Water-Soluble Pentosans of Wheat Flour. I. Viscosity Properties and Molecular Weights Estimated by Gel Filtration[1]

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ABSTRACT

Crude and purified water-soluble pentosans were obtained from flour and freeze-dried doughs (nonrested or rested 3 hr. at 30°C.; with and without added oxidants). Yields of pentosans increased in the order, flour, nonrested dough, rested dough. Specific viscosity of pentosans from nonrested doughs equaled that of those from flour, but adding potassium bromate and iodate (1 μeq. oxidant per g. flour) to doughs during mixing increased pentosan viscosity. Presence of bromate in flour-water dough apparently did not, however, recover the gelling ability of pentosan lost during resting of the doughs. The increased viscosity of pentosans obtained from doughs treated with oxidizing agents indicated that fraction II (obtained from chromatography of the water-soluble pentosans on DEAE-cellulose) might be involved in the interaction with oxidizing agents (bromate and iodate) added during mixing. Gel filtration of individual DEAE-cellulose fractions from flour pentosans showed that fraction II, a glycoprotein responsible for gelation, contained a high-molecular-weight component (MW 95,000, as estimated by gel filtration), representing 26.7% of the total purified pentosans. About 46% of the purified pentosans had MW >85,000, and about 91% had MW >40,000.

Water-soluble pentosans of wheat flour are important in breadmaking because of their effect on water-binding capacity (1,2) and the consistency (3) of dough and also their possible involvement in oxidation of flour (4,5). Interest in the role of water-soluble pentosans in breadmaking originated with Baker et al. (6), who found that solutions of water-soluble pentosans form gels after minute amounts of oxidizing agents (dough improvers) have been added. Fractionation of water-soluble pentosans of wheat flour on a column of DEAE-cellulose yielded five components, the second of which (fraction II) was found to be responsible for the gelation (7). Neukom et al. (8) postulated the glycoprotein nature of water-soluble pentosans, and Pomeranz (9) pointed out that the large size and complexity of molecular structure of pentosans and/or glycoproteins may contribute to their important water-binding role and interaction with other flour constituents and also to their possible involvement in helping oxidize flour. Such possibilities suggest the need for additional research on the nature of water-soluble pentosans in dough and their relationship to changes in oxidation resulting from flour improvers (such as potassium bromate and iodate).

Recent work by D'Appolonia et al. (10) indicates that among the fractions isolated by chromatography of water-soluble flour pentosans on DEAE-cellulose, those containing protein improve loaf volume of starch-gluten breads. But no studies have determined whether that improvement results from the presence of protein *per se* or the enhanced molecular size and complexity of structures involving pentosans (11).

To estimate the molecular size of individual fractions obtained from chromatography of flour pentosans on DEAE-cellulose, we adopted a gel filtration technique. Because pentosan molecules have expanded shapes and are

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distinctly different from standard globular proteins (12), it was considered realistic to use dextran fractions of known molecular weights and expanded structure to calibrate the gel filtration chromatographic column.

In this paper we report the results of a part of the studies characterizing components of water-soluble pentosans and glycoproteins from wheat flour and doughs mixed under various conditions.

MATERIALS AND METHODS

Flours

A typical hard red winter wheat flour, Scout-69 (protein 11.2%, 14% moisture basis), untreated and experimentally milled, was used to study water-soluble pentosans from flour and doughs. Scout R-70 (protein 9.5%, 14% m.b.) was used for the detailed study of DEAE-cellulose fractions of flour pentosans including viscosity, gelling properties, and molecular weight estimation by gel filtration.

Dough

Doughs (300 g. flour, 14% m.b.) were mixed in a farinograph bowl at 60 r.p.m. and 30°C. Absorptions were adjusted to give a dough consistency of 500 Brabender Units (B.U.). Some doughs were mixed with 1 μeq./g. flour of potassium bromate, potassium iodate, *N*-ethylmaleimide, or cysteine-HCl and were lyophilized. Also, doughs without any additives (plain doughs) and with added bromate (bromated doughs) were rested at 30°C. and 95% r.h. for 3.0 hr. Lyophilized doughs were ground to pass through a 60-mesh sieve and were stored at −20°C.

