Glycosidases of *Aspergillus niger*

II. PURIFICATION AND GENERAL PROPERTIES OF 1,2-α-L-FUCOSIDASE*

OM P. BAHL

From the Department of Biochemistry, Faculty of Health Sciences, State University of New York at Buffalo, Buffalo, New York 14214

(Received for publication, September 8, 1969)

SUMMARY

A highly purified preparation of 1,2-α-L-fucosidase, free of β-galactosidase and β-N-acetylglucosaminidase activities, has been obtained from a commercial preparation of *Aspergillus niger* by a simple isolation procedure involving ammonium sulfate precipitation, pressure dialysis, repeated gel filtration on Sephadex G-150, and chromatography on diethylaminoethyl Sephadex A-50. The enzyme has a pH optimum of 3.8 ± 0.2 and \( K_m \) and \( V_{max} \) values of 8.3 × 10⁻⁵ M and 16.0 μmoles per mg per hour, respectively, at 37° for methyl 2-O-α-L-fucopyranosyl-β-D-galactoside as substrate.

The detailed specificity studies indicate that it is highly specific for nonreducing terminal α-fucose residues linked to D-galactose residues by 1 → 2-α linkage. It hydrolyzes fucose residues quantitatively from 2-O-α-L-fucopyranosyl-β-D-galactose, 2-O-α-L-fucosyllactose, and lacto-N-fucopentaose I. It does not split β-nitrophenyl-α-L-fucoside, 2-O-, 3-O-, and 4-O-α-L-fucopyranosylfucoses, and 3-O- and 4-O-α-L-fucopyranosyl-β-D-galactoses. It also does not release any fucose from oligosaccharides of human milk such as lacto-N-fucopentaose II, lacto-N-fucopentaose III, and 3'-O-α-L-fucosylactose. The enzyme liberates 80 to 90% of fucose residues from porcine and canine submaxillary mucus. It has no action on orosomucoid, human chorionic gonadotropin, and fucan sulfate. The enzyme is extremely active against human blood group substance H, destroying virtually all of the detectable activity.

L-Fucose is frequently located on the nonreducing termini of the carbohydrate moiety of several biologically active molecules. These include serum glycoproteins, immunoglobulins, gastric and submaxillary mucus, gonadotropic hormones, and blood group substances (1). The serological activity of certain blood group substances is associated with L-fucose residues (2). It is also present in oligosaccharides of human milk and of bovine colostrum (3), marine algae polysaccharides such as fucan sulfate, and plant gums (4).

L-Fucosidases from various sources have been found to release fucose from these molecules by splitting terminal α-L-fucosidic bonds. Levy and McAllan (5) have studied the distribution of α-L-fucosidase in rat and mouse tissues. The epididymis and kidney showed the highest activity of the enzyme toward p-nitrophenyl-α-L-fucopyranoside. A cell-free extract of *Trichomonas fetus* was found to release fucose from blood group substances but failed to hydrolyze p-nitrophenyl and methyl α-L-fucosides (6). Obviously, the mammalian and bacterial enzymes possess different substrate specificity. No extensive purification and characterization of the enzymes from any of the above sources has been carried out. Recently, Aminoff (7) has reported the purification of α-L-fucosidase from *Clostridium perfringens* which exhibited substrate specificity similar to that of *T. fetus* enzyme. More recently, Tanaka et al. (8) have partially purified two α-L-fucosidases from abalone livers which hydrolyzed p-nitrophenyl α-L-fucoside, but only one of them liberated fucose from porcine submaxillary mucin. We have previously reported (14) the purification and characterization of β-acetylglucosaminidase and α- and β-galactosidases from a commercial enzyme product of *Aspergillus niger*. This communication describes the procedure for the purification of a highly specific enzyme, 1,2-α-L-fucosidase, and its kinetic and detailed substrate specificity properties, including its action on p-nitrophenyl α- and β-L-fucopyranosides, various fucose-containing oligosaccharides, porcine and canine submaxillary mucins, orosomucoid, human chorionic gonadotropin, and finally on fucan sulfate.

