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Catalytic Depolymerization of Chitin with Retention of *N*-Acetyl Group

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Chitin, a polymer of *N*-acetylglucosamine units with β-1,4-glycosidic linkages, is the most abundant marine biomass. Chitin monomers containing *N*-acetyl groups are useful precursors to various fine chemicals and medicines. However, the selective conversion of robust chitin to *N*-acetylated monomers currently requires a large excess of acid or a long reaction time, which limits its application. We demonstrate a fast catalytic transformation of chitin to monomers with retention of *N*-acetyl groups by combining mechanochemistry and homogeneous catalysis. Mechanical-force-assisted depolymerization of chitin with a catalytic amount of H_2SO_4 gave soluble short-chain oligomers. Subsequent hydrolysis of the ball-milled sample provided *N*-acetylglucosamine in 53% yield, and methanolysis afforded 1-*O*-methyl-*N*-acetylglucosamine in yields of up to 70%. Our process can greatly reduce the use of acid compared to the conventional process.

The transformation of biomass to value-added chemicals with particular functional groups is an attractive alternative to multistep functionalization of hydrocarbons from fossil fuels.[1] The most abundant terrestrial and marine biomasses are cellulose and chitin, respectively. Notably, chitin is a characteristic biopolymer consisting of nitrogen-containing monomer units, *N*-acetylglucosamine (GlcNAc), with β-1,4-glycosidic linkages (Scheme 1). Therefore, it is an attractive renewable feedstock for organic nitrogen compounds.[2] However, unlike cellulose conversion, in which thermal and mechanocatalytic depolymerization can both yield monomers,[3] chitin requires selective depolymerization of glycosidic bonds to prevent the loss of *N*-acetyl groups for wide application.

Hydrolysis of the glycosidic bonds of chitin gives GlcNAc, which is useful as a biologically active agent, an ingredient for cosmetics, and a raw material for *N*-containing organic compounds.[4] Meanwhile, methanolysis of chitin can afford 1-*O*-methyl-*N*-acetylglucosamine (MeGlcNAc), which inhibits hemagglutination, and thus can be used for suppression of influenza and cancer.[5] MeGlcNAc is also applicable to the synthesis of biodegradable polyesters and polyamides,[6] organocatalysts,[7] ligands,[8] and gelators.[9] Additionally, MeGlcNAc is a useful molecule for further transformations in glycoscience, as the hemiacetal group is protected by a methyl group.[10]

Despite a great deal of effort in various fields, efficient conversion of chitin to *N*-acetylated monomers remains a challenge. In biochemistry, chitin is depolymerized by enzymes to form GlcNAc in yields of 64–77% under ambient conditions; however, it takes 10 days for the reaction to be completed owing to low catalytic activity.[11] Conventional chemical/mechanical pretreatments slightly improved the reactivity of chitin by amorphization, but a harsh pretreatment (supercritical water with converge milling) was necessary to accelerate the reaction.[12] In synthetic organic chemistry, HCl provides GlcNAc in approximately 65% yield, but only when HCl is used in large excess (i.e., with a molar ratio of HCl to GlcNAc units in chitin of 100) and at high concentrations of 15–36%.[4a, 12a] Therefore, the serious corrosiveness of HCl becomes an issue, and a huge amount of acidic waste is produced. Dilution of HCl in the depolymerization reaction of chitin results in low selectivity for GlcNAc.[13] Moreover, the use of acid results in deacetylation of chitin,[13] but preservation of the *N*-acetyl group is essential for high physiological activity and wide application of the resulting chitin monomers.[14] The synthesis of MeGlcNAc was not studied to the same extent as that of GlcNAc; in fact, its production from chitin is not yet reported. Fischer glycosidation of GlcNAc was necessary to obtain MeGlcNAc.[15] Herein, we report catalytic depolymerization of chitin to GlcNAc and MeGlcNAc (Scheme 1) in a process that reduces the use of acid by 99.8% and is completed within one day (including all handling).

