Scanning Electron Microscopy of Bread Wheat Proteins Fractionated by Gel Filtration[1]

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ABSTRACT

Flour proteins separated by gel filtration were studied by scanning electron microscopy. All fractions studied were heterogeneous in their microscopic structure. The highest molecular-weight fraction contained disc-shaped particles joined together by stringy fibrous strands of protein. The second highest molecular-weight fraction appeared as large sheeted protein particles of various size. The third fraction comprised many small spherical particles associated with a few fibrous protein strands. The lower molecular-weight gliadins (fraction IV) contained small aggregates of amorphorous protein particles. When compared with purified glutenin, the highest-molecular-weight fraction from gel filtration appeared highly heterogeneous.

Gel filtration in dissociating solvents is now a widely used method of fractionating wheat flour proteins (1-3). This technique, although useful for following changes in molecular-weight (MW) distribution, does not yield completely distinct fractions as a preparative method (3,4). The present article presents

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physical evidence obtained with the scanning electron microscope (SEM) of the heterogeneity of the fractions from gel filtration.

MATERIALS AND METHODS

The flour used as the source of protein for this study was of straight grade milled from a variety (Manitou) of Canadian hard red spring (HRS) wheat on a Buhler experimental mill after tempering to 15.5% moisture.

The protein was extracted from the flour with AUC solvent (0.1M acetic acid, 3M urea, and 0.01M cetyltrimethylammonium bromide) and chromatographed on Sephadex G-100 using the same solvent (3).

Appropriate fractions were pooled, dialyzed against distilled water, and freeze-dried.

A sample of purified glutenin was prepared by a pH precipitation procedure (3) from the same flour as follows. Gluten was prepared by hand-washing the dough from 100 g. of flour. The wet gluten obtained was dispersed in 500 ml. of AUC by overnight stirring. After centrifugation at 20,000 X g for 30 min., the supernatant was made 70% (v./v.) in ethanol and adjusted to pH 6.4 by addition of 1N sodium hydroxide. The resulting precipitate was further purified using SE-Sephadex C-50, as previously described (3), and freeze-dried.

For electron microscopy, the freeze-dried samples were mounted on circular (12 mm. diameter) specimen stubs with double-sided tape and coated with gold to a

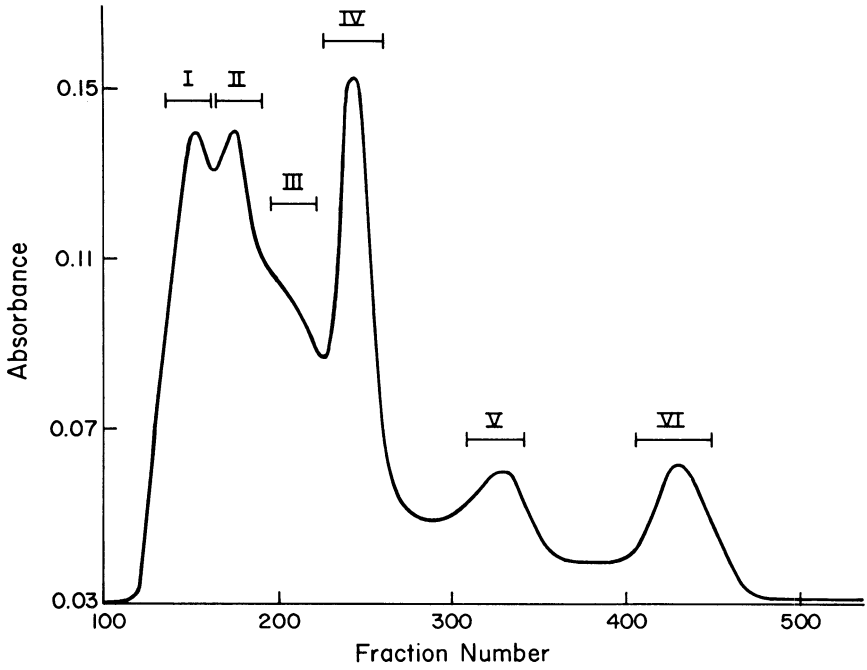

Fig. 1. Elution profile of AUC extract of Manitou (HRS) flour on Sephadex G-100 using AUC as eluting solvent (absorbance at 280 nm.).

