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Carbohydrate Research 328 (2000) 539–547

CARBOHYDRATE RESEARCH

Studies on the structure of a lithium-treated soybean pectin: characteristics of the fragments and determination of the carbohydrate substituents of galacturonic acid

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Received 26 October 1999; received in revised form 14 April 2000; accepted 17 April 2000

Abstract

Two galacturonic-acid-containing polysaccharide fractions (ChSS and P) were isolated from soybean meal and subjected to lithium treatment. The fragments obtained were analyzed by using monosaccharide and methylation analyses, and NMR spectroscopy. Lithium degradation of ChSS, followed by sodium borodeuteride reduction, hydrolysis, sodium borohydride reduction, and acetylation afforded alditol acetates, of which the labeled ones reflected residues linked to GalA. As followed from quantifications of the labeled and non-labeled alditols from each constituent monosaccharide by GLC–EIMS, 6 mol% of Ara, 22 mol% of Fuc, 13 mol% of Gal, 53 mol% of Rha, and 57 mol% of Xyl are glycosidically linked to GalA. Analysis of the lithium-treated polymer revealed that it contains arabinogalactan side chains linked to Rha O-4, which consist of a β-(1→4)-linked galactan substituted with highly branched arabinan chains. On average, an arabinogalactan chain contains up to 29 Gal and 25 Ara residues. Surface plasmon resonance was used to determine conditions for affinity chromatography. Furthermore, this technique confirmed the presence of terminal α-Fuc residues in ChSS. Polysaccharide P turned out to be relatively resistant to lithium degradation.

Keywords: Pectin; Structural analysis, lithium degradation; Surface plasmon resonance; NMR spectroscopy; *Anguila anguila* agglutinin

1. Introduction

The three major plant cell-wall polysaccharides are cellulose, hemicellulose, and pectin. These polymers can be separated using a sequential extraction protocol as described [1,2]. The backbone of plant pectins contains 2-O-substituted α-L-rhamnose and 4-O-substituted α-D-galacturonic acid residues. These polysaccharides consist of galacturonan and rhamnogalacturonan regions that can contain side chains originating from O-4 of rhamnose [3].

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0008-6215/00/$ - see front matter
PII: S0008-6215(00)00130-0

The branched structures are also referred to as hairy regions. Schols et al. [4] reported the presence of xylose substituents at galacturonic acid in a pectic xylogalacturonan fraction extracted from apple.

Structural investigations of pectins are difficult in view of their high molecular masses, the lack of homogeneity, and the absence of repeating units [3]. Even with such advanced techniques as high-resolution NMR spectroscopy or mass spectrometry, native pectins are difficult to analyze. Structural analysis generally starts with fragmentation of these complex molecules by enzymatic digestions, partial acid hydrolysis, or alkali-catalyzed elimination reactions. A drawback to these methods is that some structural information gets lost.

Previously, a pectic polymer (chelating agent soluble solids, ChSS) was isolated from the water unextractable solids (WUS) obtained from dehulled, defatted, untoasted soy beans (*Glycine max*.) by using CDTA [2]. Incubation of ChSS with different pectolytic enzymes resulted in the release of Ara and Gal from the polymer. The resulting polysaccharide residue was denoted P [5].

As has been demonstrated previously, treatment of a pectin with lithium in 1,2-diaminoethane causes degradation of the glycosyluronic acid residues, leaving the neutral glycosidic linkages intact [6,7]. This treatment can yield oligo- and monosaccharides in reducing and non-reducing form. Here, the pectic polysaccharide fractions ChSS and P were subjected to lithium degradation in order to generate fragments of these polymers for structural analysis purposes, making use of monosaccharide and methylation analyses and NMR spectroscopy. Surface plasmon resonance has been used to generate structural details for ChSS via interaction studies with *Anguila anguila* agglutinin. The latter technique is especially suitable to study lectin–carbohydrate binding [8–11].

2. Results and discussion

Lithium degradation of ChSS and P.—The optimal reaction time for lithium degradation of ChSS was determined from the recovery of GalA residues from lithium-treated material. Samples were taken at reaction times varying from 0 to 60 min. The monosaccharide composition and the polymer size of the starting material (ChSS) and of the reaction products ('Li 0 min' to 'Li 60 min') are shown in Table 1 and Fig. 1, respectively. After 10 min of lithium treatment of ChSS, GalA was degraded completely and less than 1 mol% of reduced mono- or oligosaccharides was formed. The average mass of the fragments obtained barely decreased after all GalA residues had been removed (approximately 8 kDa). The actual size of the fragments is slightly larger than indicated in Fig. 1, as the column was calibrated with pectin standards, which have larger hydrodynamic volumes than a neutral polysaccharide with the same molecular mass [12].

