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Adhesion of β-D-glucans to cellulose

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Abstract

Schizophyllan, a *Schizophylium commune* β-D-glucan, a *Tamarindus* xyloglucan, locust bean gum, a galactomannan, a barley β-D-glucan, and chitosan show specific adhesion to microcrystalline cellulose (cellulose I). Xyloglucan, locust bean gum, barley β-D-glucan, and chitosan also show the ability to adhere mercerized cellulose (cellulose II), while schizophyllan does not. As the molecular weight of schizophyllan decreases, both its ability to form triple-helical structures and its adhesion to cellulose I diminish and finally disappear, indicating that the adhesion of schizophyllan to cellulose I depends on high-molecular-weight domains that adopt the triple-helical structures. On the other hand, the adhesion of locust bean gum, chitosan, and xyloglucan to celluloses was found to be largely independent of molecular weight. Furthermore, it is thought that the adhesion of barley β-D-glucan occurs because it belongs to a group of xyloglucans.

Keywords: Cellulose-adhesive polysaccharides; Schizophyllan; Xyloglucan; Locust bean gum; Barley β-D-glucan; Chitosan

1. Introduction

Schizophyllan, secreted from a fungus, *Shizophyllum commune*, is a water-soluble (1→3)-β-D-glucan with side chains consisting of single β-D-glucosyl residues attached to O-6 of the backbone residues [1,2]. Schizophyllan adopts a triple helical structure in water [3–5]. The polysaccharide shows antitumor activity against Sarcoma 180 [6–9] and is effective in promoting the regeneration of protoplast cells of *Saccharomyces cerevisiae* [10,11]. Recently, we reported that schizophyllan adheres to yeast glucan and curdlan gel and that the specific adhesion requires high-molecular-weight molecules with a triple-helical conformation [12]. Xyloglucans occur in primary plant-cell walls and in the seeds of *Tamarindus indica*, and consist of a cellulosic backbone in which up to 75% of the (1→4)-linked β-D-glucose residues are substituted at O-6 with α-D-xylose residues [13–17]. The α-D-xylose residues are frequently substituted at O-2 with β-D-galactose residues or α-L-fucosyl(1→2)-β-D-galactose moieties [16]. These xyloglucans adhere strongly to cellulose microfibrils through hydrogen bonds between the xyloglucan backbone and

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cellulose [13,14,18]. Barley β-D-glucan is one of the major polysaccharides of grass and cereal and has complex structures with random distribution of (1→3)- and (1→4)-linkages [19]. Locust bean gum, a galactomannan, forms a rigid gel by boiling with xanthan gum [20]. Xanthan gum, an acidic extracellular polysaccharide produced by *Xanthomonas campestris*, consists of pentasaccharide repeating units and contains a (1→4)-β-D-glucan backbone like xyloglucan [20–22]. Succinoglycan, an acidic extracellular polysaccharide produced by *Agrobacterium* and *Rhizobium melilotii*, consists of octasaccharide repeating units in which three out of four residues of the main chain are (1→4)-linked β-D-glucose [23].

Recently, we found by chance that schizophyllan can adhere not only to yeast glucan but also to microcrystalline cellulose. It may be possible to classify polysaccharides by their ability to bind to cellulose. This type of analysis may reveal interesting functions of these polysaccharides. In the present paper, various polysaccharides were tested for adhesion to cellulose in order to determine general properties of polysaccharides that associate with cellulose.

2. Materials and methods

Materials.—Microcrystalline cellulose (cellulose I, Funakoshi Co. Ltd, Tokyo) was autoclaved at 120 °C for 20 min for promotion and stabilization of crystalline structures and then decanted with distilled water to remove small fibers and particles. In order to prepare mercerized cellulose (cellulose II), cellulose I was suspended in 4 M NaOH, left overnight in a refrigerator, neutralized with 4 M NaOH, and rinsed with distilled water. Barley β-D-glucan and larch wood arabinogalactan were purchased from Sigma Chemical Co. (USA). Chitosan was purchased from Tokyo Chemical Industry Co. Ltd (Tokyo). Cellulase ONOZUKA RS was purchased from Yakult Honsha Co. Ltd (Tokyo). Pullulan was purchased from Nakalai Teque (Kyoto). Schizophyllan, *Tamarindus* xyloglucan, xanthan gum, locust bean gum, and chito-oligosaccharides were kindly provided by Taito Co. Ltd (Kobe), Dainippon Pharmaceutical Co. Ltd (Osaka), San-Ei Gen FFI Inc. (Osaka), and Yaizu Suisan Kagaku Industries Co. Ltd (Shizuoka), respectively. Pustulan was provided by courtesy of Dr T. Nakajima (Tohoku University). Cyclosophoran [24], succinoglycan [23], yeast mannan, and malto-oligosaccharides were prepared in this laboratory.

