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Kinetics of Maltooligosaccharide Hydrolysis in Subcritical Water

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The kinetics of the hydrolysis of maltooligosaccharides with a degree of polymerization (DP) of 3−6 in subcritical water was studied using a tubular reactor at temperatures between 200 and 260 °C and at a constant pressure of 10 MPa. The maltooligosaccharide disappearance and product formation at residence times shorter than 50 s could be expressed by first-order kinetics. The rate constants for the hydrolysis of each maltooligosaccharide were evaluated. There was a tendency that the exo-site glucosidic bond was hydrolyzed faster than the endo-site one irrespective of the DP of the maltooligosaccharide. The hydrolysis of the maltooligosaccharides was consecutively preceded, and the time dependence of the hydrolysis for maltooligosaccharides with different DPs could be calculated by simultaneously solving the mass balance equations for all the possible saccharides.

KEYWORDS: Subcritical water; maltooligosaccharide; site-dependent hydrolysis; exo and endo sites of glucosidic bond

INTRODUCTION

Water heated to its critical temperature at a sufficient pressure to maintain its liquid state is called subcritical water. As the temperature of water increases from ambient to the near-critical point, the ion product of water increases by more than 3 orders of magnitude, making subcritical water a strong source of hydrogen and hydroxide ions far better than ambient water and allowing water in the sub- and near-critical regions to act as an effective acid catalyst (*1*−*3*). Sub- and supercritical waters have received much attention recently as novel media for chemical processes of environmental and biological importance. The most important advantages of using subcritical water as a reaction medium are the relatively low cost, improved safety, and replacement of environmentally undesirable acid catalysts.

Many reactions can be conducted in sub- or supercritical water without the addition of an acid or base because protons or hydroxyl ions resulting from the high ion product of water catalyze the reaction under these conditions. The degradation or decomposition of monosaccharides (*4*−*6*), hydrolysis of disaccharides (*7*−*11*), and dissolution and hydrolysis of cellulose (*12*, *13*) as model substances for biomass and food wastes have been treated in sub- and supercritical water. Although some kinetic studies have been reported during the past decade, information on the kinetics is still limited.

In our previous study (*7*), the susceptibility of disaccharides to hydrolysis in subcritical water was found to depend on the type of glucosidic bond. However, there could be a possibility that the susceptibility of the same types of glucosidic bonds is different depending on the length and position for the oligomers and polymers with different degrees of polymerization (DP). Because maltooligosaccharides are homogeneous oligomers in which glucose molecules are linked by α-1,4-gucosidic bonds to form a linear chain, they would be suitable substances for investigating the susceptibility.

This study aims to examine the effects of the DP and temperature on the hydrolysis kinetics of maltooligosaccharides in subcritical water. The site specificity for the hydrolysis was also determined by evaluating the rate constants for the exo- and endo-site glucosidic bonds for maltooligosaccharides with DPs of 4−6.

MATERIALS AND METHODS

Materials. Maltotriose (abbreviated G_3), maltotetraose (G_4), maltopentaose (G_5), and maltohexaose (G_6) were purchased from Wako Pure Chemical Industries, Osaka, Japan. The water used in the preparation of the sample solutions and as a mobile phase in the HPLC analysis of the maltooligosaccharides was distilled water.

Hydrolysis of Maltooligosaccharides. A continuous flow-type reactor in which a substrate solution was rapidly heated and then quickly quenched was used to react the maltooligosaccharide. The experimental apparatus and procedures are almost the same as those in our previous studies (*7*, *10*). A feed solution was prepared by dissolving each maltooligosacharide in distilled water at a concentration of 0.5% (w/v), and the solution was sonically degassed under reduced pressure. The solution in the bottle (ca. 500 mL) was connected to a helium gas bag to prevent the redissolution of oxygen into the solution and was

