Detection of Anisotropy in Cartilage Using $^2$H Double-Quantum-Filtered NMR Spectroscopy

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Double-quantum-filtered (DQF) NMR spectroscopy of $I = 1$ spin systems is a diagnostic tool for the detection of anisotropy in macroscopically disordered systems. For deuterium, this method reveals the presence of a residual quadrupolar interaction for D$_2$O in bovine nasal cartilage. This tissue is not macroscopically ordered and the quadrupolar splitting is not resolved. Fitting the calculated spectral line shapes to the experimental results was possible only when a distribution of the residual quadrupolar interaction, $\omega_q$, was assumed. The series of DQF line shapes obtained for different creation times in the DQF experiment could be fitted using a single set of three parameters: the average residual quadrupolar interaction $\Delta \omega_q/2\pi = 110$ Hz, its standard deviation $\Delta \omega_q/2\pi = 73$ Hz, and the transverse relaxation rate of 63 s$^{-1}$. Separate deuterium DQF measurements for the constituents of the cartilage, collagen, and chondroitin sulfate indicated that the DQF spectra of cartilage are the result of anisotropic motion of D$_2$O due to binding to the fibrous collagen in the tissue. © 1995 Academic Press, Inc.

INTRODUCTION

The use of multiple-quantum (MQ) NMR spectroscopy of quadrupolar nuclei for the investigation of molecular dynamics has been practiced in a variety of studies during the past two decades. Primarily, most attention was addressed to $^2$H in a nematic liquid-crystalline phase in which the quadrupolar splitting is well resolved ($I = 4$). Those experiments have also demonstrated the feasibility of MQ techniques for the study of relaxation rates. The use of MQ techniques was later extended to quadrupolar nuclei with higher spins such as $I = \frac{3}{2}$, $I = \frac{5}{2}$, and $I = \frac{7}{2}$ in liquid-crystal and isotropic media ($5$-$7$).

The question of studying $^2$H in cartilage tissue was motivated by the discovery that the motion of $^{23}$Na ($I = \frac{3}{2}$) in cartilage ($8$, $9$) and in red blood cells ($10$) is locally anisotropic, leading to a nonzero average of the quadrupolar interaction. The observed double-quantum-filtered (DQF) NMR line shape may be due to the formation of third- or second-rank tensors or both. The third-rank tensor is formed in both isotropic and anisotropic phases, whereas the second-rank tensor is formed only in anisotropic phases. For $^2$H ($I = 1$), a three-level system, the situation is more favorable. Since no tensor with a rank higher than two can be formed, the DQF signal stems exclusively from the anisotropic motion and no DQF signal is expected for isotropic systems. Thus, the detection of $^2$H DQF spectra is diagnostic of the presence of anisotropy.

THEORY

The $^2$H DQF spectrum of heterogeneous systems such as cartilage represents a sum of spectra arising from many different noninteracting sites. The overall motion of water molecules in each site is anisotropic and can be characterized effectively by a local residual quadrupolar interaction, $\omega_q$, and an orientation $\theta$ of the local symmetry axis relative to the external field. In a coordinate system rotating at a frequency $\omega$ about the laboratory-fixed $z$ axis, the time-independent Hamiltonian is given by

$$\mathcal{H}_{\omega q} = -\Delta \omega \hat{I}_z + \omega_{q,\theta} (\hat{I}_z^2 - \frac{1}{2} \hat{I}^2).$$  \[1\]

where $\Delta \omega = \omega_0 - \omega$ is the offset of the RF carrier frequency $\omega$ from resonance frequency $\omega_0$, and

$$\omega_{q,\theta} = \omega_q \frac{1}{2} (3 \cos^2 \theta - 1)$$  \[2\]

is the residual quadrupolar interaction in the laboratory frame of reference. In Eq. [1] it was assumed that the asymmetry parameter is zero.

