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Proteolytic Stability of Amphipathic Peptide Hydrogels Composed of Self-Assembled Pleated β-Sheet or Coassembled Rippled β-Sheet Fibrils

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Hydrogel networks composed of rippled β-sheet fibrils of coassembled d- and l-Ac-(FKFE)\(_2\)-NH\(_2\) amphipathic peptides exhibit proteolytic stability and increased rheological strength compared to networks of self-assembled l-Ac-(FKFE)\(_2\)-NH\(_2\) pleated β-sheet fibrils. Modifying the ratios of l and d peptides in the coassembled rippled β-sheet fibrils alters the degradation profiles of these hydrogel networks.

Self-assembled peptide nanofibrils composed of amphiphilic, α-helical, and β-sheet peptides have been exploited in bottom-up approaches to create novel bionanomaterials.\(^1\) These materials include hydrogels for \textit{ex vivo} tissue engineering, wound healing, and drug delivery.\(^2\) Amphilathic β-sheet peptides with alternating polar and nonpolar residues are a privileged class of self-assembling peptide that have found wide use as noncovalent hydrogel biomaterials.\(^3\) Amphipathic peptides of this class readily assemble into bilayer nanoribbons with a hydrophobic core and a hydrophilic exterior face (Figure 1A) that is solvant exposed, providing water solubility and facilitating formation of entangled hydrogel networks.\(^4\) These self-assembled hydrogels have been engineered to be responsive to environmental stimuli to facilitate triggered assembly, disassembly, or degradation. Hydrogels with novel emergent mechanical and degradation properties will enable next-generation applications as functional biomaterials.

We recently reported a novel class of peptide-based nanofibril composed of equimolar mixtures of enantiomeric amphipathic peptides that coassemble into “rippled β-sheet” structures as predicted by Pauling.\(^5\) We found that d and l-Ac-(FKFE)\(_2\)-NH\(_2\) peptides coassemble into β-sheets with alternating d and l-sequences to form rippled β-sheets (Figure 1B) that are distinguishable from self-assembled, single enantiomer, pleated β-sheet fibrils (Figure 1A).\(^6\) It was found that coassembly of enantiomeric peptides into rippled β-sheets was enthalpically favorable, presumably due to the altered packing orientation of rippled β-sheets relative to pleated β-sheets. Schneider \textit{et al.} have also recently reported that coassembly of enantiomeric (VKVK)\(_n\) hairpin peptides provides hydrogels with enhanced mechanical rigidity relative to the single enantiomer self-assembled fibrils; their data suggests that rippled β-sheet formation occurs with this peptide sequence as well.\(^7\) Wetzel \textit{et al.} have also demonstrated rippled β-sheet formation with enantiomeric poly-Gln amyloid peptides.\(^8\) Herein, we report on the emergent rheological and degradation properties of hydrogels derived from rippled β-sheets of d and l-Ac-(FKFE)\(_2\)-NH\(_2\) peptides. Specifically, we have found that rippled β-sheet hydrogels of coassembled d and l-Ac-(FKFE)\(_2\)-NH\(_2\) have moderately enhanced rigidities relative to the corresponding pleated β-sheet materials and that rippled β-sheets are stable to proteolytic degradation. Pleated/rippled sheet hybrid fibrils composed of varying ratios of d- and l-peptide exhibit modified hydrogel degradation profiles, demonstrating the tunability of noncovalent amphipathic peptide hydrogel clearance rates.

Based on the proposed use of amphipathic peptide nanofibrils for in \textit{vivo} applications, we sought to characterize the proteolytic degradation profiles of rippled β-sheet nanofibrils relative to single enantiomer pleated β-sheet counterparts. It has been previously demonstrated that self-assembled pleated sheet fibrils composed of natural l-enantiomeric peptides can be...

Fig. 1 Schematic representation of assembled β-sheets fibrils composed of amphipathic Ac-(FKFE)\(_2\)-NH\(_2\) peptides. A. Pleated β-sheet fibrils composed of l-Ac-(FKFE)\(_2\)-NH\(_2\); at the top is shown the β-sheet bilayer structure and the out-of-register β-sheet packing is portrayed on the bottom. B. Rippled β-sheet fibrils composed of coassembled enantiomeric D- and L-Ac-(FKFE)\(_2\)-NH\(_2\) peptides (D shown in green).
exhibited varying susceptibilities to protease degradation (Figure 2). After 24 h, l-Ac-(FKFE)2-NH2 fibrils showed significant degradation with all three proteases with nearly half the peptide consumed. After five days, less than five percent of the initial 0.5 mM l-Ac-(FKFE)2-NH2 peptide remained (17 ± 2 µM for chymotrypsin, 17 µM ± 3 µM for trypsin, and 38 ± 15 µM for proteinase K). In contrast, pleated β-sheet fibrils of d-Ac-(FKFE)2-NH2 were only minimally degraded over five days (555 ± 49 µM for chymotrypsin, 530 µM ± 33 µM for trypsin, and 486 ± 21 µM for proteinase K). Significantly, 1:1 coassembled l/D-Ac-(FKFE)2-NH2 rippled β-sheet fibrils also resisted degradation, indicating that rippled sheet packing offers protection to protease-catalyzed hydrolysis for the susceptible l-peptides (553 ± 35 µM for chymotrypsin, 527 µM ± 23 µM for trypsin, and 538 ± 39 µM for proteinase K). This also indicates that dissociation of peptides from these assembled fibrils occurs slowly.

