ELSEVIER

Carbohydrate Research 337 (2002) 2505–2511

CARBOHYDRATE RESEARCH

www.elsevier.com/locate/carres

Note

Structure of the oligomers obtained by enzymatic hydrolysis of the glucomannan produced by the plant *Amorphophallus konjac*

Paola Cescutti, Cristiana Campa, Franco Delben, Roberto Rizzo*

Dipartimento di Biochimica, Biofisica e Chimica delle Macromolecole, Università di Trieste, via L. Giorgieri 1, I-34127 Trieste, Italy

Received 15 May 2002; accepted 14 August 2002

Abstract

Dimers and trimers obtained by enzymatic hydrolysis of the glucomannan produced by the plant *Amorphophallus konjac* were analysed in order to obtain information on the saccharidic sequences present in the polymer. The polysaccharide was digested with cellulase and β-mannanase and the oligomers produced were isolated by means of size-exclusion chromatography. They were structurally characterised using electrospray mass spectrometry, capillary electrophoresis, and NMR. The investigation revealed that many possible sequences were present in the polymer backbone suggesting a Bernoulli-type chain.

Keywords: Konjac; Glucomannan; Oligomers; Structure; NMR; Capillary electrophoresis

1. Introduction

Glucomannans are carbohydrate polymers widely distributed in both hardwood and softwood plants, where they have either storage or structural functions. The polymeric sequence is linear and it is composed of (1→4)-β-D-Glc*p* and (1→4)-β-D-Man*p* sugar residues. However, the presence of few short side-chains, which may also contain galactose residues, has been reported.[1] Native polymers exhibit some degree of acetylation, which depends on the plant source. The mannose to glucose molar ratio also depends on the plant species, and usually the content of Man is higher than that of Glc one, but 1:1 molar ratios have also been described.[2] For example, the Man:Glc ratio is 1.6:1.0 in glucomannan from *Amorphophallus konjac*,[3] 2.0:1.0 in that from *Lilium* sp.,[4] and 3.0:1.0 in glucomannan from *Orchis mascula* (salep) (data from our laboratory).

Few data exist on the structure of saccharidic sequences present in the glucomannan chain. In the past, oligomers were obtained by degrading glucomannan from *A. konjac* by means of mannanases. Takahashi et al.[5] used a β-mannanase from *Streptomyces* sp. and did not find evidence for Man- or Glc-blocks in the polymeric chain. Shimahara et al.[6] used two β-mannanases extracted from konjac tubers and suggested the presence of repeating motifs dominated by short oligo-Man stretches. Brownsey et al.[7] excluded the presence of both regular repeat-units and blocks on the basis of X-ray fibre diffraction data.

In the present study, cellulase and β-mannanase were used to cleave the polymeric chain with the aim of better characterising the sequences present in the glucomannan produced by *A. konjac*. The structural investigation of dimers and trimers was carried out using modern techniques such as NMR, electrospray mass spectrometry, and capillary electrophoresis.

2. Results and discussion

The compositional characterisation of the glucomannan produced by *A. konjac* revealed only Man and Glc residues in a molar ratio equal to 1.6:1.0, which was confirmed by ^{1}H NMR spectroscopy. ^{1}H NMR spec-

Abbreviations: CE, Capillary electrophoresis; GG, β-D-Glc*p*-(1→4)-D-Glc*p*; GM, β-D-Glc*p*-(1→4)-D-Man*p*; MG, β-D-Man*p*-(1→4)-D-Glc*p*; MM, β-D-Man*p*-(1→4)-D-Man*p*. Given the abbreviations for dimers, those for trimers are self-explanatory.

* Corresponding author. Tel.: +39-040-6763695; fax: +39-040-6763691

E-mail address: rizzor@bbcm.univ.trieste.it (R. Rizzo).