Representing the problem in the Liouville operator space, the equation of motion of the spins is given by the differential equation of the density operator $\sigma(t)$,

$$\frac{d}{dt} \sigma(t) = -i\mathcal{H} \sigma(t) + \mathbf{\dot{\hat{f}}} (\sigma(t) - \sigma_0).$$  \[3\]

where $\mathbf{\dot{\hat{f}}}$ and $\mathbf{\hat{f}}$ are the superoperators for the free precession and the relaxation, respectively, and $\sigma_0$ is the equilibrium value of the spin-density matrix.
The irreducible spherical-tensor operators were chosen as the basis operators of the Liouville space. When the dominant relaxation mechanism is of quadrupolar origin, it is possible to obtain an analytical solution for Eq. [3]. The solution can be written as a time-evolution propagator \( \Gamma(t) \) in the Liouville space, whose explicit expression is given in the Appendix (Eq. [A.1.5]). The evolution of the density matrix during a time interval \( \tau \) can be evaluated by \( \sigma(t + \tau) = \Gamma(\tau) \sigma(t) \).

**CALCULATION OF SPECTRAL LINESHAPES**

In order to generate multiple-quantum coherences, the following sequence of nonselective pulses is employed (5, 11, 12):

\[
\frac{\pi}{2} - \tau/2 - \pi - \tau/2 - \pi/2 - t_1 - \pi/2 - t_2 \quad \text{(Acq)}.
\]

[4]

Contributions from the \( z \) magnetization and the single-quantum coherences during the evolution period, \( t_1 \), are suppressed using appropriate phase cycling (13). The coherence-transfer pathway diagram for the DQF experiment is given in Fig. 1.

The result of the DQF experiment [4] can be expressed in the Liouville space as

\[
\begin{align*}
\rho_{0} & \rightarrow \hat{R}\left(\frac{\pi}{2}, 0\right) & \rightarrow \hat{R}\left(\tau/2, 0\right) & \rightarrow \hat{R}(\pi, 0) \\
\rightarrow \hat{T}\left(\frac{\tau}{2}\right) & \rightarrow \hat{R}\left(\frac{\pi}{2}, 0\right) & \rightarrow \hat{T}(t_1) \\
\rightarrow \hat{F}_{\text{DQ}} & \rightarrow \hat{R}\left(\frac{\pi}{2}, 0\right) & \rightarrow \hat{T}(t_2) & \rightarrow \sigma(\tau, t_1, t_2).
\end{align*}
\]

[5]

The rotation propagator \( \hat{R}(\beta, \varphi) \) represents the nonselective RF pulses, where \( \beta \) is the tilt angle and \( \varphi \) is the phase of the rotation axis in the transverse plane. The double-quantum-filter propagator \( \hat{F}_{\text{DQ}} \) represents the effect of the phase cycling which eliminates all coherences except the double-quantum coherences during the evolution period \( t_1 \) (see Appendix A.2). It is of computational convenience to make use of the commutation relation \( [\hat{F}_{\text{DQ}}, \hat{T}(t)] = 0 \), enabling the change of the order of application of the two propagators, and thus eliminating all coherence pathways other than those described in Fig. 1.

Successive application of the propagators listed in Eq. [5] on the equilibrium-density matrix \( \sigma_{0} \) leads to an expression for the FID signal of a single site characterized by \( \omega_{q} \) and \( \theta \).

\[
\text{FID}_{\omega_{q}, \theta}(\tau, t_1, t_2) = M_{0} \frac{2}{(\omega_{q,0})^2 - (\frac{1}{2} + 2 \text{J}_1(\omega_{0}))^2} \exp(-R_2\tau) \times \sin\left( 2\text{J}_1(\omega_{0}) \right) \exp\left( (\frac{1}{2} + 2 \text{J}_1(\omega_{0})) \right) \times \exp(-R_1t_1) \times \exp(2\Delta\omega t_2) \times \exp((2\Delta\omega - R_2)t_2) \times \exp((2\Delta\omega - R_2)t_2).
\]

[6]

\[
R_1 = \frac{1}{2} \left\{ 3\text{J}_0(0) + 3\text{J}_1(\omega_{0}) + 2\text{J}_2(2\omega_{0}) \right\},
\]

[7]

\[
R_2 = \frac{1}{2} \left\{ 3\text{J}_0(0) + 3\text{J}_1(\omega_{0}) + 2\text{J}_2(2\omega_{0}) \right\}.
\]