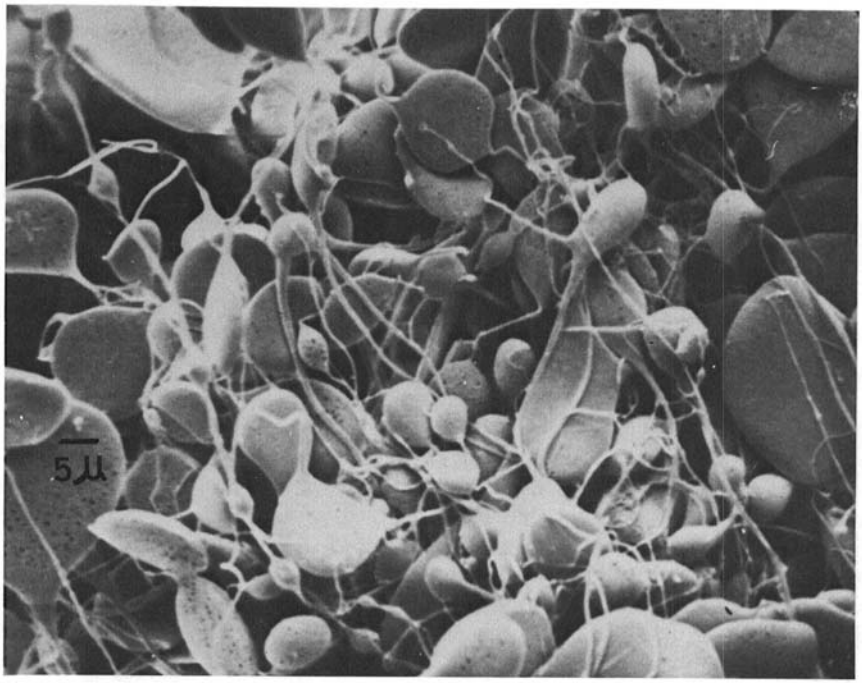

Fig. 2. SEM micrograph of fraction I from gel filtration fractionation of AUC extract of flour.

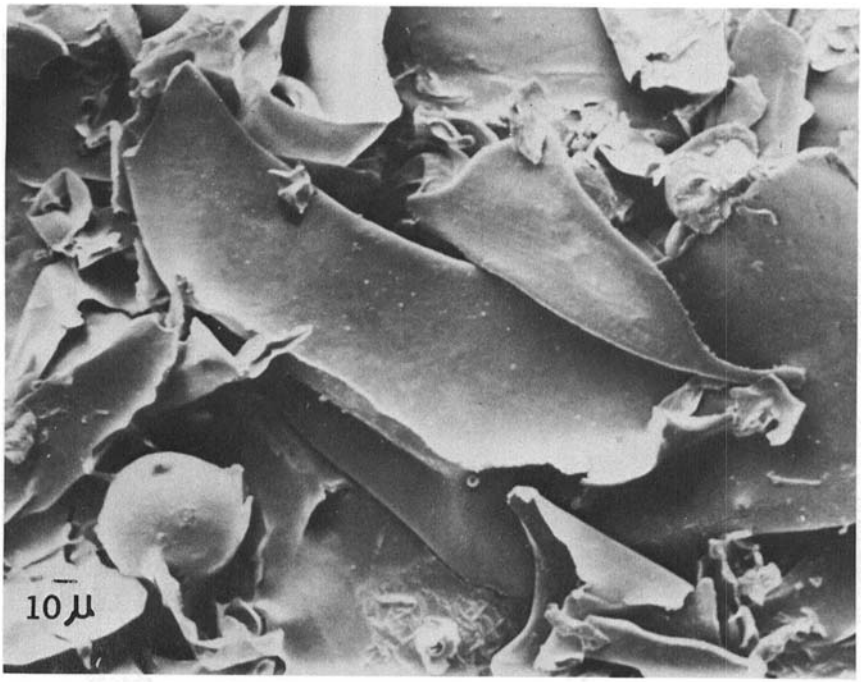

Fig. 3. SEM micrograph of fraction II.

