Thermally gelling, thermally responsive elastin-mimetic triblock hydrogels

D. S. Hart[1], A. J. M. D'Souza[2], C. R. Middaugh[3], and S. H. Gehrke[1,3]
[1]*Dept. of Chemical & Petroleum Engineering, The University of Kansas, Lawrence, KS 66045*
[2]*Department of Molecular Biology, University of Wyoming, Laramie, WY 82071*
[3]*Department of Pharmaceutical Chemistry, The University of Kansas, Lawrence, KS 66045*

Introduction:

Elastin-mimetic triblock (EMT) polypeptides can thermally gel in aqueous solution upon heating to form a stable 3-dimensional polymer network. This transition is reversible and the gel liquefies upon cooling. The sequence of this 135 kDa polypeptide is based upon the VPGVG pentamer repeat of elastin and has been constructed as a BAB block copolymer [1]. The B-blocks are modified to be more hydrophobic than VPGVG with alanine (A) and isoleucine (I) incorporated into pentamers as VPAVG[$(IPAVG)_4(VPAVG)]_{16}$IPAVG, while the center A-block is modified to be more hydrophilic than elastin with some glutamic acid (E) substituted as VPGVG[$(VPGVG)_2$VPGEG$(VPGVG)_2]_{30}$VPGVG. The network forms through aggregation of the B-blocks, and swells to a stable equilibrium in excess water [1, 2].

Determination of the specific stabilizing interactions of this network is significant to the *de novo* design of biomaterials for drug delivery and tissue engineering applications. Laser Raman spectroscopy and ATR-FTIR (attenuated total reflectance-Fourier transform infrared) spectroscopy were used to investigate the changes in secondary structure associated with the sol-gel transition in H_2O and D_2O. The Amide I regions of these deconvoluted spectra suggest that the stabilizing interactions are intermolecular β-sheets of the B-blocks with significant fractions also containing unordered structures with α-helical character and β-turns.

Experimental:

Elastin-mimetic triblock polypeptides were expressed in *E. coli* and purified as described elsewhere [1]. Lyophilized EMT was dissolved in either DIUF (deionized ultrafiltered) H_2O (Fisher Scientific) or D_2O (Sigma Aldrich) at 4°C for 2 hours to make 10% (w/w) solutions. The sol-gel transition occurred at about 18°C in both H_2O and D_2O. Laser Raman spectra were obtained from a Chromex Raman 2000 system with a 300 mW solid state diode laser at 785 nm equipped with Peltier temperature control. Each Raman spectrum was collected at a discrete temperature after the sample had attained thermal equilibrium for 20 min. Fourier-transform Infrared spectra were collected with an ABB Bomem PROTA with a ZnSe ATR crystal. The solution temperature was measured in the ATR cell as it warmed from 4°C to room temperature. Spectra from FTIR and Raman were Fourier deconvoluted on GRAMS/AI software and peaks were fit with Gaussian and Lorentzian functions in the Amide I region (1600-1700 cm^{-1}).

Results:

Raman and FTIR were run as complementary probes of the events associated with the gelation of EMT. Raman spectra were collected on EMT solutions and the H_2O or D_2O background spectra were subtracted at each temperature. The spectra in D_2O are shown in Figure 1 a). In D_2O, four peaks were fit with average values of 1669, 1651, 1631, and 1609 cm^{-1}, whereas H_2O spectra gave peaks of 1676, 1650, 1630, and 1614 cm^{-1}. Figure 1 b) depicts the peak area trends associated with gelation. Peaks at 1669 and 1631 both increase with temperature, while the peak at 1609 substantially decreases significantly in the temperature range of gelation. Thomas *et al,* working with a linear poly-(VPGVG) peptide (~100 kDa) obtained nearly identical peaks in H_2O, namely 1673 and 1650 cm^{-1}[5]. These peaks were attributed to type II β-turns based on calculations of turns residing between 1657-1693 cm^{-1}[6]. Studies of the Raman spectra of insulin have assigned a peak at 1655 cm^{-1} to

distributions of α-helices and β-turns [7]. Susi and Byler attribute a 1632 cm^{-1} peak to β-sheet through deconvolution, stating that this peak is often very weak in Raman spectra, so much so that it is usually not recognized in non-deconvoluted spectra [4]. This suggests that the β-sheet present reflects a strong interaction during gelation, given by aggregation of the B-blocks.

