ISOLATION AND CHARACTERIZATION OF A HIGH-MOLECULAR-WEIGHT PROTEIN FROM WHEAT GLIADIN[1]

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ABSTRACT

Gliadin, the alcohol-soluble protein of wheat gluten, was found to contain a small amount of a high-molecular-weight (MW) fraction when analyzed by gel filtration on columns of Sephadex G-100. The chromatograms were quite similar when either $0.01M$ or $0.1M$ acetic acid was used as eluting solvent. The resolution obtained with $3M$ urea-$0.01N$ acetic acid as eluting solvent, was not so good as with $0.1M$ acetic acid. Increasing the temperature from $25°$ to $40°C$. had no effect upon the resolution pattern of the fractions. The heaviest fraction had a MW of about 100,000, whereas the lightest had a MW of about 30,000 in a strongly deaggregating solvent system. Moving-boundary and starch-gel-electrophoretic patterns of the heaviest fraction as well as amino acid composition, solubility, viscosity, sedimentation, and diffusion constants were suggestive of a low-MW glutenin. The moving-boundary and starch-gel-electrophoretic patterns of the lightest contained the major gliadin components — alpha, beta, and gamma. Alpha-gliadin obtained by chromatography on carboxymethyl cellulose also contained components similar to the heaviest gliadin fraction obtained by gel filtration on Sephadex G-100.

Following the work of Osborne (1), the major protein components of wheat gluten have been grouped into two fractions: gliadin, which is soluble in neutral 70% ethanol solution, and glutenin, which is not. Differences in molecular weights observed for the two protein classes may be partly responsible for their different solubilities and other physical properties. Ultracentrifugation has shown that glutenin contains a wide range of molecular sizes and that its average molecular weight (MW) is 1.5–2.0 million in pH 3.1 aluminum lactate buffer (2) and is 300,000 in $1M$ urea plus $0.03M$ ammonium hydroxide (3). All glutenin components are so large that they will not electrophoretically penetrate a starch gel containing pH 3.1 aluminum lactate buffer plus $3M$ urea (4).

In contrast, the gliadin proteins migrate as sharp bands in starch-gel electrophoresis (4). Some of the major individual proteins of the gliadin complex isolated by column chromatography on carboxymethyl cellulose have low MW's. The gamma-gliadin has a MW of 31,000 in $6M$ guanidine-hydrochloride plus $0.1M$ acetic acid (5), and the beta-

gliadin an apparent MW of 31,000 or less in pH 3.1 aluminum lactate buffer (2). However, Jones et al. (2) observed that an alpha-gliadin preparation obtained by carboxymethyl-cellulose chromatography of gliadin was in the 150,000-MW range and did not appear homogeneous. Thus, no generalization concerning the MW of gliadin components could be made on the basis of previous studies.

Recently, gel-filtration chromatography has been successfully utilized to separate proteins according to differences in MW. Jones et al. (6) first applied this technique to separate wheat gluten protein on Sephadex G-75 columns. Sharp separations into gliadin and glutenin fractions as distinguished by gel electrophoresis were not achieved. Wright et al. (7) chromatographed wheat flour extracts on Sephadex G-100. They found fractions that were not retarded on the columns, i.e., MW's over 100,000, but that contained proteins which are mobile in starch-gel electrophoresis, as are gliadin proteins. These observations suggested that species intermediate between gliadin and glutenin may exist.

It has been shown that cleaving the disulfide bonds of glutenin causes a sharp decrease in its solution viscosity and MW (3). A slight decrease in the viscosity of gliadin solutions upon reductive cleavage of disulfide bonds in the protein was reported by Pence and Olcott (8), and later by Beckwith et al. (9). An explanation of this decrease in viscosity is that gliadin contains some larger-MW components consisting of peptide chains associated by disulfide bonds such as are found in glutenin.