Table 1
Sugar composition of ChSS and lithium-treated ChSS expressed as mol% [a]

Fraction	Ara	Fuc	Gal	GalA	Rha	Xyl	Alditols [b]
ChSS	24.5	2.9	37.8	23.0	4.3	7.5	0
Li 0 min	25.1	2.9	38.6	21.1	4.7	7.6	0
Li 2 min	38.4	3.1	42.0	4.5	4.9	7.1	0
Li 4 min	39.9	3.5	41.3	2.2	5.0	8.1	0
Li 6 min	37.1	2.7	47.2	tr	3.7	6.5	tr
Li 10 min	41.7	2.4	42.8	0	4.6	8.6	tr
Li 20 min	42.3	2.2	40.9	0	4.5	8.3	1.8
Li 40 min	38.0	2.3	46.2	0	3.7	7.9	1.9
Li 60 min	43.2	2.1	41.0	0	1.2	7.1	5.4

[a] Analyses were carried out in duplicate.
[b] Mol% of total amount of all alditols (i.e., Ara-ol + Fuc-ol + Gal-ol + Rha-ol + Xyl-ol).

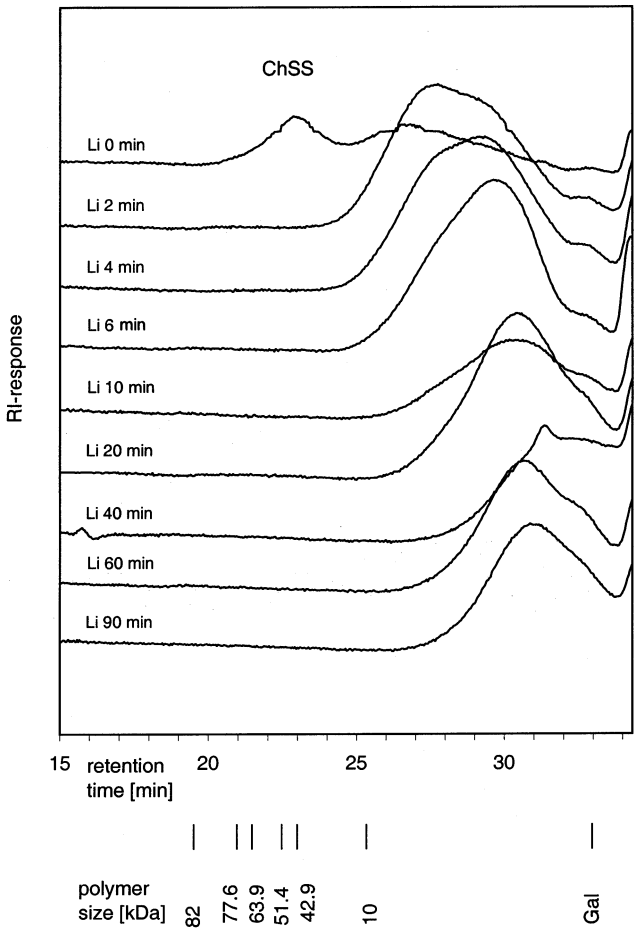

Fig. 1. HPSEC profiles of the reaction products from lithium-treated ChSS with different reaction times. ChSS has a molecular mass corresponding to the position labeled in the profile of 'Li 0 min'. The column was calibrated with pectin standards, as indicated.

