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Preparation of bacterial cellulose containing *N*-acetylglucosamine residues

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The incorporation of *N*-acetylglucosamine (GlcNAc) to bacterial cellulose (BC) was achieved by the use of *Acetobacter xylinum* strains, which were adapted to GlcNAc by the successive transfer to GlcNAc as a liquid medium (containing GlcNAc as carbon source, without glucose) or by incubation on GlcNAc as a solid medium. The incorporation of GlcNAc residues was detected by the amino-acid analysis of acid hydrolysate, and 4 mol% of GlcNAc among the glucose residues was the highest content observed. The GlcNAc-incorporated BC was hydrolyzed by cellulase [EC 3.2.1.4], lysozyme (EC 3.2.1.17], and chitinase [EC 3.2.1.14] even at low GlcNAc content. The specificities toward enzymes suggested the random distribution of GlcNAc in the BC main chain and incorporation of GlcNAc residues through β-1,4 glycoside linkages.

INTRODUCTION

The bacterium *Acetobacter xylinum* is known to produce a cellulosic pellicle of high purity at the surface of the nutrient medium. Although bacterial cellulose (BC) has a variety of desirable physical properties, such as high crystallinity, high Young's modulus, etc. (Hori *et al.*, 1987; Yamanaka *et al.*, 1989), the mechanical strength is not high enough because of the random arrangement of relatively weak hydrogen bonds among microfibrils. On the other hand, chitin, a glucosaminoglycan of β-1,4 glycoside linkage, is known to have a very tight crystalline structure owing to the possession of strong hydrogen bonds by the acetamido group at the C-2 position of GlcNAc and is known also as a native mucopolysaccharide with biodegradability (Carlstorem, 1957). In order to produce a novel BC with new functions, we have embarked on research to introduce GlcNAc residues into BC by *A. xylinum* strains. The modified BC is expected to become a multifunctional polymer with both chitinous and cellulosic properties when GlcNAc residues are introduced into the BC main chain.

This paper examines an adaptation of *A. xylinum* strains to GlcNAc by several stages of transfer to a Schramm–Hestrin (SH) GlcNAc liquid medium (instead of glucose in SH medium, Hestrin & Schramm, 1954) or by culturing on an SH GlcNAc agar medium. It was found that *A. xylinum* strains, adapted to GlcNAc, produced pellicles containing 0·5–4·0 mol% of GlcNAc residues (N-AcGBC) at the surface of the medium consisting of various ratios of glucose (Glc) and GlcNAc. The turbidity of the resulting N-AcGBC suspension in buffer solution was reduced remarkably by the addition of cellulase or lysozyme and slightly by adding chitinase. These specificities toward enzymes suggest the random distribution of GlcNAc residues in the BC main chain and the β-1,4 glycoside linkages of GlcNAc residues.

MATERIAL AND METHODS

Bacterial strains and culture conditions

All cultures were incubated statically at 28°C in liquid or solid medium; the medium compositions are shown in Table 1. Bacterial strains, activated as follows, were used for pellicle production.

(1) *A. xylinum* ATCC 10245 and NBI 1051 were maintained on Schramm–Hestrin (SH) Glc medium (Hestrin & Schramm, 1954) (wild type).
(2) A 0·5-ml aliquot of the 3 days' culture (1) was transferred to 15 ml of SH GlcNAc medium (Table 1(c)). These cultures were serially transferred to SH GlcNAc medium every 3 days of culture (STG-strains).

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Table 1. Composition of medium components

Components of medium	Concentration (w/v%)						
	SH Glc medium (a)	SH mixture medium (b)					SH GlcNAc medium (c)
Glc	2·0	1·8	1·4	1·0	0·6	0·2	0·0
GlcNAc	0·0	0·2	0·6	1·0	1·4	1·8	2·0
Bacto peptone	0·5						
Yeast extract	0·5						
Disodium hydrogenphosphate	0·27						
Citric acid	0·115						

The initial pH value of all media is 6·0.

(3) The 3 days' cultures of STG-strains were transferred to the surface of SH GlcNAc agar slants by the use of sterile inoculation loops. Colonies, grown on agar slants for 3 days at 28°C, were reinoculated to a flask containing 15 ml of SH GlcNAc medium and were then serially transferred to SH GlcNAc medium by a similar procedure to (2) (STGC-strains).
(4) A volume of 20 μl of the 1000-fold-diluted 2 days' culture in GlcNAc medium was inoculated to an SH GlcNAc agar plate. A single colony (CG-strains), grown on agar plate for 2 days at 28°C, was transferred again to a flask containing 15 ml of SH GlcNAc medium and then transfers to SH GlcNAc medium were repeatedly made every 3 days (CSTG-strains).
(5) A 0·5-ml aliquot of STGC-strain cultures was transferred to SH Glc medium, and the transfer procedures to SH Glc medium were repeated every 3 days (GC-strains).