0008-6215/02/$ - see front matter
PII: S0008-6215(02)00332-4

troscopy was also used to determine the degree of acetylation, which was 0.07 per sugar residue. The weight average molecular weight of the polymer was 5.83×10^5 g/mol and the polydispersity index was 2.9, in agreement with literature data.[3]

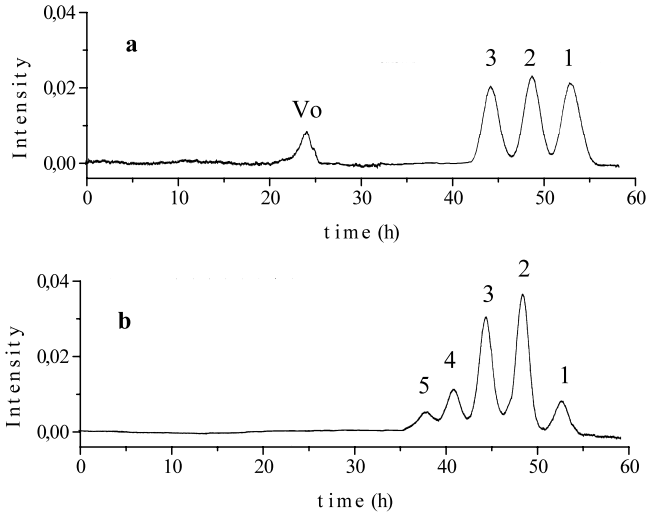

Fig. 1. Elution profiles of the cellulase (a) and β-mannanase (b) hydrolysates obtained from two Biogel P2 columns in series. The numbers indicate the size of the oligosaccharides; Vo = exclusion volume.

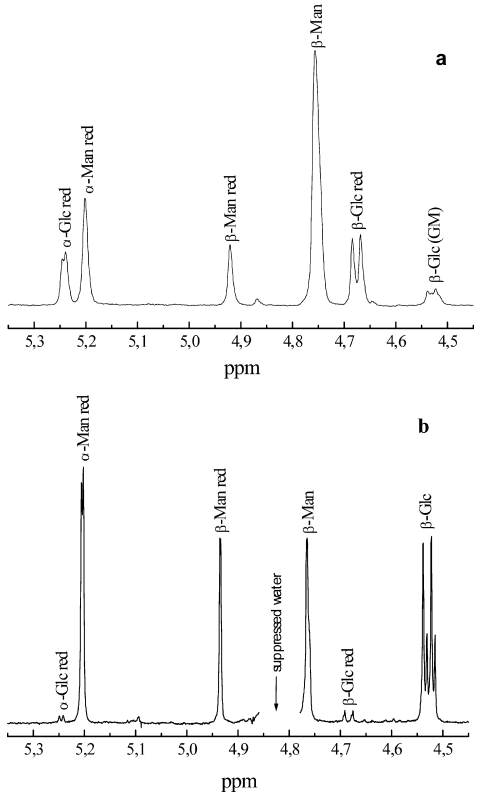

Fig. 2. Anomeric regions of the ^{1}H NMR spectra of the dimers obtained from cellulase (a) and β-mannanase (b) treatment. The suffix red indicates the residue at the reducing end.

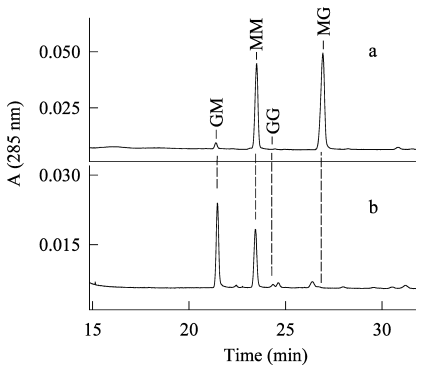

Fig. 3. Electropherograms of the dimers obtained from cellulase (a) and β-mannanase (b) treatment.

The size-exclusion chromatography fractionation of the oligomers obtained by enzymatic digestion of the glucomannan with cellulase and mannanase is shown in Fig. 1(a) and (b), respectively. In both digestions, mono-, di-, and tri-saccharides were present, while mannanase also produced tetramers and a small amount of pentamers. In addition, the hydrolysis with cellulase produced a fraction eluting at the void volume of the column. It has to be remembered that cellulase is a mixture of different enzymes, often referred to as a cellulase complex,[8] which includes (1→4)-β-D-glucosidase activity.[9] The large amount of monomers shown in Fig. 1(a) might come from the activity of the latter enzyme. The alditol acetate analysis of this monomer fraction gave a Man:Glc molar ratio equal to 0.15:1.0, ten times lower than that of the native polymer.

The primary structures of the oligomers obtained were investigated by means of NMR and capillary electrophoresis, because each chromatographic fraction was a mixture of oligomers having the same molecular weight but different composition.