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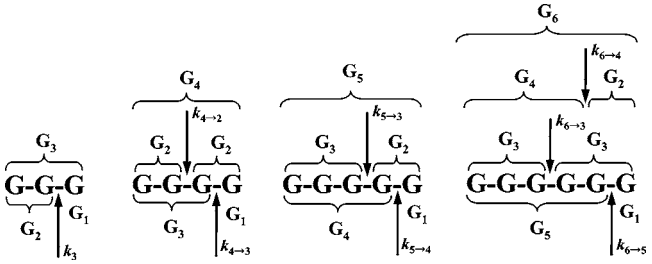

Figure 1. Chain conformations of maltooligosaccharides with DPs of 3–6 and definition of the rate constants. The arrows indicate the possible cleavage positions on the structure of each maltooligosaccharide.

delivered at a constant flow rate to give a specific residence time of 6−210 s in the reaction coil. The solution was fed to the reaction coil made of stainless steel (SUS 316) tubing (1.6 mm o.d. × 0.8 mm i.d.). The reactor was immersed in an oil bath filled with SRX310 silicone oil (Toray Dow Corning Silicone, Tokyo, Japan), and the temperature was regulated in a range of 200−260 °C. To rapidly stop the reaction, the reaction mixture leaving the reactor was passed through a cooling coil immersed in an iced water bath. The back-pressure valve (model 26-1761-24, Tescom Crop., Elk River, MN) was connected to the line after the cooling coil to control the pressure in the system and was regulated at 10 MPa. The effluent was filtered through an inline filter with a 40−55 μm pore size and collected in a sampling vessel for analysis.

Analysis. The concentrations of the maltooligosaccharides and glucose in the effluent were analyzed using an LC-10ATvp HPLC (Shimadzu, Kyoto, Japan) with a refractometer (Shodex RI-101, Showa Denko, Tokyo, Japan). The column used for the analysis was a hydrosphere ^{18}C column (250 mm × 4.6 mm i.d.), and the eluent was distilled water at a flow rate of 1 mL/min. The determination was repeated at least 3 times and averaged. The pH of the effluent was measured at room temperature using a F-13 pH meter (Horiba, Kyoto, Japan).

RESULTS AND DISCUSSION

Hydrolysis of Maltooligosaccharides. Maltooligosaccharides are homopolymers of glucose molecules that are bonded to each other by α-1,4-glucosidic linkages. As the hydrolysis proceeds, the molecular mass of the maltooligosaccharide would be progressively reduced, which leads to the accumulation of maltooligosaccharides with a lower DP and also a constituent glucose.

Figure 1 shows the chain conformation of maltooligosaccharides with DPs of 3−6 and the possible positions for the glucosidic bond cleavage of each maltooligosaccharide. Although G_3 has two possible glucosidic bonds that are hydrolyzed to produce G_2 and G_1, the cleavages of the bonds are not distinguished. Therefore, the rate constant k_3 was the sum of the rate constants for the hydrolysis of both bonds. The situation is the same for other maltooligosaccharides with DPs of 4−6, and the rate constants shown in **Figure 1** except for $k_{4\rightarrow2}$ and $k_{6\rightarrow3}$ are estimated in the same manner.

Figure 2a−d shows the concentrations of the saccharides during the hydrolysis of G_3, G_4, G_5, and G_6, respectively, as a function of the residence time τ at 220 °C and 10 MPa. The hydrolysis of G_3 yielded the equivalent mol of G_2 and G_1 at the residence times of 0−50 s, and then the successive hydrolysis of G_2 proceeded at residence times longer than 100 s (**Figure 2a**). During the hydrolysis of G_4 (**Figure 2b**), almost equivalent mol of G_3, G_2, and G_1 were produced at residence times of 0−50 s, and the successive hydrolysis of G_3 and G_2 occurred at the longer residence times. During the hydrolysis of G_5 and G_6, the concentrations of G_4 and G_5, respectively, were equivalent to that of G_1 but were slightly higher than those

