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TEMPO-mediated oxidation of (1 → 3)-β-D-glucans

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ABSTRACT

The 2,2,6,6-tetramethylpiperidine-1-oxyl radical (TEMPO)-mediated oxidation was applied to water-insoluble (1 → 3)-β-D-glucans, paramylon and curdlan, to prepare water-soluble oxidized products. When the addition level of NaClO used as the primary oxidant was 15 mmol per gram of the polysaccharide in the combination with catalytic amounts of TEMPO and NaBr under aqueous conditions at pH 10, water-soluble TEMPO-oxidized products were obtained quantitatively within 100 min. ^{13}C NMR analysis of the TEMPO-oxidized products revealed that the C6 primary hydroxyl groups of both paramylon and curdlan were completely converted to carboxylate groups by the oxidation. Thus, new (1 → 3)-β-D-polyglucuronic acid sodium salts having almost homogeneous chemical structures can be obtained. The highly crystalline paramylon took longer time for the complete oxidation of the C6 primary hydroxyls to carboxylate groups than curdlan. However, remarkable depolymerization occurs on main chains during the oxidation, and the degrees of polymerization of the water-soluble TEMPO-oxidized products prepared from paramylon and curdlan were only 68 and 86, respectively.

1. Introduction

(1 → 3)-β-D-Glucans widely exist in living organisms such as plants, fungi, yeasts and some bacteria (Clarke & Stone, 1960; Manners, Masson, & Patterson, 1973). Both paramylon and curdlan are linear (1 → 3)-β-D-glucans, and insoluble in water at room temperature. Paramylon is present as granular form in the alga *Euglena gracilis*, and has high crystallinity of about 90%, whereas curdlan is an extracellular bacterial polysaccharide with low crystallinity of about 30% (Marchessault & Deslandes, 1979). Some (1 → 3)-β-D-glucans produced by fungi have branch structures, which sometimes lead to increases in water-solubility and have some bioactivities (Barbosa, Steluti, Dekker, Cardoso, & da Silva, 2003; Hirokawa et al., 2008; Johansson et al., 2000; Tada et al., 2007). Crystal and solid-state structures of dry and hydrated paramylon and curdlan have been studied by X-ray diffraction and solid-state ^{13}C NMR analyses, showing that these (1 → 3)-β-D-glucans have triple-helical structures (Chuah, Sarko, Deslandes, & Marchessault, 1983; Deslandes, Marchessault, & Sarko, 1980; Fyfe et al., 1984; Kiss, Roberts, Brown, & Triemer, 1988; Marchessault & Deslandes, 1979).

Many researches have been carried out on (1 → 3)-β-D-glucans over the past two decades mainly because of their biological behavior such as immunomodulation and anti-tumoral activities and the corresponding potential applications to the biomedical fields (Bohn & BeMiller, 1995; Kataoka, Muta, Yamazaki, & Takeshige, 2002; McIntire & Brant, 1998; Ooi & Liu, 2000; Vismar, Vestri, Frassanito, Barsanti, & Gualtieri, 2004; Wood, 1994). Even though (1 → 3)-β-D-glucans are promising natural polysaccharides having such biological activities, their water-solubilities are quite low. Particularly, crystalline and liner (1 → 3)-β-D-glucans such as native paramylon and curdlan are insoluble in water. Derivatizations such as partial carboxymethylation and sulfation of hydroxyl groups of paramylon or curdlan have been thus studied to increase their biological activities with increasing water-solubility (Gao et al., 2008; Ohya, Nishimoto, Murata, & Ouchi, 1994; Suzuki et al., 1991; Usui, Matsunaga, Ukai, & Kiho, 1997).

Meanwhile, not only substitution reactions of hydroxyl groups with carboxymethyl ether or sulfate ester groups but also oxidation of primary hydroxyls of polysaccharides to carboxylate groups can increase water-solubility. Recently, regioselective oxidation of C6 primary hydroxyls of water-soluble and water-insoluble polysaccharides using 2,2,6,6-tetramethylpiperidine-1-oxyl radical (TEMPO) and NaBr as catalysts under aqueous conditions has been developed, and this TEMPO-mediated oxidation has pioneered a new field of carbohydrate chemistry (Bragd, van Bekkum, & Besemer, 2004; De Nooy, Besemer, & van Bekkum, 1995; Desai & Blackwell, 2003). Especially, water-insoluble β-cyclodextrin, chitins and regenerated celluloses become water-soluble by the TEMPO/NaBr/NaClO oxidation system through partial or complete conversion of the C6 primary hydroxyls to carboxylate groups (Fraschini & Vignon, 2000; Isogai & Kato, 1998; Kato, Kaminaga, Matsuo, & Isogai, 2004; Muzzarelli, Muzzarelli, Cosani, & Terbojevich, 1999).

Although water-soluble (1 → 4)-β-D-polyglucuronic acids (cellouronic acids) consisting of anhydroglucuronic acid units alone

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can be obtained quantitatively from regenerated celluloses by the TEMPO-mediated oxidation under specific conditions, remarkable depolymerization occurs on cellulose chains during the oxidation. The degrees of polymerization (DP) of cellouronic acids prepared from regenerated celluloses with the original DP of 380 and 680 decreased to only about 40 (Isogai, Yanagisawa, & Isogai, 2009; Shibata, Yanagisawa, Saito, & Isogai, 2006). Such depolymerization may take place by β-elimination of glycoside bonds at the C6 aldehyde groups formed as intermediate structures under alkaline conditions (de Nooy, Besemer, van Bekkum, van Dijk, & Smit, 1996) and/or by some active species such as hydroxyl radicals formed *in situ* as side reactions (Shibata & Isogai, 2003).

