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Model studies on methyl amyloses: correlation between reaction conditions and primary structure

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Abstract

Methyl amyloses have been prepared under various conditions and studied as model compounds for the determination of the substitution pattern in the polymer chain. After permethylation with iodomethane-d_3 the glucosidic linkages were statistically cleaved by partial methanolysis or reductive cleavage. The distribution of substituents in the dimer-, trimer-, and tetramer fraction was determined by FAB-MS and MALDI-TOF-MS and compared with that calculated from the monomer composition. While a homogeneous methylation in water gave the expected random distribution, a reaction in Me_2SO solution with sodium hydroxide and iodomethane yielded a methyl amylose with a surprising bimodal substitution pattern. A third example indicates a ds gradient in the sample as a result of topochemical reaction control. © 1996 Elsevier Science Ltd.

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1. Introduction

The physical and biological functions of polysaccharide derivatives, e.g. methyl celluloses, hydroxyethylstarches or sulfates of β-glucans, are greatly affected by the distribution pattern of their substituents. While the positions of substitution in the monomer anhydro glucose units (AGU) seem to be important, especially for properties where molecular recognition is involved, e.g. for cellulose sulfates as heparinoidic materials, the distribution along the polymer chains is mainly responsible for the physical properties such as solubility, flocculation, etc.

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The ratio of 2-, 3-, and 6-*O*-substitution can be determined by NMR spectroscopy [1] or after complete degradation by separation of appropriate monomer derivatives [2–8]. However, there are only a few papers dealing with the investigation of the homo- or heterogeneity of substitution in the polymer chains. Gelman [9] investigated the enzymatic degradability of carboxymethyl celluloses and compared the number of cleaved glucosidic linkages with the expected value calculated for a random distribution of unsubstituted glucose units and an assumed enzyme specificity. Steeneken and Woortman [10] found significant differences for the product composition obtained after enzymic degradation from methyl starches prepared in solution or in a starch slurry. Recently, Arisz et al. [11] reported on the investigation of methyl celluloses after partial hydrolysis and FAB-MS. In our model studies on methyl amyloses reported here we used a similar approach including partial methanolysis or partial reductive cleavage, FAB-MS and MALDI-TOF-MS (matrix-assisted laser desorption/ionization time-of-flight mass spectrometry), and statistical evaluation.

2. Results and discussion

Synthesis of model compounds.—The α-1,4-linked glucan amylose was used as a linear model compound. Methylation was performed under homogeneous and heterogeneous conditions in protic or aprotic solvent systems. Aliquots were taken at different reaction times to obtain samples with increasing degrees of substitution (ds) from the same reaction. Conditions applied to four selected samples are summarized in Table 1.

Monomer analysis.—The monomer composition of all methyl amyloses was determined after hydrolysis, reduction, and acetylation by GLC of the partially methylated glucitol acetates, or directly from the hydrolysate by high pH anion-exchange chromatography with pulsed amperometric detection (HPAEC–PAD) [12]. Methylation of the 2-OH group in 1,4-glucans is known to enhance the reactivity of the 3-OH. Therefore, the relative molar ratios of the eight constituents were compared with those calculated from the kinetic model of Reuben [13], which includes this intramonomeric effect. Table 1 shows a good agreement of the experimental and the calculated data for MA 1 from the homogeneous reaction in water. In contrast, the heterogeneous process in a concentrated slurry (MA 2) yielded significant deviations for all types of *O*-methylglucose moieties. A completely different pattern — an unusual 'concave' distribution with mainly mono- and trisubstituted units — was obtained for MA 3, which had been methylated in Me_2SO solution with pulverized sodium hydroxide as the base. Similar observations have recently been reported by Heinze et al. [14] from the monomer composition of carboxymethyl celluloses prepared in a protic solvent system with NaOH as the base. MA 4 which was prepared with Li-dimsyl in Me_2SO/tetramethylurea solution showed a relative good agreement with the model of Reuben.

