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Efficient hydrolysis of chitosan in ionic liquids

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ABSTRACT

Effective hydrolysis of chitosan, the *N*-deacetylated product of chitin, remains challenging. Here, we report acid-promoted hydrolysis of chitosan in imidazolium based ionic liquids with good total reducing sugars (TRS) yield under mild conditions. TRS yield reached over 60% in the presence of about 6.0 wt% concentrated hydrochloric acid at 100 °C within 7 h. Kinetic modeling of a typical experimental data set suggested that the hydrolysis most likely followed a consecutive first-order reaction sequence, where k_1 and k_2, the rate constants for TRS formation and degradation, were determined to be 0.01372 and 0.00015 min^{-1}, respectively. Our method may be useful to explore new applications of natural chitin resources.

1. Introduction

Chitin is a naturally occurring biopolymer composed of β-(1,4)-linked *N*-acetylglucosamine (GlcNAc) unit. It is the second most abundant natural polymer after cellulose and has a similar structure to cellulose. Chitin exists in several forms depending on its crystalline structure. Two of these crystalline polymorphic forms are α-chitin and β-chitin (Muzzarelli, Ilari, Tarsi, Dubini, & Xia, 1994). The β-chitin crystallizes in a monoclinic cell. However, the packing structure of α-chitin is strongly stabilized by intrachain, intrasheet, and intersheet hydrogen bonds in three-dimensional configuration (Gardner & Blackwell, 1975). Therefore, α-chitin exhibits lower reactivity, swelling, and solubility than β-chitin (Kurita, Ishii, Tomita, Nishimura, & Shimoda, 1994; Saito, Okano, Gaill, Chanzy, & Putaux, 2000; Saito, Putaux, Okano, Gaill, & Chanzy, 1997). Yet, α-chitin extracted from crab shells or shrimp is much more abundant due to its marine product origin and is more widely utilized. Chitosan is the *N*-deacetylated product of chitin and its major resource is also from marine product. Recently, chitosan has attracted much attention owing to its broad rang of applications in the food, medicine, cosmetic, material etc. (Laudenslager, Schiffman, & Schauer, 2008; Li, Dunn, Grandmaison, & Goosen, 1992), but it remains undervalued partially due to its poor solubility. Hydrolysis of chitosan to gulcosamine and its oligomers perhaps is the most common way to improve its application.

Several methods, including enzymatic hydrolysis, acidic hydrolysis or oxidative depolymerization, have been applied to hydrolyze chitosan to produce glucosamine and its oligomers (Fig. 1). The enzymatic process takes place under mild conditions, yet the hydrolysis rate is slow. Furthermore, the prices of the enzymes are high and the enzymes lose activity easily (Konieczna-Molenda, Fiedorowicz, Zhong, & Tomasik, 2008; Ming, Kuroiwa, Ichikawa, Sato, & Mukataka, 2006). Acid hydrolysis is routinely practiced to attain glucosamine and oligochitosans. Chitosan hydrolysis with concentrated hydrochloric acid requires excess acid loading, complex reactors, and has major waste disposal problems (Einbu & Vårum, 2008; Horowitz, Roseman, & Blumenthal, 1957). Furthermore, an excess of acid treatment results in the breakdown of glucosamine, which significantly lowers the yield and interferes with downstream applications. Oxidative depolymerization in concentrated nitrous acid provides chitosan oligomers with 9–18 monomeric units, and the final products contained 2,5-anhydromannose residues by deamination (Allan & Peyron, 1995; Furusaki, Ueno, Sakairi, Nishi, & Tokura, 1996). Up to now, effective hydrolysis of chitin and chitosan remains challenging.

Ionic liquids (ILs), combining good and tunable solubility properties with a negligible vapor pressure and excellent thermal stability, have recently been used for dissolving biological macromolecules including cellulose, wool keratin and silk fibroin that are linked together by intermolecular hydrogen bonds (Cuissinat, Navard, & Heinze, 2008; Swatloski, Spear, Holbrey, & Rogers, 2002; Zhang, Wu, Zhang, & He, 2005). Early data showed that chitosan had a good solubility in 1-butyl-3-methylimidazolium chloride ($[C_4mim]Cl$), and up to 10 wt% of chitosan can dissolve in this media to form a viscous solution (Xie, Zhang, & Li, 2006).

