Absolute Size Exclusion Chromatography of BSA

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We present results from Bovine Serum Albumin (BSA) measured in an analytical size exclusion chromatography (SEC) system including the Brookhaven BI-MwA static light scattering system. BSA is used ubiquitously as a test protein for concentration and for system calibration and testing. Typical results are presented.

Introduction

The extraction of insulin from blood plasma was initially performed by Cohn in the late 1930's. This blood and insulin work was an important war effort during World War II and Cohn publish his treatise on protein in 1943. The extraction is done in five steps. The Fraction V (Roman numeral 5) gives the pure form of albumin. Bovine Serum Albumin (BSA) is now purified using more efficient methods but still has the mention Fraction V.

Since pure BSA was a readily available protein, it found itself the de-facto protein for concentration standards. BSA is used in many immunoassays and for biochemical applications such as ELISA. BSA is also used to stabilize protein, DNA and enzyme broths. Although it has been one of the most studied proteins, it hasn't been crystallized to hold a diffraction pattern that would allow obtaining its complete amino acid sequence and tertiary structure.

Size exclusion chromatography (SEC) is routinely used to characterize the percentage of monomer (purity) of a protein. The detector used can be a conductivity meter or a UV detector to assess the protein concentration. Analytical methods to study protein solutions include the integration of a Static Light Scattering (SLS) detector into the existing SEC system to

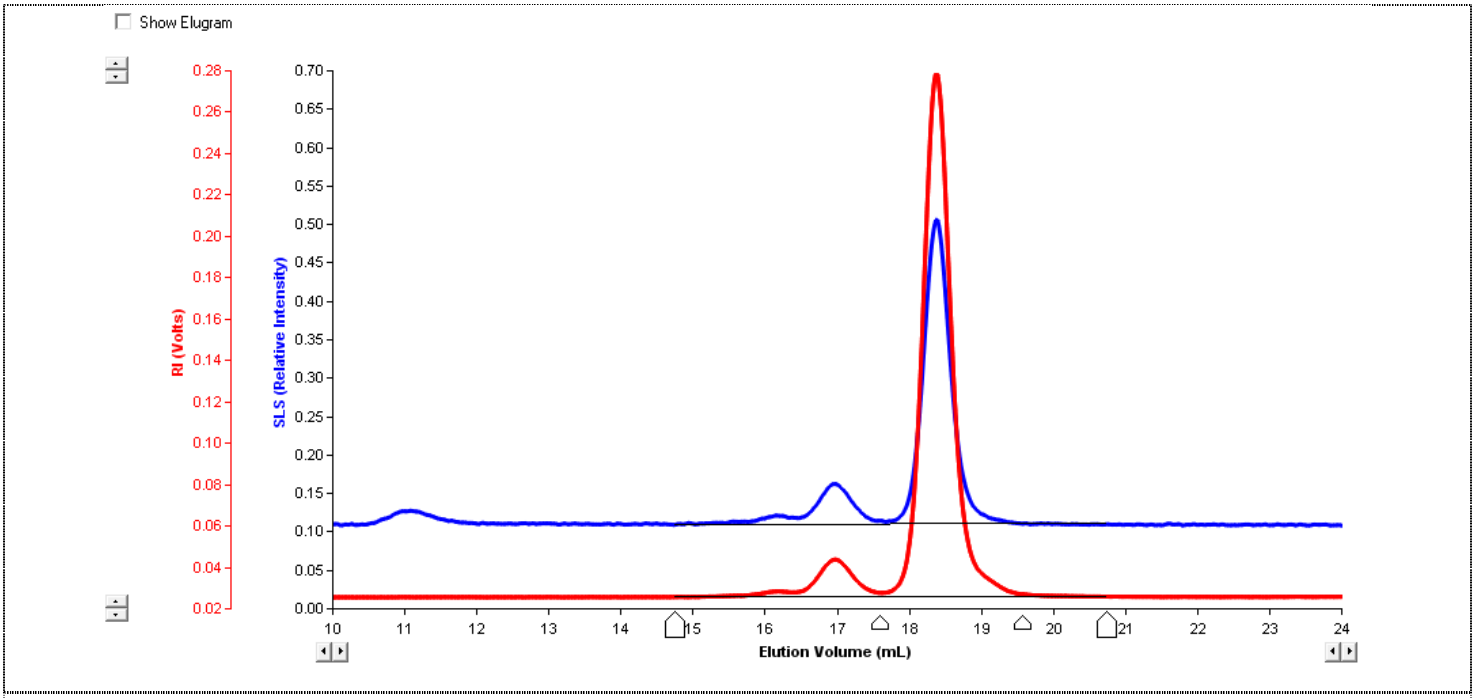

Figure 1: Chromatogram of BSA showing in red the RI signal and in blue the static light scattering intensity.

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measure the molecular weight of the protein as it elutes out of the column. The term absolute molecular weight is associated with the use of a SLS detector. It is noted that the molecular weight of the protein of interest is usually known and the analytical SEC system is used to find the minor peaks in order to reveal the nature of these proteins. For this reason the quality of the analytical system is assessed by the ability to measure the BSA dimer and trimer peaks as they mimic the trace amounts of secondary peaks in the protein sample studied.

In this application note we measured BSA and are presenting typical results.

Material Method

The SEC instrument is composed of an Agilent 1100 series isocratic system including a degasser, pump, autosampler and column heating compartment. The eluent was pre-filtered phosphate buffered saline solution (PBS) flowing at a rate of 0.7 ml/minute. The columns used were two Tosoh TSKgel with a Tosoh guard column.

