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Rheological properties of keto-sugars with high-density carbonyl groups

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ABSTRACT

Keto-dextran with high density of carbonyl groups was synthesized by the reactions of three enzymes (pyranose 2-oxidase, catalase, and dextransucrase). To evaluate the structure and interaction of keto-dextran with carbonyl groups in water, the viscosity of keto-dextran was compared with that of dextran. At a dilute concentration, the viscosity of keto-dextran is lower than that of dextran, because keto-dextran contracted due to intra-molecular interactions via hydrogen bonding with the carbonyl groups. Dynamic light scattering results showed that the hydrodynamic radii of dextran and keto-dextran were 24.1 and 16.7 nm, respectively. To evaluate only the inter-molecular interactions of the carbonyl groups, viscosities of glucose and keto-glucose were determined. The difference in the viscosity between keto-glucose and glucose was apparent within the concentrated region due to the inter-molecular interactions of the carbonyl groups in keto-glucose. The coefficients estimated by the extended Jones–Dole equation confirmed that keto-sugars have stronger hydration and association characteristics.

1. Introduction

Hydrogen bonding (H-bonding) between polysaccharide–water and polysaccharide–polysaccharide is formed, because polysaccharides have plural hydroxyl groups. Such H-bonding has played an important role in determining the conformation of polysaccharides. Hydrated amylose (α-1,4 glycoside linkage) is water-soluble, whereas crystallized cellulose (β-1,4 glycoside linkage) is water-insoluble. The presence of ionic, hydrophobic, and covalent functional groups in the polysaccharide backbone leads to the formation of stronger interactions than the H-bonding of the hydroxyl groups. For example, when a divalent metal salt is added into a solution of alginate consisting of mannuronic acid and glucuronic acid with carboxylic groups, the alginate is cross-linked with the divalent metal ion by ion-exchange interactions. This cross-linking structure is known as the egg-box model (Grant, Morris, Rees, Smith, & Thom, 1973). The formation of such interactions at the intra-molecular level causes the three-dimensional structure of the polysaccharide to shrink. In the case of inter-molecular interactions, the polysaccharide is inversely associated, and eventually forms an insoluble or gelated structure. Therefore, the strength and location (intra- or inter-) of the interactions is a characteristic that can identify the conformation of a polysaccharide in solution.

Dextran, a homopolysaccharide of glucose linked by α-1,6 glycoside linkages, is produced from sucrose by dextransucrase (DSase) extracted from the strains of *Leuconostoc* and *Streptococcus* (Robyt, Yoon, & Mukerjea, 2008). Since dextran also possesses the plural hydroxyl groups, H-bonding is formed with other hydroxyl groups in dextran and water molecules. High concentrations and high molecular weights promote the overlap of dextran random coils due to inter-molecular H-bonding and steric entanglement, resulting in a highly viscous solution (Morris, Cutler, Ross-Murphy, Rees, & Price, 1981; Tirtaatmadja, Dunstan, & Boger, 2001). In these studies, the critical overlap concentrations (C^*) of a commercial dextran with molecular weight of 2000 kDa were found to be 8 w/v% and 12 wt.%, respectively. The viscous property of dextran is useful for a thickener and a stabilizer in the food industry. Clinical dextran, with a controlled molecular weight of 75–100 kDa to have a lower viscosity, has been used as a blood plasma volume expander due to this compound possessing the same viscosity and osmotic pressure as blood plasma (Terg et al., 1996).

Introduction of functional groups into dextran also facilitates the formation of strong interactions. Carboxymethyl (CM)-dextran is cross-linked by amide bonds between carboxyl groups in CM-dextran and amino groups in protein using carbodiimide compound (Zhang, Tang, Bowyer, Eisenthal, & Hubble, 2006). CM-dextran can be used for bioseparation, biosensor production, and drug delivery system. Since dextran sulfate, a sulfate ester, resembles heparin in structure and binding ability with antithrombin, this compound is used as an anticoagulant agent instead of heparin (Mähner, Lechner, & Nordmeier, 2001).

