APPLICATION NOTE

AN1602: Characterizing lipid membrane protein complexes by SEC-UV-MALS-dRI

Membrane proteins—together with lipids—make up biological membranes that are essential for life. In order to understand the role of membrane proteins in assisting membranes to carry out many different functions, it is of great importance to understand the structure of those proteins.

Membrane protein is generally soluble only in the presence of micelles, with varying amounts of lipids bound to the hydrophobic domain; thus, it is very difficult to characterize the oligomerization state of the membrane protein in a lipid-containing solvent. In this application note we demonstrate the use of multi-angle light scattering (MALS) detection in combination with UV absorption and differential refractive index (dRI) detection to determine the molar masses (MW) of both the core protein and the entire protein-lipid complex.

In order to perform the analysis, a DAWN® MALS detector was coupled to two online detectors, UV (λ=280 nm) and dRI. The triple-detector set was placed downstream of a size-exclusion chromatography (SEC) system (ÄKTA analytical FPLC) which served to separate the solution components for determination of the distributions of molar mass and composition. A membrane protein was injected and the signals acquired by ASTRA®. The resulting traces are shown in Figure 1.

In order to keep the membrane protein in solution, it was necessary to use a mobile phase that contained lipids at greater than the critical micelle concentration. Since the membrane protein-lipid complex has quite a different conformation and probably different adsorption characteristics to the column packing than globular standard proteins, the elution property of membrane proteins and globular proteins are very different. As a result, the traditional column calibration method fails to provide any estimation on molar mass using elution time.

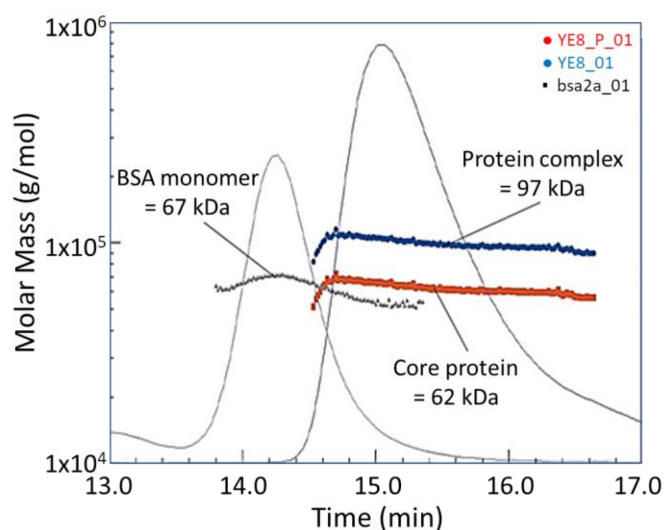

Figure 1: The analysis based on data from LS, UV, and dRI detectors reveals molar masses for the core protein and protein-lipid complex are 62 and 97 kDa, respectively. The results from BSA demonstrate that the SEC properties of these two protein samples are very different.

ASTRA's protein conjugate algorithm analyzes the data from the MALS, UV, and dRI detectors to determine the molar masses of the core membrane protein, lipid micelle, and protein-lipid complex. These data indicate that the membrane protein is in a monomeric state (62 kDa).

This example demonstrates clearly that the combination of MALS, UV, and dRI detection is a unique and powerful tool in characterizing membrane proteins in particular, and other modified proteins—such as pegylated and glycosylated proteins—in general.

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