Dependence of the Spin-Spin Relaxation Time of Water in Collagen Gels on Collagen Fiber Directions

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In this study, we investigated the effect of structural differences in collagen fibers in relation to the spin-spin (T2) relaxation time of surrounding water molecules. We propose a simple experimental model of the magic angle effect based on magnetically oriented collagen gels. Experiments were performed with a 4.7T magnetic resonance imaging (MRI) system with a quadrature radio frequency coil operated at 200 MHz for 1H resonance. Collagen gels were polymerized from collagen solutions exposed to a 4.7T magnetic field for 120 min. The T2 relaxation time was measured with the Carr-Purcell-Meiboom-Gill (CPMG) sequence. The apparent diffusion coefficient (ADC) was measured with the stimulated echo acquisition mode (STEAM) sequence with a motion-probing gradient (MPG). Orienting the collagen fibers at an angle of about 55 degrees to the main magnetic field caused an increase in the T2 relaxation times of water molecules in the collagen gels. The ADC in the direction parallel to the fibers was larger than that in the direction perpendicular to the fibers. The increase in the T2 relaxation time and ADC are attributed to a change in the magnetic interaction between the water molecules and collagen fibers.

Keywords: spin-spin relaxation time, apparent diffusion coefficient, hydration, anisotropic diffusion

Introduction

Measurement of the spin-spin (T2) relaxation time of biological tissues with magnetic resonance is useful in the study of bonding interactions between macromolecules and water. Although many studies have reported that the T2 relaxation time of protons in water molecules is affected by bonding interactions between water molecules and surrounding macromolecules,1-3 the effect of orientation of macromolecular structures on T2 relaxation times remains largely unknown. In previous studies, we investigated the effect of orientation of fiber structures on T2 relaxation. Using fibrin gels as a model of a macromolecule with structurally oriented fibers, we measured the T2 relaxation times of fibrin gels with and without magnetic orientation.4 In another study, we measured the T2 relaxation times and the apparent diffusion coefficient (ADC) of collagen gels with and without magnetic orientation.5

Fibrous polymers, such as collagen, have a structural orientation in living bodies. The T2 relaxation time of water molecules in living bodies differs between tissues. Because the molecular motion of water is significantly affected by macromolecules, the variation in relaxation times between tissues is attributed to the effect of macromolecular interactions. Collagen is a fibrous protein that serves as a supporting structure, connective tissue, and border between tissues. Collagen is largely restricted to the extracellular fluid compartment composing the structure of a fiber or a membrane. Water plays a critical role in maintaining the conformation of the collagen molecule and mechanical properties of collagen fibers.

Collagen microfibrils are composed of tropocollagen, a protein consisting of three polypeptide chains arranged in a triple helix. The microfibrils form fibers that are embedded into an amorphous ground substance. The fibers are oriented in
parallel bundles, resulting in a highly ordered structure (structural anisotropy). As a result, the motion of water molecules binding to collagenous tissue is greatly restricted, which enhances dipole-dipole interaction considerably. It is known that some macromolecules, such as fibrin and collagen, have high anisotropy in magnetic susceptibility and that static magnetic fields cause torque on molecules with anisotropic susceptibility. If magneto-static energy is higher than thermal motion energy, the molecule is oriented and stabilized in a particular direction. Collagen, a protein with a linear structure, is polymerized to form a gel by the enzyme lysyl oxidase. Under normal polymerization conditions, collagen forms a randomly oriented structure; however, if the polymerization process occurs in a strong magnetic field, collagen fibers are oriented perpendicular to the magnetic field. If the muscle fibers and collagen fibrils of a tendon are oriented at about 55 degrees to the direction of the static magnetic field, the T2 relaxation time of that portion increases. It is interesting that dipole-dipole interaction disappears at this angle. The orientation of fibrous macromolecules at 55 degrees, the “magic angle,” with respect to an externally applied magnetic field causes an increase in the T2 relaxation time. However, clear measurement of the magic angle effect is difficult because of the complex composition of biological tissues.

The purpose of this study is to clarify how the orientation of macromolecular structures affects T2 relaxation times. Magnetically oriented collagen gel, with a final collagen solution concentration of 2.5 and 3.6 mg/ml, was used as the model for a macromolecule with structurally oriented fiber. We measured the T2 relaxation times of collagen gels with magnetic orientation. Furthermore, we investigated the magic angle effects in a collagen fiber.

