APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Apr. 1990, p. 928–933
0099-2240/90/040928-06$02.00/0

Vol. 56, No. 4

Formation of Deglycosylated α-L-Fucosidase by Endo-β-*N*-Acetylglucosaminidase in *Fusarium oxysporum*

YASUNOBU TSUJI,[1] KENJI YAMAMOTO,[2]* AND TATSUROKURO TOCHIKURA[2]

Institute of Soysauce, Higashimaru Shoyu Co. Ltd., Tatsuno, Hyogo 679-41,[1] and Department of Food Science and Technology, Faculty of Agriculture, Kyoto University, Kyoto 606,[2] Japan

Received 24 August 1989/Accepted 3 January 1990

Two forms of α-L-fucosidase, deglycosylated and glycosylated, were found in the fucose-inducing culture broth of *Fusarium oxysporum*. Endo-β-*N*-acetylglucosaminidase was also found in the same culture broth. The deglycosylated α-L-fucosidase was purified from the culture broth to homogeneity on polyacrylamide disc gel electrophoresis and analytical ultracentrifugation. Purified deglycosylated α-L-fucosidase was compared in chemical composition and immunological homology with glycosylated α-L-fucosidase which had been reported previously (K. Yamamoto, Y. Tsuji, H. Kumagai, and T. Tochikura, Agric. Biol. Chem. 50:1689, 1986). Both enzymes had nearly the same amino acid compositions and were immunologically identical. Glycosylated α-L-fucosidase had mannose, galactose, and *N*-acetylglucosamine residues. In contrast, the deglycosylated enzyme had only *N*-acetylglucosamine residues. These results suggest that the deglycosylated α-L-fucosidase is formed by the release of sugar chains from the glycosylated form by *Fusarium* endo-β-*N*-acetylglucosaminidase. Furthermore, various enzymatic properties were compared: the two α-L-fucosidases were found to exhibit similar catalytic activities and thermal stability profiles. The deglycosylated enzyme, however, was slightly unstable in the acidic pH range compared with the glycosylated enzyme.

α-L-Fucosidase (EC 3.2.1.51) cleaves α-linked L-fucosidic bonds from the nonreducing ends of sugar chains in various glycoproteins, oligosaccharides, and glycolipids. The enzyme is important in the metabolism of several biologically active molecules containing L-fucose and is also useful in the structural and functional studies of many glycoconjugates (3, 11).

We have already reported that a novel α-L-fucosidase was produced in the culture fluid of *Fusarium oxysporum* S252 isolated from a soil sample, and its production was highly induced by L-fucose (17, 19). We also reported the purification and properties of the enzyme in a previous paper and showed that the enzyme was a glycoprotein (17–19).

During the investigation of *Fusarium* α-L-fucosidase, we found two α-L-fucosidases in the inducing culture broth. One of them was glycosylated and the other was deglycosylated (carbohydrate depleted). In the same culture broth, we also found endo-β-*N*-acetylglucosaminidase, which releases sugar chains from glycoproteins. To investigate the metabolism and function of the glycoprotein enzymes in microorganisms, we studied the formation of deglycosylated α-L-fucosidase and compared various properties of the two *Fusarium* α-L-fucosidases. We consider that the two *Fusarium* α-L-fucosidase are useful for studying the role of carbohydrate moieties of glycoenzymes.

MATERIALS AND METHODS

Chemicals. *p*-Nitrophenyl α-L-fucoside, L-fucose, and porcine gastric mucin were purchased from Nakarai Tesque Ltd. Concanavalin A-Sepharose 4B, Sephadex G-100, and Sephadex G-200 were products of Pharmacia Fine Chemicals. Toyopearl HW 55F and DEAE-Cellulofine AM were obtained from Tosoh Co. and Chisso Co., respectively. 2′-Fucosyllactose was purchased from Sigma Chemical Co. All other reagents were of the highest grade available.

Microorganism. *F. oxysporum* S252, which was isolated from a soil sample as an α-L-fucosidase producer (19), was used throughout this study.

Inducing cultivation. The fungus was cultured in a liquid medium consisting of 0.5% glucose, 0.5% peptone, 0.5% yeast extract, and 0.5% NaCl (pH 7.0) with shaking at 30°C. The mycelia were collected by filtration, washed twice with 20 mM Tris hydrochloride buffer (pH 8.5), and then suspended in an inducing medium of 20 mM Tris hydrochloride buffer (pH 8.5) containing 0.2% L-fucose at a cell concentration of 40 to 50 mg (wet weight)/ml. The flask was incubated for 24 h at 30°C with shaking (17).