MATERIALS AND METHODS

A commercial enzyme product from *A. niger*, Rhozyme HP-150 (without diluent), was obtained from Rohm and Haas Company, Philadelphia, Pennsylvania. p-Nitrophenyl α- and β-L-fucopyranosides, other p-nitrophenyl glycosides, and fucan sulfate were purchased from Pierce Chemical Co., Rockford, Illinois. The disaccharides, 2-O-, 3-O-, and 4-O-α-L-fucopyranosyl-β-N-fucopyranosides were prepared essentially by Côté's method (9), except that the final purification of the disaccharides was carried out on a charcoal column using stepwise elution with ethanol-water mixtures ranging in concentration from 0 to 10% alcohol. Porcine submaxillary mucin (10), orosomucoid (11), and human chorionic gonadotropin (12) were prepared according to the published procedures. Canine submaxillary mucin and 2-O-α-L-fucopyranosylactose were generous gifts of Drs. R. J. Winzler and J. P. Bahl.

1 The term "fucose" refers to α-L-fucose throughout.
and Saul Roseman, respectively. The oligosaccharides 3'-O-α-L-fucopyranosylactose, lacto-N-fucopentaose I, lacto-N-fucopentaose II, and lacto-N-fucopentaose III were kindly provided by Dr. V. Ginsburg.

The syntheses of 2-O-α, 3-O-β, 4-O-α, and 6-O-β-L-fucopyranosyl-N-galactoses or their corresponding methyl glycosides were achieved by the condensation of tri-O-acetyl-L-fucopyranosyl chloride with appropriately blocked D-galactose or methyl D-galactopyranoside in acetonitrile in the presence of mercuric cyanide and mercuric bromide as catalysts (13). The anomeric linkage was established by optical rotation, by nuclear magnetic resonance, and enzymatically. The details of the syntheses will be described elsewhere.²

**Gas Chromatographic Determination of Fucose**

Fucose liberated during the enzymatic hydrolysis of various substrates was quantitatively determined by gas-liquid chromatography. The enzymatic digest was deionized and freed of the unreacted substrate and protein by passing through a column of charcoal (1 × 0.25 cm) and a mixed bed resin, MB-3 (1 × 0.5 cm). The column was eluted with 8 to 10 ml of 10% ethanol (14). The eluate was dried in a Vortex Biodryer (The Virtis Company, Inc., Gardiner, New York), and fucose in the residue was determined as its trimethylsilyl ether derivative by gas-liquid chromatography (15). A 25-μl portion of the silylating agent (prepared by mixing 1 ml of pyridine, 0.2 ml of hexamethyldisilazane, and 0.1 ml of trimethylchlorosilane) was added to the residue, and the reaction mixture was stirred by a Vortex mixer for 10 min. The solution was evaporated to dryness at room temperature under reduced pressure, the residue was dissolved in 25 μl of cyclohexane, and a 1 μl aliquot was injected into a Varian Aerograph model 1500 gas chromatograph, equipped with flame ionization detector. A 5% coating of SE-52 on hexamethyldisilazane-treated Chromosorb (60 to 80 mesh) was employed for column packing (1/8 inch × 10 feet). The column was programmed isothermally at 175°C. The retention times for α- and β-peaks of fucose were 3.2 and 3.7 min. The use of cyclohexane eliminates the problem of tailing, as encountered with pyridine, and consequently reduces drastically the time of each run.

The coconut charcoal (Fisher Product 50-250 mesh), used above in the procedure for the gas chromatographic analysis of fucose, was treated as follows. After the removal of the fine particles by screening through a 100-mesh sieve, the charcoal was boiled with 6 N HCl for 1 hour and washed with water. It was then boiled with 1 N NaOH for 1 hour, neutralized with dilute HCl, and finally washed with water, ethanol, and acetone.

**Assay for 1,2-α-L-Fucosidase**

The assay for 1,2-α-L-fucosidase was based on the estimation of the release of fucose from either methyl 2-O-α-L-fucopyranosyl-β-D-galactopyranoside or 2-O-α-L-fucopyranosyl β-D-galactose by gas-liquid chromatography. To a 100-μl sample of a 5.5 mm solution of the substrate in 0.01 M sodium acetate buffer, pH 4.0, 10 μl of the enzyme solution were added. After incubation of the reaction mixture for 1 hour at 37°C, the fucose released was determined as described above. During the purification procedure, the assay of the enzyme in the fractions from the columns was slightly modified by using 200 μl of 1 mm solution in 0.25 N sodium acetate buffer, pH 4.0, and 10 to 25 μl of the enzyme solution.

