ORIGINAL ARTICLE

Production of disaccharides from glucose by treatment with an ionic liquid, 1-ethyl-3-methylimidozolium chloride

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Abstract The detailed reaction behavior of cellulose in an ionic liquid, 1-ethyl-3-methylimidazolium chloride ([C2mim][Cl]), was investigated. Glucose is a component of cellulose; its oligomerization was studied during treatment with [C2mim][Cl] at around 100 °C. Glucose was polymerized to produce oligosaccharides such as disaccharides and trisaccharides. These oligosaccharides, however, disappeared upon prolonging the treatment time. Maltose, nigerose, kojibiose, laminaribiose, isomaltose, and gentiobiose were identified by gas chromatograph–mass spectrometer in the disaccharides produced. The yield of these disaccharides increased as the initial concentration of glucose in [C2mim][Cl] increased. [C2mim][Cl], therefore, is a reagent capable of producing various oligosaccharides from glucose in the absence of a catalyst.

Keywords Glucose · Ionic liquid · 1-Ethyl-3-methylimidazolium chloride · Oligomerization · Oligosaccharides

Introduction

Recently, energy and environmental problems caused by fossil resources have been of concern. To overcome these problems, the establishment of a sustainable society based on renewable resources such as biomass is important. Among various biomass resources, wood is promising because of its availability and its inedible components. It is mainly composed of cellulose, hemicellulose, and lignin. For the chemical use of these components, various methods such as acid hydrolysis [1], enzymatic saccharification [2], pyrolysis [3, 4], and supercritical fluid treatment [5] have been studied.

Recently, treatment with ionic liquids has been proposed to be an attractive novel technology for wood processing. Ionic liquids are organic salts composed of cations and anions only and have melting points close to ambient temperature [6]. They are not volatile and have good solvation power towards various chemical species. Furthermore, ionic liquids are regarded as "green solvents" because of their negligible vapor pressure, non-flammability, and recyclability. Many fundamental and applied studies on the ionic liquid treatment of wood have been carried out. It has been reported that the ionic liquid, 1-ethyl-3-methyl imidazolium chloride ([C2mim][Cl]), can liquefy both softwood and hardwood by depolymerizing wood components. Additionally, the reaction mechanism of this ionic liquid treatment has been determined [7, 8]. Many studies have been conducted on the ionic liquid treatment of cellulose, which is the main component of wood. Studies have been conducted into the solubility of cellulose in ionic liquids [9–12], the derivatization of cellulose in ionic liquids [13–15], and the rheological properties of cellulose in ionic liquids [16, 17]. We have previously studied the reaction behavior of cellulose in [C2mim][Cl] [18]. The treatment of cellulose with [C2mim][Cl] at 120 °C leads to its decomposition into various products such as oligosaccharides, cellobiose, cellobiosan, glucose, levoglucosan, and 5-hydroxymethylfurfural. Recently, the decomposition mechanisms of cellulose in [C2mim][Cl] have been studied [19]. The authors found that hydrolysis is the main reaction because [C2mim][Cl] can retain water at higher than 100 °C, which is the boiling point of water.

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Glucose polymerizes into various oligosaccharides upon heating. In cellulose science, this oligomerization reaction is known as reversion. The reversion of glucose occurs during the acid hydrolysis of cellulose to produce various oligosaccharides. It has been reported that various disaccharides such as gentiobiose, isomaltose, cellobiose, maltose, sophorose, and β,β-trehalose have been produced by the heat treatment of D-glucose in HCl aq. at around 100 °C [20]. Helm and Young [21] reported that the reversion of glucose occurs during the high-temperature hydrolysis of Avicel cellulose between 160 and 250 °C with dilute sulfuric acid. The most abundant disaccharides were isomaltose and gentiobiose. Recently, the reversion of glucose in dilute sulfuric acid has been studied and the kinetics of conversion into various disaccharides has been modeled [22]. In food science, the oligomerization of glucose upon heating is recognized as a reaction that occurs during caramelization. Sugisawa and Edo [23] reported that various disaccharides such as kojibiose, sophorose, nigerose, laminaribiose, maltose, cellobiose, isomaltose, and gentiobiose were isolated from caramel upon heating glucose at 150 °C in the absence of a catalyst.

However, not much attention has been paid to the oligomerization of glucose in ionic liquids although glucose can be obtained by the decomposition of cellulose in ionic liquids, as mentioned above. In this study, therefore, we investigated the oligomerization of glucose in [C2mim][Cl]. This fundamental information is crucial in understanding the reaction behavior of various biomass sources and for the application of ionic liquids to the effective use of biomass. Glucose was selected because it is one of the most important monomers found in various biomass resources.