Characterisation of dimers obtained by cellulase and β-mannanase treatment.—The mixtures of disaccharides obtained after enzymatic hydrolysis were analyzed by means of capillary electrophoresis (CE) and ^{1}H NMR spectroscopy. The assignment of the CE peaks were achieved by comparing the results obtained for the two mixtures of dimers and from ^{1}H NMR data. The anomeric regions of the ^{1}H NMR spectra of the mixtures of dimers are shown in Fig. 2(a) and (b), where the peak assignment, based on literature values and on the evaluation of the H-1,H-2 coupling constant, is also reported. CE experiments, carried out on the cellulase digest, revealed the presence of three molecular species at migration times 21.5, 23.4, and 26.8 min (Fig. 3a), which were attributable to the dimers MM, MG, and GM. No cellobiose was present, as proved by using this disaccharide as a standard (retention time 24.2 min, data not shown). CE experiments carried out on the β-mannanase digest (Fig. 3b) showed the presence of

two main peaks at migration times 21.5 and 23.4 min, together with some very low intensity peaks, one of which (24.2 min) was attributed to GG. In particular, the peak at migration time of 26.8 min was absent in the mannanase digest, indicating that only three dimers constituted the mixture. At the same time, the anomeric region of the ^{1}H NMR spectrum of the mannanase digest (Fig. 2b) showed that very low amounts of dimers exhibiting reducing Glc, GG, and MG, (doublets at 5.24 and 4.69 ppm) were present. Therefore, these data revealed that no MG dimers were present in the mannanase digest; consequently the CE peak at 26.8 min present in the cellulase digest (Fig. 3a) was attributed to MG. The remaining two peaks in both electropherograms (21.5 and 23.4 min), attributable to GM and MM, were assigned after inspection of the anomeric region of the ^{1}H NMR spectrum of the β-mannanase digest (Fig. 2b). The signal at 4.72 ppm was assigned to non-reducing Man, and therefore it could only be attributed to MM dimers, since MG = 0. At the same time, the two doublets in the ppm range 4.50–4.55 relative to non-reducing terminal Glc were attributed to GM, since the very low of GG dimers was deduced from integration of the signals at 5.30 and 4.68 ppm. The presence of GM as two doublets was attributed to the α,β-pyranose equilibrium of the terminal reducing mannose. The integration values are reported in Table 1. By comparison with the NMR integration values, CE peaks were assigned (Fig. 3b) and their integration is also reported in Table 1. In the ^{1}H NMR spectrum of the cellulase digest (Fig. 2a), taking into account that no cellobiose was present, the signals belonging to reducing Glc had to be entirely attributed to MG dimer, while the signal at 4.55 ppm was assigned to GM. It easily followed that the amount of MM was calculated subtracting from the integration of the peak at 4.75 ppm assigned to non-reducing Man, the amount attributed to MG dimers. The integration values obtained from NMR data together with those obtained from CE experiments are reported in Table 1.

Table 1
Relative concentrations of dimers as obtained by NMR and capillary electrophoresis

Cellulase digestion		
Dimers	NMR	CE[a]
MM	1.0	1.00
MG	0.9	1.31
GM	0.1	0.06
GG	0.0	–
Mannanase digestion		
Dimers	NMR	CE[a]
MM	1.0	1.00
MG	0.0	–
GM	1.3	1.42
GG	0.1	0.04

[a] The resolution of the CE peaks allowed better accuracy than NMR data.

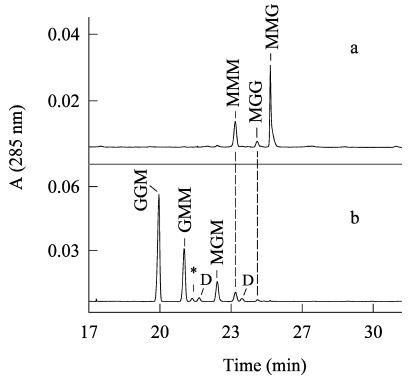

Fig. 4. Electropherograms of the trimers obtained from cellulase (a) and β-mannanase (b) treatment; in (b) the * indicates either the GGG or the GMG trimer.

