Extraction of Unreduced Glutenin from Wheat Flour with Sodium Dodecyl Sulfate

G. DANNO, Department of Agricultural Chemistry, Faculty of Agriculture, Kobe University, Nada-ku, Kobe 657, Japan

Cereal Chem. 58(4):311-313

ABSTRACT

Glutenin from wheat flour was almost completely extracted with 0.5% sodium dodecyl sulfate (SDS), pH 7.0, without prior reduction of its disulfide bonds. Wheat flour was first extracted with 0.05*M* sodium phosphate, pH 7.0, containing 0.5% SDS at a 1:20 flour/solvent ratio. About 77% of the total flour nitrogen solubilized into the clear solution. The remaining proteins in the residue, mainly glutenin, were almost completely extracted by the second extraction with 0.5% SDS, pH 7.0, by stirring with a Waring Blendor for 2 min. No foam was produced by this procedure. This extraction procedure may be useful as a simple and rapid method for separation of glutenin.

Wheat glutenin is a mixture of high molecular weight proteins containing interpolypeptide disulfide bonds. Preparation of purified glutenin is difficult because of its high molecular weight and insolubility in buffers commonly used for protein separation. The complete solubilization of native glutenin into aqueous solution is one of the most important problems in the studies on the structure and characterization of glutenin. Glutenin has been obtained by precipitation with 70% ethanol from whole gluten (Jones et al 1959) and by extraction with various solvents such as acetic acid (Bietz et al 1975), AUC-solvent (0.1*M* acetic acid, 3*M* urea, and 0.01*M* cetyltrimethylammonium bromide, Orth and Bushuk 1973), acetic acid plus mercuric chloride (Mecham et al 1972), and sodium dodecyl sulfate (SDS) plus 2-mercaptoethanol (Danno et al 1976). Because of possible contamination during extraction and purification, data on the subunit compositions of the separated glutenin reported in the literature are conflicting. In this article, a new procedure using SDS as a solvent for the extraction of the glutenin from wheat flour without prior reduction of its disulfide bonds is described.

0009-0352/81/04031103/$03.00/0

MATERIALS AND METHODS

Materials

Wheat flour experimentally milled to a 60% extraction from Canada western wheat (No. 1) was used in this study. Flours from U.S. hard red winter and U.S. western white wheat were compared with the above flour. Wheat flour was defatted by being extracted several times with dry *n*-butanol and then air-dried. SDS (99% pure) was purchased from Nakarai Chemicals, Kyoto. All other reagents were either of guaranteed reagent grade or of the best grade available.

Extraction of Glutenin

Defatted wheat flour (100 g) was suspended in 2 L of 0.05*M* phosphate buffer (Na_2HPO_4-NaH_2PO_4), pH 7.0, containing 0.5% SDS. The suspension was gently stirred with a magnetic stirrer for 50 min at room temperature and was centrifuged at 18,000 × *g* for 30 min at 15°C. A clear supernatant was obtained and was designated Fraction A. The residue was dispersed in 80 ml of 0.5% SDS, pH 7.0, by stirring at high speed for 2 min in a Waring Blendor. The slurry was diluted with 300 ml of 0.5% SDS, pH 7.0, and then gently stirred with a magnetic stirrer for 20 min and centrifuged at 22,000 × *g* for 30 min. The clear supernatant obtained was designated Fraction B. No antifoaming agent was

required because no serious foaming occurred throughout the extraction procedure. The residue was dispersed again in 200 ml of 0.5% SDS, and the slurry was centrifuged. The supernatant and the final residue were designated Fraction C and Fraction D, respectively. Fraction A and B were further fractionated by ethanol precipitation. Ethanol was added to a final concentration of 70% for Fraction A and 75% (v/v) for Fraction B. The procedure is summarized in Fig. 1.

Protein Determination and SDS Analysis

Protein was determined by micro-Kjeldahl method (N × 5.7). The SDS contents of the proteins were analyzed by the gas chromatographic method of Sigrist (1974).

