Gel Filtration and Characterization of Neutral Salt Extracted Wheat Gluten Proteins Varying in Hydrophobic Properties[1]

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ABSTRACT

Defatted gluten proteins, extracted separately with various concentrations of simple monovalent sodium salts of the lyotropic series (F, Cl, Br, ClO_4, I, SCN) were fractionated by gel filtration on Sephacryl S-300. Four fractions with apparent average molecular weights greater than 300,000, 38,000, 18,000, and 14,500, as well as a low molecular weight fraction, were characterized by electrophoresis and amino acid analysis. Large differences in both the quantitative and qualitative properties of the extracts due to anion type and concentration were obtained. With 1.0*M* nonchaotropic salts, low molecular weight gliadinlike proteins and high molecular weight gluten proteins with high charge densities were extracted, indicating that these fractions had a low tendency to interact hydrophobically. As the chaotropic nature of the salts was increased, the 38,000 molecular weight gluten fraction with gliadinlike properties became extractable, suggesting that this fraction had a strong tendency to undergo interprotein hydrophobic interactions. High concentrations of the most chaotropic salt, NaSCN, were required to extract the gluteninlike high molecular weight fraction, indicating a very strong tendency to interact hydrophobically.

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Theoretical protein studies indicate that at ionic strength levels sufficient to minimize electrostatic interactions, changes in protein properties due to variations in the concentration and nature of simple monovalent anions of the lyotropic series can usually be attributed to hydrophobic interactions (Von Hippel and Schleich 1969; Melander and Horvath 1977). In a previous study (Preston 1981), wide variations were found in defatted wheat gluten protein extractabilities and in turbidities of dilute acetic-acid soluble gluten proteins extracted with varying concentrations of simple neutral sodium salts. Increasing concentrations of salting-out anions such as fluoride and chloride reduced gluten protein extractability, whereas increasing concentrations of salting-in chaotropic anions such as iodate and thiocyanate increased extractability to levels approaching those of dilute organic acids. These results and those obtained in turbidity experiments suggested wide variations in the hydrophobic properties of gluten proteins.

To further study their hydrophobic properties, defatted gluten proteins extracted with various concentrations of simple neutral sodium salts of the lyotropic series have been separated by gel filtration on Sephacryl S-300, and the resulting fractions have been characterized by electrophoresis and amino acid analysis.

MATERIALS AND METHODS

Preparation of Defatted Gluten

The defatted gluten used in the present study was the same as that used previously (Preston 1981). It was prepared from a Canadian hard red spring wheat flour (Neepawa) by the procedure of Doguchi and Hlynka (1967) after defatting the flour with chloroform according to the method of MacRitchie and Gras (1973). The defatted gluten had a moisture content of 9.0%, a protein content of 82.7% (N × 5.7, as-is basis), and a lipid content of 0.78% (as-is basis), as determined by the procedure of Drapron (1975).

Extraction of Defatted Gluten

For extraction, 500 mg of defatted gluten and 25 ml of salt solution were mixed in a 50-ml capped centrifuge tube with a vortex mixer to suspend the gluten and then rotated at 50 rpm on a Roto-Torque Rotator (Cole-Parmer) for 1 hr at room temperature. Following centrifugation at 40,000 × *g* for 15 min at 10°C, supernatants and residues were either dialyzed against dilute lactic acid (0.05*M*) to remove salt and lyopholized for determination of Kjeldahl nitrogen or supernatants were stored at 4°C until further use. Before storage, all supernatants were made up to 2.0*M* sodium thiocyanate. All experiments were performed in duplicate.

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Gel Filtration on Sephacryl S-300

For gel filtration, Sephacryl S-300 superfine (Pharmacia) was packed into a 2.5 × 100-cm column (LKB no. 2173) with 2.0*M* sodium thiocyanate as previously described (Preston 1982). Final gel dimensions were approximately 2.5 × 75 cm. For column analysis of salt-extracted gluten proteins, 5 ml of supernatant from each separate salt extract was loaded onto the column and eluted with 2.0*M* sodium thiocyanate, using an upward flow rate of 35 ml/hr. Fractions of approximately 8 ml per tube were collected, and on the basis of absorbance (280 nm) were combined into five peaks of varying molecular weight as described previously (Preston 1982). The absorbance of each combined peak was then measured and compared against total extract (volume × absorbance) supernatant values for estimating protein distributions and recoveries. All experiments were performed in duplicate.

Electrophoresis and Amino Acid Analysis

Supernatants and gel filtration peaks were dialyzed against dilute lactic acid and lyophilized. Electrophoresis was performed in polyacrylamide gels with 0.0085*M* aluminum lactate-lactic acid buffer followed by staining with Coomassie brilliant blue R-250 as described by Tkachuk and Mellish (1980). Amino acid analysis was performed in duplicate on 6.0*M* HCl-hydrolyzed samples (24 hr at 110°C), using a 0.3 × 25 cm column of DC-5A resin (Dionex) with Na^+ buffers as previously described (Preston 1981). For peaks A and B (from gel filtration), 5 mg/ml of lyophilized material was used for electrophoresis. Because of small amounts of peaks C and D, the total lyophilized material from two column runs were made up to 500 μl with electrophoresis buffer.

RESULTS

To obtain more concentrated protein solutions for gel filtration, each gluten sample was extracted only once. Table I shows extractable Kjeldahl nitrogen, total supernatant absorbance at 280 nm, and the ratio of the latter to the former for 1.0*M* neutral salt and water extracts. Nitrogen extractabilities with the 1.0*M* salt solutions and water were somewhat lower but of similar relative magnitude compared to a previous study utilizing a more exhaustive extraction procedure (Preston 1981). Gluten protein extractability increased dramatically across the lyotropic series (F<Cl<Br<ClO_4<I<SCN), as evidenced by increases in nitrogen extractabilities and supernatant absorbances. Sodium thiocyanate, the most chaotropic salt, extracted 49.0% of the gluten nitrogen, whereas sodium chloride, a nonchaotropic salt, extracted only 4.2% of the gluten nitrogen.

