Lens Protein: Conformational Changes

Protein biochemists often need to know the molar mass distributions *and* conformation of their samples. Using conventional GPC/SEC techniques, it is impossible to determine conformation because molar mass is assumed to decrease monotonically with elution volume. Meaning, therefore, that the conformation appears to remain constant throughout the separation.

This application note reveals how you *can* determine conformation, and conformational changes by coupling a MALS detector (either a DAWN or miniDAWN) to the liquid chromatograph.

Figure 1 comes from an SEC separation of purified α-Crystallin, which is a protein found in the eye. It had been assumed historically that the shoulder observed in the leading edge of the UV peak comprised material with a more extended structure than that found in the main peak, but there had been no way of confirming this. When the absolute molar mass distribution across this peak was determined using a DAWN DSP equipped with a 10mW Argon-ion laser, the highly non-linear nature of the MW vs. Elution Volume plot revealed that the shoulder did *indeed* have a different conformation. Moreover, in the trailing edge of the UV trace, another compact structure was identified by observing an increasing molar mass slope as the elution volume decreased.

It is possible to determine the conformation of the proteins using the ASTRA software by plotting MW vs. radius, as shown in Figure 2, for the region of the distribution surrounding the leading edge shoulder. Two distinct slopes can be seen, with a transition region between. The steepness of the slope can easily be related to the conformation of molecules, with extended structures giving larger values than compact molecules. For the α-Crystallin, it could be confirmed that the shoulder did indeed contain molecules with a more extended structure.

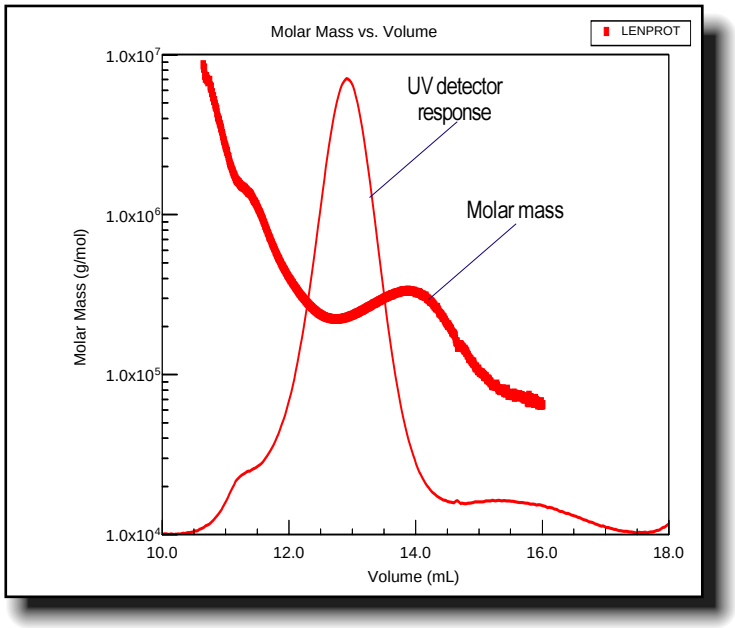

Figure 1. Marked changes in the relationship between molar mass and elution volume can be seen across the peak, despite the fact that the UV response shows nothing out of the ordinary.

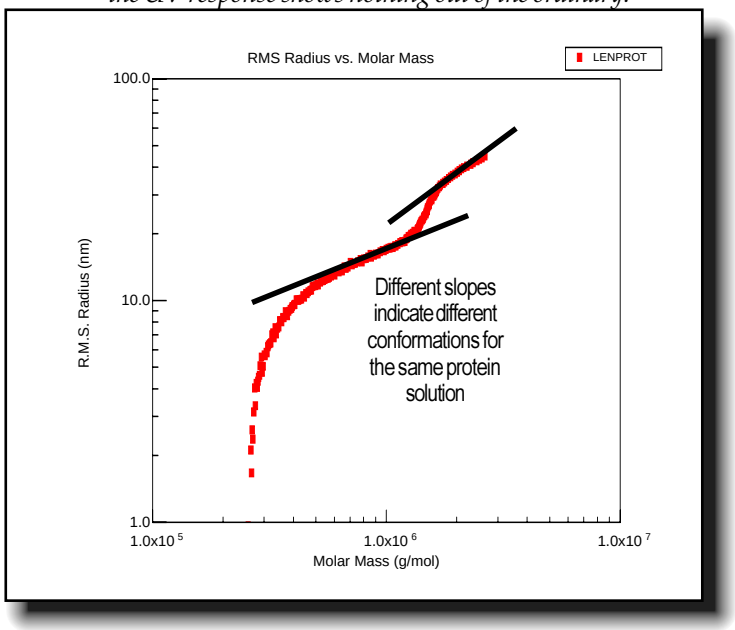

Figure 2. The slope differences in the region of the UV detector's shoulder, confirm the presence of different conformations of the protein molecule.

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