The use of dextran and its derivatives in the various fields requires a clear understanding of their rheological properties, i.e. viscosities. The rheological properties of dextran in water have been reported by many researchers (Kuge, Kobayashi, Kitamura, & Tanahashi, 1987; McCurdy, Goff, Stanley, & Stone, 1994; Padmanabhan, Kim, Pak, & Sim, 2003; Sabatié, Choplin, Doublier

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et al., 1988; Sabatié, Choplin, Moan et al., 1988). The rheological properties of polysaccharides such as dextran depend on both the structure and nature. In other words, the viscosimetry enables the estimation of the shape, size, and interaction of carbohydrate polymers.

We have previously reported that the synthesis of keto-dextran containing high-density carbonyl groups, in which carbonyl groups were prospectively introduced to sucrose as a substrate, subsequently keto-dextran was enzymatically polymerized from the substrate contained carbonyl group (Seto, Kawakita, Ohto, Harada, & Inoue, 2008). Carbonyl groups of keto-dextran create novel H-bonding with water molecules and hydroxyl groups. Such novel H-bonding enables the formation of a novel conformation of the dextran backbone via H-bonding. This is because keto-dextran introduced into all glucose units increases the amount of interaction points. To use keto-dextran as an alternative to dextran in the various fields, e.g. food industry, pharmaceutical science, and analytical engineering, the realization of their novel conformation is important. To apply keto-dextran as a carbohydrate material with the desired functionality, keto-dextran having the controlled-introduction percentage is useful for the design of novel rheological behavior, such as the hydration, the association, and gelation via H-bonding and coordination with metal ions.

In this study, the existing model of keto-dextran in water and the interaction formed by carbonyl groups were clarified from rheological properties of keto-dextran containing high-density carbonyl groups. Keto-dextran with a high percentage of introduced carbonyl groups can be synthesized by combining the reactions of pyranose 2-oxidase (P2Ox), catalase, and DSase. Since the successive enzymatic reactions allow the regioselective introduction of functional groups at each glucose unit in dextran backbone, the investigation of influence of the functional groups on the structure of polysaccharides can be easily monitored. The viscosities of dextran and keto-dextran obtained by the successive enzymatic reactions were determined. Intra-molecular interactions of monosaccharides like glucose is negligible due to the lack of glycosidic connections. In order to measure the only inter-molecular interaction of the carbonyl groups, the viscosity of keto-glucose synthesized by the P2Ox reaction was compared with that of glucose.

2. Methods

2.1. Reagent

D-(+)-Glucose (minimum 99.5%, Lot No. 21K0010), pyranose oxidase from *Coriolus* sp. (E.C. 1.1.3.10, specific activity: 2.7 U mg^{-1}, Lot No. 087K1233), catalase from bovine liver (E.C. 1.11.1.6, specific activity: 45,400 U mg^{-1}, Lot No. 096K7035), and dextransucrase from *Leuconostoc mesenteroides* (E.C. 2.4.1.5, specific activity: 185.4 U mg^{-1}, Lot No. 018K4014) were purchased from Sigma Chemical Co. Sucrose and dinitrophenylhydrazine were purchased from Wako Chemical Co. Dextrans (M_w: 8.5, 64, 400, and 2000 kDa) from Sigma Chemical Co. were used as standards for the calibration of the size exclusion chromatography (SEC) column.

