Preparation and characterization of a novel chitosan-based hydrogel using sucrose as a cross-linking agent

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Oxidized sucrose is expected to be applied as a non-toxic and highly reactive cross-linking agent in biomaterials such as proteins and polysaccharides. The purpose of this study was to analyze the structure of oxidized sucrose in detail and to evaluate its performance as a cross-linking agent for polysaccharides. FT-IR and NMR spectroscopic analysis of the oxidized sucrose revealed their precise structures at first time. The oxidized sucrose reacted with carboxymethyl chitosan (CMC) dissolved in water without any catalyst to form hydrogels. The physical and thermal properties of the hydrogel improved with increasing the feed amount of oxidized sucrose in the reaction mixture, indicating that the oxidized sucrose is suitable cross linking agent for CMC. In addition, the oxidized sucrose reacted with neat chitosan to form hydrogel without catalyst. From these observations, oxidized sucrose is useful and convenient cross-linking agent to prepare hydrogels from polysaccharides containing amino sugar residues
Key words: Cross-linking agent, Oxidized sucrose, Chitosan, Hydrogels, Amino sugar

1. INTRODUCTION

Polysaccharide hydrogels are attracting attention as biomaterials such as carriers for drug delivery systems due to their high biocompatibility [1–3]. However, many of the cross-linking agents required for gel formation are generally highly toxic, limiting their use for biological applications [4]. On the other hand, oxidized sucrose, which is a sucrose oxide, is known as a non-toxic and highly reactive reagent due to the presence of up to four aldehyde groups in its structure. Because of these characteristics, oxidized sucrose is expected to be applied as a cross-linking agent for biomaterials such as proteins and polysaccharides. However, the exact structure of oxidized sucrose has not been elucidated due to the formation of various intermediates during its production [5]. The detailed reaction mechanism of oxidized sucrose with these biomaterials has also not been clarified, despite the fact that oxidized sucrose has the excellent feature of being safe for the environment. In order to solve these problems, in this study, FT-IR and NMR were used to elucidate the detailed structure of oxidized sucrose containing effective aldehyde content, the formation of crosslinking reaction with carboxymethyl chitosan (CMC), and the reaction mechanism of chitosan as crosslinking agents.

2. EXPERIMENTAL

2.1 Materials

CMC (DS = 0.84) was purchased from Cosmo Bio Co., Ltd., Japan. Chitosan (95% of deacetylation degree) was kindly provided from Hokkaido Soda Co., Ltd., Japan. Other chemicals were of high purity, and all solutions were prepared with deionized water.

2.2 Preparation and structural characterization of oxidized sucrose

Oxidized sucrose was prepared according to the previous report. Briefly, to 200 mL of water in which 6.00 g of sucrose was dissolved, 11.25 g of sodium periodate was added, and the mixture was stirred at room temperature for 26 hours. Then, 6.42 g of barium chloride dehydrate was added and the mixture was stirred at 5°C for 1 hour. It was filtered and the byproducts in the filtrate were removed using an ion exchange resin. The resulting oxidized sucrose was subjected to structural analysis using FT-IR and NMR. The amount of aldehyde in the oxidized sucrose was also determined by titration method.

2.3 Aldehyde determination

Hydroxylamine hydrochloride 8.69 g was dissolved in 75 mL of pure water. A methyl orange solution 3

mL was added dropwise and the volume was fixed at 500 mL. In addition, 0.1 g of oxidized sucrose was dissolved in 25 mL of the above solution and stirred for 2 hours. The solution was then titrated with 0.100 mol/L NaOH. Aldehyde concentration was calculated using the following equation.

$$\Delta V \times 0.001 \times n\mathrm{NaOH} = n\mathrm{CHO},$$

where ΔV is the volume of NaOH consumed (in mL), nNaOH is the molar concentration of NaOH (in mol/L), and nCHO is the molar concentration of CHO (in mol/L). nCHO indicates the number of moles of aldehyde groups on the oxidized sucrose. Wo indicates the mass (g) of oxidized sucrose [3].

