Production of *N*-Acetyl-D-glucosamine from β-Chitin by Enzymatic Hydrolysis

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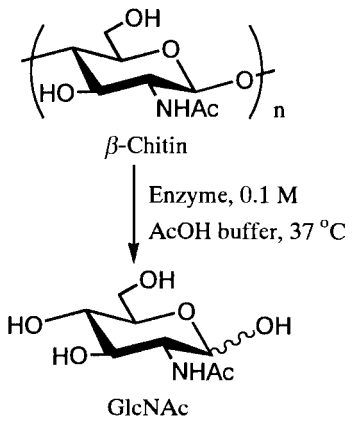
N-Acetyl-D-glucosamine, which is a focusing material for the improvement of osteoarthritis, was obtained from β-chitin by enzymatic hydrolysis in high yield (76%).

N-Acetyl-D-glucosamine (GlcNAc) has been a focusing material for the improvement of osteoarthritis as well as D-glucosamine (GlcN) hydrochloride or sulfate, which are already commercialized for this disease.[1] These GlcN salts, however, are not suitable for oral administration owing to their bitter tastes. In contrast, GlcNAc will be able to apply for oral administrated drug, because of its sweet taste. Until now, GlcNAc is mainly produced by acid (concd HCl) hydrolysis of chitin, which is a mucopolysaccharide composed of repeating GlcNAc unit and exists in crab shell (α-chitin) or squid pen (β-chitin). This procedure, however, has some problems such as high cost, low yield (below 65 %),[2] and acidic wastes by use of concd HCl, etc. Although *N*-acetylation of GlcN is also possible to produce GlcNAc, this product is not approval as a natural type material owing to its chemical modification process (*N*-acetylation). Therefore, more effective and milder process to produce natural type of GlcNAc is required, although there is no report for these processes. We report herein the enzymatic production of GlcNAc from β-chitin by use of crude enzymes like cellulase (Scheme 1).[3]



Scheme 1.

Typical procedure is as follows: β-chitin[4] (100 mg) was suspended in 10 mL of 0.1 M AcOH buffer (pH = 4.8). To the suspension, enzyme[5] (100 mg) was added and shaken at 37 °C for 4 or 8 days. A part (0.1 mL) of the reaction mixture was taken out, diluted with H_2O (0.4 mL) and CH_3CN (1.0 mL), filtered, and directly analyzed by HPLC.[6] The amount of GlcNAc in the reaction mixture was estimated from the calibration curve of commercial GlcNAc.

Table 1. Production of *N*-acetyl-D-glucosamine (GlcNAc) from β-chitin by various enzymes[a]

Run	Enzyme	Origin	Yield[b] / %
1	Cellulase T. v.	*Tricoderma viride*	26
2	Cellulase A.	*Acremonium*	29
3	Hemicellulase	*Aspergillus niger*	5
4	Papain	*Carica papaya L.*	2
5	Lipase	*Aspergillus niger*	7
6	Pectinase	*Aspergillus niger*	5

[a]Conditions: [β-Chitin]=10 mg/mL; [Enzyme]=10 mg/mL; pH=4.8 (0.1 M AcOH buffer); 37 °C; 4 days. [b]Yield was calculated as follows: Yield (%) = GlcNAc obtained (mg/mL)/ β-Chitin added(mg/mL).

In general, β-chitin has more swelling property in water than α-chitin. Moreover crude enzymes, which well degrade partially *N*-acetylated chitosans,[3] have some advantage to produce GlcNAc owing to their low cost and including both *endo*- and *exo*-type of chitinases. So we selected β-chitin and crude enzymes to produce GlcNAc. Table 1 shows the production of GlcNAc from β-chitin by various enzymes. Among these crude enzymes, cellulase *Tricoderma viride* (T. v.) and cellulase *Acremonium* (A.) were effective for the production of GlcNAc (runs 1 and 2). Hemicellulase, papain, lipase, and pectinase were not very effective to produce GlcNAc under these conditions. Although the crude preparations of cellulase T. v. and cellulase A. essentially degrade cellulose, they also degrade chitin[7] or partially *N*-acetylated chitosans[3] owing to including chitinase, and produce monomer or oligomers. Since the yield of GlcNAc by cellulase T. v. was almost the same degree as that by cellulase A., we selected cellulase T. v. for the next experiment. The yield of GlcNAc by cellulase T. v. was increased with increasing reaction time or the amount of enzyme (Table 2). The highest yields (74–76%) of GlcNAc were shown by 8-day hydrolysis with 2 or 4 folds of cellulase T. v. against chitin (runs 5 and 7). Instead of the above advantage in crude enzyme, a large amount of enzyme is required for the production of GlcNAc within short period (ca. 8 days). From the ^1H and ^{13}C NMR (in D_2O) analyses, purified GlcNAc[8] produced by enzymatic hydrolysis showed the same ^1H and ^{13}C signals as those of a commercial GlcNAc, which suggests that the chemical structure of the enzymatic

Table 2. Effect of the amount of enzyme (cellulase T. v.) on the production of GlcNAc[a]

Run	Enzyme mg/mL	Time day	Yield %
1	2	4	7
2	10	4	26
3	10	8	49
4	20	4	47
5	20	8	76
6	40	4	60
7	40	8	74

[a]Conditions: [β-Chitin]=10 mg/mL; pH = 4.8; 37 °C.

hydrolysate was confirmed to be GlcNAc.

In conclusion, GlcNAc could be obtained from β-chitin by hydrolysis with crude enzyme like cellulase T. v. in high yield (76%). This simple and mild procedure would be useful for the industrial production of a natural-type GlcNAc.

We are indebted to Sunfive Co. for supplying powdered β-chitin, Meiji Seika Co. for Cellulase A., and Amano Pharmaceutical Co. for other enzymes.

References and Notes

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4 Particle size = 3 μm; mole fraction of *N*-acetylated units (F_A) = 0.95 (determined by ^{1}H NMR in 20%(w/v) DCl/D_2O).

5 Cellulase T. v. and Cellulase A. were purchased from Wako Pure Chemical Industries Ltd, and supplied from Meiji Seika Co., respectively. Other enzymes were supplied from Amano Pharmaceutical Co., Ltd.

6 HPLC analysis was performed on a Tosoh LC-8020 apparatus (column, Shodex Asahipak NH2P-50; rt; CH_3CN/H_2O = 7/3; flow rate = 1.0 mL/min; injection, 0.1 mL; detection, UV at 210 nm).

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8 After enzymatic hydrolysis, insoluble materials were removed by centrifugation (3,000 rpm). Water-soluble fraction was purified by HPLC under the above conditions. A part of eluates corresponding to GlcNAc (t_R = 5 min) was collected and dried (5 mg).