X-ray diffraction diagram.—Diffraction patterns of powdered samples were recorded with an X-ray diffractometer (Miniflex, Rigaku Denki, Tokyo).

Adhesion of water-soluble polysaccharides and oligosaccharides to celluloses.—Cellulose I and cellulose II were packed in a column (2.0×15 cm) and rinsed with distilled water. Samples (0.1% w/v, 1 mL) were applied to the column and eluted with distilled water (200 mL) at a flow rate of 0.5 mL/min. In the case of polysaccharides, samples soaked in distilled water overnight in a refrigerator were used. The amount of carbohydrate eluted from the column was estimated colorimetrically.

Langmuir adhesions.—In a screw-capped vial (1.6×12 cm), 50 mg of cellulose I was mixed with distilled water (2 mL) containing an appropriate concentration of a water-soluble polysaccharide. The vial was attached to a board and rotated at 8 rpm at room temperature for 6 h. The vial was then centrifuged at 3000 rpm for 5 min, and an aliquot (1 mL) of the supernatant was transferred in an Eppendorf conical centrifugation tube and centrifuged again at 14 000 rpm for 10 min to remove small floating particles.

Preparation of depolymerized schizophyllan (DS).—DS was prepared as previously described [11], except that the amount of schizophyllan was scaled up 100-fold.

Measurement of molecular weight (M_r) of the DS samples.—The M_r values of samples were determined by HPLC, using tandem columns (7.8×300 mm each) of TSKgel α-M (Tosoh, Tokyo). Phosphate buffer (0.1 M, pH 6.5) containing 0.02% sodium azide and 95% Me_2SO were used as the mobile phase to estimate M_r in water and in Me_2SO, respectively. The column was eluted at a flow rate of 0.5 mL/min. Molecular-size marker pullulans (Shodex, Tokyo) were P-400 (M_r 380 000), P-100 (M_r 100 000), P-50 (M_r 48 000), P-10 (M_r 12 200), and P-5 (M_r 5800).

Preparation of oligosaccharides from locust bean gum.—Locust bean gum (500 mg) was dissolved in distilled water (250 mL) containing 0.02% sodium azide and incubated with cellulase (ONOZUKA RS, 2.5 mg) at 37 °C for 15 h. The digest was concentrated to 10 mL, applied to a column (2×50 cm) of Toyopearl HW-40S (Tosoh, Tokyo) and eluted with distilled water at a flow rate of 1 mL/min. Carbohydrate in the eluate was detected

with a refractive index monitor, and fractions (5 mL) containing oligosaccharides were pooled and lyophilized. Further separation of the oligosaccharides was conducted by HPLC on a column of TSKgel Amide-80 (Tosoh, Tokyo) using 55% acetonitrile as mobile phase at a flow rate of 0.8 mL/min.

Colorimetric assay.—Carbohydrates of most samples were analyzed by the phenol–H_2SO_4 method [25], and the amount of each polysaccharide was estimated by changing the value of OD to that of weight using a conversion table. In the case of chitosan and chito-oligosaccharides, the Blix method [26] was used. The reducing strength of oligosaccharide samples was analyzed by the modified Park–Johnson method [27].

