ELSEVIER

Carbohydrate Polymers 42 (2000) 123–131

Carbohydrate Polymers

www.elsevier.com/locate/carbpol

Water-soluble *p*-carboxybenzylated beechwood 4-*O*-methylglucuronoxylan: structural features and properties

A. Ebringerová[a,*], J. Alföldi[a], Z. Hromádková[a], G.M. Pavlov[b], S.E. Harding[c]

[a]*Institute of Chemistry, Slovak Academy of Sciences, 842 38 Bratislava, Slovakia*
[b]*Institute of Physics, State University of St. Petersburg, 198904, St. Petersburg, Russian Federation*
[c]*National Centre for Macromolecular Hydrodynamics, University of Nottingham, Sutton Bonington LE12 5RD, UK*

Received 7 July 1999; received in revised form 5 August 1999; accepted 14 September 1999

Abstract

The structural features of the water-soluble *p*-carboxybenzyl 4-*O*-methylglucuronoxylan derivative (CBGX), prepared by reaction of xylan with *p*-carboxybenzyl bromide in aqueous alkali, were characterised by various one-dimensional (1D)- and 2D-NMR techniques applied to the intact derivative as well as to both the oligosaccharide (XO) and resistant (XR) fractions obtained by digestion of CBGX with endo-β-D-xylanase. The real substitution of the xylan backbone with carboxybenzyl (CB) groups was unambiguously determined by the HMBC-NMR technique. The CB groups were located predominantly at the O-2 position of the xylosyl residues. The degrees of substitution of CBGX with 4-*O*-methylglucuronic acid units (DS_U) and CB groups (DS_{CB}) were 0.10 and 0.11, respectively. Thus, every fifth xylose unit of the main chain, on an average, bears either an acidic side chain or a CB substituent. The different proportions of both the substituents in XO and XR fractions suggested the distribution along the xylan backbone to be blockwise rather than uniform. The physicochemical properties of CBGX were characterised by means of viscometry, sedimentation velocity and HPGPC giving a relatively low intrinsic viscosity (53 ml g^{-1}) and similar values of the mean molecular mass (M_w) 27,000 g mol^{-1} and (M_{app}) 34,000 g mol^{-1}, respectively. CBGX exhibited remarkable emulsifying and protein foam-stabilising activities.

Keywords: Beechwood; 4-*O*-methylglucuronoxylan; Carboxybenzyl derivative; Structure; NMR spectroscopy; Molecular properties; Surfactant; Emulsifier

1. Introduction

During the last years, considerable interest has been directed to polymer amphiphiles because of the variety of their possible pharmaceutical, biotechnological and industrial applications (Shalaby, McCormick & Butler, 1991). Several polysaccharides have been used to build the hydrophilic backbone of the amphiphilic macromolecule. The preparation of water-soluble biopolymers with surface-active properties has been reported such as alkyl esters of alginate (Sinquin, Hubert & Dellacherie, 1993) and pectin (Klavons & Bennet, 1995), and long alkyl chain ethers of sulphoethyl cellulose (Talába, Sroková, Hodul & Cík, 1996), cellulose sulphate (Talába, Sroková, Ebringerová, Hodul & Marcincin, 1997) and 4-*O*-methylglucuronoxylan (Ebringerová, Sroková, Talába, Kacuráková, & Hromádková, 1998). Oxidised polysaccharides have been coupled with alkylamines to yield surface-active derivatives of starch (Salomonsson & Theander, 1992), xyloglucan (Lang et al., 1992), and dextran (Zhang & Marchant, 1994).

The partial etherification of the 4-*O*-methylglucuronoxylan from beechwood with *p*-carboxybenzyl bromide have been reported to yield water-soluble derivatives able to depress the surface-tension of water (Ebringerová, Novotná, Kacuráková, & Machová, 1996). In this paper, we describe the structural features of the *p*-carboxybenzyl glucuronoxylan derivative (CBGX) determined by ^1H and ^{13}C NMR spectroscopy, and the hydrodynamic and surface-active properties of the derivative.

2. Experimental

2.1. Materials and methods

The 4-*O*-methyl-D-glucurono-D-xylan (GX) used in this study was isolated from beechwood meal in a semi-technical scale (Ebringerová & Toman, 1986). It contained 83.7% of xylose and 12.4% of 4-*O*-methyl-D-glucuronic acid (U), representing a degree of substitution of the xylan chain $DS_U = 0.10$. Bovine serum albumin

* Corresponding author.
E-mail address: chemebri@nic.savba.sk (A. Ebringerová).

0144-8617/00/$ - see front matter
PII: S0144-8617(99)00150-2

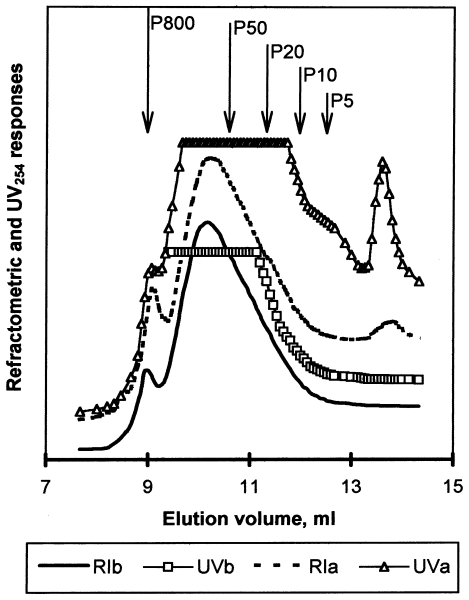

Fig. 1. HPGPC chromatograms of CBGX before (upper curves) and after (lower curves) purification recorded by refractometry (RI) and UV_{254}-detection. Arrows indicate elution time of pullulan standards of various molecular weights.

(BSA) and endo-1,4-β-xylanase of *Trichoderma viride* (EC 3.2.1.8) were purchased from Sigma Chemical Co. (St Louis, USA).

The analytical methods used for characterisation of the derivative by potentiometric titration, elementary analysis and HPGPC on Separon HEMA BIO columns were described in detail in the previous paper (Ebringerová et al., 1996).