Enzyme assay. α-L-Fucosidase activity was assayed by using *p*-nitrophenyl α-L-fucoside as the substrate, as described previously (19). One unit of the enzyme was defined as the amount of enzyme which hydrolyzed 1 μmol of substrate per min at 37°C. Endo-β-*N*-acetylglucosaminidase activity was determined with dansyl asparagine oligosaccharides prepared from ovalbumin as substrates, as described previously (14). One unit was defined as the amount of enzyme that yielded 1 μmol of dansyl asparagine-*N*-acetylglucosamine from dansyl asparagine oligosaccharides per min at 37°C. Protease activity was determined with milk casein as the substrate (6).

Gel electrophoresis. Polyacrylamide disc gel electrophoresis was performed by the method of Davis (2) with 7.5% polyacrylamide gel and Tris-glycine buffer, pH 8.3. Sodium dodecyl sulfate-polyacrylamide slab gel electrophoresis was performed with 12.5% acrylamide and 0.1% sodium dodecyl sulfate with a discontinuous Tris-glycine buffer system by the method of Laemmli (7). For molecular-weight (in parentheses) determination, phosphorylase *b* (94,000), bovine serum albumin (67,000), ovalbumin (43,000), and carbonic anhydrase (30,000) were used as molecular-weight markers. The gels were stained for protein with Coomassie brilliant blue R and destained with 7% acetic acid.

Molecular weight determination. The molecular weight of the enzyme was estimated by gel filtration on a Sephadex G-200 column (1.0 by 115 cm) by the method of Andrews (1).

* Corresponding author.

The column was calibrated with (molecular weight in parentheses) catalase (232,000), bovine serum albumin (67,000), ovalbumin (43,000), and RNase (13,700).

Amino acid analysis. The enzyme protein was hydrolyzed with 6 N HCl in an evacuated sealed tube at 105°C for 24 h. The amino acid content of the hydrolysate was determined by the method of Spackman et al. (12) with Kyowa amino acid analyzer K-101. Cysteine and half-cystine were determined from the amount of cysteic acid a described by Moore (9). Tryptophan was measured spectrophotometrically by the method of Goodwin and Morton (4).

Determination of sugar. Glucosamine was analyzed after acid hydrolysis (4 N HCl, 100°C for 6 h) with an amino acid analyzer. Neutral sugars were analyzed by gas-liquid chromatography (column, 2% OV-17; 75 cm) after acid hydrolysis (2 N H_2SO_4, 100°C for 5 h) followed by trimethylsilylation.

Analytical methods. Protein was determined by the method of Lowry et al. (8), with crystalline egg albumin as the standard. The protein elution patterns from column chromatography were determined on the basis of the A_{280}. Ultracentrifugal analysis was performed with a Spinco model E ultracentrifuge at 59,780 rpm and 20°C.

Immunological method. Antiserum to glycosylated α-L-fucosidase was prepared by immunizing two rabbits with intradermal injections of the antigen in Freund complete adjuvant. The rabbits were injected four times at 6-day intervals and were bled on day 65. An Ouchterlony double-immunodiffusion test (10) was carried out on 1.2% agar in sodium phosphate-buffered saline containing 0.1% sodium azide at 20°C.

Partial purification of endo-β-*N*-acetylglucosaminidase. Partial purification of endo-β-*N*-acetylglucosaminidase from *F. oxysporum* was carried out by the following procedure. After 3 days of cultivation in 28.3 liters of glucose medium described above, the fungal culture broth was filtered through filter paper and centrifuged at 10,000 × *g* for 10 min. The supernatant obtained was brought to 80% saturation with solid ammonium sulfate and left at 4°C overnight. The precipitate was collected and dissolved in 10 mM potassium phosphate buffer (pH 7.0), followed by dialysis against the same buffer overnight. The dialyzed solution was put through a column (6.5 by 38 cm) of DEAE-Cellulofine AM previously equilibrated with 10 mM potassium phosphate buffer (pH 7.0). The column was washed with the same buffer, and then elution was performed with a stepwise gradient of NaCl in the same buffer. The active fractions (eluted with 0.2 M NaCl) were collected and brought to 80% saturation with ammonium sulfate. The precipitate was dissolved in a small volume of 10 mM potassium phosphate buffer (pH 7.0). The concentrated enzyme solution was applied to a Sephadex G-100 column (2.2 by 120 cm) previously equilibrated with 10 mM potassium phosphate buffer (pH 7.0) containing 0.1 M NaCl and eluted with the same buffer. The active fractions were pooled and concentrated by ultrafiltration with an Amicon PM-10 membrane. The concentrated enzyme solution was used as partially purified endo-β-*N*-acetylglucosaminidase. No exoglycosidase and protease activities were detected in the enzyme preparation. The partially purified enzyme had an optimum pH of 4.0 to 4.5 and was stable in the pH range of 4.0 to 7.0. Moreover, the enzyme was substrate specific, acting on both high-mannose and hybrid types of sugar chains of glycoproteins. Details of the enzymatic properties will be reported elsewhere.

