Isolation and Fractionation of Carbohydrate-Containing Proteins from Wheat Gluten[1]

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ABSTRACT

Examination of the carbohydrates of hard red spring wheat gluten showed that the levels of carbohydrate in three fractions, sodium dodecyl sulfate (SDS)-insoluble, SDS-soluble–70% ethanol-insoluble, and SDS-soluble–70% ethanol-soluble, were 11.63, 1.27, and 0.5% (w/w), respectively. The major sugars in the SDS-insoluble fraction were glucose, xylose, and arabinose; galactose and mannose were present in lesser amounts. The SDS-soluble fractions contained glucose, galactose, and mannose as the major sugars. The ethanol-insoluble fractions contained more sugars than the ethanol-soluble fractions for each neutral sugar component. SDS-polyacrylamide gel electrophoresis (SDS-PAGE) of the three protein fractions demonstrated that the carbohydrate was associated with all protein subunits with molecular mass greater than 32,000 Da. The carbohydrate-containing fractions isolated by an SDS solubility fractionation procedure were further fractionated by gel filtration chromatography on Sephadex G-200 and Sephacryl S-400. The carbohydrate components of the SDS-soluble–70% ethanol-insoluble and SDS-soluble–70% ethanol-soluble fractions were associated with the high molecular weight subfraction obtained by gel filtration chromatography. Coincident stains for carbohydrate and protein after electrophoresis of the protein fractions indicated that these components were closely associated. The glucose in wheat gluten resulted mainly from endosperm starch, whereas other nonstarchy carbohydrate may be involved in the glycoprotein structure of wheat gluten.

The gluten proteins are largely responsible for the viscoelastic properties of wheat dough, which determine the suitability of the dough for breadmaking (Wall 1979). It is important for an understanding of gluten functionality to study not only the major protein components but also the minor components and their interactions on a molecular level. Wheat gluten is composed of protein (80–85%) and lipids (5–10%), with the remainder consisting largely of carbohydrates (Kasarda et al 1971). Several researchers (Olcott and Mecham 1947, Udy 1957, Lee and Wan 1963, Kasarda et al 1971) reported that small amounts of carbohydrate were present in gluten preparations. Inouye et al (1974) isolated two carbohydrate-rich fractions from gluten containing 3.2 and 7.2% carbohydrate. Khan and Bushuk (1979) reported the presence of carbohydrate in peak I (void volume peak) of reduced alkylated glutenin obtained by gel filtration on Sephadex G-200. McMaster and Bushuk (1983) studied the proportion and composition of carbohydrates in the alcohol-soluble and alcohol-insoluble fractions of a gluten preparation. They found that the alcohol-soluble fraction contained 0.6% (w/w) carbohydrate, which was 55.5% galactose, 20.7% glucose, 16.1% arabinose, 6.2% mannose, and 1.2% xylose. The alcohol-insoluble fraction contained 17.0% carbohydrate, which was 97% glucose with only trace amounts of arabinose, xylose, galactose, and mannose. D'Appolonia and Giles (1971) isolated the pentosans from the gluten fraction of wheat flour and found that all of the glutens extracted from different hard red spring wheat flours, durum semolinas, and soft wheat flours contained pentosan material. The pentosan material was fractionated by diethylaminoethylcellulose chromatography into five fractions. Fraction 1 was essentially an arabinoxylan with only small amounts of protein. The pentosans associated with gluten were similar to the pentosans extracted from flour.

The results of the above studies indicated that the carbohydrate associated with gluten was nonstarchy in nature; however, it is still not known whether the carbohydrate was covalently or noncovalently linked with gluten protein. Thus, more information about the carbohydrate portion of the gluten proteins at the molecular level is needed to better understand its role in dough functionality. The purpose of this study was to isolate carbohydrate-rich fractions from wheat gluten, to quantitatively and qualitatively characterize the carbohydrate portion, and to determine the association of carbohydrates with the gluten protein.

MATERIALS AND METHODS

Materials

Hard red spring wheat variety Wheaton was used in this study. The wheat was milled on a Buhler experimental mill into straight-grade flour.

Biochemicals used in this study were purchased from Sigma Chemical Company (St. Louis, MO) and Aldrich Chemical Company (Milwaukee, WI). Chemicals, reagents, and solvents were obtained from American Scientific Products (McGaw Park, IL) and were analytical grade. Gel filtration media were obtained

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from Pharmacia Fine Chemicals (Uppsala, Sweden) and Bio-Rad Laboratory (Richmond, CA).

