Application Brief

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Resolution of Adeno-Associated Viral Vector Aggregates and Fragments with Agilent Bio SEC-5

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Introduction

Adeno-associated viruses (AAVs) are a promising new class of biotherapeutic with many proven applications.[1] AAVs are large molecular complexes consisting of approximately 60 copies of capsid protein(s) encapsulating a single stranded DNA genome. Individual AAV virions may exceed 5 MDa[1], and are approximately 250 Å in size. AAV aggregates are therefore challenging to resolve using standard SEC columns, which typically have pore sizes ≤300 Å. Conventional wisdom indicates that an SEC pore size at least three times larger than the molecule of interest should be chosen, which would indicate a pore size ≥750 Å.

This application note demonstrates chromatographic resolution of AAV aggregates and fragments on an Agilent Bio SEC-5 column with 1,000 Å pore size. Bio SEC-5 columns are packed with 5 µm silica particles coated with a proprietary hydrophilic layer for efficiency and stability, and are thus well suited for analysis of large, complex biological molecules such as viral particles.

Methods

AAV samples of serotypes 2 and 9 were purchased from Vigene Biosciences, and had particle concentrations of 1.27×10^{12} and 1.76×10^{12} VP/mL respectively as determined by ELISA. Agilent AdvanceBio SEC 300Å Protein Standard (part number 5190-9417) was used for calibration and confirmation of relative mass of each AAV serotype.

Size-exclusion chromatography was conducted on an Agilent 1290 Infinity II Bio LC equipped with a binary pump and an Agilent 1260 Infinity II fluorescence detector set to Ex = 280 nm, Em = 340 nm. The mobile phase consisted of 50 mM phosphate buffer + 400 mM NaCl, pH 7.4. An Agilent Bio SEC-5 column (4.6 × 300 mm, 5 µm, 1000 Å) was chosen, with a constant flow rate of 0.4 mL/min.

Prior to analysis, 15 µL of unstressed AAV-2 or AAV-9 was diluted in 100 mM phosphate-buffered saline, pH 7.4, to a final volume of 65 µL. Injections of 20 µL were then performed in triplicate.

Published reports indicate that AAV aggregation is driven primarily by electrostatic interactions and is favored by low ionic strength and the presence of residual DNA.[2] Aggregation was induced by diluting 15 µL of AAV-9 in deionized water to a final volume of 65 µL in 0.5 mL Eppendorf Protein LoBind tubes. Two microliters of PCR-amplified dsDNA (length = 3,800 nt, concentration = 0.2 µg/ µL) was then added, and the samples were incubated overnight at 37 °C. Injections of 20 µL were then performed in triplicate the following day.

Results

Based on the reported hydrodynamic radius of monomeric thyroglobulin[3] (85.8 Å), AAV virions were expected to elute between thyroglobulin monomers and their aggregates, which was indeed observed (Figure 1).

Unstressed AAV-2 and AAV-9 had similar purity values of 98.7% and 98.8% respectively, each containing 1.2 to 1.3% aggregates.

In contrast, stressed AAV-9 showed extensive formation of aggregates and substantial levels of degradation. Stressed AAV-9 was 83.6% pure, containing 7.5% aggregates and 8.8% fragments. Notably, the total peak area of stressed samples was only approximately 60% that of unstressed samples, possibly indicating the formation of insoluble aggregates (Figure 2).

