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Constitution of konjac glucomannan: chemical analysis and ^{13}C NMR spectroscopy

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Abstract

The constitution of konjac glucomannan was determined by methylation analysis and ^{13}C NMR spectroscopy. The results of methylation analysis showed that the branching point is C-6 carbon of glucosyl units. ^{13}C NMR spectroscopy (1D and DEPT) of konjac glucomannan supported the presence of β-C-1-linked C-6 carbon of glucosyl units as the branching units. This result differs from previous investigations. The ^{13}C NMR spectra indicated that the ratio of terminal glucosyl units to terminal mannosyl units is ca. 2 and branching frequency is ca. 8%, supporting the results by Smith et al. From the splitting of main chain signals the sequences of glucosyl and mannosyl units in konjac glucomannan are estimated and a model structure for the glucomannan is proposed.

Keywords: Konjac glucomannan; ^{13}C NMR spectra; Methylation analysis

1. Introduction

Constitution of konjac glucomannan was the object of many investigations in 1920s (Mayeda, 1922; Ohtsuki, 1929). Hydrolysis of methylated konjac glucomannan was reported to give a mixture of 2,3,4-tri-*O*-methyl-D-glucose, 2,3,4-tri-*O*-methyl-D-mannose and 2,3,6-tri-*O*-methyl-D-mannose, and a formula was proposed for the polysaccharide (Nishida & Hashima, 1930). Smith and Srivastava (1959) proposed that the glucomannan has β-(1 → 4)-linked D-glucose and D-mannose residues as the main chain with branches joined through C-3 carbon of D-glucosyl and D-mannosyl residues. The repeating unit contained on the average 13 hexose residues, that is, ca. 8%. Afterwards Kato and Matsuda (1969) and Kato, Watanabe, and Matsuda (1970) fractionated and identified oligosaccharides obtained by mild acid hydrolysis or enzymatic hydrolysis of the glucomannan. They proposed that β-(1 → 4)-glycosidically linked D-glucose, cellobiose, D-mannose and D-mannotetraose units compose the main chain structure as shown in the following.

–G–G–M–M–M–M–G–M– or –G–G–M–G–M–M–M–M–

Shimahara, Suzuki, Sugiyama, and Nishizawa (1975); Takahashi et al. (1984) isolated β-(1 → 4)-linked oligosaccharides of the following structures besides those identified by Kato and Matsuda. (1969) and Kato et al. (1970) by partial acid and enzymic hydrolysis.

M–M–M–M–M, G–G–M and G–G–M–M

As for the branching point, Kato and Matsuda (1973) isolated and identified several oligosaccharides containing β-D-mannopyranosyl-(1 → 3)-*O*-β-D-mannopyranosyl

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linkage. Maeda, Shimahara, and Sugiyama (1980) confirmed the branching structure through C-3 carbon of both D-glucosyl and D-mannosyl residues by analysis of the hydrolyzate of the permethylated glucomannan by g.l.c.-m.s.

In our studies on syntheses of branched polysaccharides (Matsuzaki, Yamamoto, Sato, & Oshima, 1985) konjac glucomannan was used as one of starting materials. The constitution of konjac glucomannan was reexamined by methylation analysis and it was found that the structure was somewhat different from that proposed by Smith and Srivastava (1959) in branching frequency, constitution of nonreducing end groups and significantly in branching position. In order to prove the structure of glucomannan which we obtained, ^{13}C NMR spectra of konjac glucomannan were determined to investigate the main chain and the branch structure of the polysaccharide. So far ^{13}C NMR spectra including solid-state spectra of konjac glucomannan have been reported (Gidley, McArthur, & Underwood, 1991), but detailed assignments were not given.

After Nishinari, Williams, and Phillips (1992) published an overall review on konjac glucomannan, notable investigations on its chemical structure and NMR spectroscopy have not been reported, except mannose/glucose ratio (Kohyama, Iida, & Nishinari, 1993).

2. Materials and methods

2.1. General methods

Gas–liquid chromatography was performed with a Hewlett–Pachard S790 instrument equipped with a flame-ionization detector on fused-silica capillary columns; injection and detector temperature, 300 °C. Peak areas were determined by a Hewlett–Pachard 3390 integrator and converted into molar ratios on an effective carbon-response basis. G.l.c.-m.s. was carried with a Hewlett–Pachard 5985B g.l.c.-m.s. system. E.i.-m.s. data were obtained at the ionization voltage of 70 eV.