Ratios of supernatant absorbance to extractable Kjeldahl nitrogen were similar for the more chaotropic anions (SCN, I, ClO_4) but showed large increases with the nonchaotropic anions such as fluoride and chloride. These increases were probably due to

the decreases in the extractability of the gliadin and gluteninlike proteins, resulting in a much higher proportion of more strongly ultraviolet absorbing (higher tryptophan levels) globulinlike and/or albuminlike proteins and low molecular weight peptides and amino acids. Thus, estimates of relative protein recoveries by absorbance used in the rest of the study probably overestimate the relative recoveries of nonchaotropic salt extracts compared to the more chaotropic salts and the relative proportions of lower molecular weight fractions from gel filtration compared to higher molecular weight fractions.

Gel Filtration and Properties of 1.0*M* Neutral Salt Extracted Gluten Proteins

In a previous study (Preston 1982), it was shown that 2.0*M* sodium thiocyanate-extracted gluten proteins could be separated on Sephacryl-300 into four major fractions having average molecular weights greater than 300,000 (fraction A), 38,000 (fraction B), 18,000 (fraction C), 14,500 (fraction D), and a low molecular weight fraction consisting of peptides and amino acids. Figure 1 and Table II show Sephacryl gel filtration profiles and distributions based upon absorbances at 280 nm, respectively, of corresponding 2.0*M* sodium thiocyanate eluted fractions for 1.0*M* neutral salt- and water-extracted gluten proteins. From the gel-filtration profiles (Fig. 1), it was evident that large differences in the distribution of gluten protein fractions occurred, depending upon the salt used for extraction.

The high molecular weight fraction A gluten proteins were not extractable with water or sodium fluoride. Sodium chloride, bromide, chlorate, and iodide extracted similar amounts of fraction A proteins. This fraction accounted for approximately 20% of the total sodium chloride-extractable proteins and approximately 10% of the total extractable proteins for the other three salts. With 1.0*M* sodium thiocyanate, a large increase of approximately 300% occurred in the extractability of this fraction compared to the above salts. In fact, this was the major fraction contributing to the increased extractability of gluten proteins in 1.0*M* sodium thiocyanate.

Fraction B proteins (38,000 mol wt) were not extractable with sodium fluoride or chloride. However, as the chaotropic nature of the anions increased, large increases occurred in the extractability of this fraction with sodium thiocyanate, the most chaotropic salt, giving the highest value. Similar increases also occurred in the relative proportion of fraction B proteins to total extract up to sodium iodate. The decrease in the proportion of this fraction with sodium thiocyanate (39%) compared to sodium iodate (45%) was due to the large increase in fraction A extractability with the former salt. Fraction B accounted for 59% of the total water extractables, although the amount extracted was less than that with the more chaotropic anions.

Fraction C (18,000 mol wt) proteins were unextractable with sodium fluoride. Water and sodium chloride extracted a similar amount of this fraction, whereas sodium bromide extracted still more. No further increases in extractability occurred with the more chaotropic salts. With the exception of sodium fluoride, fraction C proteins accounted for 9–15% of the total extract absorbances.

Fraction D (14,500 mol wt) protein extractabilities increased from fluoride to bromide but did not increase further with the more chaotropic salts. Water extracted an amount similar to sodium fluoride. Fraction D proteins accounted for a much higher proportion of the total extract with the nonchaotropic salts than with the more chaotropic salts. The extractabilities of the low molecular weight fraction (peptides and amino acids) were similar with all solutions.

For determining electrophoretic profiles, several columns of each 1.0*M* salt and water extract were run, and corresponding fractions were combined, dialyzed, and lyophilized. In addition, gluten proteins were extracted with 0.05*M* acetic acid and lyophilized to serve as an electrophoretic control. Unfortunately, photographs of some gel patterns were faint because of very limited amounts of these fractions, although visual identification of bands was not difficult. Since 1.0*M* sodium fluoride did not extract any of fractions A, B, and C and only small amounts of fraction D, no electrophoresis patterns could be obtained. Similarly, no patterns were available for fraction B proteins from 1.0*M* sodium chloride

TABLE I
Kjeldahl Nitrogen Recoveries and Total Absorbances (280 nm) of 1.0*M* Neutral Salt Extracted Defatted Gluten Supernatants

Salt	Nitrogen Recovery[a] (%)	Absorbance[a] (280 nm)	Ratio[b]
H_2O	18.7	48	2.6
NaF	0.7	9	12.9
NaCl	4.2	42	10.0
NaBr	15.6	73	4.7
$NaClO_4$	25.9	92	3.6
NaI	42.7	127	3.0
NaSCN	49.0	145	3.0

[a] Average of duplicates. All duplicates were within 5% of the value of the mean.
[b] Absorbance/nitrogen recovered.