To examine the possibility that classic gliadin preparations contain proteins of considerably different MW's, we chromatographed them in columns of Sephadex G-100. The protein was eluted in at least two peaks in all the solvent systems tested. The two major fractions were compared as to MW, sedimentation and diffusion constants, viscosities, moving-boundary and gel-electrophoretic mobilities, solubilities, and amino acid composition.

Materials and Methods

Materials. Gluten was isolated from Ponca hard red winter wheat flour by the procedure of Jones et al. (10), as modified by Woychik and associates (4) for the removal of water-soluble proteins. In this procedure flour was extracted with n-butanol to remove lipids, and a gluten ball was prepared by washing a dough with 0.1% sodium chloride. The gluten ball was dispersed in 0.01M acetic acid with a Waring Blendor, and the dispersion was centrifuged at 20,000 × g to remove starch and a small amount of insoluble protein. The solution

was then briefly heated to 98°–100°C. to inactivate proteolytic enzymes. The solution was then made 0.2M with sodium chloride to precipitate gluten leaving water-soluble impurities in solution, and the gluten was freeze-dried after redispersion in dilute acetic acid. (Gluten prepared by such a procedure has the same electrophoretic components as gluten prepared from a nondefatted flour and gluten prepared by a procedure that does not involve heating (10).) Glutenin was then separated from gliadin by adjusting the pH of a 70% ethanol-0.01M acetic acid solution of gluten to pH 6.5–6.7 (10), allowing the solution to stand overnight at 4°C., and centrifuging off the glutenin. After the major portion of alcohol was removed under a vacuum, the clear gliadin solution was dialyzed against 0.01M acetic acid to remove salt and alcohol and then freeze-dried. Alpha-gliadin was isolated from gliadin by the carboxymethyl-cellulose chromatographic procedure of Woychik et al. (11). Cross-linked dextran, Sephadex G-100, was obtained from Pharmacia Fine Chemicals, Inc., and had a bead size of 40–120 μ; aluminum lactate was purchased from Z. D. Gilman, Inc.; all other chemicals were analytical grade commercial chemicals.

Gel Filtration. Since adsorption, ion-exchange, and aggregation effects are known to accompany chromatography on cross-linked dextrans, four different solvents were tested to determine which one minimized these influences.

Sephadex G-100 was equilibrated with 3M urea plus 0.01M acetic acid plus 0.1M sodium chloride for 72 hr. and then poured into a column 2.2 × 180 cm. The column was washed with about 1 liter of fresh solvent before application of 150–200 mg. of gluten, gliadin, or alpha-gliadin dissolved in 2 ml. of solvent. The protein was then eluted with the solvent at a flow rate of 12–15 ml. per hr. The effluent was collected in 8- to 10-ml. portions by means of a drop-counting fraction collector. The absorbance of each tube was measured at 280 mμ in a Beckman DU spectrophotometer. The total absorbance eluted from the column was compared to the total absorbance applied. The contents of tubes were combined, dialyzed against 0.01M acetic acid, freeze-dried, and weighed. An aliquot of stock protein solution was also dialyzed, dried, and weighed to estimate the total recovery on a weight basis. On a weight basis the recovery was 95–100%.

The column was then washed with 3M urea plus 0.01M acetic acid containing no sodium chloride until the effluent gave a negative test for chloride ion. As before, 150–200 mg. of protein was applied to the column and eluted with 3M urea plus 0.01M acetic acid but containing no salt. The flow rate, fraction size, and treatment of fractions was the same as before. The gliadin recovery was only 75% on a weight

basis. The solvent therefore was again made 0.1M in sodium chloride and the elution continued. The adsorbed gliadin was eluted just before the chloride front.