In this study, therefore, the TEMPO-mediated oxidation was applied to paramylon and curdlan to prepare water-soluble (1 → 3)-β-linked polyglucuronic acids (Fig. 1), and chemical structures and molecular weights of the TEMPO-oxidized products thus obtained were analyzed in detail.

2. Materials and methods

2.1. Materials

Commercial paramylon and curdlan (Wako Pure Chemicals Co., Japan) were used as the starting (1 → 3)-β-D-glucans. TEMPO, sodium bromide, 12% sodium hypochlorite solution, and other reagents and solvents were of laboratory grade (Wako Pure Chemicals Co., Japan), and used without further purification. Distilled water of HPLC grade was purchased from Wako Pure Chemicals.

2.2. TEMPO-mediated oxidation

Curdlan or paramylon (2 g) was suspended in water (200 ml) at pH 10 containing TEMPO (0.032 g, 0.2 mmol) and sodium bromide (0.2 g, 2 mmol), and the suspension was stirred at room temperature and 500 rpm. TEMPO-mediated oxidation was started by adding a designed amount of the 12% NaClO solution. The oxidation was carried out in a beaker loosely covered with a wrapping film. The pH of the mixture was maintained to be 10 by adding 0.5 M NaOH using a pH stat until no NaOH consumption was observed. Then, the mixture was dialyzed with de-ionized water, and freeze-dried. Yields of the oxidized products, which were calculated based on their chemical structures, were about 90%.

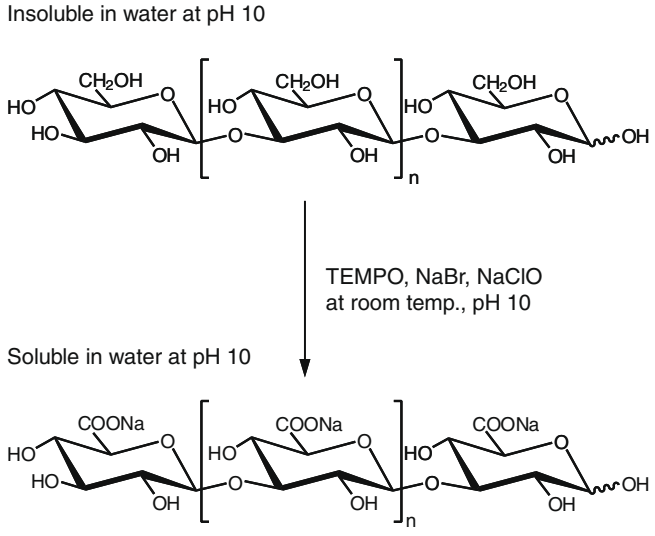

Fig. 1. TEMPO-mediated oxidation of (1 → 3)-β-D-glucan to prepared water-soluble (1 → 3)-β-D-polyglucuronic acid sodium salt.

2.3. Acid hydrolysis

Paramylon or curdlan (0.5 g) was suspended in 2 M HCl (50 ml), and the mixture was heated at 80 °C for 4 h. Then, the residue was washed thoroughly with water by centrifugation and finally with acetone. The acid-hydrolyzed products were air-dried and then vacuum-dried at 60 °C for 1 day. Yields of the acid-hydrolyzed products were 66% and 82% for paramylon and curdlan, respectively.

2.4. SEC-MALLS analysis

The original and acid-hydrolyzed paramylon and curdlan were dissolved in 1% LiCl/1,3-dimethyl-2-imidazolidinone (LiCl/DMI) at 0.1% concentration by heating the mixture at about 100 °C for 10 min. The (1 → 3)-β-D-glucan solutions thus obtained were then subjected to size-exclusion chromatography with multi-angle laser-light scattering method (SEC-MALLS, DAWN EOS, k = 690 nm; Wyatt Technologies, USA) using 1% LiCl/DMI as an eluent. A polystyrene-divinylbenzene copolymer gel (KD-806 M, 8 mm φ × 30 cm, Shodex, Japan) was used as the SEC column. The solvent and (1 → 3)-β-D-glucan solutions were filtered using 0.2 μm polytetrafluoroehtylene (PTFE) membranes (Millipore, USA) before use. Weight and number average molecular weight values were calculated from the SEC-MALLS data by ASTRA software (Wyatt Technologies, USA) with a specific refractive index increment (dn/dc) value of 0.087 ml/g for cellulose dissolved in 1% LiCl/DMI (Yanagisawa & Isogai, 2005), which consists of the same anhydroglucose units as those of (1 → 3)-β-D-glucans. A pullulan standard (M_w 22,800; Shodex, Japan) was exclusively used to normalize the MALLS photo-detectors (ASTRA for Windows user's guide version 4.90). On the other hand, water-soluble TEMPO-oxidized products were dissolved in 0.1 M NaCl at 0.1% concentration, and the solutions were subjected to the SEC-MALLS analysis using a SEC column for aqueous systems (DB-806MHQ, 8 mm φ × 30 cm, Shodex, Japan) and 0.1 M NaCl as the eluent. The dn/dc value of 0.125 ml/g for cellouronic acid (Isogai et al., 2009; Shibata et al., 2006) was used for the water-soluble TEMPO-oxidized products. Details of the SEC-MALLS system used and operation conditions were described elsewhere (Shibata et al., 2006; Yanagisawa & Isogai, 2005).

