Characterisation of high M_r wheat glutenin polymers by agarose gel eletrophoresis and dynamic light scattering

T.A. Egorov[a,*], T.I. Odintsova[b], P.R. Shewry[c], A.S. Tatham[c]

[a] *Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, 32 Vavilova Str., Moscow 117984, Russia*
[b] *Vavilov Institute of General Genetics, Russian Academy of Sciences, 3 Gubkina Str., Moscow 117809, Russia*
[c] *IACR Long Ashton Research Station, Department of Agricultural Sciences, University of Bristol, Long Ashton, Bristol BS18 9AF, UK*

Received 24 June 1998; revised version received 20 July 1998

Abstract Agarose gel electrophoresis has been used to separate the complex mixture of wheat gluten polymers into fractions ranging in M_r, determined by dynamic light scattering, from about 500 000 to over 5×10^6. The separation is reliable and reproducible and well suited to the routine analysis of multiple samples.

Key words: Wheat; Seed protein; Gluten; Protein polymer; Glutenin

1. Introduction

The gluten proteins of wheat are of immense importance in the food industry as they confer unique biomechanical properties (a balance of elasticity and extensibility) that allow dough to be processed into bread and other food products [1]. These properties are determined largely by the glutenin group of proteins, which form high M_r polymers stabilised by interchain disulphide bonds. These polymers have been described as 'nature's largest polymers' [2] and this size (estimated as ranging up to 10×10^6), combined with their low solubility even in strongly denaturing solvents, has hindered attempts at detailed characterisation. Recent work has, therefore, focused on the application of novel approaches, such as field flow fractionation, for separation [3,4] and photon correlation spectroscopy for size determination [5]. Although the results of these analyses are promising the instrumentation is not widely available and it is unlikely that the approaches will achieve widespread use. We therefore describe here a simple system, based on agarose gel electrophoresis in the presence of SDS [6]. This separates polymers of M_r up to about 6×10^6, as determined by light scattering, providing a basis for routine analyses (e.g. of polymer size distribution in relation to processing properties), as well as more detailed characterisation of polymer structure. It may also be of wider use for separating other protein polymers of similar dimensions.

2. Materials and methods

2.1. Protein extraction

Protein were extracted from milled grain by stirring for 12 h at 37°C with either 50% (v/v) aqueous propan-1-ol or 0.0125 M Tris-HCl buffer, pH 8.5, containing 2% (w/v) SDS, precipitated by the addition of 9 volumes of ice-cold acetone and collected by centrifugation at 15 000 rpm at 4°C. In some experiments, the meal was pre-extracted to remove gliadins by stirring for 2×30 min with 70% (v/v) aqueous ethanol.

2.2. Electrophoresis

Proteins were dissolved in Laemmli [7] sample buffer with (2% v/v) 2-mercaptoethanol and heated at 90°C for 3 min. Agarose gel electrophoresis was as described by Preobrazhensky [6] with minor modifications. Agarose (450 mg, Sigma Type II-A, medium EEO) was dissolved in 15 ml of electrode buffer (0.1 M boric acid, pH adjusted to 8.0 with solid Tris, containing 0.02 M EDTA and 0.1% (v/v) SDS), and cast as a 2.5 mm thick slab using a Horizon 58 (Bethesda Research Laboratories, Life Technologies) apparatus. Electrophoresis was at 25 V for 10–12 h at 6°C. The gel was then stained with Coomassie brilliant blue R250 in 40% (v/v) aqueous methanol containing 10% (w/v) trichloroacetic acid or cut into slices. Protein was extracted from slices by homogenisation with 150 μl of Laemmli sample buffer followed by incubation at 30°C for 6 h. Proteins were precipitated from the supernatant with 9 volumes of ice-cold acetone. SDS-PAGE was carried out using a Laemmli [7] system.

2.3. Polymer size determination by dynamic light scattering

Fractions were eluted from agarose gels with 0.02 M Tris-HCl buffer, pH 8.0, containing 4 M urea and filtered through a 0.1 μm Whatman Anotop filter. Molecular mass was determined using a DynaPro-801 Molecular Sizing Instrument taking at least eight measurements for each fraction. The scattered light intensity was measured with an infrared semiconductor laser (780 nm). Photons scattered at 90° to the incident laser beam were collected by a lens and conducted to an avalanche photodiode via an optical fibre, producing a single pulse for each photon received. These were stored and correlated by an integral computer. Experiments were run at 19.9 ± 0.1°C.