Isolation of Crude Pentosans

Crude, water-soluble pentosans were isolated in duplicate from flour and ground lyophilized doughs according to the procedures of Cawley (13) and Kuendig et al. (14). Crude pentosans were purified further by treating them with bacterial α-amylase to remove water-soluble starch according to the procedure of Kuendig et al. (14), with some modification in the amount of trichloroacetic acid (TCA) used to precipitate the enzyme after the treatment. To ensure a meaningful comparison among various treatments, the amount of 5% TCA required to precipitate the enzyme was estimated by the following procedure, using crude pentosans from two flours: i) Scout-69 (HRW); and ii) Red River-68 (HRS, protein 14%, 14% moisture basis). Five milligrams of twice crystallized bacterial α-amylase (Sigma Chemical Co.) was dissolved in 10 ml. 0.02M sodium phosphate buffer, pH 7.2, containing 0.4N sodium chloride and incubated at room temperature in a dialysis bag with 200 mg. lyophilized, crude pentosans dissolved in 10 ml. water. The mixture was then dialyzed at room temperature for 48 hr. against diluted (1:1) buffer solution. The enzyme was removed by precipitation with various amounts (5 to 200 mg. per mg. enzyme) of 5% TCA, then centrifuged at 20,000 × g for 10 min. The supernatant solution then was dialyzed against distilled water for 48 hr. and lyophilized.

After the α-amylase treatment, protein content was determined by the AACC micro-Kjeldahl method (15); for our experiment, we chose the amount of TCA yielding a protein content in the purified pentosans similar to that of the crude pentosans initially used. Crude, water-soluble pentosans from flour and doughs

were treated with α-amylase as described above, except that 15 mg. enzyme and 600 mg. of crude pentosans were used. Six milliliters of 5% TCA for every 600 mg. crude pentosans was used throughout this work.

Viscosity Measurements

Specific viscosities of 5 ml. solutions (0.2 to 1.0%, w./v.) of crude pentosans and DEAE-cellulose fractions were determined by measuring the flow time with an Ostwald viscometer equilibrated in a 30°C. water bath (16). To measure gelling properties, 0.10 ml. of 3% hydrogen peroxide was mixed with 10 ml. of pentosan solution and the resulting solution quickly transferred to the viscometer.

Fractionation of Purified (α-Amylase Treated) Water-Soluble Pentosans on a Column of DEAE-Cellulose

DEAE-cellulose (Cellex-D, Bio-Rad Laboratories, Richmond, Calif., exchange capacity 0.90 μeq./g.) was prepared in the borate form according to the method of Neukom and Kuendig (17).

The α-amylase-treated pentosans (400 mg.) were dissolved in 40 ml. water and applied to the top of the column. After the sample had been allowed to penetrate into the DEAE-cellulose (3.4 × 4.5 cm.), elution was accomplished in a stepwise manner in this sequence: I, distilled water; II, 0.0025M $Na_2B_4O_7$; III, 0.025M

TABLE I. YIELDS AND PROTEIN CONTENT (d.b.) OF CRUDE AND PURIFIED (α-AMYLASE-TREATED) PENTOSANS FROM FLOUR AND LYOPHILIZED DOUGHS

Source of Water-Soluble Pentosans	Mixing Time min.	Crude Pentosans		Purified Pentosans	
		Yield %	Protein %	Yield %	Protein %
Flour (Scout-69)		0.66	17.6	0.48	16.4
Plain dough (P.T.[1])	4.5	0.98	16.0	0.68	15.6
Plain dough (D.T.[2])	8	0.98	18.2	0.63	17.5
Bromated[3] dough (P.T.)	4.5	0.88	19.9	0.63	22.0
Bromated dough (D.T.)	9.5	0.88	16.8	0.64	18.4
Iodated[3] dough (P.T.)	5	0.85	20.2	0.64	21.9
Iodated dough (D.T.)	9	0.88	18.9	0.63	20.5
Plain dough (P.T., rested[4])	4.5	1.32	18.3	0.80	18.4
Plain dough (D.T., rested)	7.5	1.12	14.4	0.68	14.4
Bromated dough (P.T., rested)	4.5	1.15	16.8	0.79	19.4
Bromated dough (D.T., rested)	9.5	1.17	12.2	0.77	14.2
Dough (1 μeq. NEMI[5]/g. flour)	4.5	1.00	18.2	0.67	22.2
Dough (1 μeq. cysteine-HCl/g. flour P.T.)	2.5	1.10	24.3	0.67	28.5

[1]P.T. = mixed to peak time.
[2]D.T. = mixed to departure time.
[3]Bromated and iodated = 1 μeq. oxidant/g. flour and mixed.
[4]Rested = dough placed in fermentation cabinet at 30°C. and 95% r.h. and rested for 3.0 hr.
[5]NEMI = *N*-ethylmaleimide.