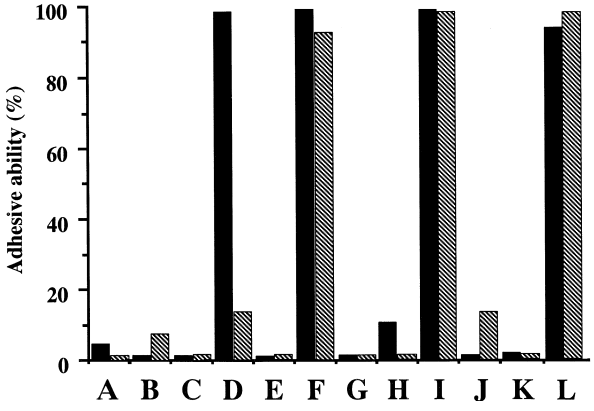

Fig. 1. Adhesive properties to cellulose I and cellulose II of water-soluble polysaccharides: A, glucose; B, pullulan; C, cyclosophoran; D, schizophyllan; E, xanthan gum; F, xyloglucan; G, pustulan; H, succinoglycan; I, locust bean gum; J, yeast mannan; K, arabinogalactan; L, barley β-D-glucan. Left black bar, cellulose I; right shaded bar, cellulose II.

3. Results and discussion

Adhesion of various water-soluble polysaccharides to cellulose.—The xyloglucans, which are structurally related to cellulose in the backbone, noncovarently adhere to cellulose microfibrils [13]. The binding to cellulose is so specific that xyloglucans have been separated from other components by cellulose column chromatography [28]. It was found that schizophyllan adheres to yeast glucan and curdlan gel [12], and our preliminary examination showed that the water-soluble (1→3)-β-D-glucan bound to cellulose. These associations might occur between polysaccharides having a complementary conformation. It might be useful in terms of the function of polysaccharides to determine the structural characteristics of cellulose-binding polysaccharides. Therefore, various branched, water-soluble polysaccharides were tested for adhesion to cellulose. Furthermore, the effect of differences in the crystallinity of cellulose was also examined.

The adhesion of various water-soluble polysaccharides to two cellulose columns is shown in Fig. 1. From these results, it was found that schizophyllan adheres quantitatively to cellulose I, but only sparingly to cellulose II, while xyloglucan, locust bean gum, and barley β-D-glucan are able to adhere quantitatively to both types of cellulose. The specific adhesion was not observed with the following polysaccharides: pullulan [29], cyclosophoran [24], pustulan [30], yeast mannan [29], arabinogalactan [21], xanthan gum [22], and succinoglycan [23]. Although the main chain of xanthan gum is the same as that of xyloglucan, the former has a side-chain with the acidic residues of D-glucuronic and pyruvic acids [22]. Carboxymethylcellulose also did not adhere to cellulose (data not shown). As all of the tested, water-soluble polysaccharides that adhered to cellulose are neutral, neutrality might be one of the necessary conditions for adhesion to cellulose.

When the adhesive polysaccharides (100 μg each) were mixed with 50 mg of cellulose I, the amounts of adhered schizophyllan, xyloglucan, locust bean gum, and barley β-D-glucan were 61, 89, 72, and 97 μg, respectively. When schizophyllan (100 μg) was mixed with 50 mg of curdlan gel, the bound amount was 36 μg. Furthermore, Langmuir plots were calculated to confirm the specificity in the binding of schizophyllan, xyloglucan, locust bean gum, and barley β-D-glucan to cellulose I. As shown in Fig. 2, their bindings could be expressed as Langmuir adsorption isotherms, suggesting that adhesion types of these polysaccharides were monomolecular layers with adhesion at the surface of the cellulose molecule [18,31].

Effects of solvents on binding strengths of schizophyllan and xyloglucan to cellulose I.—The binding data of water-soluble polysaccharides described above were conducted in distilled water. In order to make the degree of specific binding properties clear, the amounts of schizophyllan (100 μg) and xyloglucan (100 μg) binding to cellulose I (50 mg) in buffers covering wide pH range and 1 M NaCl were compared with amounts that were bound in distilled water. Buffer systems (100 mM) consisting of piperazine HCl–glycylglycine–NaOH