Purification of glycosylated α-L-fucosidase. Glycosylated α-L-fucosidase was purified from the inducing culture broth, as described previously (17).

Purification of deglycosylated α-L-fucosidase. The purification procedure prior to concanavalin A-Sepharose 4B column chromatography of deglycosylated α-L-fucosidase was essentially the same as the steps used in the purification of glycosylated α-L-fucosidase.

A 5-liter portion of the culture broth obtained after 24-h induction with 0.2% L-fucose medium was filtered and centrifuged at 10,000 × *g* for 10 min to remove the mycelia. The supernatant was used as a crude enzyme preparation. All subsequent steps were carried out in a cold room at 4°C, and potassium phosphate buffer (pH 7.0) was used throughout the purification procedure. The supernatant (5,590 ml) was concentrated by ultrafiltration with a membrane (PTGC; Millipore Corp.), and then the concentrated enzyme solution was dialyzed against 10 mM phosphate buffer. The dialyzed enzyme solution was put on a DEAE-Cellulofine AM column (6 by 23 cm) previously equilibrated with 10 mM phosphate buffer. The column was washed with the same buffer, and then the enzyme was eluted from the column with a stepwise gradient of NaCl from 0 to 0.5 M in the same buffer. The active fractions (eluted with 0.2 M NaCl) were collected and concentrated by ultrafiltration with a membrane (PM-10; Amicon Corp.). The concentrated enzyme solution was put on a Toyopearl HW 55F column (1.5 by 110 cm) previously equilibrated with 10 mM phosphate buffer and then eluted with the same buffer. The active fractions were concentrated by ultrafiltration with a membrane (PM-10; Amicon), and the concentrated enzyme solution was dialyzed overnight against 10 mM phosphate buffer containing 0.1 M NaCl. The dialyzed enzyme solution was applied to a column (1.8 by 10 cm) of concanavalin A-Sepharose 4B equilibrated with 10 mM phosphate buffer containing 0.1 M NaCl. The enzyme was eluted with the same buffer. All fractions containing α-L-fucosidase activity were pooled and concentrated by ultrafiltration with a membrane (PM-10; Amicon).

RESULTS

Presence of deglycosylated α-L-fucosidase in the culture filtrate of *F. oxysporum*. When a concentrated inducing culture filtrate of *F. oxysporum* was applied to a concanavalin A-Sepharose 4B column (1.0 by 12 cm) previously equilibrated with 10 mM potassium phosphate buffer (pH 7.0) containing 0.1 M NaCl, two distinct α-L-fucosidase fractions were found: one fraction was eluted with the same buffer, and the other was eluted with the same buffer but containing α-methyl-mannoside (Fig. 1). The results show that there were two forms of α-L-fucosidase in the culture broth, deglycosylated and glycosylated, although the glycosylated form was predominant.

Formation of deglycosylated α-L-fucosidase in inducing broth. To investigate the formation of deglycosylated α-L-fucosidase by inducing *F. oxysporum*, the time course of α-L-fucosidase production was examined in an inducing medium containing 0.2% L-fucose. An aliquot of the culture broth was withdrawn at intervals, and separation of deglycosylated α-L-fucosidase and the glycosylated form was carried out by concanavalin A-Sepharose 4B column chromatography to assay both enzyme activities. Figure 2 shows time courses for the production of both enzymes and for decreases in L-fucose in the inducing medium. The activity of endo-β-*N*-acetylglucosaminidase was also determined. The activity of glycosylated α-L-fucosidase was detected after a 6-h induction and reached a maximum at about 15 h.

