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ARABINOXYLANS FROM RYE AND WHEAT SEED THAT INTERACT WITH ICE

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ABSTRACT

Arabinoxylans that interfere with growth of ice crystals have been purified from rye (*Secale cereale* L., Rosen) and two varieties of wheat (*Triticum aestivum* L., Genesee and Hillsdale) seed. The most active polysaccharide from each seed type was homogeneous in the sense that all the molecules were in the same size range, they contained the same sugar residues, and they reacted similarly in chemical characterization experiments. Structural studies showed that the polysaccharides consist of a xylan chain to which are attached side-chains that contain a single, terminal arabinose residue. The polysaccharides differ with respect to the number of arabinose residues. The xylose:arabinose ratios in the most active fractions from rye, Genesee wheat, and Hillsdale wheat are 1.26, 1.54, and 2.08, respectively. Gel-permeation column chromatography showed that the most active polysaccharide from each seed type has a molecular weight greater than 2×10^6 and that the rye polysaccharide is slightly larger than the Hillsdale wheat polysaccharide. The rye polysaccharide is a better inhibitor of ice-crystal growth than is the Hillsdale wheat polysaccharide.

INTRODUCTION

The rate of formation, location, size, and amount of ice crystals that form in the intercellular space of cold-hardy cereal plants are typically major factors in winter survival[1,2]. Ice formation in the lower crown tissue of winter cereals can rupture xylem vessels and cause lesions that affect the viability of lateral crown meristem[2]. These meristems are essential for production of new roots in spring. The lower the temperature to which water has supercooled and the greater the quantity of water in the supercooled state in plants, the greater is the disruptive effect of ice-crystal growth[3]. Freeze injury from such conditions is especially prominent after short, mid-winter thaws that are followed by a rapid drop in temperature. At such times crown tissue has a high water content and freezes rapidly.

A study of freezing processes in a cellulose model system indicated that a

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water-extractable, cell-wall polysaccharide fraction from rye affected ice formation and that the fraction from rye had a greater effect than did a similar fraction from wheat or barley[3]. An examination of the kinetics of freezing showed that the material from rye interacted strongly with the ice–liquid interface and greatly altered the kinetics of freezing while having little effect on the freezing point[3]. Characterization experiments indicated that a polysaccharide containing arabinose and xylose was the active component[4]. Until now active polysaccharide* has not been obtained in pure form. It has been detected in crown tissue[3], seed[5], and meristem tissue culture[6] of cereal plants. Our intent is to compare the structure and relative activity of pure, active material from different tissues of the same plant and from different cereal plants and to understand the mechanism of their interaction with ice. The purification of active polysaccharide from rye and wheat seed and their characterization are reported here.

EXPERIMENTAL

Materials. — Rye (*Secale cereale* L., Rosen) and wheat (*Triticum aestivum* L., Genesee, Hillsdale) seeds were produced at East Lansing and were not treated. Orcinol, 3-hydroxydiphenyl, gel-permeation media and standard dextrans, dialysis tubing, and AG 3-X4A resin (acetate, 100–200 mesh) were obtained from Sigma, ICN-K and K Laboratories, Pharmacia, Spectrum Medical Industries, and Bio-Rad Laboratories, respectively. Spectra/Por 4, a standard cellulose dialysis tubing with a stated molecular weight cutoff of 12,000–14,000, was used.

General methods. — Solutions were concentrated at water-pump pressure by rotary evaporation at <40°, unless stated otherwise. Centrifugation was for 10 min at 22,000*g*. Freeze-inhibitor activity was determined by the flow (ice drum)[5] and infrared[6] tests and fractions were rated as described elsewhere[4]. The same rating scale and rating criteria were used in both tests[4]. A rating scale of 0–9 was used, the highest (9) indicating greatest interaction of a polysaccharide with ice[4]; a rating of 0 means no interaction, and such material is excluded from the advancing ice. Standard polysaccharide fractions were run as controls each time a test was performed. Both tests involve the uniform growth of a basically flat ice-face through the test solution. The extent of interaction of polymers with the growing ice-face can be regulated by regulating the rate of crystal growth and the intensity of interfacial agitation. The former is regulated by the rate of removal of latent heat while the latter is regulated by the flow rate of solution across the ice face in the flow test and by the intensity of the i.r. light in the i.r. test. The slower the rate of crystal growth and the greater the interfacial agitation, the more likely a polymer will be excluded. The conditions used in all tests reported were the same except as noted

