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Glycoprotein Enzymes Secreted by *Aspergillus niger*: Purification and Properties of α-Galactosidase

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An α-galactosidase (α-D-galactoside galactohydrolase [EC 3.2.1.22]) was purified to homogeneity from the culture filtrate of *Aspergillus niger*. The enzyme had an apparent molecular weight of 45,000 and was a glycoprotein. Radioactive enzyme was prepared by growing cells in [^{14}C]fructose and this enzyme was used to prepare ^{14}C-labeled glycopeptides. The glycopeptides emerged from Sephadex G-50 between stachyose and the glycopeptide from ovalbumin. Based on calibration of the column with various-sized dextran oligosaccharides, the glycopeptides appeared to have a molecular weight of 1,200 to 1,400. Analysis of the glycopeptide(s) indicated that it contained mannose and *N*-acetylglucosamine (GlcNAc) in an approximate ratio of 3 or 4 to 1. Assuming that there are two GlcNAc residues in the oligosaccharide and based on the molecular weight of the glycopeptide, the oligosaccharide probably contains eight to nine sugar residues. Alkaline treatment of the glycoprotein indicated that the oligosaccharide was probably attached to the protein by a GlcNAc → asparagine linkage. The purified α-galactosidase was most active on raffinose ($K_m = 5 \times 10^{-4}$ M, $V_{max} = 3$ μmol/min per mg of protein), but also showed good activity on *p*-nitrophenyl-α-D-galactoside and somewhat less activity on stachyose and melibiitol. The enzyme also hydrolyzed guar flour and locust bean gum, but did not attack the *p*-nitrophenyl glycosides of β-galactose, α- or β-glucose, or α- or β-mannose.

When the fungus *Aspergillus niger* is grown on a polysaccharide substrate such as guar flour, it produces and secretes a number of glycosidases into the medium. We have identified at least five different enzymatic activities in the medium, including an α-glucosidase, a β-glucosidase, an α-galactosidase, a β-galactosidase, and a β-mannosidase. Two of these enzymes, the α-glucosidase (15) and the β-glucosidase (14), were purified to homogeneity and shown to be glycoproteins. In both cases, the oligosaccharide chains had the general structure mannose $(man)_n$-*N*-acetylglucosamine $(GlcNAc)_2$, which apparently was attached to the protein by a GlcNAc → asparagine linkage. This report describes the purification to homogeneity of the α-galactosidase (α-D-galactoside galactohydrolase EC 3.2.1.22) and some of the properties of this enzyme. Like the glucosidases, this enzyme is also a glycoprotein, having an oligosaccharide composed of mannose and GlcNAc apparently attached to the protein in a glucosaminyl → asparagine bond.

MATERIALS AND METHODS

Materials. Diethylaminoethyl (DEAE)-cellulose, guar flour, raffinose, stachyose, and *p*-nitrophenyl glycosides of α- and β-glucose and α- and β-galactose were purchased from Sigma Chemical Co. *p*-Nitrophenyl-α-D-mannoside was prepared by the method of Helferich and Schmitz-Hillebrecht (3) or purchased from Sigma Chemical Co. *p*-Nitrophenyl-β-D-mannoside was a generous gift of Y. C. Lee, Johns Hopkins University (13); Sephadex G-25, G-50, and G-200 were from Pharmacia Fine Chemicals, Inc. Hydroxylapatite was prepared by the method of Levin (9) or purchased from Bio-Rad Laboratories.

Growth conditions and preparation of crude enzyme. *A. niger* (this organism was mistakenly called *A. fumigatus* in previous publications [14, 15], but has since been identified as *A. niger*) was grown at 30°C in a mineral salts medium of the following composition (grams per liter): K_2HPO_4, 2; $(NH_4)_2SO_4$, 1.4; urea, 0.3; $CaCl_2$, 0.3; $MgSO_4$, 0.3; mannose, 0.1; yeast extract, 0.05; and guar flour, 5.

Two-liter Erlenmeyer flasks containing 1 liter of medium were inoculated with 5 ml of a mycelial suspension from a 2-day-old culture that had been started from a loop of conidia. After 3 days of growth at 30°C on a rotary shaker, mycelia were harvested by filtration through four layers of cheesecloth. The filtrate was used as the source of crude enzyme.

