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Oxidation process of water-soluble starch in TEMPO-mediated system

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Abstract

Water-soluble starch was oxidized with NaClO and catalytic amounts of 2,2,6,6-tetramethylpiperidinyloxy radical (TEMPO) and NaBr in water below 4 °C at pH 10.75, and the oxidation process was studied by NMR analysis of the oxidized products. The degree of oxidation at the C6 primary hydroxyl group was controlled stepwise from 0 to 100% by controlling the amount of NaClO added or the reaction time. It was possible to distinguish intermediate aldehyde and hemiacetal structures as well as carboxyl and alcohol groups at C6 by ^{1}H and ^{13}C NMR. In particular ^{1}H NMR spectra of incompletely oxidized products indicated that significant amounts of C6 hemiacetals were formed as intermediate structures. These hemiacetals were eventually oxidized to carboxyl groups by sufficient reaction time or sufficient amounts of reagents. Based on the composition ratios of C6 structures and NaOH consumption, a new scheme of the TEMPO-mediated oxidation for polysaccharides was proposed. Furthermore, ^{13}C NMR spectra of the oxidized products revealed that the α-1,6-glucoside bonds present in the original starch as branched structures were mostly cleaved during the oxidation, thus giving almost pure α-1,4-linked polyglucuronic acid.

Keywords: TEMPO; Starch; Oxidation; Aldehyde; Hemiacetal; NMR; H–H COSY; Intermediate; Nitroxyl radical

1. Introduction

Catalytic oxidation of polysaccharides using commercially available and stable nitroxyl radicals in aqueous media has opened new fields of polysaccharide chemistry. 2,2,6,6-Tetramethylpiperidine-1-oxyl radical (TEMPO) is one of the representative radical reagents for this oxidation. Nooy, Besemer, and Bekkum (1995) first applied the TEMPO-mediated oxidation to water-soluble polysaccharides such as potato starch, amylodextrin and pullulan. The results showed that in polysaccharides highly selective oxidation of C6 primary hydroxyl to carboxylic groups can be achieved in water at pH 10–11 with NaClO and catalytic amounts of TEMPO and NaBr. The corresponding polyuronic acids were thus obtained in high yields. Chang and Robyt (1996) applied this TEMPO-mediated oxidation to not only water-soluble but also water-insoluble polysaccharides such as cellulose and chitin. Their results also supported selective oxidation at primary hydroxyl groups, although degrees of oxidation or carboxyl contents of the products were inaccurate. When the TEMPO-mediated oxidation system was applied to β-cyclodextrin, only the primary hydroxyl was oxidized to a carboxyl group, and mono-carboxyl β-cyclodextrin Na salt was isolated from the mixture (Fraschini & Vignon, 2000).

Isogai and Kato (1998) were the first to prepare almost pure β-1,4-linked polyglucuronic acid sodium salt, i.e. cellouronic acid Na salt, quantitatively from either mercerized or regenerated cellulose by the TEMPO-mediated oxidation. In this case, the water-insoluble celluloses at the initial stage become water-soluble as the oxidation proceeds at the C6 primary hydroxyl group. The obtained cellouronic acid was hydrolyzed by crude cellulase to form glucuronic acid, and thus biodegradability was present (Kato et al., 2002). On the other hand, when native cellulose samples were subjected to the oxidation, only quite small amounts of carboxyl group were introduced to solid celluloses, and water-soluble polyuronic acid could not be obtained (Isogai & Kato, 1998; Kitaoka, Isogai, & Onabe, 1999; Tahiri & Vignon, 2000).

