

Studies of Glutenin. V. Note on Additional Preparative Methods ¹

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ABSTRACT

Three methods, namely, gel filtration chromatography, pH precipitation with purification on SE Sephadex C-50, and ammonium sulfate precipitation, were compared for preparing glutenins from durum wheat. Polyacrylamide gel electrophoresis, sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and amino acid analyses showed that the third method, which is considerably easier to perform, produces relatively pure glutenin.

The well-established Osborne fractionation technique (1) and newer, more elaborate methods of preparing glutenin by gel filtration (2,3) and pH precipitation (3) require lengthy dialysis to remove salt ions prior to freeze-drying. In the course of studies of the glutenin of durum wheats, a rapid method was developed based on precipitation with ammonium sulfate. Results obtained with this method are described.

MATERIALS AND METHODS

The flour was milled from Stewart 63, a variety of Canadian amber durum wheat, in a Buhler experimental mill. The flour contained 11.3% protein ($N \times 5.7$) and 0.60% ash, both on a 14% moisture basis.

Initial Extraction

AUC (0.1M acetic acid, 3M urea, and 0.01M cetyltrimethylammonium bromide)

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extracts of flour proteins were prepared by the procedure of Meredith and Wren (4) as modified by Bushuk and Wrigley (2).

Gel Filtration of AUC Extracts

The gel filtration method used to fractionate the protein extracts into glutenins, gliadins, albumins, and nonprotein nitrogen was as described by Orth and Bushuk (3). Glutenin and gliadin obtained by this method will be designated as F₁ and F₂, respectively.

pH Precipitation from AUC Extracts

The method of Orth and Bushuk (3) based on pH precipitation and ion-exchange purification (pH glutenin) was evaluated, and further modified by using trichloroacetic acid (TCA) to precipitate glutenins in place of the dialysis and freeze-drying steps (pH-TCA glutenin). This modification produced a significant saving of time.

Precipitation from AUC Extracts with Ammonium Sulfate

This method was introduced in the present study. Ammonium sulfate (1.5 g.) was slowly added with stirring to 17 ml. of extract containing the protein from 1 g. of flour. The mixture was allowed to stand overnight at 4°C. The voluminous precipitate that formed, P₁, was removed by centrifugation at 20,000 × g for 15 min. The supernatant was treated with a second portion of 1.5 g. of ammonium sulfate; the second precipitate, P₂, was allowed to settle and centrifuged. The procedure was repeated a third time to yield precipitate P₃. Each precipitate was washed three times with 10 ml. of 70% ethanol to remove residual solvent (dialysis was not required) and dried in a vacuum at room temperature.

The first precipitate was further purified by dissolving it in 17 ml. of AUC and reprecipitating with 1.5 g. of ammonium sulfate. The precipitate designated PP₁ was separated, washed, and dried as described above.

Polyacrylamide Gel Electrophoresis (PAGE)

The method of Davis (5) as modified by Chen and Bushuk (6) was used. According to this method a 7.5% polyacrylamide gel with a running pH of 2.7 is used. All runs were performed under identical conditions and for equal times. The gels were stained with Coomassie Brilliant Blue R250.

Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE)

The method of Weber and Osborn (7), as applied to glutenin by Bietz and Wall (8) and Orth and Bushuk (3), was used to determine the number and the approximate molecular weights of the subunits of each glutenin preparation after prior reduction with β-mercaptoethanol. For developing the protein bands the following aqueous solutions were used:

Solution 1	Isopropyl alcohol	25%
	Acetic acid	10%
	Coomassie Blue	0.05%
Solution 2	Isopropyl alcohol	10%
	Acetic acid	10%
	Coomassie Blue	0.005%

Solution 3	Acetic acid	10%
	Coomassie Blue	0.0025%
Solution 4	Acetic acid	10%

The treatment periods were as follows:

Solution 1	overnight with shaking
Solution 2	6-9 hours with shaking
Solution 3	overnight with shaking
Solution 4	several hours (or until background is clear) with shaking.

All staining and destaining were done at room temperature.

