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Demixing of Water and Ethanol Causes Conformational Redistribution and Gelation of the GAG Tripeptide.

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Supporting Information

1. Experimental Section

Materials.

The tripeptide, L-glycyl-L-alanyl-L-glycine (GAG) was purchased from Bachem Biosciences Inc. (King of Prussia, PA) with >98% purity and was not further purified for any experiments. The deuterated solvents, D_2O and ethan(ol)-d (EtOD), were used for vibrational spectroscopy studies. EtOD is the deuterated ethyl alcohol, with the alcoholic hydrogen replaced by deuterium. The EtOD was purchased from Sigma with a purity of >98%.

Methods

Solution preparation. Solutions of cationic GAG were prepared by dissolving the peptide in a mixture of ethanol and DI-water at mole fractions of ethanol ranging from 0 to 0.552, which equates to 0 to 80% (v/v). The pH of each solution was adjusted to about 1.5-2 by adding HCl. For NMR studies, solutions with a peptide concentration of approximately 100 mM were made by dissolving the solid GAG in a H_2O/D_2O mixture of 9 parts H_2O to one part D_2O containing 0.1% trimethylsilane (TMS). Solutions with a mole fraction of ethanol greater than 0.12 were pipetted into a double-chamber NMR tube, which became necessary in order to mix solvents without affecting the deuterium-detecting lock signal of the NMR. The inside chamber held D_2O containing 0.1% TMS used as an internal standard. Vibrational studies (FT-IR and VCD) required a peptide concentration of 200 mM and the use of deuterated solvents D_2O and EtOD to avoid any spectral overlap of amide I with the broad band assignable HOH bending

vibrations. The use of deuterated ethanol prevents the contamination of binary mixtures with H_2O and HOD owing to an H⇔D exchange of hydroxyl proton. The prepared solutions were pipetted into a CaF_2 demountable cell with a path length of 56 μm from BioTools (Jupiter, Fl). For UVCD studies, 5 mM GAG was prepared from a 200 mM stock solution.

'H-NMR. Temperature dependent 'H-NMR spectra were recorded for all GAG samples using a Varian 500 MHz FT-NMR with a 5 mm HCN triple resonance probe at Drexel University. The v. 6.1 Varian software was used for processing of all spectra, and the pre-saturation mode (presat) was used to suppress the strong signals of solvents. The sample was set at a spin of 20 Hz, and spectra were collected starting at 25°C while increasing by 5° for each measurement with the maximum temperature of 55°C. At each temperature, the sample was given approximately two minutes to equilibrate. Either 16 or 32 scans were collected for each spectrum, with higher temperature and mole fraction of ethanol causing the need for 32 scans. The fid files were opened in MestReC software, which was used for the Fourier transform and phase correction, then exported as text files. The amide proton three-bond J-coupling ($^3J(H^NH^\alpha)$) was determined using the technique described by Toal et al.[1]

Vibrational Spectroscopy. FT-IR and VCD spectra of ethanol/water with and without GAG peptide were simultaneously recorded at a temperature of nearly 25°C using a Chiral IR™ Fourier Transform VCD spectrometer from BioTools with a spectral resolution of 8 cm^{-1}. Approximately 3600 scans were collected for

each sample. Background and baseline corrections were performed as described earlier.[2]

Ultra-Violet Circular Dichroism (UVCD) Spectroscopy. Temperature dependent UVCD spectra were measured on a Jasco J-180 spectropolarimeter (model J-810-150S) purged with N_2. The temperatures ranged from 10-60 ^{o}C in 5 degree increments using a Peltier controller (model PTC-423S). A delay time of 150 s was used to ensure adjustment at each temperature. The sample was loaded into a 100 μm cell from International Crystral Laboratories. Spectra were measured from 180-300 nm using a data pitch of 0.05 nm, a scan speed of 500 nm/min, a response time of 1.0 s, and a bandwidth of 5 nm. The spectra were corrected using appropriate background subtraction, and ten accumulations were averaged at each temperature.