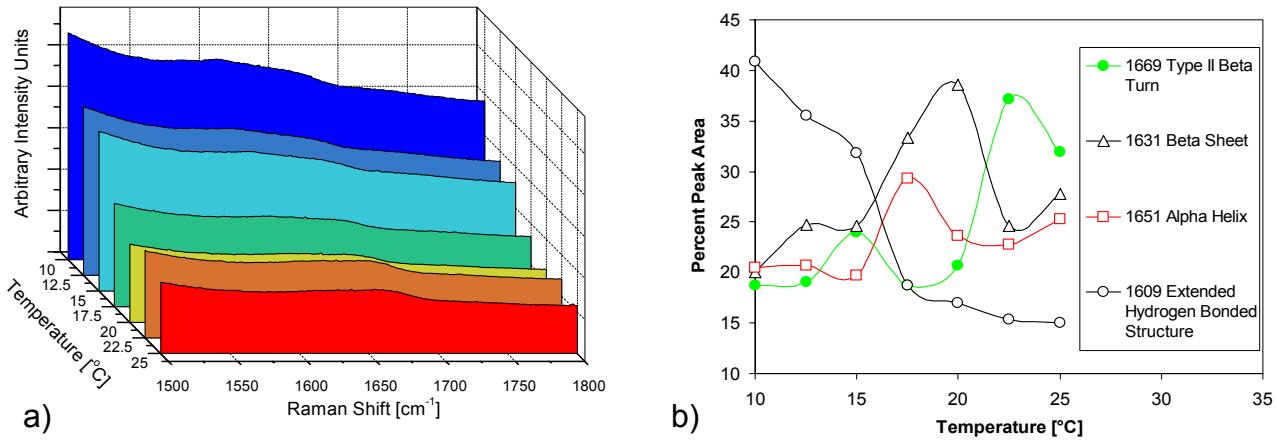

Figure 1. a) Amide I region of Raman spectra upon crossing the sol-gel transition of 10% (w/w) EMT in D_2O. b) Percent peak areas as obtained from deconvolution with structure assignments indicated. Note the adjusted scale to aid trend observation.

Also seen in Figure 1 a), is the substantial loss in Raman scattering intensity during gelation. This has been seen in other physically gelling systems and attributed to the relative strength of the H_2O/D_2O bending vibration decreasing significantly with an increase in hydrogen bonding associated with gelation [9]. Though the 1609 peak is typically only assigned to tyrosine residues in Raman, EMT contains none. In a related vibrational technique, namely FTIR, peaks in the 1610-1620 cm^{-1} region have been attributed to extended hydrogen bonded structures prior to aggregation [8]. In Figure 1 b) these extended hydrogen bonded structures dominate the secondary structure interactions in the sol-state, while showing a prominent decrease during gelation.

For additional evidence of β-sheet formation during gelation, FTIR spectra were also