To study further the effects of solvent, as well as to isolate larger portions of the various gliadin fractions, Sephadex G-100 was equilibrated with 0.1N acetic acid and poured into a column 4.0 × 110 cm. The column, jacketed and connected to a constant-temperature circulating water bath, was operated at 25° or 40°C. With this larger-diameter column, 1 to 1.5 g. of gliadin was applied in 20 ml. of 0.1N acetic acid solution. The protein was eluted with this solvent at a flow rate of 40–45 ml./hr. in 13- to 15-ml. volumes. After the absorbance was measured at 280 mμ, the contents of tubes were combined and freeze-dried without previous dialysis. The protein recovery was 90–95% on a weight basis.

During the continued operation of this column, the gel bed had packed down appreciably. The gel was therefore removed, equilibrated with 0.01M acetic acid, and then repoured into the column with this solvent. After being washed with 3 liters of fresh 0.01M acetic acid, 1.5 g. of gliadin in 30 ml. of 0.01M acetic acid was applied to the column. The elution of samples with the more dilute acetic solvent and the treatment of fractions were the same as with 0.1M acetic acid. The protein recovery was 75–80% in one run and 95% in another.

Amino Acid Analysis. Samples (10–12 mg.) from fractions at the extremes of the gliadin chromatograms (fractions I and III from the column eluted with 0.1M acetic acid) were hydrolyzed with 6N hydrochloric acid at 110°C. for 24 hr. in sealed tubes. A Phoenix Model K8000 automatic analyzer was used to determine the amino acid composition of the hydrolysates. The total nitrogen in the hydrolysates was determined by semimicro Kjeldahl analysis. The nitrogen recovered as amino acid nitrogen was 96–99% of total nitrogen.

Physical Measurements. A modified procedure of Woychik *et al.* (4) was used to compare the starch-gel-electrophoretic properties of gliadin to those of gliadin fractions obtained by gel filtration. To prepare suitable starch gels in 3M urea buffers, the amount of Connaught hydrolyzed starch recommended by the manufacturer for each batch was increased by 1 g. per 100 ml. of buffer used. A starch gel section (4 × 3 × 5/16 in.) with six (1/4 × 1/4 × 1/32 in.) slots 1/2 in. from one end was placed in the center of a Plexiglas tray (10 × 3 × 5/16 in.). The slots were filled with 25 μl. of 1–2% solutions of the various proteins in aluminum lactate buffer containing 3M urea (0.005M aluminum lactate and 0.02M lactic acid). Saran Wrap was placed over the gel, and

the ends of the tray were filled with buffer to serve as electrode com-
partments. (Some runs were made in which inserted filter-paper wicks
having adsorbed protein were substituted for sample slots.) The elec-
trophoresis was carried out by using about 100 v. potential difference
(25–30 ma.) without cooling for about 4–5 hr. After the gel was re-
moved from the tray and sliced horizontally, the fresh surfaces were
stained for 5 min. with 2% nigrosin dye in water. Excess dye was
removed by soaking in water.

Moving-boundary-electrophoretic analyses were made in a stand-
ard 0.0167M aluminum lactate-0.0695M lactic acid buffer (pH 3.0–3.1)
at about 0.4% protein concentration, with a Spinco Model H instru-
ment.

The relative viscosity of two gliadin fractions was measured at
25°C. in standard aluminum lactate buffer with Ostwald-Fenske No. 50
viscometers. Density correction of the protein solutions was neglected.
The weight intrinsic viscosity was found by extrapolating the reduced
viscosities to zero protein concentration.

Sedimentation-equilibrium analyses were made in a Spinco Model
E ultracentrifuge equipped with a rotor temperature indicator and
control system. Samples in 6M guanidine hydrochloride-0.1M acetic
acid were placed in 30-mm. double-sector cells equipped with sapphire
windows. As the system approached equilibrium, pictures were taken
in order to calculate diffusion constants by the procedure of Van
Holde (12). Sedimentation coefficient to diffusion constant ratios were
obtained from initial slopes of log concentration $vs.$ radius-squared
plots (13). A value of 0.724 for the partial specific volume of gliadin
was calculated from amino acid analysis by the procedure of McMeekin
$et\ al.$ (14). Values for sedimentation and diffusion constants obtained
in 6M guanidine hydrochloride-0.1M acetic acid were converted to
values for the constants in water at 20°C.

