NMR characterization of the C3 domain of *Streptococcus mutans* adhesin P1

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Introduction

The *S. mutans* adhesin P1 is a protein of 185 kDa, secreted by *Streptococcus mutans* gram-positive bacteria, involved in dental caries. P1 consists of a 38-residue signal sequence, an uncharacterized N-terminal region, three alanine-rich repeats (A1–3), a central domain containing a so-called variable (V) region, three proline-rich repeats (P1–3), a C-terminal region consisting of three domains (C1–3), an LPxTG sortase-recognition motif, and wall- and membrane-spanning regions (Fig 1). Recently, several publication highlighted that the C123 fragment (519 amino acids) interact with P1 on the cell surface and is involved in amyloid formation within biofilms [1]. Identifying how S. mutans interact with host components at the molecular level is essential for understanding the virulence properties. Here we focus on the NMR characterization of C3 domain of adhesion P1, which is 162 amino acids in length. The goals are to obtain functional and structural data on C3 in monomeric form with solution NMR and fibrillar amyloid forms with SSNMR.

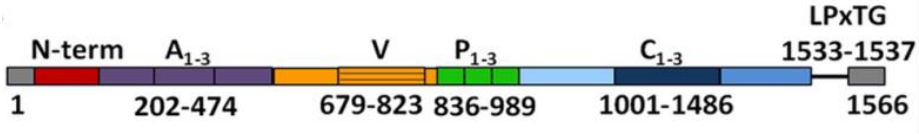

Fig 1: *S. mutans* Adhesin P1 protein is a globular protein of 185 kDa.

Experimental

For NMR experiments, we prepared U–[^{15}N] C3 and U-[^{15}N,^{13}C] C3 samples. All NMR experiments were acquired at 600 MHz with a triple-resonance cryoprobe. C3 backbone assignments were obtained by recording 2D [1H,15N]-HSQC, 3D HNCA, 3D HN(CO)CA, 3D HNCO, 3D NH(CA)CO, 3D HNCACB, and 3D CBCA(CO)NH data. Analysis is in progress with the software CCPNMR. The interaction surface of C3 was characterized by means of chemical shift perturbations (CSPs), where a series of ^{1}H-^{15}N-HSQC experiments were recorded upon addition of unlabeled A3VP1. To avoid possible aggregation of proteins, we started from a ^{15}N-labeled C3 protein concentration of 200 µM

Results and Discussion

Preliminary NMR data highlight that the C3 domain of adhesin P1 is a folded protein (Fig 2) which is able to interact specifically with the N-terminal of adhesin P1: A3VP1. NMR titrations of A3VP1 on ^{15}N-enriched C3 indicated that several C3 ^{15}N-^{1}H resonances disappear or shift with ligand addition (Fig 2). C3 NMR assignment will enable us to to define the surface interaction.

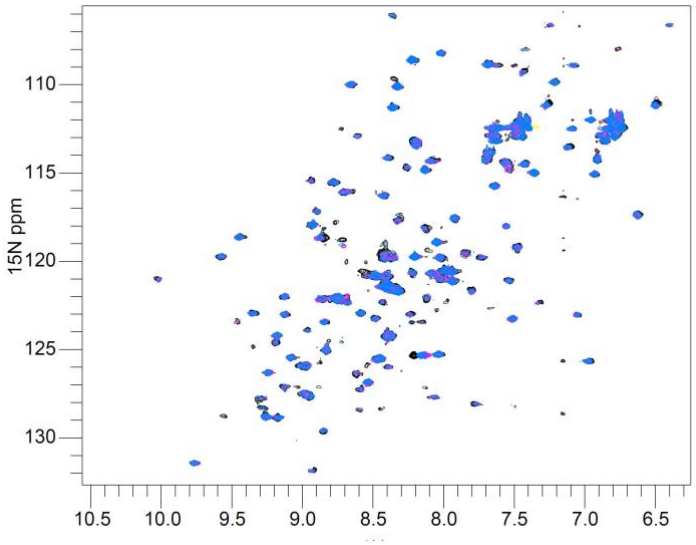

Fig 2: The **C3 domain of adhesion P1 is able to interact with A3VP1.** Chemical shift perturbation experiments for C3 in the presence of different concentrations of A3VP1.^{1}H-^{15}N HSQC of C3 alone (black), C3/A3VP1 complex at a 1:0.5 ratio C3/A3VP1 concentration (blue), and C3/A3VP1 complex 1:0.75 ratio C3/A3VP1 concentration (pink).

Conclusions

C3 assignment is very important to obtain atomic resolution information for NMR characterization of C3 alone and in presence of binding partners. If for this reason, we will next record 3D-NOESY-HSQC and 4D-HSQC-NOESY-HSQC experiments at 800 MHz.

Acknowledgements

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References

[1] Tang, W., et *al*., Journal of Biomolecular NMR, **64**, 153-164 (2016)
[2] Marley J, et *al*., Journal of Biomolecular NMR, **20**, 71–75 (2001)
[3] Larson, M.R., et al., PNAS, **107**, 5983-5988 (2010)