$Na_2B_4O_7$; IV, 0.125M $Na_2B_4O_7$; and V, 0.05N sodium hydroxide. The flow rate was mechanically maintained at 1 ml. per min., and the effluent was automatically collected in 20-ml. quantities. Protein components were located by automatically scanning the effluent at 280 nm. (Uvicord, LKB Instrument Co., Sweden). In addition, for accurate comparisons of various treatments, the protein content of each tube was measured colorimetrically using the procedure of Lowry et al. (18). Carbohydrate content in each tube was measured colorimetrically by the phenol-sulfuric acid method of Dubois et al. (19). The lyophilized fractions were used to analyze carbohydrates and proteins. DEAE-cellulose fractions, used to determine viscosity properties and gel filtration, were obtained by fractionating flour pentosans (Scout R-70) on a larger column (4.4 × 54 cm.).

Gel Filtration of Purified Water-Soluble Pentosans and DEAE-Cellulose Fractions

Gel filtration of the pentosans and calibration of the column for molecular size were carried out as described by Andrews (20) and Fisher (21) for proteins, by

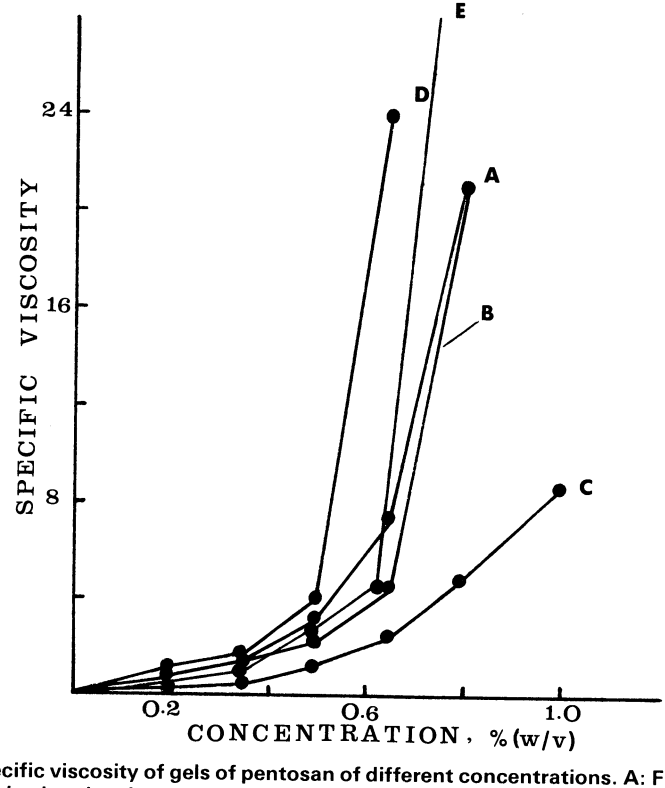

Fig. 1. Specific viscosity of gels of pentosan of different concentrations. A: Flour (Scout-69). B: Plain dough mixed to peak time. C: Plain dough mixed to departure time. D: Bromated (1 μeq./g. flour) dough mixed to departure time. E: Bromated (1 μeq./g. flour) dough mixed to peak time.

Granath and Kvist (22) for dextrans, and by Anderson et al. (23) for arabinogalactan. Fifteen grams of Bio-Gel P-150, hydrated for 24 hr. in 0.1M NaCl, was allowed to pack by gravity to a bed height of 40 cm. The column was equilibrated (using a sample tube placed on the gel surface) with 500 ml. of 0.1M NaCl. Two types of compounds (differing in shape) were used for calibration: i) globular proteins of known molecular size γ-globulin, albumin, ovalbumin, chymotrypsinogen, and bacitracin (Mann Research Laboratories, New York); and ii) dextran fractions with expanded molecular structure-dextrans T-150, T-110, T-70, and T-40 (Pharmacia Fine Chemicals, Uppsala, Sweden) (24). Calibration curves were obtained by plotting elution volumes vs. log molecular weights of proteins and dextrans.