Determination of Protein Content

Nitrogen content of the gluten was determined using the Kjeldahl method (Method 46-12, AACC 1983). Protein content of gluten was obtained by multiplying the percentage of nitrogen content by the conversion factor of 5.7 recommended by Tkachuk (1969).

Fractionation Procedure for Wheat Gluten

Flour was first defatted according to the method of McMaster and Bushuk (1983). The fractionation procedure was based on the study by Graveland et al (1980). Gluten was obtained by hand-washing a dough ball made from 10 g of defatted flour under a gentle stream of distilled water. The wet gluten was then divided into small pieces and solubilized by magnetic stirring (25 hr at 20°C) in an aqueous solvent (100 ml) containing 0.1% sodium dodecyl sulfate (SDS). The preparation was centrifuged (16,000 × *g*) at 20°C for 15 min. The precipitate was the SDS-insoluble fraction. Ethanol was added to the supernatant to a concentration of 70% (v/v). The suspension was stored for 16 hr at 4°C. The precipitate was freeze-dried to provide an SDS-soluble–70% ethanol-insoluble fraction. The supernatant then was reduced to 50% in volume by rotary evaporation under reduced pressure at 30°C and freeze-dried to provide an alcohol-soluble fraction.

α-Amylase Digestion of Wheat Gluten

Gluten (285 g) was incubated with 3,000 ml of a solution of 3.8 g of α-amylase free from proteolytic activity (α-amylase, *Bacillus subtilus* 171568, Calbiochem-Behring Co., La Jolla, CA) and 0.02 g of NaCl made up in 0.02*M* sodium phosphate buffer (pH 6.9) at 20°C. The reducing sugars released during digestion were monitored by 3,3-dinitrosalicylic acid reagent (Bernfeld 1955). When the reducing sugar concentration reached a maximum, the suspension was filtered through a glass microfiber filter (10–15 μm). The residue was washed with four volumes of absolute ethanol followed by two volumes of acetone and freeze-dried.

Carbohydrate Analysis

Monosaccharide composition was determined by gas-liquid chromatography (GLC) analysis (McGinnis 1982). Gluten fractions (10 mg) were hydrolyzed with 1*M* sulfuric acid (1 ml) in a boiling water bath for 2 hr, cooled to ambient temperature, and neutralized by the addition of barium carbonate. The resulting suspension was centrifuged, and the supernatant was used for neutral sugar analysis by GLC. The neutral sugars in the carbohydrate hydrolysates were converted to their respective aldononitrile acetates. The aldononitrile acetates were separated and identified by GLC analysis. Methyl-α-D-glucopyranoside was used as the internal standard in this procedure.

Carbohydrate contents in gluten, gluten fractions, and subfractions were estimated as total carbohydrate using the phenol-sulfuric acid method (Dubois et al 1956). Glucose was used as the standard sugar.

Amino Acid Analysis

Amino acid composition was determined by using a high-performance liquid chromatographic procedure (Simons and Johnsons 1976). Samples were hydrolyzed in 6*N* HCl for 24 hr at 105°C under vacuum. Norleucine was used as an internal standard. Results were reported as grams of amino acid per 100 g of protein.

SDS-Polyacrylamide Gel Electrophoresis with Discontinuous Buffers

SDS-polyacrylamide gel electrophoresis (SDS-PAGE) with discontinuous buffers was performed according to the method of Laemmli (1970). Samples were prepared by dissolving 10 mg of protein in 1.0 ml of Tris-SDS buffer (0.0625*M* Tris, pH 6.8) containing 2-mercaptoethanol (1%, v/v). The staining and destaining of gels were performed according to the methods of Koenig et al (1977) and Leach et al (1980) for protein and carbohydrate, respectively. Molecular weights were estimated by comparison with proteins of known molecular weight.

Gel Filtration Chromatography

The SDS-soluble material was fractionated on a column (2.5-cm diameter × 40-cm bed height) of Sephadex G-200 eluted with 0.1% aqueous SDS solution at a flow rate of 13.2 ml/hr. Fractions of 4.4 ml were collected. The carbohydrate-rich fractions were further fractionated on a column (2.0-cm diameter × 90-cm bed height) of Sephacryl S-400 eluted with 0.1% SDS solution at a flow rate of 28 ml/hr. Fractions of 10 ml were collected. Void and total volumes were determined by chromatographing blue dextran and tryptophan, respectively. The protein content in each fraction was determined by measuring the UV absorbance at 280 nm, and the carbohydrate content in each fraction was estimated as total carbohydrate (pentoses and hexoses) by using the phenol-sulfuric acid method (Dubois et al 1956) with glucose as the standard sugar.

