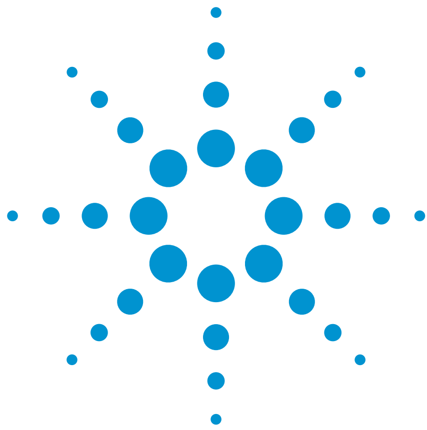

Analysis of Hsp47, a Collagen Chaperone, by Size Exclusion Chromatography (SEC)

Application Note

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Introduction

Heat shock protein 47 (Hsp47) is a glycoprotein of 47 kDa that is ubiquitous, abundant, and highly conserved. It is a collagen-specific molecular chaperone that resides in the endoplasmic reticulum. Like other chaperones, it assists in protein folding both in vivo and in vitro, as well as protecting proteins from unfolding due to external stress. Collagen is the most abundant fibrous protein in the body, therefore its correct biosynthesis and exocytosis is essential. Hsp47 has been recognised as a vital component of the collagen fibril formation pathway.

Hsp47 is a non-inhibitory member of the serine protease inhibitor (serpin) superfamily of proteins. These biomolecules are known to undergo structural conformational changes under different conditions and various conformations of the protein have been identified, each exhibiting different substrate binding and biological activity characteristics. Native Hsp47 possesses low stability and is prone to degradation and aggregation, producing molecules of lower and higher molecular weights. Monitoring of the molecular weight of native Hsp47 therefore allows differentiation between the conformation(s) present in the sample and also reveals the oligomerization state of the protein.

SEC with UV detection can be used to determine the molecular weights of proteins such as Hsp47. The native form of mouse Hsp47 was analyzed for identification of species present in the mixture.

Methods and Materials

Conditions

Sample:	Mouse Hsp47
Column:	Agilent ProSec 300S, 300 x 7.5 mm (p/n PL1147-6501)
Flow Rate:	1 mL/min
Temperature:	5 °C
Detection:	UV at 280 nm
Injection:	100 mL at 2 mg/mL conc.
Eluent:	0.1 M KH_2PO_4 containing 0.3 M NaCl at pH 7.0

Results and Discussion

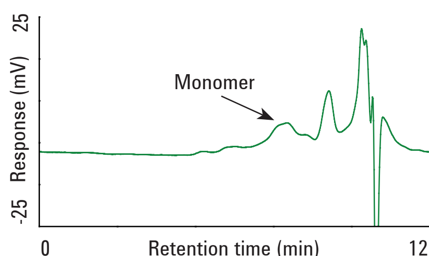

Figure 1. UV chromatogram of a sample of mouse Hsp47 comprising multiple species. The peak with a maximum at around 7.6 min denotes monomer of 47 kDa

Figure 1 illustrates the UV chromatogram for a sample of native mouse Hsp47. The sample exhibited multiple signals that were fairly broad, indicating various oligomeric states of the protein. The higher molecular weight responses suggest aggregation, whilst the lower molecular signals show some protein degradation.

Conclusion

SEC is a well-known technique for assessing protein molecular weights, a parameter that is important for determination of protein conformation, which can in turn influence their physical properties as well as biological function. The pore size of the packing allowed identification of degraded, monomeric, polymeric and aggregate species of Hsp47, an important chaperone protein found in the body.

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