Kinetics of the TEMPO-mediated oxidation of methyl α-D-glucopyranoside were investigated by Nooy and Besemer (1995) using high-performance anion-exchange chromatography, and the results suggested that significant amounts of the hydrated aldehyde group, i.e. hemiacetals, were formed as intermediate structures during the oxidation process. However, the formation of

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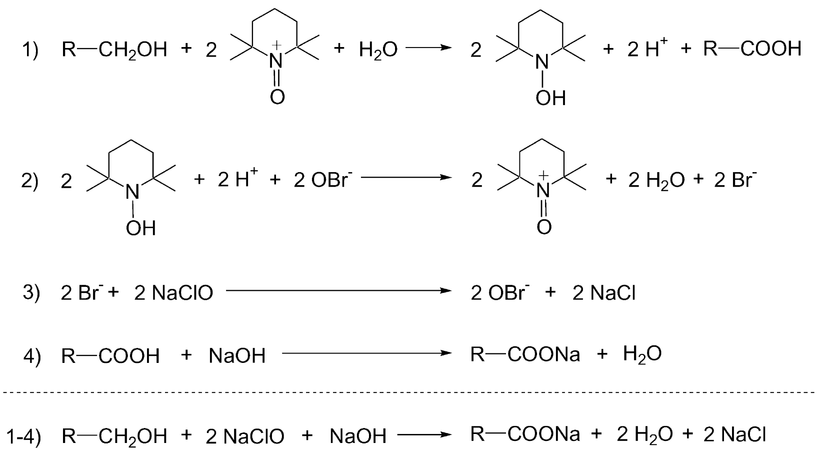

Fig. 1. Formulae for oxidation of primary hydroxyl groups of polysaccharides to carboxyl groups by the TEMPO-mediated oxidation.

the aldehyde group and/or hemiacetals in polysaccharides during oxidation has not yet been studied from a kinetic perspective. In the case of the TEMPO-mediated oxidation around pH 10–11, some depolymerization of polysaccharides owing to, for example, β-elimination, is inevitable (Isogai & Kato, 1998; Nooy, Besemer, Bekkum, VanDijk, & Smit, 1996), and thus reagents may be consumed to some extent also for side reactions.

In this study, therefore, water-soluble starch was oxidized stepwise by controlling the amount of NaClO or reaction time in the TEMPO-mediated oxidation, and the

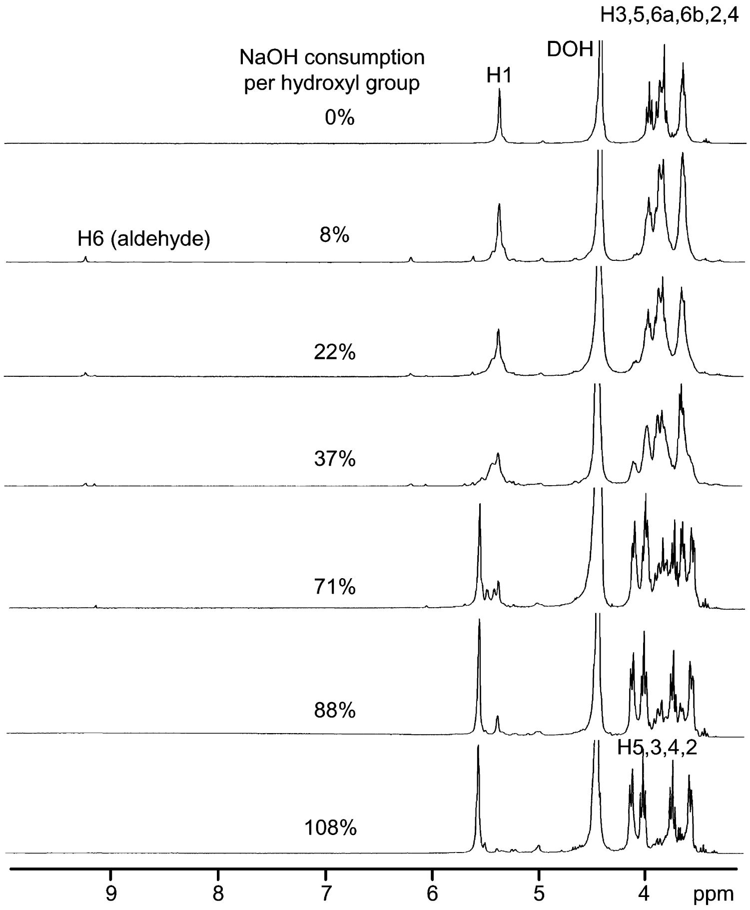

Fig. 2. ^{1}H NMR spectra of starch and its products oxidized to various degrees by the TEMPO–NaBr–NaClO system. The amount of NaOH consumed in each oxidation step is expressed as a percentage on the basis of the stoichiometric value for complete oxidation of the C6 hydroxyl group of starch to carboxyl group.