Bright Field Microscopy. The image was taken using an Olympus Model BX51 Microscope equipped with a PixeLINK PL-A662 camera. A 20x objective lens was used to provide a total magnification of 200x.

2. Conformational Analysis of GAG

In an earlier study we reported conformational distributions of GxG peptides in water based on the global analysis of amide I' band profiles in corresponding IR, polarized Raman and VCD spectra and of a set of different J-coupling constants. In this analysis each conformation was represented by a two-dimensional Gaussian distribution in the Ramachandran space.[3] For cationic GAG, this analysis yielded a pPII fraction of 0.72 and a β-strand fraction of 0.18. The remaining ten percent of the distribution is shared by various turn structures including a right-handed helical conformation. For the present study we confined our analysis on the available IR and VCD profiles and the corresponding $^3J(H^NH^\alpha)$ coupling constants of GAG in the 50 mol% ethanol/water mixture. The differences between the respective IR and VCD band profiles of GAG in D_2O and this ethanol/water mixture are modest, our analysis revealed that it mainly reflects (slightly different) red shifts of the two amide I' bands. On the contrary, the two $^3J(H^NH^\alpha)$ constants are rather different. We considered two options. First we kept the (ϕ,ψ) positions and the halfwidths of the pPII and β-distributions in the Ramachandran space constant and solely changed their statistical weight. This led to the pPII scale in Figure 4, the results suggest a substantial reduction of the pPII fraction for the considered ternary mixture. These changes have a negligible effect on the amide I' IR and VCD profiles, as one can read from the satisfactory reproduction of the amide I' profiles of the ternary mixture displayed in Figure S4. This reflects the aforementioned insensitivity of the two profiles for any changes of distributions along the ϕ-coordinate between ca. -120° and -65°.[4] The earlier analysis of GAG had yielded ϕ-values of -115° and -69° for β-

strand and pPII, respectively. Second, we kept the statistical weight constant and varied the ϕ-coordinate of pPII. This reproduced the experimentally obtained $^{3}J(H^{N}H^{\alpha})$ constant with ϕ=-79°. Changes along ψ lead to changes of both the VCD and the IR profile; they were therefore not considered. Changes of the ϕ-coordinate of the β-strand distribution can be excluded, since it sits very close to the maximum of the respective Karplus curve[5] so that any change would cause a reduction rather than an increase of $^{3}J(H^{N}H^{\alpha})$. Even though a final assessment of the ethanol-induced structural changes requires some more experiments, we decided to opt for changes of the statistical weight of the distributions as the prime effect for the following reason. A shift of the pPII conformation towards lower ϕ-values would increase the overlap with the β-strand conformation. If true, this would reflect a lowering of the barrier between both conformations. In this case fluctuations at higher temperatures would not 'see' any barrier between pPII and β. Such an effect can be expected to substantially reduce the temperature dependence of the J-coupling constant, contrary to our observation.