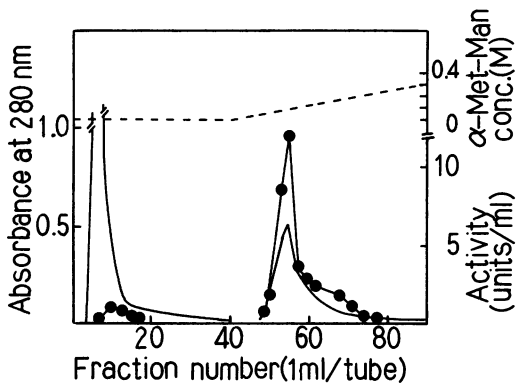

FIG. 1. Presence of deglycosylated α-L-fucosidase in the culture filtrate of *F. oxysporum*. The inducing culture broth, filtered and concentrated, was applied to a concanavalin A-Sepharose 4B column (1.0 by 12 cm) previously equilibrated with 10 mM potassium phosphate buffer (pH 7.0) containing 0.1 M NaCl. The column was eluted with the same buffer, followed by a linear gradient of α-methyl-mannoside, 0 to 0.3 M, in the same buffer. Symbols: ●, α-L-fucosidase; ——, A_{280}; – – – – –, α-methyl-mannoside (α-Met-Man) concentration.

On the other hand, deglycosylated α-L-fucosidase was first detected after a 9-h induction and its activity attained a maximum at a low level after 15 h. Though L-fucose disappeared from the medium at 13 h, the production of both α-L-fucosidases might have continued for 15 h. Since autolysis of the mold occurred at about 15 h, both α-L-fucosidase activities seemed to decrease gradually by proteolysis after 15 h. Endo-β-*N*-acetylglucosaminidase was found to maintain constant activity.

Action of endo-β-*N*-acetylglucosaminidase on glycosylated α-L-fucosidase. To examine whether endo-β-*N*-acetylglucosaminidase hydrolyzed the sugar chains of glycosylated α-L-fucosidase, partially purified endo-β-*N*-acetylglucosaminidase (67 mU) was incubated with glycosylated α-L-fucosidase (14 U, 0.12 mg) in a 1.01-ml reaction mixture containing 50 mM potassium phosphate buffer (pH 6.0) at 37°C for 7.5 h. An aliquot of the reaction mixture was taken up at intervals and applied to a concanavalin A-Sepharose 4B column (0.8 by 4 cm). The column was eluted with 10 mM potassium phosphate buffer (pH 7.0) containing 0.1 M NaCl followed by the same buffer containing 0.1 M α-methyl-

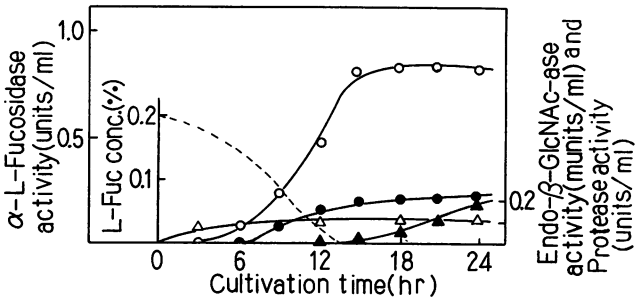

FIG. 2. Time course of enzyme production by inducing *F. oxysporum* cultivation. Deglycosylated α-L-fucosidase and the glycosylated form were separated by concanavalin A-Sepharose 4B column chromatography and were examined for total activities. Symbols: ●, deglycosylated α-L-fucosidase; ○, glycosylated α-L-fucosidase; △, endo-β-*N*-acetylglucosaminidase (endo-β-GlcNAc-ase); ▲, protease; – – – – –, L-fucose concentration.

