Solid State NMR Structural Characterization of RADA16-I Designer Peptide Nanofibers

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Introduction

The peptide RADA16-I ($COCH_3$-RADARADARADARADA-$CONH_2$) was one of the first designer peptides to self-assemble into nanofibers in aqueous solution. These nanofibers are the major component of the commercial product Paramatrix,™ which has been used in 3-dimensional cell culture and regenerative medicine. RADA16-I nanofibers exhibit the unique property of "self-healing:" nanofibers spontaneously re-grow following mechanical damage.[1] We have employed solid state nuclear magnetic resonance (NMR) spectroscopy in order to probe RADA16-I molecular structure.

Experimental

We produced synthetic RADA16-I nanofiber samples (**Figure 1a**), isotopically labeled with ^{13}C at a series of strategic sites. Solid state NMR spectroscopy was used to probe secondary structure and inter-atomic proximities (**Figure 1b**), in order to test a previously proposed structural hypothesis. Data from solid state NMR was used to constrain molecular dynamics computer simulations of RADA16-I molecular structure. NMR techniques included 2-dimensional exchange spectroscopy via fpRFDR recoupling and quantitative measurements of ^{13}C-^{13}C dipolar couplings using the PITHIRDS-CT NMR technique.[2,3]

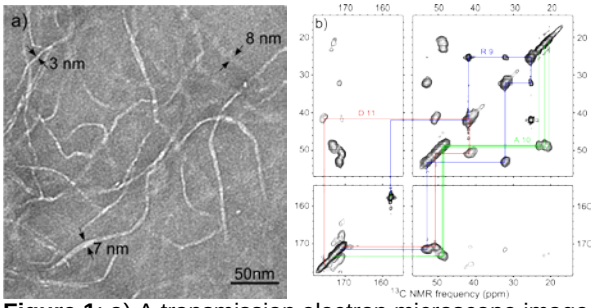

Figure 1: a) A transmission electron microscope image of RADA16-I nanofibers. b) 2D fpRFDR spectrum of uniformly ^{13}C-labeled R9, A10, and D11 RADA16-I nanofibers.

Results and Discussion

Results indicate an ordered structure composed of linear β-strands arranged into a well-defined scheme for inter-molecular packing. Dipolar coupling measurements, however, indicate that the nanofiber structure does not match that predicted in the literature. Instead of the previously proposed antiparallel β-sheet model, our results indicate that RADA16-I is composed of parallel β-sheets. The antiparallel was proposed because it avoid repulsion between like-charged arginine (or aspartate) sidechains. Our results indicate instead, that a stable configuration of oppositely charged amino acid sidechains is achieved by a registry shift between two adjacent parallel β-strands within each β-sheet.

Conclusions

Design of self-assembled peptide nanofibers is challenging because there is presently little detailed atomic structural information on stable nanofibers. While peptides can be designed with simple amino sequences to promote β-strand secondary structure, it is more difficult to predict inter-molecular order. The present results close the design loop for peptide self-assembly by establishing that the molecular structure is different from that predicted by the peptide designers. Detailed knowledge of molecular structure will enable new self-assembled peptide structure designs. Furthermore, we may now probe self-healing mechanism by isotopically labeling sites that are spatially proximate in the nanofiber structure and probing changes to inter-atomic proximity before and after self-healing.

Acknowledgements

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References

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