Materials and methods

Samples and chemicals

Glucose was purchased from Wako Pure Chemical Industries, Ltd., Osaka, Japan. It was dried in an oven at 105 °C for 24 h before use. The ionic liquid, 1-ethyl-3-methylimidazolium chloride ([C2mim][Cl]), was purchased from Tokyo Chemical Industry Co. Ltd., Tokyo, Japan. N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) was purchased from Sigma–Aldrich Co. LLC., St. Louis, MO, USA. Acetonitrile was purchased from Wako Pure Chemical Industries, Ltd., and pyridine was purchased from Nakalai Tesque, Inc., Kyoto, Japan.

Treatment with [C2mim][Cl]

Three grams of [C2mim][Cl] was heated at 100 °C or 90 °C in a 100-mL glass flask. After melting the [C2mim][Cl], glucose was added to [C2mim][Cl] at a loading of 3, 10, and 20 wt%. [C2mim][Cl] and the reaction media were gently stirred. 0 h of treatment was defined as the time when the reaction media became transparent without any glucose particles being observed in the flask. It took several minutes for the reaction media to become transparent.

Evaluation methods

The compounds solubilized in [C2mim][Cl] were analyzed by high-performance liquid chromatography (HPLC). HPLC was carried out on a Shimadzu Prominence (Shimadzu, Kyoto, Japan) equipped with a pump (LC-20AD), a column oven (CTO-20A), a refractive index detector (RID-10A), and an ultraviolet–visible detector (SPD-M20A). The samples for this analysis were prepared as follows: at a specified reaction time, 20 μL reaction medium was homogeneously mixed with 180 μL distilled water and then filtered through a 0.45-μm filter. The filtrates were analyzed under the following conditions: a Shodex Sugar KS-801 column (Showa Denko, Tokyo, Japan); a flow rate of 1 mL/min; distilled water as eluent; a refractive index detector (RID), an ultraviolet–visible detector set at 280 nm; and a column temperature of 80 °C.

To identify the compounds solubilized in [C2mim][Cl], a Shimadzu GCMS-QP2010 Ultra gas chromatograph–mass spectrometer (GC–MS) was used. GC–MS analysis samples were prepared as follows: 20 μL reaction media was homogeneously mixed with 240 μL acetonitrile. After drying with sodium sulfate, the obtained mixture was filtered through a 0.45-μm filter. Then, 120 μL filtrate was silylated at room temperature using 60 μL BSTFA mixed with 10 μL pyridine. The obtained silylated samples were analyzed by GC–MS. The capillary column was an ULBON HR-52 (diameter, 0.25 mm) from Shinwa Chemical Industries Ltd., Kyoto, Japan. The temperature program was 60 °C (0 → 1 min), 60 °C → 230 °C (1 → 21 min) and 230 °C (21 → 50 min). Helium was used as a carrier gas at a flow rate of 1.0 mL/min. The injector and detector temperature were 250 and 230 °C, respectively.

Results and discussion

HPLC analysis of the compounds solubilized in [C2mim][Cl]

Figure 1 shows HPLC chromatograms of the compounds solubilized in [C2mim][Cl] after the treatment of glucose at
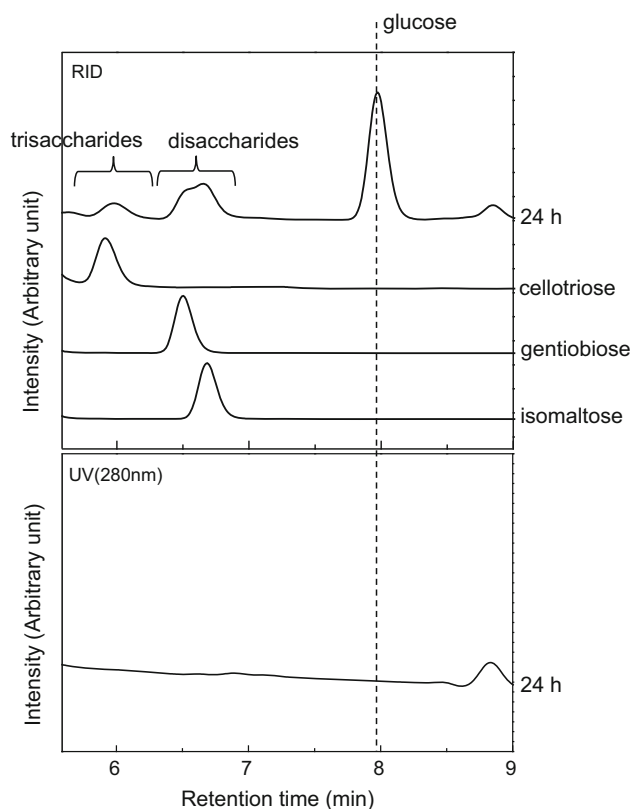
Fig. 1 HPLC chromatograms of the compounds dissolved in [C2mim][Cl] after the treatment of glucose at 100 °C for 24 h. *Top*, refractive index detector (*RID*); *bottom*, ultraviolet (*UV*) detector (λ = 280 nm)