RESULTS AND DISCUSSION

Selection of Fractionation Procedure

Selecting a fractionation procedure that would remove all the noncovalently linked starchy and nonstarchy polysaccharides from the wheat gluten without breaking any possible glycosidic linkage between the carbohydrate and protein was a major concern. In the SDS fractionation procedudure employed in this study, the formation of a gluten ball provided a relatively easy method to remove starchy and nonstarchy polysaccharides as well as water-soluble proteins that were not directly involved in the developed gluten structure. The wet gluten was then extracted with an aqueous SDS solution (0.1%). Although SDS does not dissolve all the gluten proteins, this solvent was selected because it dissociates proteins organized by noncovalent bonds. This procedure should not break glycosidic bonds between the protein and carbohydrate in the gluten.

A preliminary study was conducted to determine the best conditions for extracting gluten proteins with an SDS solution. It was concluded that the best results were obtained by extraction with a 0.1% SDS solution at a solvent-to-gluten ratio of 100:1 at 20°C and with magnetic stirring for 25 hr. Under these conditions, about 75% of the gluten protein was extracted by the SDS solution.

Three protein fractions were derived from the gluten protein by the SDS fractionation procedure: the SDS-insoluble fraction, the SDS-soluble–70% ethanol-insoluble fraction, and the SDS-soluble–70% ethanol-soluble fraction. The distribution of nitrogen in the protein fractions is shown in Table I. The SDS-insoluble fraction contained 29.1% of the total nitrogen in the gluten and the SDS-soluble fraction contained the remaining 70.9%, of which the alcohol-insoluble fraction contained 47.3% and the alcohol-soluble fraction contained 23.6% total nitrogen.

Composition of Gluten Fractions

The protein and carbohydrate contents of gluten fractions are given in Tables I and II, respectively. The SDS-insoluble fraction

TABLE I
Distribution of Nitrogen in Protein Fractions[a]

Protein Fraction	Distribution[b] (%)	Concentration[c] (%)
SDS[d]-insoluble	29.1	77.1
SDS-soluble–70% ethanol-insoluble	47.3	91.0
SDS-soluble–70% ethanol-soluble	23.6	94.1

[a] All data are expressed as the percentage of gluten fraction weight on an as-is moisture basis and as the average of two measurements.
[b] The percentage of protein weight in each gluten fraction to total protein in gluten.
[c] The percentage of protein weight in gluten fraction to the total gluten fraction weight.
[d] Sodium dodecyl sulfate.

TABLE II
Sugar Composition of Protein Fraction[a]

Protein Fraction	Total Sugar[b] (%)	Sugar Composition,[c] %					
		Ara	Xyl	Man	Gal	Glc	A.S.
SDS[d]-insoluble before α-amylase digestion	11.63	0.24	0.53	0.17	0.39	10.30	0.0013
SDS-insoluble after α-amylase digestion	1.70	0.24	0.53	0.19	0.37	0.37	...
SDS-soluble–70% ethanol-insoluble	1.27	0.05	0.09	0.19	0.14	0.80	0.0010
SDS-soluble–70% ethanol-soluble	0.50	0.01	0.01	0.09	0.19	0.20	0.0021

[a] All data are expressed as the percentage of gluten fraction weight on an as-is moisture basis and as the average of two measurements.
[b] The percentage of total sugar weight in each gluten fraction to total gluten weight.
[c] Ara = Arabinose; Xyl = xylose; Man = mannose; Gal = galactose; Glc = glucose; A.S. = amino sugar.
[d] Sodium dodecyl sulfate.