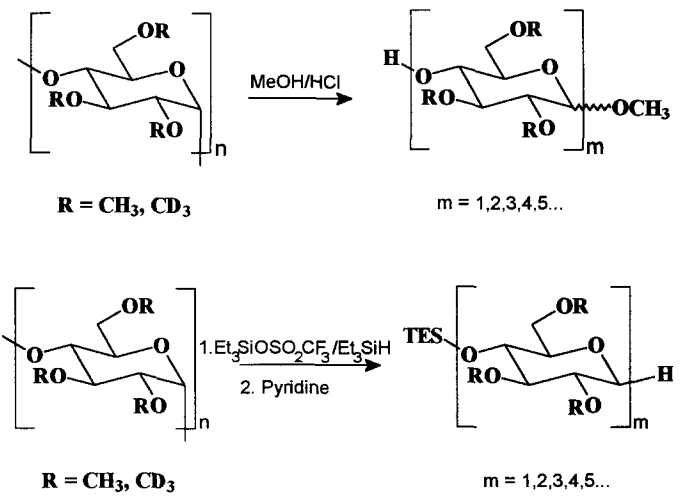
Partial depolymerization.—The methyl amyloses were permethylated using MeI-d_3 prior to partial depolymerization, to provide a statistical cleavage of the glucosidic bonds. Partial methanolysis as well as partial reductive cleavage were applied (Scheme 1).

Oligomer analysis.—The oligomeric mixtures obtained were analyzed by FAB-MS and MALDI-TOF-MS. Results of both methods are in good agreement. The quantitative

Table 1
Reaction conditions, monomer composition, and degree of substitution (ds) for methyl amyloses MA 1–4

Sample	MA 1		MA 2		MA 3		MA 4	
Reaction conditions [a]	H_2O/KCl/KOH/MeI homogeneous (dissolved at 150 °C)		H_2O/KCl/KOH/MeI heterogeneous		Me_2SO/NaOH/MeI homogeneous (NaOH partially dissolved)		Me_2SO/$(Me_2N)_2CO$/ Li-Dimsyl [b]/MeI homogeneous	
Amylose (mg/mL)	8		156		80		19	
Substitution [c]	Expt. [d]	Calcd [e]	Expt. [d]	Calcd [e]	Expt. [d]	Calcd [e]	Expt. [d]	Calcd [e]
s_0	44.23	43.34	22.84	15.09	9.22	5.89	9.95	9.44
s_2	30.62	31.42	13.36	18.92	41.14	27.67	67.29	66.70
s_3	5.31	5.20	1.52	1.25	1.35	0.93	1.93	1.94
s_6	7.69	8.58	14.38	22.13	1.20	4.53	1.22	1.73
s_{23}	3.32	3.51	2.81	5.28	4.78	22.10	5.34	6.42
s_{26}	7.02	6.22	33.32	27.76	7.84	21.31	11.64	12.23
s_{36}	0.92	1.03	1.56	1.83	0.29	0.71	0.37	0.36
s_{236}	0.89	0.70	10.21	7.74	34.18	16.95	2.26	1.18
U	44.23	43.34	22.84	16.54	9.22	5.89	9.95	9.44
M	43.62	45.20	29.26	43.65	43.69	33.13	70.44	70.37
D	11.26	10.76	37.69	33.29	12.91	44.03	17.35	19.01
T	0.89	0.70	10.21	6.52	34.18	16.95	2.26	1.18
x_2	0.419	0.419	0.597	0.597	0.879	0.879	0.865	0.865
x_3	0.104	0.104	0.161	0.161	0.406	0.406	0.099	0.099
x_6	0.165	0.165	0.595	0.595	0.435	0.435	0.155	0.155
DS	0.69	0.69	1.35	1.35	1.72	1.72	1.12	1.12

[a] 'Homogeneous/heterogeneous' is related to the amylose and the solvent.
[b] Dimsyl = $CH_3SOCH_2^-$.
[c] s_i = Relative molar ratio of glucose substituted in position i in %, $\Sigma s_i = 100\%$. x_i = Partial ds in position i, Σx_i = ds (degree of substitution). U (unsubstituted) = s_0, M (monosubstituted) = $s_2 + s_3 + s_6$, D (disubstituted) = $s_{23} + s_{26} + s_{36}$, T (trisubstituted) = s_{236}.
[d] Relative molar composition (mol%) determined experimentally.
[e] Relative molar composition (mol%) calculated according to the model of Reuben [13].