[7]

where \( \chi = e^{2\theta Q}/h \) is the quadrupolar coupling constant and \( \text{J}_n(\omega_{0}) \) are spectral densities. One should note that expression [6] is not singular at \( \omega_{q,0} = \frac{1}{2} \chi^2 \text{J}_1(\omega_{0}) \) since its limit at this point is proportional to \( (\omega_{q,0})^2 \exp(-R_2\tau) \exp(-R_1t_1) \times \exp((2\Delta\omega - R_2)t_2) \). It can be seen from Eq. [6] that for \( \omega_{q,0} > \frac{1}{2} \chi^2 \text{J}_1(\omega_{0}) \) the spectrum is expected to have oscillations with a fre-

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**FIG. 1.** DQF coherence-transfer pathway diagram (nonselective pulses. \( T_{0} \) are spherical tensors of rank \( l \) and coherence \( p \)).
frequency of $1/\tau$ since the amplitude of all spins that have resonance at frequency of $\sqrt{(\omega_0 \theta)^2 - \left(\frac{1}{2} \chi^2 J_I(\omega_0)\right)^2}$ is proportional to $\sin\left(\sqrt{(\omega_0 \theta)^2 - \left(\frac{1}{2} \chi^2 J_I(\omega_0)\right)^2}\tau\right)$.

Cartilage is an heterogeneous tissue, composed of many sites characterized by different values of $\omega_q$ and $\theta$. The former parameter is a result of averaging caused by exchange of water molecules between the various binding sites and their free state. Let us define the probability of finding deuterium nuclei in each of the sites as $P(\omega_q, \theta)$. Since no correlation is expected between $\omega_q$ and $\theta$ of each site, one can write

$$P(\omega_q, \theta) = P_q(\omega_q)P_\theta(\theta),$$  \hspace{1cm} [8]

where $P_q(\omega_q)$ is the probability of a site having a residual interaction $\omega_q$ in the local director frame of reference and $P_\theta(\theta)$ is the probability that the angle between the director of the motion and the magnetic field is $\theta$. Hence, the DQF spectrum can be evaluated by

$$S(\tau, t_1, \omega_2) = 2\pi \int_0^\pi P_\theta(\theta)\sin(\theta)d\theta$$
$$\times \int_{-\infty}^{\infty} P_q(\omega_q)S_{\omega_q}(\tau, t_1, \omega_2)d\omega_q,$$  \hspace{1cm} [9]

where $S_{\omega_q}(\tau, t_1, \omega_2)$ is a Fourier transform of FID$_{\omega_q}(\tau, t_1, t_2)$ with respect to $t_2$ (Eq. [6]).

**EXPERIMENTAL**

Four types of samples were measured: (1) a piece of fresh bovine nasal cartilage immersed in D$\text{}_2$O; (2) collagen (type I, from bovine Achilles tendon, Sigma) suspended in D$\text{}_2$O; (3) chondroitin sulfate (type C, Sigma) dissolved in D$\text{}_2$O; and (4) cartilage powder (Sigma) suspended in D$\text{}_2$O. $^2$H DQF spectra were recorded on a Bruker AM360-WB spectrometer equipped with a broadband 10 mm probe, using 10 mm tubes, at a $^2$H frequency of 55.3 MHz. All measurements were conducted at room temperature ($20 \pm 2^\circ$C).

The computer program for the fitting of the lineshapes was written in Pascal and was run on a PC386 computer equipped with a 80387 numerical coprocessor.

**EXPERIMENTAL RESULTS**

**Single-Pulse Experiments**

The single-pulse spectrum of $^2$H in cartilage consisted of three components (Fig. 2): two narrow lines ($\Delta\nu_1/2 \approx 10$ Hz each) separated by 11 Hz, superimposed on a broad line ($\Delta\nu_1/2 \approx 120$ Hz wide). An inversion-recovery experiment, $\pi-\tau-\pi/2$-Acq, yielded two relaxation times. The slow one, $T_1^* = 0.35 \pm 0.05$ s, was measured for the two narrow lines and was equal to the longitudinal relaxation time for free D$\text{}_2$O. For the broad component, we measured $T_1^* = 0.14 \pm 0.01$ s. Both narrow components of the single-pulse spectrum were tentatively assigned to contributions of free D$\text{}_2$O. The low-field peak is assigned to the free D$\text{}_2$O outside the tissue because its intensity decreased as we poured some of the D$\text{}_2$O solution out of the tube. The high-field peak arises from D$\text{}_2$O inside the cartilage. The separation of 11 Hz was assigned to the difference in the bulk susceptibility between the two environments. The origin of the lineshape of the broad component was examined using MQ spectroscopy methods.