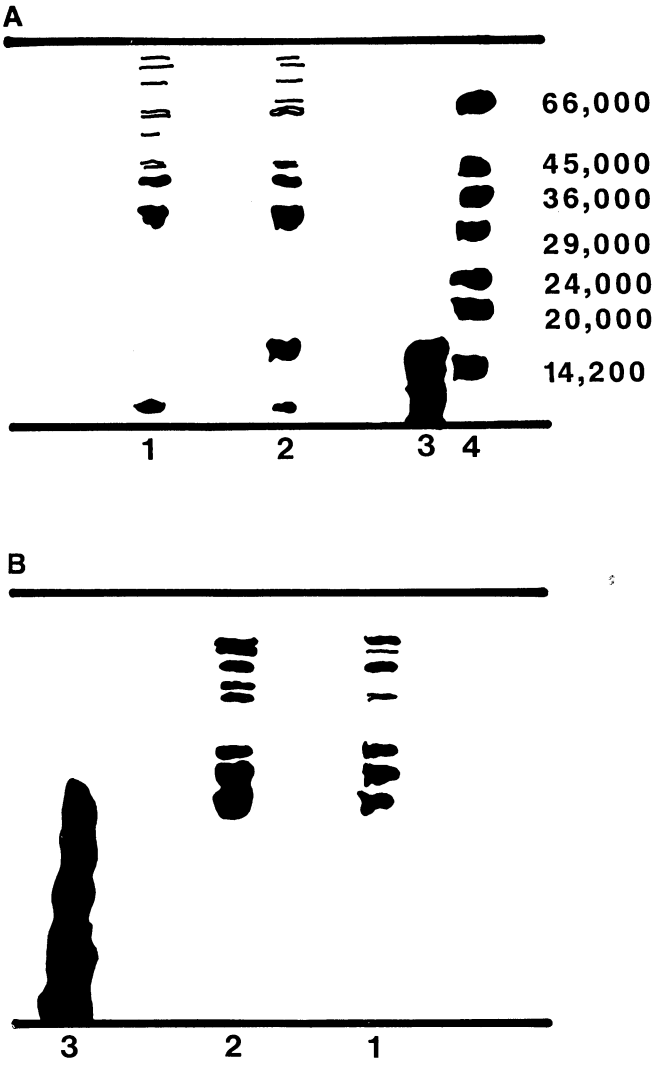

Fig. 1. Sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis patterns of wheat gluten fractions stained for proteins (**A**) and carbohydrates (**B**). Lane 1, SDS-insoluble fraction; lane 2, SDS-soluble–70% ethanol-insoluble fraction; lane 3, SDS-soluble–70% ethanol-soluble fraction; lane 4, protein standards. Molecular weights given on the right in **A**.

contained more carbohydrate than the other two fractions, and the SDS-soluble–70% ethanol-insoluble fraction contained more carbohydrate than the SDS-soluble–70% ethanol-soluble fraction. This indicated that the majority of carbohydrate was possibly associated with a high molecular weight (HMW) protein fraction that was difficult to solubilize, or that the protein fraction contained long-chain polysaccharides that were difficult to dissolve.

The sugar compositions of the protein fractions are shown in Table II. All three gluten fractions have been shown to contain carbohydrate components. The major sugars in the SDS-insoluble fraction were glucose, xylose, and arabinose; galactose and mannose were present in lesser amounts. This suggested that the SDS-insoluble fraction may contain an arabinoxylan polysaccharide that was found in gluten by D'Appolonia and Gilles (1971). The polysaccharide may be associated with the HMW protein, or it may be a HMW polysaccharide. The SDS-soluble fractions contained glucose, galactose, and mannose as the major sugars. The ethanol-insoluble fractions contained more sugar than the ethanol-soluble fractions for each neutral sugar component.

The high level of glucose in the SDS-insoluble fraction indicated that this fraction could contain a starchy polysaccharide. To verify this, the SDS-insoluble fraction was subjected to an enzymatic (α-amylase) treatment as described above. The sugar composition of the SDS-insoluble fraction after α-amylase digestion is given in Table II. After α-amylase digestion, the glucose level of the SDS-insoluble fraction was reduced significantly by about 95–98%. The small amount of glucose that was detected after α-amylase digestion was assumed to be involved in the glycosidic linkage of the side chain of the glycoprotein in this gluten fraction or the nonstarchy polysaccharide associated with this gluten fraction. The monosaccharide composition of the carbohydrate component in these fractions clearly demonstrated that the fractions were different. Their differences in solubility may well depend on their carbohydrate components.

