Dynamics and Structure of Semiflexible, Self-Assembled Peptide Chain Networks

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Hydrogels, three dimensional networks of crosslinked polymer with high water content, are ideal drug delivery vehicles for protein delivery. Their highly porous, aqueous structure provides a biocompatible, solubilizing environment in which proteins can be encapsulated and released with both spatial and temporal control to ensure optimal dosage. Key features of these hydrogels necessary for therapeutic delivery include their mechanical strength (*i.e.*, the elastic modulus), kinetics of assembly, structural stability, and rate of release of the desired therapeutics. We have engineered hydrogels that are comprised of self assembled peptides with great potential for delivery vehicles via the simple modification of the individual peptide sequence [1]. MAX1 and MAX8 are synthetic β-hairpin peptides that undergo triggered nanoscale self-assembly to form a physically crosslinked hydrogel network of fibrils with a defined cross-section, as depicted in Fig. 1.

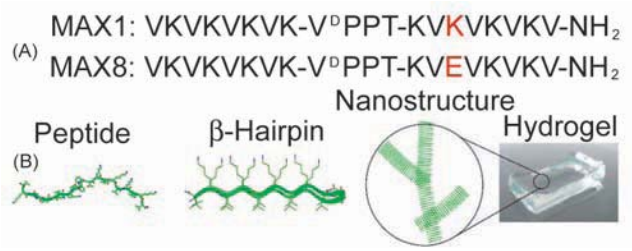

FIGURE 1: (A) Peptide sequence of MAX1 and MAX8. (B) Proposed mechanism: unfolded peptide under external stimulus folds into β-hairpins which self assemble into fibrillar nanostructures that make up the rigid hydrogel.

These peptides are freely soluble in aqueous solutions. However, when a physiological relevant concentration of salt is added at room temperature, the peptides fold into a β-hairpin, and subsequently, self-assemble to form a rigid hydrogel stabilized by non-covalent crosslinks. The sequence of MAX8 is identical to MAX1 with the exception of one single amino acid substitution (Glu replacing Lys at position 15). This reduces the net charge on the peptide and results in faster folding and self assembly kinetics for MAX8 at the same peptide concentration and identical buffer conditions. These faster folding kinetics result in more rigid gels, with the modulus for MAX8 being 2.5 kPa at 1.5 % mass fraction, which is about three times higher than for MAX1 at the same conditions [2].

Transmission electron microscopy (TEM) demonstrates that both peptides form networks of semiflexible fibrils that are composed of a bilayer of hairpins [3]. The fibrils are connected by non-covalent, interfibrillar junctions and entanglements. Imperfections in the self assembly mechanism, in which one hairpin is rotated relative to another hairpin in the bilayer, can give rise to interfibril branching. Therefore, the increased rigidity for MAX8 folded at the same peptide concentration, temperature, and buffer is hypothesized to be a consequence of structural differences either in terms of the topology of the network (*i.e.*, more imperfect branch points in MAX8) or in the compactness of the bilayer (*i.e.*, higher bending constant) [2]. Changes to these structural features can play a large role in the interaction of proteins with the peptide hydrogels and their subsequent delivery from the sterically hindering network, emphasizing the need to fully understand these structural differences between the two peptides.

In this highlight, we report the first neutron spin echo (NSE) measurements of self-assembling peptide hydrogel networks to study their dynamics on nanolength and nanotime scales. These NSE measurements were designed to explore whether these peptide fibrils can be described by the theory of semiflexible chains on length scales smaller than the persistence length of the fibrils. In addition, these studies investigated how the substitution of the Lys at position 15 with a Glu affects the nanoscale dynamics to determine whether the observed increase in viscoelasticity upon peptide substitution is due to a difference in nanoscale fibril rigidity. We follow the procedures and theoretical analysis defined in recent work on self-assembled worm-like surfactants based on the theory of Zilman and Granek [4,5]. Complementary small angle neutron scattering (SANS) measurements of the networks were also performed to assess any differences between the nanoscale structure of the two networks.

NSE measurements of the peptides demonstrate that the self-assembled peptide fibrils can be described as semi-flexible chains on nanolength and nanotime scales [2]. The normalized intermediate scattering functions $I(q,t)/I(q,0)$ obtained from

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the NSE measurements are shown in Fig. 2 for the 1.5 % MAX1 (A) and MAX8 (B). These normalized intermediate scattering functions were fitted for all samples to a stretched exponential predicted by the semiflexible chain model, $I(q,t) = I(q,0)\exp(-(\Gamma(q)t)^{\beta})$, with $\Gamma(q) = D_G q^{2\beta}$. An average β value of 0.74 ± 0.08 was measured for the peptide gels, which is consistent with the predicted value of 0.75 [4], further confirming their semi-flexible nature. From the wavevector, q, dependence of the scattering, which followed this equation, the segmental diffusivity D_G decreased upon alteration of the peptide sequence from 1.4×10^{-2} ($nm^{8/3}$ ns^{-1}) for MAX1 to 0.9×10^{-2} ($nm^{8/3}$ ns^{-1}) for MAX8. This difference indicates that the fibrils in the MAX1 network are more mobile on the range of length scales probed, which ranges from the fibril diameters to the characteristic mesh size, as determined from rheology [1]. This difference in segmental diffusivity is consistent with the change in peptide sequence, where the Glu at position 15 in MAX8 adds a salt bridge between peptides in the self-assembled fibril structure that further stabilizes the hairpin structure. However, this difference in mobility is not sufficient to fully explain the significant increase in elasticity, and therefore, we examined the nanostructure of the network itself.