**Double-Quantum-Filter Experiments**

DQF experiments were performed with the pulse sequence of Eq. [4] with an appropriate phase cycling (13) that selects the DQF coherences during the evolution period $t_1$. A series of DQF spectra with various values of the creation time $\tau$ were measured on a piece of bovine nasal cartilage immersed in D$\text{}_2$O. The evolution time was 50 $\mu$s, short enough to minimize the effect of double-quantum relaxation. The shape of the spectra varied considerably for different $\tau$ values as shown in the representative set of spectra (Fig. 3). As $\tau$ is increased, the widths of the “wings” decrease and they become more apparent, while the central line keeps growing and gets to its maximum at $\tau = 4.0$ ms. For long values of $\tau$, extra wings are added, forming ripples that decay slowly to zero. These oscillations have a frequency of $1/\tau$, as expected from Eq. [6].

The observation of DQF signal is due to the formation of a second-rank tensor during the creation time $\tau$. As can be seen from Eq. [6], such a tensor can be formed...
for a system possessing residual quadrupolar interaction $\omega_q$ resulting from the anisotropic motion of the molecules. In order to test whether the system of bovine nasal cartilage is macroscopically ordered, we repeated the experiment (a) after rotation of the sample by 90° relative to the magnetic field, and (b) after cutting the sample into small pieces (about 1 mm diameter). The observed spectra in the two cases were very similar to the original spectrum shown in Fig. 3. The DQF experiment was repeated on four samples of bovine nasal cartilage taken from two different animals, and the spectra were hardly distinguishable.

An analysis of the spectra was performed by nonlinear-least-mean-squares (14) fit of the DQF spectral lineshapes to those calculated using Eq. [9]. For the calculation of lineshapes an isotropic distribution of the sites, \( P_d(\theta) = 1 \), was assumed. This assumption is based on our experiments indicating no angular dependence of the DQF lineshapes. However, preliminary attempts to fit the experimental results with a single value of $\omega_q$ clearly indicated that some distribution of $\omega_q$ is essential. Since there was no previous knowledge about the distribution of $\omega_q$, a simplifying assumption of a normal distribution was adopted. The probability-density function is given by

\[
P_q(\omega_q) = \frac{1}{\sqrt{2\pi}(\Delta\omega_q)^2} \exp\left(-\frac{(\omega_q - \bar{\omega}_q)^2}{2(\Delta\omega_q)^2}\right),
\]

where $\bar{\omega}_q$ and $\Delta\omega_q$ stand for the average value and the standard deviation of $\omega_q$, respectively.