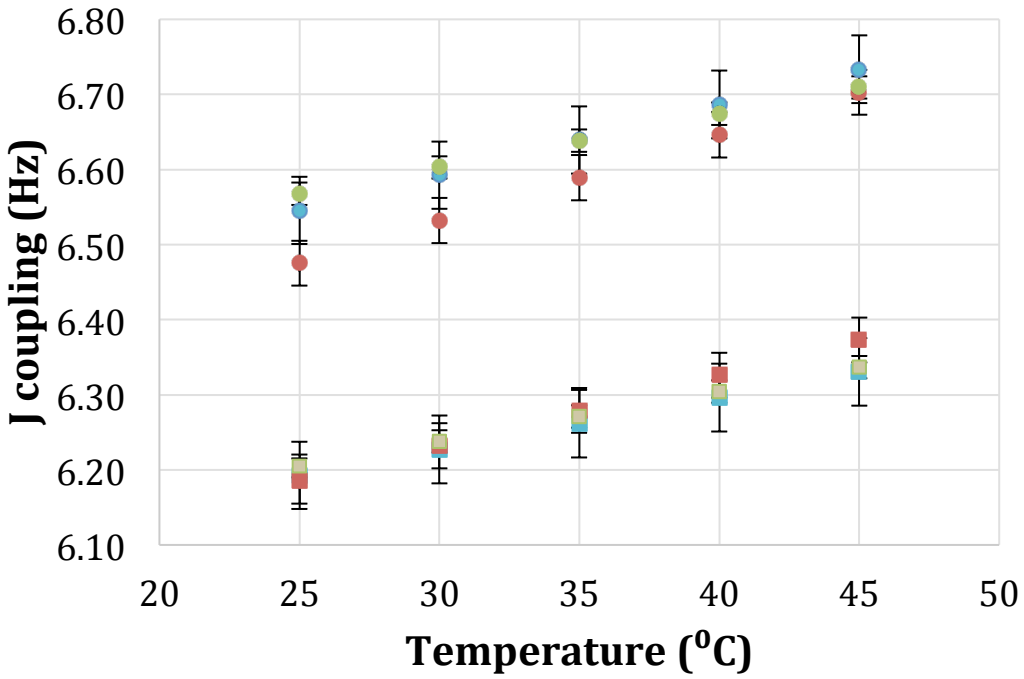

Figure S1: Temperature dependent $^{3}J(H^{N}H^{\alpha})$ of the N-terminal amide proton of cationic GAG in ethanol/water mixtures of 0.14 (squares) and 0.48 (circles) mol% ethanol taken at different concentrations of GAG (blue symbols: 50 mM GAG, red symbols: 75 mM GAG, and green symbols: 100 mM GAG). Error bars are shown to indicate that there are no statistical significant differences between $^{3}J(H^{N}H^{\alpha})$ obtained from samples with different concentrations of GAG.