*Active polysaccharide is defined as polysaccharide that interacts with ice as determined by the flow (ice drum) or infrared freeze tests, or both. Freeze-inhibitor activity is the extent to which the polysaccharide interacts with ice as indicated by the rating value obtained from the freeze tests. The tests and rating procedures are explained more fully in the Experimental.

in the Results. For both tests, the carbohydrate concentration of the solutions was approximately 1 mg/mL. The advantages of the flow test are: (*1*) more-consistent results and, (*2*) its use as a purification step in the isolation of active polysaccharide. The main advantage of the i.r. test is that it requires only 2.5% of the material needed for the flow test. Total sugar (carbohydrate) and pentose were determined by the phenol–H_2SO_4[7] and orcinol[8] methods, respectively. In the orcinol test, the amount of pentose was obtained by subtracting the absorbance at 580 nm from that at 670 nm[8]. Hexose was obtained indirectly by subtracting the absorbance contribution from the pentoses in the phenol–H_2SO_4 test from the total absorbance obtained with the test. The absorbance contribution of the pentoses was obtained from a standard curve that related absorbance at 480 nm to amount of the appropriate pentose mixture. Different pentoses have different molar extinction coefficients in the phenol–H_2SO_4 test and this was taken into account when developing the standard curve by utilizing xylose and arabinose mixtures which corresponded as closely as possible to the pentose composition of the polysaccharide being isolated. Uronic acids were determined with 3-hydroxydiphenyl[9] and protein was determined by a modified Lowry procedure[10]; NaN_3 was used to inhibit microbial growth.

Isolation of active polysaccharide. — A typical experiment is described. Ground rye seed was refluxed three times with aq. 85% (w/w) ethanol at 85°, once for 20 min and twice for 5 min. The treated meal was dried in air at 23° and ground in a Wiley mill fitted with a 40-mesh screen.

The carbohydrate concentrations given were obtained from a standard curve prepared by using a 1:1:1 molar mixture of L-arabinose, D-xylose, and D-glucose. Dry, ground rye seed (14 g) was extracted twice at 0° by reciprocating shaking, first with 100 mL of aq. 0.02% (w/v) KN_3 for 20 min and then with 80 mL for 10 min. The suspensions were centrifuged at 0°. The supernatant solutions were filtered through glass wool, combined, concentrated, warmed for 10 min at 70°, and dialyzed until the conductivity of the extract was equivalent to 0.4mM NaCl or less. Any gel that formed in polysaccharide solutions was removed by heating at 70° with swirling. Small precipitates were removed by centrifugation.

The material in the dialyzed extract was subjected to the flow test. The solution was completely frozen in plastic, drum-shaped holders, each of which held 80 mL of extract. The ice drums that formed contained a cylindrical hole through the center. Approximately 1 mm of the interior surface of each ice drum was removed at 4° by scraping and by washing with water. The initial total thickness of each ice drum wall varied between 18 and 20 mm. The material in the scrapings plus rinsings was termed the excluded material and was discarded. The material remaining in the ice was termed the included material and was rated.

After the test, the combined solution of included material was concentrated to 4 mg of carbohydrate per mL. The material in 34 mL of column buffer was applied to a column (3.2 cm, i.d. × 120 cm) of Sepharose CL-4B. The column was developed with 25mM potassium phosphate (pH 6.5) containing 0.05% (w/v) KN_3 at a rate of 0.3 mL/min and 18-mL fractions were collected. Column fractions were

analyzed for total sugar and pentose and were combined as indicated (Fig. 1a). The column fractions were selected so that the pentose material was mainly in one combined fractions, the hexose material mainly in another, and the third was a mixture. Each combined fraction was concentrated, dialyzed, and tested for freeze-inhibitor activity by both tests. The scraped ice drums were thawed and the solutions were concentrated to ~9 mL. Their carbohydrate concentrations ranged from 1.9–2.9 mg/mL. An aliquot from each was lyophilized, dried to constant weight *in vacuo* over P_2O_5, and used for sugar composition analysis.

