

Heat-Shock Proteins/Molecular Chaperones

In response to stresses, including heat, oxidants and toxins, all cells produce a common set of heat shock proteins (HSPs). Most of these HSPs are molecular chaperones, which bind and stabilize proteins at intermediate stages of folding, assembly, translocation across membranes, and degradation.

There are four classes of high molar mass HSPs and one family of small HSPs (sHSPs). The sHSPs (12-43 kDa) tend to form large multimeric complexes. Size exclusion chromatography (SEC) has often been used to characterize these complexes.

In order to understand better how HSPs behave at elevated temperatures, similar to their *in-vivo* environment, we carried out SEC measurements of an sHSP from *Methanococcus jannaschii* (Mj), a hyperthermophile, at different temperatures. Traditional SEC approaches, using column calibration with protein standards, fail since the standards denature and/or precipitate onto the column at elevated temperatures.

To obtain absolute molar masses (MM) of Mj sHSP at different temperatures, multi-angle light scattering (MALS) detection was used following SEC. The SEC system consisted of an Agilent 1100 chromatograph, a Shodex Protein 803 KW column, a Wyatt Peltier heated-cooled DAWN EOS, and a Wyatt Optilab DSP refractometer.

Molar masses of the Mj sHSP complex at all three testing temperatures, 25°, 40°, and 75° C, were found to be identical at 395 kD, as shown in Figure 1. The results confirm that the Mj sHSP complex remains intact at elevated temperatures, as expected for an HSP.

Figure 2 illustrates that the molar mass from the MALS detection is obtained from the intercept of the so-called Debye plot, and its slope yields the root-mean square radius when the radius is greater than about 10 nm.

These results with the Mj sHSP complex clearly demonstrate that MALS detection provides a simple yet powerful tool to measure the absolute molar masses of proteins, especially for HSPs at elevated temperatures—without the need for column calibration or reference standards.

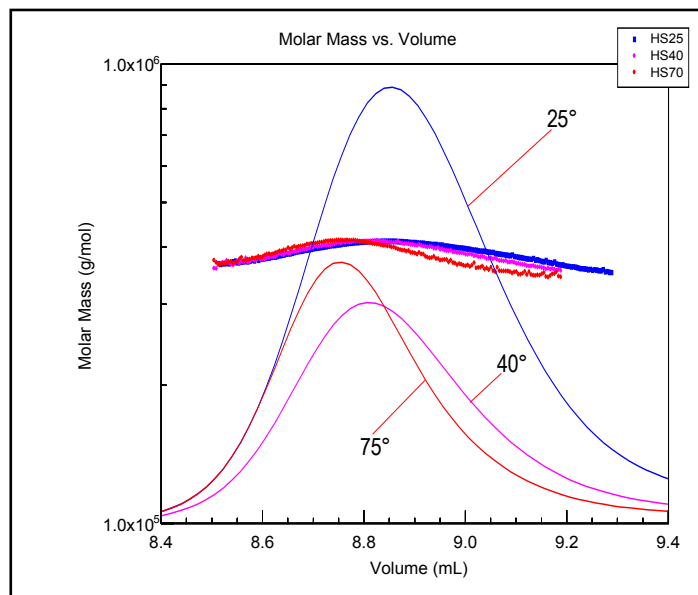


Figure 1. MM of the Mj sHSP complex measured by MALS detection at 25, 40, and 75° C with concentration traces overlaid. The sample concentration at 25°C is twice of that at the other two temperatures.

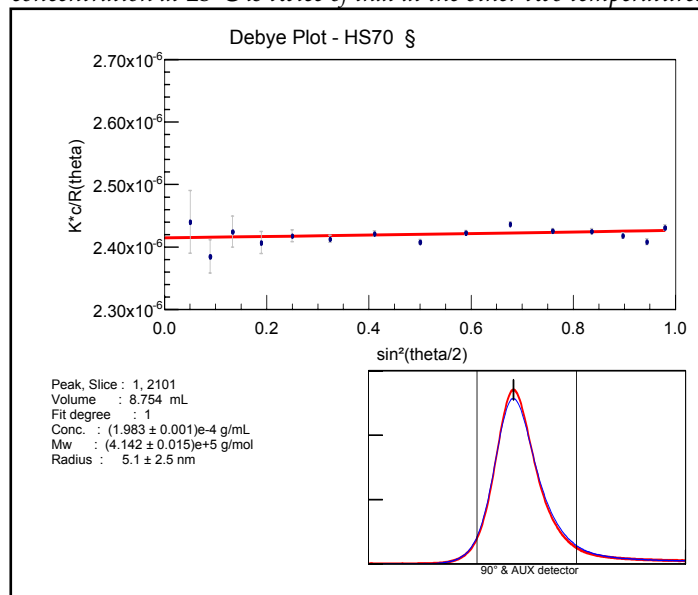
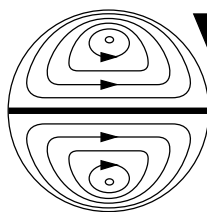


Figure 2. Debye plot of one MALS data slice from the Mj sHSP complex peak obtained from SEC at 75° C.



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