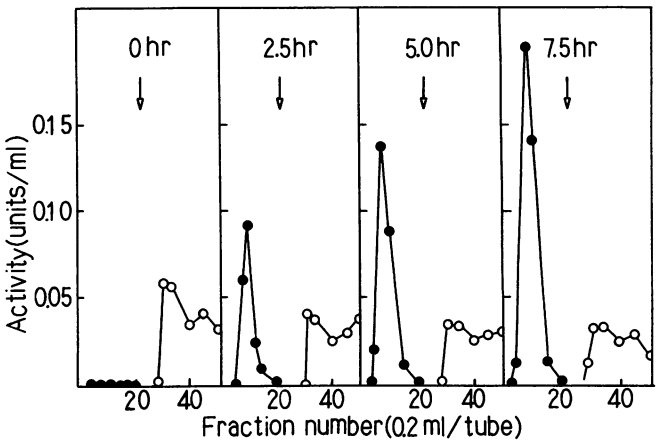

FIG. 3. Concanavalin A-Sepharose 4B column chromatography of α-L-fucosidases hydrolyzed by endo-β-*N*-acetylglucosaminidase from *F. oxysporum*. Glycosylated α-L-fucosidase, 14 U, was incubated with 67 mU of endo-β-*N*-acetylglucosaminidase in 50 mM potassium phosphate buffer (pH 6.0) at 37°C. An aliquot of the reaction mixture was taken up at intervals and diluted five times with 12.5 mM potassium phosphate buffer (pH 7.0) containing 0.125 M NaCl. The diluted solution was applied to a concanavalin A-Sepharose 4B column equilibrated with 10 mM potassium phosphate buffer (pH 7.0) containing 0.1 M NaCl. The column was eluted with the same buffer. Arrow indicates changing of effluent to 0.1 M α-methyl-mannoside. Symbols: ●, deglycosylated α-L-fucosidase; ○, glycosylated α-L-fucosidase.

mannoside. Prior to incubation with endo-β-*N*-acetylglucosaminidase, α-L-fucosidase activity was not found in the unadsorbed fractions (Fig. 3). After the incubation, however, α-L-fucosidase activity was detected in unadsorbed fractions, which increased with incubation time. The amount of deglycosylated α-L-fucosidase reached 63% of the total amount of α-L-fucosidase at 7.5 h.

Purification of deglycosylated α-L-fucosidase. The deglycosylated α-L-fucosidase was purified about 15-fold from the culture filtrate with a 6% recovery of activity. At the step of concanavalin A-Sepharose 4B column chromatography, the deglycosylated α-L-fucosidase was separated from the glycosylated form: only glycosylated α-L-fucosidase was adsorbed. At the steps prior to concanavalin A-Sepharose 4B column chromatography, α-L-fucosidase-active fractions included both glycosylated and deglycosylated forms. This accounts for the low yield of total activity after the final step of concanavalin A-Sepharose 4B column chromatography.

Enzyme purity and molecular weight. The purified enzyme migrated as a single protein band on polyacrylamide disc gel electrophoresis (Fig. 4) and showed a single symmetrical peak in the sedimentation pattern via analytical ultracentrifugation. The molecular weight of the enzyme was estimated to be 72,000 by gel filtration and sodium dodecyl sulfate-polyacrylamide gel electrophoresis. It seemed that deglycosylated α-L-fucosidase had a slightly lower molecular weight than glycosylated α-L-fucosidase (75,000) (17).

Amino acid and sugar compositions. The amino acid and sugar compositions of deglycosylated and glycosylated α-L-fucosidases are shown in Table 1. There was no tryptophan residue in the two α-L-fucosidases. From this result, deglycosylated α-L-fucosidase seemed to be released sugar chains consisting of galactose, mannose, and *N*-acetylglucosamine from glycosylated α-L-fucosidase by means of endo-β-*N*-acetylglucosaminidase.

Comparison of optimum and stable pHs of deglycosylated

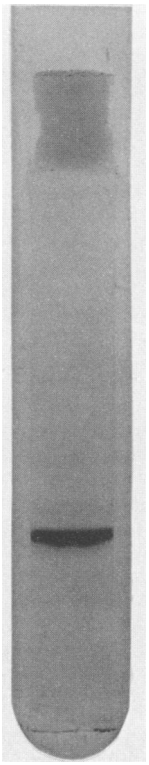

FIG. 4. Polyacrylamide gel electrophoresis of purified deglycosylated α-L-fucosidase. A 30-μg amount of the purified enzyme was applied to a 7.5% polyacrylamide gel.

and glycosylated α-L-fucosidases. Various enzymatic properties of the deglycosylated α-L-fucosidase were compared with those of glycosylated α-L-fucosidase. The effects of pH on the activity and stability of the two enzymes are shown in Fig. 5. The same pH activity profiles were obtained for the two enzymes. Both enzymes showed an optimum pH range of 4.5 to 5.5. However, the pH stability was a little different. Deglycosylated α-L-fucosidase was unstable in the acidic pH range compared with glycosylated α-L-fucosidase.