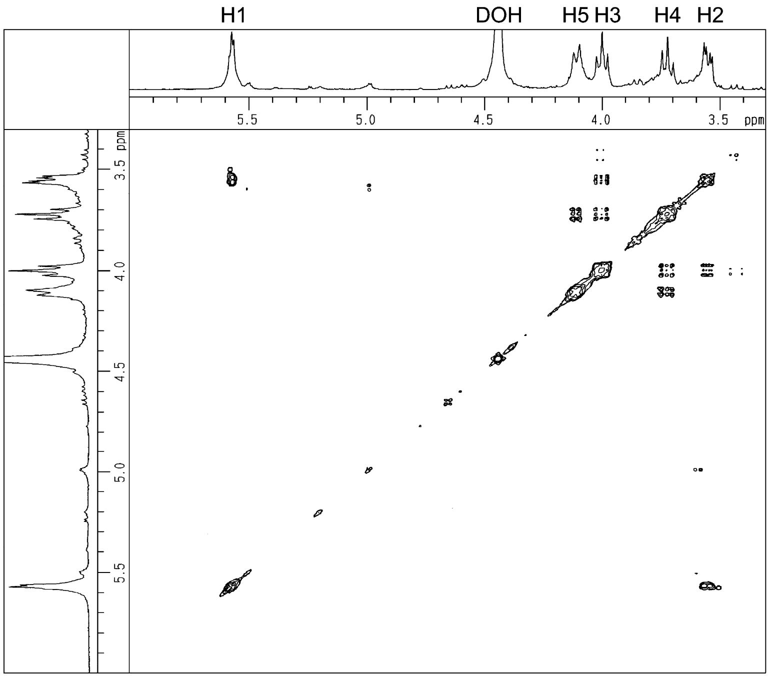

Fig. 3. H–H COSY spectrum of completely oxidized starch: α-1,4-linked polyglucuronic acid Na salt.

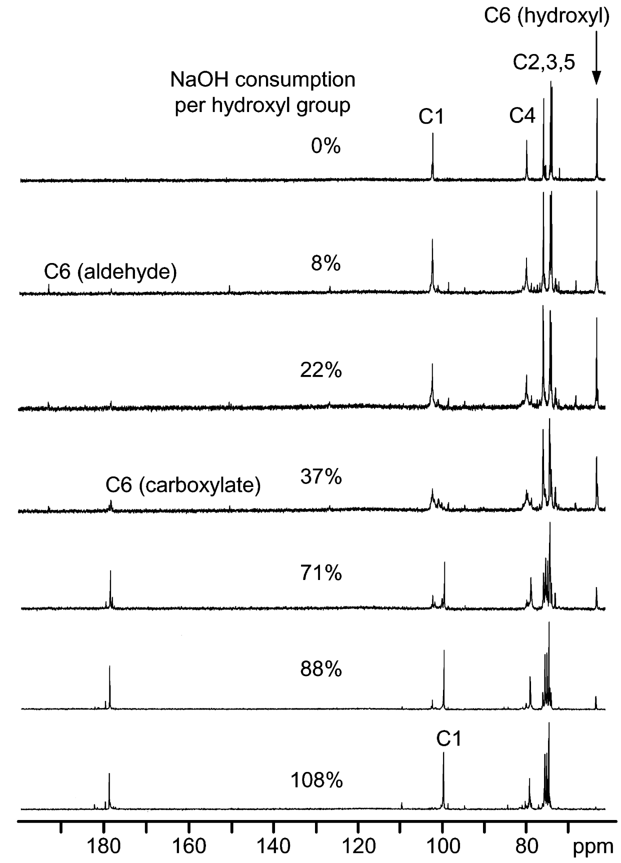

Fig. 4. ^{13}C NMR spectra of starch and its products oxidized to various degrees by the TEMPO–NaBr–NaClO system. See footnote in Fig. 2.

products at various stages of oxidation were isolated and subjected to structural analyses by NMR.

