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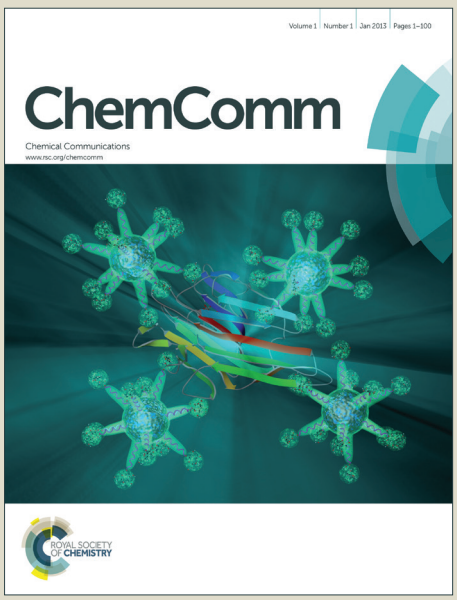

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Linear Dependence of Water Proton Transverse Relaxation Rate on Shear Modulus in Hydrogels

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It is found that hydrogelation of peptides enhances the transverse relaxation rate R_2 of water protons but has no effect on the longitudinal relaxation rate R_1 and the diffusion coefficient D. The magnitude of water proton R_2 enhancement increases linearly with the shear modulus G of the hydrogel.

In a previous publication, we reported that NMR relaxation rates of residual free gelators in hydrogels increase linearly with the shear modulus of the hydrogel.[1] The shear modulus G describes the stiffness of a material and is conventionally measured by rheometers, which requires taking a sample out of its native environment and loading it into a rheometer cell. The aforementioned linear relationship between nuclear spin relaxation rates and shear modulus suggests the possibility of using NMR or MRI to measure the shear modulus of a soft material in its native environment without coming in contact with any mechanical testing device. However, the NMR signals from residual free gelators are rather weak, making such measurements difficult. In contrast, the water proton signal in aqueous media is 10^4-10^6 times stronger than any solute proton signal. Hence if the shear modulus of a hydrogel can be determined through the water proton signal, the measurement could be carried out with ease and high accuracy. In this work, we investigate the relationship between the relaxation rates of water protons and the shear modulus of hydrogels. We found that the water proton transverse relaxation rate R_2 increases linearly with the hydrogel shear modulus G. This forms the basis of using the water proton signal to assess biomaterial stiffness noninvasively.

Since 1960, it has been observed that the relaxation rates of various water nuclei are higher in soft materials[2] and biological tissues[3] than in bulk water. This holds true not only for ^{1}H (spin quantum number I = ½), but also for ^{2}H (I = 1)[2c, 2i, 3a] and ^{17}O (I = 5/2).[2b, 2i] These early studies focused on water mobility in these materials. To the best of our knowledge, there has been no report linking water proton relaxation to material mechanical properties.

To explore the link between water proton relaxation rates and hydrogel shear modulus, we used hydrogels co-assembled from a pair of oppositely charged undecapeptides. The sequences of the two peptides, **K11** and **E11**, are shown in Table 1.

Table 1. Peptide sequence, molecular weight and net charge

Notation	sequences[a]	M. W.	Net charge[b]
K11	*ac*-KWKAKAKAKWK-*am*	1,413 Da	+6
E11	*ac*-EWEAEAEAEWE-*am*	1,419 Da	-6

a A: alanine; E: glutamic acid; K: lysine; W: tryptophan; *ac*-: acetylation; *-am*: amidation.
b Net charge refers to the number of charges at neutral pH.

Each purified peptide is dissolved in 50 mM phosphate-buffered saline. Because pH and ionic strength can affect gelation and water relaxation, the pH of all peptide solutions was adjusted to 7.4 and the conductivity to 17.0 mS/cm, equivalent to that of a buffer containing 50 mM sodium phosphate and 100 mM NaCl of pH 7.4. Note that the peptides are charged and hence contribute to conductivity. To take this into account, constant conductivity, and hence constant ionic strength, is achieved by adjusting the NaCl concentration in the solution. Gelation is induced by mixing the two peptide solutions. Compared with pH- or salt-induced gelation, mixing-induced gelation does not introduce uncertainty in the pH and ionic strength of the resulting gel, which is critical for water relaxation studies.

The shear modulus of hydrogels was monitored by a dynamic rheometer using a sealed cell under direct temperature control in the absence of any applied magnetic field. Figure 1 shows the growth of the shear modulus G of 5 hydrogels assembled from **K11** and **E11** at different concentrations. The growth of shear modulus G with gelation time is caused by the gradual incorporation of peptides into the hydrogel matrix, as shown by previous studies.[1,9] As one would expect, higher gelator concentration leads to higher shear modulus. Unless otherwise specified, all concentration refers to the total concentration of a peptide in a sample (free + gelled); all shear modulus values refer to the plateau value.

NMR measurements were conducted at 9.4 T (400 MHz for ^{1}H) in the absence of any applied mechanical force. All NMR relaxation rates and diffusion coefficients refer to those of water, not gelators.