One unit of the enzyme was defined as the amount which would liberate 1 μmole of fucose per hour at 37°C. Specific activity was expressed as units per mg of protein.

**Assay of Other Glycosidases and Proteases**

The enzymes β-galactosidase and β-acetylglucosaminidase were assayed as previously described, using appropriate p-nitrophenyl glycosides as substrates (16).

Protease activity was ascertained by using Azocoll as a substrate.² A suspension of 0.25 mg of Azocoll in 1.2 ml of 0.1 M sodium phosphate buffer, pH 7.5, was incubated with 50 μl of the enzyme (75 μg) at 37°C for 2 to 12 hours. A blank of the substrate was run concurrently under identical conditions without the enzyme.

**Purification of 1,2-α-L-Fucosidase**

All steps of purification were carried out at 4°C unless otherwise specified. Fractions from the column were monitored for protein by measuring absorbance at 280 nm (17). For the specific activity measurements, the protein was determined by the method of Lowry et al. (18). The enzyme solution at each stage of purification was concentrated by pressure dialysis using XM-50 membrane (Amicon Corporation, Cambridge, Massachusetts) as previously described (14). Since the commercial enzyme product contained large amounts of β-glucoosidase or cellulase, prolonged dialysis of the enzyme solutions in cellophane tubing was avoided because of the hazard of rupturing the dialysis tubing.

**Step 1: Extraction and Ammonium Sulfate Precipitation**—A sample of 250 g of the commercial enzyme product (Rhozyme HP-150) was extracted with 2 liters of cold 0.1 M NaCl solution over a period of 2 hours. The extract was centrifuged for 1 hour at 16,500 rpm in a Servall refrigerated centrifuge, and the residue was further extracted twice with 1 liter of 0.1 M NaCl solution each time. The combined supernatant solution (4 liters, 72 g of protein) was treated slowly by stirring with 2,808 g of ammonium sulfate to bring about 100% saturation. After allowing the precipitate to settle overnight, the solution was centrifuged again at 16,500 rpm. The precipitate was collected in 1 liter of water, and the resulting solution was further subjected to another ammonium sulfate treatment (702 g). Finally, the precipitate was dissolved in 1,000 ml of 0.04 M sodium phosphate buffer, pH 6.8, and concentrated to 400 ml by pressure dialysis. Subsequently, two additions of the buffer were made, and the solution was concentrated to a final volume of about 400 ml.

**Step 2: Column Chromatography on DEAE-Sephadex**—A column (10 x 110 cm) was packed with DEAE-Sephadex A-50 in 0.04 M phosphate buffer, pH 6.8. After equilibration of the column with the same buffer, 400 ml of the solution (17.0 g of protein) from the preceding step were applied to the column. The column was eluted with a stepwise salt gradient from 0 to 1 M NaCl in 0.04 M sodium phosphate buffer, pH 6.8. The buffer changes are indicated in Fig. 1. Fractions of 22 ml were collected. Fractions 350 to 450, containing the enzyme, were pooled and concentrated by pressure dialysis to 40 ml. To the

² Used according to manufacturer’s directions.

Vol. 245, No. 2
concentrated solution, 800 ml of 0.25 m sodium acetate buffer were added, and the solution was concentrated to 40 ml again.

**Step 3: Gel Filtration on Sephadex G-150**—The above solution (3.4 g of protein) was applied to a column of Sephadex G-150 (5 x 110 cm) packed in 0.25 m sodium acetate buffer, pH 4.6, as described previously (14). The column was eluted with the same buffer. Fractions of 4.8 ml were collected.

**Step 4: DEAE-Sephadex A-50**—A column was packed with DEAE-Sephadex in 0.01 m sodium phosphate buffer, pH 6.55. A 2-ml sample (100 mg of protein) was applied to a column (2.5 x 40 cm), and the column was eluted with a continuous salt gradient between 200 ml of 0.04 m sodium phosphate buffer, pH 7.2, and 200 ml of 1 m NaCl in the same buffer. Fractions of 4.5 ml were collected. Fractions 67 to 75, containing the enzyme 1,2-α-L-fucosidase, were pooled and concentrated by pressure dialysis to 4 ml. The enzyme solution was diluted with 25 ml of 0.25 m sodium acetate, pH 4.6, and concentrated to 4 ml again.