FTIR spectra were measured in KBr pellets (2 mg sample/200 mg KBr) using the Nicolet-Magna 750 spectrophotometer operating at 4 cm^{-1} resolution. The NMR spectra were recorded at 25°C on a Bruker DPX AVANCE-300 spectrometer equipped with a selective excitation unit and gradient enhanced spectroscopy kit (GRASP) for generation of *Z*-gradients operating at 300 MHz for ^{1}H and 75.46 MHz for ^{13}C. Acetone was used as an internal standard ($\delta = 2.225$ ppm for ^{1}H and 31.07 ppm for ^{13}C). The samples were dissolved in D_2O (99.99 at%) and measured in 5 mm tubes. For the identification of CH_2 groups, the DEPT sequence was used from the standard Bruker software library. The following pulse programs were used: 2D-DQF COSY sequence with pulse field gradients (Davis, Laue, Keeler, Moskau & Lohman, 1991). In HSQC experiments, the signal from water was suppressed using pulse sequence with pulse field gradients (Schleucher et al., 1994). The HMBC pulse program was applied with low-pass J-filter to suppress single bond correlations (Bax & Summers, 1986). The data matrices for COSY experiments were processed with a sine window function in both dimensions. For HSQC and HMBC experiments, a multiplication with a squared sine function was applied. All processing was performed using Bruker software XWIN-NMR version 1.3 on a Silicon Graphics INDY computer system.

2.2. Preparation of CBGX

The *p*-carboxybenzylated beechwood xylan derivative was prepared as previously described (Ebringerová et al., 1996). The derivative was purified by reprecipitation from an aqueous medium with four volumes of 96% ethanol. The precipitate was washed with 80–96% ethanol, then dispersed in water and stirred at room temperature for 4 h. After centrifugation, the soluble portion was separated and freeze-dried yielding the purified derivative CBGX.

The degree of substitution with CB groups of CBGX (DS_{CB}) was calculated from the potentiometrically (Kohn, Hromádková, Ebringerová & Toman, 1986) determined carboxyl group content of $Q = 1.282$ mmol g^{-1}, on the basis of

$$DS_{CB} = Q(0.132 + 0.055 \times DS_U)/(1 - 0.135Q) - DS_U.$$

The DS_{CB} value obtained was 0.11. CBGX (H^+ form) for $DS_{CB} = 0.1$: $[C_{5.7}H_{8.89}O_{4.6}(C_8H_7O_2)_{0.11}$, calcd. C 47.44%, H 5.8%; found C 47.33% and H 5.85%].

2.3. Physicochemical methods

The hydrodynamic measurements were carried out in the "Paley-buffer": $(Na_2HPO_4 \cdot 12H_2O + KH_2PO_4) - 0.05$ M; pH = 7, used for proteins. The ionic strength was changed by supplementary addition of 0.15 M NaCl. The experiments were performed in 0.2 M solvent which had the following characteristics at 25°C: density $\rho_0 = 1.0060$ g ml^{-1} and viscosity $\eta_0 = 0.91$ cP. Viscometric measurements were made at 25.0°C using a 2 ml Schott-Geräte automatic Ostwald glass capillary viscometer. The flow time of the solvent $\tau_0 = 84.5$ s. The Huggins relationship (Tanford, 1961; Tsvetkov, Eskin & Frenkel, 1970) was employed for calculating the values of intrinsic viscosity $[\eta]$ and Huggins parameter k': $\eta_{sp}/c = [\eta] + k'[\eta]^2 c + \ldots$

Sedimentation velocity analysis was performed using the analytical Beckman Model-E ultracentrifuge with a rotor speed of 47,660 rpm in a single-sector cell of optical path 12 mm. Sedimentation coefficients (*s*) were calculated from the displacement of unimodal Schlieren curve maxima *X* as a function of time. Semi-automatic data capture was employed using a graphic digitising tablet (Seifert, Heinevetter, Coelfen & Harding, 1995). Photographs were taken at 4 min intervals during 56 min, and negatives enlarged directly onto a graphics tablet interfaced to an IBM PC. The resulting records of the position of the sedimenting boundaries could then be analysed directly to yield the sedimentation coefficient s_t. The s_t values were corrected to 25°C (s_{25}) using the standard procedure (Tanford, 1961; Tsvetkov et al., 1970; Cantor & Schimmel, 1980). The dependence of s_{25} on *c* which corresponded to the equation: $s^{-1} = s_0^{-1}(1 + k_s c)$ was studied in the concentration range of $(0.03–0.47) \times 10^{-2}$ g ml^{-1}. The buoyancy factor or

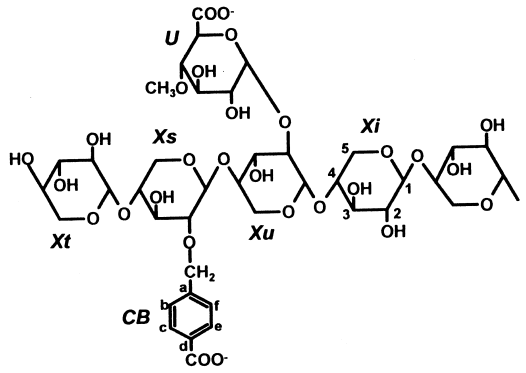

Fig. 2. Possible structural element of CBGX. U, 4-*O*-methyl-α-D-glucopyranosyl uronic acid; Xi, internal (1→4)-linked β-D-xylopyranose; Xt, non-reducing β-D-xylopyranose end unit; Xu, β-D-xylopyranose substituted at position O-2 with U; Xs, β-D-xylopyranose substituted at positions O-2 or O-3 with CB group.

density increment $(1 - \upsilon\rho_0) = \Delta\rho/\Delta c = 0.333$, where υ, the partial specific volume of the polymer, was measured with an Anton Paar (Graz, Austria) densitometer according to the procedure of Kratky, Leopold and Stabinger (1973).

2.4. Tensioactive properties

The tensioactive properties, including minimum surface tension (γ_{min}), critical micelle concentration (c.m.c.), foamability, emulsifying activity and protein foam-stabilising effect, were characterised by methods described in detail in a previous paper (Ebringerová et al., 1998).