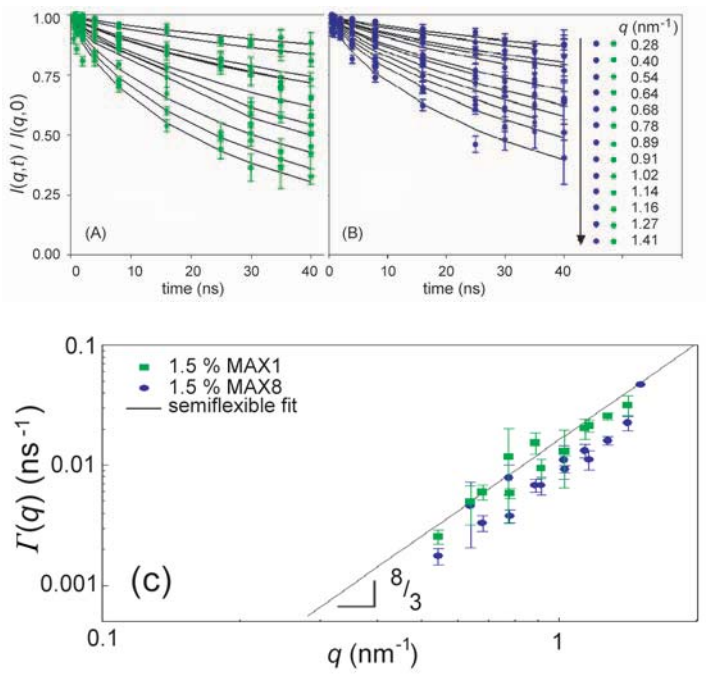

FIGURE 2: Normalized intermediate scattering functions for 1.5 % mass fraction MAX1 (A) and MAX8 (B) in pH 7.4, 50 mmol/L BTP, 150 mmol/L NaCl at 25 °C. Solid lines are fits to a stretched exponential model for semiflexible chains. (C) Relaxation rate ($\Gamma(q)$) as a function of q. The solid line represents the scaling expected for the bending modes of semiflexible chains.

A difference in the number of crosslink junctions between fibrils should manifest as a change in the small angle scattering. A look at SANS data for MAX1 and MAX8 gels supports this assessment [2,6]. As shown in Fig. 3, the scattering for both peptides is the same at high q, corresponding to the length scales of the individual fibril widths. This is expected as both peptides have the same overall length. The spectra differ, however, at lower q, which probes length scales similar to the nanoscale mesh size of the networks. The scattering intensity of MAX8 is significantly greater than MAX1 at the same concentration of peptide. The increased scattering is consistent with a heterogeneous network with a tighter mesh. Indeed, fitting these spectra to Teixeira's model yields apparent fractal dimensions of 1.26 and 1.62 respectively, which correspond with the increase in elastic modulus. This increase in network junctions for MAX8 is consistent with the very rapid rate of self-assembly as compared to MAX1, which is a direct consequence of the peptide substitution that lowers the net peptide charge [2].

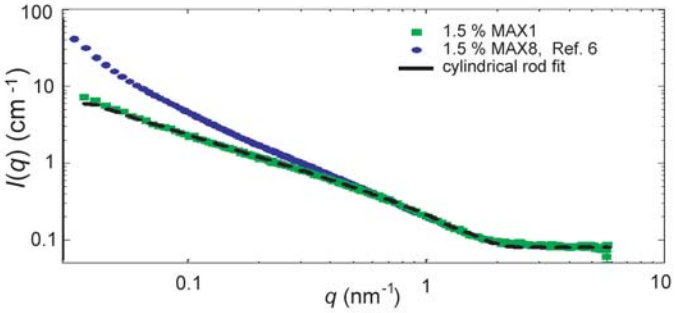

FIGURE 3: SANS intensity $I(q)$ for 1.5 % mass fraction MAX1 and MAX8 (Ref. 6) hydrogels in pH 7.4, 50 mmol/L BTP, 150 mmol/L NaCl, D_2O, 25°C.

In conclusion, combining NSE and SANS measurements yields a physical picture of the nanoscale dynamics and topology of these self-assembled peptide hydrogels that is consistent with a network of entangled and branched semiflexible fibrils. Through controlled modifications of the peptide sequence, we find that the nanoscale dynamics can be altered as well as the network topology itself. These changes in nanoscale dynamics and structure lead to substantial differences in bulk properties, such as the elastic modulus [1-3,6]. As shown, analysis of NSE and SANS data with models developed specifically for semiflexible polymers enables the quantification of the nanoscale properties of these networks and the development of molecular structure-property relations. These relations enable the rational engineering of peptide sequences to synthesize and assemble hydrogels appropriate for specific drug delivery applications.

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