Equation [9] was calculated by numerical integration for each value of $\tau$ and a set of parameters $R_2$, $\bar{\omega}_q$, and $\Delta\omega_q$. In this way it was possible to fit the lineshapes of the DQF spectra for a series of $\tau$, using a single set of parameters (Fig. 3). This set included the relaxation rate $R_2 \approx 63.0$ s$^{-1}$, an average residual quadrupolar interaction $\bar{\omega}_q/2\pi \approx 110$ Hz, and the corresponding standard deviation $\Delta\omega_q/2\pi \approx 73$ Hz. Equation [6] includes in addition to $R_2$ one of its components, $\frac{1}{2} x^2 J_1(\omega_0)$. During the fitting procedure it was found that the results were quite insensitive to the choice of $\frac{1}{2} x^2 J_1(\omega_0)$. This is easily explained by the fact that, since the three motional parameters $x^2 J_0(0)$, $x^2 J_1(\omega_q)$, and $x^2 J_2(2\omega_q)$ in Eq. [7] are necessarily positive, the value of $\frac{1}{2} x^2 J_1(\omega_0)$ must be less than the value of $\frac{1}{2} R_2$, i.e., the value of $\frac{1}{2} x^2 J_1(\omega_0)$ is smaller than 7 Hz. Thus, since $\frac{1}{2} x^2 J_1(\omega_0)$ has a significant contribution only for a narrow range of $\theta$ values where $\omega_q, \omega_0 \sim \frac{1}{2} x^2 J_1(\omega_0)$, and since $\omega_q, \omega_0$ at this range is very small, the effect of $\frac{1}{2} x^2 J_1(\omega_0)$ in Eq. [6] is negligible. A comparison between the calculated and the observed spectra reveals that the fitting is very good for intermediate and long values of $\tau$ and relatively poor for very short ones. Apparently, these deviations are due to our simplifying assumptions of normal distribution of $\omega_q$ and a lack of distribution of $R_2$ throughout the sample. A skewed distribution with more weight to larger values of $\omega_q$ would have given a better fit, but our results did not warrant the addition of more parameters. It should be mentioned at this point that an independent measurement of $R_2$ using a double-quantum-filtered quadrupole echo on another sample of bovine nasal cartilage gave a value of 63 s$^{-1}$ (15).

Longitudinal-Decay-Rate Measurements

In order to measure the longitudinal relaxation time of those deuterium nuclei which are in anisotropic sites, the pulse sequence of Eq. [4] was used, preceded by a 180° pulse: $\pi-\tau-DQF(Aq)$. The value of $\tau$ (4 ms) was chosen to give the maximum signal intensity of the DQF spectra (see Fig. 3). The value of $T_1$ obtained in this way was 0.14 ± 0.01 s. This value is identical to that obtained for the broad component of the single-pulse experiment ($T_1'$). This result may suggest that the broad component of the single-quantum experiment is due mainly to the anisotropic sites. In order to test this hypothesis the single-pulse spectrum was calculated using the set of values for the $\bar{\omega}_q$, $\Delta\omega_q$, and $R_2$ obtained from DQF measurements (previous section). The calculation was accomplished using Eq. [9] with
\[ S_{\omega_{\theta}}(\omega) = \frac{R_2}{[R_2^2 + (\omega - \omega_{\theta})^2]} + \frac{R_2}{[R_2^2 + (\omega + \omega_{\theta})^2]} \]  

[11]

The calculated lineshape was very similar to the experimental result with a width of \(\Delta \nu_{\text{pump}} \approx 120 \text{ Hz} \) (see Fig. 2, insert). Thus we conclude that the broad component of the single-pulse experiment originates mostly from the anisotropic sites. It is interesting to note that although the average residual quadrupolar interaction, \(\omega_q/2\pi = 110 \text{ Hz} \), is more than six times the natural linewidth \(R_2/\pi \approx 16 \text{ Hz} \), there is no resemblance to a powder-like spectrum. The explanation for this phenomenon lies in the considerable distribution of \(\omega_q \) (large \(\Delta \omega_q \)) resulting from the heterogeneity of the tissue.

**DQF Spectra of Collagen and Chondroitin Sulfate**

Cartilage is composed of two major macromolecules: fibrous collagen and proteoglycans (PG) which are complexes of polysaccharides (chondroitin sulfate) and proteins. Series of deuterium DQF spectra in separate samples of chondroitin sulfate, collagen powder, and cartilage powder all immersed in \(\text{D}_2\text{O} \) were measured using the DQF pulse sequence [4]. Chondroitin sulfate dissolved in \(\text{D}_2\text{O} \), while both collagen and cartilage powder are insoluble and remained suspended. For the solution of chondroitin sulfate the DQF experiment yielded a weak narrow Lorentzian line. It was assigned to an artifact that was not canceled by the phase cycling of the DQF experiment, since upon varying the time interval \(\tau \) it varied in a complex manner and did not oscillate at twice the offset frequency as expected from double-quantum coherence (the “offset test”).