Enzyme assays. Unless otherwise stated, reaction mixtures contained the following components in a total volume of 0.3 ml; substrate, 0.25 μmol; sodium acetate buffer (pH 5.0), 5 μmol; and enzyme. During the purification of α-galactosidase, the various *p*-nitrophenyl glycosides were tested as substrates to follow the removal of other glycosides.

After incubation at 37°C for various times, the reaction was stopped in one of two ways: (i) when *p*-nitrophenyl glycoside was used as substrate, 1.0 ml of a 0.4 M glycine buffer (pH 10.5) was added and the amount of liberated *p*-nitrophenol was measured at 405 nm (an optical density [OD] of 1.2 is equal to 0.1 μmol of *p*-nitrophenol); or (ii) when other substrates (i.e., raffinose, stachyose, etc.) were used, the reaction was stopped by adding reducing sugar reagents and the amount of galactose was measured by the reducing sugar method (11). Controls, in which heat-killed enzyme was used or in which enzyme was added at the end of the incubation, gave OD values of 0.2 or less, whereas incubations gave OD values of 0.3 to 1.7, depending on the time of incubation or the amount of enzyme used.

Analytical methods. Reducing sugars were measured by the method of Nelson (11), hexoses by the anthrone procedure (17), hexosamines by a modification of the Elson-Morgan method (5), and protein as described by Lowry et al. (10).

Analytical gel electrophoresis. Polyacrylamide gels (7%) were prepared and run as described by Davis (2). To visualize the protein bands, gels were stained with Coomassie brilliant blue for at least 2 h and destained electrophoretically. Carbohydrate was detected on duplicate gels by the periodate-Fuchsin stain (21). Gels containing radioactive proteins were sliced into 1-mm sections, dissolved in Protosol (New England Nuclear Corp.) at 55°C overnight, and counted in toluene scintillator fluid.

Paper chromatography. Descending paper chromatography of hexoses, hexosamines, and glucosaminitol was done on Whatman 3 MM paper in the following solvent systems: (i) *n*-butanol–pyridine–water (6:4:3); (ii) *n*-butanol–pyridine-0.1 N HCl (5:3:2); (iii) ethyl acetate-formic acid-acetic acid-water (18:1:3:4); (iv) methyl ethyl ketone-saturated boric acid-water (9:1:1); (v) *n*-propanol–ethyl acetate–water (7:1:2). Sugars and hexitols were located with the periodate-permanganate stain (8), and radioactivity was located on a radiochromatogram scanner.

Molecular weight determination. The molecular weight of α-galactosidase was estimated by gel filtration on a Sephadex G-200 column equilibrated and run in 0.02 M acetate buffer (pH 5.0) (16). The column was standardized with blue dextran, serum albumin, ovalbumin, hexokinase, and ribonuclease.

Preparation of radioactive α-galactosidase. Because it was difficult to obtain sufficient amounts of enzyme for colorimetric analysis of sugars, it was decided to make a ^{14}C-labeled enzyme for analysis of radioactive sugars. Thus, *A. niger* was grown in mineral salts for about 48 h, at which time 5 μCi of [U-^{14}C]fructose was added to each flask, and cells were allowed to grow for an additional 24 to 36 h. Cells were harvested by filtration and enzyme was purified as described for unlabeled enzyme.

Analysis of sugar composition. (i) Preparation of oligosaccharide and glycopeptide. Oligosaccharide was released from the glycoprotein by treatment with 1 N NaOH in the presence of 1 M $NaBH_4$ at 100°C for 6 h. In some cases this was done in the presence of [^3H]$NaBH_4$. Mild alkaline treatment (0.2 N NaOH at 37°C for 24 h) did not release the oligosaccharide chain. The liberated oligosaccharide was desalted on Sephadex G-10 and then purified on Sephadex G-25 and G-50. Glycopeptides were released from the glycoprotein by Pronase digestion as previously described (14, 15). Glycopeptides were purified on Sephadex G-25 and G-50 columns.

(ii) Identification of sugars. Oligosaccharides and glycopeptides were hydrolyzed in 4 N HCl at 100°C for 4 h. The hydrolysates were dried on a rotary evaporator, and HCl was removed by repeated addition and evaporation of 50% methanol. Neutral sugars were separated from amino sugars by passage through Dowex 50 H^+ columns. Neutral sugars were eluted with water, and amino sugars were eluted with 1 N HCl. Neutral sugars and amino sugars were then identified by paper chromatography.

