Solid-State NMR Spectroscopy Provides Atomic-level Insights Into the Dehydration of Cartilage

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Supporting Information

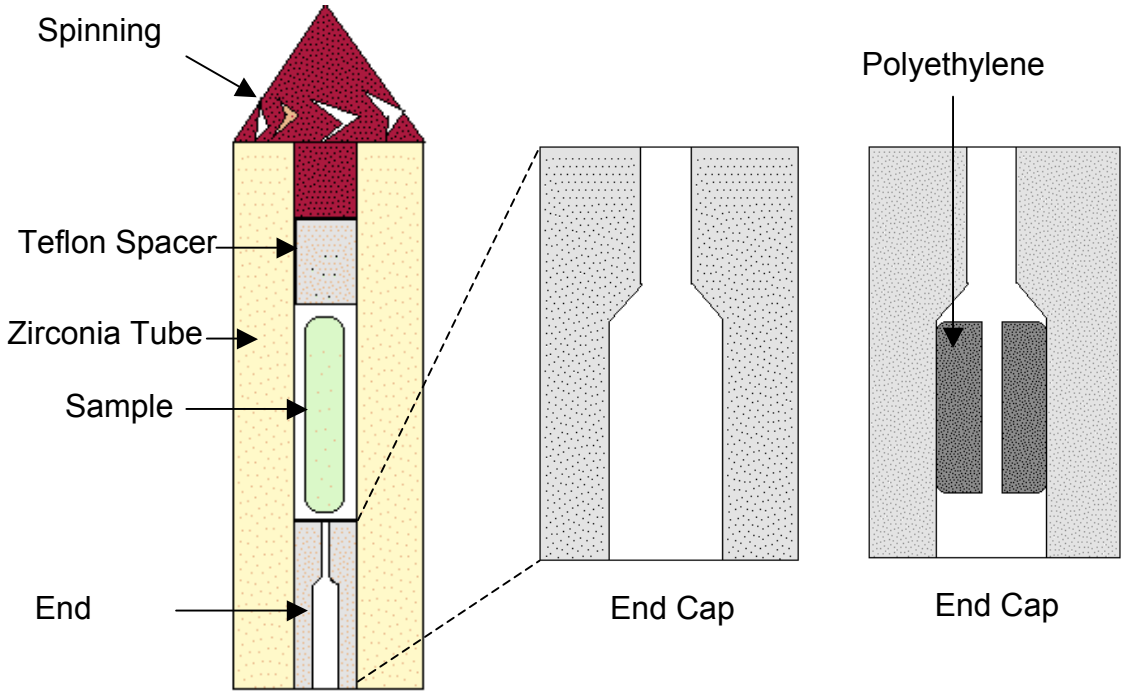

Figure S1. A sketch of NMR rotor and insert for a controlled slow dehydration of cartilage samples used in this study.

Raman microspectroscopy

A Nikon E600 epi-fluorescence microscope (Nikon, Inc., Melville, New York) was modified for NIR Raman spectroscopy in house. A 785-nm Kaiser Invictus laser was line focused (Kaiser Optical Systems, Inc., Ann Arbor, Michigan) onto cartilage specimen using a 20×/0.75 NA S Fluor objective. Raman-scattered light was dispersed through a spectrograph (HoloSpec f/1.8, Kaiser Optical Systems, Inc.) and collected for 10 min on a charge-coupled device (CCD) detector optimized for NIR wavelengths (DU401-BR-DD, Andor Technologies, Belfast, Northern Ireland). Because the instrument used a line focused laser, 126 Raman spectra were simultaneously collected on the specimen (Figure S2). Raman data were imported into MATLAB

software (v. 7.0, The Math Works, Natick, Massachusetts) and corrected for image curvature, dark current, and variations in the CCD quantum efficiency.