The sample (25 mg.) dissolved in an appropriate volume (10 ml.) of eluting solvent was applied in the sample tube of the column and was allowed to penetrate the gel. Automatic collecting and monitoring (280 nm.) of the column effluent were begun when half of the sample solution was judged to have entered the column (20). After all of the sample had entered the gel, 5 ml. of 0.1M NaCl was allowed to pass into the gel. The column, after being filled with 0.1M NaCl, was connected to a solution metering pump and the flow rate adjusted to 18 ml. per hr.

Analytical Methods

The protein content in lyophilized (crude and purified) pentosans was determined by the AACC micro-Kjeldahl method (15). Protein content in column effluent and lyophilized DEAE-cellulose fractions was determined by the Lowry procedure (18) using a standard curve of bovine serum albumin (BSA). Total carbohydrate content in the effluents from anion exchange and gel filtration columns and in lyophilized DEAE-cellulose fractions was determined by the phenol-sulfuric acid method of Dubois et al. (19) using a standard curve of xylose.

RESULTS AND DISCUSSION

Extraction and Purification of Pentosans

To avoid errors caused by slight changes in the amount of TCA used and thereby to permit better comparisons of various treatments, it was necessary to standardize the amount of 5% TCA required to precipitate α-amylase. Slight increases in the amount of 5% TCA used, above 20 mg. per mg. enzyme protein to be precipitated, pronouncedly reduced the protein content of purified pentosans. The results also support the observation of Lin and Pomeranz (25) that the TCA used to precipitate α-amylase may also precipitate proteins or glycoproteins (14, 25) associated with pentosans. Those authors believed such precipitation was responsible for their failure to obtain an increase in protein content in pentosans treated with α-amylase to remove starch. Using about 20 mg. TCA per mg. enzyme protein to be precipitated, we precipitated the α-amylase with minimal precipitation of pentosan protein; that amount was considered sufficient to reduce the risk of incomplete removal of the enzyme.

For the purpose of discussion, pentosans treated with bacterial α-amylase to remove soluble starch will be referred to as "purified pentosans." The yield and protein content of crude and purified pentosans extracted from flour and from

lyophilized and ground doughs are shown in Table I. Generally, substantially higher yields of crude and purified pentosans were obtained from dough than from flour. In addition, resting the dough (3 hr.) increased the yields, probably as a result of increased solubility. Increased solubility of pentosans during mixing and resting could markedly enhance their contribution to the distribution of water in dough (1) and to dough "water balance" (2). Higher solubility might also increase pentosan reactivity with other flour constituents in maintaining a three-dimensional dough structure (11). Among nonrested doughs, adding oxidizing agents reduced the yields of crude pentosans, which agreed with the observation of Baker et al. (6) who found that increased oxidation lowered the solubility of pentosans. Adding cysteine-HCl increased the yields of crude pentosans, compared with plain doughs and doughs mixed with *N*-ethylmaleimide (NEMI).

Among the nonrested doughs, adding bromate and iodate increased the protein content of crude and purified pentosans (compared with plain doughs), except that in bromated dough mixed to departure time, protein content of crude pentosans decreased. Adding cysteine-HCl greatly increased the protein content of crude and purified pentosans. This effect of cysteine-HCl was expected because of its drastic action on dough structure. Tsen (26) has shown that

