NMR Studies of the AAV Capsid Minor Viral Protein 1 Unique Region (VP1)

Poole, K.M.; Lins, B.; Smith, A.; Tang, W.; Long, J.R.; Agbandje-McKenna, M. (UF, Biochemistry and Molecular Biology)

Introduction

The overall goal of our study is a comprehensive characterization of a region of the Adeno-associated virus (AAV) capsid minor viral protein (VP) 1 unique region (VP1u, 1-137aa), which is absolutely essential for host infection. The VP1u contains a phospholipase A2 (PLA2) domain encoded within the first 131aa that serves to modify lipid membranes and carries nuclear localization signals (NLS) for trafficking of the intact genome-filled capsid to the nucleus during infection (3, 4, 7). The PLA2 functions to enable the virus to escape from the endocytic pathway enroute to the nucleus for genome replication and must undergo structural rearrangements to carry out its function because it is reportedly localized inside the assembled caspid. In addition, a recent report indicates that VP1u also plays a role in endosomal sorting and signal transduction. However, obtaining structure-function information on VP1u has been elusive because previously employed approaches lacked the resolution needed to elucidate its structure and structural transition dynamics (1, 2, 5, 6). Thus at present, nothing is known about its structure or the structural calisthenics, mediated by pH, which must confer its functional state on the capsid surface. We aim to fill this gap by unveiling the physical properties of this essential capsid domain under host environment conditions encountered during infection. Both NMR and X-ray crystallography will be used for these studies. We will also study the dynamics of the protein and the triggers for insertion into liposomes mimicking the endosomal membrane which must be breached by its PLA2 function. NMR is the perfect application for these latter studies, hence this application for instrument time allocation to enable us to initiate these studies. Three clinically relevant AAVs with sequence variation in their VP1u sequence and disparate tissue tropism and transduction efficiencies serve as our models.

For this proposal, we have one focused aim:

To characterization the structure and solution dynamics of the AAV2 VP1u by NMR. We will use the allocated nuclear magnetic resonance spectroscopy (NMR) time to collect preliminary HSQC data on labeled protein to show that the expressed VP1u is correctly folded. This will be followed by experiments aimed at structure determination and the characterization of the dynamics of this protein at pH/ Ca^{++} conditions encountered during infection. We will also characterize the interaction(s) of this protein with natural lipid substrates. These studies will provide the first set of information for VP1u in its functional state(s).

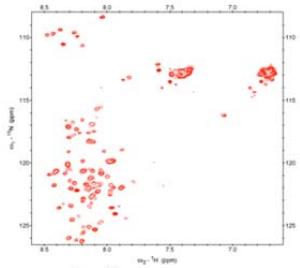

Fig 1. ^{1}H-^{15}N HSQC spectra for ^{15}N B19 VP1u (1mg/ml). After experiment, small amount of aggragated protein observed.

Experimental - Samples of ^{15}N-labeled and ^{13}C-labeled VP1u will be prepared at 0.4mM concentration in PBS buffer over a range of pHs. Samples will be prepared by the Agbandje-McKenna lab (UF) and sent to Long lab (UF) for collection and analysis of NMR data by Long, Smith and Tang. Initial screening of NMR sample conditions will be done via 15N-HSQC measurements. If successful, a standard set of 2D/3D NMR spectra will be obtained for structural characterization and resonance assignment of VP1u. NMR: 600 MHz NMR spectrometer with a 5mm triple resonance cryo probe.

Results and Discussion - Since the VP1u protein of B19 contains 227 amino acids, the size falls between that of a small and large protein. Thus, we preformed both a ^{15}N HSQC (Fig. 1) experiment (pulse optimized for small proteins) and ^{15}N TROSY (Fig. 2) experiment (pulse optimized for large proteins) to see if the sample was folded or aggregating. The resulting spectra show that the solution conditions and protein concentration are close to being optimized for structural studies using this method.

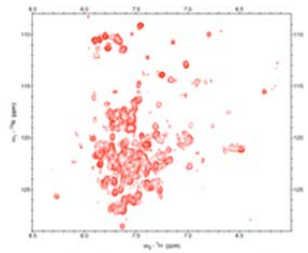

Fig 2. ^{1}H-^{15}N TROSY of ^{15}N B19_VP1u sample (1 mg/ml). Supernatant from ^{15}N HSQC experiment used for ^{1}H-^{15}N TROSY.

Acknowledgements - 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, the State of Florida, and the U.S. Department of Energy.

References

[1] Agbandje-McKenna, M., and Kleinschmidt, J. 2011. AAV capsid structure and cell interactions. Methods in molecular biology 807:47-92.

[2] Chapman, M. S. a. A.-M., M. 2006. Atomic Structure of Viral Particles, p. 107 - 123. In J. R. Kerr, Cotmore, S. F., Bloom, M. E., Linden, R. M., and Parrish, C. (ed.), Parvoviruses. Edward Arnold Ltd., New York.

[3] Girod, A., Wobus, C. E., Zadori, Z., Ried, M., Leike, K., Tijssen, P., Kleinschmidt, J. A., and Hallek, M. 2002. The VP1 capsid protein of adeno-associated virus type 2 is carrying a phospholipase A2 domain required for virus infectivity. The Journal of general virology 83:973-978.