Electron Microscopy of Endosperm Protein from Hard and Soft Wheats[1]

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ABSTRACT

Protein either in flour particles or in thin sections spreads on water as a surface dispersion. These protein dispersions were transferred to plastic-covered specimen grids and examined by transmission electron microscopy. Examinations showed that wheat flour contained lipid which formed small droplets ranging from 0.01 to 0.35 μ in diameter. Protein bodies like those found in immature wheat were not observed in any of the preparations. A purified fraction of gliadin contained particles 20 to 80 A in diameter, representing molecular weights between 17,000 and 216,000; whereas a glutenin fraction did not form small particles. Protein dispersions from hard and soft wheat had particles 65 to 250 A in diameter; hard wheat had smaller particles than the soft wheat. Mild treatment with $NaHSO_3$ increased the size of hard wheat protein particles; those in soft wheat did not change much. From these observations, hard wheat appears to have compact protein particles which swell in $NaHSO_3$, whereas the structure of soft wheat particles is less dense and they swell in distilled water.

In the electron microscope, no morphological variations have been observed which would indicate that wheat protein is a complex mixture of various molecular types. The developmental work by Buttrose (1) and Jennings et al. (2) indicates that all the protein is deposited in distinct bodies and that at maturity, these protein bodies fuse to form the matrix protein of the endosperm. Because ordinary methods were ineffective in differentiating wheat protein, a surface dispersion method was investigated. This work has been directed toward finding basic differences in wheat protein from various wheat varieties.

MATERIALS AND METHODS

Wheat samples used in this study were: Bison (HRW), Justin (HRS), Thorne (SRW), Avon (SWW), and Leeds (durum). Approximately 5-lb. samples were milled on a Buhler laboratory mill to 70% extraction.

Gliadin and glutenin were fractionated from Ponca (HRW) wheat gluten by the method of Jones et al. (3).

Preparation of Surface Dispersions

A few flour particles were dropped onto the surface of a 0.05% $CaCl_2$ solution contained in a small (1.5-in. diam.) glass beaker. An immediate spreading of the protein was observed under a dissecting microscope. A carbon-stabilized Formvar-coated copper grid was placed on a selected area to stabilize the protein from further movement. The grid was then picked up, placed on a drop of staining solution, rinsed with distilled water, air-dried, and examined in the electron microscope (RCA-EMU3F) at 50 kv.

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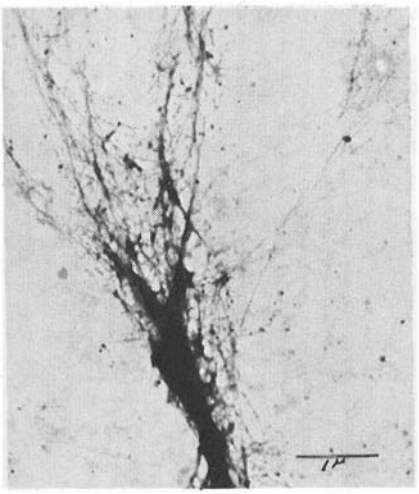
For more uniform samples, thin sections of wheat endosperm were used instead of flour particles. Selected kernels typical of the variety being studied were mounted in a pin vise. A small area about 150 by 300 μ midway up the cheek region was exposed for sectioning. The pin vise was modified so that it could be screwed into the head of a Porter-Blum ultramicrotome. Sections approximately 0.25 to 0.50 μ thick were cut with a glass knife. The untreated sections were transferred to the surface of a large drop of water (0.05% $CaCl_2$) resting on Parafilm. The protein film was transferred to a specimen grid as described above. The liquid in the drop was replaced with staining solution so that the specimen grid was not disturbed. Staining solutions consisted of 2% osmium tetroxide, 1% uranyl acetate, and 1% potassium permanganate.