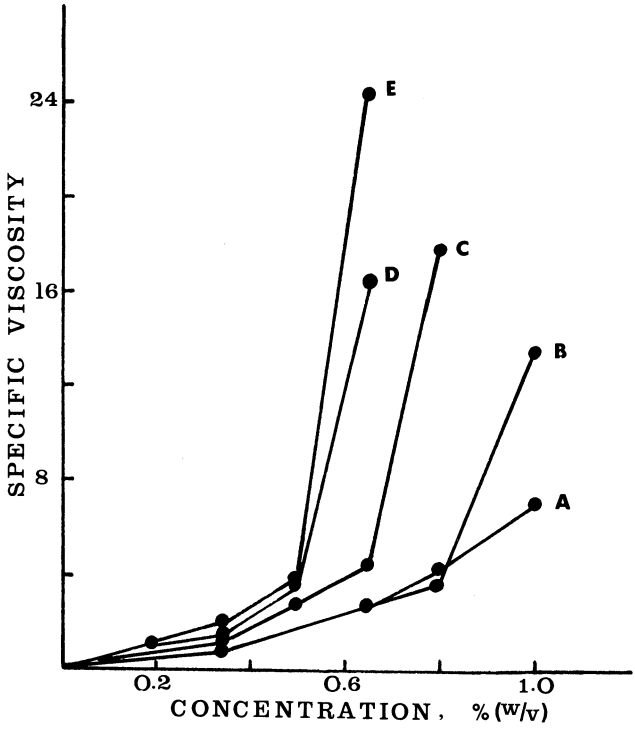

Fig. 2. Specific viscosity of gels of pentosans obtained from doughs mixed with various additives. A: Dough (1 μeq. *N*-ethylmaleimide/g. flour) mixed to peak time. B: Dough (1 μeq. cysteine-HCl/g. flour) mixed to peak time. C: Iodated (1 μeq. KIO_3/g. flour) mixed to peak time. D: Iodated (1 μeq. KIO_3/g. flour) dough mixed to departure time. E: Bromated (1 μeq. $KBrO_3$/g. flour) mixed to departure time.

cysteine-HCl increases the amount of low-molecular-weight proteins. In general, an increase in the protein content of purified pentosans from doughs containing added oxidizing agents might indicate that these agents reduce the susceptibility of glycoproteins (14, 25) to precipitation by TCA.

Viscosity and Gelling Properties of Water-Soluble Pentosans

When about 0.1 ml. of 3% hydrogen peroxide and 0.1 ml. of 0.1% sodium chlorite were added, crude pentosans from flour (10 ml. of 1% pentosan solution) formed a solid gel. Viscosities and the gelling properties of crude water-soluble pentosans isolated from flour and lyophilized dough treated with $KBrO_3$, KIO_3, NEMI, and cysteine-HCl (1 μeq. per g. flour) are shown in Figs. 1 and 2.

Adding bromate (Fig. 1) and iodate (Fig. 2) markedly increased the specific viscosity of pentosan gels from the nonrested doughs. Nonrested doughs mixed to departure time with added bromate and iodate gave the largest increases in these values.

Cysteine-HCl and NEMI reduced the viscosity of solutions and gels (Fig. 2) of pentosans, which agreed with the results of Baker et al. (6) who observed lower viscosity of flour pentosans when glutathione was added to the solutions Treating doughs with cysteine (SS reducing agent) (26) and NEMI (SH blocking agent) caused a gel to form only at high concentrations (1% w./v.). It should also be noted (from Fig. 2) that bromate was more effective than iodate in increasing the viscosity of pentosan gels.

The effects of iodate could be attributed to its rapid oxidative action (27) during dough mixing; in addition, iodate and NEMI (especially NEMI) have been known to cause a dough breakdown during mixing (28). Tsen (26,29), in his study on the effect of those agents and cysteine-HCl during dough mixing, has demonstrated that cysteine-HCl causes rapid dough breakdown by depolymerizing large protein aggregates resulting from reduction of disulfide bonds. A recent study by Tanaka (30) also has shown that adding NEMI and cysteine during mixing causes increased solubility of proteins in 0.05N acetic acid, with subsequent decrease in the amount of high-molecular-weight proteins. Recently Bloksma (31) has shown that bromate reacts more effectively with rheologically active fractions than does the more rapidly acting iodate and NEMI. The action of these oxidizing agents on dough structure might also be responsible for changes observed in properties of water-soluble pentosans and glycoproteins. Gelling ability of pentosans was lost when doughs were rested for 3.0 hr.; adding bromate did not restore this property, perhaps because of enzymes degrading pentosans. Such enzymes are known to be present in flour (32) and, together with the action of proteolytic enzymes during resting, might alter a highly specialized gelling complex of polysaccharide and protein (33). A large increase in the viscosity of the gel, even at low pentosan concentrations (0.5 to 0.65%), when bromate was added to dough, supports the observation of Udy (34), who found that adding potassium bromate to mixtures of soluble polysaccharides and gluten accelerated the gelation process. He concluded that interaction of high-molecular-weight, soluble polysaccharides, and gluten was responsible for the gelation.

Fractionation of Flour Pentosans (Scout R-70) and the Viscosity and Gelling Properties of the Fractions Obtained

To obtain pentosan fractions in sufficient quantities to study viscosity, gelling

properties, and molecular size in detail, fractionation was carried out on a large column of DEAE-cellulose as described previously. The pentosans yielded five main peaks; in addition, fractions II and III were each subdivided into two, based on carbohydrate and protein content, respectively.