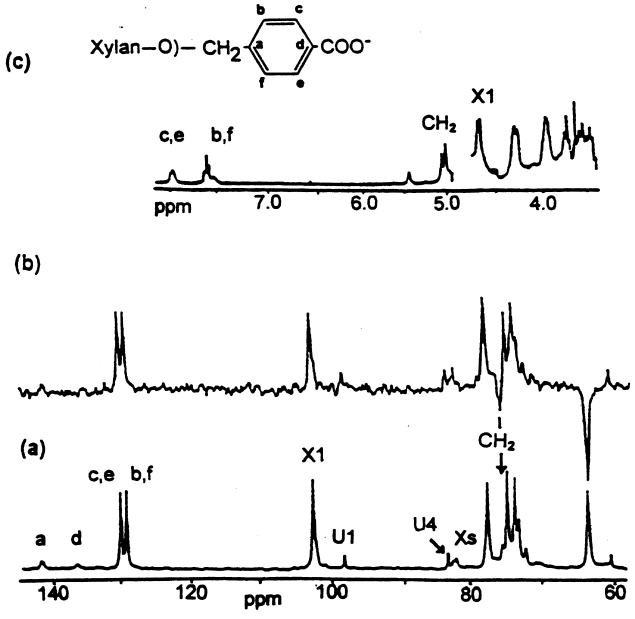

Fig. 3. NMR spectra of CBGX in D_2O: (a) ^{13}C NMR spectrum; (b) the DEPT experiment and (c) ^{1}H NMR spectrum.

3. Results and discussion

3.1. Compositional analysis

The HPGPC records (Fig. 1) of CBGX reveal the same pattern after detection by refractometry and UV-absorption at 254 nm, thereby indicating that CB substituents were distributed over all xylan molecules. The FTIR spectrum of CBGX (not shown) exhibited new absorption bands at ~1551 and 777 cm^{-1}, attributed to $\nu_{as}(COO^-)$ and benzene ring vibrations, respectively, of the CB substituent (Ebringerová et al., 1996). However, the unpurified CBGX also contained a small amount of UV_{254}-absorbing low-molecular mass components. They contribute to the intensity of these absorption bands, thus giving an overestimate for the DS_{CB} of the derivative. The removal of such byproducts was achieved by reprecipitation of the crude CBGX and washing with ethanol. The DS_{CB} (0.11) of CBGX determined by potentiometric titration accorded well with the value of 0.1 calculated from the elemental analysis data.

3.2. NMR studies

A theoretical structural unit for CBGX is shown in Fig. 2. The ^{1}H and ^{13}C NMR spectra of CBGX (Fig. 3) show intense signals from the unsubstituted (internal) (1→4) linked β-D-xylopyranosyl (Xi) and (1→2) linked 4-*O*-methyl-α-D-glucopyranosyl uronic acid (U) residues, whereas those of Xi substituted at position O-2 with such groups (Xu) are not well resolved. Xylosyl residues substituted at positions O-2 or O-3 with CB groups were signed as Xs. The presence of CB substituents is indicated by the appearance of aromatic carbon and proton signals in the regions of 128–145 and 7–8 ppm, respectively. Two singlets at δ 4.8–4.9, partially overlapped by the HDO signal, can be assigned to the benzyl CH_2 group. The corresponding carbon signals of CH_2 were shown to be at $\delta \sim 75$ by means of the DEPT experiment. The two bands at $\delta \sim 82.1$ and 82.4, not seen in the spectrum of the parent xylan (not shown), indicate substitution of both free positions (O-2, O-3) of the xylosyl residues with CB, which exerts similar effects on the α-carbons (downfield shift of ~8.5 and 7.6 ppm) as reported for methyl substituents (Azuma & Koshijima, 1983).

Assignment of the proton COSY spectrum of CBGX (not shown) was straightforward, the assignments being obtained in sequence starting from the anomeric protons, and the data are given in Table 1. The shifts and connectivities of Xi and U residues were clearly observable, whereas those of Xu and Xs gave weak cross-peaks. As expected, the singlets of the benzyl CH_2 group gave no off-diagonal peaks in the symmetrised contour plot. The ^{13}C NMR assignments of CBGX were made using the $^{1}H/^{13}C$ HSQC NMR techniques. The contour plot of the 2D spectrum of CBGX, displayed in Fig. 4, gave the cross-peak data collected in Table 1.

Table 1
^{1}H and ^{13}C NMR data of CBGX (for abbrevations, see Fig. 2; na, not assigned)

Sugar residue	Chemical shift (ppm) H/C						
	1	2	3	4	5	COOH	OCH_3
Xi	4.46	3.28	3.53	3.76	4.15/3.42		
	102.59	73.65	74.80	77.31	63.84		
Xu	4.59	3.41	3.55	3.35	4.08/3.38		
	102.41	77.42	73.30	na	63.84		
Xs		na	Na				
		82.1	82.4				
U	5.28	3.57	3.75	3.21	4.31		3.48
	98.44	72.23	73.13	83.32	73.13	177.8	60.78
CB group	CH_2	a	b,f	c,e	d	COOH	
	4.83, 4.91		7.53	7.91			
	75.2, 74.9	141.43	129.5	130.4	136.94	176.2	

Due to the rather poor solubility and high viscosity, unmodified glucuronoxylans usually give no well-resolved NMR spectra. Therefore, most of the hitherto published carbon chemical shifts for 4-*O*-methylglucuronoxylans have been assigned on the basis of data reported for structurally related oligosaccharides (Azuma & Koshijima, 1983; Cavagna, Deger & Puls, 1984; Uttile, Kováč, Saurio & Perlin, 1986; Kardošová, Matulová & Malovíková, 1998; Bazus, Rigal, Gaset, Fontaine, Wieruszeski & Fournet, 1993). Whereas, the established proton chemical shifts of the GX polymer in Table 1 accord well with the data published for GX-derived xylo-oligosaccharides, differences occur for shifts of C-2/C-5 atoms of the uronic acid residues. The results suggest that samples measured in the carboxylate form, compared to their acid form, have the signal of U-5 downfield-shifted.

Partial hydrolysis of the polysaccharide chains, particularly with the aid of endo-glycanases (Comtat & Joseleau, 1981), is often used to lower the viscosity and thus improve resolution of the NMR spectra. Digestion of CBGX with endo-1,4-β-xylanase from *T. viride* yielded an oligosaccharide fraction (XO) and a resistant, non-dialysable fraction (XR). In the ^{1}H and ^{13}C NMR spectra of both fractions (not shown), proton and carbon signals typical of terminal non-reducing (Xt) and reducing (Xα and Xβ) xylosyl end units were present. They were partially assigned using the COSY spectra and data reported for glucuronoxylan-derived oligosaccharides (Kováč, Alföldi, Kočiš, Petráková & Hirsch, 1982; Cavagna et al., 1984; Kardošová et al., 1998).

