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Note

Quantitative production of 2-acetamido-2-deoxy-D-glucose from crystalline chitin by bacterial chitinase

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Abstract

Finely powdered α- and β-chitin can be completely hydrolyzed with chitinase (EC 3.2.1.14) and β-*N*-acetylhexosaminidase (EC 3.2.1.52) for the production of 2-acetamido-2-deoxy-D-glucose (GlcNAc). Crude chitinase from *Burkholderia cepacia* TU09 and *Bacillus licheniformis* SK-1 were used to digest α- and β-chitin powder. Chitinase from *B. cepacia* TU09 produced GlcNAc in greater than 85% yield from β- and α-chitin within 1 and 7 days, respectively. *B. licheniformis* SK-1 chitinase completely hydrolyzed β-chitin within 6 days, giving a final GlcNAc yield of 75%, along with 20% of chitobiose. However, only a 41% yield of GlcNAc was achieved from digesting α-chitin with *B. licheniformis* SK-1 chitinase.

Keywords: Chitin; Chitinase; *N*-Acetyl-D-glucosamine; 2-Acetamido-2-deoxy-D-glucose; *Bacillus licheniformis*; *Burkholderia cepacia*

2-Acetamido-2-deoxy-D-glucose (*N*-acetyl-D-glucosamine, GlcNAc) and 2-amino-2-deoxy-D-glucose (D-glucosamine, GlcN) have recently been promoted as a treatment or as nutriceutical agents for patients with osteoarthritis and inflammatory bowel disease.[1,2] In contrast to GlcN hydrochloride or sulfate, both of which have a bitter taste, GlcNAc has a sweet taste that facilitates its use in daily consumption. However, GlcNAc has not been widely commercialized mainly due to the lack of an economical process for its production that is acceptable for food and medicine. The current acidic hydrolysis of chitin using concentrated HCl is inefficient and poses environmental and technical concerns.[3] On the other hand, hydrolysis of chitin with enzymes can produce GlcNAc under mild and environmentally friendly conditions. An approach whereby commercially available crude enzymes were used to hydrolyze amorphous chitin substrate has been carried out.[4] Unfortunately, this method added an additional substrate preparation step into the production of GlcNAc. The work on commercially available crude enzymes has also been extended to a production of GlcNAc by direct hydrolysis of β-chitin powder.[5,6] These reports have shown that enzymatic hydrolysis of chitin can produce GlcNAc in relatively higher yields than acid hydrolysis. Nevertheless, the remaining major impediment of an enzymatic hydrolysis process is the extremely low hydrolytic susceptibility of the natural chitin substrate, due to its high crystallinity. We show herein for the first time that crystalline chitin in both the α- and β-forms can be cleanly hydrolyzed, producing GlcNAc in virtually quantitative yield.

Powdered α-chitin (14 μm in size) from crab shells and β-chitin (3 μm in size) from squid pens were used as substrates for digestion by crude bacterial chitinase from *Burkholderia cepacia* TU09 and *Bacillus licheniformis* SK-1. A typical reaction contained 100 mU/mL (1 unit = the amount of enzyme that produces 1 μmol of GlcNAc per min from colloidal chitin) of the enzyme and 10–40 mg/mL of the substrate, unless indicated otherwise. Digestion reactions were carried out in 3–5

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Table 1
Production of GlcNAc from β-chitin by chitinase from *B. licheniformis* SK-1

β-Chitin/enzyme [a] (mg/U)	Digestion time (day)	% Yield [b]		
		GlcNAc	$(GlcNAc)_2$	Total
400	1	9	22	31
	3	18	27	45
	6	25	22	47
200	1	16	18	34
	3	34	38	72
	6	46	29	75
100	1	28	50	78
	3	53	40	93
	6	75	20	95

[a] [E] = 0.1 U/mL in 0.1 M citrate–phosphate buffer, pH 6.0.
[b] HPLC yield.

mL of 0.1 M citrate–phosphate buffer, pH 6.0, in 10-mL glass vials. The reactions were incubated in a shaking water bath, with moderate shaking, at 37 and 50 °C, respectively, when the enzyme from *B. cepacia* TU09 and *B. licheniformis* SK-1 were used. At each time point, a portion of the reaction mixture was withdrawn, diluted with water and then mixed with CH_3CN (at the ratio 31:69), filtered, and analyzed by HPLC (column: Shodex Asahipak NH2P-50; flow rate: 1 mL/min; mobile phase: 31:69 water/CH_3CN; detection: UV at 210 nm). The amount of GlcNAc in the reaction mixture was determined from a calibration curve of GlcNAc standard.