2.4 Preparation of CMC hydrogels (CMCG)

Oxidized sucrose (36.5 mg) was added to a 6-mL of aqueous CMC solution (6 mL), and the reaction mixture was stirred at 298 K for 30 min to obtain the CMCG 1. Similarly, by changing the feed amount of oxidized sucrose to 72.9, 146, and 292 mg, the CMCG 2–4 were obtained, respectively. The amount of oxidized sucrose added in the synthesis conditions of CMCG1-4 is equivalent to 0.3, 0.6, 1.2, and 2.4 moles of aldehyde group to the amino group of CMC.

2.5 Structural characterization

FT-IR spectra were measured using a PerkinElmer Spectrum Two spectrometer (PerkinElmer Inc., USA). The samples were ground to powder and mixed thoroughly with KBr powder before recording the FTIR spectra. The powder mixture was compressed into transparent discs and scanned from 4000 to 500 cm^{-1} using an average of 16 scans at a resolution of 1 cm^{-1}. NMR spectra were acquired using a Bruker AVIII500 spectrometer (Bruker BioSpin GmbH, Germany).

2.6 Rheological characterization

The rheological properties of the gels were evaluated using a Physica MCR 301 rheometer (Anton Paar GmbH, Graz, Austria) equipped with a Rheoplus 32 data analyzer and Peltier temperature control. Gap was controlled by a vertical force of 0.2 N. The temperature for all measurements was kept constant at 25°C and the strain at 1%.

2.7 Thermal gravimetric (TG) analysis

TG analysis was performed using a TG-DTA 8120 (Rigaku). Approximately 15 mg of powdered sample was placed in an Al sample pan. The sample was heated from room temperature to 500°C at a rate of 5°C/min in an air atmosphere of 25°C. Al_2O_3 powder was used as a reference.

3. RESULTS AND DISCUSSION

3.1 Synthesis of oxidized sucrose

When sucrose was oxidized, 6.05 g of oxidized sucrose was obtained as white crystals. A comparison of the FT-IR spectra of sucrose and oxidized sucrose (Fig. 1) showed that only oxidized sucrose showed a large absorption of C=O bond derived from aldehyde at 1630 cm^{-1}. This indicates that the ring of sucrose is cleaved by oxidation and aldehyde is formed at the end. Sucrose also shows a small absorption at 1630 cm^{-1}, which is the absorption of water.

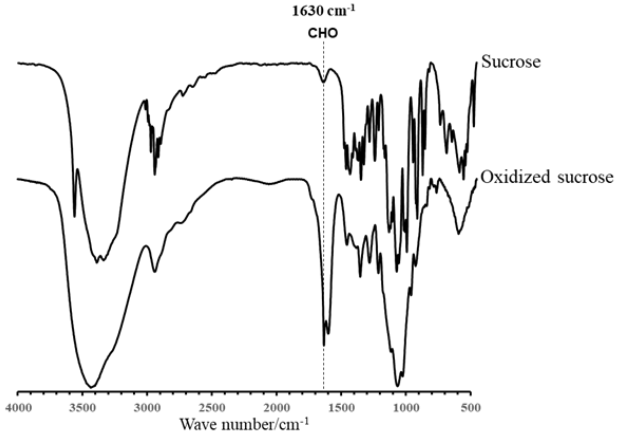

Fig. 1. FT-IR spectra of oxidized sucrose and the starting material of sucrose

Fig. 2 shows the ^{13}C NMR spectra of the oxidized sucrose. Assignment of the ^{13}C resonances were performed by the two-dimensional NMR, and the result of the assignment was also shown in this figure. The spectroscopic analysis revealed that there are two major components of the oxidized sucrose and that C3-C4 of the pentose ring and C2-C3 or C2-C4 of the hexose ring in sucrose were cleaved and oxidized. The resonance line at 172 ppm was attributed to aldehydes, and the presence of aldehydes was confirmed by ^{13}C NMR results. The aldehyde content of oxidized sucrose was precisely determined by titration method to be 7.52 $\times 10^{-3}$ mol of aldehyde/g-product.

