Isotopic control over self-assembly in supramolecular gels

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ABSTRACT: It is common to switch between H$_2$O and D$_2$O when examining peptide-based systems, with the assumption being that there are no effects from this change. Here, we describe the effect of changing from H$_2$O to D$_2$O in a number of low-molecular-weight dipeptide-based gels. Gels are formed by decreasing the pH. In most cases, there is little difference in the structures formed at high pH, but this is not universally true. On lowering the pH, the kinetics of gelation are affected and, in some cases, the structures underpinning the gel network are different. Where there are differences in the self-assembled structures, the resulting gel properties are different. We, therefore, show that isotopic control over gel properties is possible.

Low-molecular-weight, or supramolecular, gels are formed by the self-assembly of small molecules into fibers that subsequently entangle.$^{1,2}$ The assembly is driven by noncovalent interactions including hydrogen bonding, hydrophobicity, and π-stacking. As such, very small changes in molecular structure often lead to dramatic differences. It is therefore unsurprising that each molecule has solvent-dependent gelation efficiency.$^1$

For hydrogels, hydrophobicity and hydrogen bonding are dominant noncovalent interactions.$^5$ On changing from H$_2$O to D$_2$O, a number of properties change, including density, viscosity, and hydrogen bond strength.$^6$ Additionally, the hydrophobic effect has also been reported to be more pronounced in D$_2$O than in H$_2$O.$^7$ In some systems, substituting H$_2$O for D$_2$O can lead to a change in properties. For example, the persistence length of elastic peptides is higher in D$_2$O than that in H$_2$O, ascribed to stronger hydrogen bonding.$^8$ Slight differences in dimensions have been reported for nanotubes formed from a small peptide in H$_2$O or D$_2$O.$^9$ For biopolymer-based gels, the melting temperature of gelatin gels is higher in D$_2$O as compared to H$_2$O,$^{10}$ and the gels are formed at high pH, but this is not universally true. On lowering the pH, the kinetics of gelation are affected and, in some cases, the structures underpinning the gel network are different. Where there are differences in the self-assembled structures, the resulting gel properties are different. We, therefore, show that isotopic control over gel properties is possible.$^{10}$

$^\circ$C.$^{16}$ The rheological data were stated to be essentially the same. Variations in hydrophobicity were assigned as the dominant reason for changes in the melting point.

In addition to the suggestions that it might be possible to change the gel properties when using D$_2$O instead of H$_2$O, there are also important implications for a number of experimental techniques. It is common, for example, to carry out infrared spectroscopy in D$_2$O instead of H$_2$O to minimize the absorbance of water.$^{17,18}$ Likewise, NMR experiments are typically carried out in D$_2$O. Small-angle neutron scattering (SANS) is most often carried out in D$_2$O to allow contrast with the gelators.$^{19,20}$ In all cases, the often implicit assumption is that this change has no effect.

Here, we focus on a small library of dipeptide-based gelators (Scheme 1).$^{4,21−26}$ These form gels in water using a pH-switch. Typically, a solution of one of the gelators is prepared by dispersing the molecule at high pH (pH 10−11) at a concentration of 5 mg/mL. Decreasing the pH results in gelation. The kinetics here control the homogeneity of the gel and so we commonly exploit the hydrolysis of glucono-δ-lactone (GdL) to gluconic acid to lead to a slow, controlled decrease in pH.$^{27,28}$ This leads to very reproducible gels.$^{21}$ The rate of hydrolysis of GdL has been reported to differ in H$_2$O and D$_2$O.$^{29}$

There are therefore primarily two states to be considered where there might be differences in H$_2$O and in D$_2$O: the high-pH (solution) phase and the low-pH (gel) phase. It can be difficult to probe these states effectively. It is common to use electron microscopy to image the underlying structures.