The first key step in our reaction is mechanocatalytic conversion of chitin to soluble oligomers. It is known that mechanical force enhances chemical reactions under mild conditions.[16] Chitin was impregnated with a small amount of H_2SO_4 [substrate to catalyst ratio (S/C) = 8.1], and the resulting mixed solid was ball-milled at 500 rpm for 6 h to achieve mechanocatalytic hydrolysis (Scheme 1, product name: Chitin-H_2SO_4-BM). The water used for hydrolysis should be physisorbed water (1.5 wt%) in chitin. The process quantitatively converted chitin to soluble compounds such as GlcNAc (4.7% yield), $(GlcNAc)_2$ (7.8% yield), $(GlcNAc)_3$ (11% yield), $(GlcNAc)_4$ (9.7% yield), and $(GlcNAc)_5$ (8.6% yield), where $(GlcNAc)_n$ denotes an oligomer consisting of *n* GlcNAc units (Figure 1, Chitin-H_2SO_4-BM). Other products were longer and branched oligomers, as

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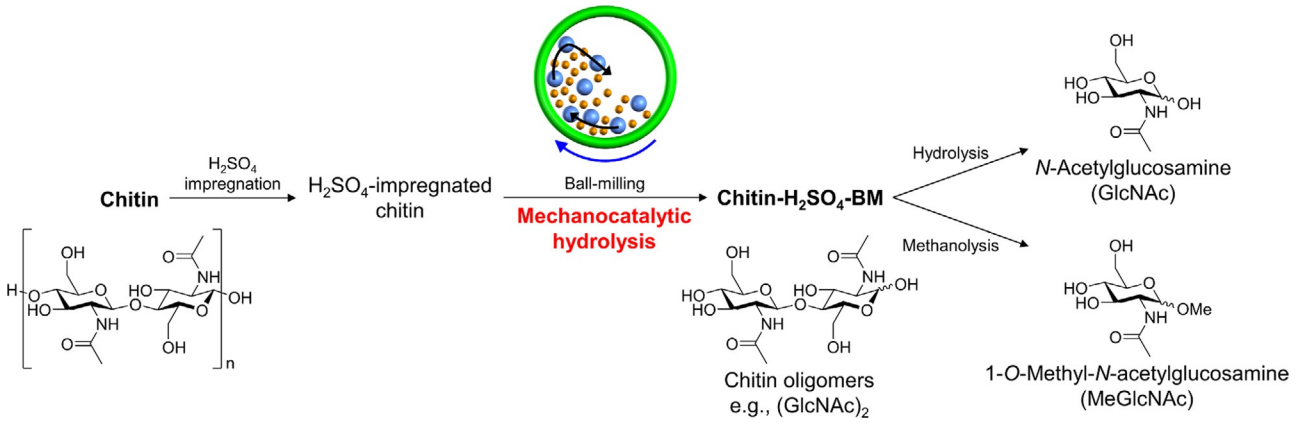


Scheme 1. Two-step depolymerization of chitin to *N*-acetylated monomers, catalyzed by H_2SO_4. The first step is mechanocatalytic depolymerization of chitin to water-soluble oligomers, and the second is solvolysis of the oligomers to *N*-acetylated monomers.

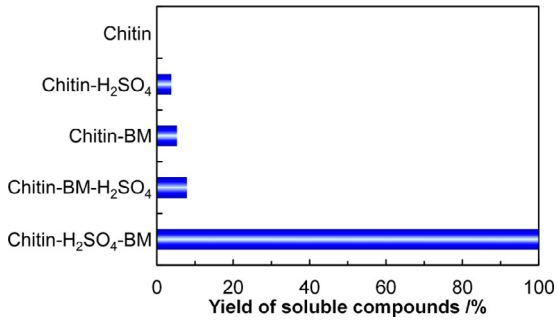
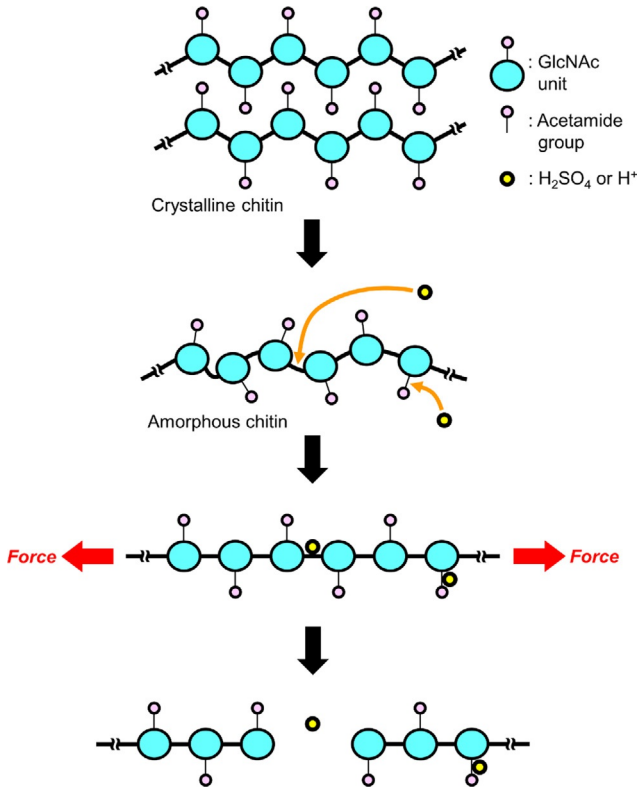