P was subjected to lithium treatment in a similar way as described for ChSS. The monosaccharide composition of P during the

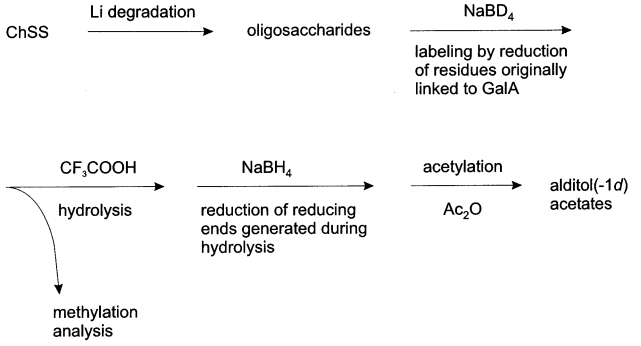

Fig. 2. Reaction scheme for the determination of carbohydrate substituents of GalA in ChSS.

reaction is shown in Table 2. Remarkably, the majority of GalA residues in P could not be degraded, not even after a reaction time of 90 min. The degradation of 8.8 mol% of the GalA residues (from 44.7 to 35.9 mol% for P and 'Li 90 min', respectively) reduces the polymer mass of only part of the material. The mass of the fragments obtained from P in the lithium degradation (90 min) varied between 80 kDa (the starting size) and 10 kDa, therefore they were not further analyzed.

Analysis of the carbohydrate fragments generated from ChSS by lithium treatment. Determination of the carbohydrate substituents of GalA in ChSS.—Part of the carbohydrate material 'Li 10 min' (17%) obtained from the sample used for the determination of the optimal reaction time for the lithium treatment of ChSS was analyzed for the monosaccharides linked to GalA. These monosaccharides were identified from the fragmentation pattern of their alditol acetates in GLC–EIMS [13] (Fig. 2). As is evident from the reaction scheme in

Table 2
Sugar composition of P and lithium-treated P expressed as mol% [a]

Fraction	Ara	Fuc	Gal	GalA	Rha	Xyl	Alditols [b]
P	5.6	7.6	9.8	44.7	11.7	20.6	0
Li 0 min	5.1	6.5	10.5	44.4	13.5	20.0	0
Li 3 min	6.8	5.3	14.6	36.7	17.2	19.4	0
Li 6 min	10.4	7.6	14.7	33.0	17.0	17.3	0
Li 10 min	8.0	7.7	14.9	37.3	14.4	17.7	0
Li 20 min	9.0	6.9	11.5	39.5	17.4	15.7	0
Li 30 min	8.9	7.1	13.8	37.6	16.0	16.6	0
Li 60 min	8.9	7.5	15.9	36.8	14.8	16.1	0
Li 90 min	9.2	7.8	15.7	35.9	14.9	16.5	0

[a] Analyses were carried out in duplicate.
[b] Mol% of total amount of all alditols (e.g., Ara-ol + Fuc-ol + Gal-ol + Rha-ol + Xyl-ol).

Fig. 2, residues originally linked to GalA are reduced with $NaBD_4$, and the other residues with $NaBH_4$. To determine which monosaccharides were originally linked to GalA, the following peak intensity ratios were used: (m/z 73)/(m/z 74), (m/z 145)/(m/z 146) and (m/z 217)/(m/z 218). The fragments corresponding to these m/z values contain C-1 and depending on the type of alditol also C-6. For each monosaccharide two sets of ratios can be obtained: one for the $NaBH_4$- and one for the $NaBD_4$-reduced carbohydrate, as indicated in Table 3. These values are referred to as H-ratio and D-ratio, respectively. For each peak intensity ratio the following equation is valid:

$$\text{Exp. value} = a \times \text{H-ratio} + b \times \text{D-ratio} \qquad (1)$$

wherein the exp. value = experimentally determined ratio of the peak intensities of two m/z values, H-ratio = value for the ratio of interest in the non-labeled alditol acetate, D-ratio = value for the ratio of interest in the labeled alditol acetate, a = mole fraction of a monosaccharide not being linked to GalA, b = mole fraction of a monosaccharide being linked to GalA, and $a + b = 1$.

The experimentally determined ratio (exp. value) and the mole fraction of each monosaccharide linked to GalA in ChSS (b) are listed in Table 4. From the quantifications of the labeled and non-labeled alditols from each constituting monosaccharide, it was concluded that on average in ChSS 6 mol% of Ara, 22 mol% of Fuc, 13 mol% of Gal, 53 mol% of Rha, and 57 mol% of Xyl are glycosidically linked to GalA. The intensities of the signals at m/z 289 and 290 are generally low, therefore, these values were not included in the calculations. Furthermore, the average mole fraction of Rha linked to GalA (53%) does not include the value calculated from the peak intensity ratio (m/z 73)/(m/z 74) (18%), as it is probably influenced by an impurity. The variation in the values calculated for Fuc can be explained by the overlap of the intense GLC signal of Ara-ol acetate with that of Fuc-ol acetate. Therefore, the mass spectrum of Fuc-ol acetate will be contaminated considerably