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We now discuss the gels. Gelation was then induced in all cases by the addition of GdL,\textsuperscript{27,28} leading to protonation of the terminal carboxylates. The rate of pH decrease is different in H\textsubscript{2}O and D\textsubscript{2}O, being slower in D\textsubscript{2}O (Figure S3, Supporting Information) in all cases. As a result, the times at which gelation begins (where the storage (\(G'\)) begins to deviate strongly from the loss (\(G''\)) modulus) as well as the profiles of \(G'\) and \(G''\) are different. In all cases, gelation begins and achieves plateau values at earlier times in H\textsubscript{2}O as compared to D\textsubscript{2}O, correlating with the slower hydrolysis of GdL in D\textsubscript{2}O. The rate of hydrolysis of GdL is catalyzed by many acids and bases, with the relative rate depending on the catalytic species.\textsuperscript{29} Since we have a complex solution where aggregates exist and are changing, as well as an evolving pH, the exact species catalyzing the hydrolysis is difficult to determine. Nonetheless, we observe that the hydrolysis in these systems is faster in H\textsubscript{2}O than in D\textsubscript{2}O (Figure S3) and this directly links to faster gelation in the H\textsubscript{2}O compared to that in D\textsubscript{2}O. The final gels are visually similar in both H\textsubscript{2}O and D\textsubscript{2}O (Figure 2). For 1, although the underlying structures are very similar at high pH (see the discussion above), the viscosities are different, which may be a result of the higher Kuhn length in D\textsubscript{2}O as compared to H\textsubscript{2}O. This manifests in the sample in D\textsubscript{2}O at early times having a storage modulus (\(G'\)) that is higher than the loss modulus (\(G''\)) (Figure 2a). The SAXS data can be used to determine the structures present but will not be easily able to pull out information about interactions between these structures. In H\textsubscript{2}O, whilst still viscous, \(G''\) dominates at early times. Since the hydrolysis of GdL is faster in H\textsubscript{2}O, changes in \(G'\) and \(G''\) occur earlier in the sample in H\textsubscript{2}O compared to that in D\textsubscript{2}O (Figure 2a). However, the final rheological values of \(G'\) and \(G''\) are similar in H\textsubscript{2}O and D\textsubscript{2}O for the gels formed from 1 (Figure S4). This is expected; we have previously found little differences for gels formed from 1 in both H\textsubscript{2}O and D\textsubscript{2}O.

However, the final values of \(G'\) and \(G''\) differ for gels formed from 2, 3, and 4 in H\textsubscript{2}O and D\textsubscript{2}O. For 2, the initial solutions are very similar in terms of the values of \(G'\) and \(G''\) (Figure 2b) and, whilst the rates of change in the moduli differ in H\textsubscript{2}O and D\textsubscript{2}O, the moduli for the final gels are relatively similar (Figure S4). For 3, the initial values of \(G'\) and \(G''\) are different, with \(G'\) being higher for the solutions in H\textsubscript{2}O. The differences in rheological data at early times for 3 show that the interactions between the structures must be stronger in H\textsubscript{2}O as compared to those in D\textsubscript{2}O since the SAXS data implies that the structures present at high pH are very similar. The final gels are stiffer in H\textsubscript{2}O as compared to those in D\textsubscript{2}O. For 4, the initial solutions have higher values of \(G'\) and \(G''\) in H\textsubscript{2}O compared to those in H\textsubscript{2}O.
Figure 1. SAXS data and fit for solutions of 1–4 in H$_2$O (open symbols) and D$_2$O (closed symbols), with fits as red lines: (a) 1, (d) 2, (g) 3, and (j) 4. Also shown are example cryo-TEM data for solutions of 1–4 in H$_2$O and D$_2$O: (b) and (c) 1 in H$_2$O and D$_2$O, respectively, (e) and (f) 2 in H$_2$O and D$_2$O, respectively, (h) and (i) 3 in H$_2$O and D$_2$O, respectively, and (k) and (l) 4 in H$_2$O and D$_2$O, respectively. All data was collected at a concentration of 5 mg/mL and a pH of 11. For the cryo-TEM data, the scale bars represent 200 nm in each case.
D₂O, and G’ dominates over G” from time zero. This correlates with the SAXS data showing that the structures are different at high pH. There are differences in the profile of the changes in G’ and G” with time for 4 (Figure 2d), with the sample in H₂O showing a steady change in G’ and G”, whilst that in D₂O shows a two-stage process. We have previously ascribed such two-stage processes to initial fiber formation and then lateral bundling.