McMaster and Bushuk (1983) used a solvent containing acetic acid, urea, and cetyltrimethyl ammonium bromide to extract wheat gluten protein. They found that the alcohol-soluble fraction contained 0.6% (w/w) carbohydrate, whereas the alcohol-insoluble fraction contained 17.0% carbohydrate. The results from this study indicated that the SDS-soluble–70% ethanol-soluble fraction contained about the same amount of carbohydrate as the alcohol-soluble fraction from their study, whereas the SDS-soluble–70% ethanol-insoluble fraction had a lower carbohydrate content than the alcohol-insoluble fraction from McMaster and Bushuk's study. Compared with the results of McMaster and Bushuk, the SDS-soluble–70% ethanol-soluble fraction from this study contained lower amounts of galactose and arabinose and higher amounts of glucose, mannose, and xylose than the alcohol-soluble fraction from their study. The SDS-soluble–70% ethanol-insoluble fraction from this study contained lower concentrations of glucose but higher concentrations of other neutral sugars than the alcohol-insoluble fraction from McMaster and Bushuk's study. The differences in sugar content between the two studies may be due to the different extraction solutions used. SDS may have dissociated the gluten proteins so that they had a more open structure than did the proteins extracted with the cetyltrimethyl ammonium bromide solution; thus, noncovalently linked carbohydrates could be removed easily from the gluten proteins.

SDS-PAGE of Carbohydrate-Containing Proteins

The gluten fractions were subjected to electrophoresis on polyacrylamide gels. The electrophoretic patterns showing the stains for protein and carbohydrate of the three gluten fractions are shown in Figure 1. When stained for protein, the electrophoretic pattern was heterogeneous, with many components entering the gel ranging in molecular weight from 14,000 to 100,000. The SDS-

insoluble and SDS-soluble–70% ethanol-insoluble fractions contained larger numbers of HMW polypeptide bands than the SDS-soluble–70% ethanol-soluble fraction. Polypeptide bands with a molecular mass of more than 30,000 Da from the SDS-soluble–70% ethanol-soluble fraction were very faint and quickly faded upon destaining the gel. When stained for carbohydrate (Fig. 1B), several dark pink bands were observed, indicating a positive periodic acid-Schiff reaction and the presence of carbohydrate. All of the carbohydrate comigrated with HMW polypeptide bands of all three fractions. The comigration of carbohydrate and protein in electrophoresis gave an indication that the carbohydrate in wheat gluten may be covalently linked. The electrophoretic pattern stained for carbohydrate in this study showed differences from the results of McMaster (1982), who found only one or two HMW polypeptides that had a positive reaction with the carbohydrate staining procedure of Fairbanks et al (1971). This can be explained by the increased sensitivity of the carbohydrate staining procedure of Leach et al (1980) used in this study. Leach et al (1980) reported that the procedure of Fairbanks et al failed to detect some glycoprotein because of its low sensitivity. Thus, in McMaster's (1982) study, it was possible that this procedure was not sensitive enough to detect small amounts of carbohydrate associated with the HMW polypeptide bands.

Amino Acid Composition

The amino acid composition of the three gluten fractions (Table III) is typical for wheat endosperm proteins in that they show high contents of glutamic acid and nonpolar amino acids (Kasarda et al 1971). The amino acid composition of these fractions was examined for any enrichment of amino acid residues that are known to occur in covalent linkages between carbohydrate and protein, as in glycoproteins. No abnormally high values were observed for the proportion of serine or threonine. The SDS-insoluble and SDS-soluble–70% ethanol-insoluble fractions contained a large proportion of aspartic acid. The amide of aspartic acid is a common amino acid linked to *N*-acetyl glucosamine in the high-mannose type of glycoproteins found in mammalian tissues. However, this class of glycoproteins is not widely distributed in plants.

TABLE III
Amino Acid Composition[a] of the Protein Fraction of Gluten of the Hard Red Spring Wheat Wheaton

Amino Acid	SDS[b]-Insoluble	SDS-Soluble–70%-Ethanol-Insoluble	SDS-Soluble–70%-Ethanol-Soluble
Asp-H	1.15	1.56	0.67
Glu-H	6.94	13.36	7.51
Ser	1.54	2.50	1.21
His	0.54	0.84	0.47
Gly	1.68	2.53	0.78
Thr	0.99	1.37	0.64
Arg	1.49	2.24	0.88
Ala	0.97	1.40	0.59
Tyr	1.16	1.90	0.77
Met	0.41	0.65	0.19
Val	1.09	1.74	0.88
Phe	1.32	1.96	1.44
Ile	0.87	1.30	0.81
Leu	1.96	3.03	1.66
Lys	0.92	1.13	0.26

[a] Grams of amino acid per 100 g of protein.
[b] Sodium dodecyl sulfate.