The molecular weight was calculated from the measured translational diffusion coefficient, D_T, using the Stokes-Einstein equation [8]:

$$D_T = k_B T/6\pi\eta R_h$$

where k_B is the Boltzmann constant, T the absolute temperature, η the viscosity of solution (taken as 1.225 for 4 M urea) and R_h the hydrodynamic radius. The diffusion coefficient measured at a single concentration is an apparent one, D_{app}, because of non-ideality effects (finite volume and charge). These effects become small when the concentration approaches zero where the approximation is made that $D_T \approx D_{app}$ and it is assumed that the effects are the same for the calibration standards [9]. The molecular weight was estimated using

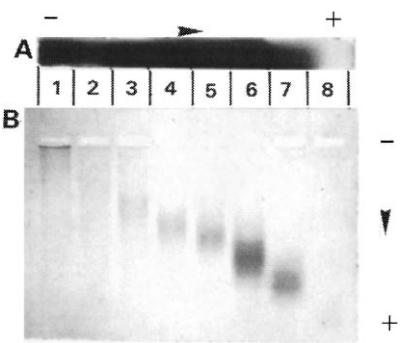

Fig. 1. Agarose gel electrophoresis of gluten proteins extracted from milled grain of wheat cv. Chinese Spring (A). The direction of migration is indicated. A gel separation similar to that shown in part A was sliced into eight sections and the proteins extracted and re-run on the same gel system (B).

*Corresponding author.

PII: S0014-5793(98)00983-1

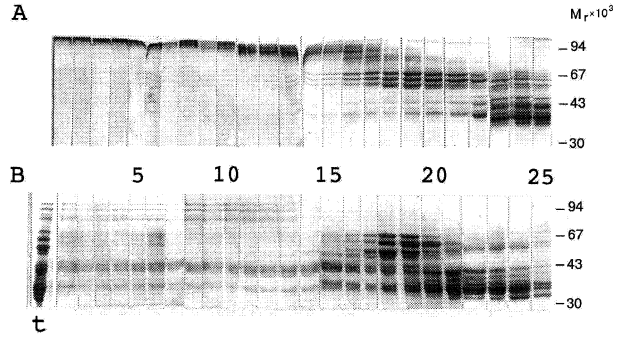

Fig. 2. SDS-PAGE of non-reduced (A) and reduced (B) proteins separated by agarose gel electrophoresis of total proteins extracted from milled grain of wheat cv. Chinese Spring with 2% (w/v) SDS. The gel strip after electrophoresis was cut into 25 parts and the proteins extracted for SDS-PAGE. t = total protein fraction applied to agarose.

the relationship between R_h and M_r for a series of standard proteins [10]. Two assumptions were made in the calculations, firstly, that the protein polymers were approximately spherical in shape and secondly, that the proteins had a constant density in relation to their size, in order to calculate the mass from the molecular volume.

3. Results

3.1. Separation of glutenin polymers by agarose gel electrophoresis

Although glutenin fractions contain some low M_r polymers and oligomers, most of the components are too large to enter a conventional polyacrylamide gel. We therefore decided to evaluate the agarose gel electrophoresis procedure which has been used by Preobrazhensky [6] to separate proteins of M_r up to 1×10^6. Preliminary analyses of standard high M_r proteins, such as thyroglobulin (M_r 670 000), ferritin (M_r 480 000) and γ-globulin (M_r 150 000) gave sharp bands after staining, demonstrating the resolving power of the system. In contrast, the non-reduced wheat glutenin fraction gave a continuous band of staining with no clear resolution into bands (Fig. 1A). However, when the gel was cut into eight slices and the protein extracted and separated a second time clear mobility differences were observed (Fig. 1B) demonstrating that the polymeric components were separated on the basis of size. The separation was not improved by altering the concentration of agarose or by using different protein extraction procedures (50% (v/v) aqueous propan-1-ol or 2% (w/v) SDS), with or without prior extraction of the gliadins.

Table 1
Estimated molecular weights of glutenin polymer fractions corresponding to fractions 1–8 of Fig. 1A

Fraction	R_h (nm)	Polydisp (nm)	$M_r \times 10^5$
1	22.6	12.5	56.3
2	19.6	10.9	39.7
3	16.6	8.1	26.3
4	13.0	7.2	14.7
5	12.4	6.9	12.9
6	9.5	5.3	6.9
7	10.2	5.7	8.2
8	9.3	5.2	6.6

In order to characterise the separation further, a total gluten protein fraction from cv. Chinese Spring (extracted with 2% (w/v) SDS) was separated by agarose gel electrophoresis and the gel sliced into 25 pieces and eluted. The proteins were then separated by SDS-PAGE, either without reduction (Fig. 2A) or after reduction of disulphide bonds with 2-mercaptoethanol (Fig. 2B). This showed that fractions 1–15 consisted mainly of polymers which, on reduction, gave both HMW and LMW subunits of glutenin. Fractions 16–25 contained increasing proportions of gliadins, mainly ω-gliadins in fractions 16–21 and γ-type and α-type gliadins in fractions 23–25. There was also some resolution of the glutenin polymers, with early fractions (1–6) only barely migrating into the SDS-PAGE gel when unreduced, while the later fractions (10–15) migrated into the gel and separated into several bands. This again confirmed that the agarose gel system separated glutenin polymers on the basis of M_r.

