Hydrolysis of Disaccharides by Metal Species in Neutral Homogeneous Solutions

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Metal species, including lanthanoid(III) ions were found to effectively hydrolyze disaccharides to the corresponding monosaccharides at 80 °C without the accompanying oxidative cleavage under neutral conditions.

Oligosaccharides play important roles in many biological events, including inflammation, cell adhesion, and signal transduction.[1] Therefore, development of artificial glycosidases for selectively controlling the specific functions of certain oligosaccharides has been a most attractive subject. They will be valuable tools for future studies in chemistry, biology, and medicine.

To date, many attempts to hydrolyze DNA and peptides using transition-metal ions such as, Cu^{II}, Co^{III}, Zn^{II}, and lanthanoide(III)/(IV), or their complexes have been reported.[2–4] In addition, as the first example, Bols et al. recently reported artificial enzymes that catalyze the hydrolysis of aryl glycosides utilizing a hydrophobic interaction between the aryl moiety and cyclodextrin.[5] However, there have been no previous reports on the hydrolysis of oligosaccharides in aqueous media under neutral conditions. On the basis of these previous findings, we expected that if metal species could properly coordinate with oligosaccharides under neutral conditions, these metal species could hydrolyze not only DNA and peptides, but also oligosaccharides. We now describe the evaluation of metal species for the hydrolysis of disaccharides under neutral conditions. To the best of our knowledge, this is the first example of the hydrolysis of disaccharides in neutral homogeneous solutions.

To evaluate metal species for the hydrolysis of maltose **1**, we carried out the first screening for active metal species using 24 different metal chloride species, Hf^{IV}, Zr^{IV}, Pd^{II}, Mg^{II}, Mn^{II}, Ni^{II}, Cu^{II}, Zn^{II}, Sc^{III}, Y^{III}, Lu^{III}, La^{III}, Ce^{III}, Pr^{III}, Nd^{III}, Sm^{III}, Eu^{III}, Gd^{III}, Tb^{III}, Dy^{III}, Ho^{III}, Er^{III}, Tm^{III}, and Yb^{III} in Tris-HCl buffer (100 mM, pH 7.0, 7.5, and 8.0) at 80 °C for 24 h. The progress of the hydrolysis was monitored by HPLC, and the conversion values were calculated based on the peak area corresponding to maltose **1**. These results are summarized in Figure 1. When Hf^{IV} and Zr^{IV} were used in the reactions, significant hydrolysis took place. However, in these cases including Pd^{II}, it was found that the pH of the reaction solutions decreased (pH 1.6, 1.8, and 2.8) owing to the hydrolysis of these metal species to metal hydroxides. In addition, when higher pH buffers were used, the hydrolysis activities decreased. Thus, these results suggested that, in the above cases, the hydrolysis of maltose **1** took place under acidic conditions. In contrast, when Y^{III}, Lu^{III}, Pr^{III}, Nd^{III}, Sm^{III}, Eu^{III}, Gd^{III}, Tb^{III}, Dy^{III}, Ho^{III}, Er^{III}, Tm^{III}, and Yb^{III} were used in the reactions, the pHs remained neutral (pH 6.8–8.0), and almost 10 mol % conversions were observed. These results clearly indicate that these metal salts hydrolyzed maltose **1** to the corresponding glucose under neutral conditions. Furthermore, it was noted that the conversion values slightly increased at higher pH for Lu^{III}, Pr^{III}, Nd^{III}, Sm^{III}, Tb^{III}, Ho^{III}, and Yb^{III}. Among these metal species, Nd^{III} provided the best result at pH 8.0.