a loading of 20 wt% for 24 h at 100 °C. Chromatograms of cellotriose, isomaltose, and gentiobiose are also included in this figure. The complex peaks observed at retention times (RTs) between 6.3 and 7.0 min by RID correspond to isomaltose and gentiobiose. No peaks were present between 6.3 and 7.0 min in the chromatograms obtained from the ultraviolet detector. The peaks obtained using the RID thus come from disaccharides. Compounds with higher molecular weights can be detected at earlier RTs using a KS-801 column. Moreover, the peak for the trisaccharide cellotriose was present at a RT of around 6 min. Therefore, the peak at around 6 min comes from trisaccharides.

GC–MS analysis of the compounds solubilized in [C2mim][Cl]

To identify the disaccharides solubilized in [C2mim][Cl], GC–MS analyses were conducted. Figure 2 shows the total ion chromatogram of the trimethylsilylated compounds in [C2mim][Cl] after the treatment of glucose at a loading of 20 wt% for 24 h at 100 °C. Although many peaks are present in this chromatogram, the peaks around the RT
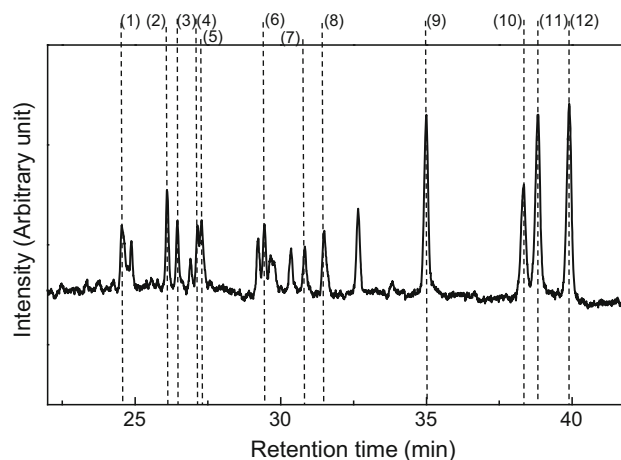
Fig. 2 Total ion chromatogram of the trimethylsilylated compounds in [C2mim][Cl] after the treatment of glucose at 100 °C for 24 h

shown in this figure come from disaccharides. Unfortunately, no trisaccharide peaks were detected because of their high boiling points. Table 1 lists the identified disaccharides in [C2mim][Cl], their RT, and their characteristic ions. The numbers in this table correspond to the numbers shown in Fig. 2. Identification was done by comparing the RTs and mass fragmentation patterns with those of authentic compounds. Six disaccharides: maltose, nigerose, kojibiose, laminaribiose, isomaltose, and gentiobiose were identified. Their chemical structures are shown in Fig. 3. These are disaccharides composed of glucose although the chemical bonds between their glucose units are different, as shown below each disaccharide's name in Fig. 3. Authentic compounds of cellobiose, sophorose and trehalose which are disaccharides composed of glucose can be detected by GC–MS analyses. However, their peaks do not match with any peaks of the chromatogram in Fig. 2. At the moment, it is not clear whether these disaccharides were not produced at all or enough to analyze by GC–MS.

The peaks in Fig. 2 present at around 30 min RT could not be identified. However, the characteristic ions of their mass fragments are very similar to the identified disaccharides. Therefore, these unknown peaks must come from disaccharides composed of glucose. These results reveal that glucose can be converted to various valuable disaccharides in [C2mim][Cl] in the absence of a catalyst.

