NOTE

Gel Filtration of Wheat Gluten Proteins on Sephacryl S-300[1]

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Cereal Chem. 59(1):73–76

Gel filtration chromatography is known to be a useful technique for the fractionation of wheat proteins (Charbonnier 1973, Jones et al 1963, Meredith and Wren 1966, Wright et al 1964). For example, Meredith and Wren (1966) extracted wheat flour with a solvent consisting of 3 *M* urea, 0.1 *M* acetic acid, and 0.01 *M* cetyltrimethylammonium bromide (AUC). Elution of the solubilized proteins on Sephadex G-200 with AUC gave four fractions with estimated molecular weights corresponding to glutenins, gliadins, albumins, and nonproteins. Similar protein elution profiles were obtained by Bushuk and Wrigley (1971) with AUC extracts of ground grain and by Simmonds and Wrigley (1972) with 6 *M* urea extracts of defatted gluten on Sephadex G-150 columns. Other gel filtration mediums such as BioGel P-150 (Lee 1968), Sephadex G-100 (Charbonnier 1973, Preston and Woodbury 1976), and BioGel P-300 (Crow and Rothfus 1968), utilizing a variety of eluting solvents, have also proved successful. Unfortunately, a major disadvantage of these methods has been the long separation times that result from the low-elution flow rates needed to prevent excessive packing of these "soft" gels. However, gels consisting of allyl dextrans cross-linked with *N*,*N*′-methylene biacrylamide (Sephacryl) have recently been introduced (Haff and Easterday 1978). These gels have relatively "rigid" properties and can withstand fairly high flow rates. They include Sephacryl S-200, which has a molecular weight fractionation range similar to that of Sephadex G-150, and Sephacryl S-300, which has a fractionation range similar to that of Sephadex G-200. The former gel has been used with formamide (Haff 1978) to fractionate prolamines from wheat (gliadins) and corn (zein). However, the resolution of wheat gliadin fractions did not appear to be as good as that obtained from previous studies with Sephadex G-100 (Charbonnier 1973, Preston and Woodbury 1976). No results are presently available for other wheat protein fractions or other cereal proteins. Studies were therefore initiated to determine whether Sephacryl gels, with their advantage of high eluent flow rates, could be used to fractionate wheat gluten proteins. This note describes a method for the fractionation of gluten proteins on Sephacryl S-300 with 2.0 *M* sodium thiocyanate.

[1]Paper 479 of the Canadian Grain Commission, Grain Research Laboratory, Winnipeg, Manitoba R3C 3G9.

MATERIALS AND METHODS

Preparation and Extraction of Defatted Gluten

A Canadian hard red spring wheat variety (*Triticum aestivum* L. cv. Neepawa) was milled to straight grade flour (approximately 75% extraction) on the Grain Research Laboratory pilot mill. Gluten was prepared by the procedure of Doguchi and Hlynka (1967) following defatting of the flour with chloroform by the method of MacRitchie and Gras (1973). The freeze-dried gluten was then ground in a coffee grinder and equilibrated with the

laboratory atmosphere for two days in open shallow glass trays. Lipid content was determined by the procedure of Drapron (1975). Protein content was determined by Kjeldahl (N × 5.7; 14% mb) or by estimation at 280 nm using a calibration curve derived from known concentrations of soluble gluten proteins in 2.0 *M* sodium thiocyanate.

For extraction, 500 mg of defatted gluten was dispersed in 25 ml of 2.0 *M* sodium thiocyanate (pH 7.0) by shaking in a 40-ml capped centrifuge tube. The tube was then rotated at 50 rpm on a rotator (Roto-Torque, Cole Parmer) for 1 hr at room temperature and centrifuged at 40,000 × *g*. Portions of the resulting supernatant were then applied to the column.

Gel Filtration

Columns of Sephacryl S-300 Superfine (Pharmacia) were packed according to a procedure similar to the manufacturer's instructions. For elution with 2.0*M* sodium thiocyanate, the preswollen gel was first equilibrated with three portions of the eluant (1:10, v/v), with excess eluant being decanted. The gel was then poured into a 2.5 × 100-cm column (LKB 2173) and allowed to pack by gravity for approximately 3 hr with the outlet closed. The upper flow adaptor was then placed in the column above the gel, and eluant was pumped through the column with a downward flow of approximately 100 ml/hr for 2 hr. After adjusting the upper flow adaptor to just above the gel, the flow rate was reduced to 40 ml/hr for 2 hr. Flow was then reversed (upward flow) and maintained at 40 ml/hr overnight. Final gel dimensions were approximately 2.5 × 75 cm.