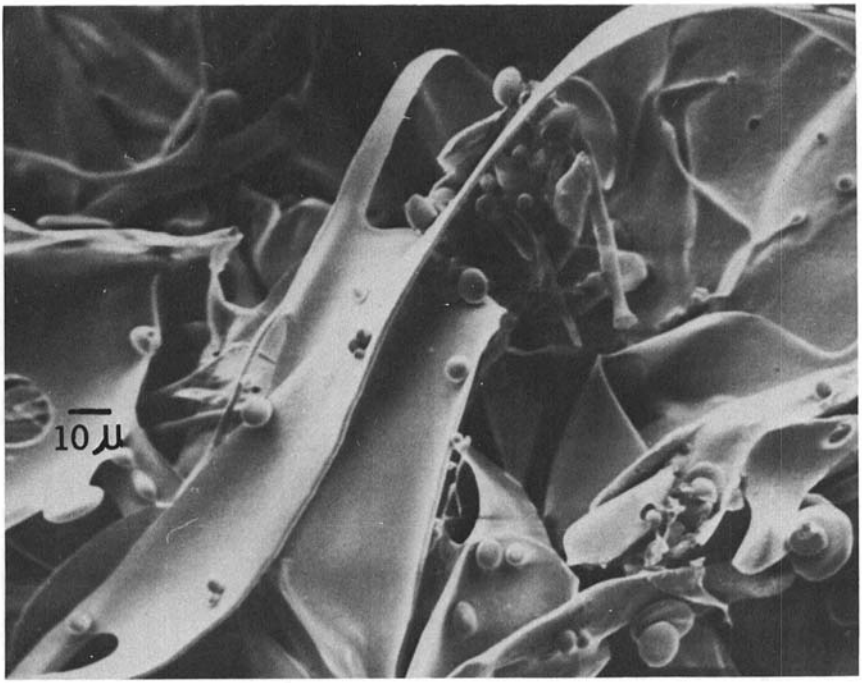

Fig. 4. SEM micrograph of fraction III.

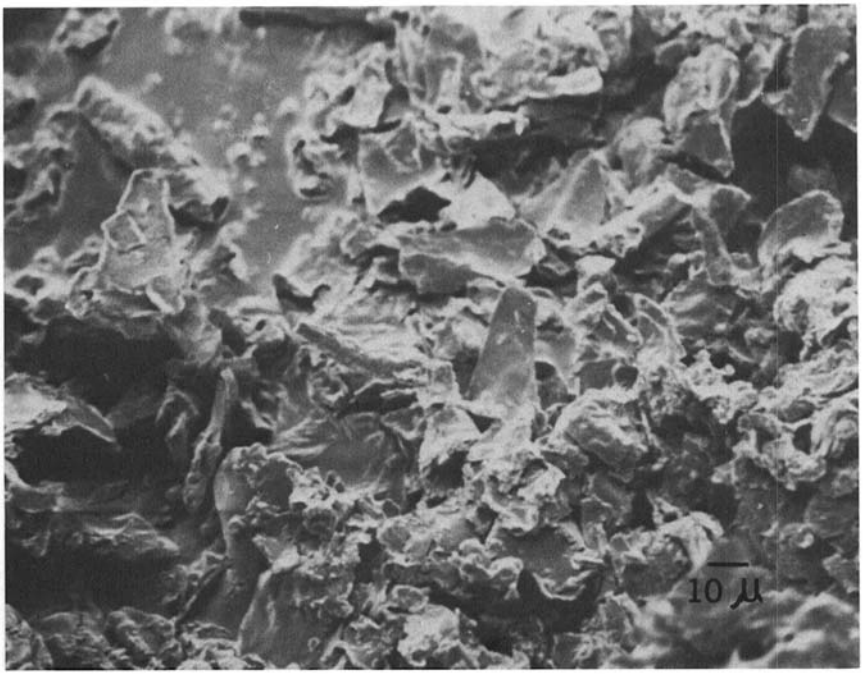

Fig. 5. SEM micrograph of fraction IV.

thickness of 20 to 25 nm. The mounted specimens were examined in a Cambridge Stereoscan MK IIa scanning electron microscope at 10 kv. accelerating potential. Samples were viewed by scanning the total specimen, and a representative area was photographed on 35-mm. Kodak Panatomic X film.

RESULTS AND DISCUSSION

Figure 1 shows the elution profile of the AUC extract of flour on Sephadex G-100. In accordance with the original suggestion of Meredith and Wren (1), fractions I and II would represent glutenin, fraction III gliadin and glutenin, fraction IV low-MW gliadin, fraction V albumin, and fraction VI would represent low-MW materials that absorb at 280 nm. These workers, and later Bushuk and Wrigley (2), implied that the glutenin and gliadin obtained by this preparative procedure were reasonably homogeneous. Later work (3,4) showed that there was considerable overlap in the electrophoretic properties of the first three fractions. Gel electrophoresis (3,4) and amino acid analysis (3) showed that fraction I was glutenin-like protein, fraction II a mixture of gliadin and glutenin, fraction III gliadin with some high-MW protein, and fraction IV gliadins with some albumin or globulin proteins. In the present study material represented by fractions I to IV was examined by SEM; fractions V and VI were not examined because the amount of material available after other analyses was not sufficient.