Growth profiles of the strain in a liquid medium were monitored turbidimetrically with a Hitachi U-3200 spectrophotometer at 660 nm.

Pellicles production and purification

A 0·5-ml aliquot of the cultures (in SH Glc or GlcNAc medium) was inoculated into 15 ml of SH Glc or a mixture medium (Table 1(a) or (b)) and incubated statically at 28°C. The pellicles produced at the surface of the mediums were harvested after 4–7 days and immersed into 2% (w/v) sodium dodecyl sulfate (SDS) aqueous solution overnight. The pellicles were boiled for 3 h in 2% SDS aqueous solution and washed under ultrasonication in 1% (w/v) aqueous NaOH followed by neutralization by the addition of 1% (v/v) AcOH. Finally, they were rinsed extensively with distilled water.

Estimation of incorporated GlcNAc in pellicle

The washed pellicles were dried at 105°C on glass or stainless-steel plates and then swollen in 85% phosphoric acid as described in the text (Reese & Mandels, 1963) and dried after extensive rinsing with deionized water. The samples were next hydrolyzed with 2N HCl for 12 h at 100°C under reduced pressure. After the hydrolysis, excess acid was removed by evaporation *in vacuo* over NaOH. The GlcNAc content in pellicles was estimated by amino-acid analysis of the hydrolysate.

Enzymes and substrates

Egg-white lysozyme (50 000 units/mg by the *M. luteus*-cell method as described by Parry *et al.*, 1965), chitinase-GODO (0·6 unit/mg by the colloidal-chitin method (Jeuniaux, 1966), Godo Shusei), and cellulase-Onozuka R-10 (1·5 units as carboxymethyl cellulase activity, Yakult) were purchased from Seikagaku Kogyo Co. Ltd, and applied without further purification. The general BC prepared in SH Glc medium by *A. xylinum* (wild type) and lyophilized β-chitin from Squid Pen were used as standard substrates for each enzyme.

Enzyme assay

Substrate suspensions were prepared by granulating solids with a Waring 7012S blender in a micro-container (No. 8575, at 14 700 r/min for 1 min × 5) at room temperature in 0·05M acetate buffers (pH 4·5 for cellulase, pH 5·2 for chitinase, pH 6·0 for lysozyme). The optical density of suspension at 540 nm was adjusted to 1·0 by each buffer. After the addition of 0·1 ml of enzyme solution into 3·0 ml of substrate suspension, the decrease in turbidity (OD at 540 nm) was measured for 10 min at 37°C. The enzymatic hydrolysates were applied for HPLC analysis after boiling for 3 min and removal of the insoluble portion. Samples (20 μl, prepared as described above) were loaded on an Asahipak GS-220 column and eluted at a flow rate of 0·5 ml/min with a mobile phase of distilled water at 60°C. Effluent was analyzed by using a Hitachi L-6000 HPLC with refractive-index detector.

RESULTS

Growth of strains

The optical density at 660 nm of the liquid medium is shown in Fig. 1 as growth curves of the wild type and STG-strains (*A. xylinum* ATCC 10245). The growth of the wild type almost reaches a maximum after 3 days' culture in SH Glc medium but does not reach a maximum after 4 days' culture in SH GlcNAc medium as shown in Fig. 1(a). On the other hand, the growth of STG-strains is depressed to two-thirds of the wild type in both SH Glc and GlcNAc medium as shown in Fig. 1(b). Although a remarkable change in pH of the SH Glc medium was observed during culture, the pH of SH GlcNAc medium was not shifted so much (in the case of both wild and STG-strains). The pH of the SH Glc medium decreased rapidly from its initial value of 6·0 to the final value of 3·0 within 2 days, whereas the pH of the SH GlcNAc medium increased slightly from 6·0 to 6·3 (Fig. 1). Thus transfer to a new medium was performed every 3 days to maintain the strains in SH GlcNAc medium.

The formation of colonies

Colonies grown on SH GlcNAc agar plate were smaller and a paler white than wild-type colonies on an SH Glc agar plate (Fig. 2(a), (b)). The number of colonies on a GlcNAc plate is also less than that of the colonies on a Glc plate. These observations seem to relate to the lower growth rate on GlcNAc than on Glc (Fig. 1).