The absence of GG dimers in the cellulase digest was explained by the presence of (1→4)-β-D-glucosidase in the cellulase complex. In fact, 97% of the products present in the mixture had Man as non-reducing terminal residue. This suggested that the *exo*-glucanase present in the cellulase complex could easily split also the GM sequence, because of the very close stereochemical similarity around the glycosidic bond of both GG and GM sequences.

Characterisation of trimers obtained by cellulase treatment.—CE experiments (Fig. 4a) showed that the cellulase produced mainly three trimers and only traces of some of the five remaining oligomers that might potentially be obtained. The anomeric region of the ^{1}H NMR spectrum (Fig. 5a) was quite complicated for evaluation of the relative concentration of the trimers present in the mixture. The necessary information was achieved by examining the anomeric region of the ^{1}H NMR spectrum of the reduced trimers (Fig. 5b) and from literature data.[10,11] In fact, taking into account that the non-reducing Man H-1 signals are located at higher ppm values than those of the non-reducing Glc H-1 and that the β-Glc H-1 peaks exhibit a ^3J coupling constant of 8.2 Hz, the two clusters centered at 4.76 and 4.83 ppm (Fig. 5b) were assigned to Man anomeric protons. The triplet centred at 4.63 ppm (Fig. 5b), resulting from the superimposition of two doublets, was attributed to two Glc anomeric protons. The analysis described by Harjunpää et al.[10] on oligomannans showed that anomeric protons of terminal non-reducing Man residues resonate at lower ppm values than those of inner Man residues. Therefore, the cluster at 4.83 ppm was attributed to inner Man residues and that one at

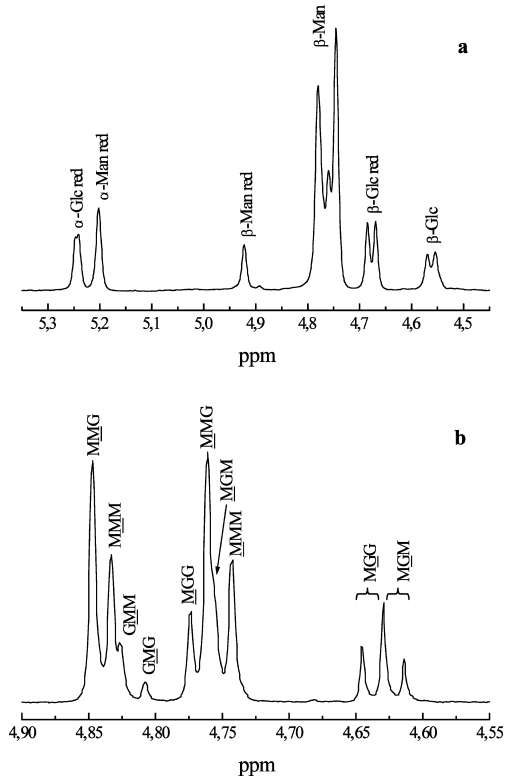

Fig. 5. Anomeric regions of the ^{1}H NMR spectra of the trimers obtained from cellulase treatment (a). The suffix red indicates the residue at the reducing end. In (b) the spectrum of the reduced trimers is reported. The underlined letters indicate the residue which gives rise to the anomeric signal.

4.76 ppm to non-reducing terminal Man. Moreover, twinning of the signals in the two clusters was observed for two pairs of peaks. This was explained by attributing the two pairs to the MMM and MMG oligomers, which are the only ones to have Man residues in both positions. As with mannose, anomeric protons of inner Glc residues resonate at higher ppm values than non-reducing terminal Glc.[11] The assignment of the cluster at 4.63 ppm was achieved considering NMR data of the reduced trimers obtained by means of β-mannanase (see the next paragraph and Fig. 6b). In that case, two clusters relative to Glc residues were present at 4.53 and 4.63 ppm and they were attributed to the non-reducing terminal and the inner Glc residues, respectively. The NMR spectrum of Fig. 5(b) indicated that the trimers with Glc residues in the non-reducing terminal position were either not present in the mixture or were under the detectable limit of the NMR spectroscopy. Actually, the resonances of some of these trimers could be detected in the ppm range pertaining to Man resonances because the very low coupling constant of H-1,H-2 in the mannose ring gives rise to a single signal of higher intensity. The low proportion of trimers exhibiting Glc in the non-reducing terminal position could be explained considering that MG dyads are not easily split by cellulase, as shown in the dimer analysis. Therefore, trimers starting with G could mainly come from the hydrolysis of XG-GY sequences resulting in trimers starting with GG or GM, which were further degraded as previously explained.