The samples in volumes of column buffer that ranged from 8–9 mL were separately applied to a column (2.2 cm, i.d. × 120 cm) of Sepharose CL-2B, and the column was developed with 25mM potassium phosphate (pH 6.5) containing 0.05% (w/v) KN_3 at a rate of 0.16 mL/min and 9-mL fractions were collected. Column fractions were treated as already described for the Sepharose CL-4B column. A portion of each combined fraction (Fig. 1b) was tested by the i.r. test. The remainder was lyophilized, dried to constant weight *in vacuo* over P_2O_5, and used in the sugar composition analysis and methylation experiments. For certain analyses, a portion of the active polysaccharide was left in solution.

The experiments with the two varieties of wheat seed were performed basically in the same way as those with rye seed; however, more seed was used since the amount of active polysaccharide per unit dry weight of seed was less. In the Hillsdale wheat experiment, 50 g of dry, ground wheat seed was extracted with 360 mL and then with 200 mL of aq. 0.02% (w/v) KN_3. Solutions containing 9.0 mg/mL and 4.2 to 4.4 mg/mL were applied to the Sepharose CL-4B and CL-2B columns, respectively. The size of the columns and the volumes applied were basically the same as in the rye experiment. Each of the isolation experiments with rye and the two wheats was repeated at least once in its entirely and the results of the repeat experiments were essentially the same as the initial experiments. In the wheat experiments, only those combined CL-4B fractions that had a significant freeze rating were applied to the CL-2B column.

Sugar composition of polysaccharides. — Into a small reaction vessel with a cone-shaped interior and with a cap fitted with a Teflon liner was placed a weighed amount of dried polysaccharide (0.25–0.30 mg), water, and sufficient 13.0M trifluoroacetic acid to give a final acid concentration of 2M in a final volume of 0.3 mL. With each set of polysaccharide samples analyzed, a standard containing known amounts of D-xylose, L-arabinose, D-galactose, D-glucose, and *myo*-inositol was prepared and treated identically. *myo*-Inositol was used as the internal reference. Samples were heated at 121° for 1 h, reduced, and acetylated[11]. Alditol acetates in Ac_2O were injected into a Varian Aerograph, Series 2100, gas–liquid chromatograph equipped with a 2 mm (i.d.) × 1.83 m glass column containing 3% SP-2340 on Supelcoport (100–120 mesh). A temperature program of 173° for 17 min followed by a 1°/min rise to 195° and a Hewlett–Packard Reporting Integrator, Model No. 3390A were used. Sugars were identified by comparing the retention times of the derivatives from the polysaccharide sample with those from the standard. The

standard was also used to calculate the mole percent sugar composition in the samples[12].

Molecular size of active polysaccharide. — Active polysaccharide from rye and Hillsdale wheat seeds, purified through the Sepharose CL-2B step, was used immediately without being lyophilized. A 1% (w/v) solution of purified polysaccharide in 0.25 mL of column buffer was applied to a column (1.0 cm, i.d. × 55.0 cm) of Sephacryl S-500. The column was developed with 25mM potassium phosphate (pH 6.5) containing 0.05% (w/v) KN_3, the flow rate was 0.17 mL/min, and 1.0-mL fractions were collected. Standard dextrans were dissolved in the same buffer and applied to the column in the same way as the samples. Polysaccharide was detected by the phenol–H_2SO_4 method.

Partial acid hydrolysis of active polysaccharide. — Portions of an aqueous solution of active polysaccharide that had been purified through the Sepharose CL-2B column were made 0.01M in trifluoroacetic acid. Each acidified sample contained 2.5 mg of polysaccharide and had a final volume of 3.0 mL. The samples were heated at 100° for 0, 10, and 30 min, cooled, made neutral with NaOH, and dialyzed in water. For the 0 time sample, neutralized acid was added. The retained material was tested for freeze-inhibitor activity with the i.r. test. The sugar composition of the retained and non-retained material was determined as described in the preceeding section. The retained material was applied to a column (1.0 cm, i.d. × 56 cm) of Sephacryl S-500, the column was developed with 50mM potassium phosphate (pH 6.5) containing 0.05% (w/v) KN_3, and carbohydrate was detected by the phenol–H_2SO_4 method.

Methylation analysis. — The Hakomori[13] methylation method as described by Sandford and Conrad[14] and Harris *et al.*[15] was used. Methylated polysaccharides were hydrolyzed either with 88% formic acid (1 h) followed by 0.125M H_2SO_4 (16 h)[16], in which case the hydrolyzate was passed through AG 3-X4A (acetate) resin and the resin was washed with methanol[17], or with 2M trifluoroacetic acid for 1 h at 121°. Methylated alditol acetates were identified by gas chromatography–mass spectrometry[18].