The HSQC spectra of XO and XR are presented in Figs. 5 and 6. For the identification of the substitution sites, the region at δ 80–85 is informative. In this region, the C-4 of the 4-*O*-methylglucuronic acid (U4) appeared in a manner similar to the intact xylan at $\sim\delta$ 83.30 and was correlated with proton signals at δ 3.21 and 3.24 for XO and XR, respectively, indicating different locations of the

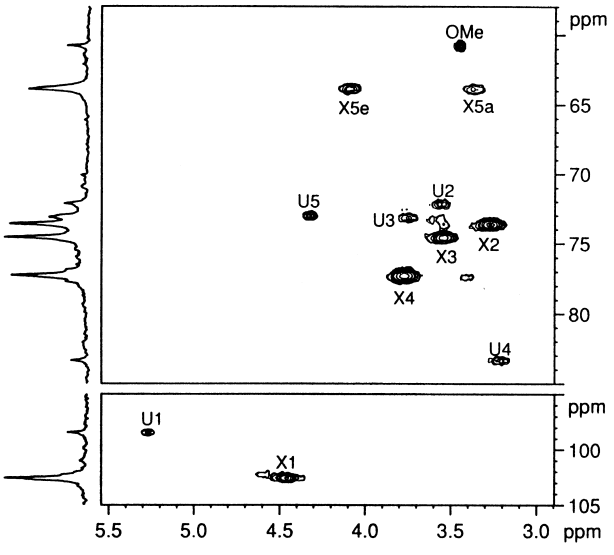

Fig. 4. $^{1}H/^{13}C$ NMR COSY spectrum of CBGX.

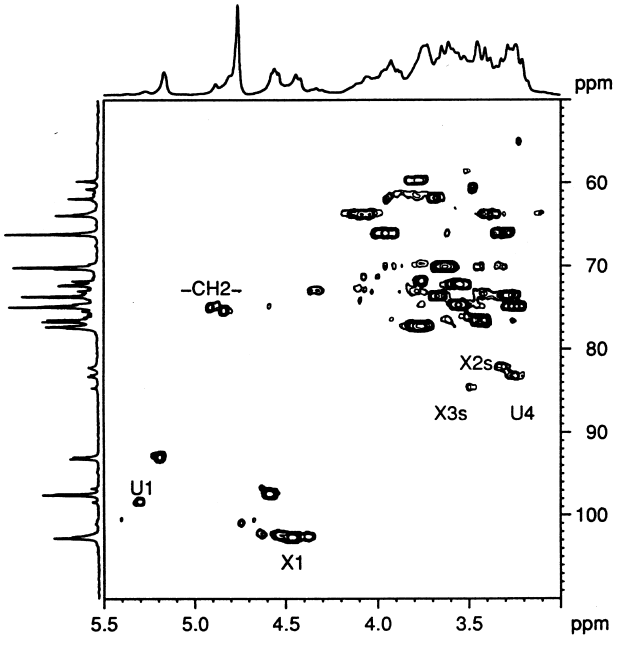

Fig. 5. $^{1}H/^{13}C$ NMR (HSQC) spectrum of the oligosaccharide fraction (XO) obtained by digestion of CBGX with endoxylanase.

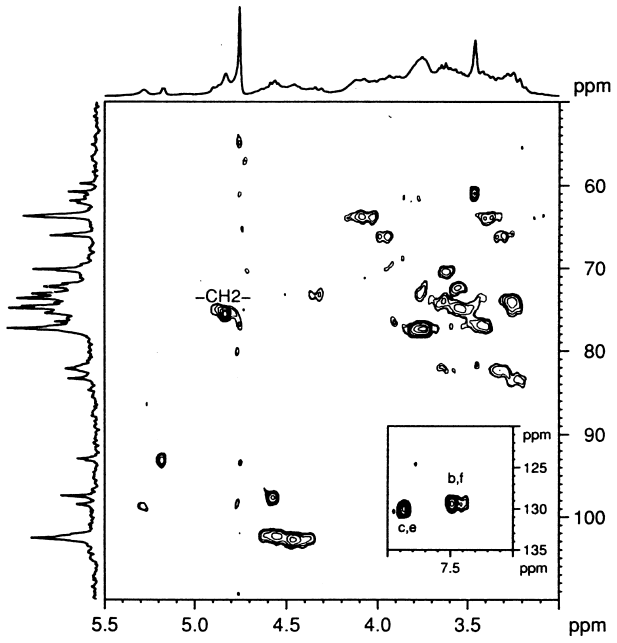

Fig. 6. $^{1}H/^{13}C$ NMR (HSQC) spectrum of the endoxylanase-resistant fraction (XR) of CBGX.

uronic acid side chain on the xylan fragments. In contrast to the intact CBGX, both XO and XR gave several carbon signals in this region. On the basis of the alkylation shifts and also the fact that the proton of the alkylated carbon is downfield-shifted, the signals for carbons of the O-2 and O-3 substituted xylose residues could be tentatively assigned. The results are summarised in Table 2. The proton chemical shifts of position O-2 in neutral and acidic xylooligosaccharides (Cavagna et al., 1984; Kardošová et al., 1998) ranged between δ 3.24 (Xβ, Xi, Xt) and 3.52 (Xα) and those for O-3 position between δ 3.42 and 3.74. In the case of XO (Fig. 5), there are three cross-peaks at δ 82.2/3.33 (prevailing), 83.1/3.27 and 84.7/3.50, corresponding to O-2 substituted Xt and Xβ, and O-3 substituted Xt, respectively. The ratio of the reducing xylosyl end groups, determined by the integral area ratios of the anomeric carbons indicated a DP of 2.5. Therefore, the substituted Xi units should be present in minor amounts. The HSQC spectrum of XR (Fig. 6) showed three xylose units (Xi, Xt and Xβ) bearing a CB group at O-2 position. The substitution at O-3 was seen for Xi, whereas it was too low for the Xβ anomer (seen in the ^{13}C NMR spectrum at δ 84.6) to give a detectable cross-peak. The results indicate a considerable substitution of Xt units (Table 2).