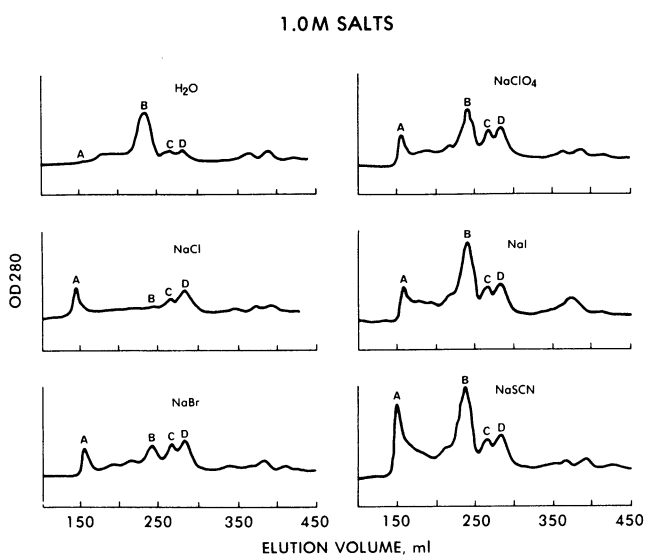

Fig. 1. Gel filtration profiles on Sephacryl S-300 of 1.0*M* neutral salt extracted gluten proteins. For each column run, 5 ml of extract was loaded and eluted with 2.0*M* sodium thiocyanate (35 ml/hr).

TABLE II
Distribution of 1.0*M* Neutral Salt Extracted Gluten Fractions from Sephacryl S-300

Fraction[a]	H_2O	NaF	NaCl	NaBr	$NaC10_4$	NaI	NaSCN
Abs	11.3	2.3	9.3	15.9	20.0	27.3	31.8
A							
Abs	0	0	1.9	1.9	2.0	2.4	8.9
Extract, %	0	0	20	12	10	9	27
B							
Abs	6.6	0	0	3.7	7.5	12.2	12.7
Extract, %	59	0	0	23	37	45	39
C							
Abs	1.1	0	1.3	2.5	2.7	2.7	2.8
Extract, %	10	0	14	15	13	10	9
D							
Abs	1.3	0.9	2.8	3.9	3.6	3.7	3.4
Extract, %	11	39	30	25	18	13	10
Low mol. wt.							
Abs	2.0	1.2	1.9	1.7	1.7	2.3	1.5
Extracts, %	18	52	20	10	9	9	5
Recoveries							
Abs	11.0	2.1	7.9	13.7	17.5	23.3	29.3
Extract, %	98	91	84	85	87	86	90

[a] Abs = average total absorbances (280 nm) of duplicate experiments. All were within 5% of the mean.

and fraction A proteins from water.

Fraction A (Fig. 2) proteins gave electrophoretic patterns characterized by a heavily stained origin and a "streaked" upper region of low mobility similar to that normally obtained with glutenins and a streaked lower region of high mobility. The presence of streaky rather than distinct protein bands in both regions may indicate the presence of strongly aggregating proteins. Although not readily clear from the photographs, visual inspection of the gels indicated wide differences in the relative staining intensities of the high- and low-mobility regions. Fraction A proteins from 1.0*M* sodium thiocyanate extracts stained more intensely in the low-mobility region, whereas extracts from 1.0*M* sodium chloride, bromide, and chlorate stained more intensively in the high-mobility regions. Sodium iodate gave relative staining intensities for the two regions intermediate to thiocyanate and the other salts.

Fraction B proteins (Fig. 3) from the 1.0*M* salt and water extracts gave electrophoretic patterns similar to Osborne gliadins. Some variations in the relative staining intensities of specific bands, however, were evident, with the more chaotropic salts appearing to have a higher proportion of lower mobility bands. In addition, the relative staining intensity of the higher mobility bands appeared to be lower than normally associated with Osborne gliadins for all extracts.

Fraction C proteins (mol wt 18,000) gave similar electrophoretic patterns for all extracts, although variations in the relative staining intensities of specific bands were evident (Fig. 4). Similar results were also obtained for fraction D proteins (not shown). In general, patterns for fractions C and D were similar and showed similarities to Osborne gliadins. Compared to fraction B proteins, however, both fraction C and D proteins had higher relative staining intensities in the high-mobility regions.

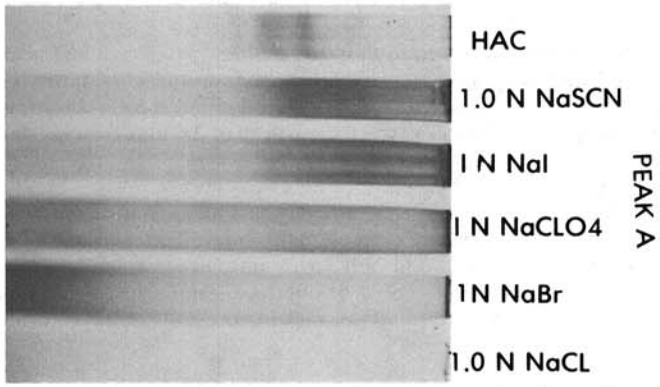

Fig. 2. Aluminum lactate polyacrylamide gel electrophoretic profiles of 1.0*M* neutral salt extracted fraction A gluten proteins from Sephacryl S-300.

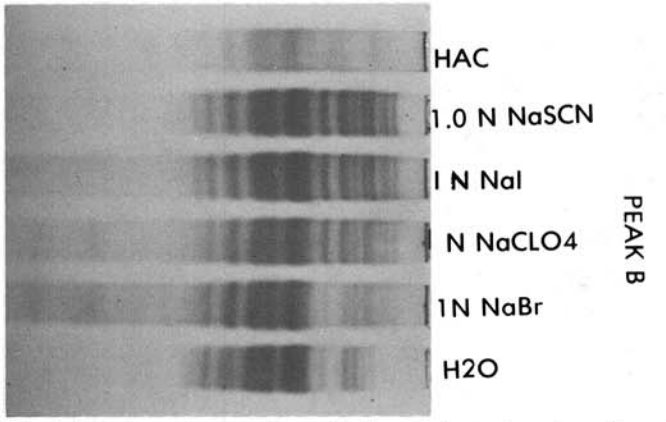

Fig. 3. Aluminum lactate polyacrylamide gel electrophoretic profiles of 1.0*M* neutral salt extracted fraction B gluten proteins from Sephacryl S-300.

