NMR structural characterization of Vp1u

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Introduction

The B19 parvovirus is a small non-enveloped single-stranded DNA virus implicated in the childhood disease *erythema infectiosum. The virus enters the cells via cadherin mediated endocytosis and it undergoes several conformational changes in its capsid structure as it is trafficked through the endosome to the nucleus for viral replication. The B19 viral capsid is composed of 60 similar capsid proteins; 95% are VP2 and 5% are a longer variant VP1.* The B19 VP1u is the N terminal unique region of VP1 that is required for viral infectivity and has PLA2-like activity, suggestive of a mechanism for virus escape from the endosome during viral trafficking. To date, no data is available on its structure and crystallization trials have failed. Therefore, we are hoping to get a structure of this protein using NMR spectroscopy.

Experimental

Isotopically enriched B19 VP1u protein was expressed in *E.coli* cells, purified, concentrated to 9 mg/mL and exchanged into a phosphate buffer containing EDTA to minimize protein degradiation (Figure 1). NMR experiments were acquired at 25°C on solution NMR instruments in the AMRIS and NMR facilities. We recorded 2D [1H,15N]-HSQC, 3D- TROSY-HNCA, 3D TROSY-HN(CO)CA, 3D TROSY-HNCO, 3D TROSY-NH(CA)CO, 3D TROSY- HNCACB, and 3D TROSY- CBCA(CO)NH data for backbone assignments. Data analysis is ongoing.

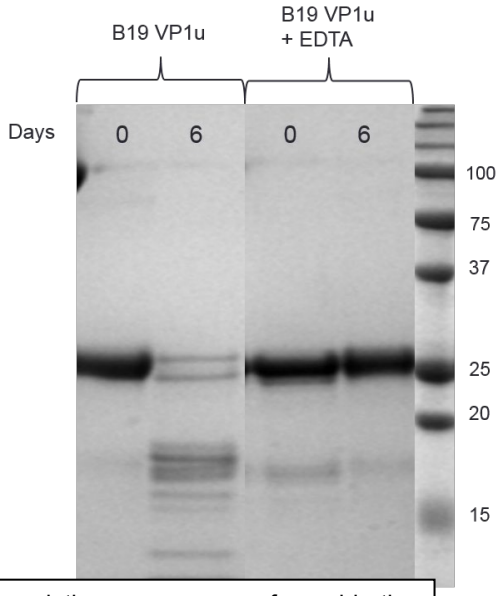

Fig 1: A degradation assay was performed in the presence or absence of EDTA. In the presence of EDTA, VP1U has sufficient stability.

Results and Discussion

Since the B19 Vp1u protein has never been studied by NMR or other structural techniques, no structural information is available. We showed in presence of EDTA, VP1U is stable enough to collect all the needed NMR assignment experiments. VP1u consist of 210 amino acids, including 16 proline. Only 90 ^{1}H-^{15}N resonances are observable on the ^{15}N-^{1}H-HSQC. We also observed a heterogeneity of the ^{1}H-^{15}N resonance intensities demonstrating the presence of the rigid and flexible regions. To date we have assigned 16 amino acids which have a longer T2 relaxation time and are predicted to make up loop regions based on homology modeling (Figure 2). The remaining unassigned residues have shorter T2 relaxation times. To continue the assignment, we propose further experiments at a lower pH as well as making use of carbon-detected NMR experiments.

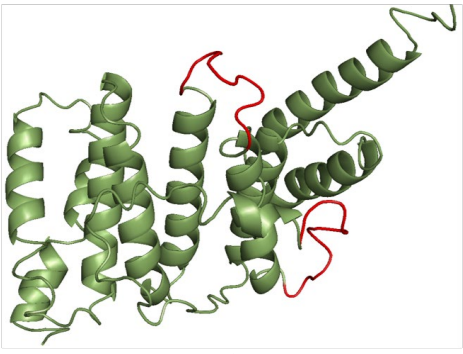

Fig 2: A model of predicted VP1u structure based on homology modeling with PLA-2. The 16 residues assigned thus far via NMR are represented in red.

Conclusions

Vp1u is a small protein of 28.75 kDa with a high helical composition leading to many overlapping NH resonances; its relaxation behavior presents further challenges. However, several NMR tools can be used to collect further structural information.

Acknowledgements

This work was supported by NIH R21AI126583. A portion of this work was performed at the National High Magnetic Field Laboratory, which is supported by National Science Foundation Cooperative Agreement No. DMR-1157490 and the State of Florida.