2.2. Isolation of konjac glucomannan and its methylation

Konjac flour was dissolved in 10% NaOH solution to give 1% solution and the glucomannan was precipitated by adding Fehling solution. The precipitate was decomposed with 1% HCl solution in ethanol, filtered, successively washed with 50% ethanol, ethanol and ether, and dried in vacuo. Due to strong alkali treatments the purified glucomannan did not accompany acetyl groups which are present in native konjac glucomannan (Maekaji, 1978). The glucomannan was hydrolyzed with 4 M trifluoroacetic acid, reduced with sodium borohydride and acetylated with acetic anhydride/pyridine. G.l.c. analysis of the alditol acetates was carried out on a WCOT column coated with methyl silicone (0.2 mm × 12.5 m) at column temperatures 150–250 °C increasing at the rate of 10 °C/min.

The glucomannan was permethylated by Hakomori (1964) method. The methylated polysaccharide was derived into partially methylated alditol acetates by the usual method and was analyzed by g.l.c.-m.s. on the same column as above. The identification of partially methylated alditol acetates was carried out from their m.s. pattern and by comparison with R_f values and m.s. pattern of partially methylated alditol acetates obtained from methylated ivorynut mannan and branched (mainly at C-6) ivorynut mannan and konjac glucomannan (Matsuzaki et al., 1985).

Separation of 2,3,4,6-tetra-*O*-methyl glucitol acetate and mannitol acetate was carried out with the same apparatus on a fused capillary column coated with Carbowax 20 M (0.2 mm × 25 m) at column temperatures 150–190 °C increasing at a rate of 0.5 °C/min.

2.3. ^{13}C NMR measurement

^{13}C NMR spectra of konjac glucomannan (the same sample as used for methylation analysis) were determined with an ECA 600 spectrometer (JEOL) operating at 600 MHz (150 MHz for ^{13}C) in a JEOL laboratory. In order to detect branched C-6 or C-3 carbon in glucosyl units, the apparatus was carefully checked to operate in the best condition. Konjac glucomannan was dissolved in 0.7N sodium hydroxide solution in D_2O at 30 °C and kept at that temperature during the measurement, to prevent complete gelation which will result in disappearance of the signals. The concentration of the solution was 3%. Neither sonification nor hydrolysis (enzymatic or acid) to reduce viscosity of the solution was not applied to keep the sample intact.

The DEPT spectrum was first determined. The spectrum was accumulated for 60 h (120,000 scans) with repetition time of 1.8 s. Then the ordinary 1D spectrum with NOE were accumulated for 90 h (145,000 scans) with repetition time of 2.2 s.

3. Results and discussion

3.1. Chemical analysis

G.l.c. analysis of alditol acetates derived from purified glucomannan confirmed that the component sugars are D-mannose and D-glucose in a ratio of 1.6:1 with no other sugars. The result of analysis of partially methylated alditol acetates is shown in Table 1. The presence of 2,3-di-*O*-Me-Glc acetate was confirmed by comparison with partially methylated alditol acetates obtained from branched (mainly at C-6) cellulose and absence of 2,3-di-*O*-Me-Man acetate was confirmed by comparison with partially methylated alditol acetates obtained from branched (mainly at C-6) ivorynut mannan and konjac glucomannan (Matsuzaki et al.,

Table 1
Mole ratios of partially methylated alditol acetates obtained from fully methylated konjak glucomannan

Partially methylated alditol acetates	Present data	Smith et al.[a]
2,3,4,6-Me_4-Glc	1.0	2
2,3,4,6-Me_4-Man	2.0	1
2,3,6-Me_3-Glc	61	10.2
2,3,6-Me_3-Man	98	15.6
2,3-Me_2-Glc	2.6	0
2,3-Me_2-Man	0	0
2,6-Me_2-Glc + 2,6-Me_2-Man	0	2.8

[a] Smith and Srivastava

1985). The branching explicitly joins through C-6 position of D-glucosyl units in the main chain, which is composed of β-(1 → 4)-linked mannosyl and glucosyl units. It is noted that the D-mannosyl units do not participate in the branching.