SDS-Polyacrylamide Gel Electrophoresis (PAGE)

SDS-PAGE was performed on 10% polyacrylamide gel slabs (0.2 cm thick) using the discontinuous buffer system of Laemmli (1970). The protein samples (3 mg/ml) were mixed with an equal volume of a solution containing 2% SDS, 4% 2-mercaptoethanol, 0.125*M* Tris (pH 6.8), and 20% glycerol and then were heated at 80° C for 5 min before application on the gels. The gels were fixed in 10% trichloroacetic acid for 2 hr, stained with 0.04% Coomassie brilliant blue R-250 in 10% trichloroacetic acid for at least 20 hr, and then destained with 7% acetic acid. The amount of proteins was estimated with scanning at 565 nm, using a Toyo densitometer, model DMV-33C.

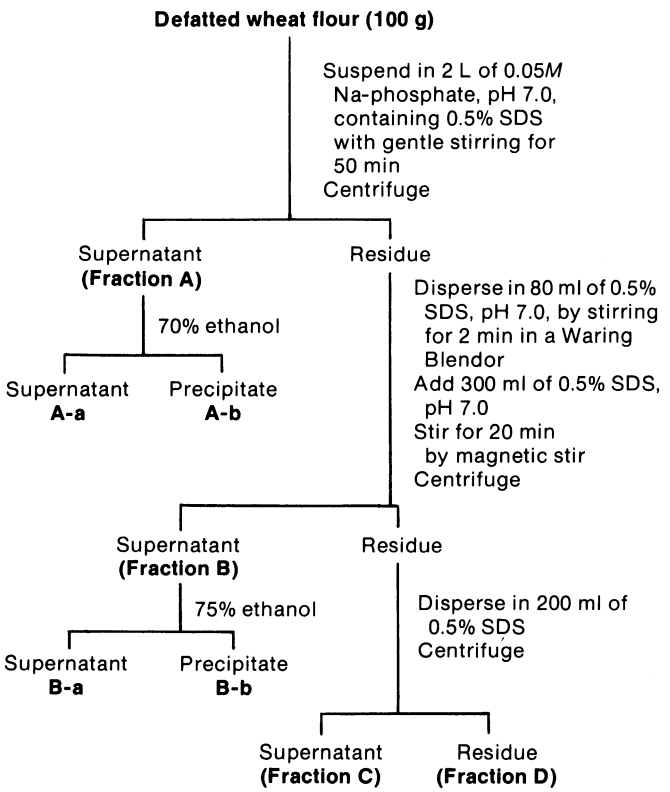

Fig. 1. Extraction of proteins from wheat flour.

TABLE I
Percentage of Nitrogen Extracted from Wheat Flour

Flour	Fraction A	Fraction B[a]	Fraction C[a]	Fraction D
Canada western wheat	77.8	19.8	0.8	1.5
Second lot	77.3	20.0	0.8	1.4
Stored two years, 5° C	76.4	21.7	1.0	1.0
Dough, lyophilized	74.2	22.6	1.0	2.2
Hard winter wheat	71.8	24.0	1.0	3.2
Western white wheat	77.8	19.0	0.9	2.4

[a] Glutenin.

Gel Chromatography of Fraction B

A Bio-Gel P-300 column (2.5 × 45 cm) was equilibrated with 0.1*M* phosphate buffer (sodium salt, pH 7.0) containing 0.5% SDS and 0.02% sodium azide. Two milliliters of the unreduced protein solution (Fraction B) was applied to the column. The elution was conducted at 25° C with the same buffer. The flow rate was 6 ml/hr, and the effluent was collected in 4-ml portions. The protein concentration of the effluent was measured at 276 nm.

Amino Acid Analysis

The protein was hydrolyzed in an evacuated sealed tube in 6*N* hydrochloric acid at 110° C for 24 hr. Analysis was performed in a Hitachi automatic amino acid analyzer, model KLA-3B.