**Gel Filtration on Sephadex G-150**—A 4-ml sample (70 mg of protein) from the preceding step was applied to a Sephadex G-150 column (2 x 150 cm). A head pressure of 40 cm was used, and the column was developed as described above.

**Substrate Specificity Studies**

**Action of 1,2-α-L-Fucosidase on Oligosaccharides**—A 100 μl solution of the oligosaccharide, containing 50 to 100 μg of the material and 10 to 20 μl of the enzyme solution (25 to 50 μg of protein), was added, and the reaction mixture was incubated at 37° for 1 to 6 hours. The digest was deionized and freed of the oligosaccharide and protein by passing through a bed of charcoal and mixed bed resin (MB-3) as described above. The eluate was evaporated to dryness on a rotary evaporator, and the resulting residue was estimated for fucose by gas-liquid chromatography.

**Action of 1,2-α-L-Fucosidase on Porcine and Canine Submaxillary Mucin, Orosomucoid, and Human Chorionic Gonadotropin**—A sample of 250 to 300 μl of an aqueous solution containing 0.5 to 3.5 mg of the material was treated with 50 to 250 μl of the enzyme solution (0.1 to 0.5 mg) in 0.05 m sodium acetate buffer, pH 4.0. After the addition of 20 μl of toluene, the digest was incubated from 4 to 48 hours. Aliquots of 50 to 100 μl were withdrawn at regular time intervals and applied to a column (1 x 0.5 cm) of charcoal and mixed bed resin (MB-3). The column was washed with 5 to 10 ml of 5% ethanol. The washings were evaporated to dryness, and the fucose in the residue was estimated by gas-liquid chromatography.

**RESULTS**

**Purification of 1,2-α-L-Fucosidase**—The purification scheme described above yielded a preparation which was almost free of β-acetylglucosaminidase, β-galactosidase, and protease activities. The scheme was based on precipitation with ammonium sulfate, pressure dialysis, and repeated chromatography on DEAE-Sephadex A-50 and Sephadex G-150. A 250-g sample of the commercial enzyme product was employed in the preparation. After three extractions of the sample with a total of 4.0 liters of 0.1 m NaCl solution, the combined extracts were subjected to precipitation twice with ammonium sulfate at 100% saturation. During the ammonium sulfate precipitation step, not only did enrichment of the enzyme occur, but also the polysaccharide contaminants were eliminated (19). The recovery of the enzyme units after the precipitation step was higher than that in the extract. This discrepancy was probably due to inaccuracy in the fucose determination by gas-liquid chromatography, which may be due to the presence of carbohydrate contaminants in the crude extract which interfered with the fucose peaks in the gas chromatogram. However, the possibility of the presence of an enzyme inhibitor in the crude material can not be ruled out. The partially purified enzyme was applied to a DEAE-Sephadex A-50 column which was developed by a linear stepwise salt gradient. The enzyme appeared in the first peak shown in Fig. 1, along with β-galactosidase and β-acetylglucosaminidase. The fractions containing the enzyme were pooled, concentrated by pressure dialysis, and further purified by gel filtration on Sephadex G-150, which effected a partial separation of 1,2-α-L-fucosidase from β-acetylglucosaminidase and β-galactosidase (Fig. 2). The resulting enzyme fraction...
FRACTION NO.

FIG. 3. Chromatography on DEAE-Sephadex A-50 of enzyme fraction from Senhadex G-150. A 2-ml sample (100 mg of protein) was applied to a DEAE-Sephadex column (2.5 × 40 cm) equilibrated with 0.04 M sodium phosphate buffer, pH 6.5. The elution was carried with 300 ml of a continuous linear salt gradient between 0.04 M phosphate, pH 6.5, and 1 M sodium chloride. Fraction size was 4.5 ml. O—O, 280-mA absorbance; A—A, β-acetylglucosaminidase; m—m, β-galactosidase; O—O, 1,2-α-L-fucosidase; I, fractions pooled.

was subjected to chromatography on DEAE-Sephadex A-50, using continuous linear salt gradient (Fig. 3) instead of the step-wise gradient used above, and finally to the gel filtration on Sephadex G-150 (Fig. 4). Fractions pooled in each case are represented in Figs. 3 and 4. A summary of the purification is given in Table I.

