FRACTIONATION OF WHEAT GLUTEN BY GEL FILTRATION[1]

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ABSTRACT

Wheat gluten was fractionated into glutenin and gliadin on columns packed with a special cross-linked dextran (Sephadex[2]). The glutenin was essentially pure electrophoretically, while the gliadin was contaminated with traces of glutenin. Gliadin components moved at slightly different rates, but resolution was not sufficient to yield separate components.

That wheat gluten contains a number of distinct components has been demonstrated by both moving-boundary (3) and starch-gel electrophoresis (10). The gluten most extensively studied at this laboratory was obtained from Ponca hard red winter wheat. A starch gel electrophoretic pattern of this gluten in aluminum lactate buffer is shown in Fig. 1. Gliadin components of gluten migrate into the gel while

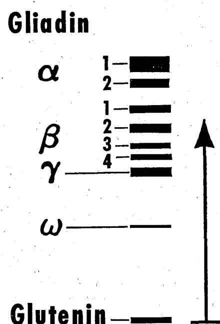

Fig. 1. Diagram of gel electrophoretic pattern of Ponca wheat gluten in an aluminum lactate buffer containing 3*M* urea.

glutenin remains at the origin. Gliadin components are grouped into α, β, γ, and *w* fractions because of their behavior in moving-boundary electrophoresis. In free electrophoresis three major peaks (α, β, γ) and a minor peak (*w*) are observed. Although these peaks show evidence of heterogeneity, especially in the β region, they are not resolved into distinct components as with the more sensitive gel electrophoresis. Grouping of gliadin components is justified also because the identity

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[2]Reference to specific equipment or an organization does not necessarily constitute endorsement by the U.S. Department of Agriculture.

of the α, β, γ, and w fractions is retained on separation in carboxymethylcellulose columns (12).

In addition to the components shown in Fig. 1, there are a number of faster-moving minor components. This fast material is water-soluble and considered to be nongluten in nature. It differs from gluten in solubility, electrophoretic mobility, physical properties (lack of elasticity and cohesiveness), and amino acid composition. In these respects it resembles the albumins and globulins of wheat flour (3,11).

Although glutenin is a charged molecule and in free electrophoresis has essentially the same mobility as α_1 gliadin, it fails to migrate into the starch gel because of its large molecular size. The exclusion of glutenin from the gel provides a new basis for defining the classic gluten fractions, gliadin and glutenin (10). Glutenin contains a broad spectrum of molecular weights extending from about 50 thousand to many millions, for which the weight-average molecular weight is about 2 to 3 million (4,8). Gliadin components have molecular weights in the 40 thousands — β-gliadin 42,000, γ-gliadin 47,000 (4).

Porath (7) has shown that substances of different molecular weights can be separated by gel filtration on columns of cross-linked dextrans. Therefore, applicability of gel filtration to the separation of glutenin and gliadin was investigated. Although suitable methods have been available for preparing gliadin (5,6), glutenin separation has not been entirely satisfactory based on either insolubility in alcohol (6) or precipitation from acidic solution by increasing pH or ionic strength (3). The alcohol procedure renders glutenin insoluble in dilute acid; glutenin prepared by precipitation is a difficultly soluble protein. Both glutenin preparations generally have shown retention of a little gliadin as judged by starch gel electrophoresis. The use of Sephadex for fractionating wheat gluten was explored to determine whether glutenin could be prepared free of gliadin and with better solubility than by previous methods. Attention also was given to possible fractionation of gliadin components, since factors other than molecular weight are involved in separations with Sephadex (2,7).

Materials and Methods

Gluten from Ponca flour was prepared as described previously (3). It was fractionated on Sephadex G-75 columns at room temperature. Columns varied in size from 4 × 30 to 6 × 93 cm. Solvents were: acetic acid (0.01–0.05M), aluminum lactate-lactic acid (μ = 0.1, pH = 3), and 9% sec-butyl alcohol-91% acetic acid (0.1M). Gluten concentrations were 2–10%. It required 1 to 6 days to complete a fractionation. Coarse

G-75 was used in some cases to speed the very slow flow rate. Fourteen-milliliter aliquots were collected in an automatic fraction collector. The absorbance (optical density) of solution in each tube was determined at 280 mμ. The electrophoretic methods are described in previous papers (3,10).

Molecular weights of fractions were determined in an ultracentrifuge by the approach to sedimentation equilibrium technique. Data were handled by means of the Trautman plot (9) as modified by Erlander and Foster (1). This method has been discussed in more detail previously (4).

Elution and electrophoretic patterns from the various experiments were essentially the same. Details of the conditions for a typical column are as follows: Suspend ungraded Sephadex G-75 in 0.05*M* sodium chloride and add to a column 6 cm. in diameter (Sephadex height about 93 cm.). Wash with water followed by 0.05*M* acetic acid. Washing requires 3 days and 3 liters of solvent. Dissolve 2 g. of gluten in 30 ml. of 0.05*M* acetic acid and add to column. Adjust the flow rate to 25 ml. per hour and wash the column with 0.05*M* acetic acid. Protein emerges from the column after 476 ml. of solvent have been collected. At this point the flow rate drops sharply to 9 ml. per hr. Gluten is quantitatively recovered in 6 days in 1 liter of solution.

Results

The elution pattern obtained is shown in Fig. 2. Tube contents were combined into six fractions as shown in the diagram. Percentage of total protein in each cut is also indicated (percent of total area under curve).