RESULTS

In the developing wheat endosperm, storage protein is deposited in globular bodies varying in size from a fraction of a micron to more than 15 μ (1). Some protein bodies are formed within a membrane enclosure; others form in the cytoplasm without a recognizable membrane. At maturity, the protein bodies fuse to form the protein matrix without any structural features which would indicate that it was composed of separate bodies. Except for scattered lipid inclusions, the protein matrix appears homogeneous with standard electron microscope techniques (4). By using a surface dispersion of protein, a different approach can be taken for examining wheat protein. Figure 1 is an example of a protein surface dispersion as seen in the electron microscope. Here, untreated flour particles were dropped onto



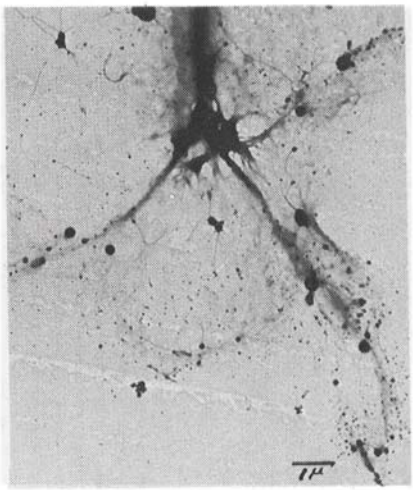


Fig. 1 (left). Surface dispersion of Justin (HRS) protein from wheat flour on 0.05% $CaCl_2$ solution. Stained with 1% uranyl acetate (10,000X).

Fig. 2 (right). Surface dispersion of Justin (HRS) protein from wheat flour on 0.05% $CaCl_2$ solution. Stained with 2% osmium tetroxide (5,000X).

a water (0.05% $CaCl_2$) surface, then stained with 1% uranyl acetate. The outer portion of the protein mass quickly extends over the surface to form a network and long strands. At the extremities, the protein may disperse to form a monomolecular layer.

A small percentage of lipid material in wheat protein has been recognized as one factor influencing dough-forming properties. Grosskreutz (5) proposed a lipoprotein model for doughs, describing platelets separated by lipid; however, he was not able to confirm this model by electron microscopy. To investigate this proposal further, a surface dispersion was stained for lipid material (Fig. 2). This preparation was stained with osmium tetroxide, which is a general fixative and stain for most organic material. Unsaturated lipids are the most osmiophilic substances in this preparation and appear as small round droplets ranging from 0.01 to 0.35 μ in diameter. Because of the abundance of these microdroplets, it is believed that they originate from the germ and aleurone and that during milling they are smeared on the flour particles. When wheat endosperm was dissected, no droplets were observed. The lipid nature of the round droplets was confirmed when similar material was extracted with petroleum ether and no droplets were present. Lipid layers or coatings on protein strands were not observed in these preparations.

The major wheat protein fractions are gliadin and glutenin. Gliadin is extracted from gluten with 70% ethanol, whereas glutenin is insoluble in this solvent. Grosskreutz (6) examined purified samples of these proteins by spraying dilute

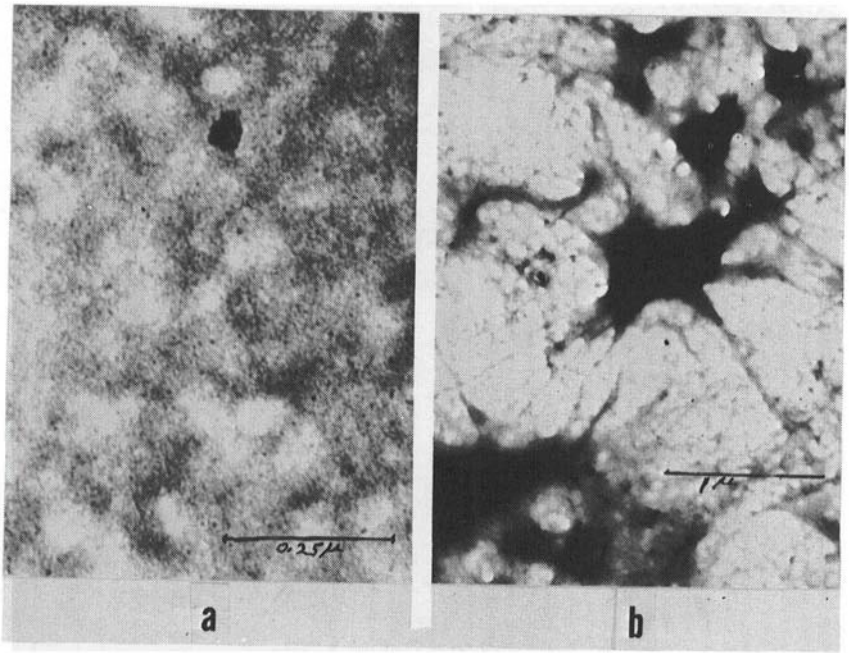


Fig. 3. a, Purified gliadin spread on 0.05% $CaCl_2$ solution. Stained with uranyl acetate (93,000X). b, Purified glutenin spread on alkaline solution, KOH (pH 9). Stained with 1% uranyl acetate (21,000X).