The percent yield of GlcNAc production increased with the reduction of substrate/enzyme ratio. Although, at the substrate/enzyme ratio of 100 mg/U, chitinase from *B. licheniformis* SK-1 completely hydrolyzed β-chitin, it gave a mixture of GlcNAc and *N*,*N*′-diacetylchitobiose [$(GlcNAc)_2$] (Table 1). The gradual increase of the GlcNAc/$(GlcNAc)_2$ product ratio with incubation time implied the presence of low β-*N*-acetylhexosaminidase (EC 3.2.1.52) activity in the crude enzyme from *B. licheniformis* SK-1 under the reaction conditions. On the other hand, hydrolysis of β-chitin with chitinase from *B. cepacia* TU09 gave mostly GlcNAc with a trace amount of chitotriose. At the substrate–enzyme ratio of 100 mg/U, a 90% yield of GlcNAc was obtained within 1 day, and a quantitative yield was realized upon prolonged incubation (Table 2).

The tightly packed chitin strands of α-chitin are known to have low susceptibility to enzymatic hydrolysis. We found that when chitinase from *B. licheniformis* SK-1 was used, it was unable to completely hydrolyze α-chitin. Only 41% of α-chitin were hydrolyzed in 6 days, even when the concentration of the enzyme used in the reaction was tenfold over the amount that was used to completely hydrolyze β-chitin (Table 3). We speculate that the crystalline domains in α-chitin are completely resistant to digestion by chitinase from *B. licheniformis* SK-1. The GlcNAc produced was probably liberated from the amorphous regions of the substrate. Chitinase from *B. cepacia* TU09 showed superior characteristics in hydrolyzing α-chitin as an 85% yield

Table 2
Production of GlcNAc from β-chitin by chitinase from *B. cepacia* TU09

β-Chitin/enzyme [a] (mg/U)	Digestion time (day)	% Yield [b] GlcNAc
400	1	31
	3	57
	6	65
200	1	62
	3	81
	6	84
100	1	90
	3	96
	6	100

[a] [E] = 0.1 U/mL in 0.1 M citrate–phosphate buffer, pH 6.0.
[b] HPLC yield.

Table 3
Production of GlcNAc from α-chitin by chitinase from *B. licheniformis* SK-1

α-Chitin/enzyme [a] (mg/U)	Digestion time (day)	% Yield GlcNAc [b]
10	1	32
	3	40
	6	41

[a] [E] = 1 U/mL in 0.1 M citrate–phosphate buffer, pH 6.0.
[b] HPLC yield.

Table 4
Production of GlcNAc from α-chitin by chitinase from *B. cepacia* TU09

α-Chitin/enzyme [a] (mg/U)	Digestion time (day)	% Yield [b] GlcNAc
100	1	37
	3	54
	7	57
33	1	41
	3	57
	7	85

[a] [E] = 0.1 U/mL in 0.1 M citrate–phosphate buffer, pH 6.0.
[b] HPLC yield.

of GlcNAc was achieved after 7 days of incubation (Table 4). It is worth noting that the hydrolysis of α-chitin with chitinase *from B. cepacia* TU09 consists of two steps. First, there is a rapid hydrolysis step in the first 24 h, during which we believe the amorphous portion ($\sim 40\%$) of the chitin particle is hydrolyzed. The second step is a slower step, where the remaining tightly packed chitin is slowly hydrolyzed. Because of this slower degradation rate, 300 mU/mL of the enzyme was used to ensure sufficient amount of active enzyme present throughout the hydrolysis. The isolation and characterization of the enzymes used here will be published elsewhere.

We have demonstrated here for the first time that chitinase from certain bacteria can completely hydrolyze both powdered α- and β-chitin to give GlcNAc in very high to quantitative yields. The cleanliness of the reaction, mild conditions, ease of substrate preparation, and high-production yield undeniably render the approach of using enzyme more attractive than the current acid-hydrolysis process for the production of GlcNAc. Despite all these beneficial factors in using bacterial chitinase, care must be taken in further development to ensure food safety and enhance cost efficiency for the industrial production of GlcNAc.

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