2. Materials and methods

2.1. Materials

A commercial water-soluble starch (Acros Co. Ltd, USA) was used without any purification treatments. A solution of 5% starch was prepared by heating at more than 80 °C for 30 min. The solution was then cooled down to room temperature, and subjected to the following oxidation. TEMPO, sodium bromide, about 11% sodium hypochlorite solution, and other chemicals and solvents were of laboratory grade (Wako Pure Chemicals Co. Ltd, Japan), and used without further purification.

2.2. Oxidation procedure

A solution of 5% starch (100 g) was put in a beaker and held at a temperature below 5 °C using an ice bath. TEMPO (0.048 g, 0.01 mol per anhydroglucose unit of starch) and sodium bromide (0.635 g, 0.2 mol per anhydroglucose unit of starch) were dissolved in 100 ml water. After TEMPO was completely dissolved, this solution was added to the starch solution at 5 °C. The pH of this solution was adjusted to 10.75 with 0.5 M NaOH. Then, a desired amount of sodium hypochlorite solution from 4.5 to 44 g was added to the starch solution, and the pH was maintained at 10.75 by continuous addition of 0.5 M NaOH using a pH-stat (automatic titrater, Hiramuma Co. Ltd, Japan). The volume

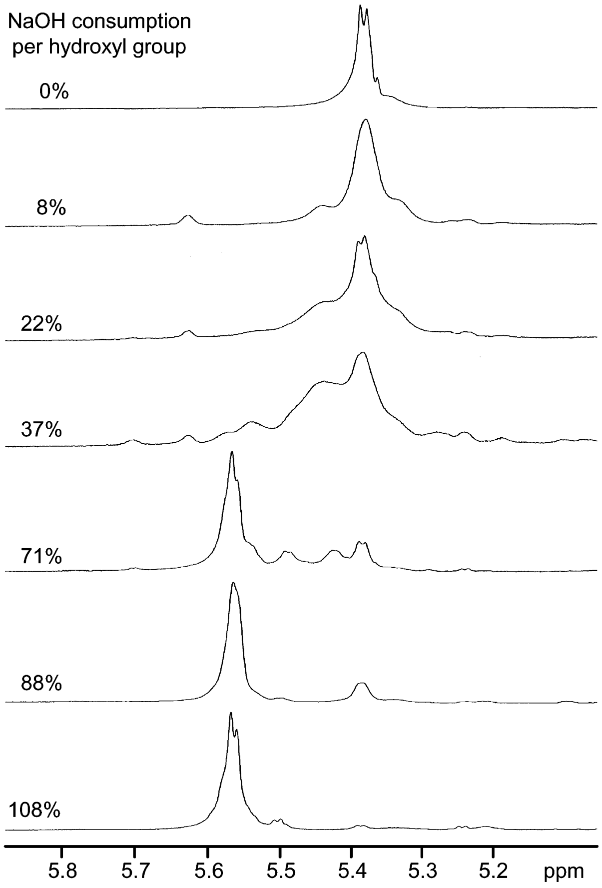

Fig. 5. Expanded ^{1}H NMR spectra of starch and its oxidized products in the range from 5.2 to 5.8 ppm.

of the 0.5 M NaOH solution added was monitored. The degree of oxidation was controlled by the following two methods: (A) a desired amount of NaClO insufficient for complete oxidation of the primary hydroxyl group of starch was added to the starch solution containing TEMPO and NaBr. The total volume of 0.5 M NaOH consumed to maintain pH 10.75 was noted after a plateau level of the 0.5 M NaOH consumption was attained; and (B) the amount of NaClO required for complete oxidation of primary hydroxyl group of starch, i.e. two moles of NaClO per mole of primary hydroxyl group of starch, was added at once, and a part of the solution was sampled several times, before the complete oxidation was attained. The 0.5 M NaOH consumptions at these sampling times were noted. The oxidation in the sampled solution was immediately quenched by adding ethanol to the solution.