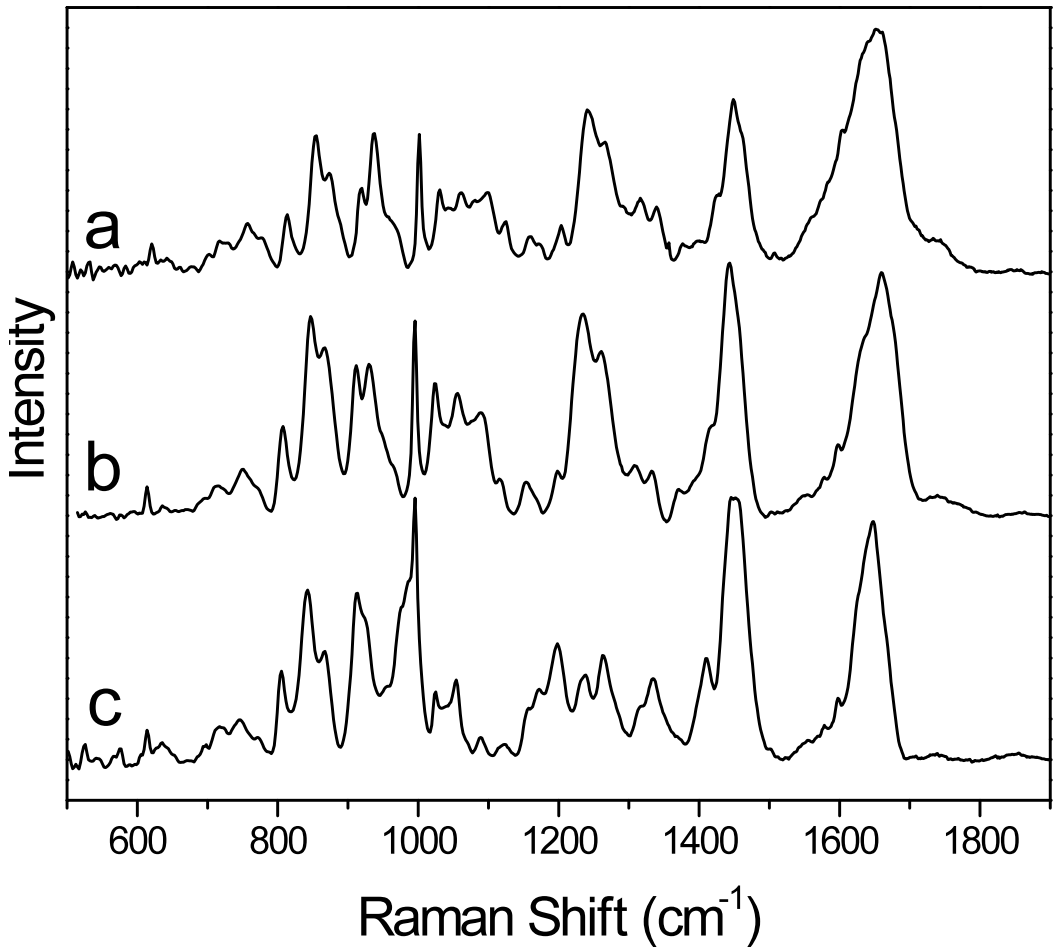

Figure S2. Raman spectra of (a) hydrated, (b) dehydrated, and (c) D_2O exchanged bovine cartilage contain contributions primarily from collagen protein with minor bands assigned to chondroitin sulfate. Dehydration or D_2O-exchange had a pronounced effect on collagen carbonyl groups, as evidenced in the amide I envelope at 1600-1700 cm^{-1}. The amide I envelope becomes narrower, reflecting a loss of intermolecular hydrogen bonding.

Table S1. Assignment of ^{13}C peaks from the RAMP-CP spectrum of hydrated cartilage.

Chemical Shift relative to TMS, δ_C (ppm)		Assignment	
Hydrated	Dehydrated	Functional Group	Type of Carbon
182.1,175.1, 171,168.7	175.9	Carboxylate, Carbonyl	C=O
157.9	161.2	Arg C_δ	CH
129.7	130	His,Phe,Trp,Tyr ring	CH
103.7,101.7	105	GAGs C1	
76.2	74.2	GAGs	CH_2
71.1	71.2	Hyp C_γ	CH
62.0		Ser C_β; Val C_α	CH
59.5	59.3	Pro C_α; Hyp C_α	CH
54.4	52.9	Hyp C_δ; Glu C_α	CH
49.8	49.2	Ala C_α	CH
47.8	47.6	Pro C_δ	CH_2
43.0	42.6	Gly C_α; Arg C5	CH_2
38.2		Hyp C_β, Asp C_β	CH_2
34.7		Val C_γ	
30.5	29.7	Pro C_β, Arg C_β	CH_2
28.9		Glu C_γ; Lys C_β	CH_2
25.3	24.2	Pro C_γ; Glu C_β	CH_2
17.5	16.3	Ala C_β	CH_3

Table S2. Assignment of ^{13}C peaks from the RINEPT spectrum of cartilage.

Chemical shift relative to TMS (ppm)	Carbon	Type of Carbon
102.5,100.1	C1	CH
50.0	C2	CH
75.5	C3,	CH
79	C4	CH
71.4,72.7	C5	CH
66.6,60.1	C6	CH
21.6	-$NHCOCH_3$	CH_3