Comparison of other properties of deglycosylated and glycosylated α-L-fucosidases. The effects of temperature on the stability of the deglycosylated and glycosylated α-L-fucosidases were examined, and the same thermal stability profiles were obtained for the two α-L-fucosidases. Both enzymes retained their full activities at temperatures below 40°C. The Michaelis constants of deglycosylated and glycosylated α-L-fucosidases for *p*-nitrophenyl α-L-fucoside, calculated from Lineweaver-Burk plots, were 0.59 and 0.48 mM, respectively. Effects of various compounds, including metal ions, sulfhydryl reagents, and sugars, on activity of either enzyme were not found. In addition, both enzymes hydrolyzed 2′-fucosyllactose and porcine gastric mucin at the same velocity.

Immunological similarity of deglycosylated and glycosylated α-L-fucosidases. To investigate whether deglycosylated and glycosylated α-L-fucosidases were immunologically the same, Ouchterlony double-diffusion analysis, using antiserum against glycosylated α-L-fucosidase, was carried out. Purified glycosylated α-L-fucosidase gave a single precipitin line against the antiserum (Fig. 6a). Then, we examined the ability of the antiserum to cross-react with deglycosylated α-L-fucosidase. An immunoprecipitin line was also observed with deglycosylated α-L-fucosidase and a single continuous precipitin line was formed with glycosylated α-L-fucosidase without any spur (Fig. 6b). These results indicated that deglycosylated α-L-fucosidase was immunologically the same as glycosylated α-L-fucosidase.

TABLE 1. Amino acid and carbohydrate compositions of deglycosylated and glycosylated α-L-fucosidases[a]

Amino acid or carbohydrate	Residues per mol of protein	
	Glycosylated α-L-fucosidase	Deglycosylated α-L-fucosidase
Amino acid		
Aspartic acid	72	74
Threonine	37	35
Serine	37	37
Glutamic acid	67	65
Proline	52	52
Glycine	64	63
Alanine	37	37
Valine	41	41
Methionine	6	6
Isoleucine	27	27
Leucine	32	32
Tyrosine	34	34
Phenylalanine	31	32
Lysine	36	36
Histidine	17	18
Arginine	18	17
Tryptophan	0	0
Half-cystine	6	6
Carbohydrate		
Mannose	12	0
Galactose	2	0
Glucosamine	6	2

[a] The molecular weights of deglycosylated and glycosylated α-L-fucosidases were taken as 72,000 and 75,000, respectively.

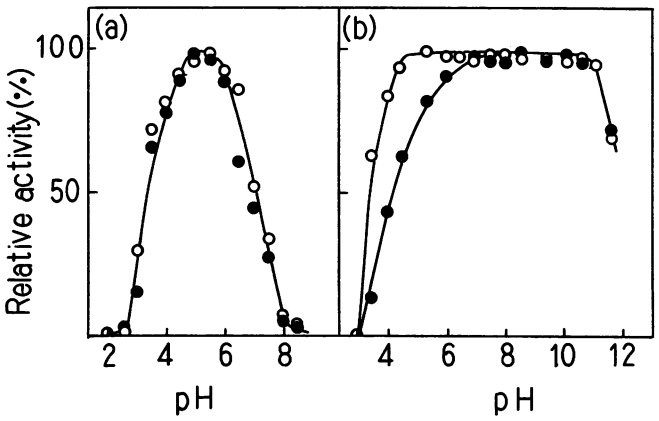

FIG. 5. Effects of pH on activity and stability of deglycosylated and glycosylated α-L-fucosidases. (a) Enzyme activity at various pHs was measured under standard conditions, using various buffer systems (50 mM), and expressed as a percentage of maximum activity. (b) The enzyme was kept at various pHs at 4°C for 2 days, using various buffer systems (500 mM), followed by assay of the remaining activity under standard conditions. Buffer systems: sodium citrate hydrochloride (pH 2.0 to 4.5), sodium citrate-citric acid (pH 4.5 to 6.5), potassium phosphate (pH 6.5 to 7.5), Tris hydrochloride (pH 7.5 to 8.5), glycine-NaOH (pH 8.5 to 10.0), borate-NaOH (pH 10.0 to 11.5). Symbols: ●, deglycosylated α-L-fucosidase; ○, glycosylated α-L-fucosidase.