The observed DQF spectral lineshapes for the suspension of collagen and cartilage powders were very similar. A set of DQF spectra of the collagen suspension for various \(\tau \) values is given in Fig. 4. By performing the offset test, we confirmed the signal to be a DQF signal and not an artifact. The observation of DQF signals clearly indicates the presence of a nonvanishing average of the quadrupolar interaction resulting from the anisotropic motion of the molecules in the suspensions of collagen and cartilage powder.

**DISCUSSION**

In this work we report the observation of a \(^3\text{H} \) DQF spectrum of \(\text{D}_2\text{O} \) in cartilage. As was discussed under Theory, the detectable DQF signal stemmed exclusively from the second-rank tensor which was formed during creation period \(\tau \) (Fig. 1). For an \(\ell = 1 \) spin system, no DQ coherences can be formed in isotropic medium, and therefore, the observation of the DQF spectrum indicates the presence of anisotropic motion of the water molecules. This is in contrast to \(\ell > 1 \) spin systems where the observed signal of the DQF experiment may evolve from DQ coherences of higher-rank tensors that pass the filter. In particular, for \(\ell = \frac{3}{2} \), the tensors \(T_{3,2}\), which are created during evolution time \(\tau \), are selected by the phase-cycling procedure. The monitoring pulse transfers \(T_{3,2}\) to \(T_{3,-1}\), which during free precession is converted into the observable \(T_{1,-1}\). Contributions from the third-rank tensors \(T_{3,2}\) can be suppressed by using a tilt angle of \(54.7^\circ\) for the last two pulses in the DQF pulse sequence (Eq. [4]) (5, 8). However, this method suffers from reduced intensity and is highly sensitive to \(T_1\) inhomogeneities. Although these problems are significantly reduced by a method based on the use of the Jeener–Broekaert pulse sequence (16, 17), they do not affect the measurement of anisotropic motion of \(\ell = 1 \) nuclei. In general, it can be shown that the observed DQF spectra of any \(\ell > 1 \) nuclei will always contain contributions evolving from higher-rank tensors, \(T_{\ell,2}\), where \(2 \leq \ell \leq 2\ell \) (18, 19). If the relaxation mechanism is governed by a second-rank interaction, the odd-rank tensors \((\ell = 1, 3, 5, \ldots , 2n - 1)\) where \(n\) is a positive integer) will be generated whenever sequence [4] is employed, for both isotropic or anisotropic motions. However, in quadrupolar nuclei with integer spins \((\ell = 1, 2, 3, \cdots)\) relaxing by a second-rank interaction, experiments selecting the highest coherence \((2\ell)\) will give vanishing results unless the motion is anisotropic.

According to the deuterium DQF measurements on the constituents of the cartilage, collagen powder, and chondroitin sulfate, it appears that the observed anisotropic mo-
tion of the $^2$H is associated with the fibrous collagen macromolecules. Similar results were reported for sodium DQF measurements in cartilage \cite{8}. This conclusion stands in good agreement with our knowledge of the structure of the cartilage tissue. The bulk of triple-helix collagen molecules arranged in fibrils have a definite spatial director. However, there is a random orientation of the fibrils in the cartilage, in contrast to tendon, where they are aligned along the major axis. On the other hand, chondroitin sulfate tends to adopt highly extended, random-coil conformations. Thus it is not surprising that it does not contribute to the residual quadrupolar interaction.

Studies of proton magnetization transfer in articular cartilage by Kim \textit{et al.} \cite{20} revealed that the magnetization transfer between macromolecules and water, which is sensitive to the correlation time, is dominated by collagen in the cartilage. It was also reported that the PG moiety with its abundant hydroxyl groups on the saccharide rings does not exhibit magnetization transfer. The phenomenon was tentatively explained by assuming fast motion of the PG hydroxyl groups. The formation of $^2$H DQF signal in cartilage reported in the present work originates also from the collagen in the tissue. However, the important factor in our case is the anisotropic nature of the motion.

The magnitude of the quadrupolar interaction constant $e^2 q Q/\hbar$ for $^2$H in solid hydrates is about 240 kHz \cite{21}. This quadrupole coupling constant is reduced in biological systems by four averaging processes: (1) averaging of the quadrupolar interaction by anisotropic reorientations of the bound water molecules; (2) diffusion of water molecules on the surface of the macromolecules; (3) fast chemical exchange between bound and free water molecules; and (4) exchange between bound water molecules with different orientations via the exchange with the surrounding free water molecules. The result of these averaging processes is a residual quadrupolar interaction; its magnitude in the local frame of reference is $\omega_q$. As the sample is heterogeneous, variation of any one of these factors may lead to a distribution of the resultant $\omega_q$, as was found in the analysis of our experimental results.

