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Journal of Chromatography A, 749 (1996) 41–45

JOURNAL OF CHROMATOGRAPHY A

Rapid method for the determination of the substitution pattern of O-methylated 1,4-glucans by high-pH anion-exchange chromatography with pulsed amperometric detection

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Received 6 March 1996; revised 7 May 1996; accepted 9 May 1996

Abstract

A rapid method has been developed for the determination of the substitution pattern of methyl-starches, -amyloses, -celluloses and -cyclodextrins in the anhydro glucose unit. All eight constituents possible for this type of copolymers could be separated by high-pH anion-exchange chromatography with pulsed amperometric detection (PAD). Peaks were assigned by comparison with synthesized standard compounds. For quantitative evaluation the relative response factors of the O-methyl-glucose derivatives were determined.

Keywords: Carbohydrates; Glucans

1. Introduction

Polysaccharides as cellulose are permethylated to improve their water solubility and their rheological properties. The total degree of substitution (DS) is usually determined by the Zeisel-method [1]. The partial DS values in the positions 2, 3 and 6 of the anhydro glucose units (AGU) can be determined by ^{13}C NMR [2]. For a more differentiated analysis of the monomer composition chemical degradation combined with chromatographic separation has to be applied. The methyl ethers of polysaccharides can be investigated by GLC–flame ionization detection (FID) after hydrolysis, reduction and acetylation as the well known partially methylated alditol acetates [3] or after peralkylation e.g. with ethyl groups and reductive cleavage as the 4-O-acetyl-1,5-anhydro-O-ethyl-O-methylalditols [4]. For a more rapid access Erler et al. [5] investigated the complementary methyl pattern of trialkylsilyl celluloses after permethylation and hydrolysis by HPLC with refractive index (RI)-detection. However, no complete separation of all O-methyl-α- and β-glucopyranoses was possible, but at least a quantification of the un-, mono-, di- and tri-substituted units, which is a good supplement to NMR analysis.

High-pH anion-exchange chromatography (AEC) is known as a very efficient separation method in carbohydrate analysis. Kragten et al. [6] had applied this technique to carboxymethyl (CM) celluloses and sulfoethyl (SE) celluloses to determine the substituent distribution after hydrolysis. These anionic derivatives are very convenient to AEC, since every CM- or SE-group enhances the retention time significantly. During model studies of the distribution of substituents in the polymer chain [7], we had to

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0021-9673/96/$15.00
PII S0021-9673(96)00468-2

analyse a large number of methyl amyloses, which prompted us to look whether high-pH AEC–pulsed amperometric detection (PAD) can also successfully be applied to this type of derivative.

2. Experimental

2.1. Materials

Methyl amyloses were prepared from amylose (Sigma) under various conditions including protic and aprotic solvents, NaOH, KOH and Li-dimsyl as the base and methyl iodide (Merck) as the methylation agent.

2.2. GLC analysis

A Carlo Erba GC6000 Vega Series 2 instrument equipped with an on-column injector and an FID system, a CPSil8CB capillary column (25 m×0.25 mm I.D., Chrompack) and a retention gap (2 m) and a Merck–Hitachi D-2500 integrator were used. Carrier gas: hydrogen, 80 kPa, temperature program: 70°C, 1 min isotherm, then with 20°C/min to 130°C and with 4°C/min to 290°C.

All samples were analyzed after hydrolysis, reduction and acetylation by GLC–FID as described [8]. For cellulose ethers methanolysis was performed prior to acid hydrolysis [9]. Response factors for the partially methylated glucitol acetates were calculated according to the effective-carbon-response concept (ECR) [10].

At least two mixtures of each partially methylated glucose ether with glucose were analyzed by the GC method and high-pH AEC for comparison.

2.3. High-pH AEC–PAD analysis

A system consisting of a gradient pump Merck–Hitachi L-6200, a CarboPac PA-1 anion-exchange column (250×4 mm, Dionex), a PAD-2 detector with a gold electrode (Dionex) and an integrator Merck–Hitachi D-2500 was used.

Methyl amyloses (ca. 2 mg) were hydrolyzed with 2 *M* trifluoroacetic acid (1 ml) at 120°C in a 1-ml Reacti-vial for 3 h. After cooling to room temperature 100 ml were taken from the hydrolysate, evaporated in a stream of nitrogen and dissolved in 200 ml of bidist. water. (The main part of the hydrolysate was used for GLC analysis.) A 5–20 μl volume of this neutral solution was applied to the column. The glucose derivatives were eluted with 20 mmol NaOH (isocratic conditions). From time to time the column was washed with 200 mmol NaOH (20 min) and subsequently equilibrated again with 20 mmol NaOH. Pulse potentials of the PAD were $E_1=0.05$ V, $E_2=0.60$ V, $E_3=-0.60$ V. Pulse times were $T_1=480$ ms, $T_2=120$ ms, $T_3=60$ ms (range 2); response time=1 s; range: 300 nA or 1000 nA.