The turbidity of gliadin fractions as a function of sodium chloride
concentration was studied in 0.03M acetic acid and in 0.03M acetic
acid plus 3.75M urea by the procedure of Beckwith $et\ al.$ (15).

Results and Discussion

$Gel\ Filtration.$ The chromatographic separation obtained with
gluten and gliadin upon Sephadex columns with 3M urea plus 0.01M
acetic acid plus 0.1M sodium chloride solvent systems is shown in
Fig. 1. Both preparations apparently contain molecules of sufficient
size (first peak) so as to be excluded from the gel matrix. The protein
under the first peak in the chromatogram for gliadin (tubes 19–26)
made up about one-third of the gliadin sample applied to the column.

With a solvent system of $3M$ urea plus $0.01M$ acetic acid (no salt), two peaks were again obtained. In this case the peak excluded from the gel comprised about one-fifth of the total. Only 75% of the applied protein was recovered. The adsorbed protein was eluted by again adding sodium chloride to the solvent. The chromatogram for the adsorbed protein consisted of two peaks which seemed to have the same relative distribution of particle sizes as found in the unadsorbed protein. This similarity indicates that there was no selective binding of either fraction.
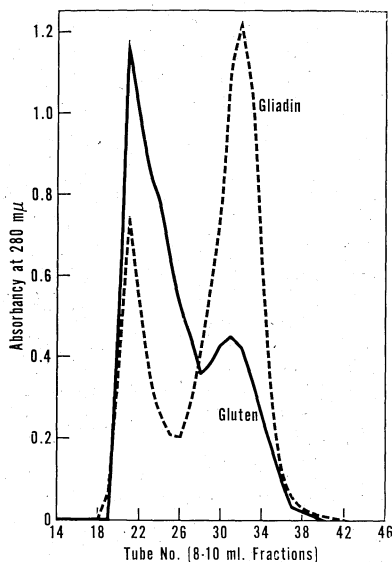

Fig. 1. Fractionation of gliadin and of whole gluten on Sephadex G-100 (2.2 × 180 cm. column). Elution with $3M$ urea plus $0.01M$ acetic acid plus $0.1M$ sodium chloride at 12–15 ml./hr.

Figure 2(a) shows the results when gliadin was eluted from a larger column of Sephadex G-100 with $0.1M$ acetic acid. This system resolved gliadin into three fractions. Fraction I contained only about 6% of the protein recovered after freeze-drying; fraction II, about 15%; and fraction III, about 80% or most of the recovered protein. The apparent amount of fraction I (fraction completely excluded from the gel) is exaggerated in the figure because turbidity in this fraction increased apparent absorbancy at 280 mμ. About 95% of the protein was recovered. Comparing these results with those given in Fig. 1 shows that $3M$ urea in the eluting system decreases resolution. This decrease may be due to the effect of urea on protein-protein interactions or
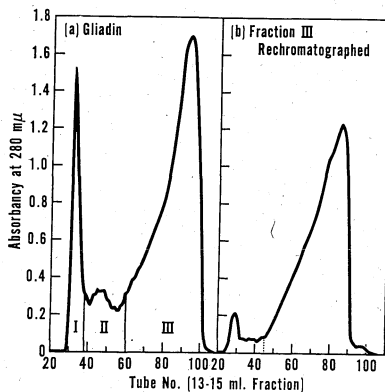
Fig. 2. Fractionation on Sephadex G-100 (4 × 110 cm. column) eluted with 0.1M acetic acid at 40–49 ml./hr.: (a) whole gliadin and (b) rechromatograph of fraction III.

protein-gel interactions or to the increased viscosity of the urea-containing system.

