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

Electronic Supporting Information

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Experimental Details

Peptide Synthesis

All peptides were synthesized as *N*-acetyl, *C*-terminal amide sequences utilizing standard solid-phase techniques with Fmoc protection and HBTU/HOBt activation on Rink amide resin (Advanced ChemTech, 100-200 mesh, 0.27 mmol/g). Following Fmoc deprotection of the final amino acid, the *N*-termini were acetylated. The peptides were side chain-deprotected and cleaved from the resin by treatment with a cleavage cocktail composed of trifluoroacetic acid, triisopropyl silane, and water (95:2.5:2.5, *v*/*v*). The cleavage cocktail was concentrated by partial evaporation *in vacuo* and the peptide was isolated by precipitation in cold diethyl ether. The precipitated peptide was collected by centrifugation and resuspension of the pellet in cold diethyl ether followed by recentrifugation. The peptide pellet was dissolved in DMSO and purified by preparatory HPLC.

Peptide Purification and Characterization

Peptide purification was conducted using a reverse phase C18 column (Waters, BEH300 10 mm, 19 × 250 mm) on a Shimadzu LC-AD HPLC system. A binary gradient of water and acetonitrile with 0.1% TFA at 10 mL min^{-1} was used and eluent was monitored by UV absorbance at 215 and 254 nm. Purity was confirmed by analytical HPLC performed using an RP-C18 column (Waters, BEH300 10 mm, 4.6 × 250 mm) and MALDI-TOF mass spectroscopy. The peptides were lyophilized prior to preparing samples for self-assembly.

Peptide Self-Assembly

Peptides were dissolved in 60% acetonitrile/water to maintain an unaggregated state in order to facilitate determination of concentration. Peptide concentrations were determined by injection onto an analytical HPLC (RP-C18 column (Waters, BEH300 10 mm, 4.6 × 250 mm) on a Shimadzu LC-AD HPLC system) and correlated to standard curves. Standard concentration curves were constructed by Wetzels's method (O'Nuallian, B.; Thakur, A. K.; Williams, A. D.; Bhattacharyya; Chen, S.; Thiagarajan, G.; Wetzel, R. *Methods Enzymol.,* **2006**, 413, 34–74) via serial dilution of purified peptides and injection of these solutions onto an analytical HPLC (reverse phase Waters column, BEH300 10 mm, 4.6 × 250 mm on a Shimadzu LC-AD HPLC system); absolute concentrations for the curve were determined by amino acid analysis (AIBioTech, Richmond, VA). Once peptide concentration of these stock solutions had been determined, aliquots of L-Ac-$(FKFE)_2$-NH_2, D-Ac-$(FKFE)_2$-NH_2, and 1:1 or 3:1 L-Ac-$(FKFE)_2$-NH_2/D-Ac-$(FKFE)_2$-NH_2 were dispensed into microcentrifuge tubes (0.5 mM total peptide for

fibril degradation studies and 8 mM total peptide for hydrogelation studies) and these solutions were frozen and lyophilized. The lyophilized peptides were then dissolved in water (Barnstead, NANOpure 0.2 mm filter, 18 Ω) and mixed by vortex for one minute to obtain optically clear, homogenous solutions. Final concentrations after lyophilization and dissolving in water for degradation studies were obtained via HPLC and reported in Fig. 2.

Degradation of Peptide Fibrils by Proteases

Lyophilized peptide (L-Ac-$(FKFE)_2$-NH_2, D-Ac-$(FKFE)_2$-NH_2, and 1:1 L-Ac-$(FKFE)_2$-NH_2/D-Ac-$(FKFE)_2$-NH_2; 0.5 mM total peptide concentration in each solution) was dissolved in water (50 μL) or solutions of 1.0 mg/ml α-chymotrypsin, trypsin, or proteinase K in water and vortexed for one minute to form transparent solutions of self-assembled peptide fibrils. These samples were allowed to stand at room temperature. Periodically (1 h, 24 h, 120 h), aliquots (10 μL) were removed and diluted into 90 μL of dimethylsulfoxide (DMSO). These DMSO solutions were sonicated for 10 minutes in order to promote disaggregation of assembled materials. The concentration of monomer was determined by immediate injection of these disaggregated peptide solutions onto analytical HPLC columns (see conditions in Table S1); integrated peak areas for the intact peptide were correlated to standard concentration curves in order to determine the concentration of monomer remaining as a function of time. Concentration curves were identical to those reported in *J. Am. Chem. Soc.* **2012**, *134*, 5556–5559).