2.4. Peak assignment

Peaks were assigned by comparison with synthesized standards. Glucose and 3-O-methylglucose were commercial samples. 6-O-Methyl-α,β-D-glucose was a gift of Professor W.A. König (University of Hamburg). 2-O-Methyl-α,β-D-glucose was obtained from methyl-α-D-glucopyranoside *via* methylation of the 4,6-O-benzylidene-2-O-TBDMS-protected derivative under rearrangement and subsequent hydrolysis as described [11]. 2,3-Di-O-methyl-α,β-D-glucose was prepared from β-cyclodextrin *via* the heptakis [6-O-TBDMS] derivative after permethylation and hydrolysis. 2,6-Di-O-methyl-α,β-D-glucose was obtained by hydrolysis of pure heptakis[2,6-di-O-methyl]-β-cyclodextrin (Cyclolab, Budapest). 3,6-Di-O-methyl-α,β-D-glucose was prepared from 3-O-methyl-α,β-D-glucose *via* the following reaction sequence: glycosylation with methanol–HCl, 4,6-O-benzylidenation, 2-O-benzylation, opening of the 4,6-O-benzylidene ring (70% 4-O-benzyl ether beside 30% 6-O-benzyl-), O-methylation, hydrogenation of benzyl groups, tritylation of O-6 of the former 6-O-benzyl isomer, separation of the methyl 3,6-di-O-methylglucoside from the 3,4-di-O-methyl-6-O-trityl derivative and hydrolysis. 2,3,6-Tri-O-methyl-α,β-D-glucose was obtained from amylose after permethylation and hydrolysis.

2.5. NMR spectroscopy

^{1}H NMR spectra were recorded with a Bruker WM400 (400 MHz) and tetramethylsilane as internal standard. Mixtures of 3-O-methyl-, 2,3-di-O-methyl-,

3,6-di-O-methyl- and 2,3,6-tri-O-methylglucose with glucose in a ratio of about 5:1 to 1:5 (w/w) were dissolved in [$^2H_2$] water (Merck). (An aliquot of each was further diluted with bidist. water and analyzed by high-pH AEC as described above.) After removal of the [$^2H_2$] water the samples were dissolved in [$^2H_6$] dimethyl sulfoxide.

3. Results and discussion

3.1.1. Qualitative analysis

The constituents of O-methylated 1,4-glucans obtained after acid hydrolysis could be separated by high-pH AEC under isocratic conditions. Fig. 1 shows the chromatogram obtained from a methyl amylose with a DS of 1.56. All peaks could be identified by coinjection with authentic standards.

With increasing number of methyl groups the glucose ethers are less retained on the anion-exchange resin, since less hydroxy groups are available for deprotonation. Therefore, the elution order is trisubstituted < disubstituted < monosubstituted < glucose (<means "before") in contrast to the anionic derivatives, where the glucose elutes first [6]. However, the di-O-methyl- as well as the mono-O-methylglucose derivatives were eluted in the same order as the CM- and SE derivatives (3.6<2.6<2.3 and 6<2), indicating that the primary 6-O$^-$ mainly contributes to the retention behaviour. Also in agreement is the observation, that the 2-O- and 3-O-substituted glucoses are only partially separated. That means that the contribution of the secondary anions is very similar. In contrast to the HPLC method [5], no problems arise from α- and β-anomers, since anomerization is very quick under the alkaline chromatographic conditions.

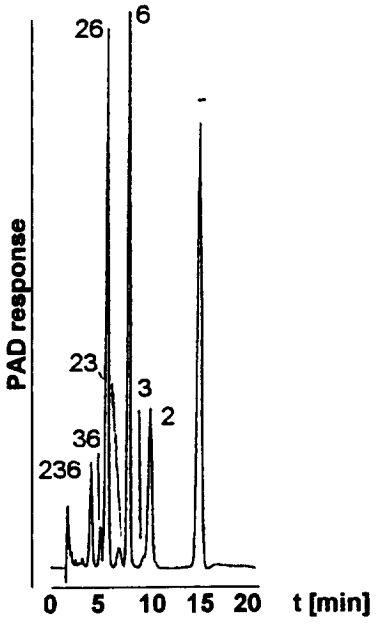

Fig. 1. Anion-exchange chromatogram of a methyl amylose hydrolysate (DS 1.56). Peaks are assigned with the positions of O-methylation in the glucose.

3.1.2. Quantitative analysis

For quantitative evaluation the relative response factors in the pulsed amperometric detector have to be determined. Glucose and one of the model compounds were mixed and the relative molar ratios were calculated from the anomeric proton signals in the NMR spectra. A substituent in position 2 causes a downfield shift for the H-1, while the resonances of the 3-O- and 3,6-di-O-methyl derivatives overlapped with signals of glucose. Therefore, additional determinations of the molar ratio were performed by GLC analysis. The relative response values determined (Table 1) are similar to the data published by Kragten et al. [6] for CM- and SE-ethers of glucose.