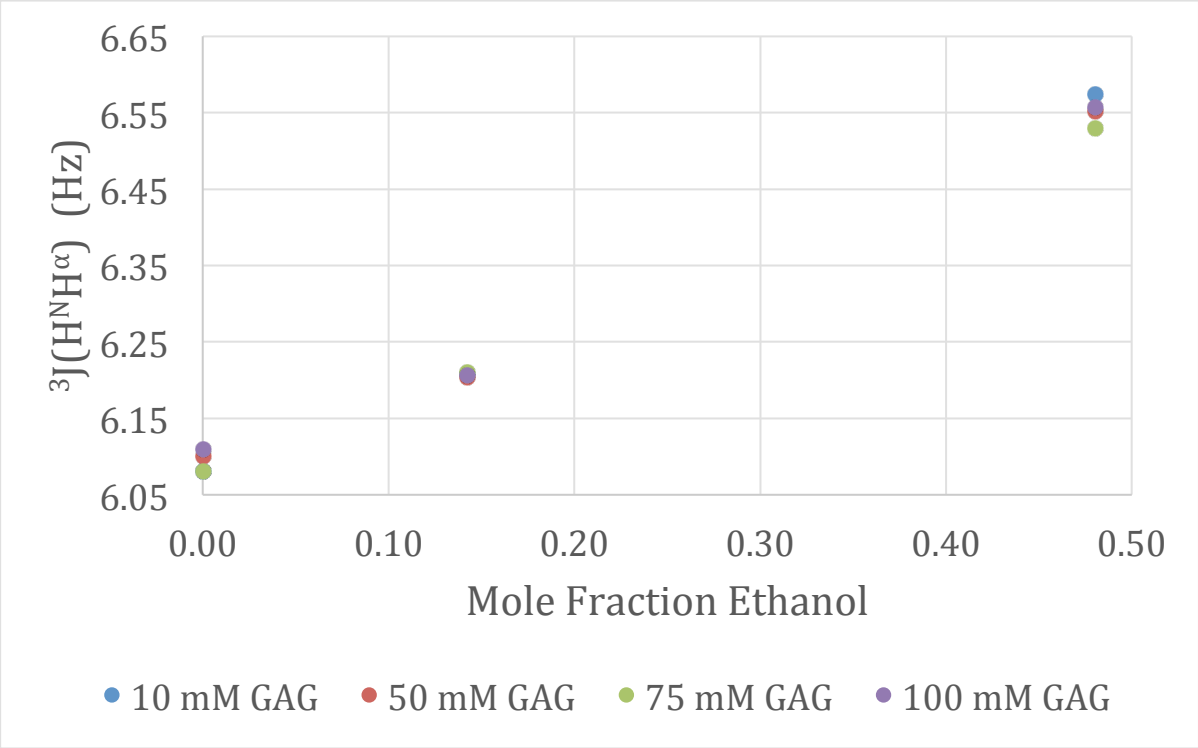

Figure S2: $^3J(H^NH^\alpha)$ of the N-terminal amide proton of cationic GAG in water and ethanol/water mixtures with 0.14 and 0.48 mole % ethanol obtained at different concentrations of GAG, as shown in figure legend, at 25°C.

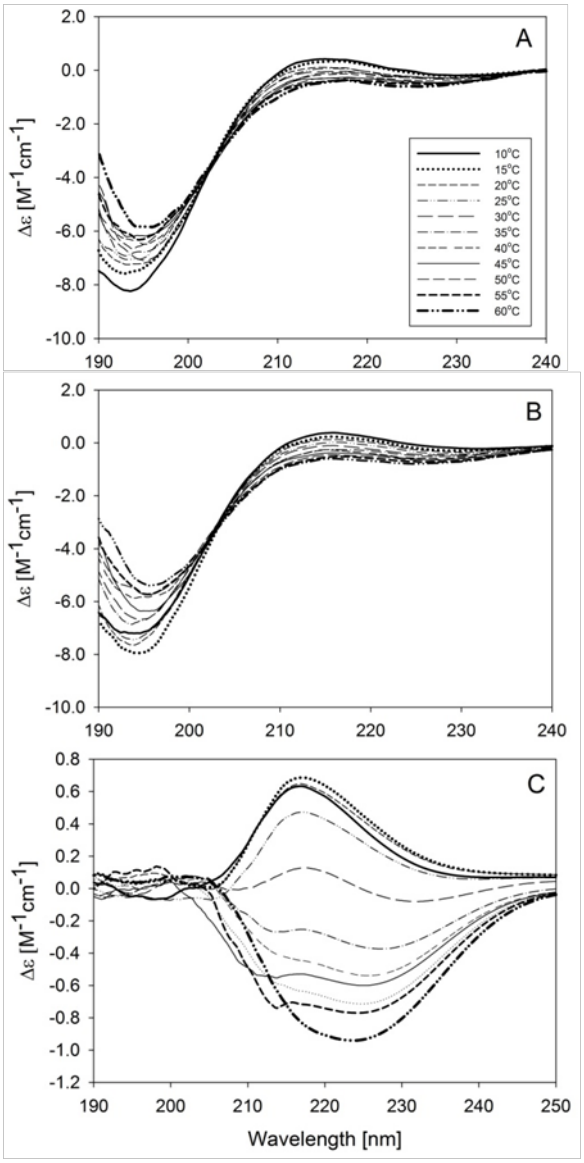

Figure S3: UVCD spectra of cationic GAG at concentrations of 5 mM (A) in 55 mol% ethanol, 10 mM (B) in 48 mol% ethanol, and 220 mM (C) in 55 mol% ethanol. Temperatures are indicated by the legend. For the 220 mM solution, the absorbance exceeded the instrumental detection limit at wavelengths below 210 nm. The spectra taken at 5 and 10 mM show the typical signal of a peptide with high pPII content.[6] At high concentration, however, the weak positive maximum at 215 nm is replaced by a negative maximum at 225 nm that qualitatively resembles spectra obtained for poly-L-proline films at high temperatures.[7]