Fig. 1 shows the chromatogram indicating the separation of 2,3,4,6-tetra-*O*-methyl glucitol acetate and 2,3,4,6-tetra-*O*-methyl mannitol acetate in the order reverse to that reported by Shibuya (1981). The constitution of end groups is D-mannose/D-glucose = 2/1. The degree of branching (number of branches for every 100 residues, *n*) is calculated from the following equations.

$$n = \frac{(2,3,4,6\text{-Man}) + (2,3,4,6\text{-Glc})}{(2,3,6\text{-Man}) + (2,3,6\text{-Glc}) + (2,3\text{-Glc})} \times 100$$

or

$$n = \frac{(2,3\text{-Glc})}{(2,3,6\text{-Man}) + (2,3,6\text{-Glc}) + (2,3\text{-Glc})} \times 100$$

where (2,3,4,6-Man), (2,3,4,6-Glc), (2,3,6-Man), (2,3,6-Glc) and (2,3-Glc) indicate relative mole ratios of partially methylated alditol acetates shown in Table 1. $n = 1.9$ or 1.6. Therefore, the branching exists for every 50–60 residues.

3.2. ^{13}C NMR measurement

Fig. 2(A) and (B) show ^{13}C NMR 1D spectra of konjac glucomannan. It is noteworthy that the spectra are well-resolved. The NMR measurements were carried out for long time (145,000 scans)with NOE, but *S/N* ratio is not enough high due to low concentration of the solution with very high viscosity. Therefore, although strict quantitative determination cannot be expected, the intensities of the signals were determined and are shown in Table 2. It is seen that semi quantitative results are observed. From the intensities and chemical shifts of the absorptions of konjac glucomannan spectrum and comparison with those of the branched cellulose (Matsuzaki et al., 1986) synthesized from cellulose as a main chain and D-glucose as side chains by orthoester glycosylation, and a β-(1 → 4)-D-mannan (Jarvis, 1990), absorptions of nos. 1, 3, 6, 7, 8 and 12 are assigned to glucosyl units and absorptions of nos. 2, 4, 5, 9, 10 and 11 are assigned to mannosyl units as shown in Table 2.

It is seen that several main chain signals, G1, G2, G4, M2 and M5 carbon signals, are split. Splittings of G3 and G5 carbons seem to be overlapped. Signals of M3 and M4 carbons do not split, although the latter is broad. Splitting of signals suggests detailed structure of the polysaccharide, as discussed later.

The methylation analysis indicated the presence of the following linkage for the branching;

-D-Glc(1 → 6)-D-Glc (main chain) and/or
-D-Man(1 → 6)-D-Glc (main chain)

^{13}C NMR measurements were especially carried out to prove the presence of (1 → 6)linkages of the branching. The absorptions of (1 → 6)-linked C-6 carbon are expected to appear between signals of free C-6 carbon and other carbons.

In Fig. 2(B) we can observe four signals **a**, **b, c** and **d** between signals of M2 carbon and (G6 + M6) carbons (signals which disappeared in the DEPT spectrum were considered as noise). The signals were assigned as shown in Table 3 comparing with the chemical shifts of disaccharides (Bock, Pedersen, & Pedersen, 1984).

Fig. 3(A) and (B) show its DEPT spectra. There are also four signals between (G6 + M6) carbons and M2 carbons. Assignments of **a** and **b** to G4 and M4 carbons in the terminal glucosyl and mannosyl units in branching, respectively, are supported. The content of **a** + **b** is about 8% of total glycosyl units and the intensity of terminal G4 carbon is about two times that of M4 carbon. The branching density is much higher than our results but the same as that

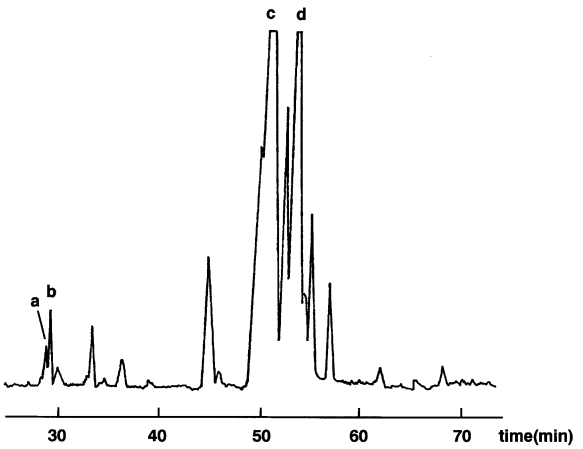

Fig. 1. G.l.c. chromatogram of partially methylated alditol acetates obtained from permethylated konjac glucomannan (a) 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol (b) 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-mannitol (c) 1,4,5-tri-*O*-acetyl-2,3,6-tri-*O*-methyl-D-mannitol (d) 1,4,5-tri-*O*-acetyl-2,3,6-tri-*O*-methyl-D-glucitol.