**Materials and Methods**

All experiments were performed with a 4.7T, 60-cm bore magnetic resonance imaging (MRI) system with a quadrature radio frequency coil operated at 200 MHz for 1H resonance. Cylindrical tubes with a 13-mm diameter were filled with collagen solutions (at final concentrations of 2.5 and 3.6 mg/ml). Collagen gels were polymerized from the collagen solution in 120 min. We prepared two collagen gels that were polymerized under 4.7T magnetic fields. The T2 relaxation time was measured with the Carr-Purcell-Meiboom-Gill (CPMG) sequence. A 90-degree pulse was applied at t = 0 ms, followed by a train of 180-degree pulses at 1-millisecond intervals. Spin echo signals were acquired at t = 256, 512, ..., 4096 ms. The delay time (TR) and the number of averages were 20 s and four, respectively. The T2 relaxation time was obtained by fitting the relaxation curve S(t) = S(0) exp (-t/T2) to a series of peaks of Fourier-transformed echo signals.

The apparent diffusion coefficients D of water molecules in the collagen gels with magnetically oriented fibers were measured with the method proposed by Stejskal and Tanner. The signal intensity S(b) of the echo signal measured with this method is given by S(b)/S(0) = exp (-bD). The attenuation factor b is defined as b = γ^2G^2δ^2 (Δ - δ/3), where γ is the gyromagnetic ratio, G is the intensity of the motion-probing gradient (MPG), δ is the pulse width of MPG, and Δ is the interval between MPG pulses. In this paper, stimulated echo signals were measured under the following conditions: TR = 1000 ms, TE = 75 ms, TM = 10 ms, d = 25 ms, D = 50 ms, b factor = 0–3000 s/mm². The above equation was fitted to the measured signals for calculating the apparent diffusion coefficient. The degree of orientation of the collagen fibers was evaluated from electron micrographs of the gels and image-processing software (NIH Image, U.S. National Institutes of Health). Thirty collagen fibers were selected from the micrographs. The angle θ of each collagen fiber to the external magnetic field was measured with the software. The degree of orientation was evaluated with the average of cos²θ. The temperature in the bore was 17 degrees Celsius.

**Results and Discussion**

The T2 relaxation times of collagen fibers oriented to the magnetic field are shown in Table 1. When

### Table 1. T2 relaxation times of water molecules in magnetically oriented collagen gels

<table>
<thead>
<tr>
<th>Orientation of collagen fibers to B₀</th>
<th>2.5 mg/ml collagen gels</th>
<th>3.6 mg/ml collagen gels</th>
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<tr>
<td>55 degrees</td>
<td>2.00 s</td>
<td>1.96 s</td>
</tr>
<tr>
<td>90 degrees</td>
<td>1.93 s</td>
<td>1.86 s</td>
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</table>
collagen fibers of the 2.5 mg/ml gel were oriented at angles of 55 degrees (magic angle) and 90 degrees with respect to the main magnetic field, water molecules in the collagen gel with magnetically oriented fibers had the relaxation times $T_2 = 2.00$ s and $T_2 = 1.93$ s, respectively (Fig. 1). When collagen fibers of the 3.6 mg/ml gel were oriented at angles of 55 degrees (magic angle) and 90 degrees with respect to the main magnetic field, water molecules in the collagen gel with magnetically oriented fibers exhibited relaxation times of $T_2 = 1.96$ s and $T_2 = 1.86$ s, respectively (Fig. 2). The ADC values of water molecules of collagen fibers oriented to the magnetic field are shown in Table 2. Figure 3 shows the relationship between the b factor and the signal intensity of water molecules in the collagen fibers of the 2.5 mg/ml gel oriented perpendicular to the magnetic field. The ADC of water molecules in the direction perpendicular to the collagen fibers was $ADC = 1.84 \times 10^{-9}$ m$^2$/s. The ADC of water molecules in the direction parallel to the collagen fibers was $ADC = 2.17 \times 10^{-9}$ m$^2$/s. Figure 4 shows the relationship between the b factor and the signal intensity of water molecules in the collagen fibers of the 3.6 mg/ml gel oriented perpendicular to the magnetic field. The ADC of water molecules in the direction perpendicular to the collagen fibers was $ADC = 1.85 \times 10^{-9}$ m$^2$/s. The ADC of water molecules in the direction parallel to the collagen fibers was $ADC = 2.04 \times 10^{-9}$ m$^2$/s. Figure 5 shows scanning electron micrographs of type-I collagen fibers.