The incompletely oxidized products thus obtained were precipitated by adding ethanol to the sampled solution at a ratio of 3:1, and repeatedly washed with water/acetone (1:7 by weight) by centrifugation until no Cl^- ion was detected in the supernatant. The wet sample was then washed with acetone followed by drying in vacuum at 45 °C for 1 day.

2.3. Analyses

^{13}C and ^{1}H NMR and H–H COSY spectra of the original water-soluble starch and the oxidized products were collected as their 5% solutions in D_2O on a JEOL JNM-LA400 at 60 °C using normal data accumulation modes. As an internal standard, 3-(trimethylsilyl)propionic-2,2,3,3-d_4 acid Na salt was used. Each peak area in ^{1}H NMR spectra was calculated after deconvolution using Lorentzian functions.

3. Results and discussion

3.1. ^{1}H NMR spectra of the oxidized products

The TEMPO-mediated oxidation of primary hydroxyl to carboxyl groups can be expressed according to the stoichiometric formulae in Fig. 1. Consequently, two moles of NaClO and one mole of NaOH are consumed for the oxidation of one mole of primary hydroxyl group, according to this scheme. Stepwise oxidation using the TEMPO–NaBr–NaClO system was applied to water-soluble starch to obtain partially oxidized products, and the relationships between NaOH consumption and carboxyl groups formed in the products were studied.

Fig. 2 illustrates ^{1}H NMR spectra of the oxidized products. As the degree of oxidation increased, clear changes in NMR patterns were observed, especially in the range of 5.2–5.7 ppm due to anomeric protons, as discussed later. Small signals due to C6 aldehyde protons were detected at 9.3 ppm for the oxidized products at consumptions of NaOH of 8–71% of the stoichiometric value although their quantities were less than 2% of the total C6. When 108% NaOH of the stoichiometric value for complete oxidation was consumed, almost complete oxidation of the C6 primary hydroxyl group of starch was attained and the α-1,4-linked polyglucuronic acid Na salt was then obtained. Thus, the NaOH consumption roughly proceeds according to the formulae in Fig. 1. Slightly excess NaOH (ca. 8%) must have been consumed for depolymerization, which is known to occur in polysaccharides to some extent during the oxidation (Isogai & Kato, 1998; Nooy et al., 1996). For assignment of C–H protons of α-1,4-linked polyglucuronic acid obtained at the 108% NaOH consumption in Fig. 2, a H–H COSY spectrum was measured (Fig. 3). The signals around 3.56, 3.72, 4.00, and 4.10 ppm were successfully ascribed to the ring protons H2, H4, H3, and H5, respectively, of the product.

3.2. ^{13}C NMR spectra of the oxidized products

Fig. 4 shows ^{13}C NMR spectra of the oxidized products. The signal due to the C6 primary hydroxyl group at 63.4 ppm gradually decreased and that due to the C6 carboxylate group at 178.6 ppm increased in turn

