

Gradient Ultracentrifugation in the Characterization of the Acetic Acid-Insoluble Proteins of Wheat Flour¹

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ABSTRACT

The acetic acid-insoluble gel proteins of wheat flour were solubilized with 0.2 mM. mercuric chloride-0.01M acetic acid. Ultracentrifugation analyses with sucrose gradients (with and without acrylamide) at pH values of 3.3 and 8.8 showed that the bulk of these proteins sedimented at a rate equal to that of ovalbumin (MW 40,000 to 44,000). Small quantities of higher-MW protein that did not migrate electrophoretically into polyacrylamide gels were also present.

When wheat flour is extracted exhaustively with 0.01M acetic acid, all but 20 to 30% of the total flour protein is solubilized. Mecham et al. (1,2) have shown that the acetic acid-insoluble proteins remaining in the highly hydrated flour residue (flour "gel" proteins) are related to the dough-mixing properties of a flour. Solubilization of these proteins in 50% acetic acid and preliminary crude fractionation of them on molecular sieves was achieved by Inamine et al. (3). By agarose column chromatography (MW exclusion limit of 30×10^6), they found that both retarded and excluded proteins were present in the "gel" fraction. Also, one intermediate fraction appeared to emerge in the 30,000-to 60,000-MW range, with polyacrylamide-gel chromatography. However, because these workers were primarily interested in protein isolation and fractionation studies, they did not define further the MW range and number of components for these gel proteins. In the present work we have solubilized these flour "gel" proteins with dilute aqueous mercuric chloride (4), a solvent unlike that used by the above workers. Also, we have found that sucrose gradient ultracentrifugation and modification, i.e., gradient ultracentrifugation by acrylamide gels (5), can be used to partially resolve these protein mixtures and obtain an estimation of their MW. The results of this study are described and discussed in this paper.

MATERIALS AND METHODS

Proteins were extracted from the acetic acid-insoluble gel fraction of an unbleached commercial spring wheat bread flour with 0.2 mM. mercuric chloride-0.01M acetic acid as solvent (4). (The higher concentration of mercuric chloride was used in this work to avoid the need for excessive volumes of solvent.) The resulting lyophilized protein preparations contained 3% carbohydrate (based on xylose). Sucrose gradients [8 to 40%, containing 7.5% acrylamide and tris-(hydroxymethyl)aminomethane (TRIS) buffer, pH 8.8] were performed in 5-ml. polyallomer tubes with a Buchler gradient mixer (5). In some runs, aluminum lactate buffer of 0.0085M final concentration, pH 3.3, was substituted for TRIS buffer. The protein dissolved in both of these solvents after preliminary

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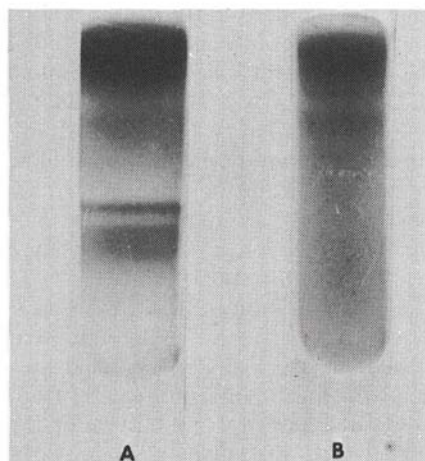


Fig. 1 (left). Acetic acid-soluble protein centrifuged in sucrose gradient (8 to 40%) containing polyacrylamide. A. Contains TRIS buffer, pH 8.8. B. Contains aluminum-lactate buffer, 0.0085M, pH 3.3.

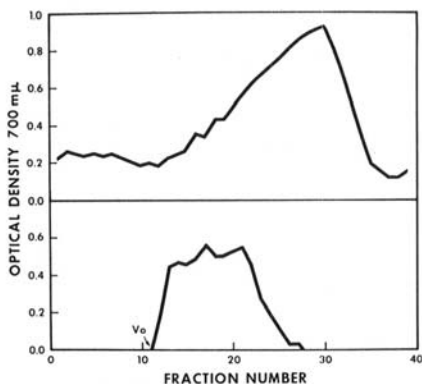


Fig. 2 (right). Wheat-flour gel-protein distribution curves. Top: Ultracentrifugation of gel protein in sucrose gradient (8 to 40%), pH 3.3. Fraction 1 was from the bottom of the centrifuge tube, fraction numbers increasing toward the meniscus. Bottom: Eluate profile of gel protein from Sephadex G-200, pH 3.3; V_0 indicates the void volume.

stirring, indicating that no deleterious effects had occurred through lyophilization. One percent solutions of the gel proteins were prepared in the appropriate buffer containing 7.5% acrylamide and layered on top of these gradients. The Beckman L-4 preparatory ultracentrifuge equipped with a SW-65 rotor was used at a force of $283,000 \times g$ for 17 hr. at 4°C . At the end of this time the tubes were removed from the centrifuge and exposed to light in order to polymerize the acrylamide, form a gel, and fix the protein bands in position (5). In other experiments the acrylamide was omitted, and at the end of centrifugation the tubes were punctured, fractions were removed dropwise from the bottom, and their protein content determined by the Lowry method (6). Protein fractions were examined by horizontal gel electrophoresis according to Inamine et al. (3), with the exception that polyacrylamide was substituted for starch as the supporting medium and 4M urea was used in a 0.0085M lactate buffer. Runs were made for 5 hr. at 400 v. For the gel-filtration experiment, Sephadex G-200 (Pharmacia) was allowed to swell for 48 hr. in excess 0.0085M aluminum lactate buffer, pH 3.3, and then packed in columns 1.4×109 cm. Two milliliters of a 2% solution of flour "gel" protein dispersed in the above buffer was applied to the top of the column, and elution was accomplished with buffer. Four-milliliter aliquots were collected and protein was determined as before. The ovalbumin used as a reference protein was obtained from Worthington Biochemical Corp.