Table 2
HSQC ^{1}H and ^{13}C NMR chemical shifts of fractions obtained by xylanase digestion of CBGX and data[a] for 4-*O*-methylglucuronoxylan-derived oligosaccharides (na, not assigned)

Fraction	Position of CB	Chemical shift (ppm) H/C			
		Xi	Xt	Xα	Xβ
XO	O-2	–	3.33	–	3.27
		–	82.2	–	83.1
	O-3	–	3.50	–	–
		–	84.7	–	–
XR	O-2	3.45	3.33	–	3.26
		82.0	82.2	–	83.4
	O-3	3.64	na	–	–
		82.3	84.7	–	–
Xylooligosaccharides[a]	O-2	3.26	3.26	3.52	3.24
		73.6	73.2	72.3	74.8
	O-3	3.56	3.42	3.74	3.57
		74.6	76.5	71.8	74.7

[a] From Cavagna et al. (1984) and Kardošová et al. (1998).

The HSQC spectra of both fractions showed at least two cross-peaks of the CH_2 group indicating different positions of CB. In XR, the signal intensity of the cross-peak for CH_2 at δ 75.2/4.88 was much lower than at δ 76.1/4.82, whereas in XO, the differences are lower and a third cross-peak can be observed at δ 75.0/4.87. All the results obtained suggest that position O-2 is preferred during *p*-carboxybenzylation of GX.

Further information about the substitution sites can be obtained using the HMBC-technique designed to detect long-range couplings. In Fig. 7, the HMBC spectrum of XR is displayed. Only weak correlation peaks of the Xs-2 carbons (the prevailing signal) with the protons of benzyl CH_2 and aromatic (c,e) ring protons were observed. However, the Hc,e signals at δ 7.86 gave strong correlation peaks with Ce,c (130.1 ppm) and Ca (141 ppm), as well as with the Xs carbons resonating at δ 82–85. In contrast, the Hb,f signals (7.49 ppm) gave strong correlation peaks with the CH_2 carbons (~76.1 ppm), Cd (136,9 ppm) and a weak cross-peak with the Xs-2 carbon. Thus, the real substitution of the xylan backbone with CB groups was unambiguously determined.

From the xylanase digestion and NMR experiments, important information becomes available on the fine structure of xylan macromolecules i.e. the distribution of the side chains of the native and modified GX. For XR, three glycosidic linkage sites, assigned to HMBC cross-peaks between the protons of Xi-1 (4.46 ppm), Xt-1 (4.38 ppm), and Xs-1 (4.53 ppm) with C-4 (77.3 ppm) of the neighbouring unsubstituted xylose residue, are seen in Fig. 7. Moreover, the proton of Xi-1 also gave a HMBC cross-peak with C-4 (76.5 ppm) of Xu and/or Xs. This indicates that the following segments are present in the xylanase-resistant xylan chains: [Xt–Xi], [Xs or Xu–Xi], [Xi–Xu], and [Xi–Xi], with a higher proportion of the last segment.

A rough estimate for the distribution of uronic acid side chains and CB groups of XO and XR xylan chains, based on the proportion of the corresponding carbon signal areas in the respective ^{13}C NMR spectra, is given in Table 3. There were significant differences in the proportion of uronic acid side chains for XO and XR, as deduced from the DS_U: 0.05 and 0.14, respectively. However, the molar ratios between CB substituents and uronic acid side chains were about the same, i.e. about 3:1 for in both fractions. The total substitution of the xylanase-resistant XR was nearly four times

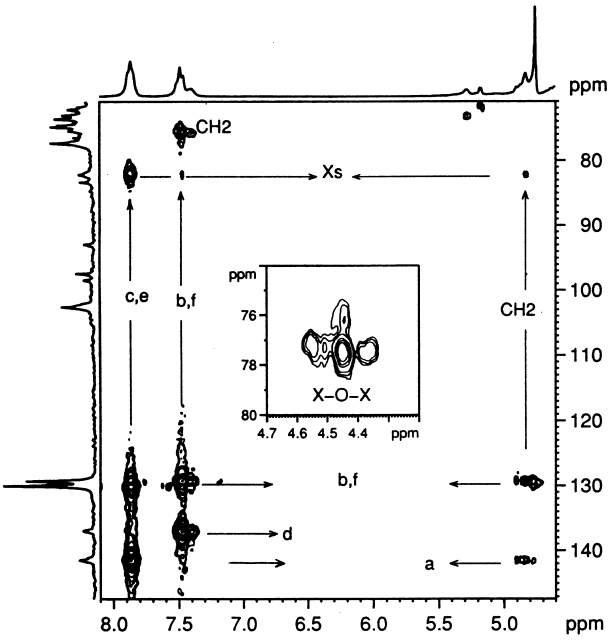

Fig. 7. $^{1}H/^{13}C$ NMR (HMBC) spectrum of the endoxylanase-resistant fraction (XR) of CBGX.

higher than in the case of the oligomeric XO. The equal CB to U ratios of XR and XO suggest that substitution with CB groups took place preferentially in the regions of a higher frequency of uronic acid side chains, probably due to their higher accessibility towards etherification in comparison to the less substituted regions with strong intermolecular H-bonding interactions. The compositional analysis of the intact CBGX revealed that every fifth xylosyl residue, on an average, bears one substituent (uronic acid or CB group). As previously reported for the beechwood 4-*O*-methylglucuronoxylan (Kohn et al., 1986), already the uronic acid side chains are non-uniformly distributed. A similar irregular distribution of uronic acid side chains has also been reported for other hardwood and softwood xylans (Shimizu, Hashi & Sakurai, 1978; Comtat & Joseleau, 1981). The different substitution patterns of XO and XR confirmed the irregular distribution pattern of both substituents in CBGX.

Table 3
Structural features of XO and XR fractions obtained by xylanase-digestion of CBGX

Molar ratios	Carbon signals[a]	XO	XR
Xyl:U	∑X-5:U-4	20:1	7:1
DS_U		0.05	0.14
Xyl:CB	∑X-5:Xs-(2 + 3)	7.7:1	2:1
DS_{CB}		0.13	0.47
CB:U	Xs-(2 + 3):U-4	3.0:1	3.3:1
$DS_U + DS_{CB}$		0.18	0.61
CB(O-2):CB(O-3)	Xs-2:Xs-3	6.3:1	5.9:1

[a] Used to estimate the approximative molar ratios from the carbon signal areas of the respective ^{13}C NMR spectra.