RESULTS AND DISCUSSION

Table I shows the nitrogen yield of Fractions A–D on a total nitrogen basis. Fraction A accounted for 77% of the total flour nitrogen and Fraction B for 20%. Fraction D was starch residue. In this procedure, the flour proteins were almost completely extracted with 0.5% SDS, pH 7.0, without prior reduction of their disulfide bonds. The yield of each fraction was relatively constant and similar among three wheat flours having different baking qualities.

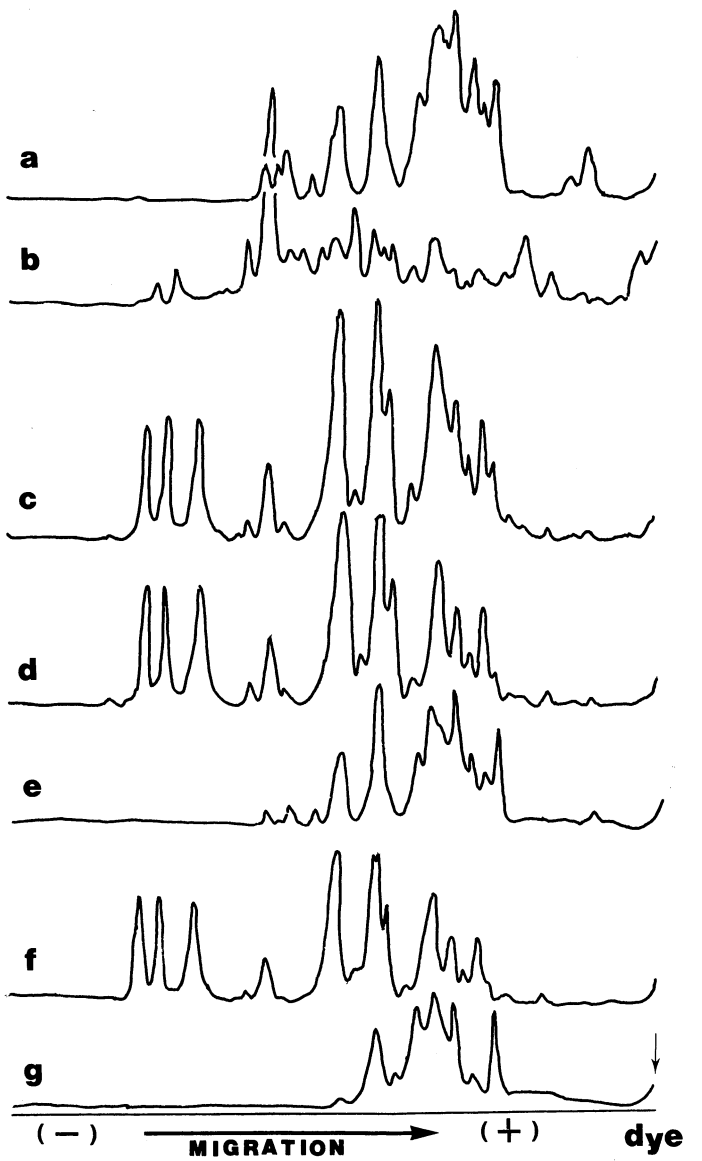

Fig. 2. SDS-PAGE patterns of reduced proteins: **a,** Fraction A-a (ethanol-soluble); **b,** Fraction A-b (ethanol-insoluble); **c,** Fraction B; **d,** Fraction B-b (ethanol-insoluble); **e,** Fraction B-a (ethanol-soluble); **f** and **g,** peaks f and g, respectively, in Fig. 3.