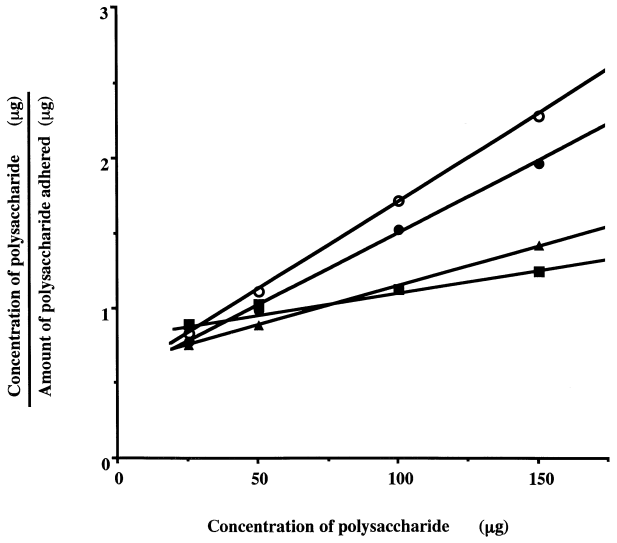

Fig. 2. Langmuir plots for the adhesion to cellulose I of schizophyllan (○), xyloglucan (●), locust bean gum (▲), and barley β-D-glucan (■).

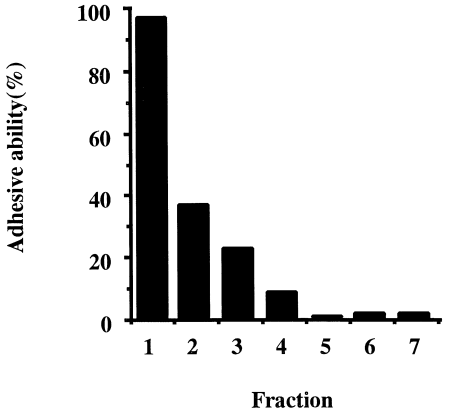

Fig. 3. Adhesive properties to cellulose I of native and depolymerized schizophyllans: 1, native; 2, **F1**; 3, **F2**; 4, **F3**; 5, **F4**; 6, **F5**; 7, **F6**.

for pH 5–10, Na_2HPO_4–NaOH for pH 11, and NaOH–KCl for pH 12–13 were used. The binding amounts of schizophyllan and xyloglucan in buffers of pH 5–11 and 1 M NaCl were almost same as that found in distilled water. The amounts of schizophyllan bound in buffers of pH 12 and 13 diminished 15 and 35%, respectively. The amounts of xyloglucan bound in buffers of pH 12 and 13 diminished 0 and 14%, respectively. From these results, it was demonstrated that these polysaccharides adhere firmly to cellulose. Furthermore, the bound polysaccharides could not be released completely without damage to the cellulose.

Properties of DS samples and their adhesive abilities to cellulose I.—Gel-permeation chromatography of the partial hydrolysate of schizophyllan resulted in the separation of six DS fractions, **F1**–**F6** [11]. The M_r values of **F1**, **F2**, **F3**, **F4**, **F5**, and **F6** measured in water were 96 000, 44 000, 22 000, 10 000, 4500, and 2400, respectively. The M_r values of these products in Me_2SO were estimated to be 28 000, 13 000, 6500, 4500, 2900, and 2200, respectively. From this data, it could be presumed that **F1**, **F2**, and **F3** exist in ordered conformations in water, while **F5** and **F6** do not, and **F4** might be a mixture of triple-helix and single strand. The adhesive abilities of DSs to cellulose are shown in Fig. 3. As the M_r of DS samples decreased, their adhesion to cellulose I also decreased [12]. Although the tendency was very similar to the adhesion of DS to curdlan gel, adhesion of comparatively high-molecular-weight DS samples to cellulose I appears to occur only about half as efficiently as their adhesion to curdlan gel.

X-ray diffraction patterns.—As shown in Fig. 1, xyloglucan, locust bean gum, and barley β-D-glucan adhere tightly to cellulose II, while schizophyllan does not adhere, indicating that their adhesive mechanisms are different. In order to understand the basis for the difference, the crystallinities of samples were analyzed by X-ray diffraction. The X-ray diffraction diagrams shown in Fig. 4 confirmed once more that the microcrystalline cellulose used for this study is cellulose I and that the cellulose treated with alkaline solution belongs to cellulose II [32]. It has been proposed that the irreversible change from cellulose I to cellulose II corresponds to different skeletal chain conformations and the chain-packing polarities (parallel and antiparallel, respectively) [33] for the