Table II shows viscosity and gelling properties for those five main pentosan fractions (0.5% w./v. in water) together with the yields and distribution of carbohydrates and proteins. Only fraction II, eluted with 0.0025M $Na_2B_4O_7$, showed a gelling reaction. That supports the observation of Kuendig et al. (7), who obtained from chromatography of water-soluble pentosans on DEAE-cellulose a fraction (fraction II), eluted by 0.01 M Na-borate, capable of gelling. A solution (0.5% w./v.) of fraction II had a high specific viscosity before gelation, which would indicate that a large molecular size may have caused the broad peak obtained from fractionation on DEAE-cellulose. Supporting evidence also was obtained from gel filtration (Fig. 4 and Table III) of individual DEAE-cellulose fractions from flour (Scout R-70) pentosans. In addition, fraction I showed a substantially higher specific viscosity than did the rest of the DEAE-cellulose fractions, except for fraction II. Fractions I and II together contained the bulk of carbohydrates (61.3%) and fractions IV and V most of proteins (73.2%) in the water-soluble pentosans (Table II).

Calibration of Gel Filtration Column

Gel filtration has proved to be useful in estimating the dimensions of proteins and polysaccharides. Although molecular weight is unlikely to be a good approximation for size in comparing such dissimilar molecules as proteins and polysaccharides, the correlation between molecular weights and gel filtration

TABLE II. YIELDS, SPECIFIC VISCOSITY, AND DISTRIBUTION OF CARBOHYDRATES AND PROTEINS IN FRACTIONS OF FLOUR[1] PENTOSANS OBTAINED FROM CHROMATOGRAPHY ON DEAE-CELLULOSE

Fraction	Yield[2] %	Specific Viscosity		Distribution[3] of	
		Before adding H_2O_2	After adding 0.1 ml. 3% H_2O_2	Carbohydrates %	Proteins %
I	17.4	3.70	3.85	26.7	1.8
II	27.9	8.20	11.20	34.6	4.7
IIIa	10.9	0.70	0.75	11.0	8.2
IIIb	2.3	...	...	0.7	11.4
IV	20.7	0.29	0.29	14.6	42.2
V	20.4	0.75	0.75	12.3	31.0
				99.9	99.3

[1]Flour: Scout-70.
[2]Percentage yields based on recovery of material eluted from the column.
[3]Based on the color reaction of carbohydrates (phenol-sulfuric acid procedure, 480 nm.) and proteins (Lowry procedure, 750 nm.) and the % yields of individual fractions.

behavior of dextrans (35) and proteins (21,36) indicates that, for a homogenous series of macromolecules, size and molecular weights are closely related. In view of the differences in size and shape between branched and expanded pentosan molecules (24,33) and globular proteins (20), the gel filtration column was calibrated with dextran fractions (of known molecular weights), considered to resemble pentosan molecules. In order to demonstrate the possible differences in elution volumes of dissimilar molecules such as indicated above and their effect on estimation of molecular weights, the column was also calibrated with globular proteins of known molecular weight.

Figure 3 shows the relationship between elution volume and log molecular weight for a series of globular proteins and dextrans of known molecular weight. Note the elution volumes of dextran fractions and globular proteins differ markedly despite close molecular weight values. These results agree with the observation of Granath and Flodin (35) that molecular weight is not a good approximation for size in comparing dissimilar molecules such as proteins and polysaccharides. Branched molecules with expanded shape are too large to enter the gel pores (21) and are eluted earlier (at lower elution volume) than the globular proteins (Fig. 3).