Amino acid compositions of the 1.0*M* salt and water extracts and of whole defatted gluten are shown in Table III. The table is arranged in order of increasing gluten protein extractability for the 1.0*M* salts and follows the lyotropic series. Changes in the amino acid compositions of the extracts from the 1.0*M* salts followed the same trend as previously shown (Preston 1981). From the nonchaotropic salts to the chaotropic salts, there were large decreases in the proportion of basic amino acids and aspartic acid (plus asparagine) and increases in glutamic acid (plus glutamine) and proline. This suggested increases in the proportion of gliadin and gluteninlike proteins with the chaotropic salts. The largest differences occurred between sodium chloride and sodium bromide. Compared to the defatted gluten, the more chaotropic salts of the lyotropic series (sodium chlorate, iodide, and thiocyanate) had higher proportions of glutamic acid (plus glutamine), proline, and phenylalanine, and lower proportions of glycine and basic amino acids. The water extract had an amino acid composition most similar to that reported for Osborne gliadins (Dronzek et al 1970).

Amino acid compositions of the fraction A proteins from 1.0*M* sodium chloride and thiocyanate are shown in Table IV. Amino acid compositions of fraction A proteins from 1.0*M* sodium bromide, chlorate, and iodide (data not shown) were very similar to that obtained with sodium chloride, suggesting the presence of

TABLE III
Amino Acid Compositions (mole %)[a] of 1.0*M* Neutral Salt Extracted Gluten

Amino Acid	H_2O	NaCl	NaBr	$NaClO_4$	NaI	NaSCN	Gluten
Aspartic acid	2.4	7.3	4.0	3.3	3.2	2.6	3.4
Threonine	2.1	3.8	2.5	2.1	2.1	2.2	2.7
Serine	5.1	6.0	5.1	4.6	4.9	5.2	5.6
Glutamic acid	37.9	20.7	35.9	37.0	37.0	37.4	33.1
Proline	18.0	9.0	15.7	17.1	17.4	17.7	15.1
Glycine	2.9	7.9	4.2	3.6	3.7	4.1	5.7
Alanine	3.0	7.3	3.7	3.6	3.3	3.4	4.4
Valine	4.5	6.6	4.6	4.4	4.3	4.2	4.4
Methionine	1.5	1.9	1.3	1.1	1.2	1.2	1.3
Isoleucine	4.2	4.0	4.0	4.0	4.0	3.8	3.5
Leucine	7.2	7.8	6.8	6.7	6.8	6.9	7.0
Tyrosine	1.6	2.7	2.1	2.0	1.9	1.9	2.2
Phenylalanine	5.1	3.5	4.9	5.2	5.2	5.2	4.4
Lysine	0.7	3.4	1.8	1.2	1.0	0.9	2.1
Histidine	1.8	2.6	2.2	1.8	1.8	1.7	1.8
Arginine	1.9	5.6	2.7	2.2	2.1	2.0	2.5
Hydrophobic	45.1	42.8	43.1	44.1	44.0	44.3	43.2
Basic	4.4	11.6	6.7	5.2	4.9	4.6	6.4

[a] Average of duplicates. Standard deviations of duplicates for each salt averaged approximately 3%.

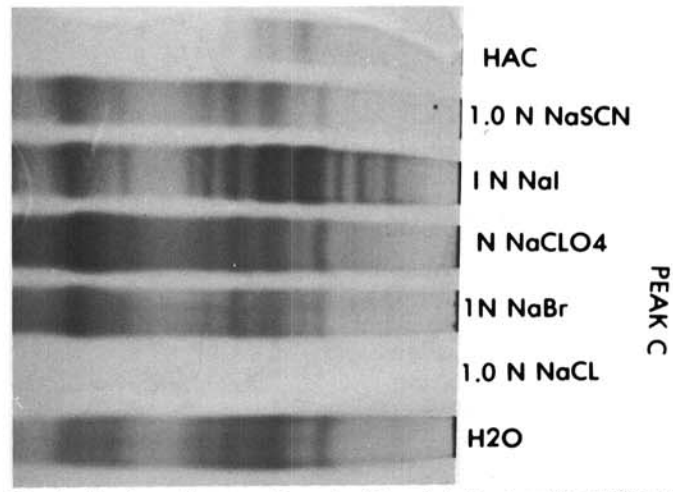

Fig. 4. Aluminum lactate polyacrylamide gel electrophoretic profiles of 1.0*M* neutral salt extracted fraction C gluten proteins from Sephacryl S-300.

similar proteins. Compared to the defatted gluten proteins, sodium chloride-extracted proteins had higher levels of aspartic acid (plus asparagine), valine, and basic amino acids and much lower levels of glutamic acid (plus glutamine) and proline. In fact, their amino acid composition was somewhat similar to Osborne albumins and/or globulins (Dronzek et al 1970). In contrast, 1.0*M* sodium thiocyanate-extracted fraction A proteins had an amino acid composition similar to the whole gluten proteins, including high levels of glutamic acid (plus glutamine) and proline and relatively low levels of basic amino acids. These latter results, the high molecular weight of this fraction determined by gel filtration (greater than 300,000), and the high relative staining intensity of the low-mobility region (including the origin) on electrophoresis (Fig. 2) suggested that the very large increase in extractability of the fraction A proteins with 1.0*M* sodium thiocyanate compared to the other salts (Table I) was due to a large increase in the extractability of gluteninlike proteins.