**Fig. 1.** At an angle of about 55 degrees (magic angle) to the main magnetic field, water molecules in the collagen gels (2.5 mg/ml) with magnetically oriented fibers had a relaxation time of 2.00 s. At an angle of about 90 degrees to the main magnetic field, water molecules in the collagen gel with magnetically oriented fibers had a relaxation time of 1.93 s.

**Fig. 2.** At an angle of about 55 degrees (magic angle) to the main magnetic field, water molecules in the collagen gels (3.6 mg/ml) with magnetically oriented fibers had a relaxation time of 1.96 s. At an angle of about 90 degrees to the main magnetic field, water molecules in the collagen gels (3.6 mg/ml) with magnetically oriented fibers had a relaxation time of 1.86 s.

**Fig. 3.** Diffusional signal attenuation of collagen fibers of the 2.5-mg/ml gel oriented perpendicular to the magnetic field. The ADC of water molecules in the direction perpendicular to the collagen fibers was $ADC = 1.84 \times 10^{-9}$ m$^2$/s. The ADC of water molecules in the direction parallel to the collagen fibers was $ADC = 2.17 \times 10^{-9}$ m$^2$/s.

**Table 2.** ADC values of water molecules in magnetically oriented collagen gels

<table>
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<tr>
<th>Orientation of diffusion to collagen fibers</th>
<th>2.5 mg/ml collagen gels</th>
<th>3.6 mg/ml collagen gels</th>
</tr>
</thead>
<tbody>
<tr>
<td>Perpendicular</td>
<td>$1.84 \times 10^{-9}$ m$^2$/s</td>
<td>$1.85 \times 10^{-9}$ m$^2$/s</td>
</tr>
<tr>
<td>Parallel</td>
<td>$2.17 \times 10^{-9}$ m$^2$/s</td>
<td>$2.04 \times 10^{-9}$ m$^2$/s</td>
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Fig. 4. Diffusional signal attenuation of collagen fibers of the 3.6-mg/ml gel oriented perpendicular to the magnetic field. The ADC of water molecules in the direction perpendicular to the collagen fibers was $\text{ADC} = 1.85 \times 10^{-9} \text{m}^2/\text{s}$. The ADC of water molecules in the direction parallel to the collagen fibers was $\text{ADC} = 2.04 \times 10^{-9} \text{m}^2/\text{s}$.

Fig. 5. SEM (scanning electron micrograph) of type-I collagen fibers. (a) Collagen gel polymerized in the absence of a magnetic field. Collagen fibers were randomly oriented. (b) Collagen gel polymerized under a 4.7T magnetic field. Collagen fibers were oriented perpendicular to the magnetic field.

The respective degrees of orientation of the magnetically oriented fibers and the randomly oriented fibers (polymerized without the magnetic field) were $[\cos^2 \theta] = 0.53$ and $[\cos^2 \theta] = 0.40$.

At an angle of about 55 degrees to the main magnetic field, the $T_2$ relaxation times of water molecules in the collagen gels with magnetically oriented fibers increased. This result can be explained as follows. The intensity of dipole-dipole interaction is related to the relative position of two nuclear spins. The magnetic field from a hydrogen atom varies the magnetic field acting on another hydrogen atom, which also depends on the relative orientation of the atoms with respect to the main magnetic field. The interaction between two spins is proportional to $(3\cos^2 \theta - 1)^4$. The dipolar interaction causes a decrease in the signal intensity due to $T_2$ relaxation. The term $(3\cos^2 \theta - 1)$ equals zero at $\theta = 54.74^\circ$.

In the two measurements with different collagen concentrations, the $T_2$ relaxation times at 55 degrees were longer than the $T_2$ relaxation times at 90 degrees. The differences in the $T_2$ relaxation times are attributed to a change of collagen fiber structure due to the magnetic orientation.

Since water diffusion in biological tissues is restricted due to the tissue microstructure, water molecules surrounded by oriented collagen fibers easily diffuse in the direction of the fibers. Therefore, the ADC in the direction parallel to the collagen fibers is larger than the ADC in the direction perpendicular to the collagen fibers. This diffusion anisotropy suggests that the motion of water is restricted by the structure of macromolecules. Nerve axons and muscles exhibit diffusion anisotropy because their fibrous structures align in a specific direction. The diffusion anisotropy observed in this study is similar to these phenomena.

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References