RESULTS

Purification of α-galactosidase. Step 1. Ammonium sulfate precipitation. Solid $(NH_4)_2SO_4$, 16 kg, was added to 30 liters of mycelium-free medium with continuous stirring. After remaining overnight, the precipitate was isolated by filtration on a pad of Celite. The Celite pad was removed and suspended in water to dissolve the enzyme. Celite was then removed by filtration and the filtrate was used in further steps.

Step 2. Acetone precipitation. The filtrate from step 1 was cooled to 0°C and 3 volumes of acetone, previously cooled to −20°C, were added with stirring. The precipitate was quickly harvested by filtration, immediately dissolved in a minimum volume of water, and dialyzed overnight against water in double-walled dialysis tubing. The dialyzed protein was then lyophilized.

Step 3. Hydroxylapatite chromatography. A total of 200 mg of lyophilized enzyme from step 2 was dissolved in 20 ml of water and applied to a column (3.5 by 25 cm) of hydroxylapatite. The column was washed with 250 ml of water and then eluted with the following linear gradients of potassium phosphate buffer (pH 7.0): 0 to 0.1 M, 200 ml each; 0.1 to 0.25 M, 200 ml each; 0.25 to 0.6 M, 200 ml each. The α-galactosidase eluted in the 0.25 to 0.6 M gradient at about 0.3 to 0.5 M buffer. Active fractions were pooled, dialyzed against water, and lyophilized.

Step 4. DEAE-cellulose chromatography. The lyophilized material from step 3 was suspended in 20 ml of water and applied to a DEAE-cellulose column (3.5 by 25 cm) equilibrated with 0.01 M potassium phosphate buffer (pH 6.5). The column was washed with 250 ml of the above buffer followed by a linear gradient of potassium phosphate buffer (pH 6.5) from 0.01 to 0.2 M (250 ml each). The α-galactosidase

eluted at about 0.15 M potassium phosphate. Active fractions were pooled, dialyzed against water, and lyophilized.

Step 5. Sephadex G-200 chromatography. A 1- to 5-mg amount of enzyme from step 4 was dissolved in 2 to 3 ml of water and applied to a column (2.5 by 70 cm) of Sephadex G-200 equilibrated with 0.02 M acetate buffer (pH 5.0). The column was run with this buffer, and active fractions were pooled, dialyzed, and lyophilized.

By the use of these procedures, the α-galactosidase was purified approximately 85-fold, with a recovery of 25%. These data are shown in Table 1. As indicated below, the purified enzyme was homogeneous by disc gel electrophoresis and gave a single band that stained with either Coomassie blue or Schiff-periodate stains.

Properties of the α-galactosidase. (i) Purity and molecular weight of the α-galactosidase. The purified α-galactosidase was found to be homogeneous by disc gel electrophoresis (Fig. 1). Only one band was seen on these gels which stained with both Coomassie blue and Schiff-periodate stains, indicating that the α-galactosidase was a glycoprotein. When the enzyme was labeled with ^{14}C by growing the cells in [^{14}C]fructose, the radioactive peak coincided with the stained areas of the gel, again suggesting a homogeneous protein. Also when subjected to either sodium dodecyl sulfate-gel electrophoresis or Sephadex G-200 gel filtration, the α-galactosidase showed only one protein band (or peak).

The molecular weight of the α-galactosidase was estimated by gel filtration on Sephadex G-200 (Fig. 2). The enzyme eluted in the same area as ovalbumin, suggesting a molecular

TABLE 1. *Purification of α-galactosidase*[a]

Procedure	Total U	Protein[b] (mg)	Sp act (U/mg of protein)
Acetone precipitation	20	181.5	0.1
Hydroxyapatite column	8.3	3.5	2.3
DEAE-cellulose column	5	1.3	3.7
Gel filtration on G-200	4.8	0.5	9.6

[a] One unit is defined as 1 μmol of p-NO_2 phenol produced per min at 37°C. Values were based on 200 mg of acetone powder as the starting material.

[b] Protein determinations were done by the Lowry method. At each step of purification, the weight of the material obtained after lyophilization was 10 to 20 times greater than expected based on protein assays. This extraneous material was mostly eliminated after using DEAE-cellulose and finally upon gel filtration.