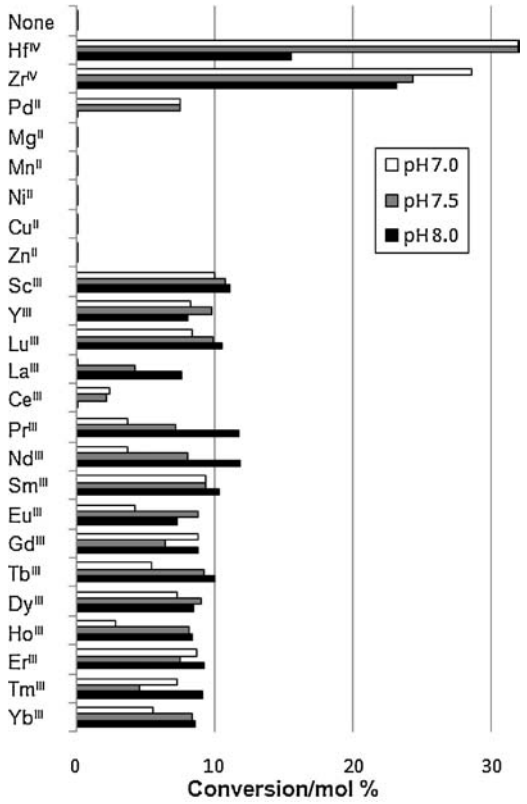

Figure 1. The hydrolysis activities of maltose **1** by metal species at 80 °C for 24 h. Maltose **1** (10 mM) was incubated with each metal salt (10 mM) in Tris-HCl buffer (100 mM, pH 7.0, 7.5, and 8.0) and analyzed by HPLC (TSKgel Amide-80, 4.6 × 250 mm; 7:3 MeCN/H_2O; flow rate 0.5 mL min^{-1}; 80 °C; detection by IR).

Table 1. Time-course of hydrolysis of maltose **1** (10 mM) using Nd^{III} (10 mM) in Tris-HCl buffer (100 mM, pH 8.0) at 80 °C

Time/h	0	6	9	24	48
Conversion/mol %	0	6.3	8.9	11.9	13.0

Next, the reaction time profile of the hydrolysis of maltose **1** using Nd^{III} was investigated as shown in Table 1. It was found that the reaction gradually proceeded as the reaction time increased. However, the reaction rate decreased as the reaction time increased. This result may suggest that the metal ion also forms a complex with glucose as the product, which is an obstacle to the hydrolysis of maltose **1**. Indeed, it was confirmed that the hydrolysis rate of maltose **1** in the presence of glucose decreased.

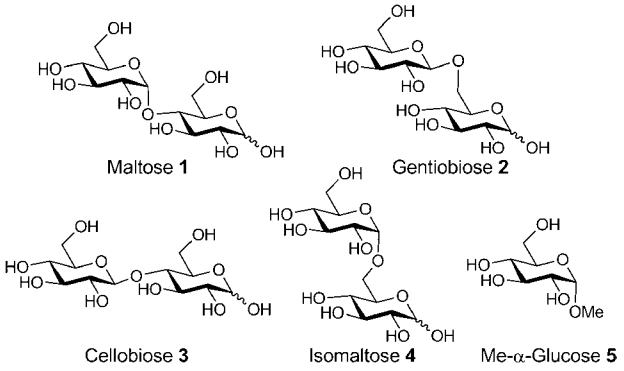

Figure 2. Chemical structures of the glycosides used in the hydrolysis.