The amino acid compositions of fraction B proteins are also shown in Table IV. Results for 1.0*M* sodium fluoride, chloride, and bromide were unobtainable due to low extractabilities. In agreement with the electrophoretic results, the amino acid compositions of the fraction B proteins from 1.0*M* sodium chlorate, iodide, and thiocyanate and water extracts were very similar to previously published values for wheat gliadins (Dronzek et al 1970). Even though the solubility of this fraction increased substantially across the lyotropic series ($SCN>I>ClO_4$), only small changes occurred in the amino acid compositions.

Table V shows the amino acid compositions of the 1.0*M* sodium thiocyanate-extracted fraction C and D proteins. Because of the large number of chromatography runs required to obtain sufficient material for each of these fractions, only single analyses of the other salt and water extracts were performed (data not shown). From the single analysis results, however, all the salt and water extracts gave fractions having amino acid compositions similar to that obtained with the corresponding 1.0*M* sodium thiocyanate fractions. Both fraction C and D proteins had amino acid compositions somewhat similar to the defatted gluten proteins, including high levels of glutamic acid (plus glutamine) and proline and relatively low levels of basic amino acids. Fraction C proteins had higher levels of threonine, valine, and total basic amino acids and lower levels of glutamic acid (plus glutamine), proline, and phenylalanine than the defatted gluten proteins. Fraction D proteins had higher levels of alanine and lower levels of glycine and total basic amino acids than did the defatted gluten proteins. Differences in the amino acid compositions of fraction C and D included threonine, serine, and total basic amino acids, which were higher in fraction C and glutamic acid (plus glutamine), proline, alanine, and phenylalanine, which were higher in fraction D. Generally, however, considering their amino acid compositions and electrophoretic profiles, both fraction C and D proteins appeared to resemble lower molecular weight gliadins (Preston and Woodbury 1976).

Effects of Sodium Chloride and Sodium Thiocyanate Concentrations on the Extractability of Gluten Protein Fractions

The effects of increasing concentrations of a nonchaotropic salt (sodium chloride) and a chaotropic salt (sodium thiocyanate) upon the extractability of the gluten protein fractions are shown in Tables VI and VII, respectively, while corresponding gel filtration profiles are shown in Figs. 5 and 6. Sodium chloride, as expected from previous studies (Preston 1981), reduced the total gluten protein extractability compared to water, as evidenced by the decrease in optical density at 280 nm. As the concentration of sodium chloride was increased from 0.05 to 0.5*M*, however, extractability increased to some extent. Part of this increase could be attributed to the increase in fraction A protein extractability. This fraction had very low extractability with low levels of sodium chloride (0–0.3*M*) but showed increases above 0.3*M* salt. These results appear to suggest a requirement for high ionic strength to solubilize these proteins. This view is consistent with the amino acid and electrophoretic data, indicating the presence of albumin and/or globulinlike proteins with much higher charge densities than the whole gluten.

In contrast to fraction A proteins, the extractability of fraction B proteins decreased with increasing sodium chloride concentration. In view of the gliadinlike nature of these proteins, as evidenced by amino acid analysis and electrophoresis data, the decrease in extractability of this fraction with increasing concentrations of sodium chloride was not surprising. Similar results are evident in the literature (Beckwith et al 1963).

Fraction C and D proteins appeared to increase in extractability as sodium chloride concentration was increased. The extractability of fraction C proteins in water, however, was as high as that for the highest (1.0*M*) salt level, whereas the extractability of fraction D

TABLE IV
Amino Acid Compositions (mole %)[a] of 1.0*M* Neutral Salt Extracted Gluten Fraction A and Fraction B Proteins

	Fraction A		Fraction B				
Amino Acid	NaCl	NaSCN	H_2O	$NaClO_4$	NaI	NaSCN	Gluten
Aspartic acid	7.8	3.1	2.5	2.9	2.6	2.4	3.4
Threonine	4.2	2.9	1.9	1.8	1.7	1.7	2.7
Serine	7.4	5.8	5.0	5.2	4.4	4.2	5.6
Glutamic acid	17.7	33.3	38.5	37.6	38.7	40.5	33.1
Proline	9.1	14.3	20.0	17.9	19.6	19.4	15.1
Glycine	11.4	7.4	2.4	2.8	2.6	2.4	5.7
Alanine	5.8	3.5	2.6	3.1	3.7	2.6	4.4
Valine	7.7	4.9	4.2	4.7	3.7	4.0	4.4
Methionine	1.8	1.5	1.4	1.1	1.0	0.9	1.3
Isoleucine	4.0	3.3	4.1	4.3	4.0	4.0	3.5
Leucine	7.8	7.2	6.9	7.2	6.6	6.6	7.0
Tyrosine	2.1	2.7	1.4	1.9	1.7	1.7	2.2
Phenylalanine	3.1	4.1	5.4	5.1	5.7	5.9	4.4
Lysine	3.5	1.3	0.7	0.8	0.6	0.7	2.1
Histidine	1.7	1.7	1.5	1.7	1.6	1.5	1.8
Arginine	4.8	1.7	1.5	1.7	1.6	1.7	2.5
Hydrophobic	41.4	41.5	46.0	45.3	46.0	45.1	43.2
Basic	10.0	5.8	3.9	4.4	3.9	3.9	6.4

[a] Average of duplicates. Standard deviations of duplicates for each salt averaged approximately 4%.