RESULTS AND DISCUSSION

Active polysaccharide, that is, polysaccharide that interacts with ice, was isolated from seeds of rye and two wheat varieties. The results from three isolation experiments, one with each type of seed, are presented in Tables I and II. In each experiment the same three-step purification procedure was used. The steps were: (*1*) the flow test, (*2*) Sepharose CL-4B, (*3*) Sepharose CL-2B. The flow test performed on the initial extract was used both for testing for freeze-inhibitor activity and as a purification step. For example, in the rye seed experiment (Table I) the material in the initial, dialyzed cold-water extract (413.4 mg) in part was included in the ice during the test (194.3 mg) and in part was excluded (183.4 mg). Gas-chromatographic sugar analyses showed that the included material (active) had a

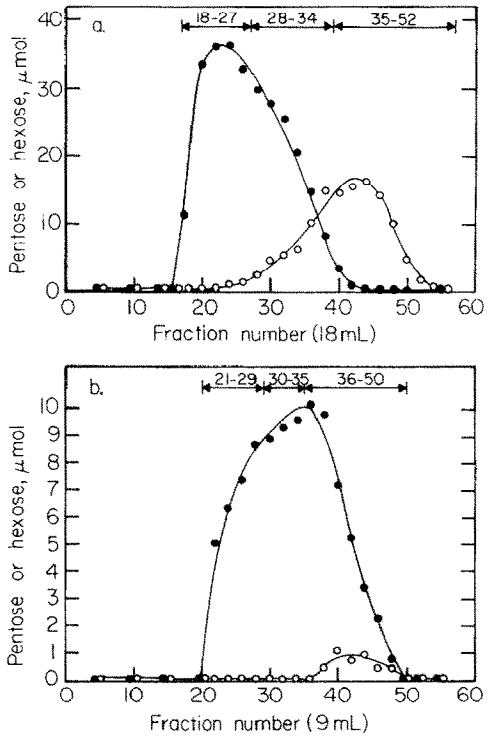

Fig. 1. Column chromatograms: (a) Sepharose CL-4B of rye included material from flow test; (b) Sepharose CL-2B of material in fractions 18–27 from the CL-4B column depicted in (a). Pentose (●) and hexose (○).

higher ratio of pentose to hexose than did either the excluded material or the starting material (Table I). The same results were obtained with the two wheat varieties (Table II). Included material was subjected to column chromatography, first on Sepharose CL-4B and then on Sepharose CL-2B (Fig. 1a and b). This resulted in additional separation of material of high activity from material of lower activity (Tables I and II). With rye seed, three purified fractions from the Sepharose CL-2B column chromatography had activity ratings >7, whereas with the wheat varieties a single fraction from each had such a high rating (Tables I and II). Colorimetric sugar analysis of the column fractions showed that the most active polysaccharides consisted mainly or solely of pentose (Fig. 1a and b and Tables I and II). Inactive material consisted mainly of hexose. The results in Fig. 1a and b are with rye seed. The material in fractions 18–27 (Fig. 1b) as well as that in fractions 28–34 and 35–52 of the CL-4B column were chromatographed on the CL-2B column and the various, combined CL-2B fractions were tested for activity (Table I). The latter two CL-2B profiles are not presented but they showed a decreasing amount of total pentose material and increasing amount of total hexose material, respectively, was present. The two wheat varieties yielded similar results (figures not presented) except the pentose and hexose profiles overlapped to a greater extent. Recovery of carbo-

TABLE I

FREEZE-INHIBITOR ACTIVITY AND SUGAR COMPOSITION OF POLYSACCHARIDE FRACTIONS FROM RYE SEED[a]