Gel Filtration on Sephadex G-200 and Sephacryl S-400

The SDS-soluble fractions obtained by the SDS solubility fractionation procedure were subjected to further studies using chromatographic techniques. The gel filtration chromatography was performed on Sephadex G-200 because of its HMW exclusion limit. The subfraction of gluten fractions from Sephadex G-200 was further fractionated on Sephacryl S-400, which has a higher molecular weight exclusion limit than Sephadex G-200. These studies were undertaken to provide background information on the behavior of both the protein and the carbohydrate components in particular fractions.

The elution profile for the SDS-soluble–70% ethanol insoluble fraction (Fig. 2) shows that protein eluted in four individual peaks. These were labeled I to IV according to the order of elution. The relative proportion of peaks (by weight), peak elution volumes, K_{av} values, and estimated molecular weights for SDS-soluble–70% ethanol-insoluble fractions are given in Table IV.

The first protein peak (peak I) was eluted in the void volume of the column, indicating that it was excluded from the Sephadex

TABLE IV
Peak Weights, Elution Volumes, K_{av} Values, and Molecular Weights of Peaks Obtained from Gel Filtration Chromatography on Sephadex G-200 of Gluten Protein Fractions

	Peak Weight[a] (%)	V_e (ml)	K_{av}	Molecular Weight
SDS[b]-soluble–70% ethanol-insoluble				
Peak I	60	135 ± 2.0	0.00	>200,000
Peak II	14	213 ± 1.8	0.24	75,000
Peak III	16	260 ± 2.8	0.39	50,000
Peak IV	10	370 ± 1.6	0.73	16,000
SDS-soluble–70% ethanol-soluble				
Peak I	30	135 ± 1.5	0.00	>200,000
Peak II	14	213 ± 1.8	0.24	75,000
Peak III	21	260 ± 2.8	0.39	50,000
Peak IV	35	370 ± 1.6	0.73	16,000

[a] Peak weight as a percentage of total weight of peaks recovered.
[b] Sodium dodecyl sulfate.

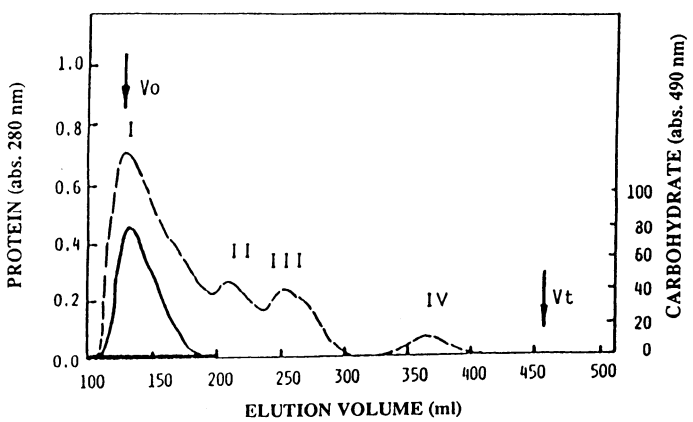

Fig. 2. Elution profile from gel filtration chromatography on Sephadex G-200 of the sodium dodecyl sulfate-soluble–70% ethanol-insoluble fraction. Protein, – – –; carbohydrate, ———

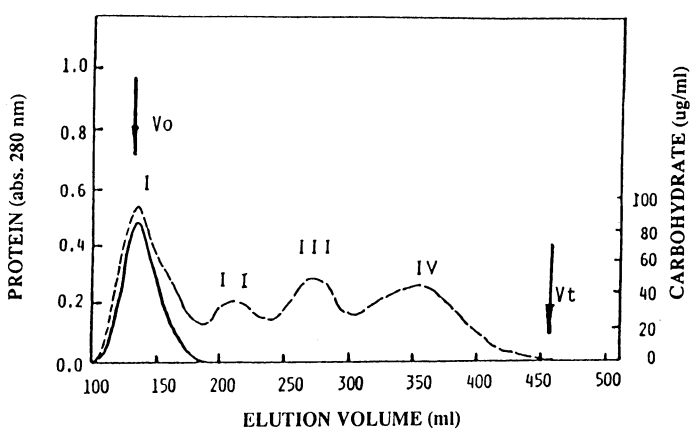

Fig. 3. Elution profile from gel filtration chromatography on Sephadex G-200 of the sodium dodecyl sulfate-soluble–70% ethanol-soluble fraction. Protein, – – –; carbohydrate, ———.

matrix and, therefore, has a molecular mass greater than 200,000 Da. The first included peak (peak II) was relatively small and eluted at 213 ml. Peak III was eluted at 260 ml, and peak IV eluted as a broad symmetrical peak (370 ml). The carbohydrate present in the SDS-soluble–70% ethanol-insoluble fraction chromatographed as a single homogeneous peak, corresponding in elution volume to the HMW protein in peak I. No carbohydrate was detected in any of the other protein peaks.