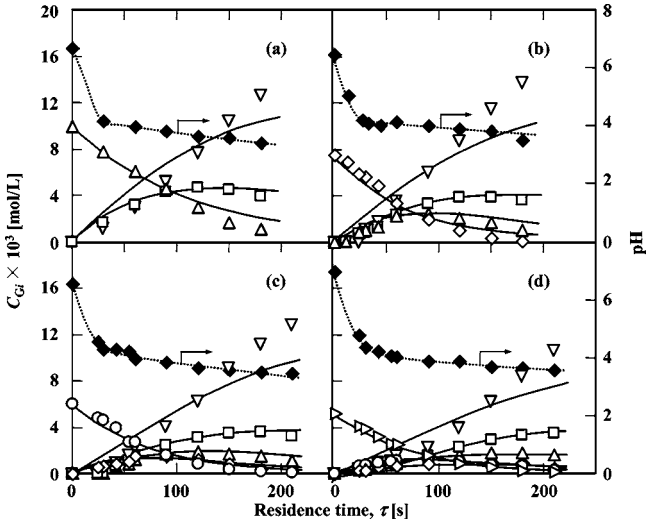

Figure 2. Hydrolysis of (**a**) maltotriose (△), (**b**) maltotetraose (◇), (**c**) maltopentaose (○), and (**d**) maltohexaose (open sideways triangle) at 220 °C and 10 MPa. C_{Gi} represents the molar concentration of the maltooligosaccharides (i = 1–6) in the effluent. The symbols □, ▽, and ◆ represent maltose, glucose, and pH, respectively. The curves were drawn based on the kinetic equations applied in this study. The dotted lines connect the experimental results.

of the other maltooligosaccharides (**Figure 2c**,**d**). For all the substrates, the successive hydrolysis of the maltooligosaccharides also occurred at residence times longer than 100 s. The pH of the effluents sharply decreased at the short residence times and further gradually decreased at longer residence times. A similar tendency in the pH change was also observed for the hydrolysis of maltose in subcritical water (*10*).

Evaluation of Rate Constants. To quantitatively investigate the susceptibility of each glucosidic bond to the hydrolysis, the rate constant for the hydrolysis of the bond was evaluated based on the mass balance at the steady state of all the possible oligosaccharides and glucose. First-order kinetics was adapted to each step for the consecutive hydrolysis of the maltooligosaccharides because of its simplicity and applicability to the hydrolysis of maltose at the short residence times (*10*). The definitions of the rate constants for the hydrolysis are shown in **Figure 1**. As mentioned previously, the glucosidic bonds at the same distance from the reducing and nonreducing ends were not distinguished in this study. Therefore, the rate constants should be the sum of the rate constants for the cleavages of both bonds, although one of the two bonds is indicated in **Figure 1**. The mass balance equations of the steady state for G_3, G_2, and G_1 are given as follows:

$$\frac{dC_G3}{d\tau} = -k_3C_{G3} \tag{1a}$$

$$\frac{dC_{G2}}{d\tau} = k_3C_{G3} - k_2C_{G2} \tag{1b}$$

$$\frac{dC_{G1}}{d\tau} = k_3C_{G3} + 2k_2C_{G2} - k_1C_{G1} \tag{1c}$$

where C is the concentration and τ is the residence time.

The mass balance equations of the steady state can also be formulated for maltooligosaccharides with DPs of 4−6 and their hydrolysates in the same manner as for G_3.

For G_4,

$$\frac{dC_{G4}}{d\tau} = -(k_{4\rightarrow3} + k_{4\rightarrow2})C_{G4} \quad (2a)$$

$$\frac{dC_{G3}}{d\tau} = k_{4\rightarrow3}C_{G4} - k_3C_{G3} \quad (2b)$$

$$\frac{dC_{G2}}{d\tau} = 2k_{4\rightarrow2}C_{G4} + k_3C_{G3} - k_2C_{G2} \quad (2c)$$

$$\frac{dC_{G1}}{d\tau} = k_{4\rightarrow3}C_{G4} + k_3C_{G3} + 2k_2C_{G2} - k_1C_{G1} \quad (2d)$$