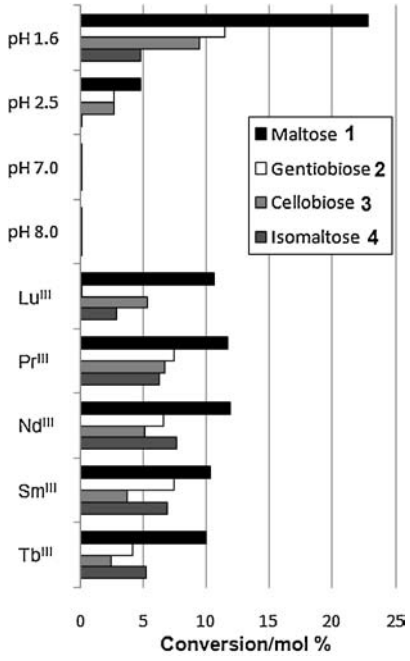

Figure 3. The hydrolysis activities of maltose **1**, gentiobiose **2**, cellobiose **3**, and isomaltose **4** by metal species at 80 °C for 24 h. Each disaccharide (10 mM) was incubated with each metal salt (10 mM) in Tris-HCl buffer (100 mM, pH 8.0) and analyzed by HPLC (TSKgel Amide-80, 4.6 × 250 mm; 7:3 MeCN/H_2O; flow rate 0.5 mL min^{-1}; 80 °C; detection by IR).

Next, we investigated the substrate dependency using gentiobiose **2** [Glc-β(1,6)-Glc], cellobiose **3** [Glc-β(1,4)-Glc], and isomaltose **4** [Glc-α(1,6)-Glc]. The chemical structures of these disaccharides are shown in Figure 2. The hydrolysis of each disaccharide (10 mM) in the presence of each metal ion (10 mM), Lu^{III}, Pr^{III}, Nd^{III}, Sm^{III}, and Tb^{III} took place in 100 mM Tri-HCl buffer (pH 8.0) at 80 °C for 24 h. In addition, the hydrolysis of each disaccharide in several different pH solutions (pH 1.6, 2.5, 7.0, and 8.0) without any metal salts was also carried out as the control reactions. These results are summarized in Figure 3.

It was found that the hydrolysis activities of the disaccharides at pH 1.6 are in the following order: maltose **1** > gentiobiose **2** > cellosibose **3** > isomaltose **4**. At pH 2.5, the activities remarkably decreased, and no hydrolysis of isomaltose **4** was observed. At pHs 7.0 and 8.0 without the metal salts, no hydrolysis of each disaccharide occurred at all. However, it was found that for the hydrolysis of maltose **1**, all the metal species showed almost twice the activity of the control reaction at pH 2.5. Isomaltose **4** was hydrolyzed by each metal salt, and the activities of Pr^{III}, Nd^{III}, Sm^{III}, and Tb^{III} were greater than the control reaction at pH 1.6. Moreover, it is interesting to note that the order of the hydrolysis of each disaccharide depends on the metal species. This result suggests that the hydrolysis activity depends on the binding affinity to each disaccharide. In order to elucidate the metal–disaccharide interaction, we performed NMR measurements with a mixture of maltose **1** and Nd^{III} in deuterated Tris-HCl buffer (pD 8.0) at 80 °C. However, no appreciable spectral change was observed owing to the extremely weak interaction.[6] Next, the hydrolysis of Me-α-glucose **5** with Nd^{III} was examined under the same conditions as a comparative study. As a result, it was found that no conversion of Me-α-glucose **5** was observed. These results suggest that the geometry of the adjacent hydroxy groups[6,7] on both pyranose rings is important for the hydrolysis of the disaccharides (Figure 4). Further investigations of the detailed mechanism and substrate specificity are now in progress.

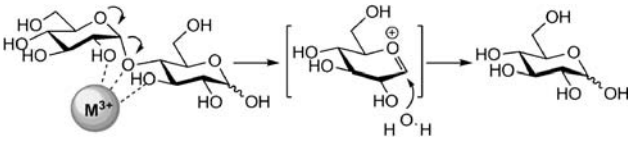

Figure 4. Proposed mechanism for the metal-induced hydrolysis of maltose **1**.

In conclusion, we describe the efficient hydrolysis of disaccharides using metal species under neutral conditions. The Nd^{III} ion is one of the most active species. The conversion values were higher than those for the conversions at pH 2.5. Furthermore, although isomaltose **4** was not hydrolyzed at pH 2.5, the metal-induced hydrolyses efficiently took place under neutral conditions (pH 7.0–8.0). Thus, we believe that the results presented here will contribute to the molecular design of novel artificial glycosidases.

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References and Notes

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