GPC/SEC–MALLS Study of Protein Denaturation

Application Note Biopharma

Author
Dr. Thorsten Hofe
contact: THofe@polymer.de

GPC/SEC-MALLS is a powerful tool to detect and follow the transition of protein structures. GPC/SEC allows the separation of the associates (dimer, trimer, etc.). Absolute molar masses can be detected for various structures, quantification is done using the concentration detector.

Introduction
The structure of a protein determines the protein function. Proteins in the native state are folded into unique 3-dimensional structures, stabilized by hydrophobic and electrostatic interactions. In addition the structure is stabilized by hydrogen bonds between the side chains of the amino acids within the structure.

Variation of environmental parameters such as pH or temperature leads to an entropically driven denaturation of the protein. The denaturation process is the transition of the native 3-dimensional structure into a random coil structure. During this process the secondary and tertiary structures are destroyed. The combination of GPC/GFC/SEC separation with light scattering detection is a powerful method to study the structure transition of proteins.

GPC/SEC can separate the proteins into monomers and higher aggregates (separation by size), the MALLS detector can simultaniously measure the molar mass and structure online.

System Requirements

	Conditions
Pump	PSS SECcurity GPC1200 isocratic pump • flow rate [mL/min]: 1.0 • mobile phase: aqueous, Phosphate buffer pH 6.6 + NaCl 0.5mol/L
Injection system	PSS SECcurity GPC1200 Autosampler • injection volume variable
Columns	• PSS PROTEEMA precolumn (8*50 mm) • PSS PROTEEMA 5µ 100 Å, 300 Å (8*300 mm each)
Sample	Thyroglobulin (bovine) M 670.000 Da (Sigma)
Loading	• 1.0 mg/mL, 20µL injection volume
Detectors	• Refractive index PSS SECcurity 1200 RI • PSS SECcurity UV detector • PSS SLD7000 7-angle MALLS
Software	PSS WinGPC Unity plus multi angle light scattering module

Procedure, Results & Discussion

A decrease of the dimer signal and a shift to lower elution volumes (as seen in Figure 1) indicates the transition of a very compact, globular structure to a less dense random coil structure. The transition occurs already during the first 30 minutes indicated by the increase of the hydrodynamic volume. Heating the protein solution up to 50ºC for 120 minutes leads to a denaturation of more than 50% of the native monomeric chains(Figure 1).

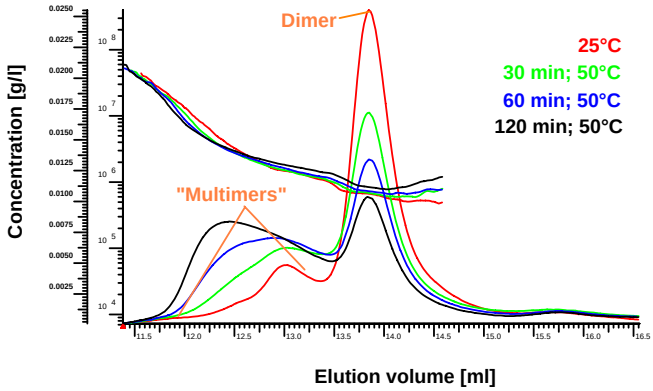

Fig. 1: Hydrodynamic volume change (concentration signal) and resulting molar mass of Thyroglobulin (bovine) as a function of the temperature. The red curve is the native protein including the associates.

Native monomers and associates tend to denaturate simultaneously. The molar mass vs elution volume behavior for the various heated samples stays constant during the transition because only the structure is changing. This is shown by the molar mass dependency in Figure 1. The general increase of the molar mass at smaller elution volume, regardless of the structure, is because of the association or agglomeration of the protein. The 90º light scattering signal (Figure 2) also shows a decrease of the native dimers and the increase of the denaturated and associated structure as a function of temperature.

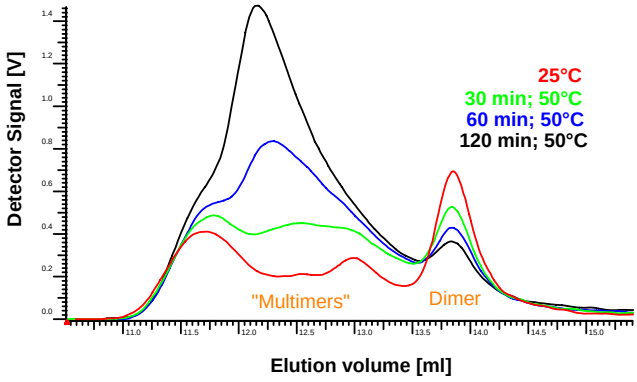

Fig. 2: Denaturation of Thyroglobulin (bovine) as a function of environmental temperature, detected with the 90˚MALLS signal. The red curve is the native protein including the associates.

Polymer Standards Service GmbH
In der Dalheimer Wiese 5 D-55120 Mainz
Tel.: +49 (0) 6131-96239-0 Fax: +49 (0) 6131-96239-11
Email: info@polymer.de www.polymer.de

Polymer Standards Service - USA Inc
160 Old Farm Rd, Suite A Amherst, MA 01002
Phone: +1 413-835-0265 Fax: +1 413-835-0354
Email: pssusa@pssgpcshop.com www.pssgpcshop.com