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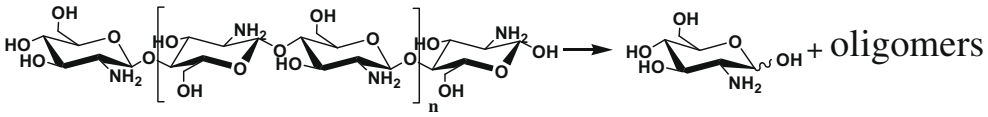

Fig. 1. Schematic illustration of chitosan hydrolysis.

We have previously demonstrated that cellulose (Li & Zhao, 2007) and lignocellulose (Li, Wang, & Zhao, 2008) can be depolymerized to afford monomeric carbohydrates and their oligomers in ILs in the presence of acid catalysts. In this paper, we would like to report our preliminary results on mineral acid-promoted depolymerization of chitosan in ILs.

2. Experimental

2.1. Materials

Low molecular weight chitosan (20–200 cP) with a degree of deacetylation (DD) value from 75% to 85%, medium molecular weight chitosan (200–800 cP) with a DD value from 75% to 85%, high molecular weight chitosan (800–2000 cP) with a DD value over 75%, and glucosamine (99%) were purchased from Sigma (St. Louis, USA), and used directly with no pretreatment. Sodium acetate (98%), 1-chlorobutane (98%) and 1-bromobutane (98%) were purchased from ABCR GmbH & Co. (Karlsruhe, Germany) and the latter two were freshly distilled before use. *N*-Methylimidazole (99%) was obtained from Zhejiang Kaile Chemicals Co. Ltd. (Hangzhou, China). Mineral acids 36.5 wt% hydrochloric acid, 65 wt% nitric acid, and 98 wt% sulfuric acid were supplied by Beijing Chemicals Co. Ltd. (Beijing, China). Sodium hydroxide (99%) was purchased from J&K Chemicals Co. Ltd. (Beijing, China). Ethyl acetate (99%) and sodium carbonate (99%) were from Guangfu Fine Chemical Research Institute (Tianjin, China). Ultrapure water used for the mobile phase and the stock solutions of the analytes was generated by Milli-Q water purification system (Millipore, Bedford, MA, USA).

2.2. Synthesis of [C_4mim]Cl

Preparation and purification of [C_4mim]Cl were done according to known procedures (Burrell, Del Sesto, Baker, McCleskey, & Baker, 2007; Webb et al., 2003) with minor modifications. Briefly, a mixture of *N*-methylimidazole (41.0 g, 0.50 mol) and 1-chlorobutane (56.0 g, 0.60 mol) was heated at 80 °C for 4 days. During the process a white solid formed, which slowly turned yellow. After cooling to room temperature, the reaction mixture formed two phases. The upper layer was removed by decanting. The lower layer was washed with ethyl acetate (3 × 30 ml) and dried in vacuum at 80 °C for 12 h. The crude product was then taken in water (150 ml) and treated with activated charcoal (5.0 g) at room temperature for 24 h. The solution was filtered through Celite, dried in vacuum at 80 °C for 12 h to give 80.2 g yellowish liquid in 92% yield, which crystallized as a white solid at room temperature. The sample had a melting point of 64–65 °C (Determined on the model 1102D Melting Point apparatus from Barnstead International), which was close to the literature data of 66 °C (Carda-Broch, Berthod, & Armstrong, 2003). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.58 (s, 1H), 7.35 (s, 1H), 7.30 (s, 1H), 4.08–4.05 (t, 2H, J = 6.0 Hz), 3.76 (s, 3H), 1.76–1.68 (m, 2H), 1.21–1.14 (m, 2H), 0.81–0.77 (t, 3H, J = 8.0 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ = 135.8, 123.4, 122.1, 49.2, 35.5, 31.1, 18.6, 12.5.