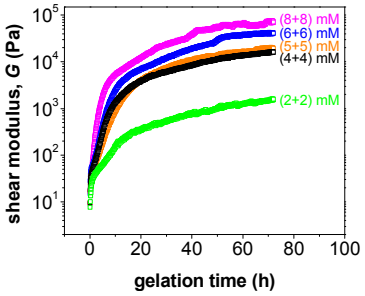

Figure 1. Growth of the shear modulus of 5 hydrogels at different peptide concentrations.

For NMR experiments, gelation took place inside a 3-mm NMR tube after mixing the two parent solutions. To lock the magnetic field, the 3-mm tube was put inside a 5-mm NMR outer tube filled with D_2O. To explore whether water proton R_1 and R_2 correlate with the shear modulus G in mature gels, R_1 and R_2 were measured for each hydrogel after *ca*. 65 h of gelation. R_1 was measured using the saturation-recovery method to avoid the radiation dumping effect[4] while R_2 was measured using the standard CPMG method.[5] To assess water mobility in mature gels, the diffusion coefficient D of water was measured using the pulsed-field gradient NMR technique.[6] To account for any concentration effect, D, R_1 and R_2 were also measured in parent peptide solutions. D, R_1 and R_2 of water in parent solutions and in gels, along with G of mature gels, are listed in Table 2. Both NMR and rheological measurements were conducted at room temperature (22.3°C).

In both solutions and gels, D decreases slightly with peptide concentration while R_1 increases slightly with concentration. Such results are in line with previous reports on water D and R_1 in protein solutions.[7] But there is hardly any difference of D and R_1 between a hydrogel and its parent solutions. In contrast, R_2 is much larger in gels than in solutions of the same concentration. By subtracting the solution value from the corresponding hydrogel value, the impact of gelation on D, R_1 and R_2 is obtained (Table 2). Figure 2 plots the concentration-corrected D, R_1 and R_2 of water vs. the shear modulus G of the hydrogel.

Table 2. NMR parameters of water and shear modulus of hydrogels

$C_{peptides}$ [a] (mM)	D (10^{-10} $m^2 \cdot s^{-1}$)			R_1 (s^{-1})			R_2 (s^{-1})			G (kPa)
	in sol.[b]	in gel	ΔD	in sol.[b]	in gel	ΔR_1	in sol.[b]	in gel	ΔR_2	at 72 *h*
2+2	21.0	21.1	0.1	0.34	0.33	-0.01	0.71	1.80	1.09	1.58
4+4	20.8	20.9	0.1	0.35	0.34	-0.01	1.02	4.90	3.88	16.17
5+5	20.8	20.8	0.0	0.36	0.36	0.00	1.19	5.97	4.78	19.82
6+6	20.6	20.8	0.2	0.36	0.36	0.00	1.36	9.16	7.65	41.32
8+8	20.4	20.5	0.1	0.36	0.36	0.00	1.67	11.45	9.78	73.36

a Refers to the concentration of each peptide in the hydrogel. For example, a hydrogel of 2 mM + 2 mM is prepared by mixing equal volumes of two parent peptide solutions, each of 4 mM. D, R_1 and R_2 of water in buffer with no peptides are respectively 21.3×10^{-10} $m^2 \cdot s^{-1}$, 0.34 s^{-1} and 0.36 s^{-1}. The shear modulus of buffer is close to that of water (at our frequency $\omega = 1$ rad/s = 0.159 Hz, the shear modulus of water is close to zero).
b Each solution data is the average of the two parent solutions, i.e., X(solution) = [X(K11 solution) + X(K11 solution)]/2. $X = D$, R_1 or R_2.

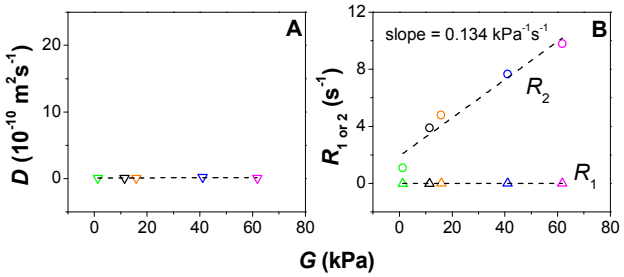

Figure 2. (A). Corrected water diffusion coefficient ($D = D_{in\ gel} - D_{in\ solution}$) vs. hydrogel shear modulus G. (B). Corrected longitudinal ($R_1 = R_{1,\ in\ gel} - R_{1,\ in\ solution}$) and transverse ($R_2 = R_{2,\ in\ gel} - R_{2,\ in\ solution}$) relaxation rates of water proton vs. hydrogel shear modulus G.

