

AN8001: Wheat protein: molar mass, size and shape by RPC-MALS

Introduction

Gluten is a mixture of two types of proteins (gliadins and glutenins) found in wheat. The quality of bread made from wheat largely depends on the gluten proteins, which form a viscoelastic network during dough mixing that leads to successful bread. For decades, extensive studies have been devoted to the characterization of these proteins; however, their exact structure and size have not been known due to their low solubility and high complexity—not to mention a shortage of appropriate analytical characterization techniques.

Reversed phase chromatography (RPC) has been widely used for analyzing wheat proteins because of its high resolution. This note shows that the concomitant use of a DAWN® multi-angle light scattering (MALS) detector and RPC constitutes a powerfully effective means to determine both molar mass and size for the wheat protein, TAM 105.

MALS determines the molar mass and size (R_g) of macromolecules from 200 g/mol and up to hundreds of millions of g/mol. Moreover, it does so online, at each elution volume in the chromatogram, from first principles, without recourse to reference standards. While typically used in conjunction with size-exclusion chromatography (SEC-MALS), MALS is also appropriate for RPC if the molecules contain UV chromophores, and the UV extinction coefficient of each peak is known (for example from the amino acid sequence) and non-zero.

Materials and Methods

MALS analysis requires concentration data, often obtained from a differential refractive index (dRI) detector such as the Optilab® in line with the MALS detector. However, in RPC the variations in solvent refractive index across the chromatographic gradient are generally very large and nonlinear, precluding the use of dRI for MALS analysis.

Hence, for the RPC-MALS measurement a DAWN MALS detector was connected in line with the UV detector of an HP 1050 (Palo Alto, CA) HPLC system. The same HPLC was used to produce a linear acetonitrile-water gradient, and the sample was separated on an ODS-silica column. ASTRA® software was used to collect and analyze the data.

In addition to concentration and MALS signals, MALS analysis also requires knowledge of the refractive increment dn/dc , which depends on both the molecule and the solvent. For SEC-MALS of proteins in aqueous buffer, dn/dc is essentially constant across nearly all proteins at a value of $(0.185 \pm 1\%)$ mL/g. For a RPC water-acetonitrile gradient, dn/dc varies across the gradient, but in the central region where most proteins elute, is relatively constant at $(0.174 \pm 3\%)$ mL/g. This degree of uncertainty increases only slightly the typical MALS uncertainty of 5% for molar mass and size.

Results and Discussion

Four closely-spaced, primary peaks were identified as in the reverse-phase chromatogram, indicated in Figure 1 (the other peaks evident are not of current interest). Narrow bands were selected around the centers of each peak as shown in Figure 2; molar mass and size were averaged across all the data points contained in each band.

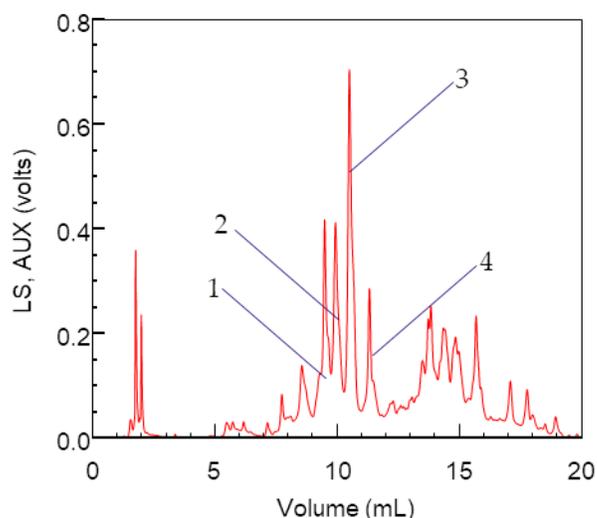


Figure 1. Reverse-phase chromatogram of gluten with the four peaks of interest identified.