Scheme 1.

reliability has been proved with maltotriose permethylated with equimolar amounts of MeI and MeI-d_3. The molar ratios of methyl derivatives in the dimeric, trimeric, and tetrameric fraction were compared with the statistical distribution of substituents in each of these oligomers as calculated from the monomer composition (Fig. 1). The values for the relative ratios of oligomers with n CH_3 groups have been connected to a graph for better visual comparison. The composition for every dp is normalized to 100%. The average ds for the oligomers ($= n(CH_3)/dp$) was in good agreement with the total ds (deviation usually less than 4%), indicating that the quantitative results are representative. Both partial reductive cleavage and partial methanolysis were appropriate degradation methods. MA 1 again shows a very nice congruity with the pattern calculated for a homogeneous distribution of substituents (= random). Deviations are within experimental error. For the methyl amylose MA 2 the ratio of oligomers with a low and high ds are enhanced, while the region around the average ds is lowered. So far, it corresponds with the heterogeneity observed by Arisz et al. for methyl celluloses [11]. In addition the distribution curve is distorted, indicating a ds gradient in the material (heterogeneity of the first type). This is simulated for a theoretical sample in Fig. 2. This type of heterogeneity can be understood from the conditions of derivatization, the amylose was swollen with water/KOH. The methyl iodide is not miscible with this pulp and therefore preferably reacts with the outer regions.

The distribution pattern of MA 3 with the unusual concave monomer composition showed two maxima, one at a ds of 1 and a second at a ds of 3. The latter represents permethylated sequences T_n with an average block length of $n > 20$ (T = trisubstituted AGU). This can be estimated from the relative amount of permethylated oligomers T_{DP} with respect to the monomer composition. From a block sequence T_n $(n - DP + 1)$ oligomers with the composition T_{DP} are available, corresponding for example with $(n - 3)100/n$ (%) of the tri-*O*-methylated units T occurring as T_4 in the tetrameric

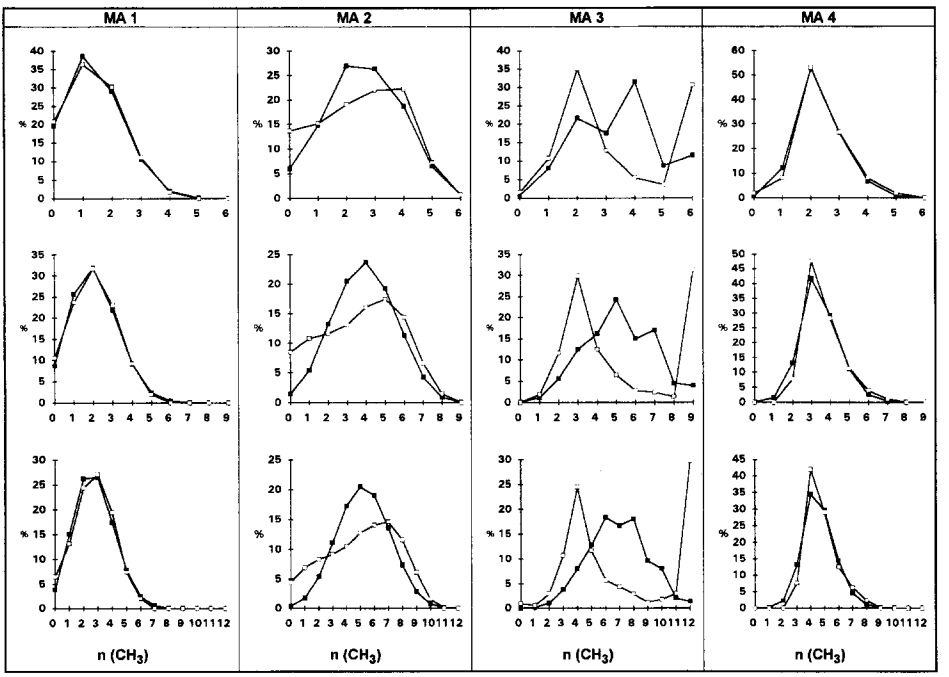


Fig. 1. Distribution of methyl groups in the dimeric (top), trimeric (middle), and tetrameric (bottom) fraction obtained from methyl amyloses MA 1–4 in comparison with the pattern calculated for a random substitution of the monomer units in the polymer chain (preparation and monomer composition see Table 1). ■ = calculated, □ = experimental.

fraction. The left part of the curve looks very similar to the distribution obtained for MA 4, which was also prepared in a polar aprotic solvent, but with the soluble base Li-dimsyl (Fig. 1, last column). This observation led us to assume that the areas with an average ds of about 1 had been formed by reaction with the dissolved part of the NaOH, while the permethylated sequences T_n are the result of local high deprotonation and subsequent methylation of the amylose OH groups after adsorption on the surface of the

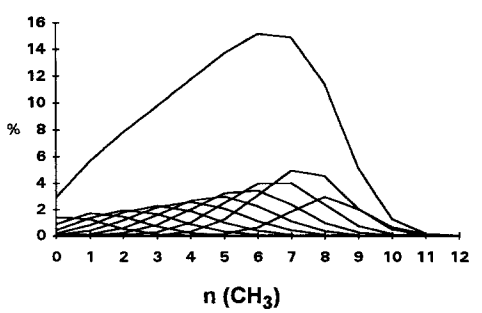


Fig. 2. Simulation of the distribution curve for a theoretical polysaccharide derivative (average ds 1.30) with a ds-gradient in the material by overlapping of distribution curves of fractions with ds 0.2–2.0.

