A Solid State NMR Constrained Structural Model for the RADA16-I Designer Self-Assembling Peptide

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RADA16-I is a small designer peptide (amino acid sequence Ac-$(RADA)_4$-NH_2) (1-2) which forms nanofiber matrices through amyloid-like cross-β self-assembly. It is the essential component of the commercial product BD Puramatrix^{TM}, which can support tissue regeneration and 3D cell culture. Unlike amyloid fibrils, nanofiber matrices of RADA16-I can dynamically re-grow following nanofiber fragmentation without addition of new peptide. The highly charged nature of the amino acid sequence at neutral pH (R^+ and D^-) has lead researchers to hypothesize that RADA16-I nanofibers are characterized by antiparallel β-sheets. In an effort to fully characterize RADA16-I nanofiber structures and understand mechanisms of self-assembly and self-healing, we have prepared isotopically labeled RADA16-I nanofiber for solid state nuclear magnetic resonance (ssNMR) measurements. Using ssNMR and supporting techniques (IR spectroscopy and X-ray scattering), we have discovered that the RADA16-I peptide does not self-assemble into nanofiber structures which have been proposed in the literature. These findings have impact on our understanding of the role of charged sidechains in peptide self-assembly. Furthermore, we will show that the peptide's behavior in solution depends on structural changes and inter-molecular organization that can occur in the solid state before the peptide is added to solution.

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