J|A|C|S
COMMUNICATIONS

Published on Web 04/07/2007

# Tunable-Alignment Chiral System Based on Gelatin for NMR Spectroscopy

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The discrimination between NMR spectra of alanine enantiomers by chiral, collagen-containing tendons has recently been reported.[1] Gelatin is partially hydrolyzed collagen where the tropocollagen strands are separated to form random coils. Gelatin gels can be formed by adding polar solvent leading to a three-dimensional polymer network that is held together by hydrogen bonds, lacking the covalent cross-links of native collagen. Stretching introduces anisotropy in gelatin, and alanine enantiomers added to it can thus be distinguished by NMR spectroscopy.[2] We recently described a simple apparatus for the rapid and reversible adjustment of the degree of alignment of NMR samples using gelatin in a silicone-rubber tube.[3] Liquid gelatin is drawn into the silicone tube that is then sealed at its lower end, inserted into a bottomless 5 or 10 mm NMR tube, and upon setting of the gel a plastic thumb-screw holds the silicone tube at various extents of stretching. Preparation of each sample takes only minutes.[4] To resolve chiral mixtures there are two adjustable properties of gelatin gels: concentration (w/v) and stretching ("extension factor" = increase in length relative to the original length). Concentration has a far larger effect in generating quadrupolar and dipolar splittings (Table 1): tuning to a given extent of splitting when stretched is done by choosing the concentration range (3−60% w/v); fine-tuning to a given splitting-value is achieved by varying the extension factor. We show here that alanine enantiomers can be distinguished by $^1$H, $^2$H, and $^{13}$C NMR.[5] This is a general property of gelatin gels, and is a notable advance on previous related work using liquid crystals.[6]

As was observed for collagen[1] in $^2$H NMR, the two enantiomers of alanine show different (L/D) quadrupolar splitting ratios for the methine (∼0.6) and methyl residues (∼3) and can easily be distinguished (Table 1, and Figures 1 and 2).[7] These ratios are only slightly affected by gelatin concentration. Quadrupolar splittings in 50% gelatin are 4−6 times smaller than in collagen,[1] but the lineshapes are much narrower and there are no residual isotropic alanine signals. Thus gelatin provides a more homogeneous chiral environment for guest molecules.

Dipolar splittings showing clear differences between L- and D-alanine were observed in $^1$H and $^{13}$C NMR spectra (Tables 2 and 3, and Figure 3). We recorded the quadrupolar splitting of the $D_2O$ solvent for each sample as a measure of its alignment. At 50% gelatin the maximum $^1$H−$^1$H dipolar splitting was 33 Hz, 9 times less than in tendons; L-alanine had larger apparent dipolar splittings than D-alanine. The L/D ratio of the apparent dipolar splittings for the methine protons was 1.8, whereas that for the methyl groups was 4.0. The ratio of the two distinct apparent proton dipolar splittings ($C\underline{H}_3$/$C\underline{H}$-$CH_3$) was 1.7 for L-alanine, and 0.7 for D-alanine. As can be seen in Figure 3, the $^1$H NMR spectral resolution of samples embedded in a matrix of 50% gelatin is remarkably high. For instance, the widths at half-height of the inner signals of the alanine methine quartet, as compared to alanine in $D_2O$, increased only 4-fold to 5.1 Hz (D-) and 6.4 Hz (L-alanine), respectively.

***Table 1.*** Quadrupolar Interactions for Deuterated L- and D-Alanine in Stretched Gelatin[a]

| sample | extension factor | $^2$H quadrupolar splitting (Hz) HDO | CD L | CD D | $CD_3$ L | $CD_3$ D |
|---|---|---|---|---|---|---|
| | | 14% Gelatin | | | | |
| L-alanine-$d_4$ | 0.8 | 62 | 39 | | 9 | |
| DL-alanine-$d$ | 0.9 | 72 | 40 | 57 | | |
| DL-alanine-$d_3$ | 0.9 | | | | 6 | |
| L-alanine-$d_4$ | 0.9 | 81 | 43 | 63 | 10 | |
| DL-alanine-$d$ | 2.1 | 138 | 75 | 110 | 18 | |
| | | 30% Gelatin | | | | |
| DL-alanine-$d$ | 0.9 | | 191 | 296 | | |
| DL-alanine-$d_3$ | 0.9 | | | | 50 | 22 |
| | | 50% Gelatin | | | | |
| DL-alanine-$d$ | 0.5 | 320 | 170 | 275 | | |
| | 1.0 | 588 | 316 | 510 | | |
| | 1.5 | 752 | 402 | 629 | | |
| DL-alanine-$d_3$ | 0.5 | 295 | | | 38 | 12 |
| | 1.0 | 526 | | | 69 | 22 |
| | 1.5 | 698 | | | 91 | 30 |
| L-alanine-$d_4$ | 0.25 | 161 | 83 | | 21 | 0 |
| DL-alanine-$d$ | 0.4 | 220 | 111 | 190 | 29 | 10 |
| DL-alanine-$d_3$ | 0.5 | 297 | 151 | 250 | 39 | 12 |
| | 0.6 | 353 | 193 | | 48 | 14 |
| | 0.75 | 418 | 244 | | 56 | 16 |
| | 1.0 | 544 | 285 | 474 | 73 | 22 |
| | 1.5 | 768 | 387 | | 100 | 29 |

[a] Dipolar splittings were too small to be resolved with $^2$H NMR.