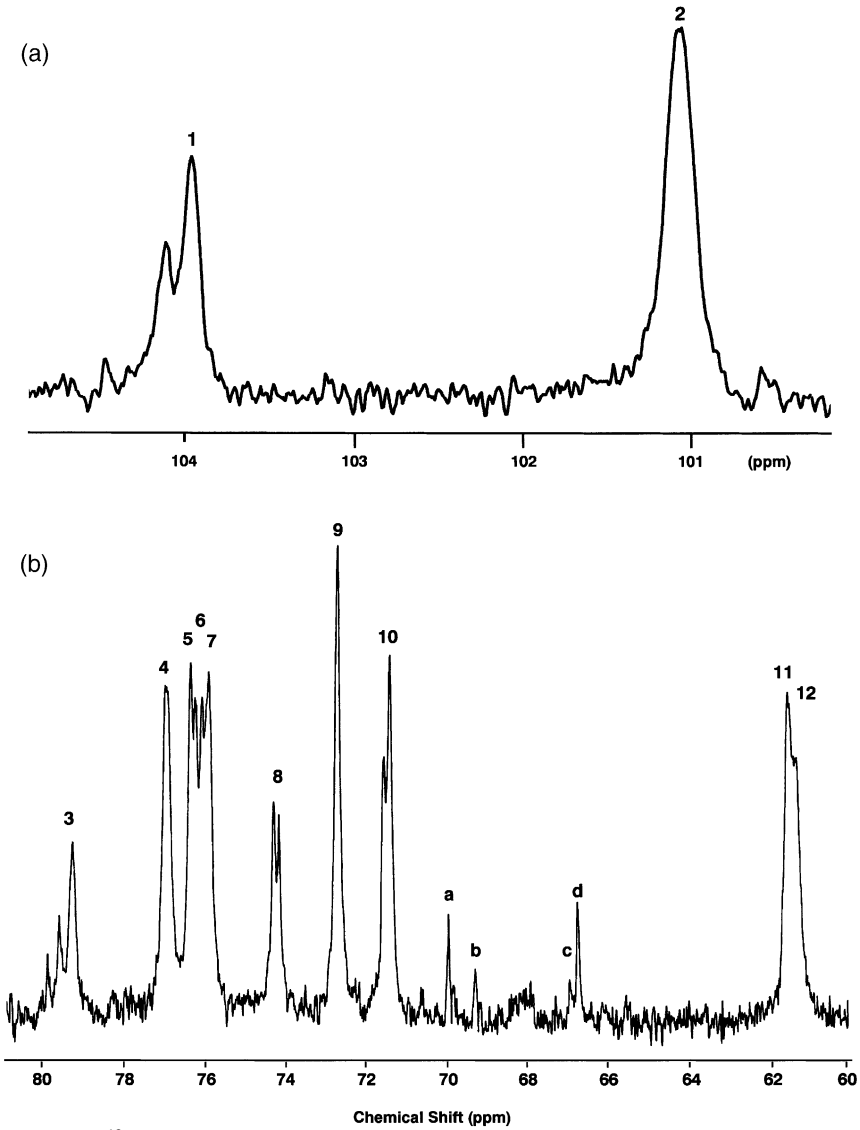

Fig. 2. ^{13}C NMR 1D spectra of konjac glucomannan (A): C1 region. (B): C2–C6 region.

Table 2
Assignments of ^{13}C NMR spectrum of konjak glucomannan

No.	Chemical shift (ppm)	Intensity	Assign.	Branched glucan[a]	Ivory nut mannan[b]
1	104.1, 103.9	0.68	G1	103.0	–
2	101.1	1.00	M1	–	101.6
3	79.6, 79.3	0.72	G4	80.0	–
4	77.0	1.04	M4	–	77.9
5	76.4, 76.3	1.03	M5	–	76.5
6	76.1, 75.9	0.38[c]	G5	75.7	–
7	75.9	0.91[c]	G3	75.3	–
8	74.3, 74.2	0.52	G2	73.2	–
9	72.7	1.04	M3	–	73.0
10	71.6, 71.4	0.97	M2	–	71.4
11	61.6	0.94	M6	–	62.0
12	61.4	0.50	G6	61.3	–

G: D-Glucose. M: D-Mannose.
[a] Matsuzaki et al., 1986.
[b] Jarvis, 1990.
[c] Signal No.7 includes a part of G5 signal.