The formation of large colonies was rarely shown on an SH GlcNAc agar plate, especially by ATCC 10245 strains (Fig. 2(d)). These colonies did not produce any pellicle even at the surface of an SH Glc liquid medium, whereas the liquid medium became turbid. These large colonies seemed to be pellicle-deficient strains, because it has been reported that smooth and large colonies obtained spontaneously were pellicle-deficient strains similar to those obtained by mutagenesis (detailed by Saxena & Brown, 1988).

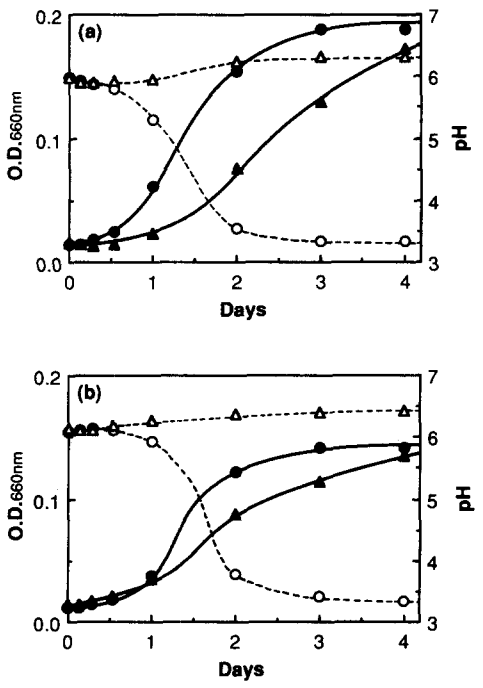

Fig. 1. The optical density at 660 nm (———) as growth curve and pH change (------) of the culture medium of *A. xylinum* ATCC 10245 wild type (a) and ATCC 10245 STG-strains (b): ●, ○ : cultured in SH Glc medium; ▲, △ : cultured in SH GlcNAc medium.

Incorporation of GlcNAc into pellicle

The estimation of small amounts of GlcNAc residues was hard to achieve among large amounts of glucose. Direct spectroscopic detection, e.g. by IR or NMR, of incorporated GlcNAc residues was not applied, because the pellicle consisted of more than 95% of Glc residues. The amount of glucosamine (GlcN) hydrochloride in acid hydrolysates of pellicle was therefore analyzed by amino-acid analysis, which was a more conventional and sensitive method than other methods for the quantification of GlcN, such as HCl-indole (Ohno *et al.*, 1985), MBTH (Tsuji *et al.*, 1969), TNBS (Satake *et al.*, 1960), and OPA (Roth, 1971) because of interference in the color development by contaminates (the GlcNAc contents obtained by these methods were 0·5–2·0 mol% higher than that by amino-acid analysis).

The incorporation of GlcNAc into a pellicle reached a maximum value in the case of the STG-strain of five transfers to an SH GlcNAc medium and culture in SH medium replacing 0·6% of Glc with GlcNAc as shown in Fig. 3. A trace of GlcNAc residue was found in a pellicle with *A. xylinum* wild type even when it was cultured in SH Glc medium as seen in Fig. 3. Though it was reported that BC from *A. xylinum* contained small amounts of amino components even if it was cultured in SH Glc medium, there are no reports on the identification of amino sugar in BC composites. Amino-acid analysis would give a clear identification of amino components when the retention time of each amino component is available on chromatographic separation. The slight increase in GlcNAc content in a pellicle was also shown by STG-strains even in Glc medium after an increment in the number of transfers to SH GlcNAc medium (Fig. 3). STG-strains after ten transfers enhanced the incorporation of GlcNAc up to 4·2 mol% by inoculation on SH GlcNAc agar slants (Fig. 4). Figure 5 shows that, in the case of CG- and CSTG-strains, the GlcNAc content of a pellicle by CG-strains (without serial transfer to an SH GlcNAc medium) was similar to that of a pellicle by STG-strains of five transfers to an SH GlcNAc medium (*c.* 2 mol%). The GlcNAc content of a pellicle by CSTG-