The 3:1 l/D-Ac-(FKFE)2-NH2 coassembled fibrils were degraded by proteases, albeit at a much slower rate and to a lesser extent than the pleated sheet l-Ac-(FKFE)2-NH2 fibrils. After five days, the overall peptide concentration in these 3:1 coassembled fibrils was reduced by approximately one half (258 ± 6 µM peptide remained with chymotrypsin, 313 ± 3 µM with trypsin, and 449 ± 12 µM with proteinase K). This result is consistent with the previous findings in that, at a 3:1 ratio of l to d peptide, it is expected that the resulting fibrils are hybrids of pleated and rippled β-sheet structures. A reduction of the total peptide by half is consistent with nearly complete degradation of the pleated β-sheet segments of these fibrils (composed of l-Ac-(FKFE)2-NH2); the rippled β-sheet segments are resistant to degradation as demonstrated by the 1:1 l to d coassembled materials. This indicates that altering the ratio of l and d peptides in hybrid pleated/rippled β-sheet fibrils can facilitate tunable proteolytic degradation of the overall peptide structure.

At high concentrations, fibrils composed of mixed amphipathic β-sheet peptides can form entangled hydrogel matrices that have been exploited as functional biomaterials. Based on the aforementioned studies, we hypothesized that rippled β-sheet fibrils would form hydrogels that resist degradation by proteases and conducted studies to test this hypothesis. Ac-(FKFE)2-NH2 fibrils have been shown to form hydrogels at concentrations above 6 mM. Hydrogels of l-Ac-(FKFE)2-NH2 pleated sheet fibrils, d-Ac-(FKFE)2-NH2 pleated sheet fibrils, and 1:1 l/D-Ac-(FKFE)2-NH2 rippled sheet fibrils were prepared by dissolving these peptides at 8 mM total peptide in water with 1 mg mL⁻¹ chymotrypsin (Figure S1). All three fibril types formed optically transparent, stable gels under these conditions. Under these conditions, significant creep of the l-Ac-(FKFE)2-NH2 pleated sheet hydrogel was observed after ~4 h, indicating a degradation of the hydrogel fibril network. After longer incubation times, the hydrogel integrity was nearly completely compromised. In contrast, the d-Ac-(FKFE)2-NH2 pleated sheet hydrogel and the 1:1 l/D-Ac-(FKFE)2-NH2 rippled sheet hydrogel maintained their integrity after 4 h. Even after several days of incubation, these hydrogels showed only minimal loss of hydrogel integrity.

We further characterized proteolytic degradation of pleated and rippled β-sheet hydrogel networks using oscillatory rheology. Quantification of hydrogel viscoelastic properties were conducted by measuring the storage modulus (G') and loss modulus (G'') of
pleated sheet hydrogels showed no loss in gel rigidity in the presence of chymotrypsin. The 1:1 L-β-sheet fibrils of self-assembled L-peptides are readily degraded by common proteases. Hybrid rippled/pleated sheet materials created by altering the ratio of L to D peptide incorporated into the fibril exhibit tunable degradation profiles. These properties are attractive for applications in biological environments in which the ability to alter degradation profiles of these materials is desired. These findings facilitate the creation of next-generation materials composed of self- or coassembled amphipathic peptides for biological applications including \textit{ex vivo} tissue culture, wound healing, and immunology.

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Fig. 3 Rheological dynamic frequency sweep with and without chymotrypsin present for L-Ac-(FKFE)$_2$-NH$_2$ hydrogels, D-Ac-(FKFE)$_2$-NH$_2$ hydrogels, and 1:1 L-β-Ac-(FKFE)$_2$-NH$_2$ rippled sheet hydrogels. Total peptide concentration was 8 mM and chymotrypsin concentration was 0.5 mg mL$^{-1}$. G$'$ values are solid lines and G$''$ values are dashed lines.

Herein, we have demonstrated that rippled β-sheet materials composed of coassembled enantiomeric amphipathic peptides are resistant to proteolytic degradation. In contrast, pleated β-sheet fibrils of self-assembled L-peptides are readily degraded by common proteases. Hybrid rippled/pleated sheet materials created by altering the ratio of L to D peptide incorporated into the fibril exhibit tunable degradation profiles. These properties are attractive for applications in biological environments in which the ability to alter degradation profiles of these materials is desired. These findings facilitate the creation of next-generation materials composed of self- or coassembled amphipathic peptides for biological applications including \textit{ex vivo} tissue culture, wound healing, and immunology.

Notes and references

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