Fraction	*Freeze-inhibitor tests*			*Relative molar composition (%)*				*Ratio, Xyl/Ara*
	Polysaccharide in test solution (%, w/v)	*Rating[b,c]*		*Ara*	*Xyl*	*Gal*	*Glc*	
		Flow test	*I.r. test*					
Water extract after dialysis				26	35	5	34	1.35
Water extract after flow test								
(a) included material (from 4 ice drums)	0.12	7,7,9,8	n.d.[d]	29	47	1	23	1.62
(b) excluded material		rating is 0		16	8	9	68	0.50
Sepharose CL-4B column chromatography of included material from water extract								
(a) col. fract. 18–27	0.047	6	6	38	62	0.1	0.7	1.64
(b) col. fract. 28–34	0.038	4	3	34	58	1	7	1.72
(c) col. fract. 35–52	0.045	2	3	20	30	3	48	1.48
Sepharose CL-2B column chromatography of combined fractions from CL-4B column								
(a) combined fractions 18–27 from CL-4B								
(1) col. fract. 21–29	0.096	n.d.	9,9	44	56	0	0.1	1.26
(2) col. fract. 30–35	0.11	n.d.	8,8	37	62	0	0.3	1.67
(3) col. fract. 36–50	0.093	n.d.	4,4	32	66	0.1	2	2.03
(b) combined fractions 28–34 from CL-4B								
(1) col. fract. 21–30	0.10	n.d.	9,9	44	56	0	0.1	1.26
(2) col. fract. 31–36	0.082	n.d.	5,3	35	64	0	0.7	1.81
(3) col. fract. 37–50	0.12	n.d.	5,5	30	61	2	6	2.01
(c) combined fractions 35–52 from CL-4B								
(1) col. fract. 21–38	0.11	n.d.	4,4	37	60	0	3	1.61
(2) col. fract. 39–52	0.12	n.d.	0,0	18	27	5	50	1.46

[a]Isolation of fractions and sugar composition analysis were performed as described in Experimental. Fourteen (14) g of rye seed was used. The amount of material in the water extract after dialysis, determined by dry weight, was 413.4 mg. The recovery of material in the CL-4B and CL-2B polysaccharide fractions, on a dry-weight basis and adjusted for material removed for analysis, ranged from 8.9–11.8% and from 1.8–5.0%, respectively, of the total polysaccharide present in the water extract after dialysis. [b]I.r. = infrared, n.d. = not determined, col. fract. = column fractions. [c]More than one value indicates multiple determinations performed on the same sample. [d]In a repeat experiment the average rating of this material was 7.4 and 8 by the flow and i.r. tests, respectively.

TABLE II

FREEZE-INHIBITOR ACTIVITY AND SUGAR COMPOSITION OF POLYSACCHARIDE FRACTIONS FROM GENESEE AND HILLSDALE WHEAT SEED[a]

Fraction	*Freeze-inhibitor tests*			*Relative molar composition (%)*				*Ratio, Xyl/Ara*
	Polysaccharide in test solution (%, w/v)	*Rating*[b,c]		*Ara*	*Xyl*	*Gal*	*Glc*	
		Flow test	*I.r. test*					
GENESEE								
Water extract after dialysis				22	19	13	45	0.86
Water extract after flow test								
(a) included material (from 3 ice drums)	0.10	2,2,3	n.d.	22	32	6	41	1.45
(b) excluded material		rating is 0		24	13	19	44	0.54
Sepharose CL-4B column chromatography of included material from water extract								
(a) col. fract. 18–27	0.11	3	5	31	55	0	14	1.75
(b) col. fract. 28–35	0.12	0[d]	0[d]	19	34	0	47	1.80
(c) col. fract. 36–52	0.12	0	0	21	9	18	53	0.43
Sepharose CL-2B column chromatography of combined fractions 18–27 from CL-4B column								
(1) col. fract. 22–32	0.11	n.d.	8,9	39	60	0.1	2	1.54
(2) col. fract. 33–36	0.089	n.d.	4,5	36	62	0.1	2	1.74
(3) col. fract. 37–52	0.11	n.d.	4,6	30	54	0	16	1.80
HILLSDALE								
Water extract after dialysis				23	31	9	37	1.35[e]
Water extract after flow test								
(a) included material (from 4 ice drums)	0.10	3,3,2,1	n.d.	23	41	6	31	1.78[e]
(b) excluded material		rating is 0		21	16	11	53	0.76[e]
Sepharose CL-4B column chromatography of included material from water extract								
(a) col. fract. 17–24	0.087	n.d.	4,3	32	65	0	3	2.04

(b) col. fract. 25–32	0.12	n.d.	3,3	27	55	1	17	2.01
(c) col. fract. 33–46	0.15	n.d.	2,0	27	29	16	28	1.06
Sepharose CL-2B column chromatography of fractions from CL-4B column								
(a) combined fract. 17–24 from CL-4B								
(1) col. fract. 18–29	0.11	n.d.	8,7	31	65	0	4	2.08
(2) col. fract. 30–35	0.11	n.d.	3,4	32	67	0.2	0.7	2.12
(3) col. fract. 36–46	0.097	n.d.	2,2	28	69	0.1	3	2.41
(b) combined fract. 25–32 from CL-4B								
(1) col. fract. 23–35	0.12	n.d.	4,3	33	65	0.1	2	2.00
(2) col. fract. 36–40	0.11	n.d.	3,3	30	68	0.2	3	2.32
(3) col. fract. 41–50	0.17	n.d.	3,2	23	54	0.9	22	2.37