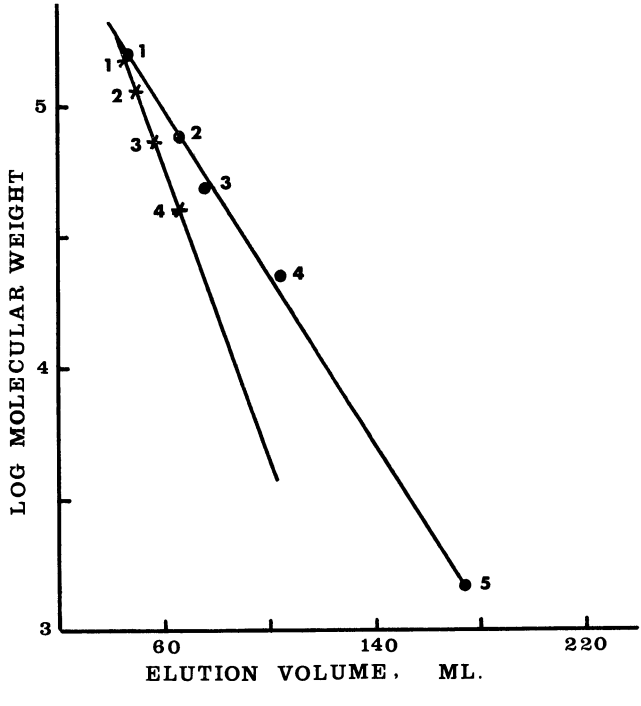

Fig. 3. Elution volume, Ve, versus log (molecular weight) for proteins (circles) and dextrans (crosses) chromatographed on a column (2.5 × 40 cm.) of Bio-Gel P-150 (100 to 200 mesh). Proteins and dextrans were eluted with 0.10M NaCl (18 ml./hr.). Proteins: 1) gamma-globulin (MW 160,000); 2) albumin (MW 67,000); 3) ovalbumin (MW 45,000); 4) chymotrypsinogen (MW 25,000); 5) bacitracin (MW 1,450). Dextrans: 1) T-150 (MW 150,000); 2) T-110 (MW 110,000); 3) T-70 (MW 70,000); 4) T-40 (MW 40,000).

Molecular-Weight Estimation of Fractions Obtained from Chromatography of Flour (Scout R-70) Pentosans on DEAE-Cellulose

Individual subfractions obtained from gel filtration on a column (2.5×40 cm.) of Bio-Gel P-150 (100 to 200 mesh) were dialyzed against distilled water and

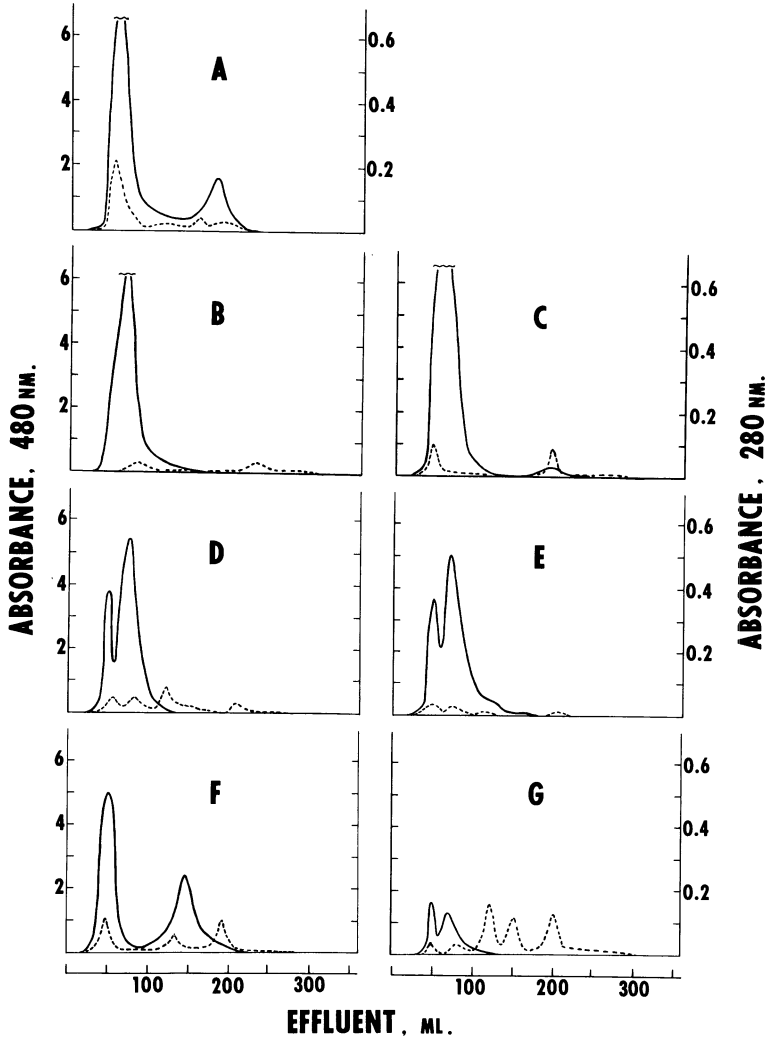

Fig. 4. Gel filtration of individual DEAE-cellulose fractions of flour (Scout R-70) pentosans through a column (2.5 × 40 cm.) of Bio-Gel P-150 (100 to 200 mesh) eluted with 0.10M NaCl (18 ml./hr.). Top curve (solid line): Carbohydrates (phenol-sulfuric acid method, 480 nm., absorbance $X10^{-1}$). Lower curve (dotted line): Proteins (280 nm.), double scale expansion. Sample size: A to F, 25 mg., and G, 15 mg. A) α-amylase-treated pentosans; B) DEAE-cellulose fraction I; C) DEAE-cellulose fraction II; D) DEAE-cellulose fraction IV; E) DEAE-cellulose fraction IIId; F) DEAE-cellulose fraction V; G) DEAE-cellulose fraction IIIb.

lyophilized to obtain the yields of each subfraction.