For G_5,

$$\frac{dC_{G5}}{d\tau} = -(k_{5\rightarrow4} + k_{5\rightarrow3})C_{G5} \quad (3a)$$

$$\frac{dC_{G4}}{d\tau} = k_{5\rightarrow4}C_{G5} - (k_{4\rightarrow3} + k_{4\rightarrow2})C_{G4} \quad (3b)$$

$$\frac{dC_{G3}}{d\tau} = k_{5\rightarrow3}C_{G5} + k_{4\rightarrow3}C_{G4} - k_3C_{G3} \quad (3c)$$

$$\frac{dC_{G2}}{d\tau} = k_{5\rightarrow3}C_{G5} + 2k_{4\rightarrow2}C_{G4} + k_3C_{G3} - k_2C_{G2} \quad (3d)$$

$$\frac{dC_{G1}}{d\tau} = k_{5\rightarrow4}C_{G5} + k_{4\rightarrow3}C_{G4} + k_3C_{G3} + 2k_2C_{G2} - k_1C_{G1} \quad (3e)$$

For G_6,

$$\frac{dC_{G6}}{d\tau} = -(k_{6\rightarrow5} + k_{6\rightarrow4} + k_{6\rightarrow3})C_{G6} \quad (4a)$$

$$\frac{dC_{G5}}{d\tau} = k_{6\rightarrow5}C_{G6} - (k_{5\rightarrow4} + k_{5\rightarrow3})C_{G5} \quad (4b)$$

$$\frac{dC_{G4}}{d\tau} = k_{6\rightarrow4}C_{G6} + k_{5\rightarrow4}C_{G5} - (k_{4\rightarrow3} + k_{4\rightarrow2})C_{G4} \quad (4c)$$

$$\frac{dC_{G3}}{d\tau} = 2k_{6\rightarrow3}C_{G6} + k_{5\rightarrow3}C_{G5} + k_{4\rightarrow3}C_{G4} - k_3C_{G3} \quad (4d)$$

$$\frac{dC_{G2}}{d\tau} = k_{6\rightarrow4}C_{G6} + k_{5\rightarrow3}C_5 + 2k_{4\rightarrow2}C_{G4} + k_3C_{G3} - k_2C_{G2} \quad (4e)$$

$$\frac{dC_{G1}}{d\tau} = k_{6\rightarrow5}C_{G6} + k_{5\rightarrow4}C_{G5} + k_{4\rightarrow3}C_{G4} + k_3C_{G3} + 2k_2C_{G2} - k_1C_{G1} \quad (4f)$$

where C_i and k_i are the concentration and rate constant for the hydrolysis of the maltooligosaccharide with DP = i (i = 3, 2, and 1), respectively, and $k_{i\rightarrow j}$ represents the rate constant for the hydrolysis of the maltooligosaccharide with DP = i (i = 4, 5, and 6) to the saccharides with DP = j and $i - j$.

The rate constant for the hydrolysis of G_2 into two G_1 molecules, k_2, was evaluated by applying the first-order kinetics to our previous results at residence times shorter than 50 s (*10*). The rate constant for the degradation of G_1, k_1, was also similarly determined using our previous data (*4*). Using these values, the rate constant for the hydrolysis of G_3, k_3, was evaluated over the entire residence time by fitting the mass balance equations in a balanced Euler method with a nonlinear regression using the Solver in Microsoft Excel for Windows (*14*, *15*) under the conditions of iteration up to 100, precision of 1×10^{-6}, 5% tolerance, and convergence of 1×10^{-4}.

Table 1. Rate Constants for the Hydrolysis of Maltooligosaccharides (DPs 3–6) with Site-Specific Directions at 220 °C and 10 MPa

rate constant	rate constant $\sim 10^3$ [s^{-1}]	rate constant per one cleaved $\sim 10^3$ [s^{-1}]	type of cleavage
k_3	8.8	4.4	exo
$k_{4\rightarrow3}$	10	5.0	exo
$k_{4\rightarrow2}$	3.7	3.7	endo
$k_{5\rightarrow4}$	8.3	4.2	exo
$k_{4\rightarrow3}$	5.3	4.2	exo
$k_{6\rightarrow5}$	8.0	4.0	exo
$k_{6\rightarrow4}$	3.4	1.7	endo
$k_{6\rightarrow3}$	2.2	2.2	endo
k_1	3.9[a]	3.9	
k_2	5.4[b]	5.4	

[a] The rate constant k_1 for the degradation of glucose was determined from our previous results (*4*). [b] The rate constant k_2 for the decomposition of maltose was determined from our previous results (*7*).