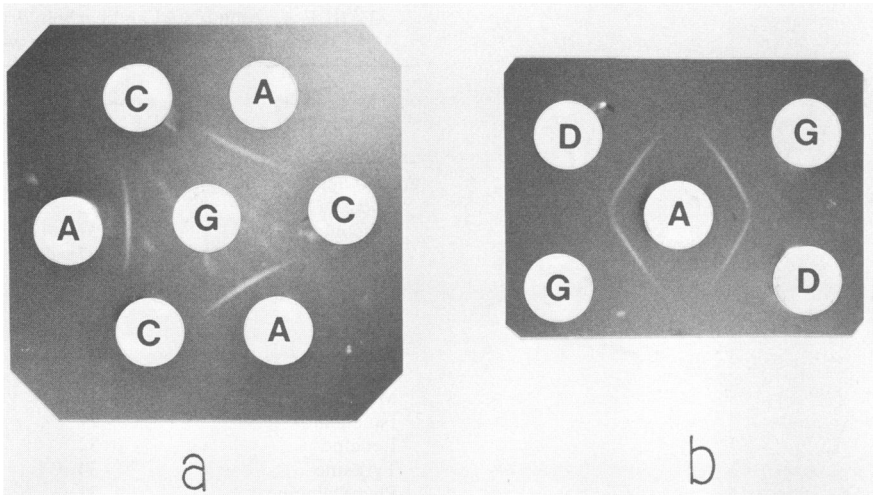

FIG. 6. Immunodiffusion pattern of rabbit antiserum against purified glycosylated α-L-fucosidase (a) and immunodiffusion test of antiserum against deglycosylated and glycosylated α-L-fucosidases (b). (a) Purified glycosylated α-L-fucosidase was placed in the center well, G. Wells C, Control antiserum; wells A, antiserum obtained on day 65 after the first booster injection. (b) Antiserum was placed in the center well, A. Wells D, Deglycosylated α-L-fucosidase; wells G, glycosylated α-L-fucosidase.

DISCUSSION

During the course of purification of α-L-fucosidase, an *F. oxysporum* glycoprotein, we obtained an α-L-fucosidase fraction containing no sugar chains. Furthermore, the deglycosylated α-L-fucosidase purified to homogeneity was found to have only *N*-acetylglucosamine residues. These findings suggest the production of an endo-β-*N*-acetylglucosaminidase by the fungus, because the enzyme hydrolytically cleaves the *N*,*N*′-diacetylchitobiose moiety in the asparagine-linked oligosaccharides of glycoproteins. Actually, we found endo-β-*N*-acetylglucosaminidase in the fungal culture fluid, though the activity was low (about 0.1 mU/ml).

Hitomi et al. presented results showing that Taka-amylase A containing only one *N*-glycosidic *N*-acetylglucosamine residue attached to an asparagine residue was obtained from Taka-diastase, a product of *Aspergillus oryzae* (5). They also found endo-β-*N*-acetylglucosaminidase in the same culture. However, they did not discuss the relationship between the formation of deglycosylated Taka-amylase A and endo-β-*N*-acetylglucosaminidase during cultivation of the fungus. We found that, when partially purified endo-β-*N*-acetylglucosaminidase from *F. oxysporum* was incubated with glycosylated α-L-fucosidase from the same fungus, deglycosylated α-L-fucosidase was formed and its level increased with incubation time. During inducing cultivation of the fungus, we did not find deglycosylated α-L-fucosidase at all after a 6-h cultivation, when a smaller amount of glycosylated α-L-fucosidase had been produced, and deglycosylated α-L-fucosidase was first detected after a 9-h cultivation. These results suggest that α-L-fucosidase at definite levels was deglycosylated gradually by endo-β-*N*-acetylglucosaminidase, which was produced from the beginning of cultivation, and that deglycosylated α-L-fucosidase accumulated in the later phase of *F. oxysporum* cultivation. The same phenomenon was found when mold had grown on medium with L-fucose as the carbon source under normal growth conditions (data not shown). We do not know, however, whether endo-β-*N*-acetylglucosaminidase is physiologically important in the metabolism of glycosylated enzymes.

Using *Flavobacterium* sp. endo-β-*N*-acetylglucosaminidase, we previously found that deglycosylated enzymes were more sensitive to proteinase and that some of them were less thermally stable than the native enzymes (13, 15, 16). To examine the role of α-L-fucosidase sugar chains, we purified the deglycosylated form to homogeneity and compared its enzymatic properties with those of the purified glycosylated form (native α-L-fucosidase) which have been reported previously (17). They exhibited similar enzymatic properties except that the pH stability profiles were a little different between the two enzymes and the deglycosylated α-L-fucosidase was more susceptible to proteases such as subtilisin, pronase, and proteases from *F. oxysporum* than the glycosylated form (data not shown). Investigations are in progress to study the role of sugar chains of *Fusarium* α-L-fucosidase further.