Table 4
Some physicochemical data of CBGX

Viscometry	
$[\eta]$ (ml g^{-1})	53.0
k' (Huggins constant)	0.34
Sedimentation velocity	
Sedimentation coefficient, s_0 (Sv)	1.63
$M_{s\eta}$ (g mol^{-1})	26,000
M_{k_s} (g mol^{-1})	28,000
HPGPC[a]	
M_{app}[b] (g mol^{-1})	34,000
M_w/M_n	1.72

[a] Measured on pullulan-calibrated Separon HEMA BIO columns.
[b] A second peak (~13 area%) shows $M_{app} > 500{,}000$ g mol^{-1}.

3.3. Physicochemical characterisation of CBGX

The molecular mass distribution of GX and CBGX was determined by HPGPC on Separon HEMA BIO columns calibrated with pullulan standards. The chromatograms (Fig. 1) indicate that the native as well as modified xylan comprised two polymer populations differing considerably in hydrodynamic volume. The apparent molecular weight (M_{app}) of the main component of GX and CBGX was about 34,000 g mol^{-1} in both cases. The minor component, representing 10–13% of the total polymer refractometric response, eluted in the very high molecular mass region ($M_{app} > 500{,}000$ g mol^{-1}). The data obtained are summarised in Table 4. Similar properties have been recently reported for maize bran heteroxylan (Chanliaud, Roger, Saulnier & Thibault, 1996) and barley arabinoxylans (Izydorczyk, Macri & MacGregor, 1998). These authors considered non-validity of the "universal" calibration for the pair heteroxylan-pullulan over the whole molecular weight range studied and overestimation of the molecular weights by SEC, respectively.

In pure water CBGX manifested the classical polyelectrolyte effects on viscosity (data not shown) typical of charged polymers (Tanford, 1961; Dautzenberg, Jaeger, Kotz, Phillip, Seidel & Stscherbina, 1994; Barrat & Joanny, 1996). Therefore, the viscometric and ultracentrifugal studies on CBGX were performed in a 0.2 M solvent where the primary polyelectrolyte effects may be considered to be suppressed. As seen in Table 4, CBGX had an intrinsic viscosity $[\eta]$ of 53 ml g^{-1}, which is comparable to data (40–70 ml g^{-1}) reported previously for beechwood glucuronoxylans (Ebringerová, Hromádková & Eremeeva, 1989), whereas, giving a much lower value in comparison to that for cereal grain arabinoxylans (190–470 ml g^{-1}; Izydorczyk & Biliaderis, 1992) and maize bran heteroxylans (100–180 ml g^{-1}; Saulnier, Marot, Chanliaud & Thibault, 1995). The Huggins constant, $k' = 0.39$, is indicative of a semi-flexible conformation.

Velocity sedimentation experiments on CBGX revealed a sedimentation coefficient s_0 of 1.63 S and a concentration

Table 5
Tensioactive properties of CBGX

Surface tension[a] (γ_{min}) (mN m^{-1})	51.0
Critical micelle concentration[a] (c.m.c.) (g l^{-1})	5.0
Foaming activity[b] (%)	40 (80 and 0)
Emulsifying activity[c] (oil layer/cream layer) (mm/mm)	
After 5 min	0/0 (0/0)
After 30 min	0/6 (0/0)
After 24 h	0/6 (0/2)

[a] Assessed by surface-tension measurements in the concentration range of 0.01–5 g l^{-1} using the Lecompte Du Nouy ring apparatus.

[b] Estimated from the foam volume of the solution ($c = 1$ g l^{-1}) measured immediately after generation of the foam in comparison to the volume of the non-shaked solution. In brackets are the values for gum arabic and CMC (Lang et al., 1992).

[c] Measured as the height (mm) of the oil and cream layers, respectively, formed after various time intervals onto the surface of the emulsion. It was prepared by mixing a 1% (w/v) solution of CBGX with paraffin oil (90/10, v/v) dyed with Sudan IV. In brackets are the values for the commercial emulgator Tween 20.

sedimentation coefficient k_s of 40 ml g^{-1}. Two possible routes for the evaluation of the molecular mass (M) were followed. The first one allows us to calculate M from s_0 and $[\eta]$ based on the concept of the hydrodynamic invariant A_0 (Mandelkern & Flory, 1952; Tsvetkov & Klenin, 1953) by the equation

$$M_{s\eta} = (R/A_0)^{3/2}(\eta/(1 - \nu\rho_0))^{3/2}s_0^{3/2}[\eta]^{1/2}.$$

Utilising the average value for A_0 of 3.4×10^{-10} (Tsvetkov, Lavrenko & Bushin, 1984), the resulting value of $M_{s\eta}$ was 26,000 g mol^{-1}.

The second route is related to the common consideration of s_0 and k_s values. In this case, the generalised relation of van Holde–Wales–Rowe (Wales & van Holde, 1954; Rowe, 1977; Pavlov & Frenkel, 1995)

$$M_{k_s} = (N_A/\beta_s)^{3/2}(\eta_0/(1 - \nu\rho_0))^{3/2}s_0^{3/2}k_s^{1/2}$$

was used. Utilising the average value for β_s of 1.0×10^7

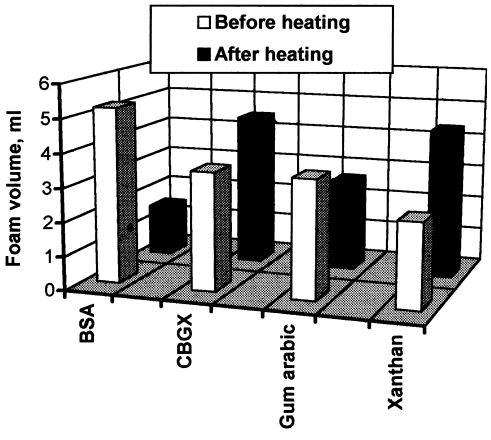

Fig. 8. Effect of CBGX and controls on the thermal stability of protein (BSA) foam, 95°C; heating at 90°C for 3 min.

(Pavlov, 1997), the resulting M_{k_s} was 28,000 g mol^{-1}. There was a good correlation between the two M values obtained.