2.3. Synthesis of [C_4mim]Br

All of the procedures were the same with those for the preparation of [C_4mim]Cl mentioned above except for minor modifications. At first 1-bromobutane was slowly added to freshly distilled *N*-methylimidazole as the reaction being exothermic. After the mixture was heated at 80 °C for 2 days, workup was done similar to that of [C_4mim]Cl to give [C_4mim]Br in 94% yield. The sample had a melting point of 77–78 °C, which was close to the literature data of 77 °C (Nishikawa & Wang, 2007). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 10.41 (s, 1H), 7.51 (s, 1H), 7.41 (s, 1H), 4.35–4.31 (t, 2H, J = 8.0 Hz), 4.12 (s, 3H), 1.94–1.86 (m, 2H), 1.41–1.35 (m, 2H), 0.97–0.94 (t, 3H, J = 6.0 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ = 138.1, 123.9, 122.3, 50.4, 37.2, 32.6, 19.9, 13.9.

2.4. Typical procedures for chitosan hydrolysis

A mixture of chitosan (255 mg) in [C_4mim]Cl or [C_4mim]Br (4.0 g) was heated at 100 °C under an atmospheric pressure with a magnetic stirrer until a clear solution was formed. To this solution were added an appropriate amount of H_2O and mineral acid. The reaction mixture was vigorously stirred with a reflux condenser. At different time intervals, samples (up to 20 samples each for ca. 50 mg) were withdrawn, weighed (recorded as M_1), quenched with cold water, neutralized with 0.50 mol/l NaOH, and centrifuged at 10,000 rpm for 5 min, measured the volume (recorded as V_1), then was 6-fold diluted with H_2O, and subjected to TRS analysis.

2.5. Total reducing sugars (TRS) analysis according to Imoto's method

The Imoto reagent was prepared as follows (Imoto & Yagishita, 1971): 0.50 g potassium ferricyanide was mixed to 1 l (0.50 mol/l Na_2CO_3) solution and stored in an amber glass bottle. A mixture containing 4 ml of Imoto reagent and 1 ml of sample was heated for 15 min at 100 °C, then cooled to room temperature. The color intensity of the mixture was measured in a JASCO V-530 Model spectrophotometer at 420 nm with a slit width of 0.06 mm. The concentration of total reducing sugars was calculated based on a standard curve obtained with glucosamine. The mass of TRS M_T and the yield of TRS were calculated as follows:

$$M_T\,(\mathrm{mg}) = \text{concentration}\,(\mathrm{umol/ml}) \times (6\,V_1) \times (M_0/M_1) \times \mathrm{Mr}/1000$$

$$\text{TRS yield} = M_T\,(\mathrm{mg})/282(\mathrm{mg}) \times 100\%$$

In which, M_T is the mass of TRS, V_1 is the volume of the sample, M_0 is the total mass of the reaction solution, M_1 is the mass of sample and Mr is the molar mass of glucosamine. The data "282 (mg)" refers to the theoretical mass of glucosamine based on an average DD value of 80% in chitosan samples.

2.6. Analysis of chitosan hydrolysis sample by ion chromatography (IC)

The IC system and components were from Dionex (Sunnyvale, CA, USA). The hardware consisted of an ICS-2500 IC system equipped with a GP50 gradient pump, an ED50A integrated amperometry detector, a CarbonPac PA10 guard column (4 × 50 mm), a

high capacity CarbonPac PA10 analytical column (4 × 50 mm), a 25 μl sample loop. Eluents were degassed via sonication under a nitrogen atmosphere, and were further purified by a borate trap column (4 × 50 mm) placed between the pump and the injection valve. Data acquisition and instrument control were performed using the Chromeleon software installed on a personal computer. Samples were eluted with the mixture solution of NaOAc and NaOH with gradient elution at a rate of 1 ml/min. Glucosamine was identified by comparing the retention time and confirmed by spiking experiments.