The elution profile on Sephadex-200 for SDS-soluble–70% ethanol-soluble fraction is shown in Figure 3. The relative proportion by peaks (by weight), peak elution volumes, K_{av} values, and estimated molecular weights are given in Table IV. The protein also eluted in four individual peaks, labeled I to IV according to the order of elution. These four protein peaks were eluted at the same volumes as the protein peaks for the SDS-soluble–70% ethanol-insoluble fraction (Fig. 2) on Sephadex G-200. The elution profiles of the alcohol-insoluble and alcohol-soluble fractions differed in that the alcohol-soluble fraction eluted more protein at the lower molecular weight range. The carbohydrate in the SDS-soluble–70% ethanol-soluble fraction also was associated with the HMW protein (peak I).

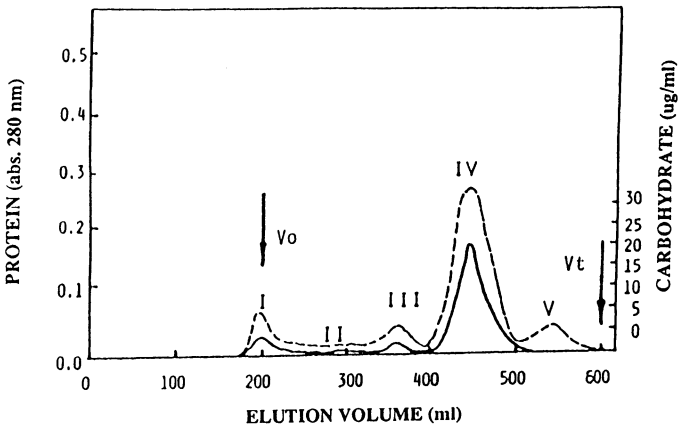

Fig. 4. Elution profile from gel filtration chromatography on Sephacryl S-400 of subfraction (peak I on Sephadex G-200) of the sodium dodecyl sulfate-soluble–70% ethanol-insoluble fraction. Protein, – – –; carbohydrate, ———.

The carbohydrate-containing peak of SDS-soluble–70% ethanol-insoluble fraction on Sephadex G-200 (peak I) was further chromatographed on Sephacryl S-400. The elution profile obtained is shown in Figure 4. The relative proportion of peak (by weight), peak elution volumes, K_{av} values, and estimated molecular weights are given in Table V. Protein eluted in five individual peaks. The carbohydrate was associated with the first four protein peaks of high molecular weight. About 85% (by weight) carbohydrate was coeluted with protein peak IV. The carbohydrate composition in this peak was examined by GLC analysis (Table VI). The major sugars in this subfraction were glucose, mannose, and galactose. When compared with the original protein fraction applied to Sephadex G-200, the total carbohydrate content in this subfraction was increased by about 11 times. However, the distribution of monosaccharide in each subfraction was very similar to that in the original protein fraction.

The carbohydrate-containing peak of SDS-soluble–70% ethanol-soluble fraction on Sephadex G-200 (peak I) was further chromatographed on Sephacryl S-400. The elution profile obtained is shown in Figure 5. The relative proportion of peaks (by weight), peak elution volumes, K_{av} values, and estimated molecular weights are given in Table V. Protein eluted in three individual peaks. When compared with the SDS-soluble–70% ethanol-insoluble fraction, the SDS-soluble–70% ethanol-soluble fraction did not have a protein subfraction in the molecular mass range of 2,000,000–320,000 Da. Only a small percentage of HMW protein peak was found in this protein fraction. The carbohydrate was associated only with protein peak II of this fraction, which had a molecular mass around 320,000 Da. The carbohydrate composition of this peak was examined by GLC analysis (Table VI). The major sugars in this peak were glucose, galactose, and mannose. The total carbohydrate content in this peak was 9.5 times larger than that in the original protein fraction applied to Sephadex G-200. However, the distribution of monosaccharide in this peak was very similar to that in the original protein fraction.