Table 3
Peak intensity ratios of alditol acetates and alditol-1*d* acetates fragments generated by EIMS (H- and D-ratios, respectively)

Ratio	Reductant	Ara-ol	Fuc-ol	Gal-ol	Rha-ol	Xyl-ol
73/74	$NaBH_4$	8.4	6.3	12.7	5.8	7.4
	$NaBD_4$	1.8	1.7	3.2	1.9	1.9
145/146	$NaBH_4$	15.1	12.1	17.5	13.3	14.2
	$NaBD_4$	1.5	0.7	1.6	0.5	1.3
217/218	$NaBH_4$	10.1	6.7	11.2	7.8	9.6
	$NaBD_4$	1.0	0.1	1.0	0.05	1.0
289/290	$NaBH_4$	7.6	5.0	8.2	6.3	6.5
	$NaBD_4$	0.5	[a]	1.0	0.02	0.9

[a] No signal intensity for m/z 290.

Table 4
Experimental peak intensity ratios (exp. value) and calculated mole fractions of monosaccharides originally linked to GalA (b) in ChSS [a]

Ratio	Ara		Fuc		Gal		Rha		Xyl	
	Exp. value	b	Exp. value	b	Exp. value	b	Exp. value	b	Exp. value	b
73/74	8.0	0.06	5.0	0.28	11.4	0.14	5.1	0.18	4.7	0.49
145/146	14.3	0.06	9.1	0.26	15.0	0.16	5.8	0.59	5.9	0.64
217/218	9.6	0.05	6.0	0.11	10.4	0.08	4.1	0.48	4.6	0.58
289/290	7.4	0.03	5.9	[b]	7.8	0.06	3.3	0.48	3.7	0.50

[a] Treated as described in Fig. 2 with a lithium reaction time of 10 min.
[b] Not calculated, as D-ratio could not be determined.

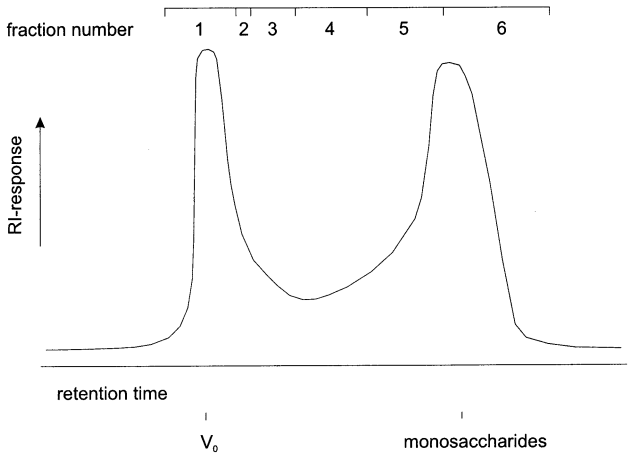

Fig. 3. Bio-Gel P-2 elution profile of ChSS treated with lithium in 1,2-diaminoethane (large scale preparation). The column was calibrated with a dextran of 40 kDa and Gal.

Table 5
Sugar composition of Bio-Gel P-2 fractions of ChSS treated with lithium for 20 min, expressed as mol% [a]

Fraction	Ara	Fuc	Gal	GalA	Rha	Xyl
ChSS	24.5	2.9	37.8	23.0	4.3	7.5
1	37.7	2.8	36.2	12.8	4.5	6.0
2	35.1	2.5	52.2	0	5.2	5.0
3	25.7	3.6	55.6	0	5.6	9.5
4	22.0	tr [b]	65.7	0	4.1	8.2
5	17.0	9.0	60.0	0	3.7	10.3
6	10.4	25.3	43.9	0	tr [b]	20.3

[a] The efficiency of the reaction has not been followed by monosaccharide analysis at different time intervals in the large scale reaction (100 mg of ChSS). The finding of GalA in fraction 1 demonstrates an inefficient reaction (compared with Table 1).

[b] tr, trace amount.

with that of Ara-ol acetate. It should be noted that the relative error in the calculated mole fractions depends on the intensity of the peaks used for the specific ratios. The higher the intensities the lower the relative error. If the percentage of reduced sugars after lithium degradation is higher than trace amounts, it is better to start with a sodium borohydride reduction, followed by hydrolysis and sodium borodeuteride reduction. In that way a smaller error in the calculated mole fractions will be present.