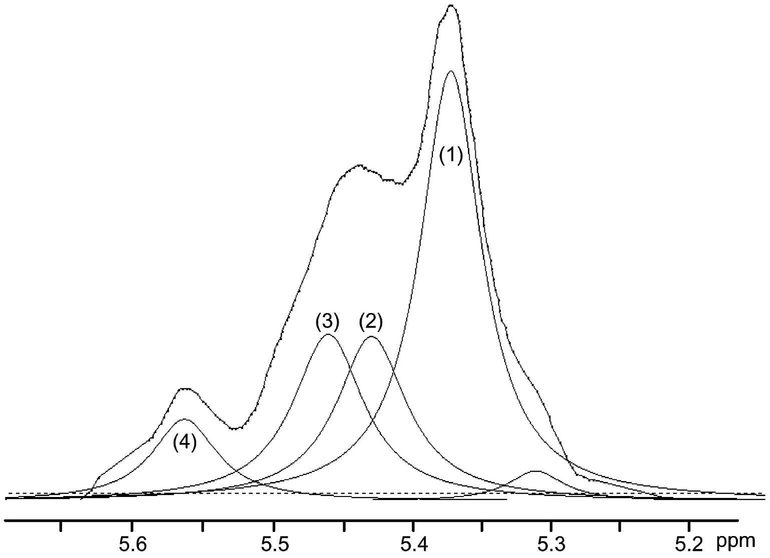

Fig. 6. An example of peak deconvolution of 1H NMR spectra in Fig. 5. (1): C1–H protons of glucose units (○); (4): C1–H protons of glucuronic acid units (□). Assumption (A) (2) + (3): C1–H protons of intermediates (△); (1) + (2) + (3) + (4) = 100% (see Fig. 7). Assumption (B) (2) + (3): C1–H protons of intermediates (△) and C6–H protons of hemiacetals; (1) + ((2) + (3))/2 + (4) = 100% (see Fig. 8); Marks ○, △ and □ correspond to those in Figs. 7 and 8.

as the oxidation proceeded. Small signals due to the C6 aldehyde group were detected in the oxidized products at 8–37% NaOH consumptions. These results revealed that aldehyde groups were clearly present in the incompletely oxidized products, although their quantities were less than 2% of the total C6. The oxidized product at the 108% NaOH consumption had an NMR pattern of almost pure α-1,4-linked polyglucuronic acid Na salt, which had no signal due to the C6 hydroxyl group.

In the ^{13}C NMR spectrum of the original starch, a signal due to C6, which formed α-1,6-glucoside bonds as branch structures, was observed at 72 ppm (Yoshizawa, Isogai, & Onabe, 1998). However, this signal disappeared in the NMR spectrum of the completely oxidized product at 108% NaOH consumption. This result indicates that the α-1,6-glucoside bonds present in the original starch are mostly cleaved and thus almost pure α-1,4-linked polyglucuronic acid having nearly no branch structures can be obtained by the TEMPO-mediated oxidation.

As the oxidation proceeded, the signal at 102 ppm due to C1 of glucose unit decreased, and that at 100 ppm due to C1 of glucuronic acid Na salt unit increased in turn. Signals due to C4 and other ring carbons also changed by the oxidation, although the chemical shift of C1 gave the greatest difference between the two units.

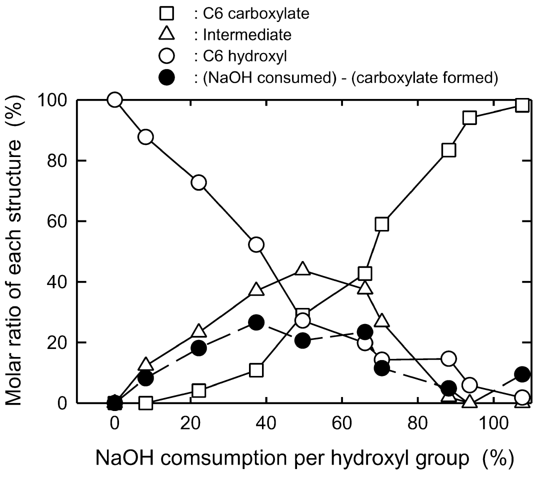

Fig. 7. Relationships between NaOH consumption and molar ratio of each structure in oxidized products. The intermediate protons between the anomeric protons due to C6 carboxyl and hydroxyl group units in Fig. 5 are postulated to be anomeric protons due to C6 hemiacetal unit.