***Table 2.*** $^1$H NMR Data of L- and D-Alanine in Stretched Gelatin

| sample | extension factor | $^2$H splitting of $D_2O$ (Hz) | $^1$H–$^1$H dipolar contribution to total splitting (Hz) $C\underline{H}$-$CH_3$[a] | $CH_3$-$C\underline{H}$[a] | $C\underline{H}_3$ |
|---|---|---|---|---|---|
| | | 30% Gelatin | | | |
| L-alanine | 1.5 | 460 | 12.1 | ∼12 | 19.7 |
| D-alanine | 1.5 | 434 | 7.4 | 7.5 | 5.3 |
| | | 50% Gelatin | | | |
| L-alanine | 0.5 | 265 | 7.2 | 5−6 | 13.0 |
| | 1 | 504 | 14.0 | ∼15 | 22.6 |
| | 1.5 | 702 | 19.7 | 20.0 | 32.6 |
| D-alanine | 0.5 | 256 | 4.1 | 4.0 | 0 |
| | 1 | 481 | 8.3 | 8.3 | 5.7 |
| | 1.5 | 677 | 11.9 | 11.8 | 8.2 |

[a] $J$ coupling (in $D_2O$) was subtracted; observed nucleus is underlined.

The spectra of two enantiomers can also be resolved in $^{13}$C NMR owing to the different (L/D) $^{13}$C−$^1$H dipolar splitting ratios for the methine (∼0.5) and methyl residues (∼6) (Table 3).

We did not observe any saturation effect on splitting values or specific binding of any guests, although high salt concentrations (>1 M) decreased the quadrupolar splittings. The $^2$H and $^1$H NMR spectra for DMSO or DMF were largely unaffected by their concentration (5% vs 50% v/v).

Differences in the quadrupolar and dipolar splittings for enantiomers most likely arise from differences in the stereochemistry of the alignment[1] (not necessarily binding) of the guests. The results presented here are by no means peculiar to alanine or any other


10.1021/ja070348v CCC: $37.00 

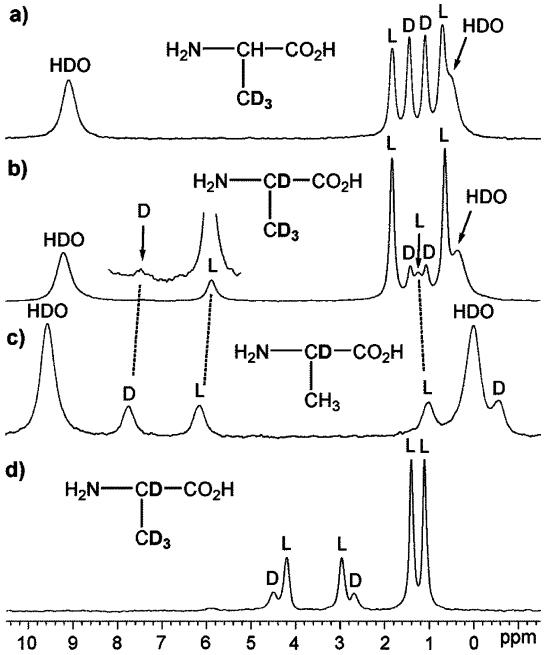


***Figure 1.*** 61.4 MHz $^2$H NMR spectra of deuterated D- and L-alanine in stretched gelatin in $D_2O$, at 15 °C: (a−c) 50% gelatin, extension factor 1.0; (d) 15% gelatin, extension factor 2.1; (a) DL-alanine-$d_3$; (b) L-alanine-$d_4$, DL-alanine-$d$, and DL-alanine-$d_3$; (c) DL-alanine-$d$; (d) L-alanine-$d_4$, DL-alanine-$d$. Note the very similar chemical shifts for the different isotopomers but clear resolution of enantiomers.

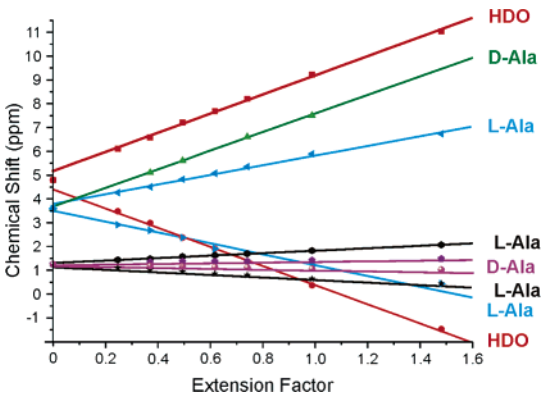


***Figure 2.*** Extension factor dependence of $^2$H NMR chemical shifts of each resonance of quadrupolar doublets observed for the L-alanine-enriched sample shown in Figure 1b (except there was only one resolved resonance for D-alanine methine; green line). A clear linear relationship exists between quadrupolar splitting and the extension factor, although not all resonances were resolved at all stretching stages. A similar linear relationship existed between splitting and gelatin concentration at constant extension factor.