The relative amount of each trimer in the mixture was calculated from integration of the resonances in the ^{1}H NMR spectra of both the native and reduced trimers. In order to compare the two spectra, the integration of the resonances attributed to H-1 of non-reducing Glc was set equal to 1 in both spectra. From peak integration of the ^{1}H NMR spectrum of the native trimers, the amount of oligomers containing reducing Glc was calculated as follows.

$$[GGG] + [MGG] + [MMG] + [GMG] = 2.47 \quad (1)$$

since

$$[GGG] + [GMG] \approx 0 \quad (2)$$

then

$$[MGG] + [MMG] = 2.47 \quad (3)$$

The two major resonances in the cluster 4.86–4.80 ppm could have been attributed to H-1 of MMG and MMM, or vice versa (i.e. [MMG] = 2 or [MMG] = 1.2).

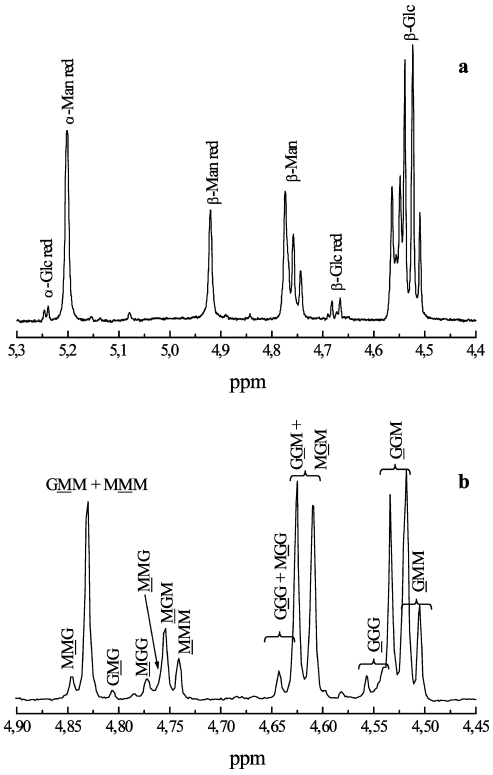

Fig. 6. Anomeric regions of the ^{1}H NMR spectra of the trimers obtained from β-mannanase treatment (a). The suffix red indicates the residue at the reducing end. In (b) the spectrum of the reduced trimers is reported. The underlined letters indicate the residue which gives rise to the anomeric signal.

Table 2
Relative concentrations of trimers as obtained by NMR and capillary electrophoresis

Cellulase digestion		
Trimers	NMR	CE[a]
MMM	1.00	1.00
MMG	1.39	1.83
MGG	0.43	0.20
MGM	0.30	–
GMM	0.08	–
GMG	0.08	–
GGM	0.00	–
GGG	0.00	–
Mannanase digestion		
Trimers	NMR	CE[a]
GGM	1.00	1.00
GMM	0.46	0.52
MMM	0.11	0.10
MGM	0.18	0.20
MGG	0.07	0.02
MMG	0.07	–
GGG	0.10	0.03[b]
GMG	0.02	0.03[b]

[a] The resolution of the CE peaks allowed better accuracy than NMR data.
[b] The value refers either to GGG or GMG (see text).

However, the assignment of the signal at 4.83 ppm to MMG ([MMG] = 1.2) would result in [MGG] = 1.3 from Eq. (3). This value would have given rise to a resonance for MGG, in the appropriate resonance cluster, as intense as the one relative to MMG, and this is not compatible with the recorded spectrum (Fig. 5b). Therefore, the signal at 4.83 ppm was assigned to MMM and that one at 4.85 ppm to MMG, and the following relative concentrations were deduced.

$$[MMM] = 1.39$$

$$[MMG] = 1.88$$

$$[MGG] = 0.60$$

$$[GGG] = 0.00$$

The relative content of non-reducing Man was then obtained from the ^{1}H NMR spectrum of the reduced trimers.