Methylation analysis.—Following the procedure depicted in Fig. 2, it is possible to use part of the reaction mixture after the first reduction step for a linkage analysis. Methylation analysis of the complete mixture of carbohydrates obtained from lithium-treated ChSS showed the presence of 4- and 4,6-substituted Gal and of 3-, 5-, and 3,5-substituted Ara.

Gel filtration followed by monosaccharide analysis.—The material (43.4 mg) obtained from a 20 min lithium treatment of 100 mg ChSS was separated into six fractions using Bio-Gel P-2 gel chromatography, as indicated in Fig. 3. Fraction 1 contained 10 mg, fraction 2 contained 2 mg, and fractions 3–6 less than 0.1 mg carbohydrate. The results of the monosaccharide analysis are shown in Table 5. It is possible that the high refractive index intensities of fractions 5 and 6 in the Bio-Gel P-2 chromatogram are caused by volatile compounds, removed by lyophilization. Note that the amounts of Fuc and Xyl are relatively high in fractions 5 and 6 compared with fractions 1 and 2. This can indicate the presence of short Fuc- and Xyl-containing side chains of GalA. Methylation analysis of ChSS [14] showed that only terminal Fuc and terminal, 2-, 4-, and 2,3-substituted Xyl (39, 44, 6, and 11%, respectively) occur. These results are in agreement with the present determination of carbohydrate substituents of GalA (see above), and with those of Aspinall et al. [15] who isolated Fuc*p*-(1→2)-Xyl via partial acetolysis, and β-Xyl*p*-(1→3)-GalA via partial acid hydrolysis, followed by enzymatic digestion of soybean cotyledon meal.

NMR analysis.—The void volume peak (major fraction 1) was taken for further investigation. Analysis of the 1D ^{1}H and ^{13}C, and 2D TOCSY, NOESY, and HSQC (Fig. 4) spectral data of fraction 1, taking into account literature data [16], led to the assignment of terminal β-Gal (C-4/H-4, 69.7/3.91) and 4-substituted β-Gal (C-4/H-4, 78.5/4.18 and 77.4/3.97), as shown in Table 6. The ratio between the cross-peak intensity of H-4,C-4 of terminal and 4-substituted Gal in the HSQC spectrum demonstrates that the average chain length of the galactan chains is 29 Gal residues. Methylation analysis indicated the presence of some 4,6-substituted Gal. However, no specific spin system for 4,6-substituted Gal could be identified, which indicates a low degree of branching at Gal O-6. Assign-

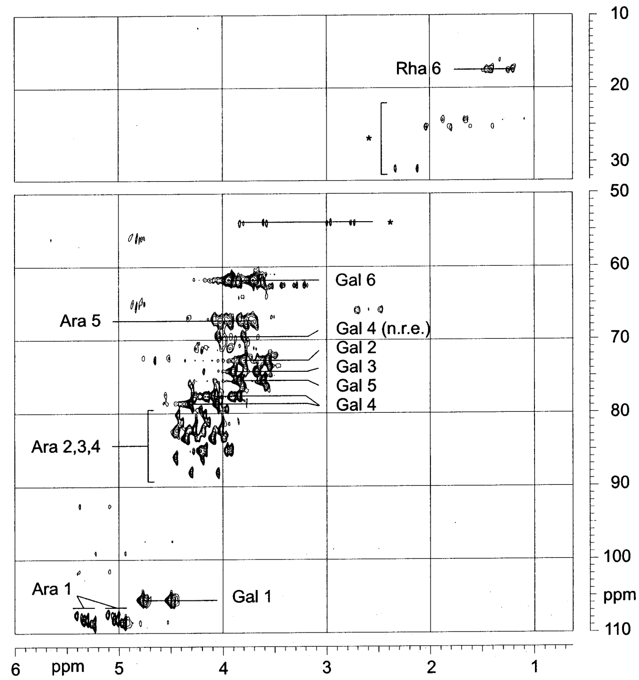

Fig. 4. HSQC spectrum of fraction 1 obtained from a Bio-Gel P-2 separation of ChSS treated with lithium, recorded at 600 MHz and 27 °C. Gal 2 refers to a cross-peak between H-2 and C-2 of Gal, n.r.e. to non-reducing end, and * to a non-carbohydrate impurity.

ments of the protons and carbons of Ara are mainly based on cross-peaks in the HSQC spectrum in combination with literature data [17]. As several spin systems for Ara exist, this residue is probably present as highly-branched arabinan.