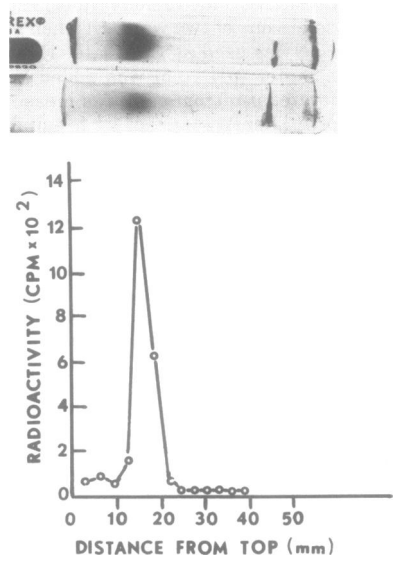

FIG. 1. *Polyacrylamide gel electrophoresis of the purified α-galactosidase. Gels were run as described and contained 50 μg of protein. The upper gel was stained with Coomassie blue, whereas the lower gel was stained with periodate-Schiff reagent. Radioactive enzyme was also run on gels which were cut into sections, solubilized in Protosol, and counted in toluene scintillator (bottom graph).*

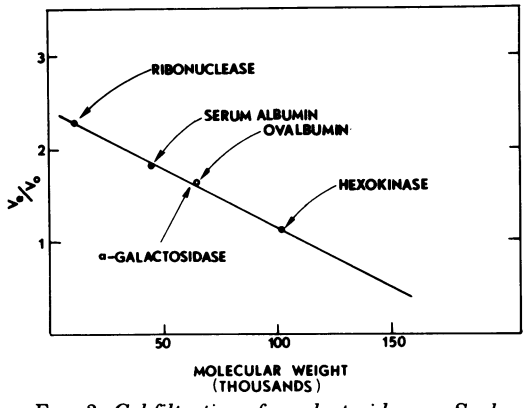

FIG. 2. *Gel filtration of α-galactosidase on Sephadex G-200. Various proteins of known molecular weights were used to calibrate a Sephadex G-200 column which was then used for the determination of the elution volume of α-galactosidase.*

weight of about 45,000. Although gel filtration has been questioned as a reliable method of molecular weight determination for glycoproteins, the fact that the α-galactosidase emerged in the same areas as ovalbumin, also a glycoprotein, suggests a similarity in molecular weights. Gel electrophoresis in sodium dodecyl sulfate also suggested a molecular weight of

about 40,000 and indicated that the α-galactosidase had only one polypeptide chain.

(ii) Effect of enzyme concentration, time, and pH on activity. As shown in Fig. 3, at protein concentrations of 2, 4, and 8 μg, the rate of hydrolysis of *p*-nitrophenyl-α-D-galactoside was proportional to protein concentration for at least 20 min. The effect of pH on catalytic activity was tested with glycine, acetate, citrate, phosphate, and tris(hydroxymethyl)aminomethane buffers of pH values ranging from 2 to 8.5. The pH activity profile shown in Fig. 4 indicated a pH optimum of about 4 to 4.5.

(iii) Substrate specificity and enzyme kinetics. The purified α-galactosidase showed good activity toward *p*-nitrophenyl-α-D-galactoside, but was inactive on *p*-nitrophenyl-β-D-galactoside, α- or β-D-glucoside, and α- or β-D-mannoside (Table 2). The enzyme was also tested on a number of galactose oligosaccharides, including melibiose (6-*o*-α-D-galactopyranosyl-D-glucose), melibiitol, raffinose [*o*-α-D-galactopyranosyl-(1 → 6)-*O*-α-D-glucopyranosyl-(1 → 2)-β-D-fructofuranoside], and stachyose [*o*-α-D-galactopyranosyl-(1 → 6)-raffinose]. The enzyme showed good activity towards raffinose, but had lower activity on stachyose and melibiitol, With each of these substrates, the release of galactose was confirmed by paper chromatographic identification of the sugar. No activity could be detected using melibiose as substrate, although this sugar was difficult to assay because of high reducing values in control tubes. The enzyme also catalyzed the release of galactose from the polysaccharides, guar flour, and locust bean gum.