Our results may be compared with the results of Migchelsen and Berendsen on the deuterium quadrupolar splitting of D$_2$O in oriented collagen fibers of rat tail tendon \cite{22, 23}. They observed a value of $\omega_q/2\pi = 1535$ Hz for 1.2 grams of D$_2$O per gram dry weight of collagen. The water concentration in cartilage equilibrated with D$_2$O was measured from the ratio of the dry and wet weight of the sample, giving a value of 85%. The collagen fibrils compose about 60% of the dry weight of (human) cartilage tissue \cite{24}. Using the tentative assumption that $\omega_q/2\pi$ is proportional to the ratio of the collagen and water concentrations gives a value of $\omega_q/2\pi$ of about 180 Hz for the ratio present in cartilage. This result and our value of 110 $\pm$ 73 Hz are of the same order of magnitude. However, such a comparison has the following limitations: (1) The collagen in the rat tail tendon (type I) is different from that in the bovine nasal cartilage (type II), and (2) since the collagen fibers in cartilage are not aligned, processes of exchange and diffusion may cause additional averaging.

In our interpretation of the DQF spectral lineshapes we assumed a distribution of $\omega_q$ but did not consider any heterogeneity in the spectral densities; i.e., the spectral densities have been regarded as constants in the integration (Eq. \cite{9}). In general, heterogeneity of any of the factors that affect the spectral density, such as the size of the quadrupolar interaction, the reorientation correlation times, the fraction of bound water, and the orientation dependence, can cause a distribution of spectral densities. A detailed description of the orientation dependence of the spectral densities has been previously given \cite{9, 25}. However, the fitting of the calculated $^2$H DQF lineshapes to the observed spectra has indicated that the modulation of the lineshapes due to distribution of the spectral densities is negligible compared to that caused by the broad distribution of the residual quadrupolar interactions. Hence we conclude that $^2$H DQF lineshape analysis can not give information about the heterogeneity of the spectral densities in the cartilage.

The results of $^2$H DQF spectra of D$_2$O in cartilage may be compared to those of $^{23}$Na in the same tissue \cite{9}. In the latter studies values of $\omega_q/2\pi = 262$ Hz, $\Delta \omega_q/2\pi = 120$ Hz, and $1/T_{2f} = 355$ s$^{-1}$ were found. The fact that the ratio $\omega_q/R_2$ is 11 for deuterium compared to 4.6 in sodium means that the DQF lineshapes of the $^2$H is determined mainly by $\omega_q$ and its distribution, while that of $^{23}$Na is more sensitive to the relaxation time $T_{2f}$. Thus the calculation of the residual quadrupolar interaction $\omega_q$ and its distribution from the DQF spectra is easier for deuterium DQF NMR measurements.

We have shown that $^2$H DQF NMR spectroscopy is a very sensitive method for the determination of the residual quadrupolar interaction resulting from local order. Unlike nuclei of spin-\frac{3}{2}, such as $^{23}$Na, no DQF spectra are obtained for spin 1 nuclei unless there is an anisotropic motion of the nuclei. The $^2$H DQF is independent of $B_i$ inhomogeneity which may only modulate the intensity of signal. Thus, DQF NMR of deuterium can be used also for imaging of anisotropies in biological tissues \textit{in vivo}.