From Figure 2, it can be seen that, in mature gels, D and R_1 are independent of G while R_2 increases linearly with G, i.e.,

$$R_2 = a + r_{shear} \cdot G \qquad (1)$$

where r_{shear} is the slope with the unit $kPa^{-1} \cdot s^{-1}$. This linear dependence of water R_2 on the shear modulus G is analogous to the linear dependence of water R_i on the gadolinium concentration [Gd] in paramagnetic relaxation enhancement:

$$R_i = R_{i0} + r_i \cdot [Gd] \qquad (i = 1 \text{ or } 2) \qquad (2)$$

where r_i is the slope with the unit $mM^{-1} \cdot s^{-1}$ and is called the paramagnetic relaxivity.[8] In light of the parallel between Eqns. 1 and 2, and the fact that the shear modulus in these hydrogels is dominated by its elastic component,[1,9] Eqn. 1 can be considered elastic relaxation enhancement and r_{shear} the elastic relaxivity. Like Eqn. 2, which is valid only within certain gadolinium concentration range, Eqn. 1 might be valid only within certain shear modulus range. Also like the paramagnetic relaxivity r_i, which depends on the structure of the gadolinium chelate, the elastic relaxivity r_{shear} might depend on the structure of the hydrogel and ultimately the structure of the gelator.

Although the validity range for Eqn. 1 is presently unknown, we notice that the range shown in Figure 1, which is 1.3 – 62 kPa, covers the stiffness range of many soft biological tissues.[10]

The negligible dependence of water D and R_1 on hydrogel G suggests that gelation has little impact on water mobility. This is not surprising since over 98% w/w of the hydrogel is water. Hence peptide fibers occupy only a small fraction of the hydrogel volume and impose little restrictions on the motions of water molecules. In fact, previous NMR relaxation studies using $H_2{}^{17}O$ have concluded that even water molecules in the hydration layer of proteins and peptides suffer a mere two-fold motion retardation.[11] The question then is what is the origin of the water transverse relaxation enhancement upon gelation?

One possible contributing factor is the exchange between water protons and labile protons in the peptides (e.g., amide protons). Such exchange couples water proton relaxation with peptide proton relaxation. Upon gelation, the peptide motions are slowed down, leading to faster peptide proton relaxation. Due to the coupling between water protons and peptide protons, water proton relaxation is also enhanced. This mechanism has been previously proposed to explain the enhancement of water R_2 upon serum albumin aggregation.[12]

Another possible contributing factor is the local magnetic field gradient around peptide fibers. During gelation, peptides aggregate into nano-scale fibers, causing structural inhomogeneity inside the hydrogel.[13] In the presence of external magnetic field, structural inhomogeneity will result in local magnetic field inhomogeneity, because macromolecules have different diamagnetic susceptibility than bulk water[14] and such difference grows with the size of the macromolecule.[15] Hence as peptide fibers form and grow, local magnetic field inhomogeneity will grow as well. It is known that local magnetic field inhomogeneity can significantly increase R_2 with little effect on R_1.[5a, 16]

Because local magnetic field inhomogeneity is caused by structural inhomogeneity in the hydrogel, R_2 should increase as peptide fibers form and grow. To test this possibility, we monitored water R_2 and peptide fiber size during gelation at a gelator concentration of 8 mM **K11** + 8 mM **E11**. Water R_2 was continuously collected as gelation proceeds. The results are shown in Figure 3A. Peptide fiber growth was monitored by small-angle X-ray scattering (SAXS) and the details have been presented in a previous publication.[9] To aid comparison, the maximum dimension of the fiber cross-section, d_{max}, at different time points are shown in Figure 3B. Of course, d_{max} captures only one aspect of the fiber network. Nonetheless, R_2 and d_{max} show similar growth patterns; rapid rise within the few hours followed by much slower growth afterwards. This result lends support to the conclusion that gelation-induced R_2 increase is caused by local magnetic field inhomogeneity resulting from peptide fiber formation and growth.

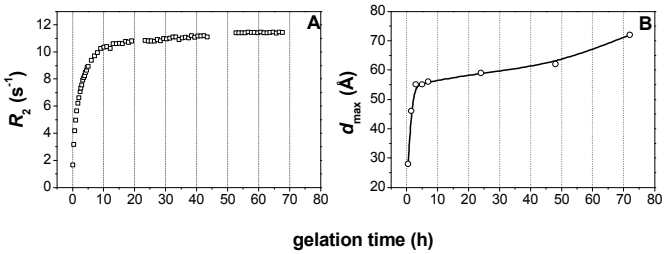

Figure 3. The growth of water proton transverse relaxation rate R_2 shows similar trend as the growth of the maximum cross-sectional dimension of peptide fibers, d_{max}.

Conclusions

In conclusion, it was found that the transverse relaxation rate R_2 of water protons in hydrogels increases linearly with the shear modulus of the hydrogel. This result suggests that it might be possible to assess biomaterial stiffness through the water R_2. Unlike magnetic resonance elastography,[17] this type of measurement does not require stimulating the material mechanically using acoustic waves. Hence standard NMR or MRI devices and techniques can be used.

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Notes and references

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† Electronic Supplementary Information (ESI) available: peptide synthesis and purification, HPLC and Mass Spectroscopy analysis, sample preparation, NMR and rheology experiments. See DOI: 10.1039/c000000x/

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