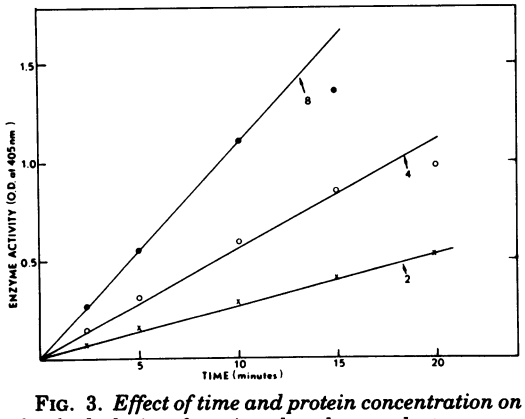

FIG. 3. *Effect of time and protein concentration on the hydrolysis of p-nitrophenyl-α-D-galactopyranoside by purified α-galactosidase. Incubations contained 2 (0.003 U), 4 (0.005 U), and 8 (0.01 U) μg of protein in assay mixtures as described in the text. At the times indicated, portions were removed and the amount of p-nitrophenyl liberated was measured.*

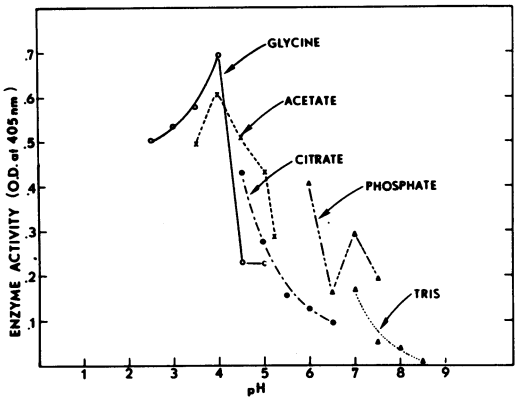

FIG. 4. *Effect of pH on α-galactosidase activity. Assays were as described in the text, except that various buffers were used (at a final concentration of 0.015 M) at the indicated pH. Activity was measured by release of p-nitro-phenol, using p-nitrophenyl α-D-galactoside as substrate. Incubations contained 4 (0.005 U) μg of protein and were incubated for 15 min.*

TABLE 2. *Substrate specificity of α-galactosidase*[a]

Substrate tested[b]	Galactose (nmol) released in (min):		
	15	30	60
p-Nitrophenyl-α-galactoside	25	75	125
Raffinose	75	135	200
Stachyose	15	40	65
Melibiitol[c]	20	45	75
Guar gum	40	55	115
Locust bean gum	60	70	115

[a] No activity on *p*-nitrophenyl glycosides of α- or β-glucose, α- or β-mannose, or β-galactose or on Karaya gum or Arabic gum.

[b] When *p*-nitrophenyl-α-galactoside, melibiitol, raffinose, or stachyose was used, incubations contained 2.5 μmol of substrate (7.5×10^{-3} M), whereas with guar gum and locust bean gum incubations contained 1 mg of substrate. In each case, controls were run without enzyme or with heat-killed enzyme. Assays were by the reducing sugar method.

[c] Melibiose was also tested as a substrate. However this substrate was difficult to assay by the reducing sugar method. No activity could be detected by using the reducing sugar method or glucose oxidase.

The effect of substrate concentration on activity was examined by using both *p*-nitrophenyl-α-D-galactoside (Fig. 5) and raffinose (Fig. 6) as substrates. Lineweaver-Burk plots of the data gave an apparent K_m of 5×10^{-4} M and a V_{max} of 3 μmol/min per mg of protein for raffinose and an apparent K_m of 1.8×10^{-4} M and a V_{max}

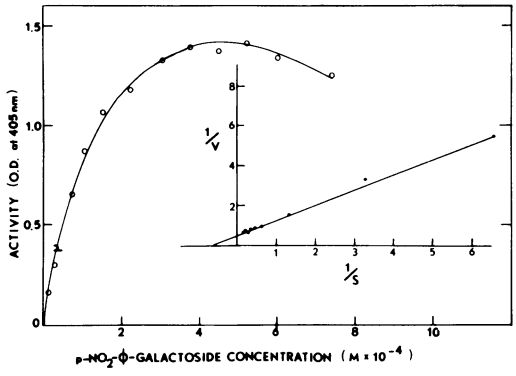

FIG. 5. *Effect of concentration of p-nitrophenyl-α-D-galactopyranoside on reaction velocity. Assays were as described in the text, except that the concentration of substrate was varied as indicated. Incubations were for 15 min with 8 (0.01 U) μg of protein.*