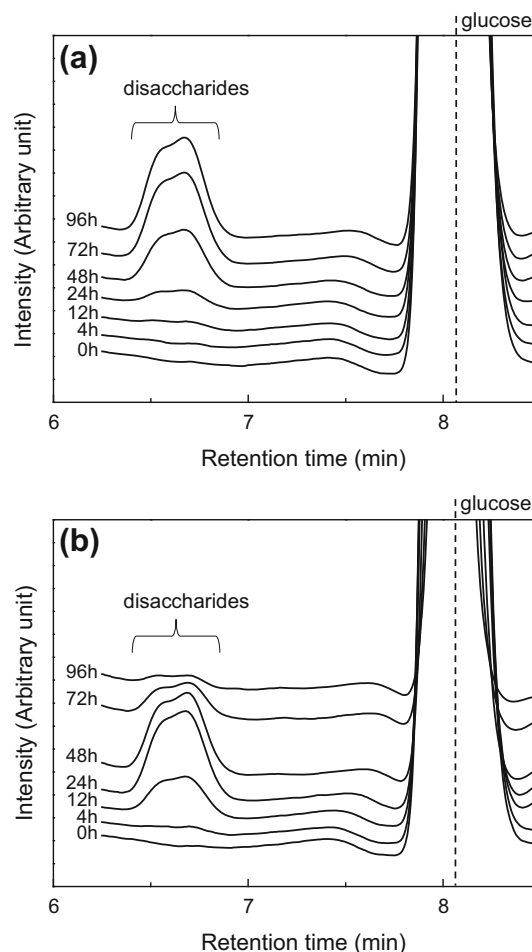Effect of reaction conditions on the yield of disaccharides

Figure 4 shows HPLC chromatograms of the disaccharides solubilized in [C2mim][Cl] after the treatment of glucose at a loading of 3 wt% at 90 °C (a) and 100 °C (b). In all the chromatograms, peaks from glucose were observed at a RT of around 8 min. At 90 °C, the disaccharide peak at around

**Table 1** List of identified disaccharides, their retention time (RT), and their characteristic ions

No.	Products	RT (min)	Characteristic ions (m/z)
1	Maltose	24.5	243, 271, 305, 361
2	Nigerose	26.0	243, 271, 319, 361, 435, 451
3	Maltose	26.4	243, 271, 305, 361, 451
4	Kojibiose	27.1	243, 271, 305, 319, 331, 361, 435, 451
5	Nigerose	27.3	243, 271, 319, 361, 435, 451, 539
6	Laminaribiose	29.2	243, 271, 305, 361, 435, 451, 569
7	Kojibiose	30.8	243, 271, 305, 319, 361, 451, 539
8	Laminaribiose	31.0	243, 271, 305, 361, 451,
9	Isomaltose	34.9	243, 271, 305, 319, 361, 451, 569, 583
10	Gentiobiose	38.3	243, 321, 451, 319
11	Gentiobiose	38.8	243, 271, 305, 361, 435, 451, 569, 583
12	Isomaltose	39.8	243, 271, 305, 319, 361, 435, 451
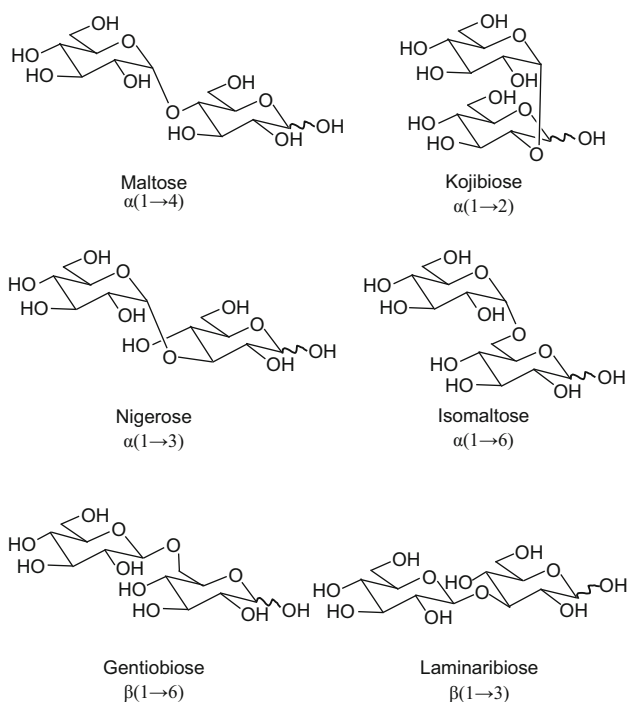
Fig. 3 Chemical structures of the various disaccharides identified

Fig. 4 HPLC chromatograms of the disaccharides dissolved in [C2mim][Cl] after the treatment of glucose at a loading of 3 wt% at 90 °C (**a**) and 100 °C (**b**)