The rheological data are determined from the mechanical properties of the primary self-assembled structures, as well as the degree of lateral association and other entanglements, which combine to give the overall gel network. The similarity in data for gels formed from 1 in H₂O and D₂O could be coincidental, with the average of very different interactions leading to an overall similar gel. Alternatively, the similarity may suggest that the primary structures and networks are not affected by the change in solvent.

Cryo-TEM of the gel phase is problematic due to sampling issues from the stiff networks (see the discussion in the Supporting Information and Figure S5). Hence, to probe the underlying structures, we again turned to SAXS (Figure 2). For gels of 1, the SAXS data are very similar. The data can be fitted to a flexible elliptical cylinder. This is as expected from previous work; primary fibers laterally aggregate to lead to structures where the scattering can be best fit to an elliptical shape. From the fitting, the radii were 2.5 and 2.7 nm in H₂O and D₂O, with axis ratios of 2.1 and 2.2, respectively. There are differences in the Kuhn length, a measure of the structures’ flexibility, with values of 25 and 95 nm for H₂O and D₂O, respectively. The lengths in both cases are again outside the range that can be probed here. These data imply that the structures in the gel phase are essentially the same in both H₂O and D₂O, with perhaps some variation in flexibility. The gels are formed at different rates and so the difference in flexibility
may represent different degrees of entanglement and lateral packing resulting from how quickly charge is removed from the structures.

For gels formed from 3, the best fit to the SAXS data is again the flexible elliptical cylinder, with the radii being very similar (4.3 and 4.6 nm in H2O and D2O, respectively), as are the axis ratios (3.1 and 3.3, respectively), and the Kuhn lengths (around 25 nm in both cases), with the overall length again being outside the range that can be probed by SAXS. Hence, for 2, the structures in the gel phase are very similar in H2O and D2O despite the small differences in rheology.

For gels formed from 3, the differences in the rheology data are reflected in the SAXS data. The data from the gels in H2O can be best fitted to a hollow cylinder model, with a radius of 22 nm and a thickness of 6.5 nm. A polydispersity in the radius of 0.11 needed to be included to ensure a good match to the kinetics of hydrolysis in H2O and D2O. Nonetheless, we show that there is a link between a single property such as hydrophobicity and whether there is an effect in moving between H2O and D2O.

For gels formed from 4, the scattering data are again different from one another. The data from the gels in H2O can be best fitted to a hollow cylinder model, with a radius of 2.9 nm and an axis ratio of 1.9. The data for the gels formed in D2O fit best to a cylinder model combined with a power law. The cylinders have a radius of 4.0 nm. Hence, again, the differences in the rheology of the gels in H2O and D2O can be ascribed primarily to different structures underpinning the network.

Hence, where the underpinning structures differ, there are concomitant differences in the rheological properties. In all cases, the kinetics of the hydrolysis of GdL and hence the rate of hydrolysis is temperature dependent.28 However, it is not possible to simply carry out experiments at different temperatures to match the kinetics of hydrolysis in H2O and D2O. For this class of gelator, there can be temperature effects. For example, 1 has a different self-assembled structure at room temperature and above 40 °C, for example.34 Likewise, it is difficult to suggest that there is a link between a single property such as hydrophobicity and whether there is an effect on changing from H2O to D2O. Nonetheless, we show that there is potential to use isotopic changes to control the properties of gels from a single gelator. This shows that the general assumption that there is no effect in moving between H2O and D2O does not always hold.

ASSOCIATED CONTENT

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.langmuir.0c01552.

Full experimental details including sample preparation methods; analysis methods; further SAXS discussion and analysis; rheology data; and further TEM data (PDF).