2.5. Other analyses

Carboxyl contents of the samples were determined by electric conductivity titration using 0.05 M NaOH (Saito & Isogai, 2004). Paramylon and curdlan were dissolved in deuterated dimethylsulfoxide (DMSO-d_6) at about 100 °C for several min, and ^{13}C NMR spectra of these solutions cooled at room temperature were recorded on an ALPHA-500 (JEOL, Japan) using tetramethylsilane (Aldrich, USA) as an internal standard for 0 ppm. Water-soluble TEMPO-oxidized products were dissolved in D_2O with 3-trimethylsilyl-2,2,3,3-d_4-propionic acid sodium salt (Aldrich, USA) used as an internal standard for 0 ppm, and the solutions were subjected to the ^{13}C NMR measurement. Data accumulation times were about 25,000. X-ray diffraction patterns of pellet samples (about 0.1 g for each) prepared using an apparatus for IR measurement at ca. 750 MPa for 5 min were collected on a RINT 2000 (Rigaku, Japan) at 0.01°/s from 3° to 30° of the diffraction angle 2θ with a Ni-filtered Cu Kα radiation at 40 kV and 40 mA. Crystallinity indices of the original polysaccharides and their TEMPO-oxidized products were determined from the ratio of the separated peak area due to crystalline diffractions to the total area from 3° to 30° of 2θ (Wada, Heux, & Sugiyama, 2004).

3. Results and discussion

3.1. TEMPO-mediated oxidation of paramylon and curdlan

Paramylon and curdlan were oxidized by designated amounts of NaClO used as the primary oxidant and catalytic amounts of TEMPO and NaBr in water at pH 10 and room temperature until no consumption of 0.5 M NaOH was observed. The stoichiometric amount of NaClO to complete oxidation of the C6 primary hydroxyl groups of the 1 g polysaccharide was 12.3 mmol. However, the maximum addition level of NaClO was set to be 15 mmol/g, *i.e.*, about 20% excess to the stoichiometric amount, being assumed that a part of NaClO added was evaporated from the reaction mixture loosely covered with a wrapping film during the oxidation.

As shown in Fig. 2, the paramylon granules 1–5 μm in size were swollen in water as the amount of NaClO added was increased in the oxidation. It seemed that the swelling or dispersion in water occurred from the outer side of paramylon granules by the oxidation. Even at the NaClO addition level of 7.5 mmol/g, the oxidized products were insoluble in water, and some granules still remained in the oxidized products without dissolution. On the other hand, the curdlan particles with crashed spherical shapes became water-soluble by the oxidation with NaClO of 7.5 mmol/g. Although the oxidized product prepared with NaClO of 3.8 mmol/g was insoluble in water, most of the original shapes of curdlan particles disappeared, and were strongly swollen in water (Fig. 3).

The relationships between the amount of NaClO added and either reaction time, ratio of C6 carboxylate groups formed, crystallinity index or water-solubility are listed in Table 1. Here, the carboxylate groups formed and determined by the conductivity titration were regarded as the C6 carboxylate groups formed by the oxidation. From both paramylon and curdlan, water-soluble oxidized products were obtained quantitatively by the TEMPO-mediated oxidation. Because all the C6 primary hydroxyl groups were converted to carboxylate groups by the oxidation with NaClO of 15 mmol/g, the reaction was likely to proceed almost stoichiometrically. In the case of curdlan, the oxidized product prepared even at the NaClO addition level of 7.5 mmol/g became water-soluble, where 62% of the C6 primary hydroxyl groups were converted to carboxylate ones.

The results in Table 1 are plotted in Fig. 4. Carboxylate groups were formed in similar manners to the NaClO addition level for both the TEMPO-oxidized paramylon and curdlan, although curdlan was slightly susceptible to the oxidation more than paramylon. Especially, the time required for complete consumption of NaClO added was twice as much as that for curdlan at the NaClO addition level of 15 mmol/g; paramylon is more restrictive to the oxidation probably because of its high crystallinity.

3.2. Structural analyses of the TEMPO-oxidized products

Fig. 5 shows ^{13}C NMR spectra of the original paramylon and curdlan dissolved in DMSO and their TEMPO-oxidized products dissolved in D_2O. ^{13}C NMR spectra of both paramylon and curdlan had six signals alone, showing that these polysaccharides are liner (1 → 3)-β-D-glucans without any branch structures. When the NaClO addition level was 15 mmol/g, no signal around 60 ppm due to the C6 primary hydroxyls of the original (1 → 3)-β-D-glucans was present in the spectra. Correspondingly, the signal due to the C6 carboxylate groups appeared at about 175 ppm. Thus, almost all C6 primary hydroxyls of paramylon and curdlan were converted to carboxylate groups by the TEMPO-mediated oxidation. When the NaClO addition level was 7.5 mmol/g, the oxidized curdlan had signals due to both the C6 primary hydroxyls and carboxylate groups in the NMR spectrum. The signal area ratio, the C6 carboxylate groups/(the C6 primary hydroxyl and C6 carboxylate groups), were about 0.7. This value is close to that of 0.62 in Table 1, which was determined by conductivity titration.