TABLE V
Amino Acid Compositions (mole %)[a] of 1.0*M* Sodium Thiocyanate Extracted Gluten Fraction C and Fraction D Proteins

Amino Acid	Fraction C	Fraction D	Gluten
Asparatic acid	4.2	3.9	3.4
Threonine	4.2	2.5	2.7
Serine	6.5	4.7	5.6
Glutamic acid	30.5	32.8	33.1
Proline	12.5	16.4	15.1
Glycine	4.7	4.1	5.7
Alanine	4.2	5.7	4.4
Valine	6.3	5.3	4.4
Methionine	2.2	1.8	1.3
Isoleucine	4.7	4.3	3.5
Leucine	7.1	7.3	7.0
Tyrosine	2.2	1.6	2.2
Phenylalanine	2.8	4.4	4.4
Lysine	1.9	1.0	2.1
Histidine	2.8	1.7	1.8
Arginine	3.2	2.5	2.5
Hydrophobic	44.0	40.8	43.2
Basic	7.9	5.2	6.4

[a] Average of duplicates. Standard deviations of duplicates for each salt averaged approximately 4%.

proteins was lowest in water.

In contrast to sodium chloride, increasing concentrations of the chaotropic salt, sodium thiocyanate, led to large increases in gluten protein extractability (Table VII). The increases were generally due to increases in the extractability of all fractions. In particular, fraction A and fraction B proteins showed the largest increases and at higher salt levels accounted for the highest percentages of the total extract. In contrast to sodium chloride and the other salts, the amino acid and electrophoretic data indicated that gluteninlike proteins were mainly responsible for increases in the extractability of fraction A proteins with sodium thiocyanate. Increases in the extractabilities of fraction C and D proteins with increasing salt concentration were also higher with sodium thiocyanate compared to sodium chloride.

DISCUSSION

In a previous study (Preston 1981), it was suggested that the effects of neutral salt solutions of the lyotropic series upon the physical properties of wheat gluten may be useful in the study of the hydrophobic properties of these proteins and their relationship to rheological and baking properties. At ionic strength levels sufficient to overcome electrostatic interactions, nonchaotropic salts such as sodium fluoride and sodium chloride only extracted low levels of gluten proteins, whereas chaotropic salts such as sodium iodide and sodium thiocyanate extracted levels approaching that of dilute organic acids. These results and those involving turbidity experiments suggested that at low salt concentrations the extractability and aggregation properties of gluten proteins are largely determined by ionic interactions, whereas in higher salt concentrations (0.5*M*) hydrophobic interactions predominate. Results from the present study support

TABLE VI
Effects of Sodium Chloride Concentration on the Extractability of Gluten Fractions from Sephacryl S-300

Fraction[a]	0	0.05	0.1	0.3	0.5	1.0
Abs	11.3	5.9	7.1	8.4	9.8	9.3
A						
Abs	0	0	0.1	0.3	1.5	1.9
Extract, %	0	0	1	4	16	20
B						
Abs	6.6	0.7	0.8	0.9	0	0
Extract, %	59	12	11	10	0	0
C						
Abs	1.1	0	0.2	0.8	1.0	1.3
Extract, %	10	0	3	10	10	14
D						
Abs	1.3	1.6	1.9	2.4	3.2	2.8
Extract, %	11	28	27	29	33	30
Low mol wt						
Abs	2.0	1.5	1.2	2.4	2.5	1.9
Extract, %	18	25	17	29	26	20
Recoveries						
Abs	11.0	3.8	4.2	6.8	8.2	7.9
Extract, %	98	65	59	82	85	84

[a] Abs = average total absorbances (280 nm) of duplicate experiments. All duplicates were within 5% of the mean.

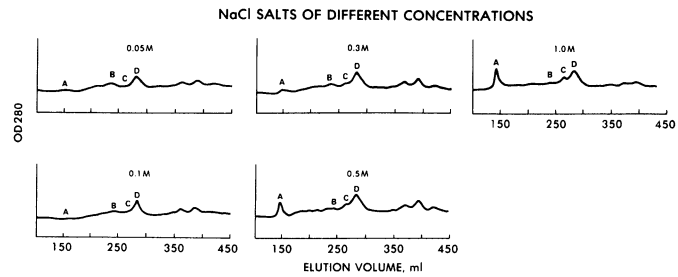

Fig. 5. Gel filtration patterns on Sephacryl S-300 of gluten proteins extracted with varying concentrations of sodium chloride. For each column run, 5 ml of extract was loaded and eluted with 2.0*M* sodium thiocyanate.

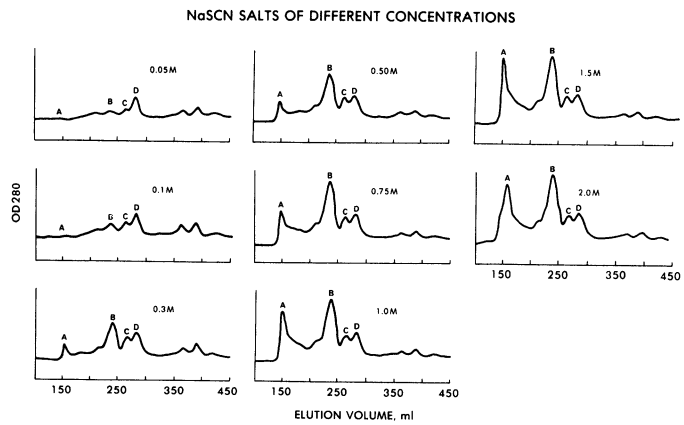

Fig. 6. Gel filtration profiles on Sephacryl S-300 of gluten proteins extracted with varying concentrations of sodium thiocyanate. For each column run, 5 ml of extract was loaded and eluted with 2.0*M* sodium thiocyanate.