Hydrogelation

Peptides were assembled as described in the self-assembly section above. At 8 mM concentrations of total peptide, these self-assembled peptides formed homogenous, optically transparent hydrogels. Peptide hydrogel samples that were analyzed in the presence of protease were dissolved in water (16 mM), mixed by vortex for one minute and allowed to stand for 10 minutes. An equal volume of 1.0 mg/mL α-chymotrypsin (giving a final protease concentration of 0.5 mg/mL and peptide concentration of 8 mM) in water was then added and the samples were again mixed by vortex for one minute. Samples were subjected to rheological analysis one hour after preparation.

Rheology

Rheology measurements to characterize the emergent viscoelastic properties of these hydrogels were conducted on a TA Instruments Discovery HR rheometer operating in oscillatory mode, with a 20 mm parallel plate geometry. Peptide gels (120 μL) were transferred to the stage and the

rotor gap was set to 225 μm. Samples were analyzed using a dynamic frequency sweep that was performed over a range of frequencies from 0.1 to 100 rad s^{-1} at 0.1% constant strain at 25 ^{o}C. This strain falls within the linear viscoelastic region for these peptide materials as previously determined (*Mol. BioSyst.* **2009**, *5*, 1058–1069; *J. Am. Chem. Soc.* **2010**, *132*, 9526–9527; *Biomacromolecules* **2011**, *12*, 2735–2745). All reported G′ and G′′ values are an average of measurements on at least three independent samples.

Figure S1. Vial inversion tests of hydrogel stability in the presence of chymotrypsin for hydrogels of (from left to right) L-Ac-$(FKFE)_2$-NH_2 pleated β-sheet fibrils, D-Ac-$(FKFE)_2$-NH_2 pleated β-sheet fibrils, and 1:1 D/L-Ac-$(FKFE)_2$-NH_2 rippled β-sheet fibrils after (A) 0 h (immediately after dissolution of peptides) and (B) 4 h. These hydrogels were optically transparent; apparent turbidity in these images is due to entrapped air bubbles within the gels.

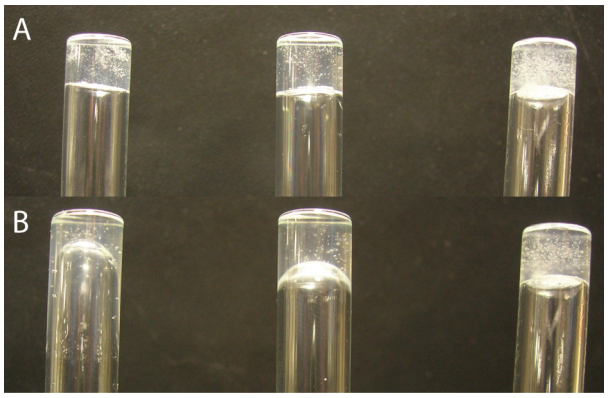

Table S1. Analytical HPLC purification conditions

Peptide	**R_t** (min)	**Gradient** (solution **A**: water/0.5% TFA; solution **B**: acetonitrile/0.5% TFA)
D-Ac-$(FKFE)_2$-NH_2	11.9	Isocratic 5% **B** 5 min, 5-95% **B** over 10 min, 95% **B** 5 min
L-Ac-$(FKFE)_2$-NH_2	11.9	Isocratic 5% **B** 5 min, 5-95% **B** over 10 min, 95% **B** 5 min

Figures S2-4. Analytical HPLC traces of synthetic peptides detected by UC absorbance at 215 nm.

Figure S2. D-Ac-FKFEFKFE-NH_2.