Figure 2 shows the SEM photograph of material representative of fraction I.

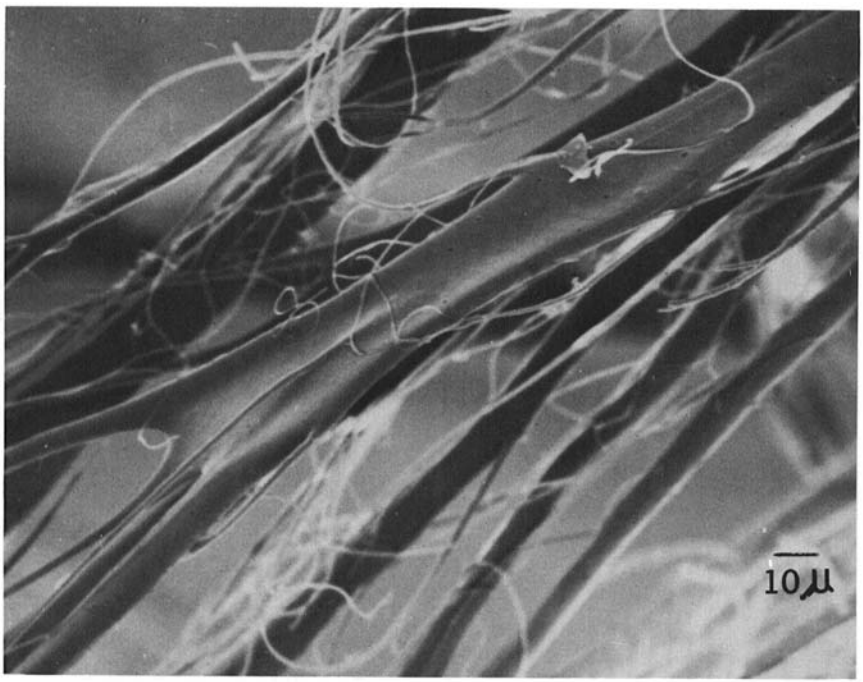

Fig. 6. SEM micrograph of purified glutenin of Manitou (HRS) wheat.

This fraction, referred to as high-MW glutenin by Meredith and Wren (1), was quite heterogeneous in microscopic structure. It consisted of numerous disc-shaped particles of approximately 5 μ diameter, joined by fibrous protein strands. The discs varied in size and shape. These discs were similar in shape to the Sephadex particles used in the chromatography, but were at least an order of magnitude smaller (size range of Sephadex G-100 was 40 to 120 μ). Associated with some of the stringy fibers and disc-shaped particles were pieces of sheetlike material.

The material of fraction II (Fig. 3) consisted mainly of sheets of various size. There was no evidence of the fibrous strands noted in fraction I. Spherical particles were noted in this material; these are probably starch granules. Small amounts of the sheetlike material characteristic of fraction II were also seen in the micrograph of fraction I (see Fig. 2).

Fraction III (Fig. 4) comprised leaflike material associated with small quantities of fibrous protein. Many small spherical particles (5 to 10 μ diameter) are seen in this material. In contrast to the structure shown in Fig. 2, the spherical particles (Fig. 4) are not linked by fibrous strands.

Fraction IV (Fig. 5) was much more uniform and characteristically different from fractions I-III. No distinct structure was evident; the material appeared to be relatively amorphous.

By way of comparison and contrast, the micrograph of purified glutenin showed a very interesting regular structure (Fig. 6). This protein comprises mainly thick fibers (10 μ diameter) entangled with thin fibrous strands (1 μ). The fibers are generally unidirectional and give the impression of a cooperative aggregation in the direction of the fibers. This glutenin, which was shown by amino acid analysis and disc-gel electrophoresis to be a much purer glutenin than fraction I obtained by gel filtration (3), appears fairly uniform under the SEM. The marked structural difference between fraction I protein (Fig. 1) and purified glutenin prepared by pH precipitation from a gluten ball (Fig. 6) is attributed to their methods of preparation. Formation of a gluten ball, and the purification steps involved in the pH precipitation procedure, yield a fibrous, fairly uniform glutenin compared to the gel filtration technique.

Although all fractions examined in Figs. 2-5 contained a variety of proteins, many distinct microscopic structures were evident. In this regard the SEM could be a useful tool for studying the structure of flour proteins in conjunction with other physicochemical methods.

Acknowledgments

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