Figure 4 shows the patterns obtained from gel filtration of various DEAE-cellulose fractions of flour pentosans; Table III, the molecular weights of the subfractions. The molecular weights (Table III) were determined from the calibration curve for the column (Fig. 3), using the elution volumes measured for each subfraction. Note (from Table III) that, as expected, when globular proteins were used to calibrate the gel filtration column, molecular weight values of pentosan fractions were considerably higher than the values obtained using a dextran-calibration curve. In view of the differences in elution volumes and estimated molecular weights shown in Fig. 3 and Table III, respectively, the molecular weights determined from dextran calibration curve (Fig. 3) will be used for reference in the following discussion.

When α-amylase-treated pentosans were chromatographed (Fig. 4, A) the majority of carbohydrates and proteins appeared as a single peak eluting near the exclusion limit of the gel having a molecular weight corresponding to 95,000 (Table III). DEAE-cellulose fractions I and II (Fig. 4, B and C, respectively) were nearly homogenous with respect to carbohydrate, as shown by their single symmetrical peak for carbohydrates. Fraction II, which contained a larger protein peak than did fraction I, also contained a minor component containing carbohydrate and protein, possibly a glycoprotein. Fraction I (Fig. 4, B), reported to be a pure arabinoxylan (14,25), appeared at an elution volume corresponding to a molecular weight of 72,000. Fraction II, a glycoprotein

TABLE III. MOLECULAR WEIGHTS OF DEAE-CELLULOSE FRACTIONS FROM WATER-SOLUBLE PENTOSANS OF FLOUR (SCOUT-R-70) ESTIMATED BY GEL FILTRATION THROUGH A COLUMN (2.5 × 40 cm.) OF BIO-GEL P-150

Fraction		Sub-fraction	% Based on Original Yields of DEAE-Cellulose Fractions	Molecular Weights	
				Using dextrans	Using globular proteins
A Purified pentosans		a	...	95,000	125,000
		b	...	<1,000	1,200
B DEAE-cellulose fractions					
	I	a	17.5	72,000	110,000
C	II	a	26.6	95,000	130,000
		b	1.2	<1,000	<1,000
E	IIIa	a	1.6	120,000	>150,000
		b	9.9	36,500	67,000
G	IIIb	a	0.2	115,000	>150,000
		b	0.7	38,000	69,000
D	IV	a	3.2	115,000	140,000
		b	16.8	30,500	63,000
F	V	a	14.1	107,000	140,000
		b	6.3	<1,000	4,500

(14,33) responsible for gelation, had an apparent molecular weight of 95,000 (Table III). Fractions I and II represented 17.5 and 26.7% of the purified pentosans, respectively. The high molecular weight of fraction II accounted for the broad peak obtained for this component during fractionation on DEAE-cellulose and the high specific viscosity shown by this fraction (Table II).

DEAE-cellulose fractions IIIa to V, on gel filtration, showed considerable heterogeneity in their molecular sizes (Table III). Fractions IIIa (Fig. 4, E) and IV (Fig. 4, D) were divided into two subfractions based on carbohydrate distribution, each with the first peak appearing near the exclusion limit of the column at elution volumes corresponding to molecular weights of 120,000 and 115,000, respectively. The second peaks of fractions IIIa and IV were of low molecular weight (36,500 and 30,000, respectively) and together represented about 26.7% of the total purified pentosans. A small protein peak (IIIb), obtained from DEAE-cellulose chromatography, was found to be the most heterogeneous among all the fractions during gel filtration (Fig. 4, G) and contained five protein peaks (two carbohydrate peaks overlapping the first two). Fraction V (Fig. 4, F) also showed considerable heterogeneity: The first peak, of high molecular weight (107,000), represented about 14% of the total pentosans; the next large peak, of low molecular weight (1,000), represented about 6.3% of the total pentosans (Table III).

Acknowledgment

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