Table 3
Assignment for signals between 60 and 71 ppm of ^{13}C NMR spectrum of konjak glucomannan

Signal	Chem. shift (ppm)	Intensity	Assignment	Literature (ppm)
a	69.9	0.09	t-G4	70.6[a]
b	69.2	0.05	t-M4	67.5[b], 68.4[c]
c	67.0	0.02	–	–
d	66.8	0.13	β-G6	69.4[d], 68.3[e]

G: D-Glc. M: D-Man. t: terminal. Chemical shifts with footnotes a–d are cited from those of disaccharides (Bock et al. (1984)).
[a] β-Glc(1 → 4)-β-Glc.
[b] β-Man(1 → 4)-β-Glc.
[c] β-Man(1 → 4)-β-Man.
[d] β-Glc(1 → 6)-β-Glc.
[e] β-(1 → 6)-glucan.

by Smith and Srivastava. The ratio of terminal glucosyl units to terminal mannosyl units also supports that obtained by Smith et al.

The signals, **c** and **d** are those of substituted C-6 carbons. Signal **d** was first assigned to α-D-Glc(1 → 6)-D-Glc based on its chemical shift (66.8 ppm), since the chemical shift of C-6 carbon of disaccharide, α-D-Glc(1 → 6)-β-D-Glc, is 66.5 ppm (Bock et al., 1984) and that of α-(1 → 6)-D-glucan is 66.7 ppm (Colson, Jennings, & Smith, 1974). However, C-1 signal of α-D-glucosyl unit (ca. 99 ppm) was not detected between 77 and 101 ppm in both 1D and DEPT spectra. Therefore, it is now assigned to C-6 carbon of β-D-Glc(1 → 6)-D-Glc, although chemical shifts of β-(1 → 6)C-6 carbon in