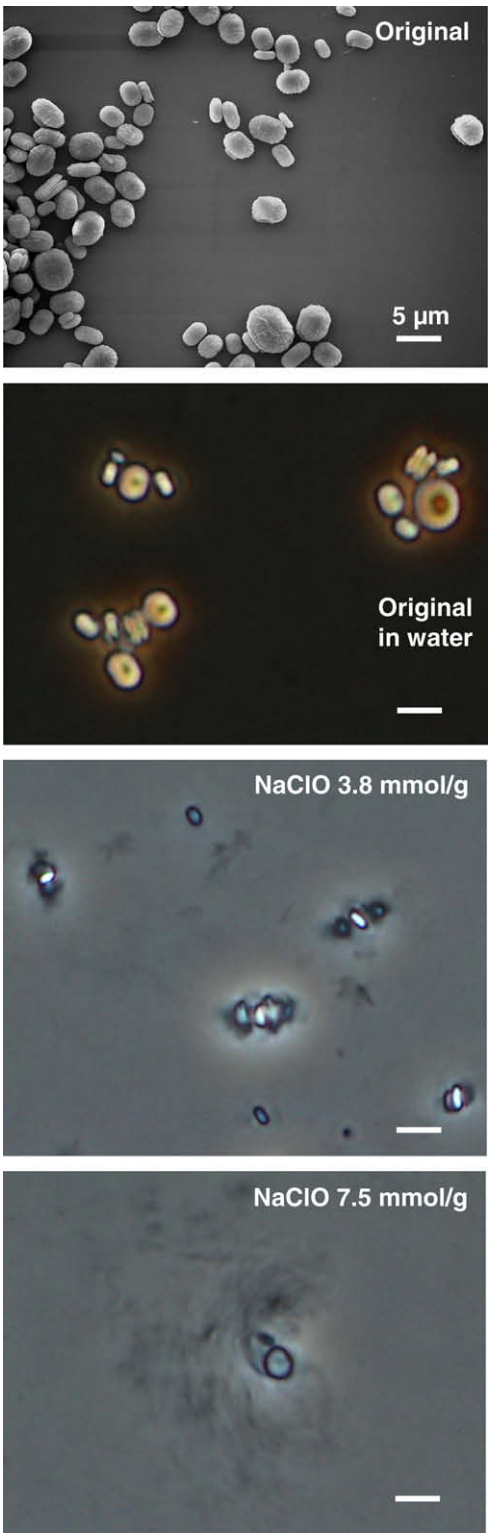

Fig. 2. SEM image of the original paramylon and phase-contrast microphotographs of paramylon and its TEMPO-oxidized products in water.

On the other hand, the oxidized products prepared with the NaClO addition level of 15 mmol/g had some additional small signals

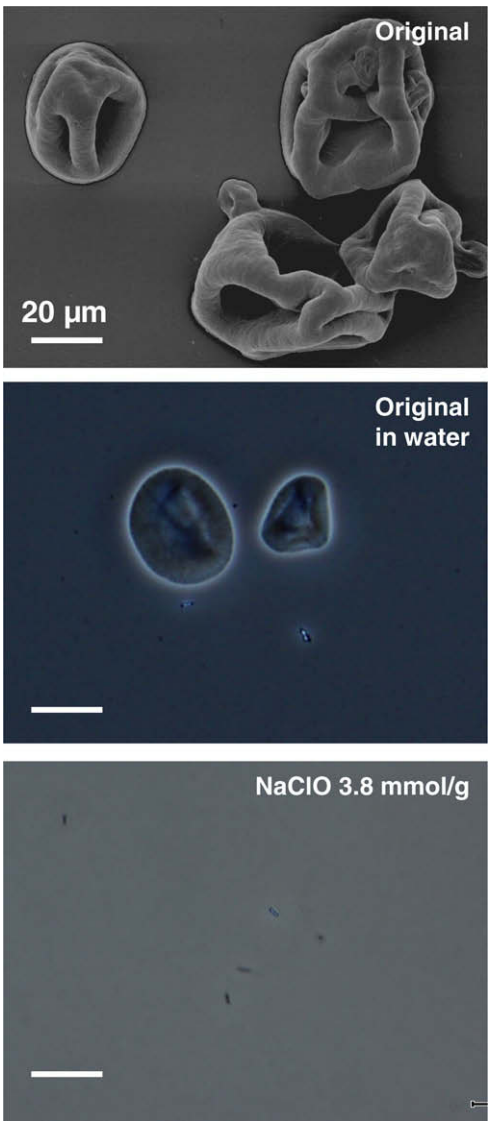

Fig. 3. SEM image of the original curdlan and phase-contrast microphotographs of curdlan and its TEMPO-oxidized product in water.

at about 71, 74, 94 and 96 ppm. The signals at 94 and 96 ppm may be ascribed to those due to anomeric carbons, indicating the occurrence of hydrolytic cleavage of glycoside bonds of the (1 → 3)-β-D-glucans during the reaction. Thus, some side reactions including oxidation at C2 or C3 hydroxyls to ketones and/or depolymerization are likely to take place in part on the glucan chains during the TEMPO-mediated oxidation under the conditions used in this study (Potthast, Rosenau, & Kosma, 2006; Röhring et al., 2001). However, it can be concluded that polyglucuronic acid Na salts mostly consisting (1 → 3)-β-linked anhydroglucuronic acid units can be prepared from paramylon and curdlan by the TEMPO-mediated oxidation.