APPENDIX

\textit{A.1. Time-Evolution Propagator} $\hat{T}(t)$

The dynamics of the spin-density operator $\sigma(t)$ are described in the Liouville-operator space by a differential
equation (Eq. [3]). The matrix elements of the free-precess-
ion superoperator are given by

$$\Omega_{\alpha\beta} = \text{Tr} \{ \hat{H}_{\omega, \beta} [\hat{B}_{\alpha}, \hat{B}_{\beta}] \}.$$  \hspace{1cm} [A.1.1]

The spherical-tensor operators were chosen as basis operators,

$$\{ \hat{B}_{\alpha} \} = \{ \hat{T}_{00}, \frac{1}{\sqrt{2}} \hat{T}_{11}, \frac{1}{\sqrt{2}} \hat{T}_{10}, \frac{1}{\sqrt{2}} \hat{T}_{1-1}, \frac{\sqrt{6}}{3} \hat{T}_{22},$$
$$-\frac{\sqrt{6}}{3} \hat{T}_{21}, \frac{\sqrt{6}}{3} \hat{T}_{20}, \frac{\sqrt{6}}{3} \hat{T}_{2-1}, \frac{\sqrt{6}}{3} \hat{T}_{2-2} \}. \hspace{1cm} [A.1.2]$$

The choice of spherical-tensor operators leads to the following matrix form of $\hat{S}$:

$$\tilde{S} = \begin{pmatrix}
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & -\Delta \omega & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 2\Delta \omega \\
\end{pmatrix}.$$  \hspace{1cm} [A.1.3]

The relaxation superoperator, $\hat{G}$, for the $I = 1$ case in the anisotropic phase was calculated by Jacobsen et al. (26, 27). The relaxation matrix is diagonal and all its elements can be expressed by three independent motional parameters,

$$\chi^2 J_0(0); \chi^2 J_1(\omega_0); \chi^2 J_2(2\omega_0). \hspace{1cm} [A.1.4]$$

where $\chi = e^2 g Q/\hbar$ and $J_0(0)$, $J_1(\omega_0)$, and $J_2(2\omega_0)$ are the spectral densities.

The differential equation (Eq. [3]) was solved analytically, and the solution is given as a propagator in Liouville space,

$$\hat{G}(t) = \begin{pmatrix}
1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & d & 0 & 0 & f & 0 & 0 & 0 \\
0 & 0 & a & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & d^* & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & c & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & e & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & b & 0 \\
0 & 0 & 0 & 0 & 0 & f^* & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & c^* & 0 \\
\end{pmatrix}, \hspace{1cm} [A.1.5]

where

$$a = \exp[-\chi^2 (J_1(\omega_0) + 4J_2(2\omega_0)) t],$$
$$b = \exp[-\chi^2 (3J_1(\omega_0)) t],$$
$$c = \exp[-\chi^2 (J_1(\omega_0) + 2J_2(2\omega_0) - 2i\Delta \omega) t],$$
$$d = \frac{\exp(ic_1 t) + (c_3)^2 \exp(-ic_1 t)}{(c_3)^2 + 1} \exp(-(c_2 - i\Delta \omega) t),$$
$$e = \frac{\exp(-ic_1 t) + (c_3)^2 \exp(ic_1 t)}{(c_3)^2 + 1} \exp(-(c_2 - i\Delta \omega) t),$$
$$f = \frac{c_3(\exp(ic_1 t) - \exp(-ic_1 t))}{(c_3)^2 + 1} \exp(-(c_2 - i\Delta \omega) t),$$
$$c_1 = \frac{1}{2} \chi^2 J_1(\omega_0)^2 - \frac{1}{4} \chi^2 J_2(2\omega_0)^2,$$
$$c_2 = \frac{1}{4} \chi^2 (3J_0(0) + 3J_1(\omega_0) + 2J_2(2\omega_0)).$$
$$c_3 = \frac{c_1 - \frac{1}{2} \chi^2 J_1(\omega_0)}{\omega_{\Delta \omega}}.$$

The time evolution of the density matrix during time interval $\tau$ can be evaluated by $\sigma(t + \tau) = \hat{G}(\tau) \sigma(t)$.

A.2. DQ Filter Propagator $\hat{F}_{DQ}$

For mathematical convenience, we introduce the DQ filter propagator, $\hat{F}_{DQ}$, which will simulate the effect of an ideal phase cycle, eliminating all coherences except for DQ. In the spherical-tensor representation, $\hat{F}_{DQ}$ gets the simple form

$$\hat{F}_{DQ} = \begin{pmatrix}
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
\end{pmatrix}. \hspace{1cm} [A.2.1]$$

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