2.2. Preparation of sugar solutions

Synthesis of keto-dextran was carried out by combination of the three enzymatic reactions (P2Ox, catalase, and DSase) as previous report (Seto et al., 2008). To control the molecular weight of dextran and keto-dextran, the polysaccharide solutions were treated by ultrasonication using an ultrasonic cleaner (Branson 3510J-DTH, Oscillating frequency; 42 kHz, ultrasonic wave; 130 W) for 12 h at 50 °C. The molecular weights of the treated dextran and keto-dextran were determined by SEC (*column*: TSKgel G4000PW_{XL} – Tosoh Co., *pump*: 515 HPLC Pump – Waters Co., *detector*: 2414 refractive index detector – Waters Co., *recorder*: C-R8A Chromatopac – Shimadzu Corp., *mobile phase*: water, *flow rate*: 1.0 mL/min). Keto-glucose was also converted from glucose by the reaction of P2Ox and catalase.

2.3. Viscosimetry of dextran, keto-dextran, glucose, and keto-glucose

The viscosities of dextran, keto-dextran, glucose, and keto-glucose were determined for three times using an ostwald viscometer (Asahi Inc., Product No. 734) in a thermostatic bath at 33 ± 0.5 °C. The viscometries of polysaccharide solutions and monosaccharide solutions were carried out at the concentration between 0.1–1.0 g/dL as the dilute region and 5–30 g/dL as the concentrated region, respectively. Specific viscosity is estimated from the following equation:

$$\eta_{sp} = \frac{t_1}{t_0} - 1 \tag{1}$$

where t_0 and t_1 are the passage times (s) of solvent and solution, respectively. The reduction viscosity is defined by dividing the specific viscosity with the concentration of the polymer, and is composed as the following equations of Huggins and Schultz–Blaschke (Huggins, 1942; Irurzun, Figini, Marx-Figini, & Grigera, 2000):

$$\frac{\eta_{sp}}{C} = [\eta] + k_H[\eta]^2 C + \cdots \tag{2a}$$

$$\frac{\eta_{sp}}{C} = [\eta] + k_{SB}[\eta]\eta_{sp} \tag{2b}$$

where $[\eta]$, k_H, and k_{SB} are the intrinsic viscosity, the Huggins constant, and the Schultz–Blaschke constant, respectively. The intrinsic viscosity is a physical quantity dependent on the molecular weight, shape, and size of individual polymer chains which are separately dissolved in a solvent. The Huggins constant indicates the hydrodynamic inter-molecular interactions. The radii of gyration r_G for dextran and keto-dextran in water are related to the molecular weight M and the intrinsic viscosity $[\eta]$ according to Flory–Fox relationship (Flory & Fox, 1951):

$$r_G = \left(\frac{M[\eta]}{\Phi' \times 6^{3/2}}\right)^{1/3} \tag{3}$$

where Φ' is universal constant of Flory–Fox ($\Phi' = 2.1 \times 10^{23}$).

The Jones–Dole equation (Jones & Dole, 1929) is usually employed to estimate the viscous coefficients for small molecules such as glucose and keto-glucose:

$$\eta_r = \frac{\eta}{\eta_0} = 1 + AC^{1/2} + BC \tag{4}$$

The coefficients A and B are the constants concerned with ion-ion and solute–solvent interactions, respectively. As Eq. (4) is applicable to only solutes with low concentrations, a number of researchers have added a quadratic term DC^2 to accommodate samples of higher concentrations (extended Jones–Dole equation) (Abdulagatov & Azizov, 2005, 2006; Abdulagatov, Zeinalova, & Azizov, 2006; Jones & Talley, 1933; Nakagawa, 1995).

$$\eta_r = \frac{\eta}{\eta_0} = 1 + AC^{1/2} + BC + DC^2 \tag{5}$$

The coefficient D is the value accounting for all solute–solvent and solute–solute interactions. Since glucose and keto-glucose are non-ionic molecules, the $AC^{1/2}$ term including ion-ion interactions is negligible. The resulting Jones–Dole equation for glucose and keto-glucose is the following:

$$\eta_r = \frac{\eta}{\eta_0} = 1 + BC + DC^2 \quad (6)$$

The coefficients B and D are estimated from the slope and intercept in the plot of $(\eta/\eta_0 - 1)/C$ against C.