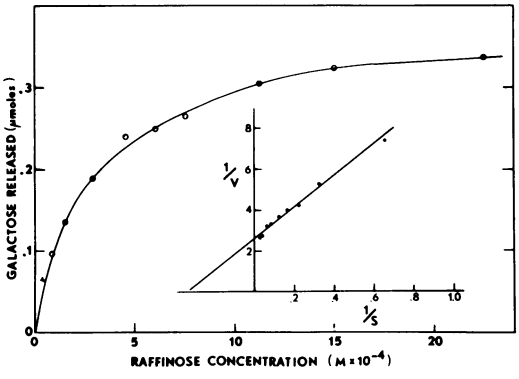

FIG. 6. *Effect of raffinose concentration on reaction velocity. The release of galactose was measured by the reducing sugar method. Assays were as described in the text, except that raffinose concentration was varied as indicated. Incubations were for 15 min with 8 (0.01 U) μg of protein.*

of 1.5 μmol/min per mg of protein for *p*-nitrophenyl-α-D-galactoside.

Carbohydrate composition of the α-galactosidase. (i) Isolation of glycopeptides and analysis of sugars. Preliminary studies on the α-galactosidase indicated that the enzyme contained the sugars mannose and GlcNAc. However, it was difficult to obtain sufficient amounts of enzyme for a complete analysis of these sugars. Therefore, radioactive enzyme was prepared by growing cells in [^{14}C]fructose, and this enzyme was purified to homogeneity as described. As shown in Fig. 1, the ^{14}C-labeled enzyme gave a single radioactive band upon gel electrophoresis that corresponded to the protein and carbohydrate staining areas of the gel. The radioactive enzyme was digested with Pronase, and the digestion mixture was passed through a column of Sephadex G-25 to isolate a crude glycopeptide fraction. The first peak of radioactivity emerged prior to the stachyose standard followed by a second radioactive peak near the glucose area of the column. The first radioactive peak was pooled, lyophilized, and rechromatographed on a Sephadex G-50 column (Fig. 7). A peak of radioactivity emerged from this column just after the glycopeptide prepared from ovalbumin and prior to the stachyose standard. This elution profile suggests an oligosaccharide of some six to eight sugar residues.

The glycopeptide from Sephadex G-50 was subjected to strong acid hydrolysis to liberate the sugars, and the hydrolysate was concentrated to dryness to remove HCl and then passed through a column of Dowex 50 H^+ to separate neutral sugars from amino sugars. The wash and eluate from the column were subjected to paper chromatography to isolate glucosamine and mannose. Based upon radioactivity and assuming that the sugars are equally labeled, the ratio of mannose to GlcNAc was about 2.8:1. The identity of these sugars as mannose and glucosamine was confirmed by further chromatography in several additional solvent systems.

(ii) Determination of the carbohydrate → peptide linkage. To determine the nature of the sugar → amino acid linkage, the α-galactosid-

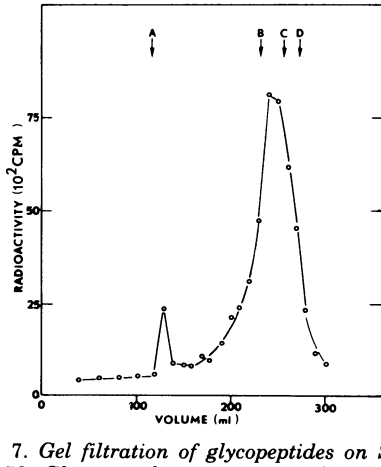

FIG. 7. *Gel filtration of glycopeptides on Sephadex G-50. Glycopeptides were prepared by Pronase digestion of radioactive α-galactosidase and partially separated on Sephadex G-25. The peak from G-25 was pooled and run on Sephadex G-50 in water. Fractions were monitored for radioactivity. The column was calibrated with the following standards: (A) blue dextran; (B) glycopeptide obtained by Pronase digestion of ovalbumin; (C) stachyose; (D) glucose.*

ase was subjected to mild and strong alkaline hydrolysis in the presence of $NaBH_4$. In each case, after acidification and lyophilization the mixture was passed through Sephadex G-50. After strong alkaline treatment a radioactive peak emerged from the G-50 column at approximately the same position observed for the glycopeptide released by Pronase digestion of the α-galactosidase (Fig. 7). This radioactive peak was followed by a second larger peak of ^3H emerging near the glucose area of the column. Complete acid hydrolysis of this oligosaccharide released all of the radioactivity as glucosaminitol, as evidenced by chromatography in several solvent systems. On the other hand, mild alkaline treatment of the α-galactosidase did not release the oligosaccharide chain since only a ^3H peak was observed on the G-50 column eluting in the glucose area. This is presumptive evidence that the oligosaccharide is attached to the protein by a GlcNAc → asparagine linkage.