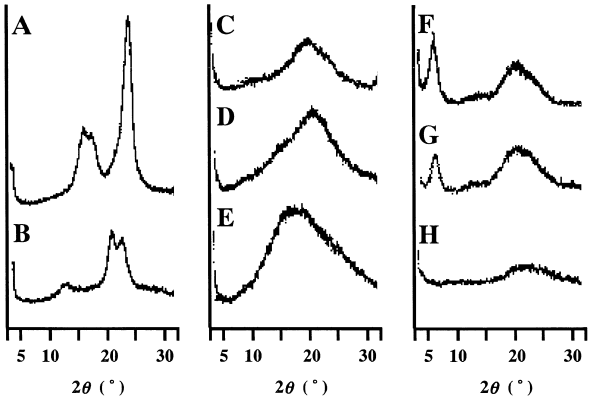

Fig. 4. X-ray diffraction patterns of cellulose I (A), cellulose II (B), xyloglucan (C), xanthan gum (D), locust bean gum (E), native schizophyllan (F), DS sample **F2** (G), and DS sample **F6** (H).

two polymorphs. The X-ray diffraction diagrams of schizophyllan, yeast glucan, and curdlan gel showed peaks near 6.4°, which corresponds to the triple-helical conformation [12], suggesting that the association of schizophyllan with cellulose might be related to their ordered domains. However, the diffraction patterns of xyloglucan and locust bean gum did not show any characteristic peaks and appeared similar to that of unbound xanthan gum (see Fig. 4). While there was insufficient sample of barley β-D-glucan for producing a clear picture, by X-ray diffraction, it was found that the diffraction pattern was similar to that of xyloglucan. The unbound DS sample **F7**, which is thought to exist in a single coil, did not show the characteristic peak near 6.4°.

Therefore, the adhesion of schizophyllan to cellulose I and curdlan gel has the common requirement for high-molecular-weight molecules with a triple-helical conformation. Thus, it is likely that adhesion of schizophyllan to cellulose proceeds by a different mechanism than that for the adhesion of xyloglucan and locust bean gum.

Properties of oligosaccharides from locust bean gum and their adhesion to cellulose I.—Oligosaccharides were obtained by digesting locust bean gum with cellulose (ONOZUKA RS). The average of degree of polymerization (dp) was estimated colorimetrically to be about 13. The elution pattern of the hydrolysate is shown in Fig. 5. The oligosaccharides (**F13**, **F14**, and **F15**) eluted in fractions 13, 14, and 15 were concentrated and analyzed by HPLC on a column of TSKgel Amide-80. The elution patterns are shown in Fig. 6 (I, II, and III). Fractions **F13**, **F14**, and **F15** were applied to a column of cellulose I and eluted with distilled water. Each eluate was concentrated and analyzed by HPLC (see IV, V, and VI in Fig. 6). Seven peaks

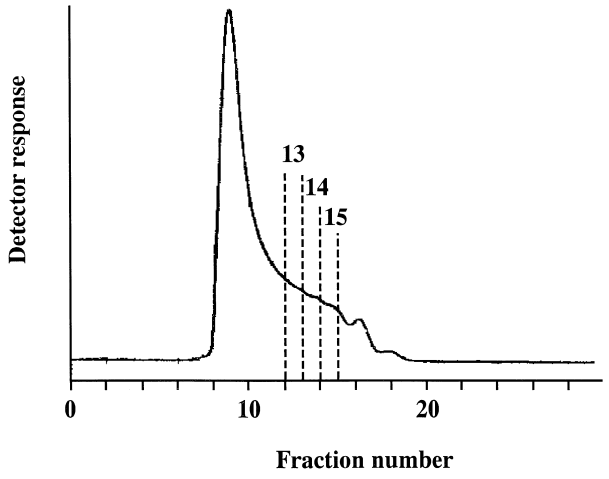

Fig. 5. Gel filtration on Toyopearl HW 40S of enzyme digest of locust bean gum.