Figure 1. Mechanocatalytic depolymerization of chitin. Dissolution conditions: chitin weight 406 mg, distilled water 40 mL, 298 K.

determined by liquid chromatography/mass spectroscopy (LC/MS) and nuclear magnetic resonance (NMR) spectroscopy (Figure S1 and S2 in the Supporting Information). It is known that side chains improve the solubility of oligomers.[16i] Chitin oligomers are useful as antitumor, immunoenhancing, and antimicrobial agents,[17] and as precursors for monomers (vide infra).

In controlled experiments (Figure 1), pristine chitin contained no soluble compounds, whereas chitin that was ball-milled in the absence of H_2SO_4 (Chitin-BM) afforded soluble compounds in 5.2% yield, with the chitin becoming amorphous (Figure S3). A H_2SO_4-impregnated chitin that was aged at 298 K for 6 h (Chitin-H_2SO_4) had a soluble fraction of only 3.7%. Hence, both ball-milling and H_2SO_4 are necessary for depolymerization of chitin. To examine the synergy between mechanical force and H_2SO_4, chitin was ball-milled, impregnated with H_2SO_4, and aged at 298 K for 6 h (Chitin-BM-H_2SO_4); this sample had a soluble fraction of only 7.8%. Therefore, mechanical force was only effective for the depolymerization of chitin in the presence of H_2SO_4.

Notably, acetyl groups in the GlcNAc units were preserved after the mechanocatalytic reaction, as Chitin-H_2SO_4-BM consisted of *N*-acetylated oligomers and did not contain acetic acid. This result is specific to the mechanocatalysis, as the conventional hydrolysis using a small amount of acid results in significant removal of *N*-acetyl groups.[13] In the mechanocatalytic reaction (Scheme 2), we propose that amorphization of chitin first occurs by the ball-milling, which enhances the accessibility of acid to chitin owing to lower steric hindrance.[18] Then, mechanical force assists the cleavage of the connecting points of polymers, in which polymers are subjected to macro-scale power as tensile stress.[19] Thus, the use of mechanical force resulted in selective activation of glycosidic bonds connecting the GlcNAc units rather than amide bonds in the acetamide



Scheme 2. Proposed schematic of mechanochemical depolymerization. The mechanical force first amorphizes chitin, which increases the accessibility of acid to the substrate. The main chain is subjected to strong mechanical force, which leads to selective dissociation of the connecting points of the chain (glycosidic bonds), whereas the side chains (acetamide groups) do not dissociate.

groups hanging from the units. This is the remarkable merit of mechanocatalysis in the selective depolymerization of chitin. The merit is different from that in the conversion of simple sugar polymers like cellulose that have only one functional group to be hydrolyzed (glycosidic bond).[16]

To produce GlcNAc, Chitin-H_2SO_4-BM was further hydrolyzed in water under rapid heating–cooling conditions (Table 1). The catalyst for hydrolysis was H_2SO_4 contained in the sample (S/C = 8.1), which gave a 32% yield of GlcNAc at 463 K in 1 h (entry 1). The yield of GlcNAc was improved to 53% when the amount of H_2SO_4 was increased to S/C = 2.0 and the reaction

Table 1. Solvolysis of chitin samples to *N*-acetylated monomers.[a]

Entry	Sample	S/C	*T* [K]	Solvent	Product[b] [%] GlcNAc	MeGlcNAc	Other[c]
1	Chitin-H_2SO_4-BM	8.1	463	Water	32	–	68
2	Chitin-H_2SO_4-BM	2.0	443	Water	53	–	47
3	Chitin	–[d]	463	Methanol	–	0.0	0.0
4	Chitin-H_2SO_4	8.1	463	Methanol	–	4.0	1.7
5	Chitin-BM	–[d]	463	Methanol	–	0.0	2.2
6	Chitin-BM-H_2SO_4	8.1	463	Methanol	–	16	4.6
7	Chitin-H_2SO_4-BM	8.1	463	Methanol	–	68	27
8	Chitin-H_2SO_4-BM	4.1	463	Methanol	–	70	28