3.4. Functional properties

The tensioactive properties of CBGX in aqueous solution were seen in Table 5. The CB substituents have a moderate hydrophobisation effect as it is manifested by the relatively weak depression of the surface tension of water. Similar behaviour was reported also for the low-substituted octyl tamarind xyloglucan derivatives (Lang et al., 1992) exhibiting a high foaming activity (200%), similar to that of a commercial whipping protein. The foamability of CBGX (40%) was lower than that of gum arabic complex (80%), a protein–polysaccharide complex. However, CBGX exhibited excellent emulsifying activity, comparable to that of the commercial emulgator Tween 20 (oxyethylated monolauransorbitol). The emulsion was very stable and no oil layer appeared even after 4 days of storage.

The effect of CBGX on the thermal stability of the protein (BSA) foam is seen in Fig. 8. By intense mixing of an aqueous solution of BSA, a high foam volume was generated, which diminished to about 30% after heating. Addition of CBGX to the solution resulted in a depression of the initial foam volume, similar to that reported for xanthan gum or gum arabic (Izydorczyk, Biliaderis & Bushuk, 1991). However, CBGX stabilised the protein foam to a similar extent as did the xanthan gum, whereas gum arabic showed no positive effect.

Usually, the foamability and protection effect in foam stabilisation is related to the viscosity of the polysaccharide and its film-forming properties. In the case of CBGX and alkylated glucuronoxylan derivatives (Ebringerová et al., 1998), which exhibit rather low viscosities in comparison to polysaccharide-based tensioactive compounds (Lang et al., 1992), this effect may be influenced by electrostatic interactions between the present carboxyl groups and the protein (Dalev & Simeonova, 1995). The CB substituent itself has an amphiphilic nature and therefore it imparts solubility to the xylan on one side and creates centres for hydrophobic inter- and intramolecular interactions on the other side which might also affect the film-forming properties of the xylan chains.

4. Conclusions

This study has permitted the identification and characterisation of the primary structure of CBGX and the fine structure of the xylan chains as well. The ^{1}H–^{1}H homonuclear and ^{1}H–^{13}C NMR heteronuclear correlation spectroscopy analyses of the intact CBGX polymer and the xylanase-released oligomeric and resistant fractions as well, suggest that the CB groups are located on the xylopyranose residues more frequently at positions O-2 than O-3. The real susbtitution of the xylan backbone was confirmed by the HMBC-correlation technique. There was no indication of substitution of the uronic acid residues. Employing the enzymic

degradation of CBGX, it was found that both the uronic acid side chains and the CB substituents are very non-uniformly distributed along the xylan chains. The mean molecular mass obtained by the analytical ultracentrifuge technique was slightly lower than the apparent molecular mass derived from the pullulan-calibrated HPGPC experiment. CBGX exhibited remarkable emulsifying and protein foam-stabilising activities.

Acknowledgements

The British Council, Slovakia, the Slovak grant agency VEGA (project no. 2/4148) and the University of Nottingham were acknowledged for financial support.

References

Azuma, J., & Koshijima, T. (1983). ^{13}C-NMR spectroscopy of carbohydrates II. ^{13}C-NMR spectroscopy of xylan and related carbohydrate. *Wood Research Technical Notes, 17*, 132–169.

Barrat, J.-L., & Joanny, J.-F. (1996). Theory of polyelectrolyte solutions. *Advances in Chemical Physics, 94*, 1–66.

Bax, A., & Summers, M. F. (1986). Proton and carbon-13 assignments from sensitivity anhanced detection of heteronuclear multiple-bond connectivity. *Journal of the American Chemical Society, 108*, 2093–2094.

Bazus, A., Rigal, L., Gaset, A., Fontaine, T., Wieruszeski, J.-M., & Fournet, B. (1993). Isolation and characterisation of hemicelluloses from sunflower hulls. *Carbohydrate Research, 243*, 323–332.

Cantor, Ch. R., & Schimmel, P. R. (1980). *Biophysical chemistry Part II*, San Francisco, CA: Freeman.

Cavagna, F., Deger, H., & Puls, J. (1984). 2D NMR analysis of the structure of an aldotriouronic acid obtained from birch wood. *Carbohydrate Research, 129*, 1–8.

Chaniliaud, E., Roger, P., Saulnier, L., & Thibault, J.F. (1996). Static and dynamic light scattering studies of heteroxylans from maize bran in aqueous solution. *Carbohydrate Polymers, 31*, 41–46.

Comtat, J., & Joseleau, J.-P. (1981). Mode of action of a xylanase and its significance for the structural investigation of the branched L-arabino-D-glucurono-D-xylan from redwood (*Sequoia sempervirens*). *Carbohydrate Research, 95*, 101–112.

Dalev, P. G., & Simeonova, L. S. (1995). Emulsifying properties of protein–pectin complexes and their use in oil-containing foodstuffs. *Journal of Science Food and Agriculture, 68*, 203–206.

Dautzenberg, H., Jaeger, W., Kotz, J., Phillip, B., Seidel, Ch., & Stscherbina, D. (1994). *Polyelectrolytes*, Munich: Haser.

Davis, L. A., Laue, E. D., Keeler, J., Moskau, D., & Lohman, J. (1991). Absorption mode two-dimensional NMR spectra recorded using pulsed field gradients. *Journal of Magnetic Resonance, 94*, 637–644.

Ebringerová, A., & Toman, R., (1986). Preparation of 4-*O*-methyl-D-glucurono-D-xylan from beechwood, Czech CS No. 231,686 (CA: 107, 47).

Ebringerová, A., Hromádková, Z., & Eremeeva, T. E. (1989). Alternative Verfahren zur Gewinnung von Hemicellulosen des D-Xylantyps aus Laubhözern. *Holz Roh-u. Werkstoff, 47*, 355–358.

Ebringerová, A., Novotná, Z., Kacuráková, M., & Machová, E. (1996). Chemical modification of beechwood xylan with *p*-carboxybenzyl bromide. *Journal of Applied Polymer Science, 62*, 1043–1047.

Ebringerová, A., Sroková, I., Talába, P., Kacuráková, M., & Hromádková, Z. (1998). Amphiphilic beechwood glucuronoxylan derivatives. *Journal of Applied Polymer Science, 67*, 1523–1530.