[a]Isolation of fractions and sugar composition analysis were performed as described in Experimental. Forty-eight (48) g of Genesee wheat and 50 g of Hillsdale wheat seed were used. The amount of material in the water extract of Genesee and Hillsdale wheat seed after dialysis, determined by dry weight, was 590.4 and 420 mg, respectively. The recovery of material in the CL-4B and CL-2B polysaccharide fractions, on a dry-weight basis and adjusted for material removed for analysis, ranged from 6.6–7.5% and 1.4–2.9%, respectively, for Genesee wheat and 10.5–18.1% and 1.6–7.4%, respectively, for Hillsdale wheat, of the total polysaccharide present in the water extract after dialysis. [b,c]Same as Table I. [d]In the repeat experiment the material had ratings of 1 and 3 by the flow and i.r. tests, respectively. [e]These sugar composition data are from a repeat experiment.

hydrate from the CL-4B and CL-2B columns in both the rye and wheat experiments as determined colorimetrically ranged from 66–99%.

Sugar composition analysis of the most active polysaccharides purified through the Sepharose CL-2B step showed they consisted of arabinose and xylose (Tables I and II). Isolation of two active rye polysaccharide fractions containing negligible amounts of glucose and galactose (Table I) and separation of hexose from pentose material at each step in the purification of the most active polysaccharides (Tables I and II) indicate that these two hexoses are from contaminating polysaccharides.

The combined fraction from the Sepharose CL-4B column that contained the most active polysaccharide from two rye, one Genesee wheat, and two Hillsdale wheat isolation experiments was tested for uronic acid and in each case none was detected [<0.5% (w/w)]. The same CL-4B polysaccharide fraction from one rye, one Genesee wheat, and one Hillsdale wheat isolation experiment was tested for protein and in each case none was detected [<0.6% (w/w)]. In these same experi-

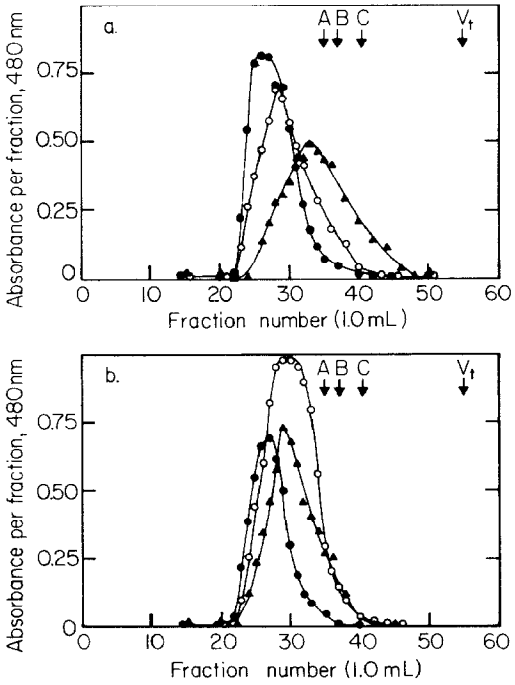

Fig. 2. Sephacryl S-500 column chromatograms of purified rye (a) and Hillsdale wheat (b) polysaccharides. The rye samples were isolated as described in Experimental and were basically the same as those reported in Table I but from a separate isolation experiment. In the rye experiment, column fractions 18–27 of the CL-4B column were combined and applied to the CL-2B column and column fractions 21–29 (●), 30–36 (○), and 37–50 (▲) from the CL-2B column were combined, tested for freeze-inhibition activity, and applied to the S-500 column. The freeze inhibitor ratings from the i.r. freeze test were 9,9 (21–29), 4,6 (30–36) and 3 (37–50). The wheat samples were those described in Table II. Column fractions 18–29 (●), 30–35 (○), and 36–46 (▲) from the CL-2B column were applied to the S-500 column. The freeze inhibitor ratings for these samples are given in Table II. A, B, and C designate the elution maximum for standard dextrans T-2000, T-500, and T-70, respectively.

ments, the included material from the dialyzed water extract of the seeds after the flow test was also tested for protein and the amounts ranged from 5.8–12% (w/w).