3. Results and discussion

At the starting point, we took low molecular weight chitosan (LMWC) as a model substrate and performed hydrolysis reactions in [C_4mim]Cl. The hydrolytic efficiency was evaluated by quantifying TRS based on the Imoto's method (modified Schales method), a specific method for analysis of glucosamine and its oligomers (Imoto & Yagishita, 1971; Li, Xie, Lin, Xie, & Ma, 2009). When 255 mg (equals to 1.5 mmol glucosamine unit based on the average molecular weight of the unit) LMWC was treated with 300 mg concentrated H_2SO_4 (3.0 mmol) and 54 mg H_2O (3.0 mmol) for 6 h, the TRS yield was 34% (Table 1, entry 1). Further increasing acid loading to 450 and 520 mg, TRS yields were 51% and 50%, respectively (Entries 2 and 3). Compared with Entries 1 and 2, it was apparent that acid loading had some influences on TRS yield. In this case, more acid loading was required than that used in cellulose hydrolysis (Li & Zhao, 2007), because chitosan is a basic polymer, and that every single glucosamine unit would consume an equivalent of acid. For this reason, it was difficult to hydrolyze chitosan with a catalytic amount of protic acid. Entries 4 and 5 listed results with different water loading. These data indicated that a longer reaction time was required to reach the maximal TRS yield as the initial water content increased. Therefore, it was important to control water loading in chitosan hydrolysis.

Hydrochloric acid-promoted hydrolysis of LMWC were listed in Entries 6–8. These reactions generally required longer reaction time to obtain similar yields to those catalyzed with sulfuric acid. Although, no additional H_2O was introduced, initial H_2O content in the reaction system reached roughly 4.2, 6.1 and 7.9 wt%, for Entries 6, 7 and 8, respectively. In contrast, initial H_2O content in Entry 2 was around 1.1 wt%. Thus, slower reaction rate catalyzed by hydrochloric acid might be partially attributed to higher water content. This was also in agreement with water content effect of sulfuric acid-promoted reactions (Entries 4 and 5). Nitric acid also showed nearly equal effectiveness to hydrochloric acid in the reaction (Entry 9).

Furthermore, we have applied this method for the depolymerization of medium molecular weight chitosan (MMWC) and high molecular weight chitosan (HMWC). Experimental data listed in Entries 10–13 showed that longer reaction time was required to reach maximal TRS yields with these materials compared to LMWC (Entries 2 and 7). It was also interesting to see that slightly better TRS yields were obtained for MMWC and HMWC.

We also found that [C_4mim]Br had similar capability as a medium for acid-promoted hydrolysis of chitosan (Entries 14–18). Although, the reaction was performed with a reduced acid loading and a short reaction time, TRS yields were comparable to those in [C_4mim]Cl. This might be due the fact that the reaction medium [C_4mim]Br reinforced the acidity of the mineral acid. Further attempts have been tried to hydrolyze chitin, but TRS yields appeared much lower than those of chitosan (Entry 19). We also noticed that the reaction mixture became more viscous, suggesting that other complex products formed under these conditions. It would take more effort to achieve efficient hydrolysis of chitin.

Imidazolium based ILs are known to have a strong ability to disrupt hydrogen bonds to dissolve cellulose (Remsing, Swatloski, Rogers, & Moyna, 2006). A similar mechanism may be involved in solubilization of chitosan. When chitosan was completely dissolved in [C_4mim]Cl/Br and formed a homogeneous solution, it made the 1,4-β-glucosidic bond more accessible to the catalytic specie H^+. This is likely the reason that the hydrolysis rate was much higher in [C_4mim]Cl/Br system when compared with those where hydrolysis occurred at the surface of chitosan (Belamie, Domard, & Giraud-Guille, 1997). Therefore, a physical barrier for hydrolysis was overcome through formation of a solution. In addition, the dissociated Cl^-/Br^- and the electron-rich aromatic system of [C_4mim]$^+$ may weaken the glycosidic linkage to facilitate hydro-

Table 1
Reaction conditions and TRS yields of chitosan hydrolysis in [C_4mim]Cl.[a]