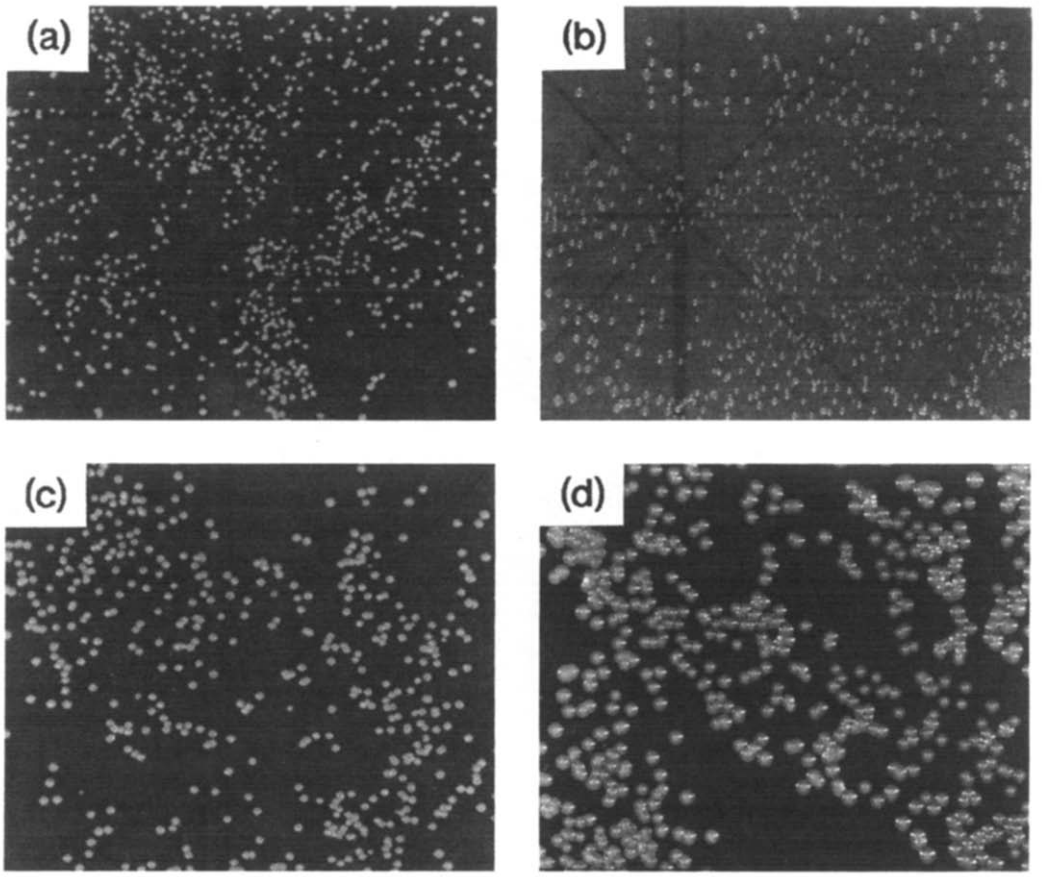

Fig. 2. Colonies grown on SH Glc and GlcNAc agar plate: (a): *A. xylinum* NBI 1051 wild type on SH Glc agar plate; (b): *A. xylinum* NBI 1051 CG-strains on SH GlcNAc agar plate; (c): *A. xylinum* ATCC 10245 wild type on SH Glc agar plate; (d): *A. xylinum* ATCC 10245 CG-strains on SH Glc agar plate shown to be defective in pellicle formation.

strains was depressed after a number of transfers to an SH GlcNAc medium. Such a depression was also shown in STGC-strains, especially ATCC 10245 (Fig. 6), and the growth on SH GlcNAc agar slants was no longer effective for an increase in GlcNAc incorporation. The strains grown on an SH GlcNAc agar medium seemed to be insensitive to the increment in GlcNAc content in the medium and to the transfer to a GlcNAc medium. However, the effective number of transfers for STG-strains was shown to be around five in the absence of an agar-slant culture, as is seen in Figs 3 and 4.

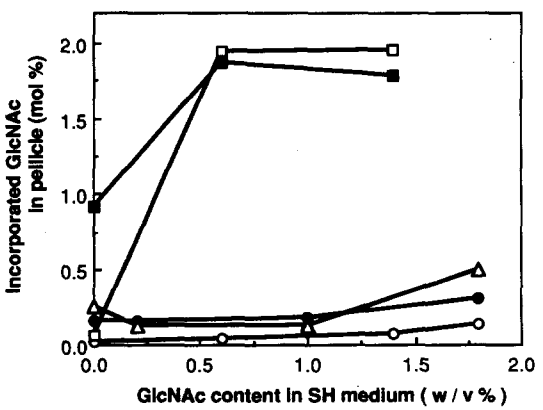

Fig. 3. The amount of GlcNAc incorporated into pellicle produced at SH Glc and mixture medium by *A. xylinum* ATCC 10245 wild type and STG-strains: —○— : without transfer to SH GlcNAc medium (wild type); —●— : 1 time of transfer to SH GlcNAc medium; —△— : 2 times of transfer to SH GlcNAc medium; —□— : 5 times of transfer to SH GlcNAc medium; —■— : 10 times of transfer to SH GlcNAc medium.