Increasing the temperature of column operation from 25° to 40°C. did not disrupt the gel bed or change the positions of elution of the gliadin fractions shown in Fig. 2(a). The flow rate increased at the higher temperature, but was readjusted to coincide with flow rate at the lower temperature.

The results of rechromatographing gliadin fraction III in Fig. 2(a) are shown in Fig. 2(b). A small amount of material is completely excluded from the Sephadex gel, and some is eluted where fraction II was eluted. We feel this result is explainable in terms of the limited resolution of the column. However, there is also the possibility of concentration-dependent aggregation or even disulfide polymers forming on passage through the column or after separation which could cause the formation of high-MW material in fraction III.

Reducing the acetic acid concentration of the eluted solvent to 0.01M did not change the position of elution for the different fractions shown in Fig. 2(a). However, the relative amounts of each fraction differed significantly, and protein recoveries ranged from 75 to 95% in replicate runs. This variation might be due to electrostatic interaction between the charged groups on the protein and the few charged groups on the gel, and this interaction would be more likely to occur at the lower acetic acid concentration.

Since nearly quantitative yields were obtained with 0.1M acetic acid and since the fractions obtained did not require additional dialysis, this solvent was used in the separation of gliadin fractions for

characterization studies; i.e., fractions I, II, and III of Fig 2(a) are the ones reported on later in this paper.

A fraction containing alpha-gliadin prepared by chromatography of gliadin on carboxymethyl cellulose (11) was separated on Sephadex G-100. The elution system employed was $3M$ urea plus $0.01M$ acetic acid plus $0.1M$ sodium chloride. Results are shown in Fig. 3. This
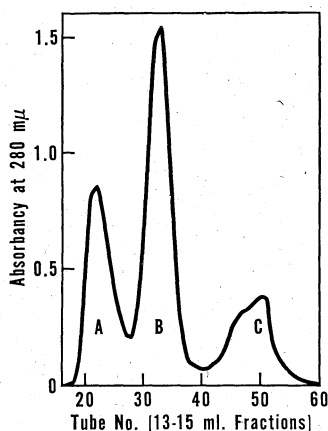

Fig. 3. Fractionation of alpha-gliadin on Sephadex G-100 (2.2 × 180 cm. column). Elution is with $3M$ urea plus $0.01M$ acetic acid plus $0.1M$ sodium chloride at 12–15 ml./hr.

alpha-gliadin preparation contains three distinct size fractions, the first fraction apparently being large enough to be completely excluded from the gel.

Amino Acid Analysis. The amino acid composition of fractions I and III of Fig. 2(a) are given in Table I. The low-MW fraction (frac-

TABLE I
AMINO ACID COMPOSITION OF GLIADIN FRACTIONS
(Moles amino acid per 10^5 g. protein)

AMINO ACID	FRAC-TION I [a]	FRAC-TION III	FR. III / FR. I	AMINO ACID	FRAC-TION I [a]	FRAC-TION III	FR. III / FR. I
Alanine	22	26	1.19	Lysine	5	4	0.74
Arginine	15	13	0.87	Methionine	13	9	0.73
Aspartic acid	13	24	1.85	Phenylalanine	39	34	0.87
Cystine	10	9	0.92	Proline	132	133	1.01
Glutamic acid	324	304	0.94	Serine	61	44	0.72
Glycine	30	23	0.78	Threonine	22	17	0.74
Histidine	14	14	1.03	Tyrosine	12	22	1.92
Isoleucine	31	34	1.09	Valine	39	37	0.95
Leucine	63	63	1.00	Ammonia	315	316	1.00

[a] Of Fig. 2.

tion III) contains almost twice as much aspartic acid and tyrosine as the high-MW fraction. In contrast, the heavier proteins (fraction I) contain slightly greater amounts of lysine and arginine as well as glycine, methionine, phenylalanine, serine, and threonine. These differences bring the amino acid composition of fraction I closer to that of glutenin as reported by Wu and Dimler (16).