From our earlier NMR study of fragments obtained from P via mild acid hydrolysis [14], it is already known that a rhamnogalacturonan in which 28% of the Rha residues is substituted with Gal or galactobiose (55 and 45%, respectively) is present:

$$\begin{array}{l} \quad\quad (\mathrm{R})_{28\%} \\ \quad\quad\quad \downarrow 4 \\ \rightarrow 2)\text{-}\alpha\text{-L-Rha}p\text{-}(1\rightarrow 4)\text{-}\alpha\text{-D-Gal}p\mathrm{A}\text{-}(1\rightarrow \end{array}$$

R = β-D-Gal*p*-(1 or β-D-Gal*p*-(1 → 4)-β-D-Gal*p*-(1 (ratio 55:45)

Combining the data of the fragments of P and the present data, it is supposed that in ChSS some Rha residues of the polymer backbone are substituted with galactan chains and a small number of Gal residues in this galactan contain highly-branched arabinan side chains. Consequently, substituent R in the model shown above, can also be an arabinogalactan. From the NMR results and the average mass of the fragments obtained from the lithium treatment of ChSS (approximately 8 kDa, see above), the average number of Ara and Gal residues in this arabinogalactan was calculated to be 25 and 29, respectively. This is in good agreement with the results of the monosaccharide analysis of fraction 1.

Determination of conditions for lectin chromatography for the isolation of Fuc-containing carbohydrates using surface plasmon resonance.—Previous results indicated the presence of terminal L-Fuc in a mild hydrolysate

Table 6
^{13}C and ^{1}H chemical shifts of Bio-Gel P-2 fraction 1 from ChSS treated with lithium [a]

Residue	C-1/H-1	C-2/H-2	C-3/H-3	C-4/H-4	C-5/H-5(a/b)	C-6/H-6a/b
		Ara-2,3,4 not specifically assigned				
*)-α-L-Ara*f*-(1 → [b]	108.3/5.26; 107.9/5.16, 5.19; 107.7/5.12, 5.10	88.1/4.18; 86.1/4.33; 85.1/4.09; 85.1/4.05; 83.3/4.22; 83.2/4.11; 82.2/4.32; 82.2/4.13; 81.3/4.28; 80.3/4.29			67.3/3.95–3.80; 65.8/3.95–3.80	
β-D-Gal*p*-(1 →	105.2/4.65	n.d. [c]	73.2/n.d.	69.7/3.91	76.1/n.d.	n.d.
4)-β-D-Gal*p*-(1 →	105.2/4.65	72.6/3.68	74.1/3.79	78.5/4.18; 77.4/3.97	75.3/3.72	61.9/3.80; 61.6/3.80

[a] Assignments are based on 1D ^{1}H and ^{13}C, and 2D HSQC, TOCSY and NOESY spectra. Chemical shifts for ^{1}H are expressed in ppm relative to internal acetone (δ 2.225), and for ^{13}C to external glucose (δ Glc*p* C-1α 92.9 [25]).

[b] *, terminal, 5-, or 3,5-substituted Ara.

[c] n.d., not determined, because of the relatively low amount of terminal Gal (t-Gal:4-Gal = 1:28). As can be deduced from the data in Ref. [16] chemical shift values of t-Gal and 4-Gal C-*x*/H-*x* except C-4/H-4 may overlap due to the great difference in intensity.