TABLE VII
Effects of Sodium Thiocyante Concentration on the Extractability of Gluten Fractions from Sephacryl S-300

Fraction[a]	0	0.05	0.1	0.3	0.5	0.75	1.0	2.0
Abs	11.3	9.9	15.8	20.1	21.5	30.6	31.8	35.0
A								
Abs	0	0.1	0.2	1.5	18	6.7	8.9	11.3
Extract, %	0	1	2	8	8	22	27	32
B								
Abs	6.6	2.5	3.2	7.4	9.9	12.9	12.7	14.4
Extract, %	59	26	26	37	46	42	39	4.1
C								
Abs	1.1	1.2	1.3	2.4	2.3	2.8	2.8	3.1
Extract, %	10	12	11	12	11	9	9	9
D								
Abs	1.3	2.67	3.2	3.8	3.6	3.1	3.4	4.4
Extract, %	11	27	26	19	17	10	10	13
Low mol wt								
Abs	2.0	2.9	3.8	3.7	1.9	3.0	1.5	1.8
Extract, %	18	29	24	19	9	9	5	5
Recoveries								
Abs	11.0	9.4	11.7	18.9	19.5	28.5	29.3	35.0
Extract, %	98	95	89	95	91	92	90	100

[a] Abs = average total absorbances (280 nm) of duplicate experiments. All duplicates were within 5% of the mean.

and extend these conclusions.

With 1.0*M* salt solutions, the nonchaotropic salts (NaF and NaCl) extracted low levels of gluten proteins. These extractable proteins probably lack the ability to undergo strong interprotein hydrophobic interactions. Gel filtration profiles showed mainly low molecular weight proteins (Fractions C and D) with medium to high electrophoretic mobilities and amino acid compositions similar to gliadins. In addition, 1.0*M* sodium chloride solutions also extracted a high molecular weight fraction. This protein fraction had high electrophoretic mobilities and a much lower proportion of glutamic acid (plus glutamine) and proline and a much higher proportion of basic amino acids than normally associated with gluten proteins. The decrease in the extractability of this fraction with decreasing sodium chloride concentration (Table VI) is consistent with this view. At lower salt levels, aggregation probably occurs because of ionic interactions. Gluten proteins with similar properties have been reported previously (Huebner and Wall 1976, 1980; Khan and Bushuk 1979; Frazier et al 1981). Recent studies by Frazier et al (1981) indicate that gluten proteins with relatively high charge densities may be very important in lipid binding.

Fraction B proteins had apparent molecular weights (mol wt 38,000), amino acid compositions, and electrophoretic mobilities consistent with the major gliadin fraction extracted with dilute acetic acid and/or 70% ethanol (Preston and Woodbury 1976). As the chaotropic nature of the 1.0*M* salt solutions was increased across the lyotropic series ($F<Cl<Br<ClO_4<I<SCN$), large increases in the extractability of this fraction occurred. The high extractability of this fraction in 1.0*M* solutions of the two most chaotropic salts (Table II) compared to the very low extractability in the nonchaotropic salts, and the increase in extractability of this fraction with increasing concentrations of sodium thiocyanate suggests strong interprotein hydrophobic interactions associated with high surface hydrophobicities. This finding is not surprising in view of previous studies that indicate the strong hydrophobic nature of gliadin proteins (Greene and Kasarda 1971; Caldwell 1979; Popineau and Godon 1978; Chung and Pomeranz 1979).

In addition to the high molecular weight fraction extractable in 1.0*M* sodium chloride, bromide, chlorate, and iodide, a second high molecular weight fraction with quite different properties was extracted with 1.0*M* sodium thiocyanate, the most chaotropic salt. This fraction had low electrophoretic mobilities and an amino acid composition, including high levels of glutamic acid (plus glutamine) and proline, consistent with wheat glutenins soluble in organic acids (Dronzek et al 1970). The inability of all but the most chaotropic salt (1.0*M* NaSCN) to extract these gluteninlike proteins and the increased extractability with a high level of sodium thiocyanate (Table VII) indicates very strong interprotein hydrophobic interactions associated with very high surface hydrophobicities. Previous studies by Chung and Pomeranz (1979) support this conclusion. Utilizing hydrophobic interaction chromatography, these authors found that glutenins generally were more strongly absorbed than gliadins, suggesting stronger hydrophobic interactions with the former. In addition, studies by Huebner (1970) and by Arakawa and Yonezawa (1975) have shown that glutenins are more sensitive than gliadins to precipitation with sodium chloride, which increases hydrophobic interactions (Von Hippel and Schleich 1969).

A high proportion of the gluten proteins was inextractable even in the highest concentration of the most chaotropic salt (2.0*M* NaSCN). This inextractability appears to be closely related to the presence of disulfide bonds, as evidenced by the high gluten extractability (more than 90%) in 2.0*M* sodium thiocyanate in the presence of 2-mercaptoethanol (Preston 1981). Whether or not this inextractability is due to the presence of very large proteins formed by intersubunit disulfide bonds or to very strong hydrophobic interactions due to conformational effects of disulfide bonding (or a combination of the two effects) is not clear. The fact that these proteins can be extracted with very strong hydrophobic disrupting solvents (Meredith and Wren 1966; Kobrehel and Bushuk 1977; Huebner and Wall 1980) suggests that the latter may play an important role.

From the present results, the low molecular weight (fractions C and D) gliadins and high molecular weight gluten proteins with high charge densities (NaCl-extractable fraction A) show the least tendency to interact hydrophobically. In contrast, the major fraction of gliadin proteins with molecular weights of approximately 38,000 (fraction B) appear capable of strong hydrophobic interactions, whereas the soluble glutenins (NaSCN= extractable fraction A) and probably the 2.0*M* sodium thiocyanate- insoluble gluten proteins are capable of very strong hydrophobic interactions. A number of factors may account for these differences.