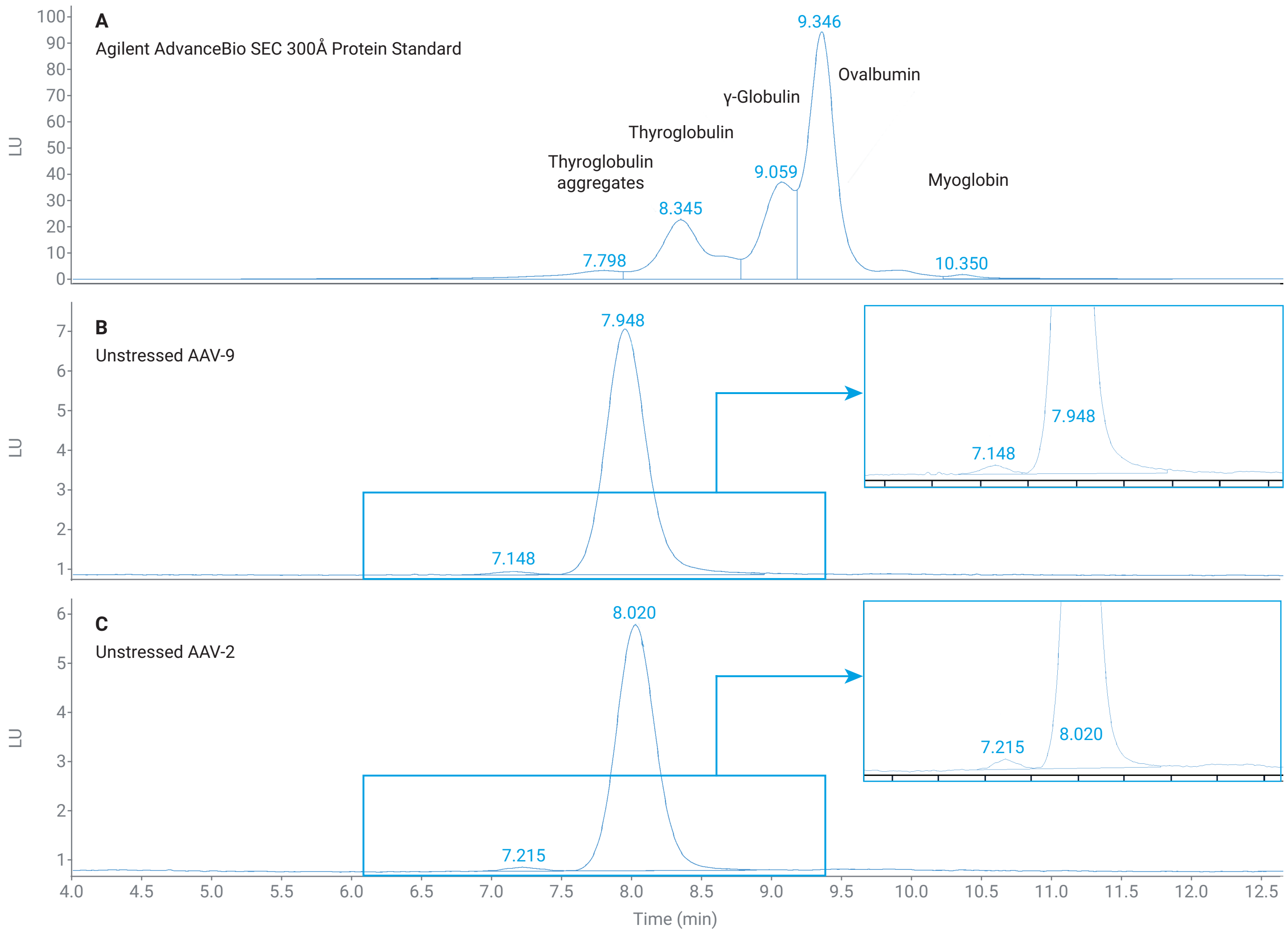

Figure 1. Fluorescence chromatograms of Agilent AdvanceBio SEC 300Å Protein Standard and unstressed AAV-9 and AAV-2. Note that the protein standard also contains insulin, which is not shown because it contains no tryptophan amino acids and is therefore nonfluorescent under the chosen excitation and emission settings.

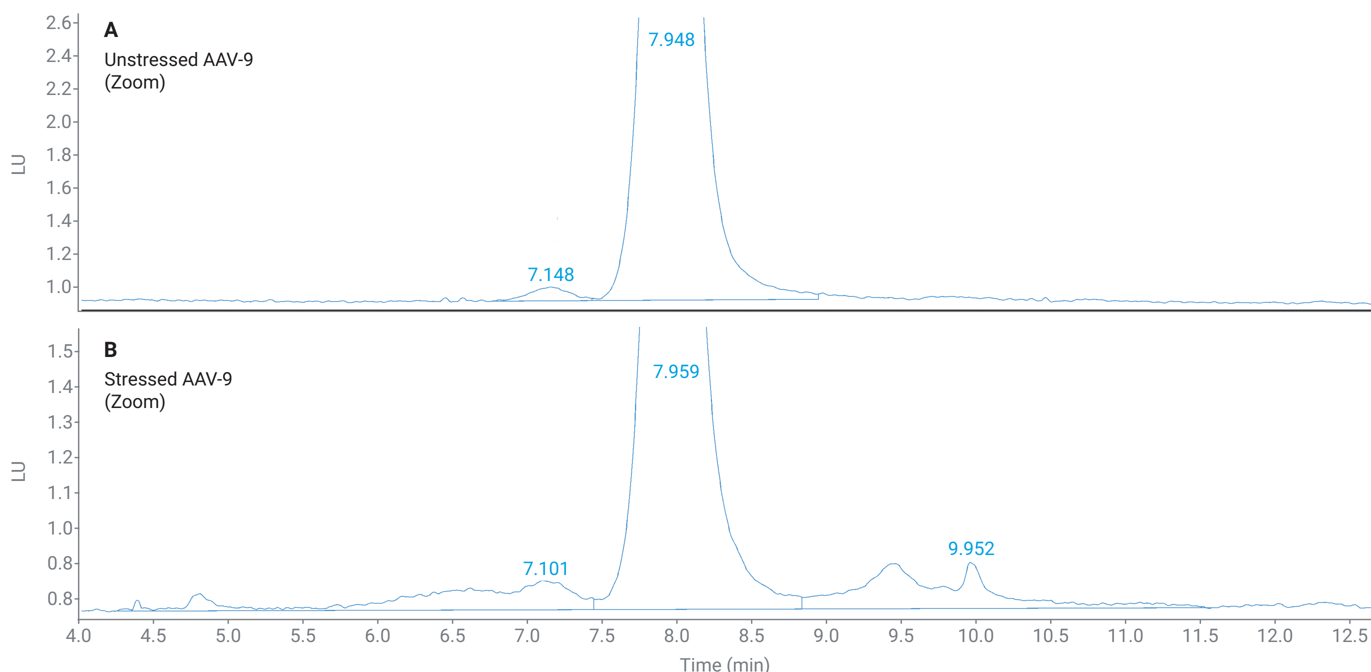

Figure 2. Fluorescence chromatograms of unstressed and stressed AAV-9.

Conclusion

Agilent Bio SEC-5 columns contain particles with 1,000 Å pore size making them suitable for AAV aggregate and fragment analysis, which is essential during AAV purification and subsequent formulation stability testing. Orthogonal methods such as bulk dynamic light scattering or analytical ultracentrifugation may be useful for addressing large, insoluble aggregates that may inevitably form under stress conditions along with the demonstrated SEC aggregate analysis for routine testing.

References

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2. Wright, J. F. *et al.* Identification of Factors That Contribute to Recombinant AAV2 Particle Aggregation and Methods to Prevent Its Occurrence During Vector Purification and Formulation. *Molecular Therapy* **2005**.
3. Nobuo, Ui. Electrophoretic Mobility and Isoelectric Point of Hog Thyroglobulin. *BBA* **1972**.

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