For column analysis, 4–8 ml of gluten extract (10 mg of protein per milliliter) or 5.0 mg of standard protein dissolved in 5 ml of 2.0*M* sodium thiocyanate were loaded onto the column and eluted at 40 ml/hr with upward flow. Fractions of approximately 8 ml were collected. Contents of tubes from each peak were then combined and protein recoveries estimated at 280 nm. Corresponding peaks from several fractionations were combined, dialyzed exhaustively against 0.005*M* lactic acid to remove salt, and freeze-dried. These fractions were then analyzed by electrophoresis with aluminum lactate buffer (0.008*M*, pH 3.1) by the procedure of Tkachuk and Mellish (1980).

RESULTS AND DISCUSSION

The defatted gluten isolated from the hard red spring wheat variety Neepawa was the same as that used in a previous study (Preston 1981). It had a protein content of 82.7% (N × 5.7, as is), a moisture content of 9.0%, and a lipid content of 0.78%.

Preliminary Studies

Because of the physical and chemical properties of wheat gluten proteins, gel filtration eluants have been mainly restricted to solvents of low ionic strength such as dilute solutions of organic acids (Huebner and Rothfus 1971) and/or denaturing agents such as urea and detergents (Meredith and Wren 1966, Simmonds and Wrigley 1972) in order to maintain high solubilities. Several of these solvents were initially investigated as eluants to determine their ability to resolve gluten protein fractions on Sephacryl S-300 columns of small diameter (1.5 × 50 cm). Results of these studies are shown in Fig. 1. Extraction and elution of the solubilized gluten proteins with 0.05*M* acetic acid, 0.05*M* acetic acid:2*M* urea, or AUC resulted in broad and poorly resolved peaks. Slightly better resolution was obtained with 2.0*M* urea at pH 7.0, but peaks were still poorly resolved (Fig. 1D). The poor resolution in the acid buffers may have been the result of protein binding. This has previously been shown to occur with Sephacryl gels at low pH through hydrophobic and/or hydrogen binding (Haff and Easterday 1978). The low ionic strength of these solvents and of the 2.0*M* urea (pH 7.0), which would favor ionic interactions, also may have been partially responsible for the lack of resolution.

Recent studies in our laboratory have shown that sodium thiocyanate in concentrations above 0.5*M* solubilizes significant proportions of wheat gluten proteins (Preston 1981). With 2.0*M* sodium thiocyanate, the solubility of defatted Neepawa gluten (66%) was similar to that obtained with 0.05*M* acetic acid (70%). Electrophoretic profiles and amino acid compositions of the solubilized proteins were also similar for both solvents. In view of these results and of the ability of this salt to minimize ionic and hydrophobic interactions (Pahlman et al 1977, Von Hippel and Schleich 1969), studies were initiated to determine whether sodium thiocyanate solutions would improve resolution of gluten protein fractions on Sephacryl S-300. Initial studies with 1.5 × 50-cm columns (data not shown) showed that elution of the extracted gluten proteins with 2.0*M* sodium thiocyanate gave greatly improved resolution of protein fractions compared to those of the other solvents tested. Further studies were therefore done on larger columns.

Studies with Sephacryl S-300 and Sodium Thiocyanate

Figure 2 shows the Sephacryl S-300 gel filtration profile (2.5 × 75-mm column) of gluten proteins solubilized with 2.0*M* sodium thiocyanate eluted at 40 ml/hr with the same solvent. Gel filtration profiles obtained at elution rates of 10 and 20 ml/hr were similar (data not shown). Even at flow rates of 60 ml/hr, little resolution was lost. Four major protein peaks were evident (A–D). Two low molecular weight fractions, probably due to peptides and amino acids, were also present. Table I shows the average molecular weights of the protein peaks, which were estimated from the standard proteins plotted in Fig. 3. Molecular weights of 300,000, 38,000, 18,000, and 14,500 were estimated for peaks A, B, C, and D, respectively. In addition, the shoulder peak following A had an estimated molecular weight of 200,000, and the shoulder peak preceding B had an estimated molecular weight of 80,000. Rechromatography of each of the four peaks (Fig. 4) gave a single peak with an elution volume and estimated molecular weight almost identical to that of the corresponding original peak.