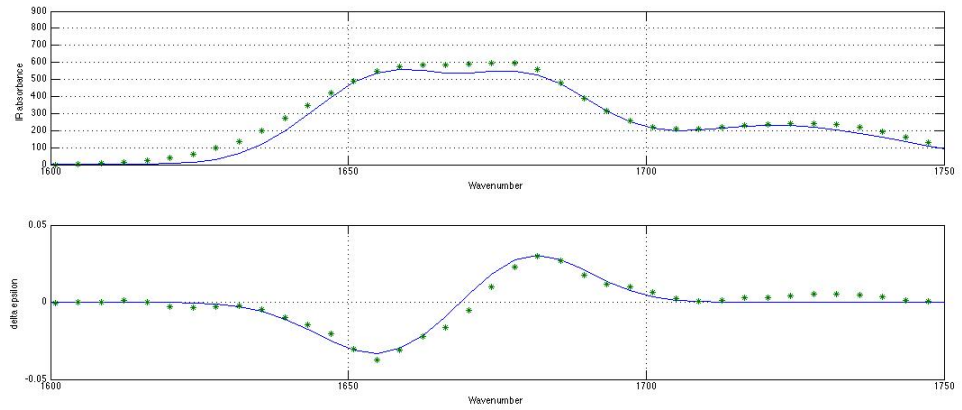

Figure S4: Simulation of the amide I' profiles of cationic GAG in 50 mol% ethanol/water by using the excitonic coupling model described in earlier publications. The solid lines are the result of the simulation. The figure has been directly produced by our Matlab software.

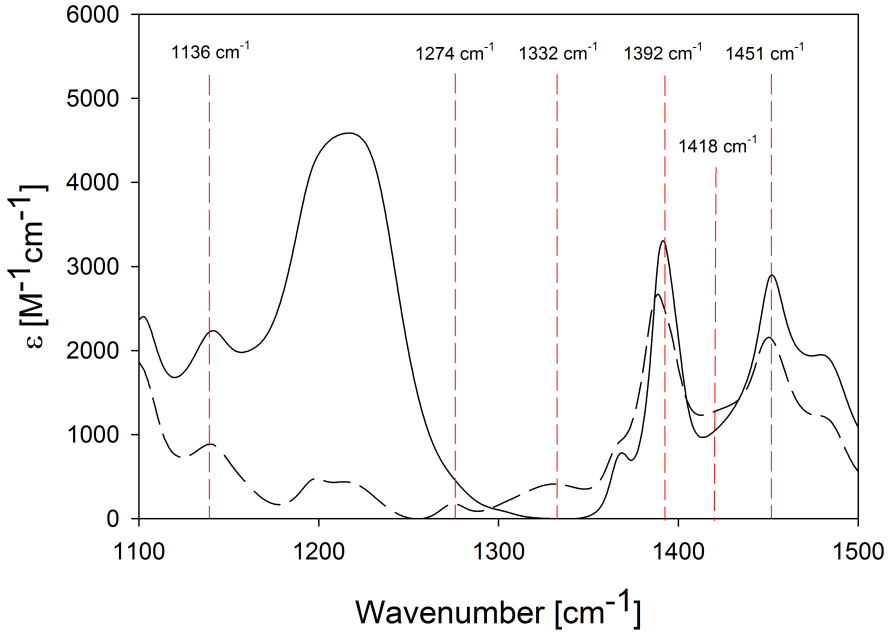

Figure S5: IR spectra bands of ethanol bending and stretching modes from 1100 to 1500 cm^{-1} after background subtraction of D_2O using our program Multifit: 17 mol% ethanol (solid line) and 55 mol% ethanol (dashed line).