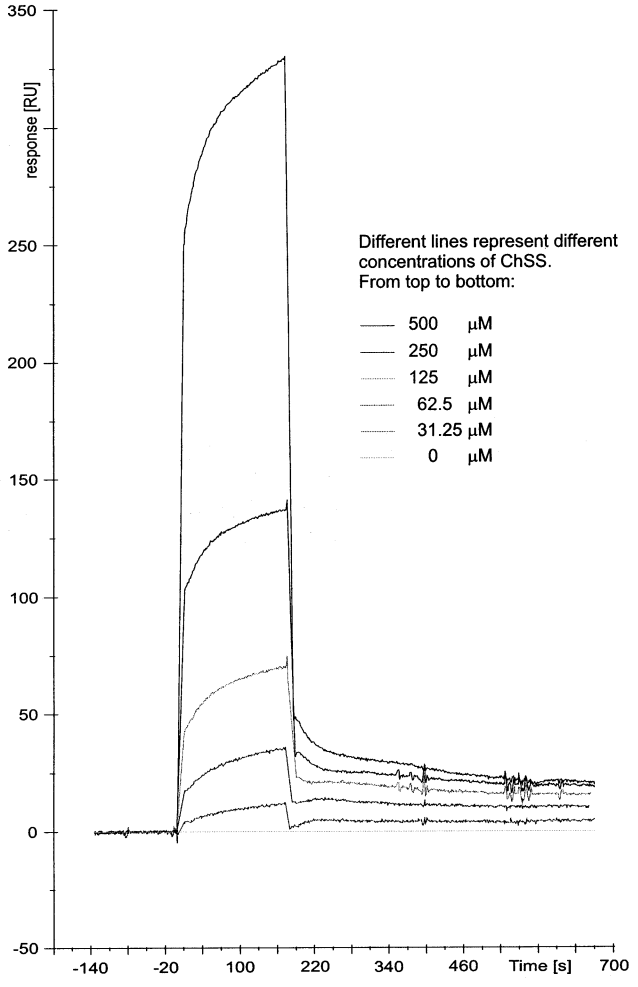

Fig. 5. Binding of ChSS to the L-Fuc specific lectin of the European fresh water eel (*Anguila anguila* agglutinin) as determined with surface plasmon resonance.

of fraction P [14]. Further insight into this item was obtained by studying the interaction of ChSS [18] with the terminal α-L-Fuc specific lectin of the European fresh water eel (*Anguila anguila* agglutinin [AAA]) using surface plasmon resonance as the detection method [19,20]. This lectin is a dimer of two identical subunits having 2 L-Fuc-binding sites each [21].

Maltohexaose was used as a negative blank and did not bind. In a model study, steady-state analysis of the interaction of Fuc-α-(1→2)-Gal-β-(1→3)[Fuc-α-(1→4)]-GlcNAc-β-(1→3)-Gal-β-(1→4)-Glc with the lectin showed two binding constants (K_D 4.58×10^{-4} and 1.43×10^{-3} M). The stronger affinity constant (K_D 4.58×10^{-4} M) was assigned to binding to the first binding site and the weaker (K_D 1.43×10^{-3} M) to binding to the second. A previous study of this lectin revealed only 1 K_D (1.6×10^{-3}) for both binding sites, as determined by equilibrium dialysis [21]. This value is comparable with the weaker binding constant in the present study.

ChSS also showed binding to AAA immobilized to the chip as indicated in Fig. 5. Different substrate concentrations did not reach a maximum in response, which is indicative of the aggregation of polysaccharide chains to a ChSS molecule that is bound by the lectin. Therefore, no binding constants could be calculated. However, it still indicates that the polymer is bound by the lectin. In conclusion, some of the branches of ChSS have α-L-Fuc as a terminal residue. These results indicate that in principle this lectin can be used to isolate Fuc-containing fragments from lithium-treated ChSS.

3. Experimental

Materials.—All chemicals used were *pro analyse* grade or purer, and chemical reactions, except the lithium degradation, were performed in tubes sealed with Teflon-lined screw caps. The lithium wire (diameter 3.2 mm) was obtained from Janssen Chimica. The BIAcore 2000 instrument, the BIAevaluation software 3.0, the sensor chip CM5, and the amino coupling kit were purchased from Pharmacia Biosensor AB (Uppsala, Sweden). The lectin of the European fresh water eel [*Anguila anguila* agglutinin (AAA)] was purchased from Sigma-Aldrich Chemie (Steinheim, Germany).

Dehulled, defatted, untoasted soybeans (*Glycine max.)* were ground and extracted with distilled cold water. The residue was extracted with a 1,4-dithiothreitol (DTT) solution containing sodium dodecyl sulfate (SDS), followed by gelatinization and several incubations with α-amylase. The residue thus obtained is called WUS (water unextractable solids). Soybean WUS was extracted with 1,2-diaminocyclohexane-*N*,*N*,*N*′,*N*′-tetraacetic acid (CDTA) to give a pectin called ChSS (chelating agent soluble solids) in the filtrate [2]. Incubation of ChSS with pectolytic enzymes yielded a pectin called P [5].