solutions on parlodion films and making shadowed and negative-stained preparations. He found that glutenin particles were about 100 A in diameter and that the gliadin particles were doughnut-shaped and were about three times as large as the glutenin. Similar fractions were spread on an aqueous surface, stained with uranyl acetate, and examined in the electron microscope. No comparison could be made with Grosskreutz' observations. In Fig. 3, a and b show the difference in the spreading properties of the two proteins. Gliadin forms a film over the water, whereas glutenin forms strands. The spreading property of glutenin increases when the water is made slightly alkaline (pH 9). Little spreading took place on distilled water. Closer observation of the gliadin preparation showed that it was composed of small particles varying in size from 20 to 80 A in diameter (Fig. 3, a). This size represents molecular weights from about 17,000 to 216,000[2]. The small particles could be the basic gliadin particle, since they are in the range reported by other workers (7). The glutenin fraction did not show any small particles when spread on a liquid surface. This lack of particles is probably due to the molecular arrangement of the protein and to the strong cohesive forces that hold it together.

Hard and soft wheat proteins were examined to determine if any differences in structure existed which would relate to protein quality. Comparable material was obtained by cutting 0.25-μ sections from the central portion of a wheat kernel. After the protein was dispersed on the water surface, it was stained with 1% potassium permanganate. In Fig. 4, a, b, c, and d show the stained protein from hard, soft, and durum wheats. In these electron micrographs, the protein can be seen spreading out from a mass to form films, strands, and small particles. Since some of the low-molecular-weight proteins may have dissolved, the high-molecular-weight particles stand out more clearly. The protein particles range in size from about 65 A to more than 250 A in diameter and represent molecular weights from 125,000 to more than 8 million. On the one hand, particles from Thorne (SRW), Avon (SWW), and Leeds (durum) were similar in size distribution (Fig. 4, b, c, d); on the other hand, Bison (HRW) had smaller particles (Fig. 3, a). The soft wheats had more patches of protein film than the hard and durum wheat proteins.

In wheat it is known that disulfide-bonding links small molecular protein species into large insoluble molecules. Treatment with reducing agents, such as bisulfite, cleaves the disulfide bonds and separates the protein into units of smaller molecular weight (8). When bisulfite was tried on dispersed protein of Bison wheat, the protein particles became larger and more distinct than untreated ones. In Fig. 5, a and b show proteins treated with 3% $NaHSO_3$ (pH 8) for 5 and 15 min. and then stained with 1% potassium permanganate. In comparing Fig. 4, a with Fig. 5, a, the clarity with which each particle is seen in the treated samples indicates that some background material had been dissolved. Increasing the time from 5 to 15 min. also increased particle size. Presumably, since only a fraction of the disulfide bonds were affected, swelling of the protein particles resulted. Except for size, there appeared to be no chemical distinction between protein particles. This observation suggests

[2]Molecular weights of protein particles were calculated on the basis of a spherical particle having a density of 1.34.