FIG. 4. Chromatography on Sephadex G-150. A 4-ml sample (70 mg of protein) in 0.25 M acetate buffer, pH 4.6, was chromatographed on Sephadex G-150. The elution was carried with 0.2 M acetate buffer, pH 4.6, maintaining a hydrostatic head of 40 cm. Fractions of 2.7 ml were collected. O—O, 280-mA absorbance; A—A, β-acetylglucosaminidase; m—m, β-galactosidase; O—O, 1,2-α-L-fucosidase; I, fractions pooled.

Table I

<table>
<thead>
<tr>
<th>Purification procedure</th>
<th>Total protein</th>
<th>Enzyme</th>
<th>Specific activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg</td>
<td>units × 10²</td>
<td>units/mg × 10²</td>
</tr>
<tr>
<td>1. Crude enzyme</td>
<td>72,000</td>
<td>50,400</td>
<td>0.7</td>
</tr>
<tr>
<td>2. Ammonium sulfate precipitation</td>
<td>3,400</td>
<td>143,400</td>
<td>42.2</td>
</tr>
<tr>
<td>3. DEAE-Sephadex A-50..</td>
<td>570</td>
<td>62,400</td>
<td>109.7</td>
</tr>
<tr>
<td>4. Sephadex G-150..</td>
<td>250</td>
<td>55,500</td>
<td>222.0</td>
</tr>
<tr>
<td>5. DEAE-Sephadex A-50..</td>
<td>124</td>
<td>50,000</td>
<td>403.2</td>
</tr>
</tbody>
</table>

* Specific activity was defined as micromoles of fucose released from methyl 2-O-α-L-fucopyranosyl-β-D-galactopyranoside per hour per mg of the enzyme at 37°.

* See text for explanation.

pH Optimum of the Enzyme—The effect of pH on the catalytic activity of the enzyme was studied by using methyl 2-O-α-L-fucopyranosyl-β-D-galactopyranoside in 0.01 M citrate-phosphate buffers ranging from pH 2.4 to 8.0. The pH activity profile
FIG. 7. Kinetics of release of fucose from 2-O-α-L-fucosyllactose (●) and from desialylated porcine (△) and desialylated canine (○) submaxillary mucins by A. niger 1,2-α-L-fucosidase. Samples of 3.75 mg of desialyzed submaxillary mucins in 500 μL of 0.01 M acetate buffer, pH 4.0, were incubated at 37°C with 250 μL of the enzyme (0.5 μg protein) in the same buffer. Aliquots of 100 μL at different time intervals were analyzed for fucose by gas-liquid chromatography. In the case of 2-O-α-L-fucosyllactose, 830 μg of the material in 450 μL of the buffer and 50 μL of the enzyme solution (50 μg protein) were used. Aliquots of 50 μL at different time intervals were analyzed for fucose by gas-liquid chromatography.

The enzyme A. niger 1,2-α-L-fucosidase hydrolyzed 80 to 90% of fucose residues from the intact as well as desialylated canine and porcine submaxillary mucins (Fig. 7), indicating that α-L-fucose is located at the nonreducing termini of the oligosaccharide chains in the mucins. Furthermore, the α-L-fucose residues are linked by 1→2 type linkage to β-D-galactose in the carbohydrate chains of both porcine and canine submaxillary mucins. Recent chemical studies based on methylation and periodate oxidation of the oligosaccharides derived from porcine submaxillary mucin have shown (20, 21) that fucose is indeed terminal and is linked to the β-D-galactose residue in the oligosaccharide chains by 1→2 type linkage. Little is known about the structure of the carbohydrate chains of canine submaxillary mucin. However, the.