Moving-Boundary and Starch-Gel Electrophoresis. Moving-boundary electrophoretic patterns in aluminum lactate buffer for fractions I and III of Fig. 2(a) and whole gliadin appear in Fig. 4. The pattern for the heaviest gliadin fraction shows that the mobility of this fraction is the same as that of alpha-gliadin and glutenin (3). The moving-boundary pattern of gliadin fraction III is similar to that of whole gliadin, except that there is an increased proportion of beta-gliadins.

Starch-gel-electrophoretic patterns of whole gliadin and gliadin fractions of Fig. 2(a) are shown in Fig. 5. To emphasize trailing in the gels, 25 μl. of 2% solution (0.5 mg. of protein) was placed in the slots. Whereas in moving-boundary electrophoresis fraction I had the mobility of alpha-gliadin or glutenin, this fraction in starch gel trails between the origin and the gamma-region, presumably because of its large molecular size. Since it does penetrate a starch gel and glutenin does not, the molecular size of fraction I must range between that of
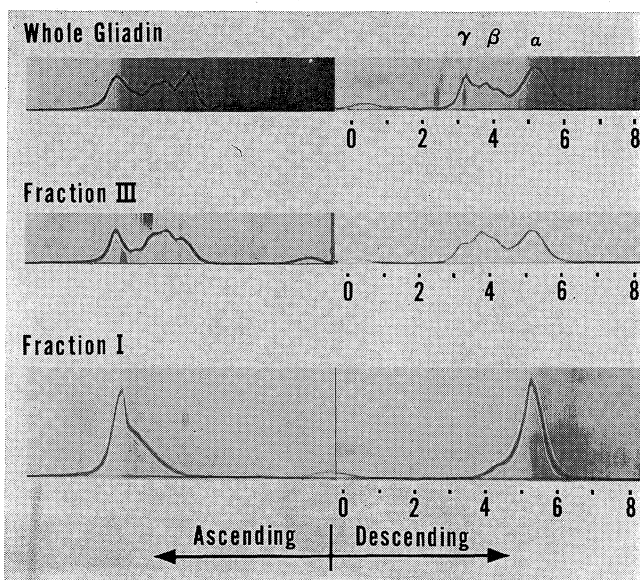

Fig. 4. Moving-boundary-electrophoretic patterns for gliadin and fractions in 0.0167M aluminum lactate plus 0.0695M lactic acid (pH 3.0). Scale below each descending pattern gives electrophoretic mobility in Tiselius units.
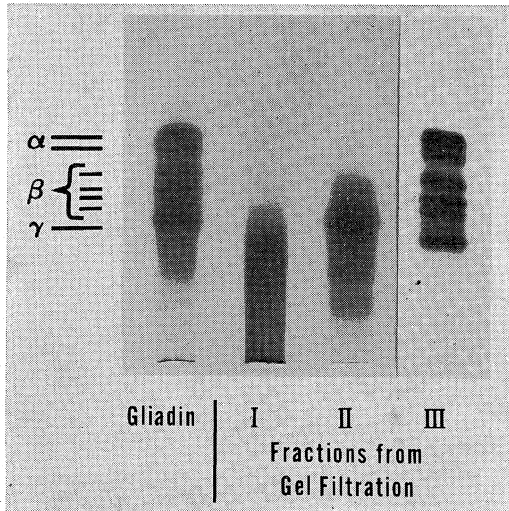
Fig. 5. Starch-gel electrophoresis of gliadin and fractions in a buffer of 0.005M aluminum lactate, 0.02M lactic acid, and 3M urea.

glutenin and that of alpha-gliadin. When a large protein sample is applied, the starch-gel pattern of whole gliadin shows the same trailing of heavier components that is seen in the pattern for fraction I. Trailing occurs when normal protein loads are applied (such as in ref. 4), but is quite hard to detect. In addition to containing proteins from both fractions I and III, gliadin fraction II also has minor gliadin components (17) that migrate appreciably more slowly than gamma-gliadin. In fraction III, obtained by gel filtration, the major gliadin components (4) predominate, i.e., alpha-, beta-, and gamma-gliadins. Even though the gel is heavily loaded, no trailing is visible.