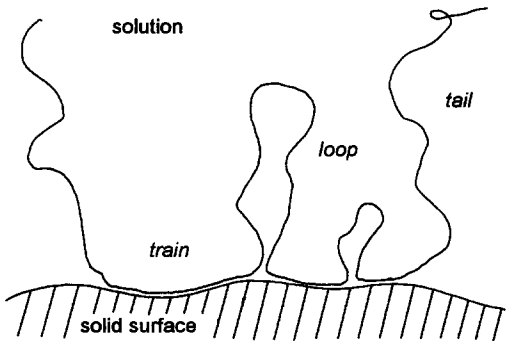


Fig. 3. Model for the adsorption of polymer molecules from solution on a surface according to Fleer and Scheutjens [15]: formation of *trains*, *loops* and *tails*.

solid NaOH. This is illustrated by the model of Fleer and Scheutjens [15] in Fig. 3. Polymer molecules are adsorbed from the solution forming *trains* interrupted by *loops* and terminated by *tails*. The average length of the *trains* should correspond to the average block length. When the tri-*O*-methylated AGUs (T) representing the blocklike sequences are subtracted from the monomer composition, the remaining pattern is in agreement with Reuben's model (data not shown in detail) and comparable to the oligomer composition of MA 4.

Water solubility.—While methyl amyloses like MA 1, MA 2 or MA 4 were mainly or even completely soluble in water, no derivative with a block like structure (ds 0.6–1.83) could be dissolved. During enzymatic degradation with α-amylase and amyloglucosidase 80% of the unsubstituted glucose units were liberated from a methyl amylose with mainly un- and tri-substituted AGUs from a heterogeneous reaction of amylose in $HCONMe_2$ with NaOH/MeI (ds 0.6). The higher molecular residue which was resistant to enzymic attack could be extracted with chloroform, a further indication for the block structure.

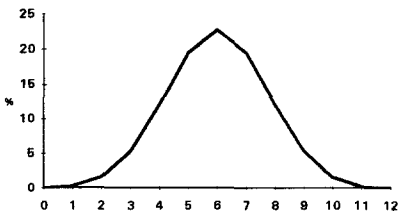
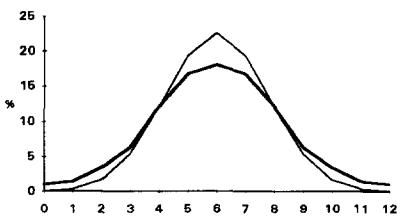
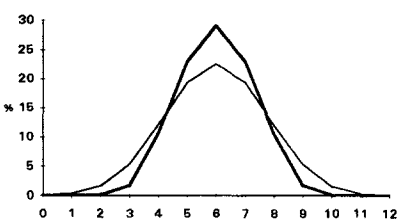
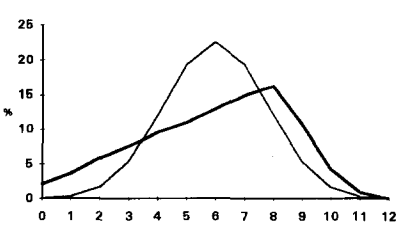
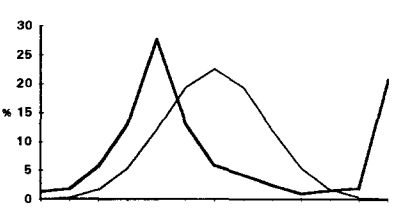
3. Conclusion

It was demonstrated that a homogeneous as well as different types of heterogeneous substituent distributions in the polymer chain of polysaccharide derivatives can be qualitatively and quantitatively described by chemical and mass spectrometric methods, and can be correlated to the conditions of their preparation. The bimodal distribution observed for derivatives prepared in a polar aprotic solvent with a partially soluble base seems to be typical for two competing or subsequent reactions under various conditions. In conclusion, typical substitution patterns are classified in Table 2.