The oligosaccharide released by alkaline treatment was subjected to strong acid hydrolysis, and the monosaccharides were reduced with $[^3H]NaBH_4$. Neutral sugars were separated from amino sugars on a Dowex 50 H^+ column, and the ^3H-labeled sugar alcohols were isolated by paper chromatography in solvent (i). In the neutral sugar fraction a peak of radioactivity was observed that corresponded to mannitol as well as a small peak of radioactivity at the origin and a rapidly migrating peak near the solvent front. In the amino sugar fraction most of the radioactivity corresponded to glucosaminitol, but again small peaks of radioactivity were observed at the origin and near the solvent front. The mannitol and glucosaminitol areas were eluted and rerun in other solvents (glucosaminitol in solvent [iii] and mannitol in solvent [iv]). In these solvents, the radioactive sugar alcohols migrated the same as the appropriate standards. The ratio of ^3H in mannitol as compared with glucosaminitol was about 3.8:1, suggesting seven to eight mannose residues and two GlcNAc residues.

DISCUSSION

The α-galactosidase (α-galactoside galactohydrolase [EC 3.2.1.22]) was purified to apparent homogeneity from the culture filtrate of *A. niger*. The enzyme gave a single band on polyacrylamide gel electrophoresis that stained with both protein (Coomassie blue) and carbohydrate (periodate-Fuchsin) stains, indicating that the α-galactosidase was a glycoprotein. Although we were not able to obtain sufficient amounts of enzyme for quantitative and qualitative identification of the carbohydrate, the sugars could be determined by the use of radioactive enzyme. After purification, the ^{14}C-labeled enzyme or glycopeptides prepared from this enzyme were subjected to complete acid hydrolysis, and the radioactive sugars were identified by paper chromatography. Two radioactive sugars, identified as mannose and glucosamine, were present and the ratio of radioactivity in these sugars was about 3:1. Since other glycosidases secreted by this fungus are also glycoproteins and contain an oligosaccharide with two GlcNAc residues linked in a chitobiose linkage (14, 15), it seems likely that the oligosaccharide of the α-galactosidase contains five or six mannose residues and two GlcNAc residues. This would be in accord with the gel filtration of the glycopeptide, which suggested a molecular weight of about 1,200. In addition, Rudick (Abstr. Am. Soc. Biol. Chem., San Francisco, 1975) has recently shown that α-glucosidase produced by this fungus is susceptible to cleavage by endo-β-glucosaminidase. This enzyme is known to cleave between two GlcNAc residues (18). Based on susceptibility to alkaline cleavage, the oligosaccharide of the α-galactosidase is probably attached to the protein by GlcNAc-asparagine linkage.

A number of glycoproteins have been characterized whose sugar composition is somewhat similar to the enzymes secreted by this fungus. Thus, thyroglobulin (1), ovalbumin (7, 19), *A. oxyzae* α-amylase (4, 20), and ribonuclease B (18) contain oligosaccharides composed of mannose and GlcNAc, and these proteins appear to have $(man)_n$-$(GlcNAc)_2$-Asn linkages. Several excellent reviews are available covering glycoenzymes (12) and glycoproteins having GlcNAc-asparagine linkages (6). Although the α-galactosidase has not been shown to be susceptible to the endo-β-*N*-acetylglucosaminidase, its similarity to the β-glucosidase and α-glucosidase suggests that it also has a $(man)_n$-$(GlcNAc)_2$-Asn linkage.

The α-galactosidase showed good activity on raffinose [*O*-α-D-galactopyranosyl-(1 → 6)-*O*-α-D-glucopyranosyl(1 → 2)-β-D-fructofuranoside] and *p*-nitrophenyl-α-D-galactopyranoside, but much lower activity on stachyose [*O*-α-D-galactopyranoside-(1 → 6)-raffinose] and melibiitol [6-*O*-α-galactopyranosyl-D-glucitol]. However, no activity could be detected toward melibiose. This substrate was difficult to assay by the reducing sugar method because of high blank values in control incubations. However, by using either this method or glucose oxidase, no activity was observed. The enzyme did release galactose from the polysaccharides of guar flour

and locust bean gum, both of which have been used as carbon sources for the growth of this fungus.

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