compound that may bind to (tropo)collagen. Both adjustable properties of the gelatin gels, concentration and stretching, yielded linear relationships with the amount of quadrupolar and dipolar splitting. Thus by varying concentration and stretching (see Figure 2) most complex spectra could be resolved. In addition $^2$H EXSY spectra obtained by using very short mixing times (∼1 ms) correlated spins in adjacent energy levels owing to exchange between these in each isolated deuterium nucleus. This phenomenon is distinct from correlations between two multiplets of quadrupolar-coupled $^2$H nuclei that can be studied by using Q-COSY and its variants.[8]

Melting the stretched gelatin and releasing it after cooling result in a compressed gel and change the sign of the residual dipolar coupling. The anisotropy in stretched gelatin is maintained even in

***Table 3.*** $^{13}$C NMR of L- and D-Alanine in Stretched 50% Gelatin

| sample | extension factor | $^2$H splitting of $D_2O$ (Hz) | $^{13}$C–$^1$H dipolar contribution to total splitting (Hz) | | |
|---|---|---|---|---|---|
| | | | $^{13}\underline{C}$–$^1$H[a] | $^{13}\underline{C}$–$^1H_3$[a] | $^{13}\underline{C}(H_3)$–$^1$H[a] |
| L-alanine | 0.5 | 281 | 22 | −7 | ∼−5 |
| | 1 | 535 | 44 | −12 | −10 |
| | 1.5 | 760 | 62 | −17 | −14 |
| D-alanine | 0.5 | 334 | 48 | −1 | ∼−5 |
| | 1 | 599 | 91 | −2 | −9 |
| | 1.5 | 806 | 121 | −3 | −12 |
| D:L = 2:1 | 1.5 | 699 | **103**, 58 | **−2**, −15 | **−10**, −14 |

[a] $J$ coupling (in $D_2O$) was subtracted; observed nucleus is underlined. A minus sign means opposite signs for J and D.

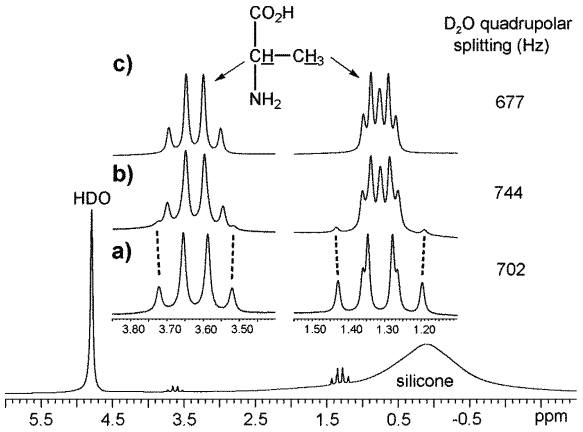


***Figure 3.*** 400.13 MHz $^1$H NMR spectra of L-alanine (full spectrum and inset a), D-alanine (inset c), and a D-enriched mixture of D- and L-alanine (inset b). All samples were in 50% gelatin with an extension factor of 1.5. Variation in the $D_2O$ splitting was due to slight differences in the extent of stretching.

the presence of intact human red blood cells[3] thus allowing us to use gelatin as a "shift reagent", resolving peaks from intra- and extracellular chemical species.

Potential limitations of the method include (1) line broadening in high gelatin concentrations, (2) excessive peak overlap owing to the induced splittings that may not be able to be removed by the available extension or compression factor, and (3) specific (un)-folding interactions between a host molecule, such as a peptide, and gelatin.

In summary, stretched gelatin gels yield significant and tunable quadrupolar and dipolar splittings that can be used to rapidly differentiate chiral and prochiral centers in bio-organic molecules.

**Acknowledgment.** The work was funded by a Discovery Grant from the Australian Research Council to P.W.K. We thank Dr. David Philp for valuable discussions.

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(4) High water solubility is not necessary since up to 50% DMSO or DMF can be used as a co-solvent.
(5) Alanine was chosen for comparison with previous published work.
(6) For example (a) Kay, L. E.; Thomson, D. S.; Prestegard, J. H. *Magn. Reson. Chem.* **1988**, *26*, 860. (b) Weise, C. F.; Weisshaar, J. C. *J. Phys. Chem. B* **2003**, *107*, 3265. (c) Canet, I.; Courtieu, J.; Loewenstein, A.; Meddour, A.; Pechine, J. M. *J. Am. Chem. Soc.* **1995**, *117*, 6520. (d) Sarfati, M.; Lesot, P.; Merlet, D.; Courtieu, J. *Chem. Commun.* **2000**, 2069.
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JA070348V