$$2[MMM] + 2[MMG] + [MGG] + [GMM] + [MGM] + [GMG] = 7.74 \quad (4)$$

According to the previous assignments, the shoulder flanking the signal at 4.83 ppm and the low intensity signal at 4.81 ppm referred to trimers having Man in the middle position. Since MMG and MMM were already assigned, the foregoing resonances could only be attributed to GMM and GMG, and they both gave an integration value of 0.11. Their assignment was achieved from the ^{1}H NMR spectrum of the trimers obtained by mannanase treatment (Fig. 6b). The absence of the signals in the Glc resonance region belonging to the shoulder at 4.83 ppm could be explained by the fact that the high coupling constant of the β-Glc H-1, that gives rise to well resolved doublets, may result, in the case of low intensity signals, in their coalescence into the baseline. As a consequence, from Eq. (4), the relative concentration of [MGM] was readily calculated to be 0.36. All of the values obtained were then re-calculated relative to the MMM trimer and are reported in Table 2. Considering the relative concentration obtained for MGG and MGM trimers, they were assigned to the doublets at 4.64 and 4.62 ppm, respectively. The complete peak assignment is reported in Fig. 5(b).

The three main CE peaks were assigned (Fig. 4a) by comparison of their integration values with those derived from NMR experiments (Table 2).

The higher abundance of MMG trimers with respect to MMM might be explained by considering that cellulase readily hydrolysed both GG and GM sequences and not MM and MG sequences. Therefore, the probability of finding a trimer ending with G is higher than that of a trimer ending with M.

Characterisation of trimers obtained by β-mannanase treatment.—The analysis of trimers obtained by means of mannanase was carried out following the same strategy used in the case of cellulase. The CE output (Fig. 4b) exhibited eight peaks of different intensity; two of which had the same migration times of two dimers (referred as 'D' in Fig. 4b), so that only six could be assigned with certainty to trimers. The ^{1}H NMR spectrum of the native trimers (Fig. 6a) indicated that the amount of saccharides exhibiting glucose as reducing end was very low, while the ^{1}H NMR spectrum of the reduced trimers (Fig. 6b) showed that the amount of glucose was higher than that of mannose.

Assignment of the resonances of the reduced trimers was achieved by taking advantage of the information obtained from the trimers produced by cellulase hydrolysis. Considering the twinning of the signals and the previous findings, the two doublets at 4.63 and 4.53 ppm could only be assigned to GGM oligomers. The low intensity signals present in the glucose region of the spectrum were assigned to GGG trimers. Similarly, the low intensity signals in the mannose region of the spectrum were assigned to MMG and GMG. The complete assignment of the resonances is shown in Fig. 6(b). The signal belonging to the GMG trimer was assigned only in the spectral region of the mannose,

and not in that one of the glucose, because of its very low abundance. It must be pointed out that superimposition of the ^{1}H NMR spectra of the reduced trimers obtained by cellulase and mannanase treatment revealed extensive overlapping. In the region of H-1 of the internal G residue, the signals of GGG trimers overlapped completely with those of MGG trimers (4.65–4.62 ppm), and the signals of GGM trimers with those of MGM oligomers. In the region of H-1 of M residues, the signal of GMM overlapped with that of MMM, and that of MGM with MMG. Despite this, integration of the peaks corresponding to each trimer was obtained, because each oligomer presented at least one non-overlapping signal.

In Table 2, the relative concentration values of the different trimers obtained from integration of the NMR signals are shown, together with the integration of CE peaks. The assignment of the electropherogram was carried out by comparing NMR and CE integration values. It is noteworthy that GGM and GMM trimers, which the NMR analysis showed to be abundant in the mannanase trimers and almost absent in the cellulase trimers, exhibited the very same pattern in CE experiments.

3. Conclusions

Useful information about the occurrence of specific oligomeric sequences was obtained combining the use of cellulase and mannanase, as cleavage agents, and the use of NMR and capillary electrophoresis as analytical tools. Although, for a quantitative analysis of the data obtained, the enzyme specificity in glycosidic-bond cleavage should be defined in detail, a number of interesting features could still be derived.