Entry	Acid[b]	Chitosan	Acid loading (mg)	Water loading (mg)	Reaction time (min)	TRS yield (%)
1	H_2SO_4	LMWC	300	54	360	34
2	H_2SO_4	LMWC	450	54	300	51
3	H_2SO_4	LMWC	520	54	240	50
4	H_2SO_4	LMWC	450	27	190	50
5	H_2SO_4	LMWC	450	108	480	53
6	HCl	LMWC	300	–	450	50
7	HCl	LMWC	450	–	350	58
8	HCl	LMWC	600	–	450	56
9	HNO_3	LMWC	290	–	540	51
10	H_2SO_4	MMWC	450	54	440	54
11	H_2SO_4	HMWC	450	54	440	56
12	HCl	MMWC	450	–	480	61
13	HCl	HMWC	450	–	480	61
14[c]	H_2SO_4	LMWC	150	54	320	49
15[c]	H_2SO_4	MMWC	150	54	330	50
16[c]	HCl	LMWC	300	–	420	55
17[c]	HCl	MMWC	300	–	420	63
18[c]	HCl	HMWC	300	–	420	61
19[d]	HCl	Chitin	450	–	420	25
20[e]	HCl	Chitosan	35 wt% HCl at 80 °C		240	56

[a] All reactions were performed with 255 mg of chitosan at 100 °C in 4.0 g of [C_4mim]Cl, unless otherwise specified.
[b] H_2SO_4, HCl and HNO_3 indicate 98 wt% sulfuric acid, 36.5 wt% hydrochloric acid and 65 wt% nitric acid, respectively.
[c] [C_4mim]Br was used in lieu of [C_4mim]Cl.
[d] Three hundred and five milligrams of chitin as a substrate.
[e] Hydrolysis with 35 wt% HCl at 80 °C and the major product had a DP value ranged form 1 to 7 (Yabuki, 1995).

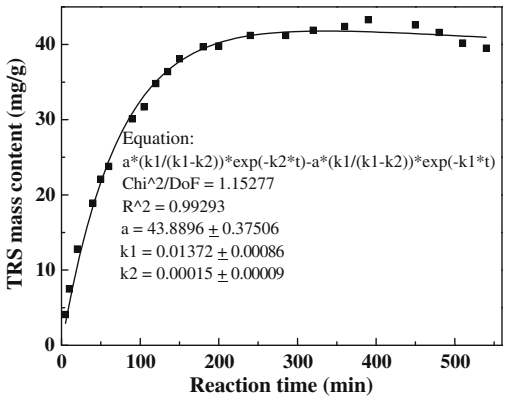

Fig. 2. Time course of chitosan hydrolysis. Reaction conditions: LMWC (0.255 g) in the mixture of 36.5 wt% HCl (0.45 g) and [C_4mim]Cl (4.0 g) at 100 °C under an atmospheric pressure.

lysis. This method produced equal or higher TRS yields when compared with procedures in an aqueous system using hydrochloric acid alone (Entry 20) (Yabuki, 1995) or hot phosphoric acid. In the later case, hydrolysis actually gave oligoglucosamine with a degree of polymerization (DP) values of 6–8 in 10–20% yield.

To better understand the kinetics of acid-promoted hydrolysis of chitosan in [C_4mim]Cl, we recorded the time course of TRS production from LMWC for 9 h in the presence of hydrochloric acid. Regression analysis of the experimental data by non-linear least squares curve fitting using a consecutive first-order reaction model with the software Origin 7.0 gave a perfect match (Fig. 2), and the parameters R^2, k_1 and k_2, representing coefficient of determination, the rate constants for TRS formation and degradation, were determined to be 0.99293, 0.01372 and 0.00015 min^{-1}, respectively. Apparently, chitosan hydrolysis proceeded significantly faster than the product degradation, as k_2 was much smaller than k_1. Therefore, the kinetics of chitosan hydrolysis in ILs was similar to that of cellulose (Li & Zhao, 2007) and most likely followed a consecutive first-order reaction sequence. When sulfuric acid was employed as a catalyst under otherwise identical conditions, k_1 and k_2 were 0.0240 and 0.0008 min^{-1}, respectively. These data were in good agreement with the observed reaction time values shown in Table 1. Noting that our previous work on hydrolysis of cellulose in ILs with sulfuric acid as the catalyst indicated that the k_1 value was 0.0730 min^{-1} (Li & Zhao, 2007), which was 3-fold higher than that for chitosan hydrolysis. These data suggested that acid-promoted hydrolysis of chitosan seemed chemically more challenging. We envisioned that protonation at the C-2 amino group of the glucosamine unit may significantly inhibit the glycosidic oxygen atom from protonation, and thus result in a decreased rate of cleavage of the glycosidic linkage (Einbu, Grasdalen, & Vårum, 2007).