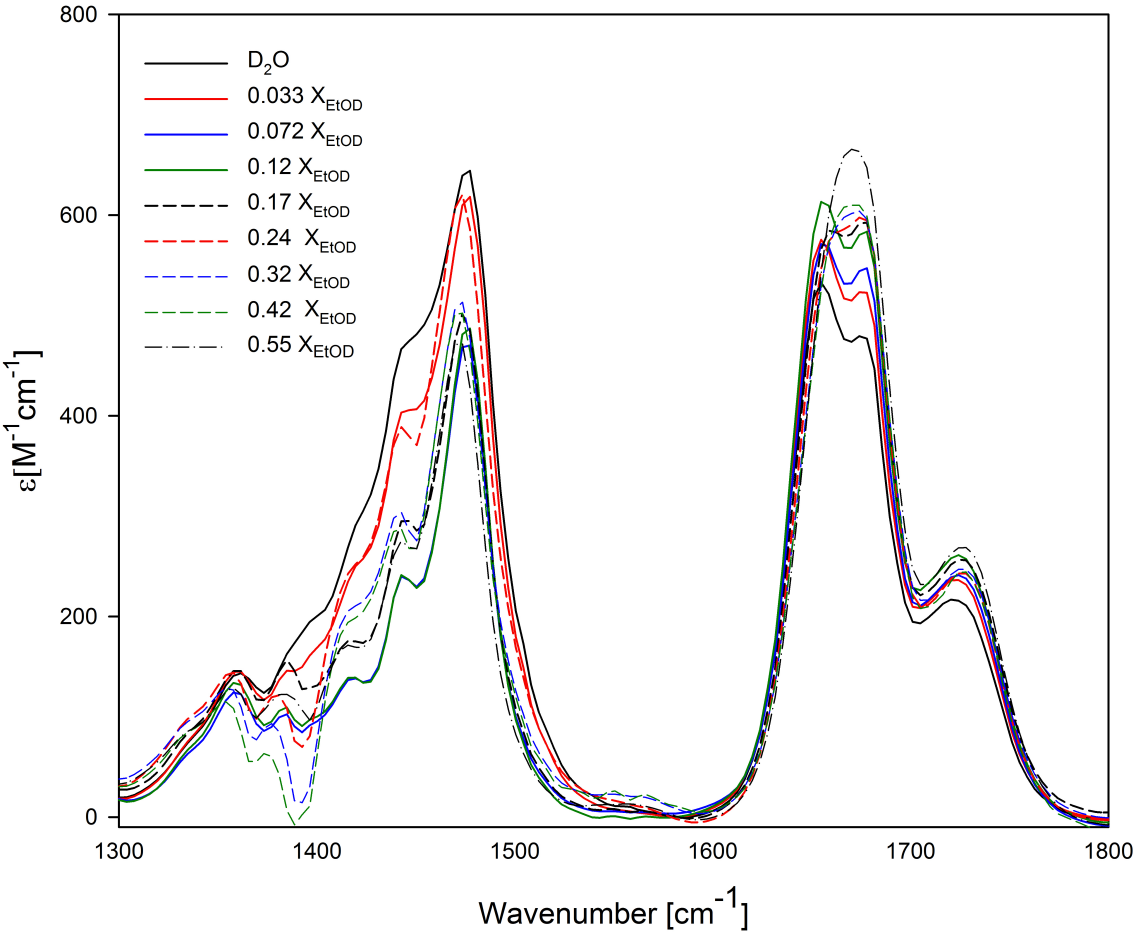

Figure S6: IR spectra of the 1300-1800 cm^{-1} region of 200 mM cationic GAG dissolved in the indicated ethanol/water mixture. The numbers reflect the molar fractions of ethanol in the mixture. The bands between 1300 and 1500 cm^{-1} result mostly from CH_2 and CH_3 deformation modes. The most intense band in this region is assignable to a mixture of amide II' and CH_3 out-plane antisymmetric bending modes. The 1600-1700 cm^{-1} region contains the amide I' band profile. The band at ca. 1700 cm^{-1} is assignable to the C-terminal C=O stretching mode.

Ref**erences**

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