The Effect of Temperature on Protein Size Exclusion Chromatography

Application Note

BioPharma

Authors

Umbreen Ahmed and Greg Saunders
Agilent Technologies, Inc.

Introduction

Proteins comprise an extremely heterogeneous class of biological molecules. They are often unstable when not in their native environment, which can vary considerably between cellular compartments and extracellular fluids. If certain conditions (such as temperature) are not controlled, extracted proteins may not function correctly or remain soluble. Proteins can lose activity as a result of proteolysis, aggregation and suboptimal experimental conditions.

The stability of many proteins is dependent on a temperature range that is distinct for each individual protein. Thermophilic proteins, for example, require relatively high temperatures for activity and can remain active at temperatures between 40-80 °C. Mesophilic proteins function best between 15-40 °C, denaturing at very high temperatures. A general rule of thumb is that the majority of proteins are more stable at reduced temperatures. Globular protein size exclusion chromatography is therefore performed at ambient temperature or ~5 °C, as elevated temperatures can result in breaking of hydrogen bonds, affecting tertiary structure interactions and ultimately leading to protein denaturation.

Size exclusion chromatography (SEC) is an excellent technique for investigating the effect of temperature on proteins, allowing analysis of aggregation and protein unfolding. This note describes the analysis of a series of globular proteins under various temperature conditions to identify unfolded and/or aggregate species.

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Methods and Materials

Conditions

Sample:	Proteins
Column:	Agilent ProSEC 300S, 7.5 × 300 mm (p/n PL1147-6501)
Eluent:	0.1 M KH_2PO_4 containing 0.3 M NaCl, pH 7.5
Flow Rate:	1.0 mL/min
Injection Volume:	100 µL
Temperature:	5 °C, 25 °C, 45 °C, 65 °C
Detector:	UV at 310 nm

Results and Discussion

Figures 1-3 illustrate SEC chromatograms of a range of globular proteins under varied temperatures.

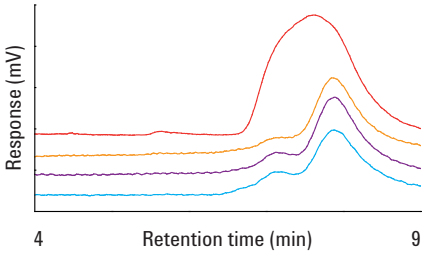

Figure 1. Overlay of UV response for a sample of γ-globulins under different temperatures, with limited effect on stability at the lower temperatures but a marked effect on retention behavior at 65 °C . Blue - 5 °C, Purple - 25 °C, Orange - 45 °C, Red - 65 °C

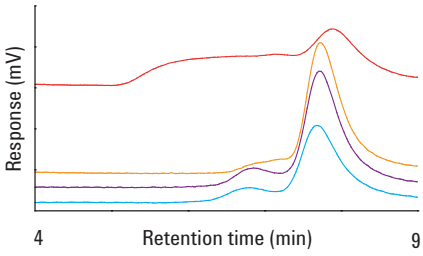

Figure 2. Overlay of UV response for a sample of BSA under various temperatures. Again, the lower temperatures had little effect on stability but there was a significant effect at 65 °C. Blue - 5 °C, Purple - 25 °C, Orange - 45 °C, Red - 65 °C

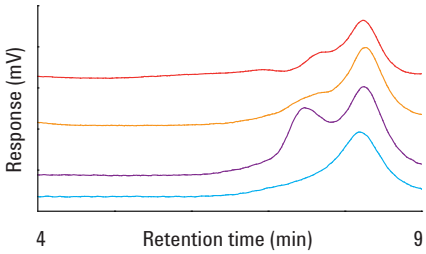

Figure 3. Overlay of UV response for a sample of ovalbumin at different temperatures, with clear changes in elution behavior above 5 °C . Blue - 5 °C, Purple - 25 °C, Orange - 45 °C, Red 65 - °C

The results shown in Figures 1 and 2 demonstrate that for BSA and γ-globulins the elution profiles at 5 °C, 25 °C, and 45 °C were generally quite similar, suggesting that in the short residence time of the protein in the column the globular proteins were reasonably stable. However, further heating to 65 °C significantly altered the elution behavior, causing an increase in the amount of aggregation and/or polymer and a shift in the position of the main protein peak.

Ovalbumin in Figure 3 showed a large change in elution behavior when heated above 5 °C, with the single peak and small amount of dimerization that then gave way to larger quantities of oligomers and aggregated material as the temperature rose. This indicates that ovalbumin is more sensitive to temperature than BSA or γ-globulins.

Conclusion

Analysis of globular proteins under varying temperatures allowed the investigation of the effect of temperature on protein elution. Measuring UV intensity identified differences in protein mixtures due to temperature and provided information on the thermal stability of the above proteins. In particular ovalbumin showed a high degree of temperature instability, illustrating the requirement of cooling to 5 °C to obtain the best quality data when analyzing proteins by SEC.

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