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Analysis of phospholipase activity in adeno-associated virus particles by liquid-chromatography/mass-spectrometry

Adeno-associated virus (AAV) belongs to the Parvoviridae, a family of small, non-enveloped isosahedral viruses. The viral capsid has T=1 symmetry and is composed of 60 subunits, made up from three proteins (VP1, VP2, VP3) in a ratio of 1:1:10. The minor proteins are the same as VP3 in their C-termini region, but they have additional domains on their N-termini that play essential roles in cellular entry and trafficking. Structural studies of AAV have shown that the N-termini of VP1 and VP2 are initially internalized in the capsid and become externalized, most likely during endocytosis. Based on sequence and structural similarity, VP1 contains a phospholipase A2 domain (PLA2) which, when mutated, dramatically reduces infectivity. Currently, little is known about the mechanism of VP1 externalization or the role of the lipase in escape of the virus particle from the endosome. Also, due to low sequence similarity, there is even concern over whether this is a true PLA2 type domain. To address these questions, we have developed a liquid-chromatography mass-spectrometry based assay for lipase activity. To date, we have tested factors such as receptor binding, heat, and pH on the externalization of the PLA2 and are addressing the question of substrate specificity.