Analysis of various Globular Proteins using Agilent ProSEC 300S Columns

Application Note

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Introduction

Proteins are of intense interest due to their role in many biological processes. Isolating, purifying and studying proteins are major goals of many laboratories around the globe, often with the intention to use the biological activity of proteins for clinical research applications. With exceedingly complex chemical and structural properties, protein analysis is very demanding, and many techniques have been developed in this area. The analysis of proteins by size exclusion chromatography is widely used as part of the purification of complex protein mixtures and also as part of studies in protein size, shape and aggregation.

Proteins can be loosely separated into three main groups, globular proteins, fibrous proteins and membrane proteins. Globular proteins or spheroproteins are globe or sphere-like in shape and are more or less universally soluble in water where they form colloidal solutions. Nearly all enzymes with major metabolic functions are globular proteins. Among the most known globular proteins is haemoglobin, a member of the globin protein family. Other globular proteins are the immunoglobulin family (IgA, IgD, IgE, IgG and IgM), and alpha, beta and gamma globulins. ProSEC 300S columns are designed for protein analysis and may be used to investigate a wide range of globular proteins, including the albumins by size exclusion chromatography (SEC). Separating molecules on the basis of their size in solution, SEC is an excellent technique for analyzing proteins of differing sizes.

Protein analysis by SEC is not straightforward. Proteins are complex materials that often contain ionic as well as hydrophobic and hydrophilic groups in a single molecule. Individual proteins are monodisperse (contain species of a single molecular weight) but may contain oligomers or other species, and range in size from small to extremely large. These factors can make the development of methods for protein analysis very complex.

This note illustrates the analysis of a range of globular proteins in buffer solution by SEC using a ProSEC 300S column.

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Methods and Materials

Conditions

Column:	ProSEC 300S, 300 x 7.5 mm (p/n PL1147-6501)
Eluent:	0.3M: 50mM KH_2PO_4-K_2HPO_4 (@ pH 6.8) containing 0.3M NaCl
Flow Rate:	1.0 mL/min
Inj. Vol:	20 µL
Sample Conc:	4 mg/mL
Temp:	25 °C
Detection:	UV at 280 nm

Results and Discussion

The figure shows overlaid chromatograms of a range of globular proteins.

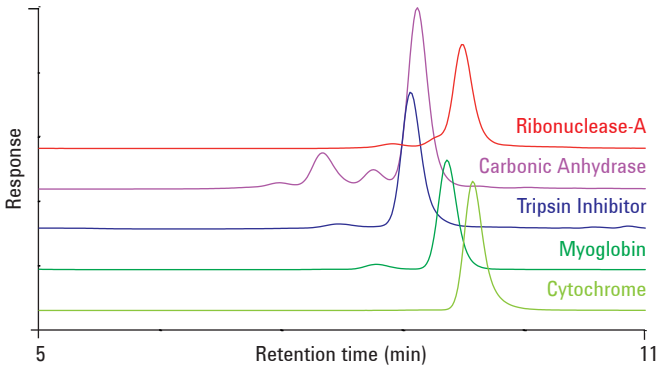

Figure 1. Chromatographs of globular proteins

Some of the proteins in this study gave monodisperse peaks indicative of a single species in solution, whereas others showed complex multimodal peaks due to either the presence of numerous components in the sample or aggregation effects.

Conclusion

A single ProSEC 300S column analyzed a selection of globulin proteins on the basis of their size in solution and molecular weight, showing differences in the nature of the materials. The ProSEC 300S column contains a packing with a surface modified for compatibility with proteins, ensuring that true size exclusion is obtained with minimal unwanted interaction effects. The pore size of the packing has been specifically selected to allow the analysis of a wide range of small to medium-sized proteins.

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