Amino Acid Analysis

The proteins were hydrolyzed under vacuum in 6N HCl for 24 hr. at 110°C. In this hydrolysis procedure, any urea or ammonium sulfate in the protein sample is converted to ammonium chloride. Since some of the samples contained traces of urea and ammonium sulfate, the amino acid contents were reported on an ammonia-free basis. Only those amino acid contents which differed by more than 6% (2 σ or 95% confidence limit) were considered significant.

RESULTS AND DISCUSSION

Glutenin Prepared by Gel Filtration

The elution profile for the AUC extract of flour on Sephadex G-100 was essentially the same as that reported by Bushuk and Wrigley (2) for whole ground Stewart 63 wheat. Four fractions (F_1 to F_4) were obtained. As suggested by Meredith and Wren (4), F_1 was regarded to be glutenin, F_2 as gliadin, F_3 the albumins, and F_4 the nonprotein nitrogen.

As expected, on electrophoresis on polyacrylamide gel, most of fraction F_1 did not enter the separating gel (results not shown). When SDS-PAGE was performed on the reduced F_1 proteins, 14 subunits were detected (Fig. 1). Comparison of the amino acid profile of F_1 and F_2 (Table I) showed that F_1 is lower in glutamic acid, proline, and phenylalanine and higher in threonine, serine, glycine, alanine, methionine, leucine, and tyrosine. These differences are in general agreement with the results obtained by Ewart (9) for glutenin and gliadin.

pH Precipitation and Purification by Ion-Exchange

As was obtained for F_1 , the glutenin prepared by pH precipitation with purification by ion exchange also had no components that entered the gel on PAGE. The SDS-PAGE patterns of these two glutenins were essentially identical (Fig. 1; compare F_1 with pH).

The glutenin prepared by precipitating solubilized, ion-exchange-treated pH precipitate with TCA (pH-TCA glutenin) was not examined by PAGE and amino acid analysis. The SDS-PAGE pattern of the pH-TCA glutenin was substantially identical to the pattern obtained for F_1 and pH glutenin (compare F_1 , pH, and pH-TCA in Fig. 1).

Glutenin prepared by pH precipitation, compared to F_1 glutenin, had a lower content of glutamic acid, proline, and methionine, and was higher in lysine,

