1.047/\omega_Q, \beta_0, \beta'_0) = -0.235i\, T_{4,-1} \tag{19a}$$

while, for the $I = 5/2$ Jeener–Broekaert experiment with $\beta = \beta' = 49.1°$,

$$\sigma^{\max}(\tau = 0.4547/\omega_Q, \beta_{90°}, \beta'_0) = 0.376 T_{2,-1} \tag{19b}$$

The modulus of the ratio of the two coefficients is thus 0.625. The theoretical relative sensitivity of the two experiments can be calculated by including a factor allowing for the maximum peak heights of the spectra associated with the $T_{n,-1}$ tensors (the largest diagonal element in the product $T_{n,-1} \cdot T_{1,+1}$). The ratio of these factors for $T_{4,-1}$ and $T_{2,-1}$ is 1.265. Therefore the theoretical sensitivity ratio of four-quantum filtration/Jeener–Broekaert experiments is 0.791, a figure which we have verified by computer simulation.

The observed ratios in Fig. 3a,b are clearly much smaller than this value. One part of the explanation of this lies in the forms of the $T_{2,-1}$ and $T_{4,-1}$ spectra (Fig. 2a). In the presence of significant homogeneous and inhomogeneous broadening the satellite resonances will overlap. In the triply-antiphase $T_{4,-1}$ spectrum this will produce significant signal cancellation, whereas in the singly-antiphase $T_{2,-1}$ spectrum co-addition occurs. The second part of the explanation lies in the fact that, although the radiofrequency field strengths, $\omega_1/2\pi$, used in the experiments in Fig. 3a,b were 12.3 and 18.5 kHz, respectively, the ^{17}O linewidth is of the same magnitude and excitation of high orders of multiple-quantum coherence is known to be an inefficient process with such a relatively weak pulse. These two factors combined mean that a four-quantum filtered spectrum is weakened relative to the Jeener–Broekaert one. Fig. 3c shows $I = 5/2$ spectra simulated using parameters that approximately match the experimental spectra in Fig. 3a,b and it can be seen that the four-quantum filtered spectrum is more than an order of magnitude weaker than the Jeener–Broekaert spectrum.

In summary, two NMR experiments designed for selective excitation of spin $I = 5/2$ nuclei with residual quadrupolar splittings have been presented in this article. The $I = 5/2$ Jeener–Broekaert experiment is strongly preferred to the four-quantum filtration experiment as it is a very much more sensitive technique in experimental practice. Both techniques have been applied to ^{17}O-enriched water in biological systems. The occurrence of water which displays a residual ^{17}O quadrupolar splitting has been demonstrated for the first time in a model biological system and an excised tissue sample. The resulting ^{17}O NMR spectra have been shown to have the characteristics predicted in computer-simulated $I = 5/2$ NMR spectra.

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