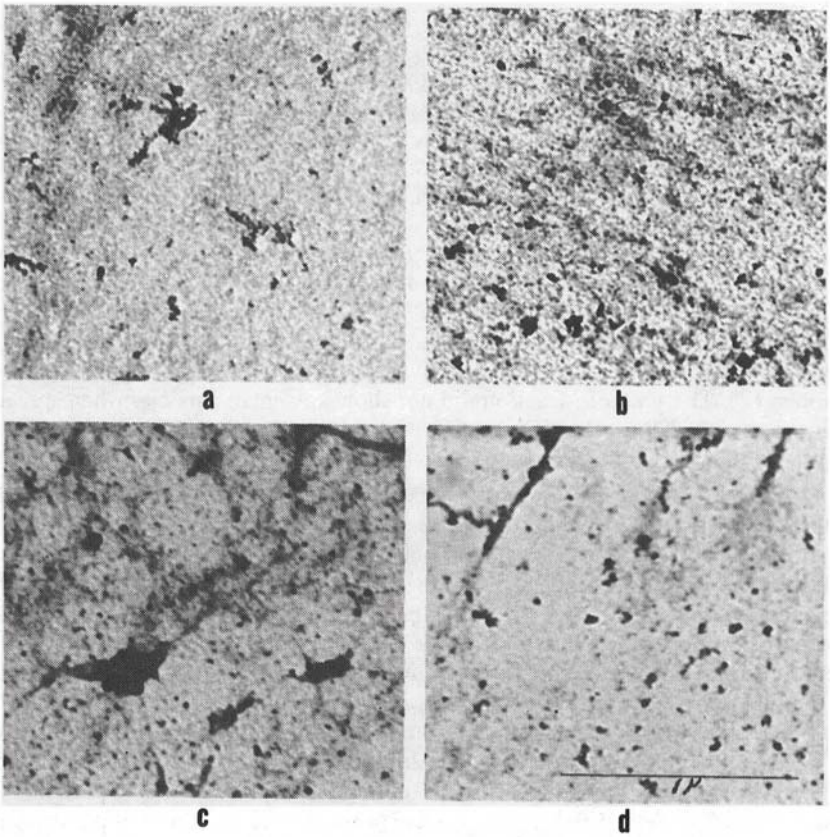


Fig. 4. Surface dispersion of various wheat proteins from thin sections on 0.05% $CaCl_2$ solution. Stained with 1% potassium permanganate (32,400X). a, Bison; b, Thorne; C, Avon; d, Leeds.

that all the particles have similar structures and are composed of similar protein units.

When soft wheat protein was treated with $NaHSO_3$, the results were similar to those for hard wheat: background protein was dissolved and the remaining particles were shown in good contrast. The size of the treated particles was not noticeably different from that of the untreated sample; however, they were smaller than the protein particles of hard wheat. From these observations it appears that bisulfite has more effect on the protein of hard wheat than on soft wheat. In these experiments there was no way to determine how much protein had been dissolved.

Another approach for examining wheat protein involves quick fixation and staining. Wheat sections were placed directly on a solution of 1% potassium permanganate to prevent excessive spreading of the protein. Figure 6 shows proteins from hard, soft, and durum wheat. The degree of spreading differed for each sample. Hard wheat protein still has the general outline of the original wedge

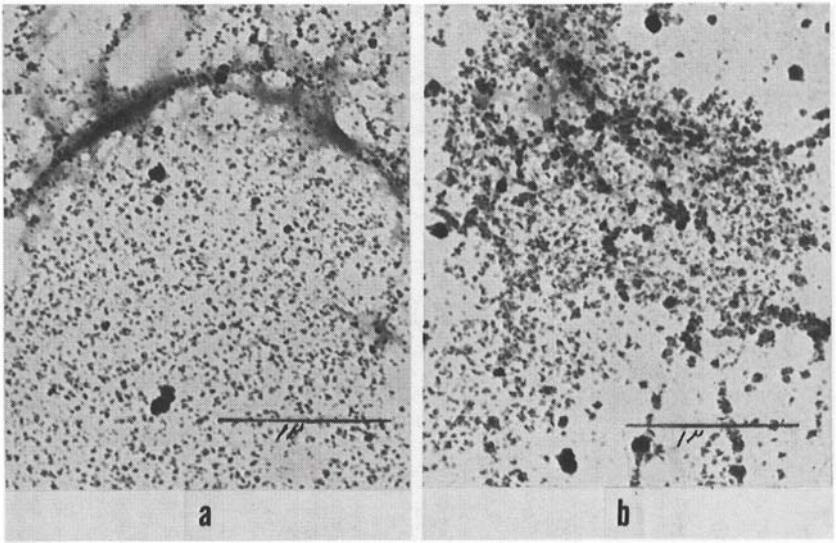


Fig. 5. Surface dispersion of protein from sections of Bison wheat on 0.05% $CaCl_2$ solution. Stained with 1% potassium permanganate (23,000X). a, 5-min. treatment with 3% $NaHSO_3$, pH 8; b, 15-min. treatment with 3% $NaHSO_3$, pH 8.

protein. The margins remained thick in contrast to the rest of the protein, which spreads out to form a network. The protein is comprised of small particles about 250 A in diameter. Durum wheat protein spread more than the hard wheat (Fig. 6, d) to form a larger network pattern. Small protein particles are not so pronounced as those of hard wheat. The more dense areas show only a smooth film of protein. Protein from soft red winter wheat (Fig. 6, b) retained its original shape but had diffused margins in contrast to hard wheat. Protein particles were hard to detect because of the lack of contrast in the protein. The most disrupted protein structures occurred with the soft white winter wheat (Fig. 6, c). The protein spreads out in a diffused mass with little resemblance to the original shape of the protein. Protein particles are not distinct; they have little contrast and blend together to form hazy patches. None of the protein samples showed any evidence of protein bodies as seen in immature wheat.