Alpha-gliadin, obtained by chromatography on either carboxymethyl cellulose (11) or sulfoethyl cellulose (18), has a moving-boundary electrophoretic pattern like that of fraction I in Fig. 4 (11). Starch-gel electrophoresis shows that alpha-gliadin contains trailing material in addition to the characteristic alpha-gliadin bands (18). Starch-gel-electrophoretic patterns for the fractions obtained from alpha-gliadin by gel filtration on Sephadex G-100 (Fig. 3) are given in Fig. 6. Fraction A (the fraction excluded from the gel) shows definite trailing because of the large molecular size of its components. Fraction B has the characteristic alpha-gliadin gel bands plus some beta-gliadin contaminants (or perhaps trailing alpha components). Fraction C contains mostly water-soluble contaminants plus an alpha-gliadin band and a faint gamma-gliadin band. Judging from the starch-gel-

Fig. 6. Starch-gel electrophoresis of alpha-gliadin fractions obtained by gel filtration (Fig. 3) in a buffer of 0.005M aluminum lactate, 0.02M lactic acid, and 3M urea.

electrophoretic pattern for fraction A, it seems that alpha-gliadin obtained by chromatography on carboxymethyl cellulose contains material similar to the heavy fraction of gliadin obtained by gel filtration on Sephadex G-100. Judging from the pattern of fraction C (the fraction with the smallest molecular size range), it is concluded that the contaminating water-soluble proteins are smaller than the major gliadin components.

Intrinsic Viscosity. Values of gliadin in standard aluminum lactate buffer solutions have ranged from 0.15 to 0.21 (3,15) depending upon the method of isolation and perhaps on the ionic strength of the solution (19). Table II lists reported values for gliadin and gives the viscosity in this solvent of fractions I and III obtained by gel filtration. The value of 0.30 dl./g. for fraction I is between the value of 0.87 dl./g. reported for glutenin (15) and that for gliadin. This value indicates

TABLE II

MOLECULAR WEIGHTS AND PHYSICAL CONSTANTS OF GLIADIN AND GLIADIN FRACTIONS

MATERIAL	INTRINSIC VISCOSITY	$s_{20,w} \times 10^{13}$	$D_{20,w} \times 10^7$	f/f_o	$\overline{M}_w$
	25°C. dl./g.				
Gliadin	0.15–0.21	2.48			46,000
Fraction I	0.30	3.55	3.00	2.55	104,000
Fraction III	0.093	1.42	4.17	2.49	30,000

that gliadin contains fairly large and perhaps asymmetric molecules. The much lower value for fraction III, or the major gliadin components, indicates that this fraction comprises smaller and perhaps more compact proteins than those in fraction I.

Sedimentation Analysis. The weight-average molecular weights, sedimentation coefficients, diffusion constants, and frictional ratios for gliadin fractions I and III and the parent gliadin also appear in Table II. The reported values for MW's show that gliadin separation on Sephadex columns did indeed take place according to differences in molecular size. The MW value of 30,000 for the major gliadin components (fraction III) is essentially the same as that for gamma-gliadin in this solvent (5) and is close to the value obtained for the beta-gliadins in a less deaggregating solvent (2) This agreement implies that all the major gliadin components have about the same MW. However, a slight upward curvature in the log concentration *vs.* radius-squared plots shows that the fraction is not entirely homogeneous with respect to MW. Log concentration *vs.* radius-squared plots for fraction I also curve upward slightly; therefore, this fraction contains proteins with several different MW's. (Trailing observed in starch-gel electrophoresis also indicates that fraction I ranges in molecular size.)