6.8 min increased gradually with an increase in treatment time. A similar trend was observed at 100 °C before the 24-h treatment. However, after the 24-h treatment, the disaccharide peak decreased. From these chromatograms, the disaccharide yield was calculated based on the calibration curve prepared using isomaltose as a standard. Figure 5 shows the change in disaccharide yield for the

glucose reaction in [C2mim][Cl] at 90 °C and at 100 °C. At 90 °C, the disaccharide yield was found to increase during the reaction and it reached 2.9 % at 96 h. At 100 °C, however, the yield was highest at 24 h and was 3.2 %. Our previous paper [18] on the heat treatment of cellulose with [C2mim][Cl] revealed that cellulose decomposes into low-molecular weight compounds via glucose without producing polysaccharides as final products. This means that the saccharides are not stable in heated [C2mim][Cl]. Therefore, once glucose is converted into a disaccharide it decomposes into a low-molecular weight compound. This is the reason for the highest disaccharide yield being obtained at 100 °C, as shown in Fig. 5. Figure 6 shows the relationship between the initial loading wt% of glucose in [C2mim][Cl] and the disaccharide yield after 24 h of treatment at 100 °C. The disaccharide yield was found to increase as the loading wt% increases. This result suggests that a high load of glucose in [C2mim][Cl] is required to maximize disaccharide production.
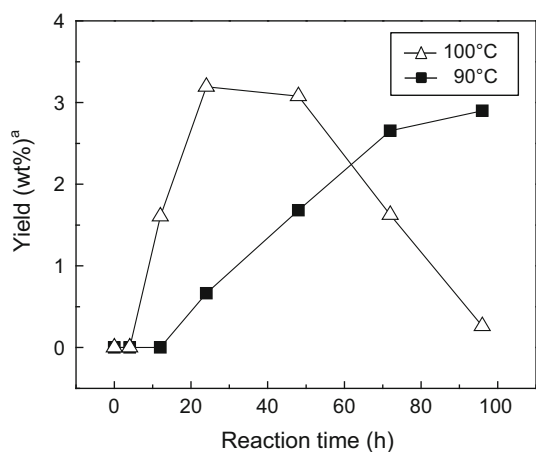
Fig. 5 Change in disaccharide yield during the reaction of glucose in [C2mim][Cl] at 90 °C (*solid square*) and 100 °C (*open triangle*). [a] The yield was calculated using the following equation: Yield (wt%) $= W_1/W_0 \times 100$, where W_0 is the amount of charged glucose and W_1 is the weight of the disaccharides
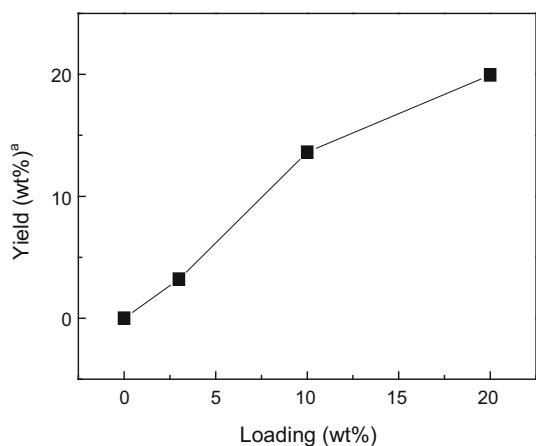
Fig. 6 Relationship between the initial loading wt% of glucose in [C2mim][Cl] and the disaccharide yield. [a] The yield was calculated using the following equation: Yield (wt%) $= W_1/W_0 \times 100$, where W_0 is the amount of charged glucose and W_1 is the weight of disaccharides

It has previously been reported that heating at 150 °C is necessary for the reversion of glucose in a reaction system without a catalyst [23]. Reversion occurs around 100 °C when using HCl or sulfuric acid as a catalyst [21, 22]. We thus carried out the heat treatment of glucose at 100 and 90 °C without [C2mim][Cl]. However, no oligosaccharides were produced in these experiments (data not shown). These results indicate that [C2mim][Cl] can cause the reversion of glucose without a catalyst. [C2mim][Cl] is thus a new reaction system for the production of oligosaccharides from glucose. Isomaltose is a disaccharide identified in this study and it is a component of new and healthy foods because it improves intestinal flora [24]. To

produce these valuable oligosaccharides the ionic liquid reaction system, [C2mim][Cl], is superior to previous methods because of the absence of a catalyst and the simple reaction procedure as well as the simple apparatus required. This is assisted by the negligible vapor pressure and non-flammability of the ionic liquid.

Conclusion

Glucose was found to be polymerized during treatment with [C2mim][Cl] at around 100 °C. Various disaccharides such as maltose, nigerose, kojibiose, laminaribiose, isomaltose, and gentiobiose were identified. The disaccharide yield tends to increase as the initial concentration of glucose in [C2mim][Cl] increases. Consequently, [C2mim][Cl] polymerizes glucose to oligosaccharides. We thus report a new and simple method for the production of various valuable oligosaccharides from various monosaccharides using an ionic liquid.

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