CONCLUSIONS

Wheat gluten protein was separated into three carbohydrate-containing fractions by using aqueous SDS solution. The levels of carbohydrate in three fractions, SDS-insoluble, SDS-soluble–70% ethanol-insoluble, and SDS-soluble–70% ethanol-

TABLE V
Peak Weights, Elution Volumes, K_{av} Values, and Molecular Weights of Peaks Obtained from Gel Filtration Chromatography on Sephacryl S-400 of Subfraction (Peak I on Sephadex G-200) of Gluten Protein Fractions

	Peak Weight[a] (%)	V_e (ml)	K_{av}	Molecular Weight
SDS[b]-soluble–70% ethanol-insoluble				
Peak I	14.5	199 ± 1.5	0.00	>2,000,000
Peak II	11.8	217 ± 0.3–336 ± 1.7	0.08–0.46	760,000–390,000
Peak III	10.0	353 ± 2.1	0.52	335,000
Peak IV	57.7	420 ± 3.1	0.54	320,000
Peak V	6.0	120 ± 2.8	1.06	200,000
SDS-soluble-70% etanol-soluble				
Peak II	21	199 ± 0.8	0.00	>2,000,000
Peak III	64	420 ± 1.3	0.54	320,000
Peak IV	15	520 ± 0.9	1.03	200,000

[a] Peak weight as a percentage of total weight of peaks recovered.
[b] Sodium dodecyl sulfate.

TABLE VI
Sugar Composition of the Protein Fractions of the Hard Red Spring Wheat Wheaton from Sephacryl S-400

Protein Fraction	Sugar Content (%)	Sugar Composition,[a] %				
		Ara	Xyl	Man	Gal	Glc
SDS[b]-soluble–70% ethanol-insoluble, peak IV	14.7	0.58	1.04	2.20	1.62	9.26
SDS-soluble–70% ethanol-soluble, peak II	6.6	0.13	1.13	1.19	2.51	2.64

[a] All data are expressed as the percentage of gluten fraction weight on an as-is moisture basis and as the average of two measurements. Ara = Arabinose; Xyl = xylose; Man = mannose; Gal = galactose; Glc = glucose.
[b] Sodium dodecyl sulfate.

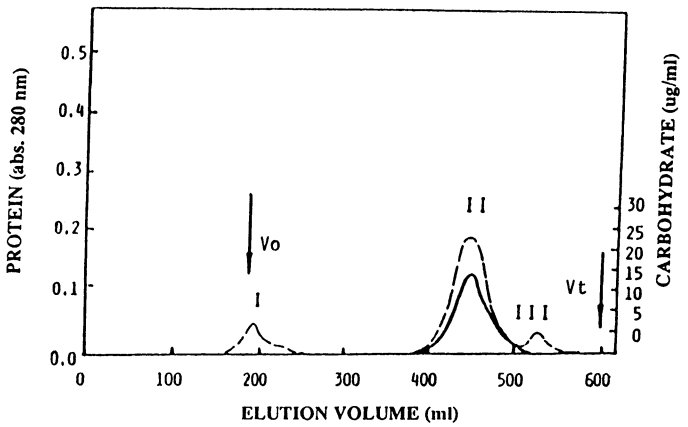

Fig. 5. Elution profile from gel filtration chromatography on Sephacryl S-400 of subfraction (peak I on Sephadex G-200) of the sodium dodecyl sulfate-soluble–70% ethanol-soluble fraction. Protein, – – –; carbohydrate, ———.

soluble, were found to be 11.6, 1.3, and 0.5% (w/w), respectively. The major sugars in the SDS-insoluble fraction were glucose, xylose, and arabinose; galactose and mannose were present in lesser amounts. The SDS-soluble fractions contained glucose, galactose, and mannose as the major sugars. The ethanol-insoluble fractions contained more sugars than the ethanol-soluble fractions for each neutral sugar component.

The carbohydrate components of the SDS-soluble–70% ethanol-soluble fractions were associated with the HMW fraction obtained by gel filtration chromatography on Sephadex G-200 and Sephacryl S-400. SDS-PAGE of the three protein fractions showed that the carbohydrate was associated with all the protein subunits with molecular weight greater than 32,000. It was found that the carbohydrate and protein in wheat gluten coeluted during gel filtration and comigrated on SDS-PAGE. These observations support the hypothesis that the carbohydrate and protein in the gluten fractions may have been covalently linked. However, additional evidence for the existence of a covalent protein-carbohydrate linkage needs to be demonstrated. In a subsequent article, Chen et al (1992) report on the use of proteolytic enzyme digestion of the gluten preparation and mass spectrometric analyses of isolated fractions to establish the nature of the relationship between carbohydrate and protein.

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