DISCUSSION

Possibly the differences in wheat protein quality depend more on structural than on chemical variations. Therefore, some aspects of macromolecular organization of wheat protein were examined with an electron microscope. Instead of the embedding-sectioning technique, a surface dispersion of protein was used. This mild treatment offers many possibilities for examining protein under various conditions. Observations were first made on plain protein in an effort to locate protein bodies. None of the preparations showed any signs of protein bodies that exist in immature wheat. There were, however, lipid droplets in flour which were released from the germ and aleurone cells during milling. If this observation is

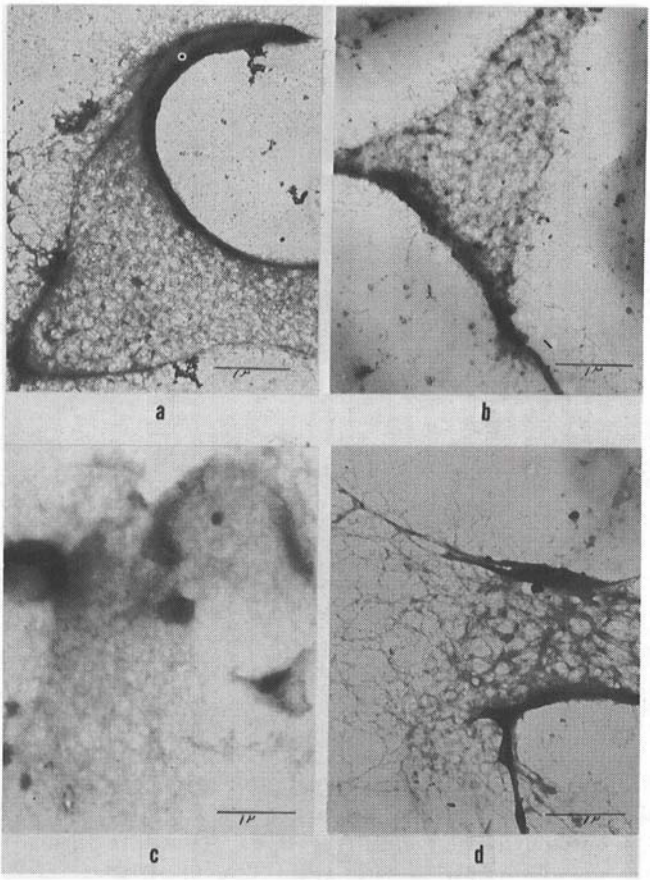


Fig. 6. Protein from wheat sections placed directly on 1% potassium permanganate solution (10,200X). a, Bison; b, Thorne; c, Avon; d, Leeds.

correct, the lipid content of flour can be influenced by making changes in the milling process.

A significant observation is the small particles which form on the water surface (Fig. 4). These particles range in size from 65 to 250 A in diameter and are similar to those found in protein bodies of immature wheat (1). It is believed that these particles are a basic unit of storage protein in wheat. The observations presented here indicate that differences exist between protein particles of the various hard and soft wheats. Particles from hard wheat are compact structures difficult to disrupt, whereas the protein particles from soft wheat are expanded and easy to disrupt. This idea has been previously mentioned by Mecham et al. (9) and more recently by Tsen (10); they report that protein of soft wheat is more soluble than protein of hard wheat. Tsen also contends that the mechanical action of dough-mixing may disrupt large protein aggregates into smaller units by tearing and shearing, by scission of noncovalent bonds, or by reduction of disulfide bonds.

Another aspect of wheat protein is noted by Morton and Raison (11) and by Jennings (12), who found that the gliadin fraction increases during the later stages of kernel maturation. Graham et al. (13) indicate that the small protein bodies contain more gliadin than the large bodies. These findings suggest that small areas of the mature protein matrix would be alcohol-soluble and that evidence of this solubility can be seen in the electron microscope. At present no evidence of gliadin-rich bodies has been found, and no distinction can be made regarding solubility properties of protein particles. It remains for future work to characterize the solubility properties of the protein particles.

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