RESULTS AND DISCUSSION

Figure 1 shows photographs of the wheat flour gel proteins sedimented in the sucrose gradient and fixed in polyacrylamide. At both pH 8.8 and 3.3 the bulk of the proteins moved slowly, indicating that most of them had a relatively low MW.

When a tube that was run at pH 3.3 (without acrylamide) was punctured and its contents monitored for protein, a small amount of higher-MW material was found at the bottom of the tube in addition to the large amounts of lower-MW protein near the meniscus (see Fig. 2, top). High-MW protein was also found in the flour-protein mixture when it was subjected to gel filtration (see Fig. 2, bottom). The appearance and rapid increase in protein concentration when the void volume was reached showed that some protein was excluded from the Sephadex G-200 molecular sieve beads. However, high- and low-MW components were not well separated, and in this respect gradient ultracentrifugation gave a clearer picture of the weight distribution of flour "gel" protein than gel filtration.

When fractions taken from the main protein band in a tube ultracentrifuged at pH 3.3 (as in Fig. 1B) were examined by gel electrophoresis, the patterns showed that partial resolution of components had occurred (see Fig. 3). This band area (fractions 17 through 32) consisted of protein components differing slightly from one another in mobilities. The high-MW protein fractions near the bottom of the centrifuge tube did not migrate electrophoretically into the polyacrylamide gel. In Fig. 3 these fractions are below fraction 16, and are not shown in the photograph.

With the run made in TRIS buffer, pH 8.8, some protein sedimented away from the bulk of the material, forming one sharp band and some more-diffuse ones (Fig. 1). Apparently part of the protein had aggregated into higher-MW species at this pH. Figure 4 shows that a reference protein, ovalbumin (MW = 42,000), sedimented at pH 8.8 at a rate almost equal to the bulk of the flour "gel" protein. If one assumes that these wheat proteins are compact in nature, then the majority of them have MWs of approximately 40,000 to 44,000. Somewhat similar protein fractions obtained from wheat flours by Stanley et al. (7) were found by standard ultracentrifugation studies to contain components with MWs varying from 14,000 to 30,000. The extracting solvent used by these workers was phenol-acetic

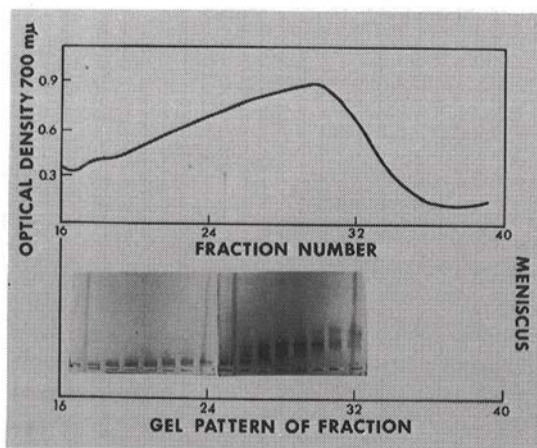


Fig. 3. Top: Protein-absorption curve of fractions taken from centrifuge tube containing acetic acid-insoluble protein sedimented in sucrose gradient (8 to 40%), aluminum lactate 0.0085M, pH 3.3. Bottom: Polyacrylamide gel electrophoresis pattern of above protein fractions. Gels run in urea, 4M-aluminum lactate, 0.0085M, pH 3.3

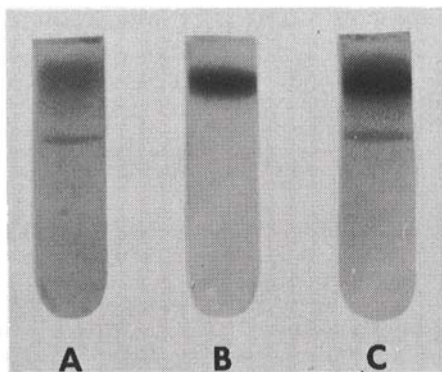


Fig. 4. Centrifugation runs in sucrose gradient (8 to 40%) containing polyacrylamide and TRIS buffer, pH 8.8. A. Acetic acid-insoluble protein. B. Ovalbumin. C. Acetic acid-insoluble protein plus ovalbumin.

acid-water, a mixture that exerts dissociative effects on proteins. The dissimilarities of extraction media being considered, our MW values for most of the gel proteins are reasonably near to the results of the above workers. However, high-MW material such as we detected was not found in their work. The faster moving protein band at pH 8.8, Fig. 1A, had a MW of 145,000 calculated according to Martin and Ames (8). Further work is underway to examine this fraction by gel electrophoresis.

To our knowledge, density gradient ultracentrifugation with acrylamide has not been used before to study the sedimentation behavior of wheat flour proteins. The extension of the technique to the study of wheat proteins other than those investigated here should prove to be of value, particularly where it is desirable to obtain rough MW estimates.

Acknowledgment

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