Yield of pellicles

Figure 7 shows the yield of a pellicle from STG-strains. The yield of a pellicle was reduced remarkably by the number of transfers to a new SH GlcNAc medium in spite of the enhancement of the GlcNAc content, and the yield did not change so much on the strains after five transfers. On the other hand, the yield of a pellicle formed in media of various Glc and GlcNAc compositions by STGC and CSTG-strains was increased slightly by the reduction in GlcNAc content. The pellicle formation in an SH GlcNAc medium was achieved, and the yield was approximately one-tenth of that of the wild-type pellicle.

Though the regeneration of pellicle formation was shown by GC-strains of both ATCC 10245 and NBI

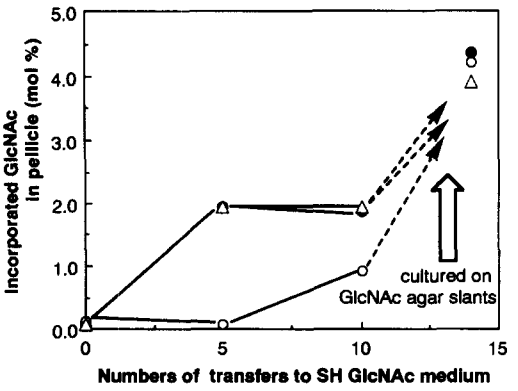

Fig. 4. The relationship between the amount of GlcNAc incorporated into pellicle produced by *A. xylinum* wild type, STG-strains, and times of transfer to SH GlcNAc medium: —○— : 2% Glc in medium (SH Glc medium); —●— : 1·4% Glc and 0·6% GlcNAc in SH mixture medium; —△— : 0·6% Glc and 1·4% GlcNAc in SH mixture medium.

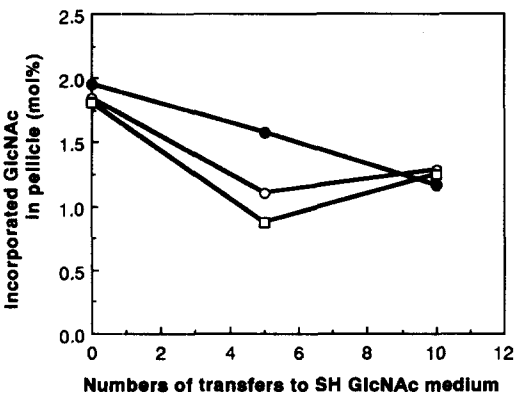

Fig. 5. The relationship between the amount of GlcNAc incorporated into pellicle and numbers of transfers to SH GlcNAc medium (*A. xylinum* ATCC 10245 CG-strains and CSTG-strains): —○— : 2% Glc in medium (SH Glc medium); —●— : 1·4% Glc and 0·6% GlcNAc in SH mixture medium; —□— : 0·6% Glc and 1·4% GlcNAc in SH mixture medium.

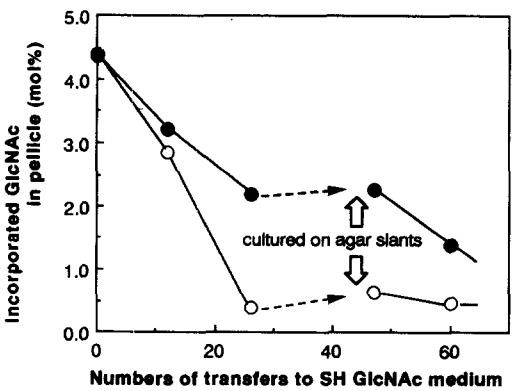

Fig. 6. The amount of GlcNAc incorporated into pellicle produced by *A. xylinum* ATCC 10245 STGC-strains (○) and NBI 1051 STGC-strains (●) at SH mixture medium containing 1·4% of Glc and 0·6% of GlcNAc.

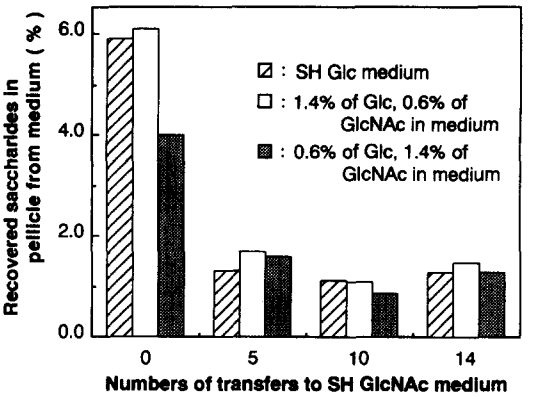

Fig. 7. Yield of pellicles produced by *A. xylinum* ATCC 10245 wild type and STG-strains at SH Glc, mixture medium: recovery of saccharides.

