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

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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 domain is hypothesized 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.

Results and Discussion

To date ^{15}N labeled VP1u has been expressed and purified. Unfortunately, the desired sample conditions for the NMR experiments: 1 mg/mL of VP1u at a pH of 6.5 has caused some of the protein to precipitate out of solution. Therefore, we are still optimizing the sample conditions for future NMR experiments. As shown in Figure 1, there is decent spectral dispersion of VP1u backbone amide resonances. However, it is obvious from the spectra that there is also some aggregated protein. We are confident that with optimization of the sample conditions further NMR experiments in line with the overall goals of this project will be carried out.

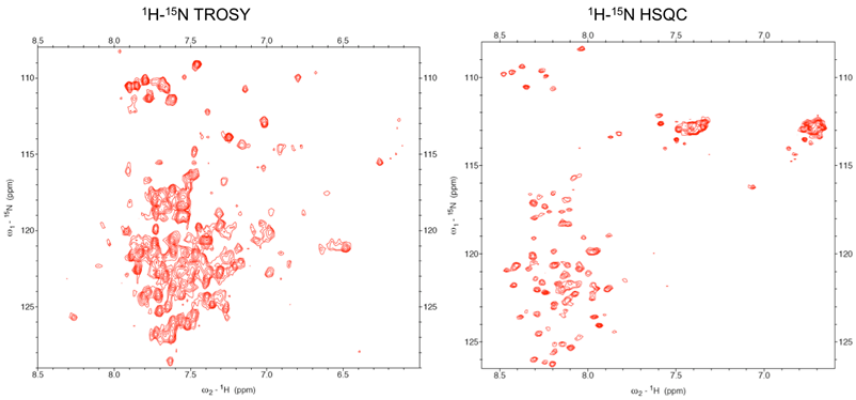

Figure 1: ^{1}H-^{15}N TROSY and ^{1}H-^{15}N HSQC of VP1u. 1 mg/mL VP1u, 50 mM HEPES, 150 mM NaCl, at pH 6.5.

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