To investigate the additive effect of metal ion, $CdCl_2$ (1 mol/L) was dissolved into molecular-weight-uncontrolled dextran and keto-dextran solutions with concentration of 0.1 g/dL, and then the viscosity of the dextran and keto-dextran solutions (approximately 2000 kDa) was determined by a viscometer (DV-II + Pro, BROOKFIELD). The efficiency of cross-linking effect is defined as the viscosity of solution after addition of metal salt per that before addition of metal.

2.4. Measurement of the polysaccharides radii using dynamic light scattering

Hydrodynamic radii of dextran and keto-dextran were measured using dynamic light scattering. After the polysaccharide solutions with the concentration of 0.1 g/dL were permeated through a microfilter membrane (0.45 μm, Advantec Co.), the size distribution of dextran and keto-dextran was determined using Otsuka electronics Co. Ltd., ELS Z (Analytical Research Center, Saga University, Japan) at 25 °C.

3. Results and discussion

3.1. Adjustment of the molecular weight of dextran and keto-dextran

To adjust the molecular weights of dextran and keto-dextran polymerized by the DSase reactions, the solutions were subjected to ultrasonication for 12 h at 50 °C. The size exclusion chromatograms of the dextran solution before and after ultrasonic treatment are shown in Fig. 1(a) and (b), respectively. The peak at around 5.5 min was shifted to 8.0 min by the ultrasonic treatment, indicating that ultrasonic treatment leads to the degradation of dextran, as previously reported (Hamdy, Gardner, Winkle, & Stahly, 1958; Hamdy, Winkle, Stahly, Weiser, & Birkeland, 1956). The disentanglement of the dextran chain might also be induced. Similar to the dextran solution, the ultrasonic treatment degraded the keto-dextran as illustrated in Fig. 1(c). The weight-average molecular weights of dextran and keto-dextran, which were obtained from the size exclusion chromatograms, were $10^{5.64}$ and $10^{5.71}$ Da, respectively. Dextran and keto-dextran with equivalent molecular weights were used for determining viscosities.

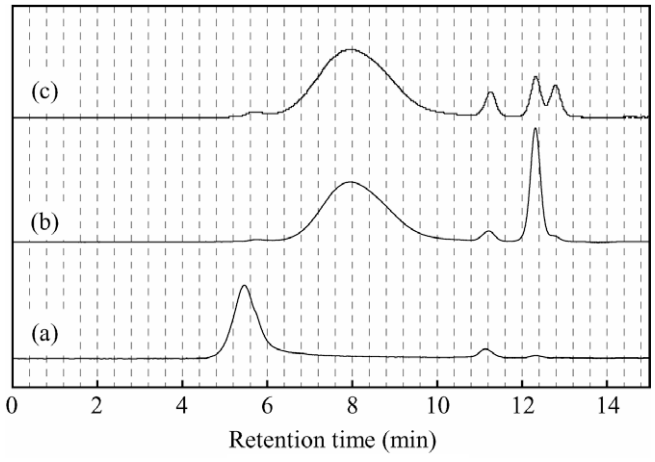

Fig. 1. Size exclusion chromatogram of polysaccharides produced by DSase; (a) dextran before sonication, (b) dextran after sonication, and (c) keto-dextran after sonication.

3.2. Rheological properties of dextran and keto-dextran in the dilute region

The polymer model in aqueous solution was estimated from the rheological properties of dextran and keto-dextran. The Huggins plots of dextran and keto-dextran solutions are presented in Fig. 2. The values of η_{sp}/C linearly increased with increasing concentration of the polysaccharides. The intrinsic viscosity $[\eta]$ and Huggins constant k_H were calculated from the slope and intercept of the lines. The $[\eta]$ was also calculated from the Schultz–Blaschke plots (Fig. 3). The $[\eta]$, k_H, and k_{SB} of dextran and keto-dextran are described in Table 1. The $[\eta]$ and the viscometric constants of dextran were slightly higher than those of keto-dextran. These results suggest that the conformation of keto-dextran had shrunk due to intra-molecular interactions via carbonyl groups in dilute region. The r_G of dextran and keto-dextran was calculated using the values of $[\eta]$ (Table 1). Dextran and keto-dextran had r_G values of 48.1 and 45.3 nm, respectively.