X-ray diffraction patterns of the original (1 → 3)-β-D-glucans and their TEMPO-oxidized products are depicted in Fig. 6. Paramylon had crystallinity clearly higher than that of curdlan. The crystallinity of the oxidized products decreased with increasing the NaClO addition level. The high crystallinity of paramylon might have brought about high resistance to the oxidation, in comparison with curdlan. The TEMPO-oxidized products prepared with the NaClO addition level of 15 mmol/g, whose C6 primary hydroxyl groups were completely oxidized to carboxylate groups, had disordered structures, which was the same as those of (1 → 4)-β-D-polyglucuronic acids, *i.e.*, cellouronic acids, prepared from regenerated celluloses by the TEMPO-mediated oxidation.

Table 1
Reaction conditions of TEMPO-mediated oxidations of (1 → 3)-β-D-glucans and properties of the oxidized products.

Sample	NaClO added (mmol/g)	Reaction time (min)[a]	Ratio of C6 carboxylate groups	Crystallinity index	Water-solubility
Paramylon	0	0	0.01	0.47	–
Oxidized paramylon	3.8	10	0.22	0.05	–
Oxidized paramylon	7.5	20	0.43	0.00	–
Oxidized paramylon	15.0	100	1.00	0.00	+
Curdlan	0	0	0.02	0.28	–
Oxidized curdlan	3.8	5	0.28	0.02	–
Oxidized curdlan	7.5	15	0.62	0.00	+
Oxidized curdlan	15.0	50	1.00	0.00	+

[a] Time required for complete consumption of NaClO added.

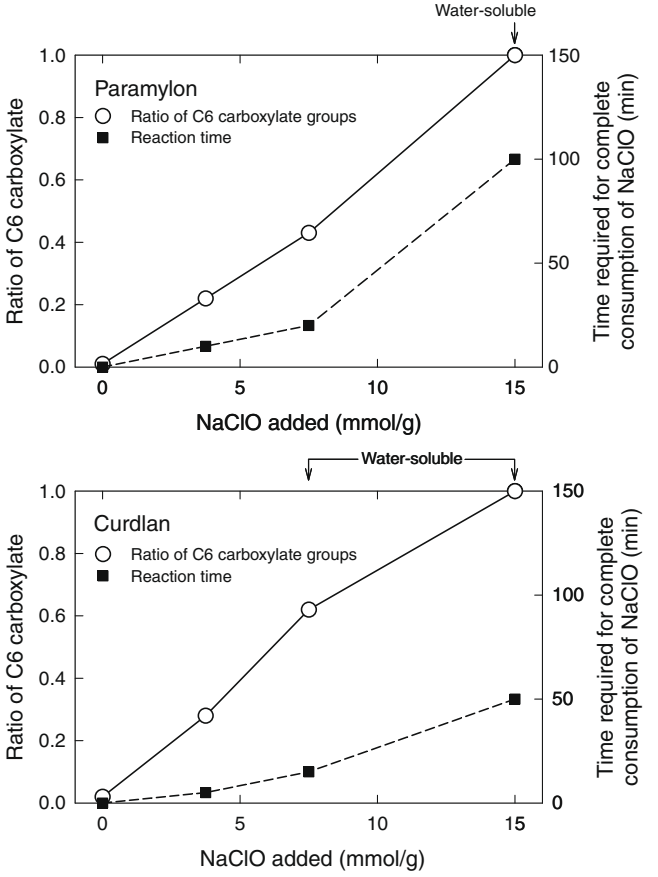

Fig. 4. Ratios of C6 carboxylate groups of TEMPO-oxidized paramylon and curdlan, and times required for complete consumption of NaClO added. See Table 1.

3.3. Molecular weights of the TEMPO-oxidized products

The TEMPO-oxidized products prepared from paramylon and curdlan with the NaClO addition level of 15 mmol/g were analyzed by SEC-MALLS to determine their molecular weight parameters. Because paramylon and curdlan as well as their acid-hydrolyzed products were soluble in 1% LiCl/DMI by heating around 100 °C and the dissolution states were maintained at room temperature after cooling, SEC-MALLS analysis of these polysaccharides were carried out using 1% LiCl/DMI as the solvent and eluent.