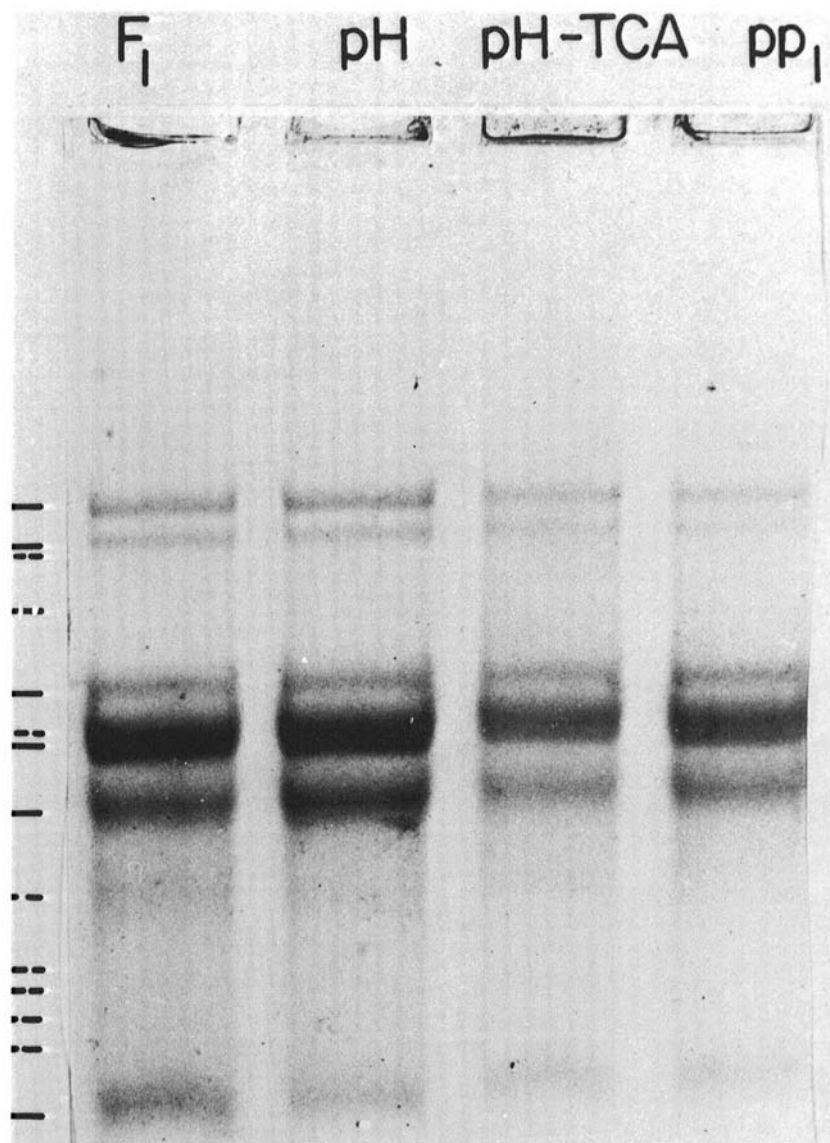


Fig. 1. SDS-PAGE patterns of reduced glutenins prepared by four methods: F₁, glutenin prepared by gel filtration chromatography on Sephadex G-100; pH, glutenin prepared by pH precipitation and purification with SE-Sephadex C-50; pH-TCA, glutenin prepared by substituting TCA precipitation for the dialysis and freeze-drying steps of the pH precipitation-ion exchange purification technique; and PP₁, glutenin prepared by fractional ammonium sulfate precipitation of P₁.

histidine, arginine, aspartic acid, threonine, alanine, valine, isoleucine, leucine, and phenylalanine (see Table I). On the other hand, F₁ protein had a higher content of

TABLE I. AMINO ACID COMPOSITION OF PROTEIN FRACTIONS EXPRESSED AS MOLE PERCENT ON AN AMMONIA-FREE BASIS^a

	F ₁	F ₂	pH	P ₁	PP ₁
Lysine	1.41	1.35	4.35	1.13	4.35
Histidine	1.60	1.72	2.02	1.48	2.20
Arginine	2.69	2.60	4.43	1.81	4.36
Aspartic acid	3.04	2.91	6.51	2.56	7.03
Threonine	3.19	2.62	4.29	3.20	4.18
Serine	6.97	5.99	6.83	7.22	6.11
Glutamic acid	34.5	38.4	23.0	37.5	20.4
Proline	12.3	13.0	8.63	13.5	8.03
Glycine	8.09	6.18	8.43	7.77	8.65
Alanine	3.97	3.36	6.44	3.00	6.96
Valine	4.14	3.76	5.26	3.37	5.97
Methionine	1.45	1.22	0.84	1.42	2.20
Isoleucine	3.30	3.36	4.25	3.12	4.12
Leucine	7.11	6.23	8.06	6.54	8.39
Tyrosine	2.88	2.47	2.72	2.72	3.15
Phenylalanine	3.50	4.79	3.90	3.61	3.85

^aTryptophan, cysteine, and cystine were not determined.

those amino acids which are characteristic of gliadin and was lower in those that are characteristic of glutenin.

Ammonium Sulfate Precipitation

PAGE patterns (Fig. 2) of P₁, P₂, and P₃ revealed that P₁ was mainly glutenin, P₂ was mainly gliadin, and P₃ was a mixture of gliadin and albumin. The impurities in fraction P₁ are believed to be gliadin proteins since this fraction had a higher content of those amino acids that differentiate gliadin from glutenin. Comparison of the amino acid composition of P₁ with those of F₁ and F₂ showed that P₁ resembles F₂ rather than F₁ (see Table I).

The gliadin-like impurities in P₁ were removed by reprecipitation from AUC. The reprecipitated glutenin (PP₁) did not enter the gel during electrophoresis (see Fig. 2). PP₁ accounted for 27% of the total flour protein. SDS-PAGE of reduced PP₁ protein gave a pattern that appeared to be identical to the patterns of reduced glutenins prepared by gel filtration and pH precipitation (Fig. 1).