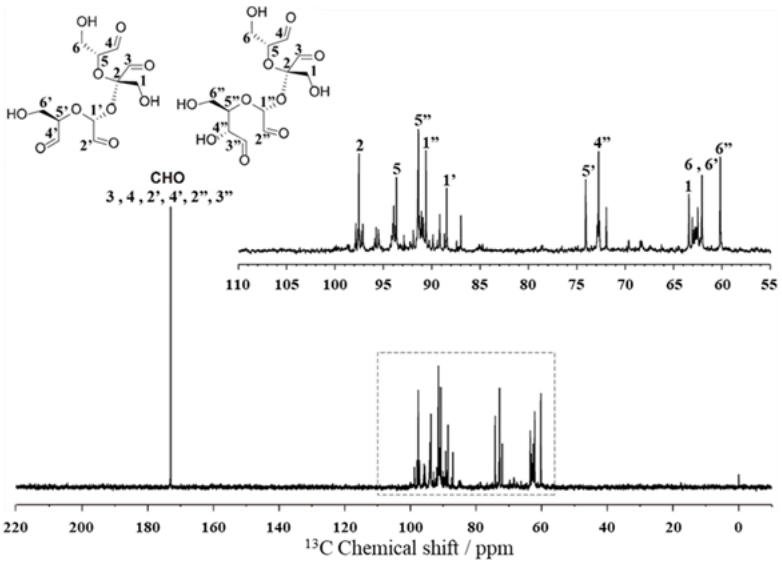

Fig. 2. ^{13}C NMR spectrum of oxidized sucrose

3.2 Carboxymethyl chitosan hydrogels (CMCG)

As a result of the cross-linking of CMC with oxidized sucrose, the reaction proceeded without the need for a catalyst at room temperature, forming a gel as shown in Fig. 3 (left). the dynamic viscoelasticity of CMCG was measured (Fig. 3, right), and the G" values, which indicate the behavior of the liquid component inside the gel, were all parallel regardless of the change in frequency. The G" values, which indicate the behavior of the liquid component inside the gel, were parallel to each other regardless of the change in frequency, indicating that the liquid component was strongly retained inside the gel in all the samples. The G" values, which indicate the physical strength of the gel, increased with the amount of cross-linking agent added, indicating that the physical strength of the gel could be controlled by the feed amount of cross-linking agent.

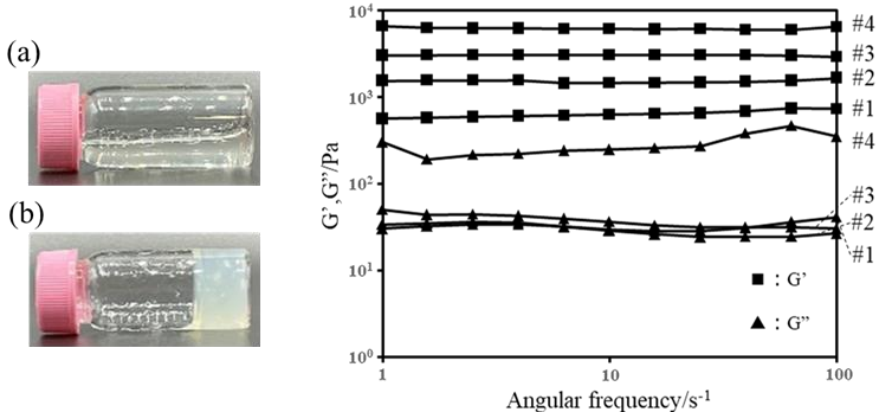

Fig. 3. Before (a) and after (b) crosslinking reaction of CMC with oxidized sucrose in aqueous solution (left), and dynamic viscoelasticity of the CMCG 1–4 (right)

3.3 Thermal property of CMCG

As summarized in Table 1, thermal analysis (Fig. 4) showed that the starting temperature of thermal decomposition (Ti) became lower as the amount of crosslinker increased. This may be due to the loss of polysaccharide ends and the decrease in intramolecular interactions due to the progress of crosslinking. On the other hand, the higher temperature at the end of pyrolysis (Tf) was probably due to the existence of localized regions where thermal resistance was greatly improved by the high density of crosslinked sites. All of these effects were due to the progress of crosslinking, and it was clear that the thermal properties also varied with the amount of crosslinking agent.