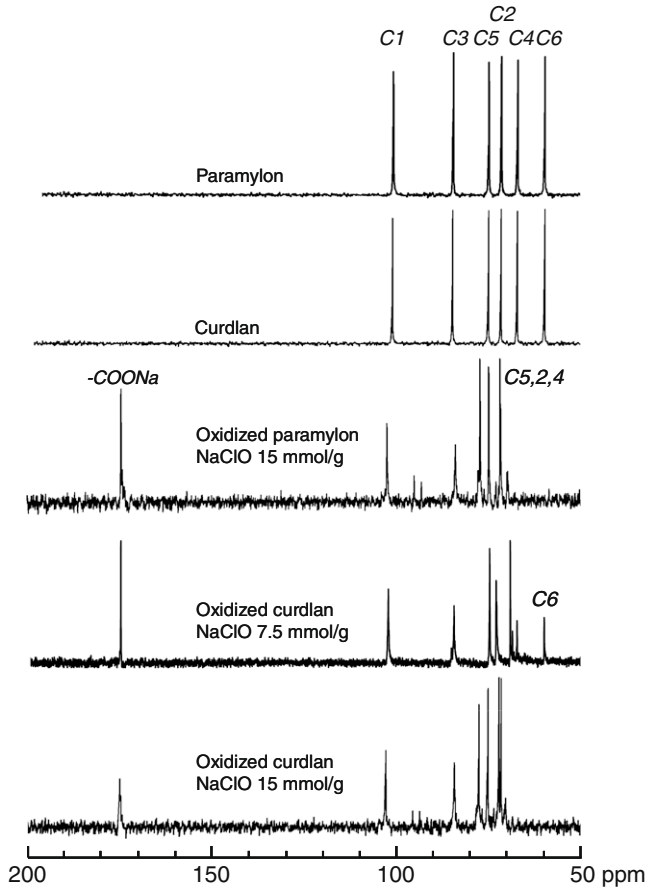

Fig. 5. ^{13}C NMR spectra of the original paramylon and curdlan dissolved in DMSO-d_6 and the corresponding waters-soluble TEMPO-oxidized products dissolved in D_2O.

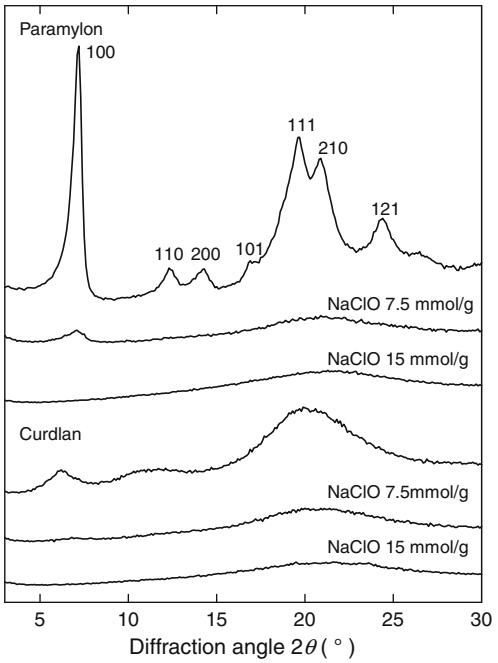

Fig. 6. X-ray diffraction patterns of paramylon, curdlan and their TEMPO-oxidized products.

The weight and number average DP values (DPw and DPn, respectively) of paramylon and curdlan and their acid-hydrolyzed products are listed in Table 2. The DPw of curdlan was as high as 6790, whereas that of paramylon was only 1670, which was about 1/4 of that of curdlan. On the other hand, the DPw of curdlan decreased to 270, *i.e.*, about 1/25 of the original value, by the acid hydrolysis, while the DPw of paramylon decreased to 1010. The high crystallinity of paramylon might have brought about such higher resistance to acid hydrolysis.

The DPw values of water-soluble TEMPO-oxidized paramylon and curdlan were only 68 and 86, respectively. Thus, remarkable depolymerization occurred on the TEMPO-oxidized products. In the case of TEMPO-mediated oxidation of regenerated celluloses at pH 10, DPw values of cellouronic acids obtained are about 40, which corresponds to their leveling-off DP values obtained by dilute acid hydrolysis (Isogai et al., 2009). In the case of paramylon and curdlan, however, the DPw values of the TEMPO-oxidized products were not related to those of the acid-hydrolyzed products. Other factors than the leveling-off DP may participate in the decreases in DP of the (1 → 3)-β-D-glucans during the oxidation. The DPw of the TEMPO-oxidized paramylon was lower than that of the TEMPO-oxidized curdlan, probably because the reaction time for the former was longer than that for the latter.

The DP distribution patterns of the original paramylon and curdlan, their acid-hydrolyzed products and TEMPO-oxidized products are shown in Fig. 7. All samples had normal distribution patterns without any additional peaks or shoulders. Even though the TEMPO-oxidized products had the DPw values of only 68–86, these products are expected to have certain levels of biological activities caused by the (1 → 3)-β-linked backbone chains and their water-solubility. These are our future research subjects.

4. Conclusions

Water-insoluble paramylon and curdlan turn to water-soluble products by catalytic oxidation using the TEMPO/NaBr/NaClO system in water at pH 10 and room temperature. When the NaClO addition level was 15 mmol per gram of the polysaccharide, almost all the C6 primary hydroxyl groups were oxidized to carboxylate groups within 100 min for both paramylon and curdlan by the oxidation. The highly crystalline paramylon took longer time for the complete oxidation of the C6 primary hydroxyls to carboxylate groups than curdlan. Thus, (1 → 3)-β-linked polyglucuronic acid sodium salts having almost homogeneous chemical structures can be obtained quantitatively by the TEMPO-mediated oxidation. However, significant depolymerization occurs on the main chains during the oxidation, and the DPw values of the water-soluble TEMPO-oxidized products prepared from paramylon and curdlan were only 68 and 86, respectively. The original DPw values of paramylon and curdlan determined by SEC-MALLS using 1% LiCl/DMI as the solvent and eluent were 1670 and 6790, respectively.

Table 2
Weight and number average molecular weights (M_w and M_n, respectively) and the corresponding DPw and DPn values of the original (1 → 3)-β-D-glucans, their acid-hydrolyzed products[a], and water-soluble TEMPO-oxidized products.