For random coil polymer solutions such as dextran, the coil overlap parameter, $C[\eta]$, characterizes the solution property. The logarithmic specific viscosity as the functional of the logarithmic $C[\eta]$ for keto-dextran is illustrated in Fig. 4. The breakpoint between $\log \eta_{sp}$ and $\log C[\eta]$ was not observed in low concentration region. In the higher concentration region, the C^* is obtained, where the overlap of keto-dextran chain is promoted. The slope of 1.2 is slightly lower than that of 1.4 reported in previous study (Morris et al., 1981). The lower slope of keto-dextran is probably due to the conformation shrunken by intra-molecular interaction of carbonyl group and the branched structure of keto-dextran compared with dextran (Seto et al., 2008).

When cadmium ion was added into dextran and keto-dextran solutions with similar molecular weight, the cross-linking effect of cadmium ion on the viscosities of dextran and keto-dextran solution were confirmed. Keto-dextran had the more cross-linking effect than dextran, in which the efficiencies of cross-linking effect for dextran and keto-dextran were 1.48 and 1.63, respectively. This would be explained by the hard and soft acids and bases rule (Pearson, 1963). Cadmium and carbonyl group are comparatively soft metal and ligand group, respectively. On the contrary hydroxyl group is hard ligand, such that cadmium ion is more likely to interact with the carbonyl groups in keto-dextran to form the six-coordinated complex.

3.3. Size distribution of dextran and keto-dextran in the dilute region

The molecular sizes of dextran and keto-dextran were also measured by dynamic light scattering. Fig. 5 shows the size distribution

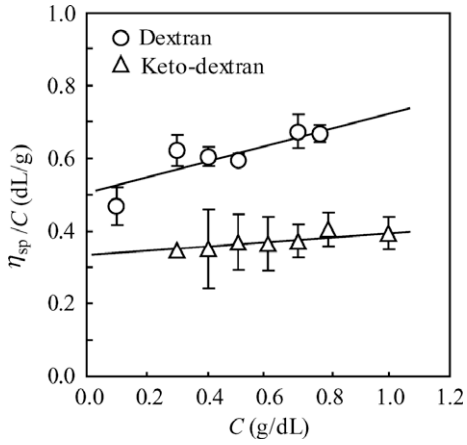

Fig. 2. Huggins plots of dextran and keto-dextran produced by enzymatic reactions. The error bar is the standard deviation determined for three times experiments.

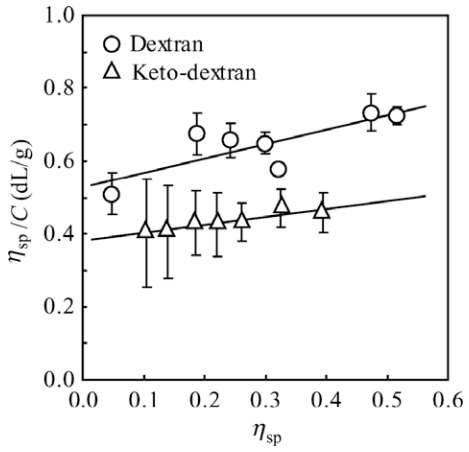

Fig. 3. Schulz-Blaschke plots of dextran and keto-dextran produced by enzymatic reactions. The error bar is the standard deviation determined for three times experiments.