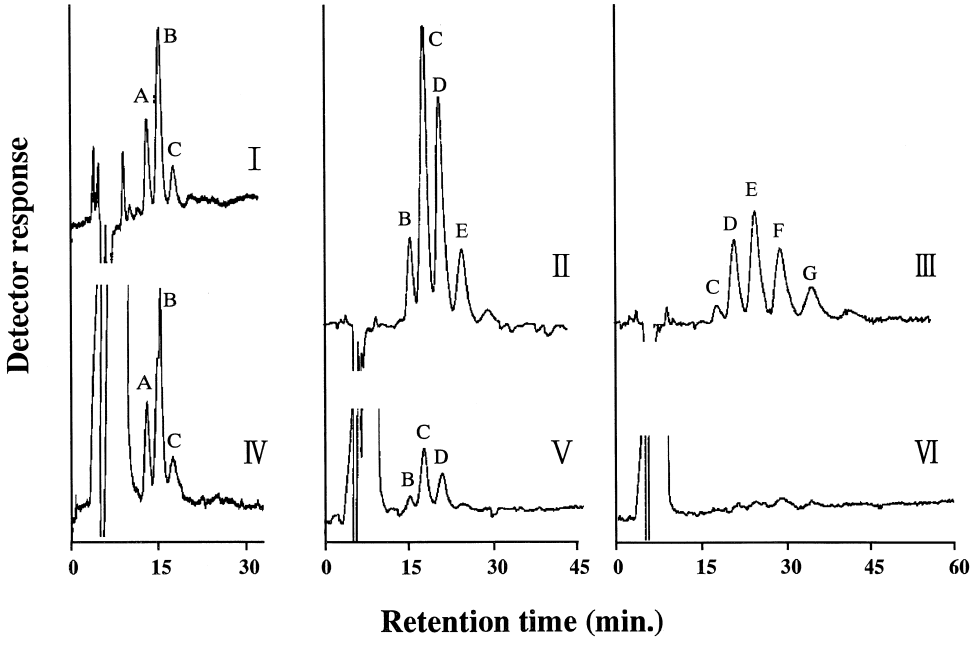

Fig. 6. HPLC chromatograms of oligosaccharides of locust bean gum. Elution patterns of fractions 13, 14, and 15 obtained in Fig. 5 are shown in I, II, and III, respectively. Samples of fractions 13, 14, and 15 were also applied to the cellulose I column and eluted with distilled water (not shown). The elution patterns of the material that did not bind to cellulose are shown in IV, V, and VI, respectively. The peaks detected in HPLC are marked A, B, C, D, E, F, and G in order of eluting sequence.

detected in Fig. 6 were marked A, B, C, D, E, F, and G and it was found that peak D bound comparatively to cellulose I and peak E did completely. By comparison to the elution positions of malto-oligosaccharides, the dp of peak E was estimated to be 6–7, which was also supported by the colorimetric method, suggesting that the main chain of peak E might be dp 5–6. Hayashi et al. reported that xyloglucan oligosaccharides, with $dp < 7$ did not bind to cellulose, indicating that the main chain of the xylooligosaccharide needs $dp > 4$ to adhere to cellulose [18]. These results suggested that the adhesion of locust bean gum to cellulose is similar to that of a xyloglucan.

Adhesion of chitosan and chito-oligosaccharides to celluloses.—The association of (1→4)-β-D-glycans with cellulose may depend on the conformation of the β-linkages between C-1 and C-4. As the linkage between C-1 and C-4 of chitosan is homologous to that of cellulose, it is expected that chitosan may also adhere to cellulose. However, chitosan is insoluble in water, and its adhesion to cellulose was examined under acidic conditions. Chitosan (1 mg) was dissolved in 0.1 M acetate buffer (1 mL, pH 4.0), applied to a cellulose column (2×15 cm) equilibrated with the buffer, and then eluted with the buffer (100 mL). Analyses of chitosan in the eluate revealed that 91 and 90% of chitosan applied were, respectively, retained to cellulose I and cellulose II, indicating a tight binding of chitosan to cellulose. As chito-oligosaccharides are water soluble, their adhesive properties were tested using a column packed with cellulose I. D-Glucosamine was eluted without adhesion, chitobiose and chitotriose were eluted at 75 and 25%, respectively, and chito-oligosaccharides longer than the tetraose adhered completely. From these results, it was found that chitosan is also a cellulose-binding polysaccharide.

4. Conclusion

Xyloglucan, locust bean gum, barley β-D-glucan, and chitosan may bind by a mechanism based on the complementary of the surfaces of these (1→4)-linked β-D-glycans to cellulose. Conversely, schizophyllan, a water-soluble (1→3)-β-D-glucan, adheres to cellulose by an entirely different mechanism that depends on the formation of a triple-helical form that is adopted only when the (1→3)-β-D-glucan has a high molecular weight.

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