NMR Characterization of C3 Fibrils From the Strep. Mutans Adhesin P1

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Introduction

S. mutans adhesin P1 is a 185 kDa protein secreted by gram-positive *Streptococcus mutans* and is involved in dental caries. Several recent publications highlight that the proteolytic C123 fragment of P1 interacts with intact P1 on the cell surface and is involved in amyloid formation within biofilms [1]. Identifying how S. mutans biofilms are formed at the molecular level is essential for understanding the virulence properties. Here we focus on NMR characterization of the C3 domain of C123, which is 162 amino acids and amenable to NMR characterization.

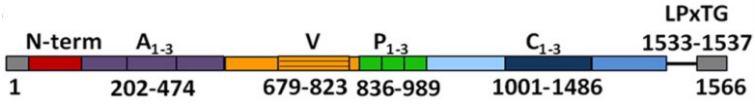

Fig 1: *S. mutans* Adhesin P1 protein is a 185 kDa protein.

Experimental

We heterologously expressed U–[^{15}N]–C3, U–[^{15}N,^{13}C]–C3, and variants which were uniformly ^{15}N enriched and selectively ^{13}C enriched at specific amino acid residues to enable collection of REDOR-filtered spectra. C3 protein was purified by affinity and size exclusion chromatography. To enable DNP enhancement of the C3 fibrils and C3 bound to cell walls, fibrils or cell walls are suspended in buffer containing 5 mM AMUPol before being packed into DNP rotors.

Results and Discussion

Significant enhancement of the protein signals is observed when using DNP at 600 MHz (Fig 2). The increased sensitivity makes possible the collection of multidimensional NMR data for assigning specific residues in C3. Due to the lower resolution observed at cryogenic temperatures, we are relying on specific isotope labeling and NMR filtering strategies to identify key residues which differ in chemical shift between the fibrils and the cell wall-bound form.

Conclusions

Assignment of some of the amino acid residues in C3 fibrils will enable us to compare the structure of C3 in amyloid fibrils, cell wall-bound, soluble and crystalline C3 in order to determine key structural changes driving the formation of amyloid fibrils.

Acknowledgements

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References

[1] Tang, W., et *al.*, J. Biomol. NMR, **64**, 153-164 (2016).

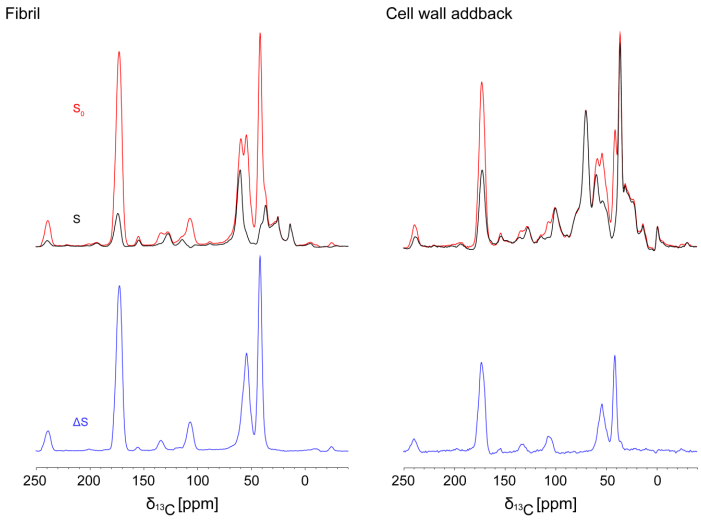

Fig 2: DNP enhanced REDOR-filtered spectra of C3 fibrils (left) and C3 bound to *S. mutans* cell walls (right). DNP spectra collected at105 K (512 scans) with samples containing 5 mM AMUPOL in a buffer made from 30:60:10 DMSO-d_6:D_2O: H_2O. A DNP enhancement of ~17-24 was observed.