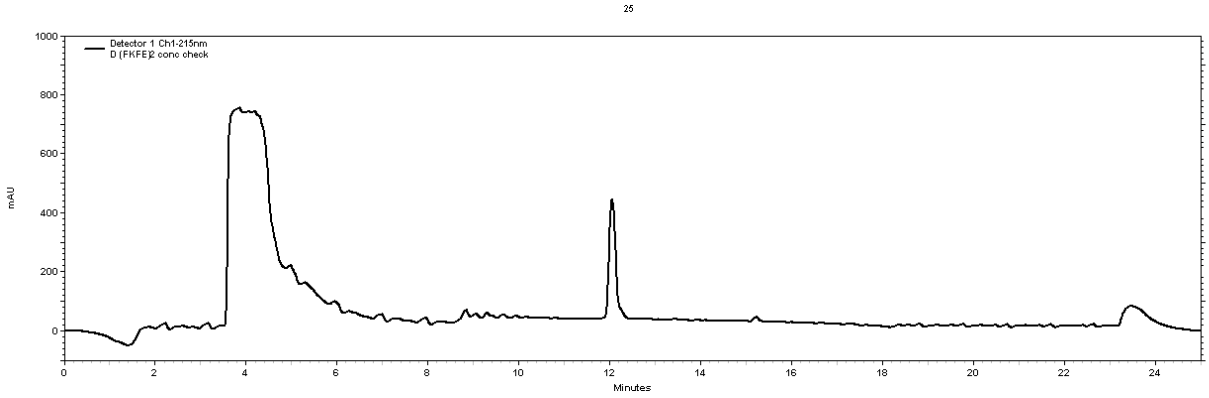

Figure S3. L-Ac-FKFEFKFE-NH_2.

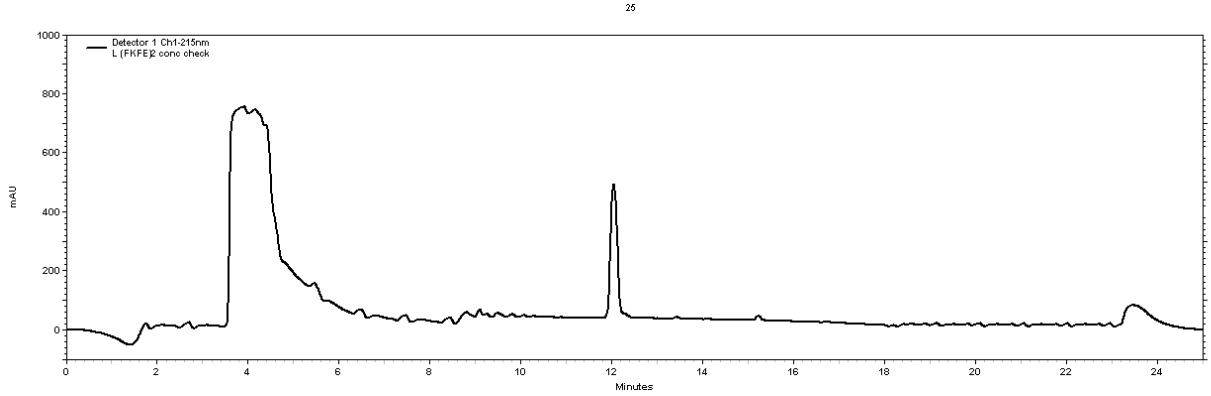

Figure S4. D- and L-Ac-FKFEFKFE-NH_2 combined.