Sample	M_w	(DPw)	M_n	(DPn)	M_w/M_n
Paramylon	270,000	(1670)	212,000	(1310)	1.37
Curdlan	1,100,000	(6790)	890,000	(5500)	1.24
Acid-hydrolyzed paramylon	164,000	(1010)	99,300	(610)	1.66
Acid-hydrolyzed curdlan	43,300	(270)	29,900	(180)	1.45
TEMPO-oxidized paramylon	13,600	(68)	9920	(50)	1.28
TEMPO-oxidized curdlan	17,000	(86)	13,500	(68)	1.26

[a] Acid hydrolysis with 2 M HCl at 80 °C for 4 h.

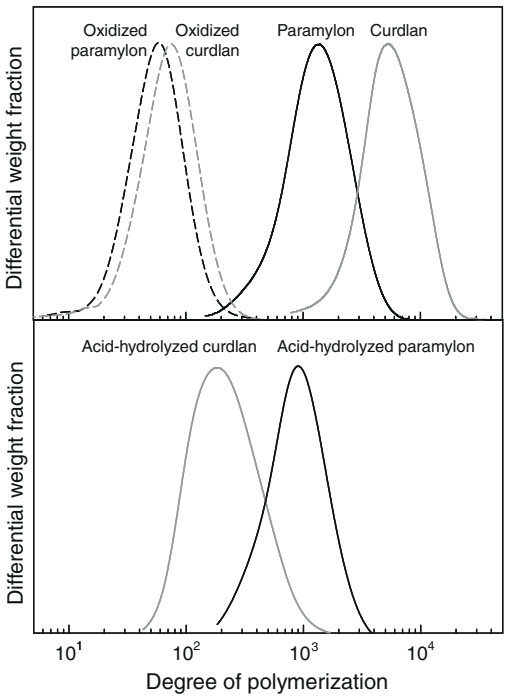

Fig. 7. DP distribution patterns of paramylon, curdlan, their acid-hydrolyzed products and water-soluble TEMPO-oxidized products.

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References

ASTRA for Windows user's guide version 4.90.

Barbosa, A. M., Steluti, R. M., Dekker, R. F., Cardoso, M. S., & da Silva, M. L. C. (2003). Structural characterization of Botryosphaeran: A (1 → 3,1 → 6)-β-D-glucan produced by the ascomyceteous fungus, *Botryosphaeria* sp.. *Carbohydrate Research, 338*, 1694–1698.

Bohn, J. A., & BeMiller, J. N. (1995). (1 → 3)-β-D-Glucans as biological response modifiers: A review of structure–functional activity relationships. *Carbohydrate Polymers, 28*, 3–14.

Bragd, P. L., van Bekkum, H., & Besemer, A. C. (2004). TEMPO-mediated oxidation of polysaccharides: Survey of methods and applications. *Topics Catalysis, 27*, 49–66.

Chuah, C. T., Sarko, A., Deslandes, Y., & Marchessault, R. H. (1983). Packing analysis of carbohydrates and polysaccharides. Part 14. Triple-helical crystalline structure of curdlan and paramylon hydrates. *Macromolecules, 16*, 1375–1382.

Clarke, A. E., & Stone, B. A. (1960). Structure of the paramylon from *Euglena gracilis*. *Biochimica et Biophysica Acta, 44*, 161–163.

De Nooy, A. E. J., Besemer, A. C., & van Bekkum, H. (1995). Highly selective nitroxyl radical-mediated oxidation of primary alcohol groups in water-soluble glucans. *Carbohydrate Research, 269*, 89–98.

de Nooy, A. E. J., Besemer, A. C., van Bekkum, H., van Dijk, J. A. P. P., & Smit, J. A. M. (1996). TEMPO-mediated oxidation of pullulan and influence of ionic strength and linear charge density on the dimensions of obtained polyelectrolyte chains. *Macromolecules, 29*, 6541–6547.

Desai, R. N., & Blackwell, L. F. (2003). TEMPO-mediated regiospecific oxidation of glycosides to glucuronides. *Synlett, 13*, 1981–1984.

Deslandes, Y., Marchessault, R. H., & Sarko, A. (1980). Triple-helical structure of (1 → 3)-β-D-glucan. *Macromolecules, 13*, 1466–1471.

Fraschini, C., & Vignon, M. R. (2000). Selective oxidation of primary alcohol groups of β-cyclodextrin mediated by 2,2,6,6-tetramethylpiperidine-1-oxyl radical (TEMPO). *Carbohydrate Research, 328*, 585–589.

Fyfe, C. A., Stephenson, P. J., Taylor, M. G., Bluhm, T. L., Deslandes, Y., & Marchessault, R. H. (1984). Hydration effects in the ^{13}C CP/MAS NMR spectra of solid (1 → 3)-β-D-glucans. *Macromolecules, 17*, 501–502.

Gao, F.-P., Zhang, H.-Z., Liu, L.-R., Wang, Y.-S., Jiang, Q., Yang, X.-D., et al. (2008). Preparation and physicochemical characteristics of self-assembled nanoparticles of deoxycholic acid modified-carboxymethyl curdlan conjugates. *Carbohydrate Polymers, 71*, 606–613.

Hirokawa, Y., Fujiwara, S., Suzuki, M., Akiyama, T., Sakamoto, M., Kobayashi, S., et al. (2008). Structural and physiological studies on the storage β-polyglucan of haptophyte *Pleurochrysis haptonemofera*. *Planta, 227*, 589–599.

Isogai, T., Yanagisawa, M., & Isogai, A. (2009). Degrees of polymerization (DP) and DP distribution of cellouronic acids prepared from alkali-treated celluloses and ball-milled native celluloses by TEMPO-mediated oxidation. *Cellulose, 16*.