The rate constants for the hydrolysis of G_4, $k_{4\rightarrow2}$, and $k_{4\rightarrow3}$ were subsequently determined by the same method using the predetermined rate constants for saccharides with a lower DP. All the rate constants were also evaluated using the step-by-step calculations and are listed in **Table 1**. Using the estimated rate constants, the concentrations versus the residence time of a maltooligosaccharide and its hydrolysates were calculated and are denoted by the solid curves in **Figure 2a−d** for G_3, G_4, G_5, and G_6, respectively. The calculated curves coincided with the experimental values for short residence times from 0−50 s. However, there were deviations at the longer residence times for every substrate. The deviation may be ascribed to the decrease in pH as shown for the hydrolysis of maltose (*10*). As shown in **Figure 2**, the initial pH was high, but it started to decrease and gradually reached an almost constant value of 3.6 as the residence time increased. A decrease in pH would be caused by the relatively small amount of organic acids formed as the degraded products of glucose. Our previous results showed that the hydrolysis of maltose was accelerated (*10*) and that the degradation of glucose slowly proceeded (*4*) at lower pH values. Consequently, the concentration of G_1 at the longer residence times was estimated as the lower value in this study. However, the kinetics analysis could well explain features for the hydrolysis of the maltooligosacharides at the short residence times.

Site Specificity for the Hydrolysis of Maltooligosaccharide. The rate constants for the hydrolysis of the maltooligosaccharides with different DPs at 220 °C and 10 MPa are summarized in **Table 1**. For the hydrolysis of G_4 to G_6, two or three rate constants were considered, and one of them corresponds to the cleavage of the exo-site glucosidic bonds. As mentioned before, the rate constants for G_3 to G_6, except for $k_{4\rightarrow2}$ and $k_{6\rightarrow3}$, were estimated as the sum of the values for two possible cleavages. Therefore, we calculated the rate constants per one cleavage, and these values are also listed in **Table 1**. The rate constants for the hydrolysis of G_4, G_5, and G_6 for the exo-site glucosidic bonds were slightly higher than those for the endo-site bonds. Although this difference was not very large, it would be interesting to know if the exo-site bond is hydrolyzed faster than the endo-site one.

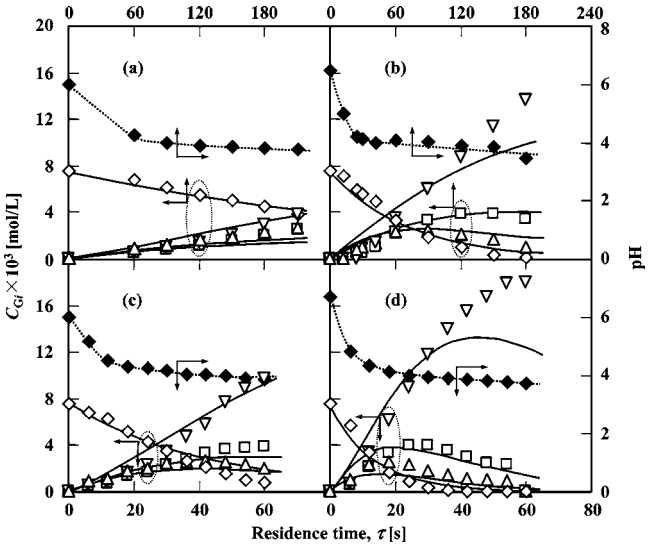

Figure 3. Hydrolysis of maltotetraose at (**a**) 200, (**b**) 220, (**c**) 240, and (**d**) 260 °C and 10 MPa. Symbols: (◇) maltotetraose, (△) maltotriose, (□) maltose, (▽) glucose, and (◆) pH. The curves were drawn using eqs 2a–d and the rate constants estimated in this study. The dotted lines connect the experimental results.

