Solid-State NMR Evidence of Coiled-Coil Interactions Within SAF Designer Peptide Nanofibers

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Introduction

The complementary self-assembling peptides $hSAF_{AAQ}p1$ and $hSAF_{AAQ}p2$ were created by rational design of amino acid sequences to self-assemble into extended coiled-coil nanofibers as a result of specific interactions between these the two peptide components.[1-2] Although previous studies of this peptide system support the formation of desired nanostructure through measurements of secondary structure and nano-scale assembly, the precise molecular organization has not been observed experimentally. Thus, we have performed tests of the structural hypothesis using solid-state NMR measurements, with the aim of determining whether or not nanofibers are stabilized by the molecular structure and interactions that motivated the molecular design.

Experimental

First, transmission electron microscopy (TEM) and circular dichroism spectroscopy (CD) were utilized to observe the fiber formation and evaluate transformations in the secondary structure of $hSAF_{AAQ}$ molecules. Then, to further understand the nature of molecular-level organization, a series of NMR experiments were taken. NMR spectra (1D ^{1}H-^{13}C CPMAS) were collected for peptide samples within and without mixture of $hSAF_{AAQ}p1$ and $hSAF_{AAQ}p2$ components, with and without waters of hydration. Multidimensional NMR experiments (2D fpRFDR and 2D DARR) were employed for samples with uniform ^{15}N and ^{13}C-labeling at the K6 and L12 residues of L-$hSAF_{AAQ}p1$ and the A4, E15, and I23 residues of L-$hSAF_{AAQ}p2$. Finally, molecular modeling was used to compare the solid-state NMR structural constraints with hypothesized nanofiber structure.

Results and Discussion

The TEM and CD measurements indicated that nanofibers were being formed and the molecules were adopting α-helical secondary structure regardless of whether or not complementary peptide components were mixed in solution. With 2D NMR experiments, interactions were observed between the residues L12 and I23 on the different peptide components. This interaction was predicted from the hypothesized sticky-ended heterodimer structure (Fig. 1). Hence, our results demonstrate the first direct molecular-level evidence for the complementarity between the two peptide components. Although the use of two distinct peptide sequences do interact in a highly specific way, we also observed nanofibers in a solution of a single peptide component, indicating that undesired self-assembly pathways are also possible in this system.

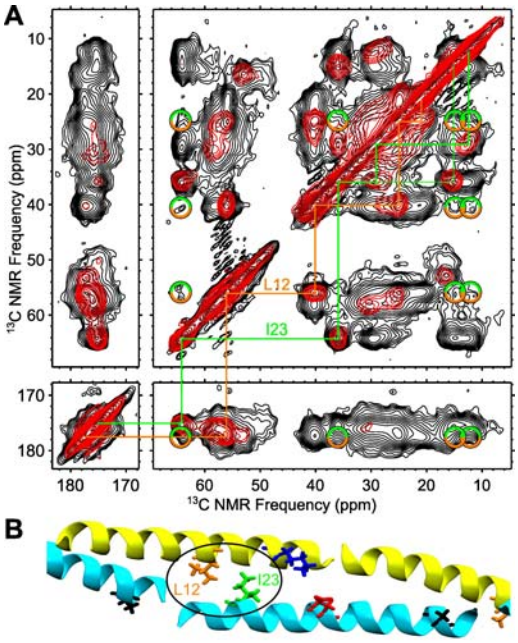

Figure 1. A) 2D DARR (black) and fpRFDR (red) spectra of uniform isotopic labeling $hSAF_{AAQ}$ nanofiber providing the inter-residue crosspeaks. B) A molecular model of a sticky-ended $hSAF_{AAQ}$ nanofiber with isotopic labeling amino acids in different colors and a predicted interaction.

Conclusions

We have demonstrated complementary inter-molecular interactions between $hSAF_{AAQ}p1$ and $hSAF_{AAQ}p2$ peptide within designed coiled-coil nanofibers. Our work has also revealed, however, that the single component $hSAF_{AAQ}p1$ can also self-assemble into nanofibers, indicating that undesired self-assembly pathways are also possible.

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References

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