Isogai, A., & Kato, Y. (1998). Preparation of polyuronic acid from cellulose by TEMPO-mediated oxidation. *Cellulose, 5*, 153–164.

Johansson, L., Virkki, L., Maunub, S., Lehto, M., Ekholm, P., & Varo, P. (2000). Structural characterization of water-soluble β-glucan of oat bran. *Carbohydrate Polymers, 42*, 143–148.

Kataoka, K., Muta, T., Yamazaki, S., & Takeshige, K. (2002). Activation of macrophages by linear (1 → 3)-β-D-glucans – Implications for the recognition of fungi by innate immunity. *The Journal of Biological Chemistry, 277*, 36825–36831.

Kato, Y., Kaminaga, J., Matsuo, R., & Isogai, A. (2004). TEMPO-mediated oxidation of chitin, regenerated chitin and *N*-acetylated chitosan. *Carbohydrate Polymers, 58*, 421–426.

Kiss, J. Z., Roberts, E. M., Brown, R. M., Jr., & Triemer, R. E. (1988). X-ray and dissolution studies of paramylon storage granules from *Euglena*. *Protoplasma, 146*, 150–156.

Manners, D. J., Masson, A. J., & Patterson, J. C. (1973). Structure of a β-(1 → 3)-D-glucan from yeast-cell walls. *The Biochemical Journal, 135*, 19–30.

Marchessault, R. H., & Deslandes, Y. (1979). Fine structure of (1 → 3)-β-D-glucans: Curdlan and paramylon. *Carbohydrate Research, 75*, 231–242.

McIntire, T. M., & Brant, D. A. (1998). Observations of the (1 → 3)-β-D-glucan linear triple helix to macrocycle interconversion using noncontact atomic force microscopy. *Journal of the American Chemical Society, 120*, 6909–6919.

Muzzarelli, R. A. A., Muzzarelli, C., Cosani, A., & Terbojevich, M. (1999). 6-Oxychitins, novel hyaluronan-like regiospecifically carboxylated chitins. *Carbohydrate Polymers, 39*, 361–367.

Ohya, Y., Nishimoto, T., Murata, J., & Ouchi, T. (1994). Immunological enhancement activity of muramyl dipeptide analogue CM-curdlan conjugate. *Carbohydrate Polymers, 23*, 47–54.

Ooi, V. E. C., & Liu, F. (2000). Immunomodulation and anti-cancer activity of polysaccharide–protein complexes. *Current Medicinal Chemistry, 7*, 715–729.

Potthast, A., Rosenau, T., & Kosma, P. (2006). Analysis of oxidized functionalities in cellulose. *Polysaccharides II, 205*, 1–48.

Röhring, J., Lange, T., Potthast, A., Rosenau, T., Sixta, H., & Kosma, P. (2001). Novel methods to determine carbobnyl functions in cellulosic substances. *Proc. Post-Symp. 11th Int. Symp* (pp. 94–97). Wood Pulping Chem., Grenoble, June 18–49.

Saito, T., & Isogai, A. (2004). TEMPO-mediated oxidation of native cellulose. The effect of oxidation conditions on chemical and crystal structures of the water-insoluble fractions. *Biomacromolecules, 5*, 1983–1989.

Shibata, I., & Isogai, A. (2003). Depolymerization of cellouronic acid during TEMPO-mediated oxidation. *Cellulose, 10*, 151–158.

Shibata, I., Yanagisawa, M., Saito, T., & Isogai, A. (2006). SEC-MALS analysis of cellouronic acid prepared from regenerated cellulose by TEMPO-mediated oxidation. *Cellulose, 13*, 73–80.

Suzuki, T., Ohno, N., Adachi, Y., Cirelli, A. F., Covian, J. A., & Yadomae, T. (1991). Preparation and biological activities of sulfated derivatives of (1 → 3)-β-D-glucans. *J. Pharmacrobio-Dyn., 14*, 256–266.

Tada, R., Harada, T., Nagi-Miura, N., Adachi, Y., Nakajima, M., Yadomae, T., et al. (2007). NMR characterization of the structure of a β-(1 → 3)-D-glucan isolate from cultured fruit bodies of *Sparassis crispa*. *Carbohydrate Research, 342*, 2611–2618.

Usui, S., Matsunaga, T., Ukai, S., & Kiho, T. (1997). Growth suppressing activity for endothelial cells induced from macrophages by carboxymethylated curdlan. *Bioscience, Biotechnology, and Biochemistry, 61*, 1924–1925.

Vismar, R., Vestri, S., Frassanito, A. M., Barsanti, L., & Gualtieri, P. (2004). Stress resistance induced by paramylon treatment in *Artemia* sp.. *Journal of Applied Physiology, 16*, 61–67.

Wada, M., Heux, L., & Sugiyama, J. (2004). Polymorphism of cellulose I family: Reinvestigation of cellulose IV. *Biomacromolecules, 5*, 1385–1391.

Wood, P. J. (1994). Evaluation of oat bran as a soluble fibre source—Characterization of oat β-glucan and its effects on glycemic response. *Carbohydrate Polymers, 25*, 331–336.

Yanagisawa, M., & Isogai, A. (2005). SEC-MALS-QELS study on the molecular conformation of cellulose in LiCl/amide solutions. *Biomacromolecules, 6*, 1258–1265.