4. Experimental

General.—Amylose was either purchased from Sigma (M_w = 960.000) or a kind gift of Dr. Vorwerg and Dr. Radosta (Fraunhofer IAP, Teltow, Germany) with M_w = 280.000

Table 2
Classification of substitution patterns demonstrated for the tetrameric fraction of a theoretical polysaccharide derivative with a ds 1.5. The plane graph shows the homogene statistical distribution as a reference. 0, 1, 2, 3,..., 12 is the number of substituents in the 2-, 3-, and 6-position

Distribution pattern	*Type*
	Homogenous distribution in the polymer chain (statistical distribution)
	More heterogeneous distribution in the polymer chain compared to a statistical distribution: regions with high and low DS enhanced, oligomers of the average DS diminished
	More regular distribution in the polymer chain compared to a statistical distribution: regions with high and low DS diminished, oligomers of the average DS enhanced highest regularity is reached for repeating units
	Distorted heterogeneous distribution, indicating a DS-gradient in the sample.
	Bimodal distribution resulting from two parallel reaction processes, e.g. for phase controlled reactions

and 48.000, respectively. Me_2SO, $HCONMe_2$, $CO(NMe_2)_2$, MeOH, $Et_3SiOSO_2CF_3$, Et_3SiH, MeI, and MeI-d_3, were from Merck and MeLi was from Aldrich. Monomer analysis was performed as described [12,16].

Methylation of amylose. General procedure.—Amylose (ca. 800 mg) was dissolved or suspended in the solvent or solvent mixture. Base (2 equiv/OH, MA 4: 1 equiv/OH) was added under ice cooling and the mixture stirred at room temperature. When sodium hydroxide or potassium hydroxide were used, MeI (2 equiv/OH, MA 4: 1 equiv/OH) was added directly after the base, but in the case of Li-dimsyl, after 30 min. After a certain time an aliquot was taken from the reaction mixture, neutralized, dialyzed, and freeze dried (3–5 fractions of different ds per reaction). For special conditions see Table 1. Permethylation with MeI-d_3 was performed with 4 equiv NaOH/OH in Me_2SO.

Partial methanolysis.—Permethylated amylose (CH_3/CD_3, 2–5 mg) was treated with 0.1 M HCl in methanol (ca. 1 mL) for 1 h at 90 °C (TLC-control, ethylacetate) and, after cooling, was evaporated to dryness in a stream of nitrogen.

Partial reductive cleavage.—Permethylated amylose (CH_3/CD_3, 2–5 mg) was dissolved in dichloromethane (200–500 μL) and $Et_3SiOSO_2CF_3$ (1.5 equiv/glucosidic bond) and Et_3SiH (3 equiv) were added. After 30 min at room temperature pyridine was added (10–25 μL), and the solution was washed with saturated $NaHCO_3$ solution. The dried organic phase was evaporated to dryness in a stream of nitrogen.

FAB-MS.—FAB mass spectra were recorded in the range m/z 200–1500 on a VG Analytical VG/70-250S instrument with a xenon-gun and *m*-nitrobenzylalcohol as matrix, saturated with NaI. The data were accumulated over 10 scans. (Acceleration voltage: 8 kV in the positive ion mode. Scan time: 20 s. Resolution: 2500). The relative signal intensities (R.I.) at m/z $[M + Na]^+$ were corrected for their signal/noise ratio with respect to the corresponding isotopic peak at $m/z = [M + 1 + Na]^+$. Th.I. = theoretical intensity of the M + 1-signal, calculated from the molecular formula. Noise = R.I. [M + 1 + Na] − (R.I. [M + Na] * 0.01 * Th.I. [M + 1 + Na])/(1 − 0.01 * Th.I. [M + 1 + Na]).

MALDI-TOF-MS.—MALDI mass spectra were recorded on a Kratos Kompact MALDI-3 (Shimadzu) in the reflectron mode. A nitrogen laser (337 nm) with a pulse width of 3 ns was used for ionization. Acceleration voltage: 20 kV in the positive ion mode. 4-Hydroxy-α-cyanocinnamic acid (10 mg/mL in dichloromethane/MeOH, 4:1, v/v) was used as the matrix. Sample preparation: 0.1 μL of NaI solution (5%) was dried on the target. Then matrix solution (0.2 μL) and the sample in dichloromethane (2 mg/mL, 0.1 μL) were added and dried. The areas under the signals were integrated.

Calculation of the statistical distribution of AGUs in the oligomeric fractions.—The relative molar composition of the oligomeric fractions for a statistical distribution of substituents was calculated according to $(U + M + D + T)^{DP}$ with DP = 2, 3, and 4. U = unsubstituted, M = mono-, D = di-, and T = trisubstituted AGUs.

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