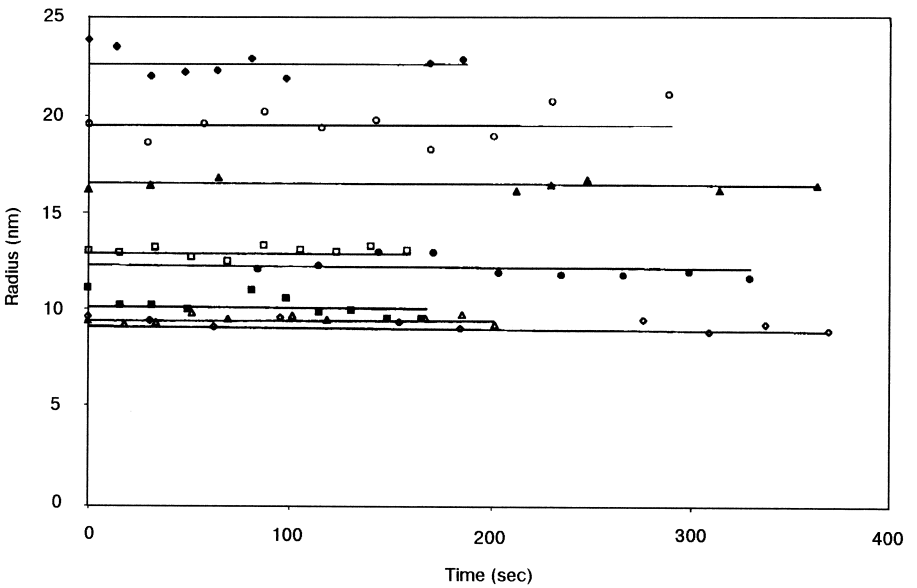

Fig. 3. R_h values determined for the different fractions with decreasing molecular weight corresponding to fractions 1–8 in Fig. 1. 1 (♦); 2 (○); 3 (▲); 4 (□); 5 (●); 6 (■); 7 (◇) and 8 (△).

3.2. Determination of polymer size by light scattering

The molecular weights of eight fractions, corresponding to those shown in Fig. 1 were determined using dynamic light scattering, from the average R_h values (Fig. 3). The results (Table 1) show that the M_rs of the polymers present in these fractions decrease from about 5.6×10^6 in fraction 1 to about 6.6×10^5 in fraction 8. Table 1 also shows values for the polydispersity of the fractions. These were high, which may result from a combination of factors relating to the nature of the polymers and to the assumptions made in the calculations. In addition, although the results are calculated to give a single M_r value for each fraction, Fig. 1 shows that these fractions can be expected to contain a continuous range of polymers varying in their M_r and dimensions. The binding of SDS to the proteins and the presence of urea may also result in some expansion due to charge repulsion and partial denaturation and result in an over-estimation of the M_r.

Nevertheless, the dynamic light scattering results confirm that the agarose gel electrophoresis procedure separates the glutenin polymers on the basis of M_r and indicates that they have M_rs ranging from about 500 000 to over 5 million. It therefore provides a simple method for the routine fractionation of glutenin and other high M_r protein polymers.

Acknowledgements: IACR receives grant-aided support from the Biotechnology and Biological Sciences Research Council of the United Kingdom. The work was supported by the Royal Society, Joint Project P695 'Covalent Structure and Disulphide Mapping of Wheat Gluten Proteins' and the Russian Fund for Basic Research (Grant 96-04-49850).

References

[1] MacRitchie, F. (1992) Adv. Food Nutr. Res. 36, 1–87.
[2] Wrigley, C.W. (1996) Nature 381, 738–739.
[3] Wahlund, K.-G., Gustavsson, M., MacRitchie, F., Nylander, T. and Wannerberger, L. (1996) J. Cereal Sci. 23, 113–119.
[4] Stevenson, S.G. and Preston, K.R. (1996) J. Cereal Sci. 23, 121–131.
[5] Sutton, K.H. (1996) In: Gluten '96 (Wrigley, C.W., Ed.), pp. 317–320, Royal Australian Chemical Institute, Australia.
[6] Preobrazhensky, A.A. (1993) Anal. Biochem. 209, 315–317.
[7] Laemmli, U.K. (1970) Nature 227, 681–685.
[8] Tanford, C. (1961) in: Physical Chemistry of Macromolecules, pp. 275–316, John Wiley and Sons, New York.
[9] Harding, S.E. (1997) in: Protein Structure: A Practical Approach, 2nd edn. (Creighton, T.E., Ed.), pp. 219–251, IRL Press, Oxford.
[10] Claes, P., Dunford, M., Kenney, A. and Vardy, P. (1992) in: Laser Light Scattering in Biochemistry (Harding, S.E., Sattelle, D.B. and Bloomfield, V.A., Eds.), pp. 66–76, Royal Society of Chemistry, Cambridge.