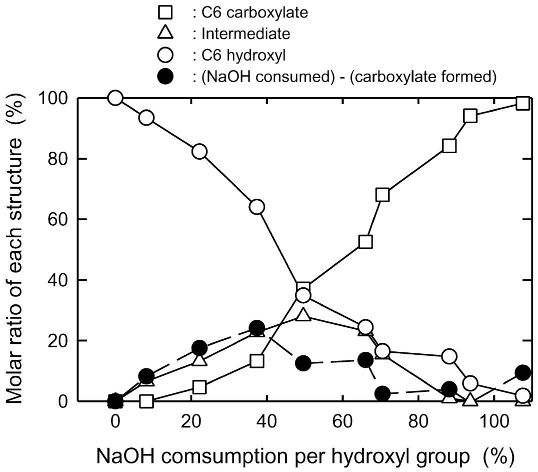

Fig. 8. Relationships between NaOH consumption and molar ratio of each structure in oxidized products. The intermediate protons between the anomeric protons due to C6 carboxyl and hydroxyl group units in Fig. 5 are postulated to be anomeric protons due to C6 hemiacetal unit and C6–H protons forming hemiacetals.

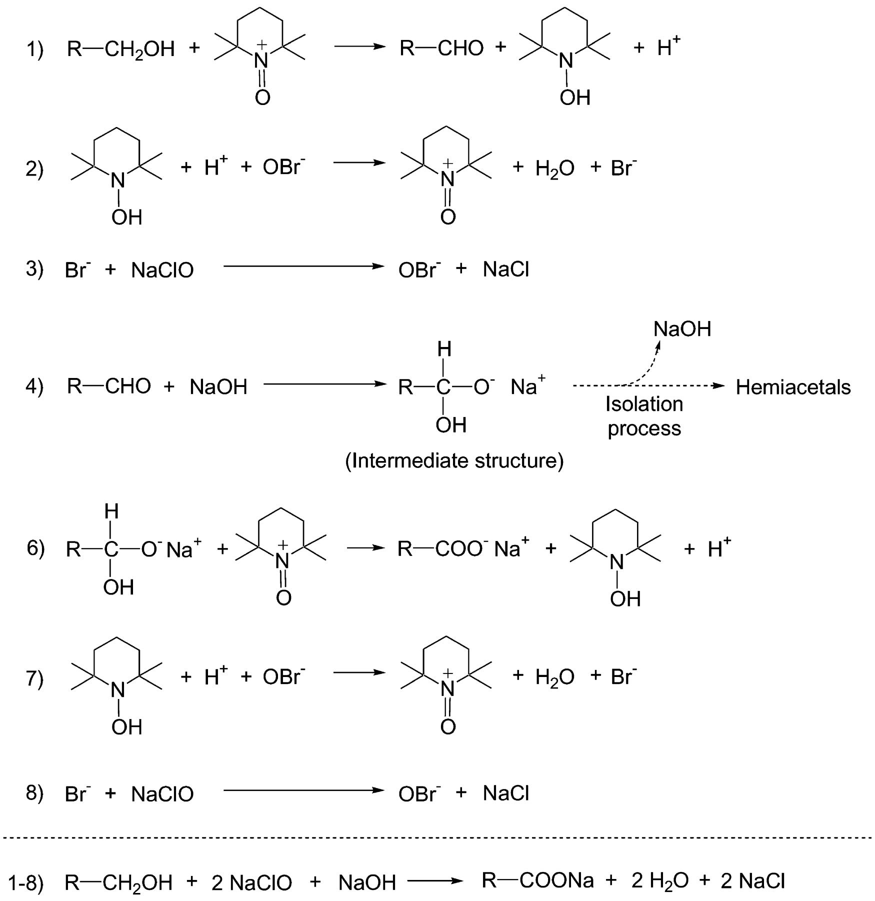

Fig. 9. Oxidation scheme of primary hydroxyl groups of polysaccharides to carboxyl ones by the TEMPO-mediated system.

3.3. Formation of intermediate structures during the oxidation

A more detailed analysis of the oxidized products was curried out using the patterns of anomeric protons in the expanded ^{1}H NMR spectra (Fig. 5). Some complicated signals other than the anomeric protons of glucose and glucuronic acid Na salt units at 5.37 and 5.57 ppm, respectively, were observed between these two signals for the incompletely oxidized products.