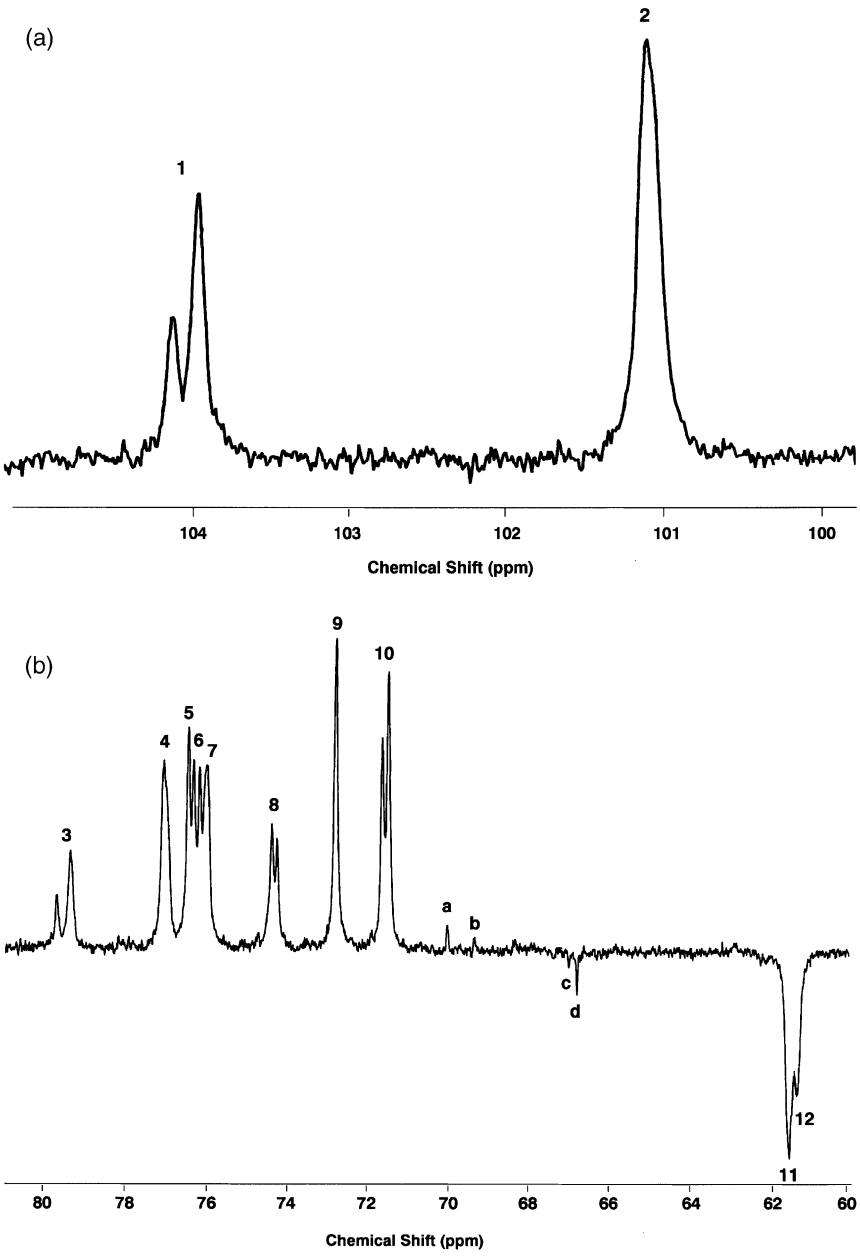

Fig. 3. ^{13}C NMR DEPT spectra of konjac glucomannan (A): C1 region. (B): C2–C6 region.

the literatures (Table 3) are high. Main chain linkage at C-4 carbon may cause a considerable downfield shift of the substituted C-6 carbon. The β-C-1 signal of linked D-glucosyl unit may overlap with G1 signal of glucosyl units (104 ppm) in the main chain.

It is also probable that the C-6 bond is linked to mannosyl unit, that is, D-Man(1 → 6)-D-Glc, as well as D-Glc(1 → 6)-D-Glc. Chemical shift of α-C-1 carbon of (1 → 6)-α-D-mannopyranan (Frechet & Schuerch, 1969; Hatanaka et al., 1991) derived from a polymer of 1,6-anhydro-2,3,4-tri-*O*-benzyl-β-mannopyranose obtained with PF_5 as catalyst is 100.8 ppm, and that of synthetic α-(1 → 6)glucomannan is 100.5 ppm (Kobayashi, Eby, & Schuerch, 1977). As stated before, there is no signal between 80 and 101 ppm. Therefore, the linkage may be β-D-Man(1 → 6)-D-Glc, if present, although direct evidence cannot be obtained. At present it is difficult to conclude that which residue, glucosyl or mannosyl, is linked to C-6 carbon of glucosyl unit in the main chain.

It seems that signal **c** shows the effect of neighboring units, such as D-Man(1 → 6)-D-Glc (or D-Glc(1 → 6)-D-Glc). The content of **c** is less than 15% of **d**.

Chemical shift of β-(1 → 3)-linked C-3 carbon is 85–86 ppm as observed for curdlan (β-(1 → 3)-glucan). Chemical shift of α-(1 → 3)-linked C-3 carbon is 83.2 ppm (Colson et al., 1974; Bock et al., 1984). No signal was observed between 80 and 90 ppm in the spectra of konjac glucomannan. Therefore, it is certain for our sample that the branching point is C-6 carbon of glucosyl units in the main chain.

3.3. Sequential distribution of glucosyl and mannosyl units in the chain

Splittings of main chain signals indicate sequences of glucosyl and mannosyl units in the main chain. Table 4 shows chemical shifts and intensities of those signals. The splittings may be caused by the difference in neighboring units, such as D-Glc-D-Glc and D-Glc-D-Man.

The ratio of split signals indicates the ratio of D-Glc-D-Glc (G–G) linkages to D-Glc-D-Man (G–M) linkages or that of D-Man-D-Glc (M–G) linkages to D-Man-D-Man (M–M) linkages. As is seen in the table most of the ratio is near 0.6 which is the ratio of glucosyl units to mannosyl units in the polysaccharide. The most simple interpretation of the results is random distribution of glucosyl and mannosyl units according to their concentrations, that is, no selectivity for the glycosyl units exerts in the formation of the polysaccharide chain.