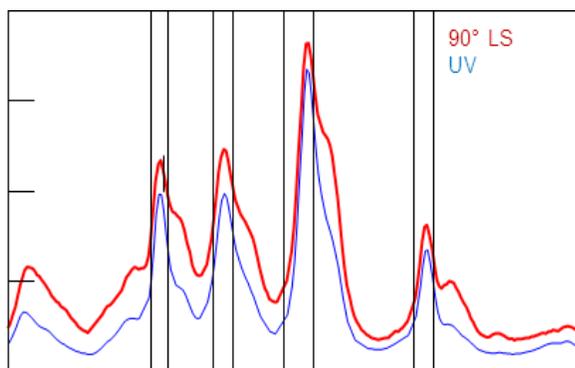


Figure 2. Selection of peak regions 1-4, showing light scattering signal at 90° (red) and UV absorbance signal (blue).

Figure 3 shows a typical Debye Plot (inverse scattering intensity against scattering angle) at a single elution point. Molar mass and size are determined by means of a linear regression, where the slope provides the mean square radius and the extrapolation to zero angle provides the molar mass. Averaging with appropriate weighting across the band, performed automatically in ASTRA, determines number-, weight- and z-averages of each protein.

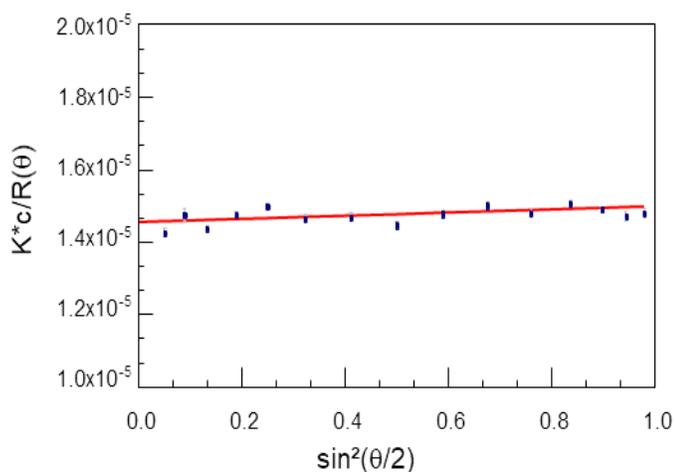


Figure 3. Debye Plot for one slice in the chromatogram, showing inverse scattering intensity vs. scattering angle. From which molar mass and size are determined.

Table 1 lists the molar masses determined by both light scattering and complementary DNA (cDNA) techniques for three proteins of greatest interest (the fourth was not analyzed by cDNA). The rms radii measured directly by the DAWN, when compared with the molar masses, indicate that the proteins exhibit elongated and possibly rod-like shapes under reverse-phase chromatographic conditions. This shape may be a factor in the dough's viscoelastic behavior.

Peak	Molar Mass [kDa]		RMS radius (nm)
	cDNA	MALS	MALS
1	68.7	67.6 ± 0.3	11.7 ± 1.5
2	87.2	87.4 ± 0.4	11.4 ± 1.5
3	83.1	79.7 ± 0.4	11.7 ± 1.5
4	N/A	70.6 ± 0.7	13.8 ± 1.5

Table 1. Wheat protein: molar mass and size values for peaks 1-4, compared with cDNA analysis.

Conclusions

Four primary gluten proteins were separated by reverse-phase chromatography and characterized by online MALS. Molar mass values determined by MALS were very close to those determined by (offline) complementary DNA sequencing. In addition, conformation estimates are obtained from comparison of size (R_g) with molar mass, fleshing out the biophysical picture.

SEC-MALS would not have separated these four proteins with very similar sizes. For proteins and other UV-active molecules, RPC-MALS is an effective means of determining molar mass, size and shape when SEC is inappropriate.

Acknowledgements

We thank the Kansas State University for providing the sample, column, and cDNA data.



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