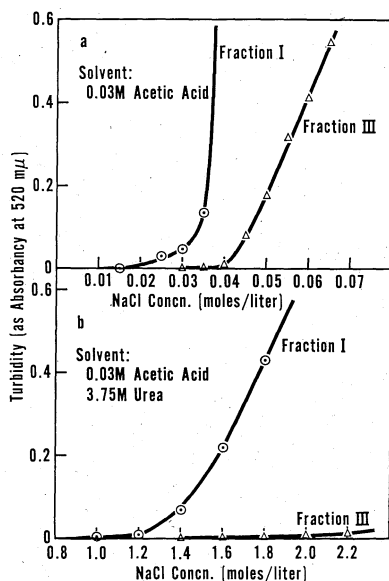The sedimentation and diffusion constants given are weight-average

Fig. 7. Effect of sodium chloride concentration on absorbance of gliadin fractions dissolved in 0.03*M* acetic acid (a) and in 0.03*M* acetic acid plus 3.75*M* urea (b).

values corrected to water at 20° and are those expected from other results presented in this paper. The high frictional ratios obtained for fractions I and III are in the same range as those for proteins known to be quite asymmetric (20). Since these frictional ratios are similar, differences between fractions I and III in intrinsic viscosity and electrophoretic mobility in starch gels reflect differences in molecular size rather than shape.

Influence of Ionic Strength on Turbidity. The influence of ionic strength upon the turbidity of gliadin fractions I and III in two different solvents is shown in Fig. 7. In both solvents fraction I is more sensitive to ionic strength than fraction III. This difference is magnified in the solvent containing urea. The difference in salt-sensitivity between the two fractions is most likely due to difference in size. Although fraction I contains more basic amino acids than fraction III, so that on that basis fraction I should be the more soluble (less salt-sensitive) in acetic acid, its larger molecular size makes it the more salt-sensitive fraction.

Conclusions

Although extraction by neutral 70% ethyl alcohol does appear to separate gluten proteins into fractions differing markedly in average MW, the separation is not clear-cut and some degree of overlapping apparently occurs. Gliadin, thus prepared, was further fractionated by gel filtration into two major components which differed significantly in MW. One fraction, approximately 6% of the total gliadin, had an average MW of around 100,000 and a higher intrinsic viscosity than whole gliadin. This material resembles low-MW glutenin in its amino acid contents, solubility characteristics, and mobility in moving-boundary and starch-gel electrophoresis. It is not homogeneous in MW, as evidenced by nonideal behavior on sedimentation in an ultra-centrifuge and by a streaked pattern on starch-gel electrophoresis. The major gliadin peak eluted from a Sephadex G-100 column constitutes 80% of the total protein and is more homogeneous in MW, averaging about 30,000. It consists of the alpha, beta, and gamma fractions characterized previously by distinct bands on gel electrophoresis.

In view of the very different behavior of these two major gel-filtration fractions of gliadin, criteria other than alcohol solubility should be chosen to define gliadin proteins. A more detailed chemical comparison of these two groups of protein, including studies on the nature of the disulfide bonds and polypeptide chains, should be made before any reclassification is considered. Such studies are presently under way at this Laboratory.

The high MW observed for alpha-gliadin obtained by column chromatography (2) is not consistent with the occurrence of alpha-gliadins in the low-MW fraction from gel filtration. Alpha-gliadin fractions eluted from a carboxymethyl cellulose column can be separated by Sephadex into components of different MW's, but the heavier components are not identical to alpha-gliadin. It is now apparent that carboxymethyl cellulose columns do not necessarily yield pure components, and several criteria, including Sephadex chromatography and gel electrophoresis, should be used to evaluate fractions. The high-MW fraction of gliadin generally is eluted with alpha-gliadin on several cellulose cation exchangers (11,18). The alpha-gliadin proteins can be purified by chromatography on Sephadex G-100 or G-75.

Acknowledgment

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