Assuming random distribution of glycosyl units, their sequential distribution was calculated. Consider that the glucomannan is an assembly of blocks, each of which is composed of one glucosyl block and one mannosyl block. For example

–(GGMMMM)(GMMM)(GGMM)–

Assuming that (G–M)/(G–G) and (M–M)/(M–G) is 1.6(= p), number of blocks with certain length of glucosyl or mannosyl units was calculated from the following equations. For mannosyl blocks, and for 100 blocks,

$$m_1 = 100p/(1+p),\quad m_{i-1} = m_{i-2}p/(1+p) - m_i,\quad i > 2$$

where m_i is number of mannosyl blocks with i mannosyl units.

Similar equations are derived for glucosyl blocks and blocks are combinations of various glucosyl and mannosyl blocks.

In Table 5, number of glucosyl or mannosyl blocks in 100 blocks is shown against the length of glucosyl or mannosyl units in a block. It is noted that G–M–G and G–G–G oligosaccharides which are predicted to be formed to a considerable extent (38.5 and 9%), were hardly observed in the fragmentation and oligosaccharide analysis. Therefore, random distribution was not applicable.

When sequential length of glucosyl units is one or two as previous investigators (Kato & Matsuda, 1969; Shimahara et al., 1975) assumed, it was found that the following model well satisfies the results of NMR spectroscopy shown in Table 4. The basic structure of the model is composed of eight blocks, each of which containing one glucosyl block and one mannosyl block. Of eight glucosyl blocks, three are

Table 4
Assignments of split signals in ^{13}C NMR spectrum of konjak glucomannan

No.	Chemical shift (ppm)	Intensiy[a]	Assignments
G1-L	104.09	58	β-D-Glc(1 → 4)-β-D-Glc
G1-H	103.94	100	β-D-Glc(1 → 4)-β-D-Man
G4-L	79.58	46(61)	β-D-Glc(1 → 4)-β-D-Glc
G4-H	79.26	100	β-D-Man(1 → 4)-β-D-Glc
G2-L	74.33	100	β-D-Glc(1 → 4)-β-D-Man
G2-H	74.20	67	β-D-Glc(1 → 4)-β-D-Glc
M2-L	71.57	55	β-D-Man(1 → 4)-β-D-Glc
M2-H	71.42	100	β-D-Man(1 → 4)-β-D-Man
M5-H	76.38	100	β-D-Glc(1 → 4)-β-D-Man
M5-L	76.27	58	β-D-Man(1 → 4)-β-D-Man

G: D-Glc; M: D-Man.
[a] Relative intensity. Specified carbon is that in underlined glycosyl units.

Table 5
Number of blocks containing certain length of glucosyl or mannosyl units in the block

Length of units	Mannosyl block	Glucosyl block
1	38.5	61.7
2	23.7	23.6
3	14.6	9.0
4	9.0	3.5
5	5.5	1.3
6	3.4	0
7	2.1	
8	1.3	
>9	2.0	

Total number of blocks: 100.

glucosyl units and five are cellobiosyl units. The length of mannosyl blocks changes from monomeric to presumably pentameric. Total number of glucosyl units is 13 and that of mannosyl units is 21 (M/G = 1.6). Number of G–G linkages is 5, M–M linkages 13 and G–M (or M–G) linkages 8. For example,

$$G-M-G-M_2-G-M_3-G_2-M-G_2-M_2-G_2-M_3-G_2-M_4-G_2-M_5-$$

Average length of mannosyl blocks is 2.6. Although the length distribution of mannosyl blocks is not known, it is deduced that number of mannobiosyl and mannotriosyl blocks may be large. (The example cannot show the length distribution of mannosyl blocks well due to its small size). The basic structure can be extended to a large molecule as far as the ratio of glucosyl-end blocks to cellobiosyl-end blocks is kept constant (1:1.6)and M/G = 1.6. The model reasonably explains the results of fragmentation as well as the results of NMR spectroscopy.

For G4 signals, the ratio is evidently low. There is a sharp signal downfield in the 1D spectrum, but it disappears in the DEPT spectrum. If this signal is included in G4-L, the ratio is 0.61. So far no explanation can be given for the low ratio and the downfield signal.

In conclusion, konjac glucomannan is composed of β-(1 → 4) linked D-glucosyl and D-mannosyl residues as the main chain with branches through β-(1 → 6)-glucosyl units. Degree of branching is about 8% and the ratio of terminal glucosyl units to mannosyl units is ca. 2.

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