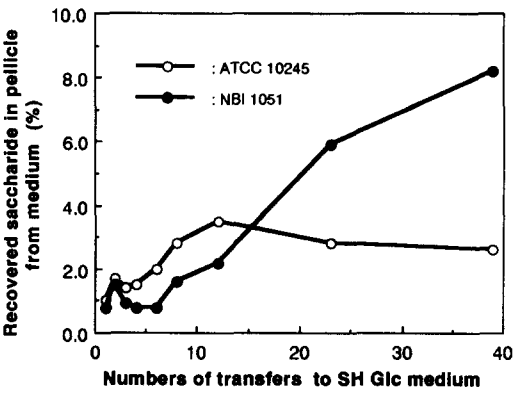

Fig. 8. The relationship between numbers of transfers to SH Glc medium and yield of pellicle produced at SH Glc medium by *A. xylinum* ATCC 10245 GC-strains (○) and NBI 1051 GC-strains (●).

1051 in an SH Glc medium, the pellicle yield in GC-strains of NBI 1051 became higher than that of ATCC 10245 at twelve transfers to the SH Glc medium (Fig. 8). These results may be related to the higher cellulose productivity or lower frequency for the production of pellicle-deficient mutation of NBI 1051 than that of ATCC 10245.

Enzyme susceptibility

Since suspensions of BC and N-AcGBC were prepared successfully by simple homogenization with the Waring blender, turbidimetry could be applied to detect enzyme susceptibilities directly and the homogeneity of saccharide residues in N-AcGBC molecules. Since lysozymic hydrolysis is a basic indication of the presence of β-1,4 linked GlcNAc residue, N-AcGBC was analyzed by lysozymic hydrolysis at OD 540 nm in a similar manner to the ML-cell assay of lysozyme (Parry *et al.*, 1965). Susceptibilities for cellulase and chitinase were also monitored in the same way, at OD 540 nm. Figures 9, 10, and 11 show the preliminary

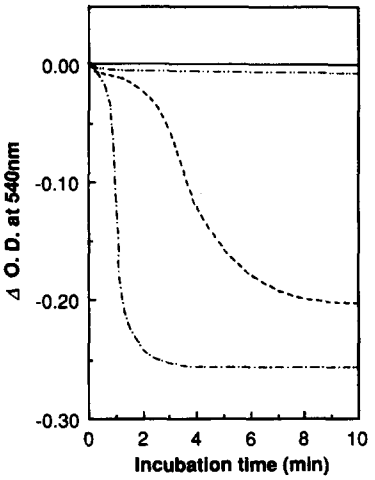

Fig. 9. Time courses of lysozyme hydrolyses of BC, chitin, and N-AcGBC: ——: BC, lysozyme 10 μg/ml; ------: N-AcGBC, lysozyme 1 μg/ml; -·-·-·-: N-AcGBC, lysozyme 5 μg/ml; -··-··-··-: chitin, lysozyme 10 μg/ml.

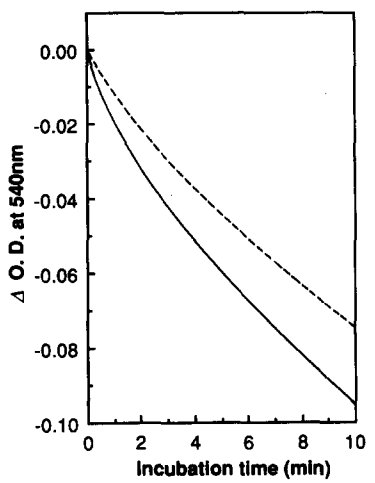

Fig. 10. Time courses of cellulase hydrolyses of BC (——) and N-AcGBC (------). (cellulase: 50 μg/ml).

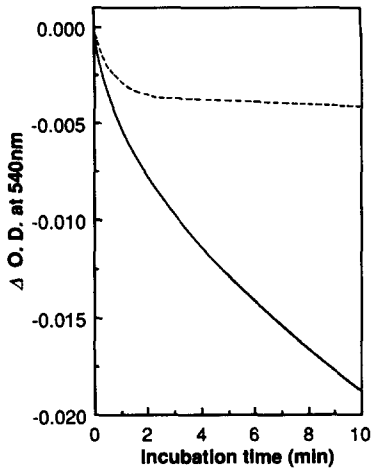

Fig. 11. Time courses of chitinase hydrolyses of chitin (——) and N-AcGBC (------). (chitinase: 100 μg/ml).