Izydorczyk, M. S., Biliaderis, C. G., & Bushuk, W. (1991). Physical properties of water-soluble pentosans from different wheat varieties. *Cereal Chemistry, 68*, 145–150.

Izydorczyk, M. & Biliaderis, C. M. (1992). Influence of structure on the physicochemical properties of wheat arabinoxylan. *Carbohydrate Polymers, 17*, 237–247.

Izydorczyk, M. S., Macri, L. J., & MacGregor, A. W. (1998). Structure and physicochemical properties of barley non-starch polysaccharides II. Alkali- extractable β-glucans and arabinoxylans. *Carbohydrate Polymers, 35*, 259–269.

Kardošová, A., Matulová, M., & Malovíková, A. (1998). 4-*O*-methyl-D-glucurono-D-xylan from *Rudbeckia fulgida*, var. *sullivanti* (Boynton et Beadle). *Carbohydrate Research, 308*, 99–105.

Klavons, J. A., & Bennet, R. D. (1995). Preparation of alkyl esters of pectin and pectic acid. *Journal of Food Science, 60*, 513–515.

Kohn, R., Hromádková, Z., Ebringerová, A., & Toman, R. (1986). Distribution pattern of uronic acid units in 4-O-methyl-D-glucurono-D-xylan of beech (*Fagus sylvatica* L.). *Collection of the Czechoslovakia Chemistry Communication, 51*, 2243–2249.

Kovác, P., Alföldi, J., Kociš, P., Petráková, E., & Hirsch, J. (1982). ^{13}C-NMR spectra of a series of oligoglucuronic acid derivatives and structurally related (4-*O*-methylglucurono)xylan. *Cellulose Chemical Technology, 16*, 261–270.

Kratky, O., Leopold, H., & Stabinger, H. (1973). *Methods in Enzymology, 27*, 98–115.

Lang, P., Masci, G., Dentini, M., Creszenzi, V., Cooke, D., Gidley, M. J., Fanutti, C., & Reid, J. S. G. (1992). Tamarind seed polysaccharide: preparation, characterisation and solution properties of carboxylated, sulphated and alkylaminated derivatives. *Carbohydrate Polymers, 17*, 185–198.

Mandelkern, L., & Flory, P. J. (1952). The frictional coefficient for flexible chain molecules in dilute solution. *Chemical Physics, 20*, 212–214.

Pavlov, G. M. (1997). The concentration dependence of sedimentation for polysaccharides. *European Biophysics Journal, 25*, 385–397.

Pavlov, G. M., & Frenkel, S. Ya (1995). Sedimentation parameter of linear polymers. *Progress in Colloidal and Polymer Science, 99*, 101–108.

Rowe, A. J. (1977). The concentration dependence of transport processes: a general description applicable to the sedimentation, translational diffusion, and viscosity coefficients of macromolecular solutes. *Biopolymers, 16*, 2595–2611.

Saulnier, L., Marot, C., Chanliaud, E., & Thibault, J.-F. (1995). Cell wall polysaccharide interactions in maize bran. *Carbohydrate Polymers, 26*, 279–287.

Salomonsson, B. A.-C., & Theander, O. (1992). Coupling of 1-aminoodecan starch by bromine oxidation and reductive amination. *Starch, 44*, 260–263.

Schleucher, J., Schwendiger, M., Sattler, M., Smidt, P., Schedletzky, O., Glaser, S. J., Sorensen, O. W., & Griesinger, C. (1994). A general enhancement scheme in heteronuclear multidimensional NMR employing pulsed field gradients. *Journal of Biomolecular NMR, 4*, 301–306.

Seifert, A., Heinevetter, L., Coelfen, H., & Harding, S. E. (1995). Characterization of gliadin–galactomannan incubation mixtures by analytical ultracentrifugation. Part I. Sedimentation velocity. *Carbohydrate Polymers, 28*, 325–332.

Shalaby, S. W., McCormick, C. L., & Butler, G. B. (1991). In S. W. Shalaby & C. L. McCormick & G. B. Butler (Eds.), *Water soluble polymers, ACS symposium series*, 467. Washington, DC: American Chemical Society.

Shimizu, K., Hashi, M., & Sakurai, K. (1978). Isolation from softwood xylan of oligosaccharides containing two 4-*O*-methyl-D-glucuronic acid residues. *Carbohydrate Research, 62*, 117–126.

Sinquin, A., Hubert, P., & Dellacherie, E. (1993). Amphiphilic derivatives of alginate: evidence for intra- and intermolecular hydrophobic association in aqueous solution. *Langmuir, 9*, 3334–3337.

Talába, P., Sroková, I., Hodul, P., & Cík, G. (1996). New alkylated *O*-(2-sulfo ethyl)cellulose and its properties. *Chemical Papers, 50*, 101–104.

Talába, P., Sroková, I., Ebringerová, A., Hodul, P., & Marcincin, A. (1997). Cellulose-based biodegradable polymeric surfactants. *Journal of Carbohydrate Chemistry, 16*, 573–582.

Tanford, Ch. (1961). *Physical chemistry of macromolecules*, New York: Wiley.

Tsvetkov, V. N., & Klenin, S. I. (1953). Diffusion of polystyrene fractions in dichlorethan. *Doklady Akademii Nauk SSSR*, *88*, 49–51.

Tsvetkov, V. N., Eskin, V. E., & Frenkel, S. Ya (1970). *Structure of macromolecules in solutions*, London: Butterworths.

Tsvetkov, V. N., Lavrenko, P. N., & Bushin, S. V. (1984). Hydrodynamic invariant of polymer molecules. *Journal of Polymer Science, Polymer Chemistry Edition*, *22*, 3447–3460.

Uttile, P., Kováč, P., Sauriol, T., & Perlin, A. S. (1986). NMR spectra of aldobiuronic and aldotriuronic acid derivatives related to 4-O-methyl-D-glucurono-D-xylan. *Carbohydrate Research*, *154*, 251–258.

Wales, M., & van Holde, K. J. (1954). The concentration dependence of the sedimentation constants of flexible macromolecules. *Polymer Science*, *14*, 81–86.

Zhang, T., & Marchant, R. E. (1994). Novel polysaccharides surfactants: synthesis of model compounds and dextran-based surfactants. *Macromolecules*, *27*, 7302–7308.