Table I also gives quantitative protein distributions of each of the four peaks and of the low molecular weight fractions from two column runs estimated by absorbance at 280 nm. Distributions of the fractions were similar for both runs, and total recoveries were

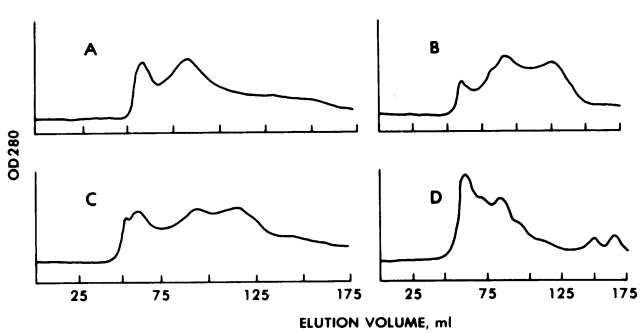

Fig. 1. Gel filtration of wheat gluten proteins in different eluants on 1.5 × 50-cm Sephacryl S-300 columns. **A** = 0.05 *M* acetic acid, **B** = 0.05 *M* acetic acid and 2.0*M* urea, **C** = 3*M* urea:0.1*M* acetic acid:0.01*M* cetyltrimethylammonium bromide, **D** = 2.0*M* urea, pH 7.0.

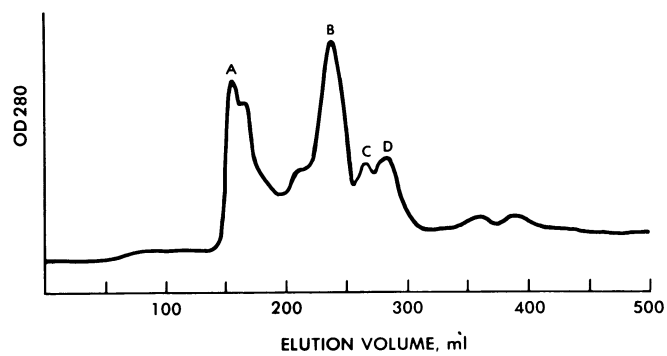

Fig. 2. Gel filtration of wheat gluten protein soluble in 2.0*M* sodium thiocyanate on a 2.5 × 75-mm Sephacryl S-300 column, using 2.0*M* sodium thiocyanate as eluant. **A–D** = Major protein peaks.

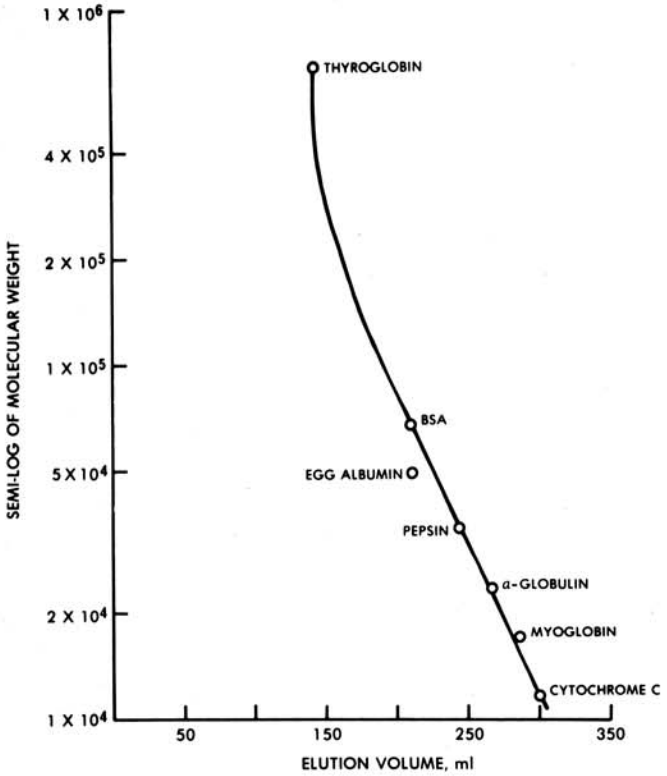

Fig. 3. Standard molecular weight curve for a 2.5×75-mm Sephacryl S-300 column. Flow rate was 40 ml/hr with 2.0*M* sodium thiocyanate.

TABLE I
Molecular Weight and Quantitative Distribution (absorption at 280 nm) of Protein Peaks Eluted with 2.0*M* Sodium Thiocyanate on Sephacryl S-300[a]

Peak	Elution Volume[b] (ml)	Molecular Weight[b]	Recovery (%) Run No. 1	Recovery (%) Run No. 2
A	150	300,000	32	30
B	240	38,000	42	44
C	272	18,000	8	8
D	289	14,500	12	16
Low molecular weight	···	···	5	6

[a] Determined on a 2.5×80-cm column with a 5-ml extract (52 mg of protein) of Neepawa defatted gluten and a flow rate of 40 ml/hr.
[b] Average of two determinations.