The analysis of dimers and trimers showed that the number of mixed saccharidic sequences present in the polymer was high enough to exclude the existence of large block structures, as suggested by other groups.[5]

Gel permeation chromatography was carried out on a known amount of different reference molecules (glucose, maltose, maltotriose, maltotetraose, maltopentaose, and maltoheptaose) and it showed a linear correlation between the amount of the oligomers loaded on the column and the response of the RI detector. At the same time, the proportionality constant slowly decreased on increasing of the oligomer size. This experiment permitted the evaluation of the absolute amount of each oligomer by using both the integration values of the chromatographic peak, relative to each oligomeric mixture, and the relative concentrations of each component obtained from NMR and CE. The foregoing information was used to calculate the relative amount of specific sequences with respect to the total amount of all the sequences.

Considering the oligomers obtained by mannanase treatment, the MM dyads were present in dimers and in trimers (MM, MMM, MMG, and GMM). The total number of millimoles of MM dyads in these oligomers was 7.1×10^{-3}, while the total millimoles of dyads were 27.3×10^{-3}, as obtained from the total weight of saccharides revealed by RI detection divided by the disaccharide molecular weight. Therefore, the occurrence of MM dyads with respect to all possible dyads was 26%. A similar calculation was carried out for GG dyads which were present in dimers and in trimers (GG, MGG, GGG, and GGM). The percentage of GG dyads with respect to all the possible dyads was 16%. These values were similar to those obtained by pure statistical considerations for a polymer containing G and M in random sequences, with a M:G ratio equal to 1.6:1.0 as in konjac glucomannan. The theoretical values were 38% for the MM dyads and 14% for the GG ones.

The same calculation was performed for the oligomers obtained by cellulase treatment. In this case, the experimental MM dyad content was 30%, in rather good agreement with the theoretical one. However, the content in GG was as low as 2%. This exceedingly low value was clearly due to the presence of (1→4)-β-D-glucosidase in the cellulase complex. As a matter of fact, 87% of the monomers present in the mixture were glucose, as found by the alditol acetate analysis. It must be pointed out that not all the monomeric glucose derived from the cleavage of GG sequences. However, in a chain exhibiting a M:G ratio equal to 1.6:1.0, the probability of finding a Glc residue next to another one is 0.14, and the probability that a Glc monomer was originally in a GG dyad is, at least, 12% ($0.87 \times 0.14 = 0.12$), a figure not far from the theoretical 14%.

Although the values given here are subject to errors due to the inherent imprecision of the evaluation, they strongly suggest that the polymer chain of the glucomannan synthesised by *A. konjac* is characterised by the occurrence of random sequences of Man and Glc residues, as in a Bernoulli-type chain.

4. Experimental

Compositional analysis.—Hydrolysis of the glucomannan was performed with 2 M trifluoroacetic acid at 125 °C for 1 h. Alditol acetates were prepared as previously described.[12] GLC analyses were carried out on a Hewlett–Packard 5890 gas chromatograph equipped with a flame ionization detector and an SP2330 capillary column (Supelco, 30 m), using He as the carrier gas. The temperature program used was 200–245 °C at 4 °C/min, and then 245 °C for 20 min.

Treatment with endo-*glycosidases.*—The polysaccharide (11 mg) was treated with cellulase from *Penicillum funiculosum* [(Sigma, EC 3.2.1.4 (15 U)] in 0.05 M

NaOAc buffer, pH 4.8 at 37 °C for 24 h. Digestion with β-mannanase from *Aspergillus niger* [Megazyme, EC 3.2.1.78 (16 U)] was carried out on 12 mg of glucomannan in 0.02 M NaOAc buffer, pH 4.5 at 40 °C for 24 h. After hydrolysis, the solutions were boiled for 10 min, centrifuged, filtered, and freeze-dried. The products were dissolved in water and separated on two columns (I.D. = 16 mm, height = 950 mm) of Biogel P2 in series at a flow rate of 6 mL/h, using distilled water as eluent. Each peak was analyzed by means of electrospray mass spectrometry to determine the size of the oligosaccharides present.

Electrospray mass spectrometry.—Mass spectra were recorded on a API-I PE SCIEX quadrupole mass spectrometer equipped with an articulated ion spray source and connected to a syringe pump for sample introduction. The instrument was calibrated using a polypropylene glycol (PPG) mixture (3.3×10^{-5} M PPG 425, 1×10^{-4} M PPG1000, and 2×10^{-4} M PPG 2000), 0.1% (v/v) MeCN and 2 mM ammonium formate in 50% (v/v) aqueous MeOH. The oligosaccharides were dissolved in 50% (v/v) aqueous MeCN and 6×10^{-5} M ammonium acetate at a suitable concentration and injected at a flow rate of 5 μL/min. The ionspray voltage was 5000 V and the orifice potential was 50 V. The spectra were recorded in the positive mode using a scan step size of 0.1 amu.