Injections of 100 microliters of 2 mg/ml BSA were done. The detectors were the Agilent 1100 series variable wavelength detector followed by the BI-RI (Brookhaven Instruments Corp, Holtsville, NY) and the BI-MwA (Brookhaven Instruments Corp., Holtsville, NY). The BI-MwA is a static light scattering instrument measuring the scattering light intensity at 7 angles in order to calculate the absolute molecular weight of the sample. The wavelength of the BI-MwA laser is 637 nm with a power of 35 mW. The BI-RI is a differential refractometer used to measure the concentration of protein. The dn/dc used was 0.185 cm^3/g for the BSA. The BI-RI has a wavelength of 620 nm matching closely the wavelength of the BI-MwA. This is an important feature for accurate molecular weight and concentration measurements as well as calibration of the instruments.

The ParSEC software was used to control some instruments, acquire the data and perform the necessary calculations. The results presented are all from the ParSEC software.

Results

Figure 1 shows the elugram of an injection of 2 mg/ml of BSA made on the 2 columns. The signal from the differential refractometer in red is proportional to the sample concentration. The BI-MwA detector signal in blue is proportional to the intensity of scattered light as well as concentration and molecular weight, as the BSA has a fixed dn/dc. In SEC, the larger molecules are expected to elute earlier than molecules of lower molecular weight, as they cannot pass through the smaller pores of the columns' packing material. In this elugram, the large peak located at an elution volume of approximately 18.5 mL is expected to be the monomer of BSA. The columns are separating the monomer peak well, as we can see baseline resolution between this monomer peak and the aggregate peak to the left. The peak that precedes the monomer peak at approximately 17 mL is expected to be the dimer of BSA. Similarly, the peak at around 16 mL is expected to be the trimer of BSA. An expert eye would probably note a smaller aggregate peak even before the trimer. It should be noted that the small detection at 11 mL on the blue trace is not seen in the BI-RI detector. This is typical for aggregates, where the concentration is too low to be measured by the RI detector but the molecular weight is high enough that the BI-MwA registers a good signal.

Figure 2 shows the calculated results for the monomer peak of the same injection. The red trace is

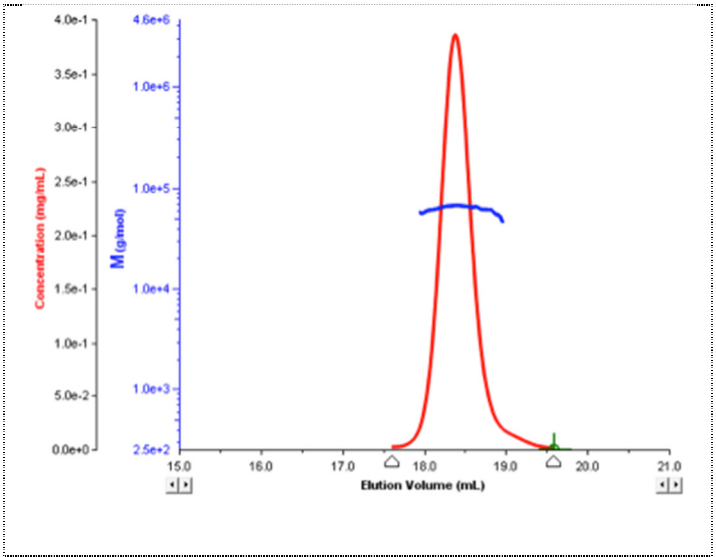

Figure 2: Molecular weight and concentration vs elution volume for the monomer peak.

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the concentration calculated from the RI signal. Since the detector is calibrated, the concentration can be calculated for every slice (at all times). It can be calculated two different ways, from the injected material, assuming 100% elution, or from the RI signal knowing the value of dn/dc. It should be noted that although 2 mg/ml of BSA was injected, the maximum concentration recorded is approximately 0.4 mg/ml at the peak. This is expected since the injected volume was 100 µL and the BSA monomer peak eluted over 2 mL.

The blue trace is the molecular weight calculated by combining the signals from the BI-RI and the BI-MwA. The ParSEC software calculates the absolute molecular weight independently for each elution slice to create the curve of M_w vs elution volume. The molecular weight is fairly constant across the peak, which is expected for a monomeric sample.

Figure 3 is from a different BSA with more dimer and trimers and even higher –mers. The molecular weight measured is showed to increase for the trimer. Obviously the ParSEC software gives all the results for these measurements and the integration limits can be set to obtain the results from only one peak.

This application note shows the typical results obtained for BSA. The results are easily calculated with ParSEC for absolute measurements of the protein.

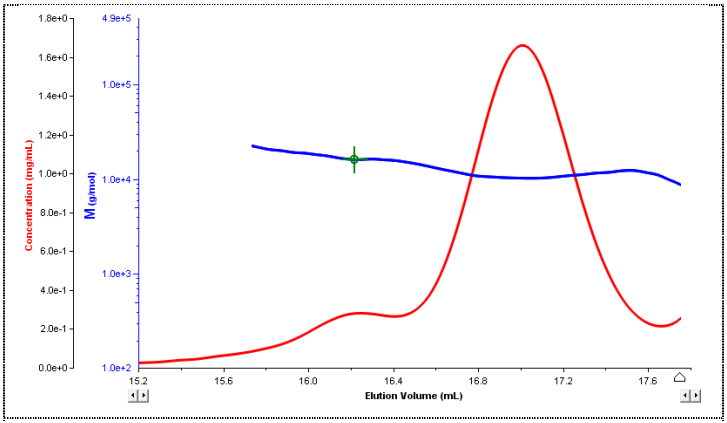

Figure 3: Molecular weight and concentration vs elution volume for the dimer, trimer and larger –mers.

Reference

1. Cohn EJ and Edsall JT. (1943) Proteins, Amino Acids and Peptides as Ions and Dipolar Ions, Reinhold Publishing, New York

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