The amino acid compositions (Table I) showed that PP₁ protein was lower in serine, glutamic acid, and proline and higher in lysine, histidine, arginine, aspartic acid, threonine, alanine, valine, methionine, isoleucine, and leucine than F₁. When compared with pH glutenin, PP₁ protein was lower in glutamic acid and higher in valine, methionine, and tyrosine. Glutenins prepared by gel filtration and pH precipitation are higher in those amino acids that characterize gliadin and lower in those that characterize glutenin when compared with glutenin prepared by the double precipitation with ammonium sulfate. Accordingly, the glutenin represented by PP₁ is considered to be a relatively pure form of glutenin.

SUMMARY

Two rapid and efficient techniques were described for preparing glutenin, the TCA modification of the pH precipitation method proposed by Orth and Bushuk (3), and precipitation from AUC extracts with ammonium sulfate. For preparative work the latter method is recommended since precipitates with ammonium sulfate

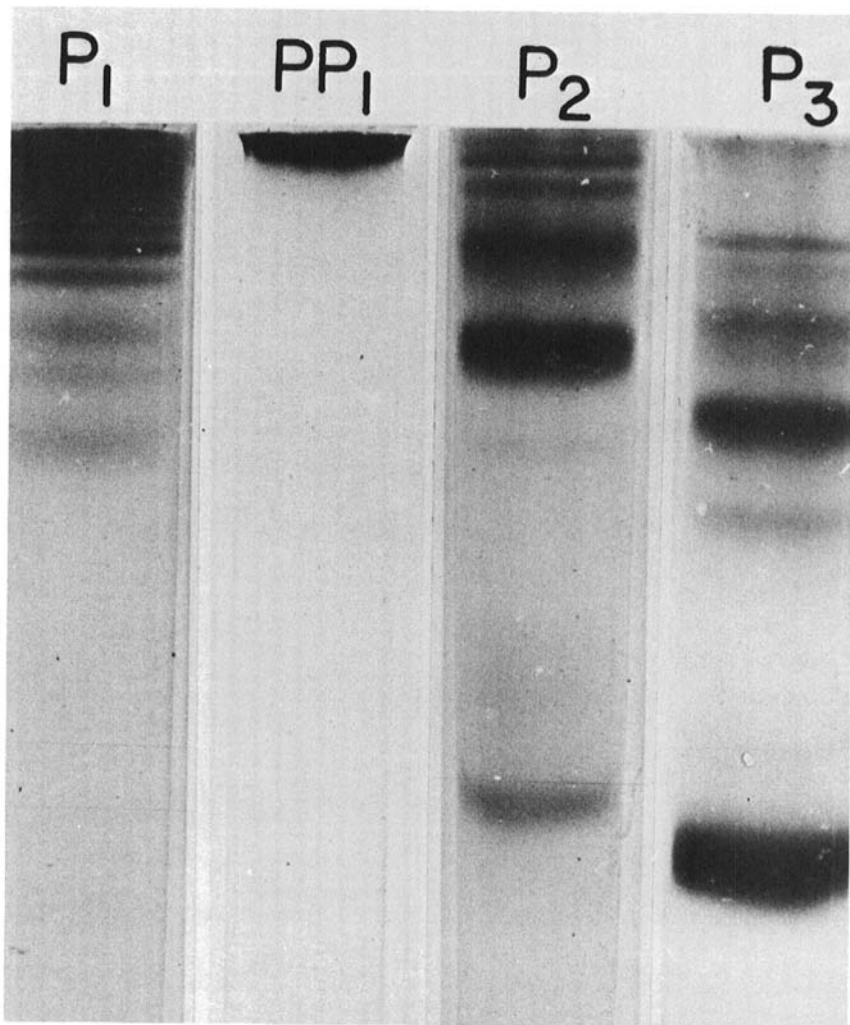


Fig. 2. PAGE patterns of proteins prepared by fractional precipitation from AUC with ammonium sulfate: P₁, first ammonium sulfate precipitate; PP₁, P₁ redissolved in AUC and reprecipitated with ammonium sulfate; P₂, second ammonium sulfate precipitate; and P₃, third ammonium sulfate precipitate.

are easily obtained and take considerably less time than other published techniques. No sophisticated equipment is required, and the procedure produces a relatively pure form of glutenin.

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