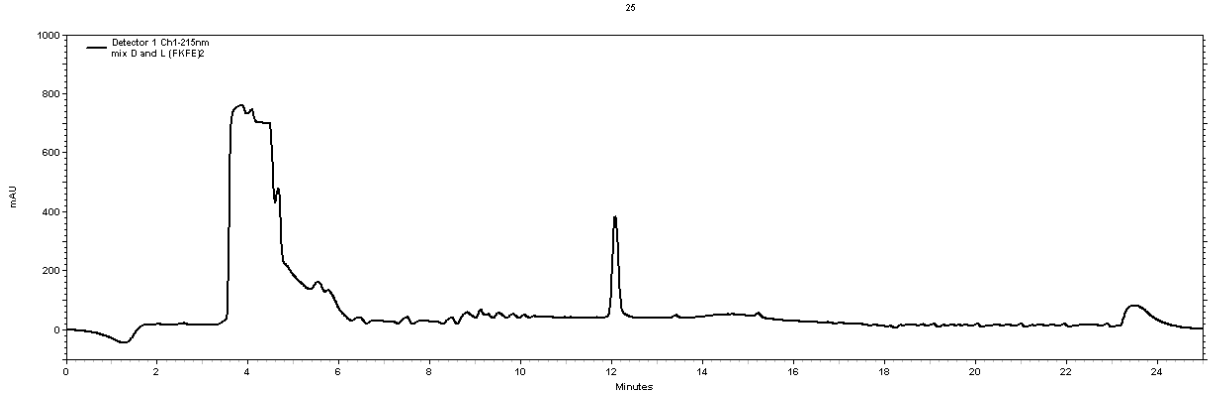

Table S2. Calculated and observed *m*/*z* for all synthetic peptides by MALDI-TOF-MS.

Peptide	calc [MH^+]	obs [MH^+]	calc [MNa^+]	obs [MNa^+]
D-Ac-$(FKFE)_2$-NH_2	1162.60	1163.59	1184.58	1185.57
L-Ac-$(FKFE)_2$-NH_2	1162.60	1163.48	1184.58	1185.30

Figure S5. MALDI-TOF-MS spectrum for D-Ac-FKFEFKFE-NH_2.

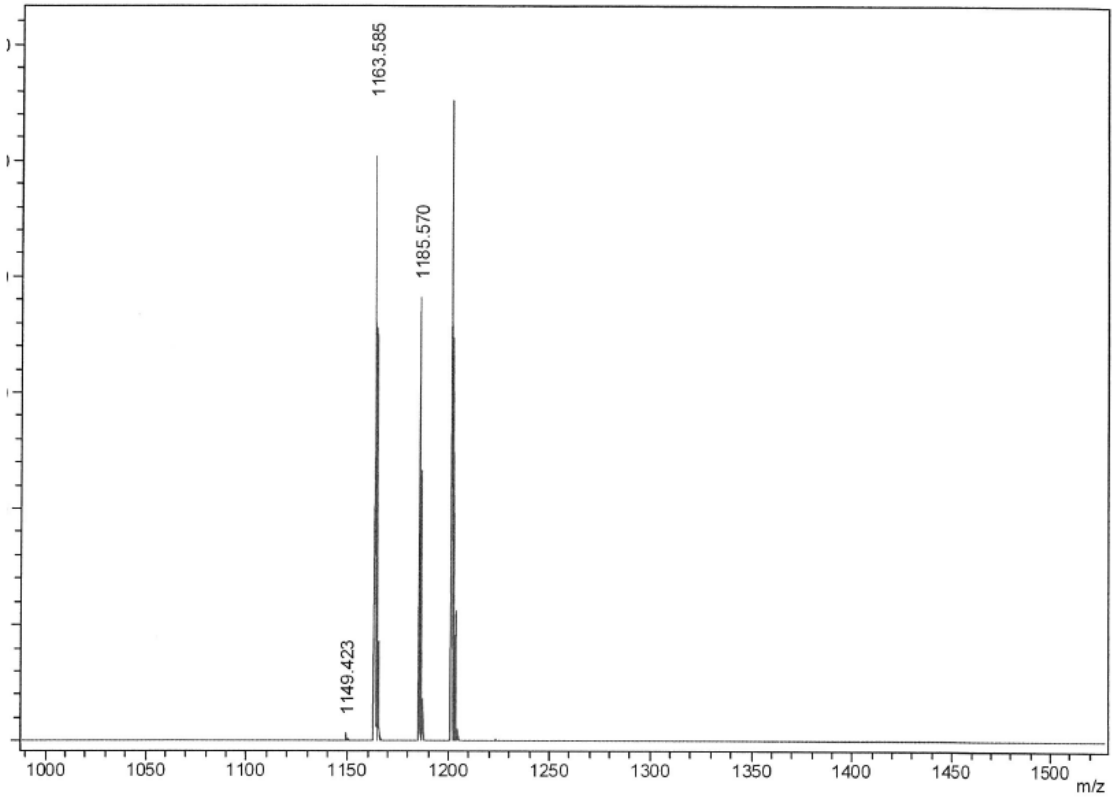

Figure S6. MALDI-TOF-MS spectrum for L-Ac-FKFEFKFE-NH_2.