In our previous study (*7*), it was demonstrated that the rate constant for the hydrolysis of various disacharides could be correlated to the electrostatic potential charge of the glucosidic oxygen atom. For the site specificity for the hydrolysis of the maltooligosaccharides used in this study, the electrostatic potential charge might also be an important factor. On the other hand, an increase in the DP induces the flexibility of fluctuation (structural hindrance) of the maltooligosaccharide molecule. This fluctuation might also induce the difference in the probability of the proton attack on the glucosidic bond. Further investigation into the importance of electrostatic potential change and chain flexibility during hydrolysis in subcritical water, while beyond the scope of this present study, might provide useful insight to enhance our understanding of these phenomena.

Hydrolysis of Maltotetraose at Different Temperatures. **Figure 3a−d** shows the changes in the concentration versus the residence time of maltotetraose and its hydrolysates at temperatures of 200−260 °C and 10 MPa. At every temperature, the concentration of the saccharides with a shorter DP increased at the longer residence times. This tendency was in agreement with that reported by Carvalheiro et al. (*16*). The concentration of G_1, which is a constituent monomer of the maltooligosaccharide, was higher for the longer residence times and higher temperatures. The rate constants, $k_{4\to3}$, $k_{4\to2}$, and k_3, were evaluated by methods similar to those described previously. Although the k_3 value was estimated for the hydrolysis of maltotriose in the previous section, it was treated as a parameter to be determined as well as the $k_{4\to3}$ and $k_{4\to2}$ values.

The temperature dependence of a rate constant, k, is in many cases expressed by the Arrhenius equation.

$$k = k_0 \exp(-E/RT) \qquad (5)$$

where k_0 is the frequency factor, E is the activation energy, R is the gas constant, and T is the absolute temperature. **Figure 4** shows the Arrhenius plots for the $k_{4\to3}$, $k_{4\to2}$, and k_3 values. For every rate constant, the plots lay on a straight line on a semilogarithmic scale. The k_0 and E values for $k_{4\to3}$, $k_{4\to2}$, and k_3 were evaluated to be 2.2×10^8 s^{-1} and 9.9×10^1 kJ/mol, 3.6×10^{10} s^{-1} and 1.2×10^2 kJ/mol, and 7.6×10^9 s^{-1} and 1.1×10^2 kJ/mol, respectively. The E value for $k_{4\to3}$ was lower than that for $k_{4\to2}$, indicating that the energy barrier for the cleavage of the exo-site bond would be lower than that for the cleavage of the endo-site bond.

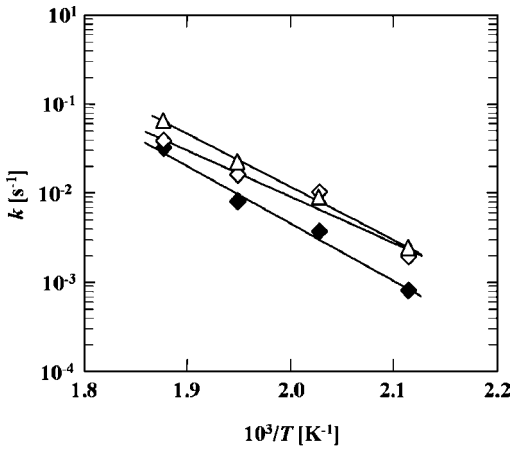

Figure 4. Arrhenius plot of the rate constant, k, for the hydrolysis of maltotetraose. Symbols: (◇) $k_{4\to3}$, (◆) $k_{4\to2}$, and (△) k_3.

Conclusion. The hydrolysis of maltooligosaccharides with DPs of 3−6 in subcritical water consecutively proceeded. The rate constants for the cleavage of each bond were evaluated by applying first-order kinetics to the experimental results at short residence times. A weak site specificity was found for the hydrolysis, and the rate constants for the exo-site glucosidic bonds were slightly greater than those for the endo-site bonds. The activation energy for the cleavage of the exo-site bond was smaller than that for the cleavage of the endo-site bond for the hydrolysis of maltotetraose.

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