Determination of the optimal reaction conditions for the lithium degradation of ChSS.—The lithium-degradation procedure was carried out according to Lau et al. [7]. Briefly, ChSS was dried in vacuo for 24 h over P_2O_5. Then, 50.4 mg of ChSS was added to 10 mL of 1,2-diaminoethane at room temperature and the suspension was stirred under an argon atmosphere. After the carbohydrate had dissolved (13 min) three 2–3 mm long pieces of lithium wire with a diameter of 3.2 mm were added. Within 10 min the mixture turned blue, and this blue color was maintained for 1 h by adding a 2–3 mm long piece of lithium wire, whenever the solution turned pale. At reaction times of 0 (the moment the mixture turned blue), 2, 4, 6, 10, 20, 40, and 60 min, 1.0 mL samples (without lithium) were taken and added to 2 mL of water, and then lyophilized. Each residue was dissolved in water at 5 °C and desalted at 5 °C on a column (8 mm × 15 cm) of Dowex AG 50W-X12 (100–200 mesh, H^+ form; Bio-Rad) using water as eluent. Eluates were lyophilized and aliquots were used for molecular-size determination (HPSEC), monosaccharide analysis, and determination of the carbohydrate substituents of GalA.

For the large scale preparation of fragments of ChSS, the same procedure was used as described above. In short, ChSS (100 mg) was dissolved in 1,2-diaminoethane (20 mL), pieces of lithium wire were added, and the reaction was quenched after 20 min by pouring the solution onto ice (100 mL). The desalting was carried out on a Bio-Gel P-2 column (80 × 2.8 cm) at 60 °C and eluted with water as eluent (RI detection). In this way 43.4 mg of carbohydrate material (56%) was obtained.

Determination of the optimal reaction conditions for the lithium degradation of P.—The same procedure as described for ChSS was followed using 20.4 mg of P dissolved in 4 mL of 1,2-diaminoethane.

High-performance size-exclusion chromatography.—HPSEC was performed on a SP8800 HPLC system (Spectra Physics) equipped with three columns (300 × 7.5 mm each) of Bio-Gel TSK in series (60, 40, and 30XL; Bio-Rad) in combination with a TSK guard column (40 × 6 mm). The elution was carried out with 0.4 M NaOAc buffer (pH 3.0) at 30 °C. Calibration of the HPLC system was performed using pectins with molecular weights in the range 10–82 kDa as determined with viscosimetry [22].

Monosaccharide analysis.—Samples were subjected to methanolysis (methanolic 2 M HCl, 24 h, 85 °C) and the resulting mixtures of (methyl ester) methyl glycosides were trimethylsilylated with 1:1:5 hexamethyldisilazane–chlorotrimethylsilane–pyridine, and quantitatively analyzed by GLC [13,23].

Methylation analysis, hydrolysis, sodium borohydride reduction, and acetylation.—Samples were methylated, hydrolyzed, reduced with sodium borohydride and acetylated as described [13,14,16,23,24]. For sodium borodeuteride reductions, $NaBD_4$ was used instead of $NaBH_4$. The mixtures of partially methylated alditol acetates were analyzed by GLC and GLC–EIMS, whereas the mixtures of alditol acetates were analyzed by GLC–EIMS.

NMR spectroscopy.—Samples were dissolved in D_2O (99.96 atom% D, Isotec, USA). NMR spectra were recorded on Bruker AC-300 (Department of Organic Chemistry, Utrecht University) or Bruker DRX-600 (NSR Center, University of Nijmegen) instruments at a probe temperature of 27 °C. NMR spectra were recorded and processed as described [14,16].

Surface plasmon resonance analyses.—AAA was immobilized to the surface of the sensor chip (flow channel 1). Deactivated lectin was obtained by boiling it in 100 mM NaOAc buffer (pH 5.0) for 15 min, and linked to the reference channel of the chip (flow channel 2). Lectins were attached to the chip via a standard amine coupling technique. Binding of carbohydrates to the lectin was performed in 100 mM NaOAc buffer (pH 6.0). Regeneration of the system was carried out with an injection of 5 mM HCl. Further details concerning these analyses are as described [11]. The binding capacity of the lectin towards α-Fuc was calculated by subtracting the binding to the deactivated lectin from the binding to the active lectin.

Acknowledgements

This research was supported by the Dutch Technology Foundation (NWO/STW), Gistbrocades, and the Product Board for Feeding Stuffs (VVR).

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