[a] Common reaction conditions: chitin weight 406 mg, solvent 40 mL, rapid heating–cooling conditions (time course is shown in Figure S4). [b] Based on moles of GlcNAc units. [c] Other soluble products. [d] No H_2SO_4 was impregnated on chitin.

temperature was lowered to 443 K (entry 2). However, the formation of by-products was still observed in these reactions because of the degradation of GlcNAc, which is thermally unstable in water; in a stability test of GlcNAc, only 17% of GlcNAc was recovered after being subjected to the same heating conditions (Table S1, entry S1). As the generation of acetic acid was limited (< 1%), the degradation must have resulted from the conversion of hemiacetal groups in our rapid reaction.

The second key point in our reaction is to overcome the stability issue by using methanolysis instead of hydrolysis, as the product MeGlcNAc does not contain a hemiacetal group. Indeed, 94% of the MeGlcNAc was recovered after heat treatment at 463 K (Table S1, entry S2). It is notable that the advantage of MeGlcNAc is not only the good stability but also the wide applications in various fields as described above.[5–10] Recently, Pierson et al. demonstrated that alcoholysis of chitin was possible in the presence of H_2SO_4 (S/C = 0.9), although the deacetylated product [i.e., 1-*O*-(2-hydroxyethyl)-glucosamine] was obtained predominantly (24% yield) owing to the harsh conditions required for direct depolymerization of the robust chitin.[20] Therefore, we can expect selective synthesis of MeGlcNAc from the reactive chitin oligomers contained in Chitin-H_2SO_4-BM.

Chitin samples were subjected to methanolysis at 463 K to demonstrate selective synthesis of MeGlcNAc. The reaction of Chitin-H_2SO_4-BM provided MeGlcNAc in 68% overall yield, even when only a small amount of H_2SO_4 was used (S/C = 8.1) (entry 7). The ratio of α- to β-anomers in MeGlcNAc was 5.0, and the high stereoselectivity is favorable for further applications (the formation mechanism of the α-anomer is discussed in Figure S5). The turnover number (TON) of H_2SO_4 for MeGlcNAc production was 5.6, showing that the acid acted as a catalyst. This result was in sharp contrast to those obtained from conventional systems, which require a large excess of HCl (TON < 0.01) to obtain *N*-acetylated monomer.[4a] With regard to by-products, only a trace amount of methyl acetate (0.90%) was detected by gas chromatography (flame ionization detector). Almost no deacetylation occurred during methanolysis under our conditions. The yield of methanol-insoluble humins was 4.7 wt% based on the weight of chitin used. The yield of MeGlcNAc increased to 70% under optimum conditions (S/C = 4.1, entry 8). In contrast, methanolysis of other samples—pristine chitin, Chitin-H_2SO_4, Chitin-BM, and Chitin-BM-H_2SO_4—gave low yields of MeGlcNAc (≤ 16%; entries 3–6). These results clearly show that mechanocatalytic depolymerization of chitin prior to methanolysis is essential for the high-yield synthesis of MeGlcNAc. The reactivity of chitin is dramatically different from that of cellulose; the methanolysis of cellulose gives a good yield of methylglucose with no pretreatment.[21]

In summary, we demonstrated the catalytic conversion of chitin to *N*-acetylated monomers, notably MeGlcNAc. The process consists of mechanocatalytic depolymerization of chitin to soluble oligomers, followed by solvolysis to monomers. Mechanical force was found to be essential to achieving selective catalytic depolymerization with retention of *N*-acetyl groups, demonstrating the remarkable merit of mechanocatalysis. The second thermocatalytic methanolysis converted oligomers to the *N*-acetylated monomer MeGlcNAc in 68–70% yield, owing to the improved reactivity of the substrate and the good stability of the product. In the overall process, the amount of acid used for the reaction was drastically reduced (by 99.8%) compared with the conventional process. Our method overcomes critical issues in conventional systems for chitin depolymerization, and opens the door to various applications of chitin-based compounds.

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