TABLE II
Amino Acid Composition[a] of Protein Fractions

Amino Acid	Fraction A-a[b]	A-b[c]	B-a[b]	B-b[c]	Whole B
Aspartic acid	3.28	6.19	3.08	2.48	2.65
Threonine	2.36	4.15	2.38	3.18	3.06
Serine	5.03	6.38	5.10	6.30	5.62
Glutamic acid	36.50	22.09	36.60	34.39	34.68
Proline	18.46	11.07	18.19	14.18	14.28
Glycine	3.40	9.27	3.37	9.24	8.14
Alanine	3.30	6.49	3.10	2.79	3.55
Half cystine	1.27[d]	0.83[d]	1.30[d]	0.81[d]	0.87[d]
Valine	4.11	5.63	4.67	4.18	4.15
Methionine	0.06	0.64	0.49	0.23	1.34
Isoleucine	3.90	3.91	4.13	3.17	3.08
Leucine	6.97	7.72	6.94	6.83	6.67
Tyrosine	2.03	2.79	1.73	3.23	3.02
Phenylalanine	4.82	3.65	4.86	3.02	3.39
Lysine	0.81	3.70	0.62	1.37	1.26
Histidine	1.63	1.53	1.30	1.55	1.61
Arginine	2.05	3.94	2.16	3.11	2.62

[a]Number of residues per 100 total residues.
[b]Ethanol-soluble.
[c]Ethanol-insoluble.
[d]Uncorrected value.

Most of the proteins of Fraction A were reported to be single-chained protein, such as albumin, globulin, and gliadin (Danno et al 1974). To investigate whether some glutenins were incorporated into Fraction A, the fraction was further fractionated by ethanol precipitation and adjusted to pH 6.5 with sodium hydroxide. About 30% of the total nitrogen of Fraction A was precipitated by the procedure. The SDS-PAGE pattern (Fig. 2b) of the precipitated proteins (Fig. 1, Fraction A-b) differed markedly from that of glutenin. Thus, Fraction A clearly did not contain appreciable amounts of glutenin, and the precipitated proteins were mixtures of albumins, globulins, and other ethanol-insoluble proteins (Table II). The supernatant protein (Fraction A-a) was mainly gliadin (Fig. 2a and Table II).

Glutenin, which remained with starch as a residue after the first extraction, has been reported to be solubilized by reduction of its disulfide bonds with 2-mercaptoethanol or mercuric chloride (Danno et al 1974). In the present study, the glutenin was solubilized into clear supernatant as Fraction B without prior reduction of its disulfide bonds.

Figure 3e shows the SDS-PAGE pattern of the reduced protein from Fraction B; the pattern closely resembles that of reduced glutenin. Similar results were also obtained on the proteins from Fraction C (data not shown). To investigate the purity of glutenin of Fraction B, Fraction B was applied on the Bio-Gel P-300 column and chromatographed. Without reduction of disulfide bonds, most of the proteins eluted at the void volume of the column (peak f). Peak g may be gliadin mixed with this fraction. The SDS-PAGE patterns are shown in Fig. 2f and g. These low molecular weight impurities were also separated by the ethanol precipitation method. About 85% of the total nitrogen of Fraction B was precipitated. The precipitated protein (Fraction B-b) was glutenin (Fig. 2d and Table II). The proteins in the supernatant (Fraction B-a) were low molecular weight proteins and may be gliadin that remained with starch and glutenin after the first extraction (Fig. 2d and Table II).

By this extraction method, glutenin with relatively high purity was obtained. This method is simple and thus may readily be scaled up. Furthermore, Fish et al (1970) reported that sulfhydryl groups on the polypeptide chain in the SDS complex were protected from each other. Thus, the glutenin preparation (Fraction B) may have negligible amounts of gliadin and water-soluble proteins incorporated into the glutenin molecules with covalent bonds by sulfhydryl and disulfide interchange.

SDS-glutenin is stable, and the inactivation step of proteolytic enzyme was unnecessary. SDS-PAGE patterns of the reduced glutenin were unaffected during long storage of SDS-glutenin.