NMR spectroscopy.—^{1}H NMR spectra were recorded on a 500 MHz Varian UNITY INOVA instrument using a probe temperature of 50 °C for the disaccharides obtained by mannanase treatment and of 70 °C for all the other samples. Integration of overlapping signals was carried out using the Origin software (OriginLab Corporation, USA) resorting to multiple lorentzian fitting.

Molecular weight determination.—Weight-average molecular weights were determined by means of size exclusion chromatography (HPLC Jasco PU-880), using three TSK-PWXL columns in series (G6000, G5000, and G3000), coupled with a low angle laser light scattering detector (Chromatix X-100).

Capillary electrophoresis.—The experiments were carried out at 30 °C on an Applied Biosystems HPCE Model 270A-HT using a fused silica column (72 cm (50 cm to detector) × 50 μm I.D. × 375 μm O.D., Supelco). Samples were derivatized with 4-aminobenzonitrile[13] and loaded under vacuum at a pressure of 16.9 kPa (1.5 s). Before sample injection, a 4-min conditioning of the capillary with the buffer (100 mM sodium tetraborate, pH 9.2) followed a 2-min washing with 0.1 M NaOH (vacuum pressure 67.6 kPa). The applied voltage was 15 kV and the UV detection wavelength was 285 nm. Peak areas were evaluated after normalization for the migration time.[14]

Acknowledgements

The Italian Ministry for University and Research and the University of Trieste are acknowledged for financial support. POLYtech s.c.ar.l. is greatly acknowledged for the use of the HP 5890 gas chromatograph. ESI mass spectra were run at the Centro Servizi Polivalenti di Ateneo (University of Trieste). Konjac glucomannan was a kind gift of Professor M. Dentini (University of Rome).

References

1. Stephen, A. M. Other Plant Polysaccharides. In *The Polysaccharides*; Aspinall, G. O., Ed.; Academic Press: New York, 1983; Vol. 2, pp 97–192.
2. Andrews, P.; Hough, L.; Jones, J. K. N. *J. Chem. Soc.* **1953**, 1186–1192.
3. Davè, V.; McCarthy, S. P. *J. Environ. Polym. Degrad.* **1977**, *5*, 237–241.
4. Andrews, P.; Hough, L.; Jones, J. K. N. *J. Chem. Soc.* **1956**, 181–188.
5. Takahashi, R.; Kusakabe, I.; Kusama, S.; Sakurai, Y.; Murakami, K.; Maekawa, A.; Suzuki, T. *Agric. Biol. Chem.* **1984**, *48*, 2943–2950.
6. Shimahara, H.; Suzuki, H.; Sugiyama, N.; Nisizawa, K. *Agric. Biol. Chem.* **1975**, *39*, 301–312.
7. Brownsey, G.; Cairns, P.; Miles, M. J.; Morris, V. J. *Carbohydr. Res.* **1988**, *176*, 329–334.
8. Wood, T. M. *Biochem. Soc. Trans.* **1992**, *20*, 46–53.
9. Sahasrabudhe, N. A.; Ranjekar, P. K. *FEMS Microbiol. Lett.* **1990**, *66*, 291–294.
10. Harjunpää, V.; Teleman, A.; Siika-Aho, M.; Drakenberg, T. *Eur. J. Biochem.* **1995**, *234*, 278–283.
11. Goldberg, R.; Gillou, L.; Prat, R.; Hervé du Penhoat, C.; Michon, V. *Carbohydr. Res.* **1991**, *210*, 263–276.
12. Albersheim, P.; Nevins, D. J.; English, P. D.; Karr, A. *Carbohydr. Res.* **1967**, *5*, 340–345.
13. Schwaiger, H.; Oefner, P. J.; Huber, C.; Grill, E.; Bonn, G. K. *Electrophoresis* **1994**, *15*, 941–952.
14. Baker, D. R. *Capillary Electrophoresis*; J. Wiley & Sons: New York, 1995; pp 201–209.