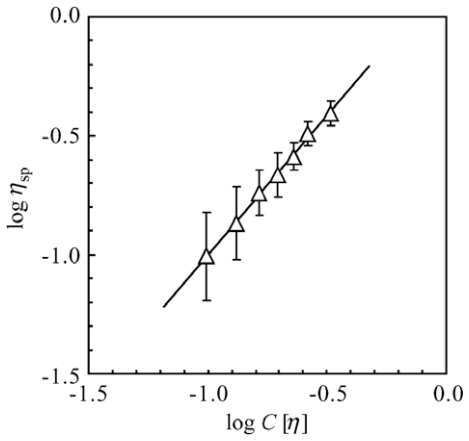

Fig. 4. Concentration dependence of specific viscosity for keto-dextran. The error bar is the standard deviation determined for three times experiments. Regression equation; $y = 1.168x + 0.169$, $R^2 = 0.997$.

of dextran and keto-dextran in aqueous solution. The r_H of dextran and keto-dextran were 12.1 and 8.8 nm, respectively. The r_H of dextran in this study closely matches the r_H of dextran measured previously (Armstrong, Wenby, Meiselman, & Fisher, 2004; Ioan, Aberle, & Burchard, 2000; Nordmeier, 1993; Nordmeier, Xing, & Lechner, 1993; Suzuki, Wada, & Suzuki, 1982). Durand et al. and Rotureau et al. found that hydrophobic dextrans aggregated in water when hydrophobic functional groups were introduced (Durand & Dellacherie, 2006; Rotureau, Chassenieux, Dellacherie, & Durand, 2005). The blue dextrans, synthesized by the coupling of Cibacron blue 3G-A and dextran, exhibited larger r_H values than the unmodified dextrans due to coulomb repulsion between the ionic dye ligands. However the r_H of blue dextran was observed to decrease with increasing ionic strength because of the shielding of the coulomb repulsion between the ionic sulfonate groups in the dye ligands (David, Stephanie, & Derek, 2008). The r_H of keto-dextran was lower than that of dextran, as well as the r_G estimated from the viscosity data. The narrow distribution of molecular sizes indicates that dextran and keto-dextran were not intermolecularly associated, when the concentrations of samples were in dilute region.

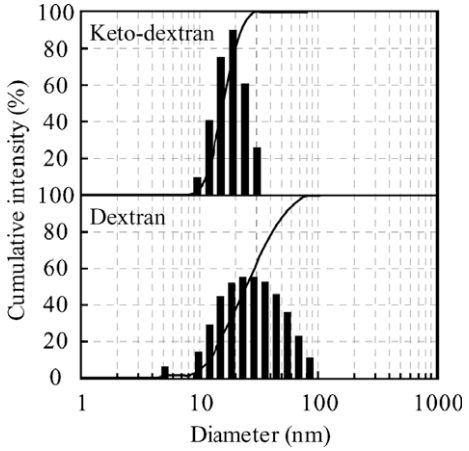

Fig. 5. Molecular size distributions of dextran and keto-dextran in water in the dilute region.

3.4. Rheological properties of glucose and keto-glucose in the concentrated region

The effects of carbonyl groups in facilitating interactions were observed to be similar between keto-glucose units and keto-dextran. Since keto-glucose is not connected by glycoside bonding, the intra-molecular interaction of keto-glucose is negligible. The relative viscosities ($\eta_r = \eta/\eta_0$) according to Jones–Dole equation and the coefficients estimated from $(\eta_r - 1)/C - C$ plot are illustrated in Fig. 6 and Table 2, respectively. The difference between the viscosities of keto-glucose and glucose increased as the concentration increased. The abilities of hydration (coefficient B) and inter-molecular interactions (coefficient D) increased when carbonyl groups were introduced.

Table 1
Viscometric constants and the radii of gyration of dextran and keto-dextran estimated from the Huggins and Schultz–Blaschke plots.