The dependence of the PAD response upon the potential of detection (E_1) is known to show a maximum at about 200 mV (at 100 mmol NaOH). The increase of sensitivity is about +30% for glucose compared to a detection at 50 mV [12]. We found a similar increase of the detector response for the O-methylglucoses, while the relative response compared to glucose as reference compound only

Table 1
Relative PAD response values of O-methyl glucose ethers

Me in position	Relative response
–	1.00
2	0.67
3	0.60
6	0.86
23	0.44
26	0.35
36	0.33
236	0.167

slightly increased or even decreased. For 2,3,6-tri-O-methylglucose the relative response decreases from 16% at 50 mV to 14% at 130 mV and then again increases to nearly 16% at 210 mV. For 2,3-di-O-methyl-glucose the response slightly increases from 44% at 50 mV to 47% at 200 mV. Therefore, the relative response factors could not be improved by changing the detector potential. The response also depends on the concentration of NaOH. If after-column addition of NaOH to a concentration of 100 mmol is applied, deviations of the relative response values have to be expected. It is recommended to control the response values with the system used.

3.1.3. Application to methyl amyloses

The monomer composition of methyl amyloses was determined by the method described. In Table 2 results are given for some examples in the DS range 0.26–1.56. Data are in relatively good agreement with GLC analysis. Obviously, the partial DS at O-2 is generally enhanced compared to the GC chromatographic method. 3,6-Di-O-methyl-glucose is sometimes overlapped by a side product, which was not identified yet, but may be hydroxymethylfurfural (HMF). This may cause a too high molar ratio for this constituent (see MA 1 and 3). Problems may also arise from the incomplete separation of the 2- and 3-O-methyl derivatives. However, the ratio of these components (3- and 3.6) are usually low due to the low reactivity of the 3-OH. The wide range of the PAD response values (Table 1) makes this detector less appropriate for samples with a low DS. However, slight undermethylation of "per-methylated" samples can be detected with very high sensitivity.

Acknowledgments

Financial support by the Bundesminister für Forschung und Technologie (Projekt-No. 0310319 A) is gratefully acknowledged.

Table 2
Molar monomer composition of methyl amyloses MA 1–5, comparison of high-pH AEC–PAD and GLC

Position	Sample									
	MA 1		MA 2		MA 3		MA 4		MA 5	
	AEC[a]	GLC[b]	AEC	GLC	AEC	GLC	AEC	GLC	AEC	GLC
–	77.1	78.2	69.1	68.8	44.8	46.9	48.4	51.7	15.2	15.4
2	12.9	13.6	7.1[c]	5.9	28.8	28.0	21.3	19.7	9.3[c]	7.7
3	2.3	2.0	[d]	0.9	5.2	5.3	3.9	4.3	[d]	0.7
6	4.6	4.6	13.3	14.4	6.8	7.0	0.7	0.7	17.5	19.3
23	0.9	0.6	0.9	1.1	3.8	4.1	1.4	1.1	1.6	1.4
26	2.3	1.0	6.7	6.3	7.1	6.4	1.3	1.1	43.1	41.9
36	n.d.	0.1	n.d.	0.4	2.4	1.0	n.d.	n.d.	n.d.	0.3
236	n.d.	n.d.	2.8	2.3	1.1	1.3	23.1	21.4	13.3	13.3
U[e]	77.1	78.2	69.1	68.8	44.8	46.9	48.4	51.7	15.2	15.4
M[e]	19.8	20.2	20.4	21.2	40.8	40.3	25.9	24.7	26.8	27.7
D[e]	3.1	1.7	7.7	7.7	13.3	11.5	2.6	2.2	44.7	43.6
T[e]	n.d.	n.d.	2.8	2.3	1.1	1.3	23.1	21.4	13.3	13.3
DS(2)	0.16	0.15	0.17	0.15	0.41	0.40	0.47	0.43	0.67	0.64
DS(3)	0.03	0.03	0.04	0.05	0.13	0.12	0.28	0.27	0.16	0.16
DS(6)	0.07	0.06	0.23	0.23	0.17	0.16	0.25	0.23	0.74	0.75
DS	0.26	0.24	0.44	0.44	0.71	0.67	1.00	0.93	1.56	1.55

[a] AEC: determination by anion-exchange chromatography after hydrolysis.
[b] GLC: determination as the partial methylated glucitol acetates by GLC.
[c] 2-O-Methyl- and 3-O-methylglucose summarized.
[d] Not separated from 2-O-methylglucose.
[e] U=unsubstituted, M=monosubstituted, D=disubstituted, T=trisubstituted.

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