Fractions were compared by gel electrophoresis. Diagrams of the gels are shown in Fig. 3. Fractions 1 and 2 contain almost pure glutenin with only minor quantities of α- and γ-gliadin. The β-components do not appear until fraction 3, which also contains a large proportion of γ-gliadin. The α-gliadins appear first in fraction 4 and the water-

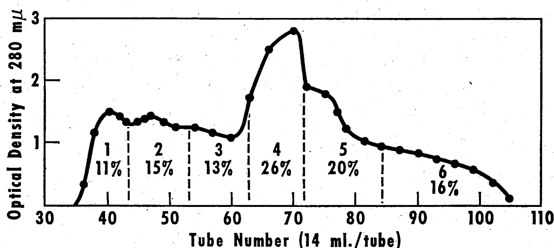

Fig. 2. Elution curve. Gluten from Sephadex column in 0.05*M* acetic acid.

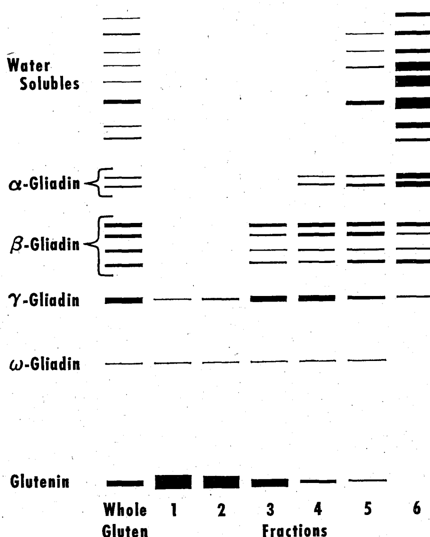

Fig. 3. Diagram of gel electrophoretic patterns of gluten and gluten fractions.

TABLE I
DISTRIBUTION OF GLUTEN COMPONENTS IN FRACTION FROM SEPHADEX COLUMN

FRACTION	PERCENTAGE OF FRACTION					
	1	2	3	4	5	6
Water-solubles	0	0	0	0	5	44
α–Gliadin	0	0	0	42[a]	37[a]	23
β–Gliadin	0	0	15	25	32	30
γ–Gliadin	2	10	26	29	23	2
w–Gliadin	2	4	5	3	2	0
Glutenin	96	86	55	2[a]	1[a]	0

[a] Arbitrary division.

solubles initially in fraction 5. Almost all the water-solubles are in the final fraction.

Width of the bands gives an approximation of the relative concentrations of components. A more quantitative determination of concentration was made with moving-boundary electrophoresis (Table I), but it does not distinguish between α-gliadin and glutenin. Since fractions 4 and 5 contain both, the quantity has been arbitrarily divided between the two. This division was based on recent measurements by Huebner and Woychik[3], which showed that the Ponca gluten added to the column contained 30% glutenin. Seventy-eight percent of the total glutenin is accounted for in the first two fractions and the remainder in the third. Fractions 4 and 5 therefore could contain only insignifi-

[3] Huebner, F. R., and Woychik, J. H. Private communication.

TABLE II
RECOVERY OF COMPONENTS FROM SEPHADEX COLUMNS

Fraction	Percentage of Total	
	Added to Column	Recovered from Column
Water-solubles	9.8	8.0
α–Gliadin	23.0	22.0
β–Gliadin	18.2	19.7
γ–Gliadin	16.5	17.5
w–Gliadin	2.4	2.4
Glutenin	30.0	31.0

cant quantities of glutenin. Estimation of relative concentration of glutenin from examination of color of the starch gel is particularly difficult because the material is packed against the cut surface of the gel at the origin.

Recovery of individual components is excellent. The amounts of each component in the sample added to the column and the amounts recovered from the column are compared in Table II.

Molecular weights of the fractions were determined in aluminum lactate-lactic acid buffer ($\mu = 0.1$, pH $= 3$). Trautman plots obtained from fractions 1–5 were very curved, indicating molecular-weight heterogeneity. The plot for fraction 6 was straight, indicating a homogeneous molecular weight. Results are given in the table below. Values

Fraction	M_w *(Average)*
1	2–3 million
2	2 million
3	½ million
4	½ million
5	¼ million
6	40 thousand

for fractions 3–6 are very approximate; owing to difficulties in measuring wide ranges of molecular weights and to the problem of estimating partial specific volume of a mixture. A separation based on molecular-weight differences was achieved. Relatively pure glutenin was obtained in fractions 1 and 2, but the gliadin was contaminated with glutenin. Gliadin components moved on Sephadex at slightly different rates, but no pure components were obtained.

The centrifuge patterns for fractions 1 and 2 have a main peak that contains 85% of the total and that has a sedimentation constant of 5.8 S at the maximum. The remaining 15% is broadly dispersed and has a greater sedimentation constant. This pattern is in agreement with the results of Taylor and Cluskey (8), who showed that four times precipitated glutenin was polydispersed and had a maximum at about 6 S.

Glutenin prepared on Sephadex is less soluble than that prepared by precipitation. Precipitated glutenin can be dissolved by gentle stirring in dilute acetic acid or aluminum lactate buffer. Glutenin from Sephadex requires much more vigorous treatment to disperse it. Apparently insolubility is a characteristic of pure glutenin and not a result of denaturation, particularly since passage through Sephadex is a very mild treatment.

As indicated under Materials and Methods, a number of different elution techniques were employed. Elution patterns and electrophoretic results from the various experiments were the same. Because the separation achieved was independent of solvent, concentration of gluten, and Sephadex particle size, detailed results of only one experiment are given. Molecular weights were determined for fractions from this one experiment.

No preservative was used to prevent action of microorganisms. A portion of the gluten in the reported experiment was on the column for 6 days. The recovery, molecular weight, and electrophoretic results indicate no microbial contamination.

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