	Huggins plot		Schultz–Blaschke plot		Mean [η] (dL/g)	r_G (nm)
	k_H	[η] (dL/g)	k_{SB}	[η] (dL/g)		
Dextran	0.93	0.530	0.74	0.530	0.530	48.1
Keto-dextran	0.70	0.375	0.59	0.380	0.378	45.3

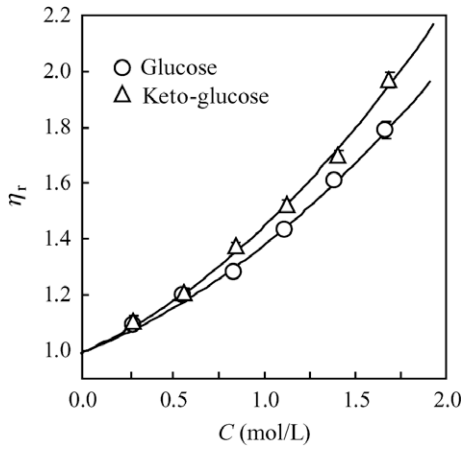

Fig. 6. Relative viscosities of glucose and keto-glucose aqueous solution as a function of concentration. The error bar is the standard deviation determined for three times experiments.

Table 2
Hydration coefficients *B* and association coefficients *D* for glucose and keto-glucose estimated using the extended Jones–Dole equation.

	Glucose	Keto-glucose
Solute–solvent coefficient *B*	0.29	0.32
Solute–solute coefficient *D*	0.10	0.14

The location of the interaction via the carbonyl groups is dominated by intra-molecules in the dilute region, and the viscosities of keto-dextran solutions decreased due to contraction effect. Conversely in the concentrated region, it is expected that the location of the interactions in keto-dextran switches from solely intra-molecular to inter-molecular interaction with the other dextran and water molecules, resulting that keto-dextran has higher viscosity and solubility due to the association and the hydration, respectively. The carbonyl group is effective in altering the conformation of carbohydrate polymers such as dextran.

The structure and solution property of a polysaccharide depends on the segment species, pattern of binding, and degree of polymerization. Introduction of carbonyl groups in a polysaccharide backbone enables to control the structure and solution property. In the case of dextran backbone, the strong interaction, the shrunken conformation, and the high water retentivity were expressed by introduction of carbonyl group with high density. When the carbonyl group is introduced into other polysaccharides, the unique change of the properties occurs. Amylose (α-1,4) with a helix structure in water is a water-soluble polymer that is similar to dextran. The structure of amylose should become rigid when a carbonyl group is introduced into the amylose backbone at high density. This rigidity is due to the H-bonding between amylose molecules and also the coordination with additives. In contrast, cellulose linked by β-1,4 glycoside bonds has a crystallographic structure via inter-molecular H-bonding, and is therefore water-insoluble. To dissolve cellulose, its crystalline structure should be reduced by the introduction of a methyl group and a hydroxyethyl group. Alternatively cellulose should be converted into a poly-electrolyte such as carboxymethyl-cellulose. It is expected that the location of the H-bonding using carbonyl groups introduced into cellulose switches the cellulose–cellulose interactions to cellulose–water molecule interactions. As a result, the crystalline nature of cellulose is reduced, i.e. cellulose is soluble in water. If various transferases are found and the successive enzymatic reactions are applied *in vitro* to synthesize other polysaccharide derivatives, the design of structure and solution property for carbohydrate polymers with a desired functionality should be realized.

4. Conclusion

Keto-dextran, in which a carbonyl group was regioselectively introduced in all glucose units, was synthesized using the reactions of three enzymes successively. From the rheology behavior of the obtained keto-dextran, it was found that novel H-bonding formed by the carbonyl groups induced the intra-molecular shrinkage in the dilute region. The inter-molecular interactions between carbonyl groups and between carbonyl groups-water molecules were dominant in the concentrated region, when monomeric keto-glucose was used to prevent the intra-molecular interactions. Therefore, keto-dextran may also be hydrated and associated in the concentrated region. Carbohydrate polymers with high-density functional groups are expected to be effective application in the field of food and analytical chemistry.

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