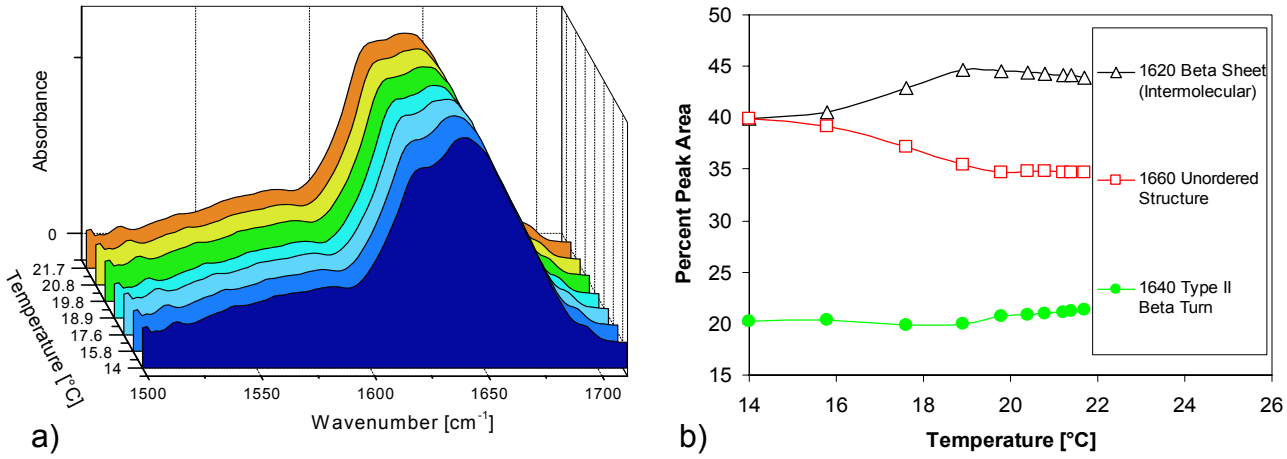

Figure 2. a) Amide I region of ATR-FTIR spectra upon crossing the sol-gel transition of 10% (w/w) EMT in D_2O. b) Percent peak areas as obtained from deconvolution with structure assignments indicated. Note the adjusted scale to aid trend observation.

striking change during gelation is the apparent increase in the peak near 1620 cm^{-1} relative to 1640 cm^{-1}. To quantify this change, spectra were deconvoluted to yield three peaks centered obtained on EMT solutions. FTIR spectra were collected on EMT in D_2O to avoid the larger H_2O bending mode interference in the Amide I region. As shown in Figure 2 a), the most at 1620, 1640, 1660 cm^{-1} and revealed only a modest change in secondary structure (Figure 2 b). However, peaks associated with β-structure did increase, while the unordered structure with α-helical character decreased. The peak at 1620 has normally been assigned as intermolecular β-sheets, which presumably occurs from the intermolecular hydrophobic association of B-blocks [3]. In model linear peptides, Hollősi *et al* have shown that types I-III β-turns yield a peak 1640±2 cm^{-1} in D_2O [10]. As shown in Figure 2 b) this peak (1641 cm^{-1}) intensity increased slightly during gelation (~ 18^{o}C). The repeat pentamer VPGVG in the A-block is known to form a type II β-turn in aqueous solution [5]. Liu *et al* demonstrated that a band at 1661 cm^{-1} indicates unordered structure with α-helical character by sequentially increasing dimethylsulfoxide (DMSO) concentration in a solution of hemoglobin in D_2O [11].

Discussion:

Raman and to a lesser extent FTIR show that β-structure increases relative to unordered structure during gelation, though the change is not as sharp as the physical observation of gelation. The presence of β-turns with the apparently strong signal of β-sheet in D_2O suggests these are the dominant stabilizing interactions, even though the literature often assigns higher frequency peaks (1660-1680 cm^{-1}) in Raman as only β-sheet. These spectra imply that the α-helical character may also reside in the B-blocks. In Raman, the slight increase in α-helical character parallels the initial rise in β-sheet, while the dichotomy between β-sheet and unordered structure (with α-helical character) is shown in FTIR. It is hypothesized that this turn is localized to the A-block due to the high percentage of VPGVG pentamer repeats, and the B-blocks intermolecular associations increase with temperature. In conclusion, the assignments from FTIR and Raman support the hypothesis that an increase in β-sheet and β-turns act to stabilize the network during gelation. The stability of the network in excess solvent also suggest that, once formed these interactions persist. Further interpretation of spectral changes is underway.

Acknowledgements:

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