LITERATURE CITED

1. **Andrews, P.** 1965. The gel filtration behavior of proteins related to their molecular weights over a wide range. Biochem. J. **96**:595–606.
2. **Davis, B. J.** 1964. Disc electrophoresis. II. Method and application to human serum proteins. Ann. N.Y. Acad. Sci. **121**: 404–407.
3. **Furbish, F. S., N. L. Krett, J. A. Barranger, and R. O. Brady.** 1980. Fucose plays a role in the clearance and uptake of glycocerebrosidase by rat liver cells. Biochem. Biophys. Res. Commun. **95**:1768–1774.
4. **Goodwin, W. T., and R. A. Morton.** 1946. The spectrophotometric determination of tyrosine and tryptophan in proteins. Biochem. J. **40**:628–632.
5. **Hitomi, J., Y. Murakami, F. Saitoh, N. Shigemitsu, and H. Yamaguchi.** 1985. Purification and characterization of endo-β-*N*-acetylglucosaminidase of *Aspergillus oryzae*. J. Biochem. **98**:527–533.
6. **Kunitz, M.** 1947. Crystalline soybean trypsin inhibitor. II. General properties. J. Gen. Physiol. **30**:291–310.
7. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
8. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.
9. **Moore, S.** 1963. On the determination of cysteine as cysteic acid. J. Biol. Chem. **238**:235–237.

10. **Ouchterlony, O.** 1949. Antigen-antibody reactions in gels. Acta Pathol. Microbiol. Scand. **26:**507–517.
11. **Prieels, J. P., S. V. Pizzo, L. R. Glascow, J. C. Paulson, and R. L. Hill.** 1978. Hepatic receptor that specifically binds oligosaccharides containing fucosyl α1→3 N-acetylglucosamine linkages. Proc. Natl. Acad. Sci. USA **75:**2215–2219.
12. **Spackman, D. H., W. H. Stein, and S. Moore.** 1958. Automatic recording apparatus for use in the chromatography of amino acid. Anal. Chem. **80:**1190–1206.
13. **Takegawa, K., M. Inami, K. Yamamoto, H. Kumagai, T. Tochikura, B. Mikami, and Y. Morita.** 1988. Elucidation of the role of sugar chains in glucoamylase using endo-β-N-acetylglucosaminidase from *Flavobacterium* sp. Biochim. Biophys. Acta **955:**187–193.
14. **Yamamoto, K., S. Kadowaki, K. Takegawa, H. Kumagai, and T. Tochikura.** 1986. Purification and characterization of endo-β-N-acetylglucosaminidase from a *Flavobacterium* sp. Agric. Biol. Chem. **50:**421–429.
15. **Yamamoto, K., K. Takegawa, H. Kumagai, and T. Tochikura.** 1986. Elucidation of the role of sugar chains in glycosylated-enzymes using endo-β-N-acetylglucosaminidase from *Flavobacterium* sp. Agric. Biol. Chem. **50:**2167–2169.
16. **Yamamoto, K., K. Takegawa, H. Kumagai, and T. Tochikura.** 1987. Effect of protease digestion on the activity of sugar-depleted enzymes prepared with endo-β-N-acetylglucosaminidase from *Flavobacterium* sp. Agric. Biol. Chem. **51:** 1481–1487.
17. **Yamamoto, K., Y. Tsuji, H. Kumagai, and T. Tochikura.** 1986. Induction and purification of α-L-fucosidase from *Fusarium oxysporum*. Agric. Biol. Chem. **50:**1689–1695.
18. **Yamamoto, K., Y. Tsuji, T. Tochikura, T. Seno, H. Yamaguchi, H. Fukuda, S. Akutsu, and M. Kawamura.** 1987. Blood group substance-degrading α-L-fucosidase produced by *Fusarium oxysporum*. Agric. Biol. Chem. **51:**1995–1996.
19. **Yano, T., K. Yamamoto, H. Kumagai, T. Tochikura, T. Yokoyama, T. Seno, and H. Yamaguchi.** 1985. Purification and characterization of a novel α-L-fucosidase from *Fusarium oxysporum* grown on sludge. Agric. Biol. Chem. **49:**3179–3187.