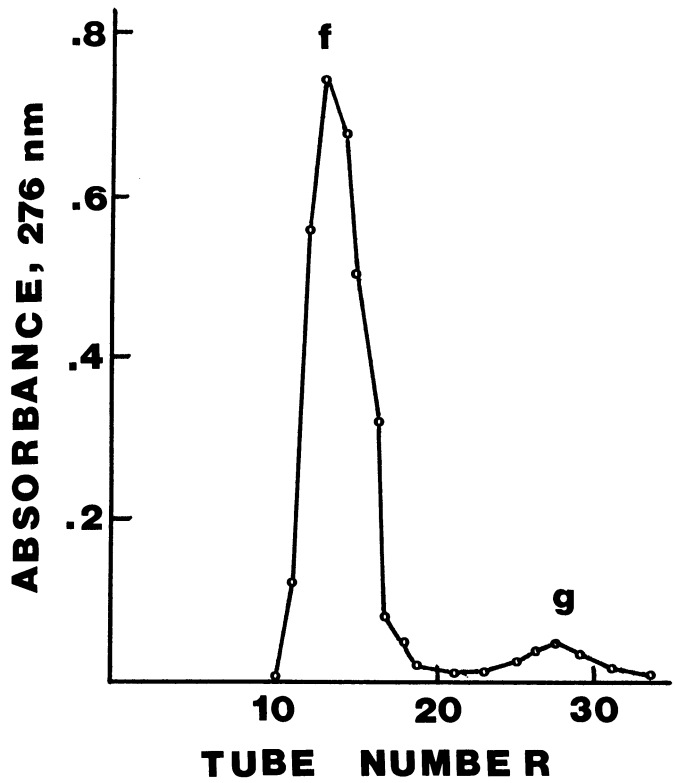

Fig. 3. Gel chromatography on Bio-Gel P-300 column of the unreduced proteins of Fraction B: **f**, peak at void volume containing most of the proteins; **g**, peak possibly containing gliadin. Peaks f and g correspond to those in Fig. 2.

Removal of the SDS from the prepared glutenin is necessary in order to study its biochemical and physicochemical properties. The glutenin (Fraction B) was precipitated with ethanol. The resulting precipitate was washed several times with 75% ethanol. The SDS contents of the washed glutenin were 0.4–0.8% (db). The washed glutenin was soluble in 0.1*N* acetic acid containing 2-mercapto-ethanol.

LITERATURE CITED

BIETZ, J. A., SHEPHERD, K. W., and WALL, J. S. 1975. Single-kernel analysis of glutenin: Use in wheat genetics and breeding. Cereal Chem. 52:513.

DANNO, G., KANAZAWA, K., and NATAKE, M. 1974. Extraction of wheat flour proteins with sodium dodecyl sulfate and their molecular weight distribution. Agric. Biol. Chem. 38:1947.

DANNO, G., KANAZAWA, K., and NATAKE, M. 1976. Identity of SDS-insoluble proteins with purified glutenin from wheat flour. Agric. Biol. Chem. 40:739.

FISH, W. W., REYNOLDS, J. A., and TANFORD, C. 1970. Gel chromatography of proteins in denaturing solvents. J. Biol. Chem. 245:5166.

JONES, R. W., TAYLOR, N. W., and SENTI, F. R. 1959. Electrophoresis and fractionation of wheat gluten. Arch. Biochem. Biophys. 84:363.

LAEMMLI, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature. 227:681.

MECHAM, D. K., COLE, E. W., and NG, H. 1972. Solubilizing effect of mercuric chloride on the "gel" protein of wheat flour. Cereal Chem. 49:62.

ORTH, R. A., and BUSHUK, W. 1973. Studies of glutenin. I. Comparison of preparative methods. Cereal Chem. 50:106.

SIGRIST, H. 1974. Gas chromatographic determination of sodium dodecyl sulfate. Anal. Biochem. 57:564.

[Received December 14, 1979. Accepted December 5, 1980]