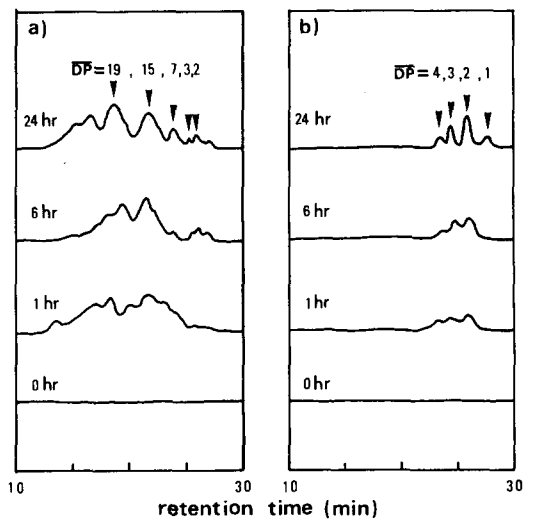

Fig. 12. Gel-permeation chromatograms of lysozymic hydrolysates (lysozyme: 5 μg/ml). (a): N-AcGBC; (b): chitin.

results of enzymatic hydrolysis of N-AcGBC. N-AcGBC was shown to have better susceptibility toward lysozyme than that of β-chitin (Fig. 9). The turbidity of the N-AcGBC suspension was rapidly reduced by the addition of lysozyme, and the rate of reduction depended on the concentration of enzyme added. The gel-permeation chromatography (GPC) of lysozymic hydrolysates of N-AcGBC and β-chitin is shown in Fig. 12 in a time-dependent manner (no water-soluble fraction was observed in lysozymic hydrolysates of BC). The lysozymic hydrolysate of chitin was mainly composed of chitobiose and chitotriose, whereas that of N-AcGBC was composed of a trace of low-molecular-weight fractions and oligomer fractions. The GPC profiles of the latter fractions showed similar values of $\overline{DP}$, independent of the reaction time. The above results may suggest that the distribution of the GlcNAc residues in N-AcGBC is random rather than block. The profiles of cellulase hydrolysis of BC and N-AcGBC are shown in Fig. 10. A slower rate of hydrolysis was shown for N-AcGBC than for BC, and the lower cellulase activity is suggested to be due to the incorporation of GlcNAc residues into the main chain of BC. Chitinase showed a very slight susceptibility to N-AcGBC and high susceptibility of chitin is unlikely in the case of lysozyme treatment (Fig. 11).

DISCUSSION

The presence of four enzymatic steps has been suggested as shown below in the pathways of cellulose synthesis (from glucose to cellulose) (Ross *et al.*, 1991).

(1) phosphorylation of Glc by glucokinase; Glc → glucose-6-phosphate (Glc-6-P) (Benziman & Rivetz, 1972);
(2) the transphosphorylation of Glc-6-P to glucose-1-phosphate (Glc-1-P) by phosphoglucomutase;
(3) the synthesis of uridine-5'-diphosphoglucose (UDP-Glc : the precursor of cellulose synthesis in *A. xylinum* (Swissa *et al.*, 1980)) by pyrophosphorylase;
(4) cellulose synthase reaction to extend the length of the polymer chain.

It has been suggested that lipid (polyisoprenol) pyrophosphoryl glucose and lipid pyrophosphoryl cellobiose are present between step (3) and (4) (Garcia *et al.*, 1974; Colvin & Leppard, 1977). Cellulose synthesis starts in *A. xylinum* from Glc-6-P synthesized through the two alternative pathways. One pathway is direct phosphorylation of exogenous Glc. The other is an indirect one via the pentose cycle and the gluconeogenesis pathway, which is induced from oxalacetate via pyruvate in *A. xylinum* (Ross *et al.*, 1991). On the other hand, some Glc are converted to gluconic acid by the catalysis of glucose oxidase binding in cell membrane (Shinagawa *et al.*, 1976), and then the resulting gluconic acid is released to medium. This release of gluconic acid causes the lowering of pH in culture medium (as shown in Fig. 1). Since the pH of SH GlcNAc medium did not decrease during cultures, gluconic acid seemed not to be produced by *A. xylinum* strains grown on an SH GlcNAc medium. It is indicated that the conversion of GlcNAc to Glc is not achieved in an extracellular pathway, and GlcNAc seems to be taken up to the intracellular pathway. The metabolism of GlcNAc may be carried out in cytoplasm through a reverse-pathway reaction of GlcNAc-6-P production from Glc via fructose-6-P and glucosamine-6-P, since the growth of *A. xylinum* strains in an SH GlcNAc medium was observed (Figs 1 and 2). Although the pellicle was produced at the surface of the SH GlcNAc medium (the GlcNAc content was 2–4 mol%), the yield of pellicle was about one-tenth that of the wild type. This result suggests that one of the pathways is predominant: either cellulose synthesis by hexose phosphate or hexose phosphate synthesis by GlcNAc.