Gel-permeation chromatography showed that the most active, combined CL-2B fraction from both rye and Hillsdale wheat were larger than the Dextran T-2000 standard (Fig. 2a and b). The results also indicated that the most active rye polysaccharide from the CL-2B column was slightly larger than the corresponding wheat polysaccharide (Fig. 2a and b).

The activity of purified rye and Hillsdale wheat seed polysaccharide after partial acid hydrolysis for 0, 10, and 30 min was 7, 2, and 0, respectively, for the rye polysaccharide and 7, 1, and 0, respectively, for wheat polysaccharide. Sugar analysis after dialysis of the 30-min rye sample showed that ~60% of the arabinose in the starting sample was present in the non-retained material and that only arabinose was found in this fraction. Analysis of the corresponding wheat sample showed that ~75% of the arabinose and 5% of the xylose in the starting sample was present in the non-retained material. Gel-permeation column chromatography of the retentate from the 0-, 10-, and 30-min hydrolyses of the rye and wheat samples showed there was a progressive decrease in polysaccharide size with increasing time of hydrolysis and that the size of each hydrolyzed sample was less than that of Dextran T-2000.

Methylation analysis (data not presented) showed that all the arabinose in the most active polysaccharide from rye and Hillsdale wheat seed was present as terminal arabinose and was in the furanose form. The percentage values for terminal arabinose (methylation analysis), branch points (methylation analysis), and arabinose content (sugar composition analysis) all were the same within ±1.5%. The products of methylation analysis were 2,3,5-tri-*O*-methylarabinose, 2,3-di-*O*-methylxylose, 2-*O*- or 3-*O*-methylxylose or both, and xylose.

With the freeze-test conditions used, the most active, purified polysaccharide fraction isolated from rye and Hillsdale wheat had a rating of 9 and 7.5, respectively (Tables I and II). As both rated at the upper end of the rating scale, the standard conditions used did not establish if their activities were really different. When the test conditions were changed by increasing the intensity of the i.r. light, thereby increasing agitation, the freeze-inhibitor rating of the most active Hillsdale wheat polysaccharide decreased more rapidly than did the corresponding rye polysaccharide (Table III). This indicates that the wheat polysaccharide is displaced more readily than the rye polysaccharide from the ice–water interface and shows that the rye polysaccharide is a more effective inhibitor of ice-crystal growth than the wheat polysaccharide.

Polysaccharides that interact with ice have been purified from rye and wheat seed. They retained their activity on purification. The most active polysaccharide fraction from each seed type was homogeneous in the sense that the molecules were in the same size range, they contained the same sugar residues, and they behaved similarly in the chemical characterization experiments. The active polysaccharides are arabinoxylans. The most active rye polysaccharide differs structur-

TABLE III

RATING VALUES OF ACTIVE RYE AND HILLSDALE WHEAT POLYSACCHARIDE UNDER VARYING INTENSITY OF INFRARED LIGHT[a]

Increase in voltage to infrared lamp (%)	*Freeze inhibitor rating*	
	rye	*wheat*
0	9	8
6	9,9	5,6
9	7	3
13	0	0

[a]Active polysaccharides were purified as described in Experimental and were not lyophilized. For each cereal, the first combined fraction eluted from the CL-2B column was used. This was obtained from chromatography of the first combined CL-4B fraction. Freeze-inhibitor rating was performed as described in Experimental. The "Increase in voltage to infrared lamp (%)" value of 0 represents the standard i.r. freeze test conditions used throughout this work. Increasing the voltage increases the intensity of the i.r. light which in turn increases interfacial agitation (Experimental).

ally from the wheat polysaccharides in that it has more side-chains and in being slightly larger in size. Each side-chain of both polysaccharides contains a single, terminal arabinose residue in the furanose form. Whether xylose is present in the side-chains was not established. The most purified, active rye polysaccharide has a higher freeze-inhibitor rating than does the Hillsdale wheat polysaccharide when test conditions are used that discriminate between them.

Differences in sugar composition and structure between these two polysaccharides may be due to genetic variance. If so, cereal lines may be selected or bred that can be used to determine the contribution of specific polysaccharide structural features to particular modifications of intercellular ice formation and structure and therefore to survival of plants in various cold environments.

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