99 and 104%. Average recoveries for each of the fractions were 31, 43, 8, 14, and 6% for A, B, C, D, and the low molecular weight components, respectively. However, the estimated distribution of the lower molecular weight protein fractions is probably overestimated; recent studies in our laboratory[2] indicate that these fractions have a much higher ratio between absorbance at 280 nm and Kjeldahl nitrogen value than do the high molecular weight fractions.

Figure 5 shows the electrophoretic patterns obtained with aluminum lactate buffer for the four gluten protein fractions from the Sephacryl column and for the original extract. Peak A gave a strongly stained origin as well as heavy streaking in the upper part of the gel. These results and the high estimated molecular weight of this fraction suggest that these proteins consist mainly of glutenins. Peaks B and C had protein patterns consistent with gliadinlike proteins, with peak B having mainly slow and intermediate moving bands and peak C having a higher proportion of fast-moving bands. Peak D consisted mainly of fast-moving bands with mobilities similar to those of low molecular weight gliadins and/or albumins. In addition, peaks B, C, and D had some staining at the origin, which indicates aggregation of some of the proteins in the

[2] Unpublished data.

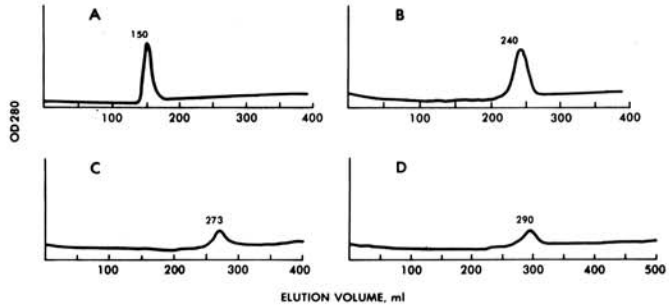

Fig. 4. Rechromatography of isolated peaks obtained from the single run described in Fig. 2 under the same conditions. **A** = peak A, **B** = peak B, **C** = peak C, **D** = peak D.

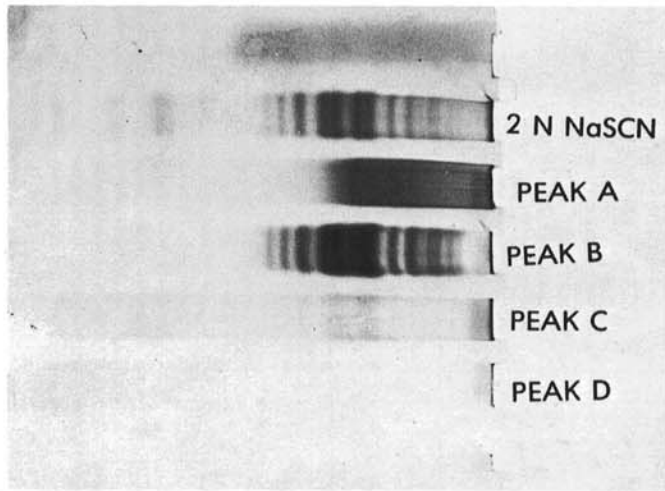

Fig. 5. Aluminum lactate electrophoresis of gluten proteins soluble in 2.0*N* sodium thiocyanate and their fractions isolated by Sephacryl S-300 chromatography.

aluminum lactate buffer. The lower staining intensity of peak D and, to a lesser extent, peak C may be related to a lower protein content in the dried fractions. However, losses due to dialyzing or to the presence of peptides of very high mobility on proteins that migrate off the gel may have also contributed.

CONCLUSIONS

A procedure was developed for the gel filtration of wheat gluten proteins on Sephacryl S-300 with 2.0*M* sodium thiocyanate. A Canadian hard red spring wheat variety showed four major protein peaks with average molecular weights of 300,000, 38,000, 18,000, and 14,500. In addition to good peak separation, the gel was able to withstand high eluant flow rates without significant losses in resolution. This latter factor may prove to be particularly important because much shorter times are required for separation of gluten proteins than are needed for previously published gel filtration methods.

ACKNOWLEDGMENT

I acknowledge the technical assistance of P. Lee.

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[Received June 18, 1980. Accepted October 20, 1981]