The following assumptions are proposed, because a trace of GlcNAc was detected in a general BC produced by wild-type-bacteria in an SH Glc medium (Figs 3 and 4).

(1) Acetamido-group-transferring enzyme is present in a cellulose synthase system.
(2) UDP-GlcNAc synthesized in cytoplasm for peptidoglycan or lipopolysaccharide synthesis becomes a substrate of cellulose synthase.

Though further work is needed to confirm the above assumptions, either enzyme-acetamido-group transferase or enzyme picking up GlcNAc as a substrate seems to be present in a cellulose synthase system. Since a slight increase in GlcNAc content in a pellicle was shown by STG-strains even in an SH Glc medium (Figs 3 and 4) and the GlcNAc content of pellicles obtained in the media of various compositions of Glc and GlcNAc by the strains grown in an SH GlcNAc agar medium were identical as shown in Figs 4 and 5, it is assumed that the activation of some enzyme system may be achieved during the adaptation process to a GlcNAc-rich medium (the repeat of transfer to a GlcNAc medium and culture on a GlcNAc solid medium).

The five times of transfer to a fresh medium seemed to make *A. xylinum* strains adapt to the new environment as shown by the observations described below.

(1) The incorporation of GlcNAc into a pellicle reached a maximum for STG-strains after five transfers (Figs 3 and 4), whereas the yield was reduced to one-third of the original one (Fig. 7).
(2) The reversion to cellulose synthesis was observed when GC-strains were cultured by more than five transfers to an SH Glc medium (Fig. 8).

The depression of the GlcNAc content in a pellicle produced by CSTG-strains (Fig. 5) and STGC-strains (Fig. 6) suggests the regeneration of cellulose synthase activity. In the case of pellicle-deficient mutation, spontaneous or by mutagenesis, the pellicle reproduction has been shown when they were cultured stationary (Valla & Kjosbakken, 1982). *A. xylinum* ATCC 10245 and NBI 1051 used in this study showed slight differences in the formation of a colony and incorporation of GlcNAc. It seems that strains of high cellulose productivity and strains of low frequency for the production of pellicle-deficient mutants may be favored rather than the incorporation of GlcNAc so far.

The resulting N-AcGBC was shown to have susceptibility for lysozyme (Fig. 9). A turbidity decrease in chitin suspensions was rather fast at the initial stage of lysozymic hydrolysis, and the rate of turbidity decrease was then suppressed (Fig. 9). This result suggests that lysozyme hydrolyzes insoluble (or the amorphous part of) chitin in the early stages of reaction and then hydrolyzes predominantly the resulting water-soluble chitin oligomers down to chitobiose and chitotriose. This suggestion is substantiated by the GPC profiles of lysozymic hydrolysates of chitin composed mainly of chitobiose and chitotriose (Fig. 12). Since the continuous decrease in turbidity was observed in an N-AcGBC suspension (Fig. 9) and the GPC profiles of N-AcGBC hydrolysates showed little change in the $\overline{DP}$ of main fractions even after a prolonged reaction time (Fig. 12), the resulting water-soluble oligomers seem to be mainly composed of glucose, and the distribution of GlcNAc in an N-AcGBC molecule should be random; further GlcNAc residues should be incorporated

through a β-1,4 glycoside linkages. The induction period in the early stages of reaction (as seen in Fig. 9) seems to suggest that conformational change in N-AcGBC is induced by the approach of lysozyme. Although the close chemical and crystalline structure of chitin is proposed to be similar to the N-AcGBC molecule owing to the lysozyme susceptibility, a slightly deviated structure from β-chitin is proposed for N-AcGBC, because only a very slight susceptibility of N-AcGBC for chitinase was observed (Fig. 11).

We have reported for the first time the production of bacterial cellulose having multi-susceptibilities for enzymes owing to the incorporation of GlcNAc by *A. xylinum* strains under slightly modified conditions with BC biosynthesis. N-AcGBC also showed a higher orienting tendency and Young's modulus than those of BC (data will be published elsewhere). A GlcNAc content of 0·5–4·0 mol% is sufficient for modification of BC to attach both cellulosic and chitinous properties. Further investigation is required to determine the fine mechanism of metabolic pathways of GlcNAc and the cellulose synthase system. Major interests are the mechanism of GlcNAc incorporation and the incorporation of other sugars.

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