As discussed in a previous study (Preston 1981), gluten proteins generally have high levels of apolar amino acids and low levels of charged amino acids, which would favor hydrophobic interactions. In general, apolar amino acids tend to reside in the interior of the protein structure to avoid contact with water because of the large free energy of transfer (up to 3 kcal per residue). Charged amino acids almost always reside on the outside to promote interaction with water, while polar amino acids can reside inside or outside with little difference in free energy, providing that the interior residues undergo H-bonding (Kauzmann 1959, Tanford 1962). As pointed out by Fisher (1964), however, proteins with a high ratio of apolar to charged and polar amino acids will have an excess of apolar residue that cannot be accommodated in the hydrophobic core; thus, surface hydrophobicity will be increased, promoting protein aggregation due to hydrophobic interactions. Moreover, proteins with low charge densities tend to have a higher proportion of apolar amino acids on the surface because polar residues cannot compete thermodynamically as effectively as charged amino acids with apolar residues for sites on the surface (Melander and Horvath 1977).

The strong tendency of the major gliadin fraction (fraction B), the soluble glutenin fraction (2.0*M* NaSCN-soluble fraction A), and perhaps the 2.0*M* NaSCN-insoluble gluten fraction to undergo interprotein hydrophobic interactions is probably largely related to their high proportion of apolar amino acids (Table IV) and their relatively low proportion of charged amino acids (Yoshino and Matsumoto 1966), which would increase surface hydrophobicity. In contrast, the inability of the high molecular weight 2.0*M* NaCl-soluble gluten fraction A proteins to undergo strong hydrophobic interactions may be related to its higher charge density. However, these factors cannot explain the apparent large differences in the relative strengths of hydrophobic interactions between the fraction B gliadins, the fraction A soluble glutenins (and insoluble gluten proteins), and the low molecular weight gliadins (fractions C and D). In fact, the higher proportion of hydrophobic amino acids and lower charge densities of the fraction B gliadins compared to the fraction A glutenins suggest that the former have higher surface hydrophobicities, which is in opposition to the results obtained.

Several possible factors may explain this apparent discrepancy. Studies by Fisher (1964) and Bigelow (1967) have shown that globular protein formation is favored over a much wider range of average residue hydrophobicities for low molecular weight proteins ($H\Phi$ ave = 900–1,200 cal per residue for mol wt 40,000) compared to high molecular weight protein ($H\Phi$ ave = 1,000–1,100 cal per residue for mol wt 80,000). This is probably related to spatial considerations in that the hydrophobic core of low molecular weight proteins can more easily accommodate apolar residues than higher molecular weight proteins. Thus, in proteins with high proportions of apolar amino acids, those with higher molecular weights would tend to have a greater proportion of apolar residues on the surface, resulting in a higher surface hydrophobicity and a stronger tendency to interact hydrophobically.

This factor may, in large part, explain the apparent stronger interprotein hydrophobic interactions of the 2*M* NaSCN-soluble fraction A glutenins (and perhaps the insoluble gluten proteins) compared to the fraction B gliadins. Even though the proportion of apolar amino acids was slightly lower and charged amino acids were probably higher, the higher molecular weights of the former, due presumably to interchain disulfide bonding, would result in a hydrophobic core less capable of accommodating the large number of apolar residues, resulting in a much greater surface

hydrophobicity. The almost complete extractabiltiy of the higher molecular weight gluten proteins with 2.0*M* sodium thiocyanate in the presence of 2-mercaptoethanol supports this conclusion (Preston 1981). Similarly, the lower molecular weight gluten proteins (fractions C and D) would be more capable of accomodating a higher proportion of apolar residues in their hydrophobic cores and thus, without considering other factors, would have a lower surface hydrophobicity and thus interact less strongly than the fraction B proteins.

In addition to its effect on molecular weight, disulfide bonding may have other important effects on gluten protein hydrophobicities. As pointed out by Bigelow (1967), covalent cross links such as disulfide bonds may prevent protein folding and thus reduce globular protein formation. This can result in a more asymetric protein shape that would increase surface hydrophobicity and increase interprotein hydrophobic interactions. Thus, the number and position of disulfide bonds in gluten proteins may be an important factor in determining their relative surface hydrophobicities. In particular, this factory may further increase the surface hydrophobicities of the 2.0*M* sodium thiocyanate-soluble glutenins and insoluble gluten proteins since a reduction in protein folding would further disrupt a hydrophobic core less capable (due to high molecular weights) of accommodating the large number of apolar residues present.

The last factor discussed that may affect the surface hydrophobicities of the gluten proteins is proline content. Although the presence of proline, on the basis of thermodynamic arguments (related to conformational entropy changes), would be expected to stabilize globular protein structures and thus reduce surface hydrophobicity this amino acid can also have a very marked effect on the rate of protein folding and unfolding. Studies by Brandts and co-workers (1975) and by Creighton (1978) indicate that in unfolded proline containing proteins, the rate of refolding is dependent upon the rate of *cis-trans* isomerism of proline residues. Thus, polypeptides containing 20 proline residues would require approximately 10 min to refold from a denatured state; with 30 residues, 100 min would be required for refolding. Because gluten proteins normally contain a very high proportion of proline, the ability of these proteins to refold following work input, such as mixing in the preparation of gluten, may be severely restricted. Thus, although not necessarily favored thermodynamically, the unfolding of the high molecular weight glutenins during work input may be to some extent irreversible and thus lead to increased surface hydrophobicity. It is also interesting to note that the effect of proline isomerism on the rate of protein folding and unfolding may partially explain such phenomena as stress relaxation in doughs and other related phenomena.

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