NMR characterization of the C3 domain of 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.

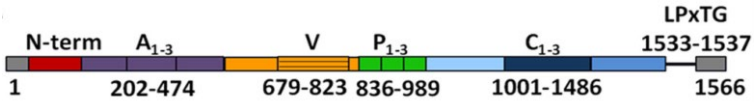


Fig 1: *S. mutans* Adhesin P1 protein is a 185 kDa protein.

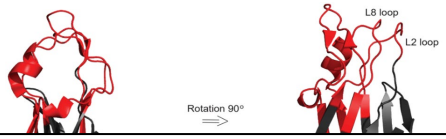
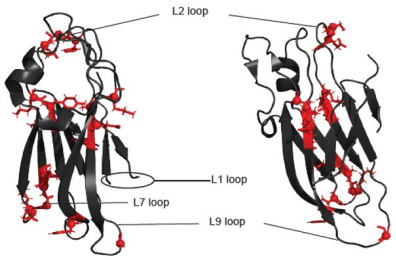


Fig 2: 66% of C3 residues were assigned via solution NMR and are shown in red.

Experimental

We heterologously expressed U–[^{15}N]–C3 and U–[^{15}N,^{13}C]–C3 using a protocol developed by Marley et al [2]. C3 protein was purified by affinity and size exclusion chromatography. A3VP1 was produced and purified as previously described [3]. To assign C3 resonanced, we collected 3D NMR assignment experiments using ^{13}C-^{15}N- C3. Protein interactions were monitored by 2D ^{1}H-^{15}N HSQCs. NMR experiments were performed on Bruker instruments in the AMRIS and NMR user facilities and assigned by CCPNMR.



Results and Discussion

We were able to assign 113 residues (66%) in the C3 domain that are primarily located in loops and/or are solvent accessible regions (Fig 2). Using these assignments, we characterized the quaternary interaction of the C3 domain with A3VP1, amyloid inhibitor and anti-P1 antibody. NMR titration of A3VP1 with C3 validated the direct interaction between these two domains. We observed that the residues most affected by A3VP1 binding are mainly located on the L1, L2, L7, and L9 loops. The mapping of C3 residues affected by A3VP1 binding highlighted two A3VP1 binding sites (Fig 3) on opposite ends of the C3 protein. Complementary NMR titration of A3VP1 with a C3/C12 complex indicated L7 and L9 are the biologically relevant binding sites for A3VP1. We also observed that the C3 domain interacts specifically with the amyloid inhibitor resveratrol (Fig 4). Moreover, we also able to identify the binding sites for anti-P1 antibodies called 6-8c, 5c-10, 5-3e (Fig 5).

Fig 3: C3 residues perturbed by A3VP1 binding are indicated in red. The C3 model is based on an X-ray structure (4TSH) of C123.

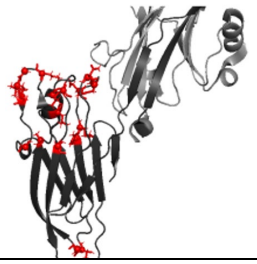


Fig 4: C3 residues perturbed by resveratrol binding are shown in red. The model is based on the X-ray structure of C123 (4TSH). C3 and C12 domains are represented in black and grey, respectively.

Conclusions

The C3 assignment enabled us to characterize the C3 quaternary interactions with proteins relevant to biofilm formation as well as an amyloid inhibor. In the future, we would like to concentrate on the characterization of the initial step of the fibril amyloid formation by solution and solid-state NMR.

Acknowledgements

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References

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

Fig 5: C3 residues perturbed by 6-8C binding are shown in red. The model is based on the X-ray structure of C123 (4TSH). C3 and C12 domains are represented in black and grey, respectively.