Self-Assembly of Heterochiral Peptides with Varied Sequence Patterns

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Statement of Purpose: Biomaterials constructed from self-assembling peptides have shown considerable promise as in vitro cell culture matrices, scaffolds for tissue engineering and drug delivery, and immune adjuvants for vaccine development[1]. Natural proteins are composed of L amino acids therefore most self-assembling peptides designed to date have utilized L amino acids[2]. D amino acid analogs of peptides and proteins are attractive for applications in biotechnology and medicine due to their reduced proteolytic sensitivity[3]. Recently, self-assembling peptides composed of all D amino acids have been described and characterized[4]. However, the design and self-assembly of heterochiral peptides with both L and D amino acids has not been investigated. In this study, we repot the effects of various L and D sequence patterns in short amphipathic peptides. We investigated how positioning D amino acids at specific sites induce structural changes in the resulting assemblies. Eight variants of the self-assembling peptide KFE8 (Ac-FKFEFKFE-Am) with patterns of L and D amino acids were compared and our results indicate that sequence pattern variation within a single peptide can result in biomaterials with widely different morphologies.

Methods: All peptides were synthesized using standard Fmoc Chemistry on a CS Bio-CS336X solid phase peptide synthesizer. The crude product was purified by reverse-phase HPLC to > 90% purity. Peptide mass was confirmed by MALDI and fibril morphology was investigated using transmission electron microscopy (TEM). Briefly, 1 mM peptides were allowed to fibrillize in water overnight at room temperature (1 mM), diluted in PBS (0.3 mM) and applied to 300 mesh copper grids (Quantifoil). The grids were negatively stained with 2% uranyl acetate, and imaged on a JEM1400 TEM (JEOL).

Results: TEM data indicated that all eight peptides self-assembled into 3-D nanostructures (Fig. 1). Peptides with alternating L and D residues (LDLDLDLD or DLDLDLDL) assembled into flat extended sheet like structures (Fig. 1C and 1D). Peptides terminating with L or D amino acids at both N- and C-terminus with a central L or D core (LLDDDDLL or DDLLLLDD) assembled into defined nanofibers with tightly controlled width (Fig. 1E and 1F). Diblock peptides composed of stretches of L or D amino acids (LLLLDDDD or DDDDLLLL) assembled into nanofibers with a helical twist. Moreover, complementary sequence patterns of L and D residues resulted in comparable nanostructures (Figa. 1A&1B, 1C &1D, and 1E&1F). These results indicate that heterochiral peptides with varied sequence patterns of L and D amino acids containing identical amino acids, net charge, and hydrophobicity assemble into nanomaterials with different morphologies and could be useful for a variety of biological and biomedical applications.

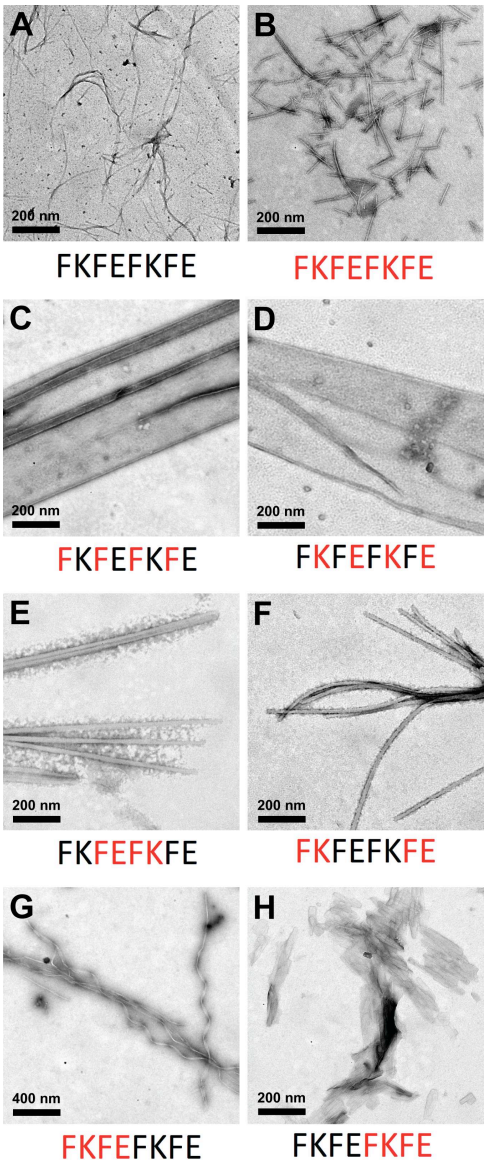

Figure 1. TEM micrographs showing nanostructures of different variants of the amphipathic peptide KFE8 sheets with varied sequence patterns of L and D residues. D amino acids are shown in red.

Conclusions: In conclusion, our findings indicate that heterochiral self-assembling peptides with varying sequence patterns of L and D amino acids assemble into nanomaterials with different morphologies and might be useful as scaffolds for a variety of biological applications.

References:

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