There are several possibilities for the assignments of these unknown signals. The ^{1}H NMR spectrum of mono-carboxyl β-cyclodextrin reported by Fraschini and Vignon (2000) supported the idea that the chemical structures of C6 in the adjacent unit had no influence on the chemical shifts of anomeric protons of the successive unit. The following two possibilities then remain for the assignment: (A) the unknown signals are due to anomeric protons, whose C6 positions form hemiacetals, (B) the unknown signals consist of two kinds of protons, anomeric protons, whose C6 positions form hemiacetals, and C6–H protons forming hemiacetals. Here, hemiacetals include hydrated C6 aldehydes together with the hemiacetals formed between C6 aldehyde and other hydroxyl groups of starch. Of course, it might be possible that a part of these anomeric protons and C6 hemiacetal protons overlapped in part with the signal at 5.37 or 5.57 ppm. Based on the above assumptions A, B and others, each ^{1}H NMR spectrum in the range from 5.2 to 5.7 ppm was deconvoluted using Lorentzian functions. One example is depicted in Fig. 6.

The relationships between the NaOH consumption and molar ratio of each postulated structure were plotted in Figs. 7 and 8 on the above two assumptions A and B, respectively. Other possibilities such as the aforementioned overlapping of signals were excluded, because, in such cases, some of the calculated ratios of the intermediate components became negative values around −40. In both Figs. 7 and 8, the molar ratio of the C6 hydroxyl group decreased roughly in proportion with the NaOH consumption from 0 to 108%. However, there are clear discrepancies between the NaOH consumptions and amounts of carboxylate formed in the incompletely oxidized products (filled circles in Figs. 7 and 8), suggesting that NaOH was consumed for the oxidation of the C6 primary hydroxyl group of starch but not for the formation of the C6 carboxylate in these regions of NaOH consumptions.

It is plausible, therefore, that the NaOH consumption corresponds to the formation of intermediate structures, i.e. hemiacetals. In fact, in both figures, the patterns of the assumed plots (open triangles) roughly correspond to those of the differences between the NaOH consumption and the ratio of the C6 carboxylate formed, although the aforementioned assumption B seems to be more suitable. These patterns for the formation of intermediate hemiacetals during the TEMPO-mediated oxidation process are similar to those reported for methyl glucoside (Bragd, Besemer, & Bekkum, 2000; Nooy & Besemer, 1995). In any cases, these results indicate that significant amounts of aldehydes, most of which form hemiacetals in the aqueous oxidation media, are formed at the C6 position of starch as intermediate structures, and NaOH is consumed during the formation process of these intermediate structures. Complete oxidation to the C6 carboxylate is then achieved through these intermediate structures by sufficient reaction time or sufficient amounts of reagents. On the basis of the obtained results, more detailed scheme of the TEMPO-mediated oxidation of the C6 primary hydroxyl group of starch is illustrated in Fig. 9.

Kitaoka et al. (1999) reported that handsheets prepared from bleached kraft pulp treated by the TEMPO-mediated oxidation had unusually high wet tensile strength without any additives. It is well known that dialdehyde starch, which form intra- and inter-molecular hemiacetals at the C2 and C3 aldehydes, has been used as a wet-strength additive in practical papermaking. The results reported by Kitaoka et al. therefore support the supposition that aldehyde groups are formed as intermediate structures in the partially oxidized pulps and the formation of hemiacetal bonds between the fibers during papermaking process brings about the remarkable increase in wet tensile strength.

4. Conclusion

During the TEMPO-mediated oxidation process of starch, a significant amount of the C6 aldehyde groups, which form hemiacetals with either water or residual hydroxyl groups of starch, are likely to form as intermediate structures. Consumption of NaOH during the oxidation at pH 10.75 occurs at this stage of C6 aldehyde or hemiacetal formation. Further oxidation by sufficient amounts of reagents or sufficient reaction time leads to the complete oxidation of the intermediate structures to the C6 carboxylic acid Na salt. Besides this selective oxidation of the C6 primary hydroxyl group, most α-1,6-glucoside bonds present in the original starch as branch structures are cleaved during the oxidation, thus consequently giving almost pure α-1,4-linked polyglucuronic acid Na salt.

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