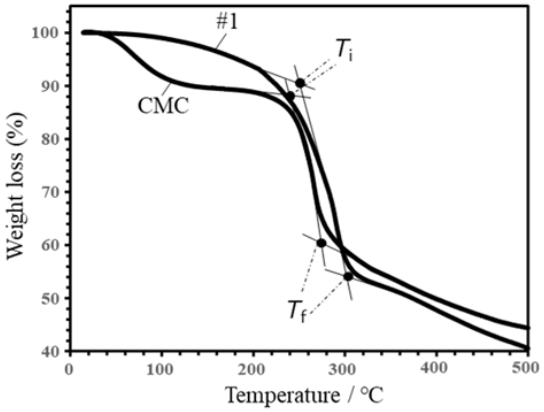

Fig. 4. TG of CMCG 1 and CMC

Table 1. Initial (T_i) and final (T_f) degradation temperatures for CMC and CMCG 1–4

Sample	T_i / °C	T_f / °C
CMC	247	283
CMCG 1	249	297
CMCG 2	241	301
CMCG 3	227	305
CMCG 4	215	309

3.4 Chitosan hydrogels (CG)

The addition of oxidized sucrose to chitosan solution dissolved in acidic solution resulted in the formation of a gel similar to CMC. The dynamic viscoelasticity was compared with that of CMCG (Fig. 6), and only CG showed a large change with the frequency of "G." CMC has a carboxymethyl group at the end, and its electrostatic repulsion expands the mesh structure inside the gel, strongly retaining the liquid component inside the gel. On the other hand, chitosan does not have electrostatic repulsion at the end of the sugar chain, and therefore cannot hold liquid components strongly in the gel.

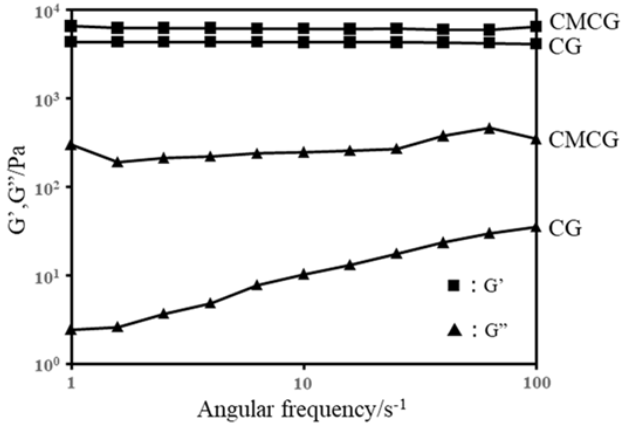

Fig. 5. Dynamic Viscoelasticity of CMCG and CG

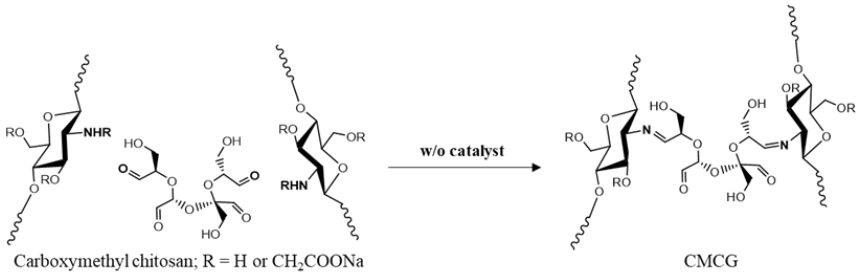

Fig. 6. Reaction of oxidized sucrose with amino polysaccharides

4. CONCLUSIONS

Structural analysis revealed that the structure of oxidized sucrose is the result of oxidative cleavage of C3-C4 of the pentose ring and C2-C3 or C2-C4 of the hexose ring of sucrose. The cross-linking of CMC, a chitosan derivative, with oxidized sucrose showed that the reaction proceeded under mild conditions at room temperature and the gel was formed without the need for a catalyst. It was concluded that the aldehyde group in the structure of the oxidized sucrose selectively reacted with the amino group of the polysaccharide to form the gel (Fig.7). Therefore, oxidized sucrose was found to be a cross-linking agent that can cross-link polysaccharides with amino groups without using a catalyst. In addition, various measurements showed that the physical properties of gels cross-linked with oxidized sucrose could be controlled depending on the amount of oxidized sucrose added. These results suggest that oxidized sucrose can be applied to biomaterials such as wound dressings.

ACKNOWLEDGMENTS

This work was supported in part by the Japan Society for the Promotion of Science (JSPS) [Grant No. JP21K05175 (H.K.)].

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