Previous studies have routinely employed soluble reducing sugar analysis method to evaluate the effectiveness of chitosan hydrolysis (Chiang, Chang, & Sung, 2003; Wang & Yeh, 2008). We also found that quantification of hydrolysis products using the Imoto's method was sensitive, acceptable and immune to interference by ILs. Yet, we further tried to analyze the hydrolysis mixture using an ion chromatography (IC) approach. A representative IC chromatogram of the reaction mixture after hydrolysis for 7 h was showed in Fig. 3. As expected, hydrolysis of chitosan gave rise to glucosamine (peak 8) and oligochitosans (peaks 9–17), and the content of glucosamine took up to 50% of all the detected products (glucosamine and oligochitosans). It was also clear that other byproducts were formed (peaks 1–7) during the process. Unfortunately, complete identification of all products would require extensive studies and be out of capabilities of our facilities.

Although, chitin and chitosan are abundant biopolymers, they have not been fully explored as a renewable carbohydrate sources. This may be partially due the fact that cost-effective hydrolysis of chitin and chitosan remains to be developed. Yet, both GlcNAc and glucosamine have been identified as potential fermentation substrates for microorganisms of biotechnological significance (Smits, Rinzema, Tramper, van Sonsbeek, & Knol, 1996; Wu et al., 2008).

4. Conclusions

In conclusion, we have demonstrated that good TRS yields can be readily achieved once depolymerization of chitosan was performed in ILs in the presence of mineral acids. Under such condi-

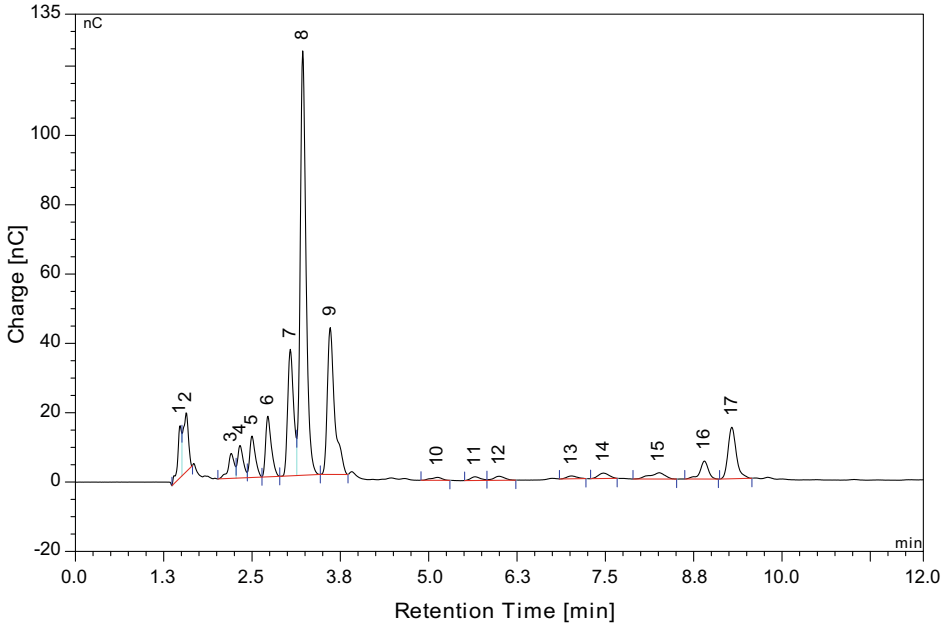

Fig. 3. Typical IC chromatogram of the mixture of chitosan hydrolysis. Reaction conditions: LMWC (0.255 g) in the mixture of 36.5 wt% HCl (0.45 g), and [C_4mim]Cl (4.0 g) at 100 °C under an atmospheric pressure.

tions, a homogeneous system is likely formed. The present work should be valuable to explore new feedstock for biofuel and bio-based chemicals.

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