### Table II

**Table II**

<table>
<thead>
<tr>
<th>Substrates tested</th>
<th>Hydrolysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. 2-O-α-L-Fucopyranosyl-β-D-galactose</td>
<td>100%</td>
</tr>
<tr>
<td>2. Methyl 2-O-α-fucopyranosyl-β-D-galactoside</td>
<td>None</td>
</tr>
<tr>
<td>3. 2-O-α-L-Fucopyranosylactose</td>
<td>None</td>
</tr>
<tr>
<td>4. Lacto-N-fucopentaose I</td>
<td>None</td>
</tr>
<tr>
<td>5. Porcine submaxillary mucin</td>
<td>80-90%</td>
</tr>
<tr>
<td>6. Canine submaxillary mucin</td>
<td>80-90%</td>
</tr>
<tr>
<td>7. Human chorionic gonadotropin</td>
<td>None</td>
</tr>
<tr>
<td>8. Orosomucoid</td>
<td>None</td>
</tr>
<tr>
<td>9. Fucan sulfate</td>
<td>None</td>
</tr>
<tr>
<td>10. 3'-O-α-L-Fucopyranosylactose</td>
<td>None</td>
</tr>
<tr>
<td>11. Lacto-N-fucopentaose II</td>
<td>None</td>
</tr>
<tr>
<td>12. Lacto-N-fucopentaose III</td>
<td>None</td>
</tr>
<tr>
<td>13. 2-O-α-L-Fucopyranosyl-β-D-galactose</td>
<td>None</td>
</tr>
<tr>
<td>14. 3-O-α-L-Fucopyranosyl-β-D-galactose</td>
<td>None</td>
</tr>
<tr>
<td>15. Methyl 3-O-α-L-fucopyranosyl-β-D-galactoside</td>
<td>None</td>
</tr>
<tr>
<td>16. Methyl 4-O-α-L-fucopyranosyl-β-D-galactoside</td>
<td>None</td>
</tr>
<tr>
<td>17. Methyl 3'-O-α-L-fucopyranosyl-β-D-galactoside</td>
<td>None</td>
</tr>
<tr>
<td>18. 3-O-β-L-Fucopyranosyl-β-D-galactose</td>
<td>None</td>
</tr>
<tr>
<td>19. 4-O-α-L-Fucopyranosyl-β-D-galactose</td>
<td>None</td>
</tr>
<tr>
<td>20. 6-O-β-L-Fucopyranosyl-β-D-galactose</td>
<td>None</td>
</tr>
</tbody>
</table>

* See text for the conditions used for enzymatic hydrolyses.

α-Fucose content of the porcine and canine submaxillary mucins employed in these studies was 8.9 and 9.5%, respectively.

* Personal communication from Dr. Arthur P. Grollman, Albert Einstein College of Medicine, Bronx, New York.
release of fucose from this mucin by 1,2-α-L-fucosidase indicates clearly the position and the linkage of the fucose residues in the mucin. Since the enzyme did not cause the hydrolysis of fucose residues in human chorionic gonadotropin and orosomucoid, this may indicate that fucose residues in these glycoproteins have a different linkage than that in porcine or canine submaxillary mucin. Structure of a glycopeptide from orosomucoid, proposed by Yosizawa and his co-workers recently (22), indicates the presence of 1,3-α-L-fucosidic linkage. Another fraction obtained in the course of the present studies contained a fucosidase which was found to release fucose from human chorionic gonadotropin and orosomucoid; this fraction showed no activity toward porcine or canine submaxillary mucins and, therefore, appears to be a different enzyme, presumably specific for a different linkage. This fraction also did not hydrolyze p-nitrophenyl-α-L-fucopyranoside, α-D-galactosidase (19, 27, 28), α-D-mannosidase (19, 28, 29), and niger 1,2-ac-L-fucosidase did not hydrolyze fucan sulfate. A summary of the substrate specificity studies is given in Table II.

**Discussion**

Recently, a great deal of interest has been aroused in glycosidases which are capable of hydrolyzing specific sugar residues from the nonreducing terminal of the carbohydrate chains. Several such enzymes as β-N-acetylglucosaminidase (14, 16, 23–26), β-D-galactosidase (19, 27, 28), α-D-mannosidase (19, 28, 29), and α-L-fucosidase (5, 8) have been highly purified and characterized. These enzymes require for their action a specific nonreducing sugar residue and a specific anomic configuration of the glycosidic bond, regardless of the nature of the aglycon. The enzyme 1,2-α-L-fucosidase, on the other hand, shows a much higher degree of specificity than the enzymes mentioned above. It requires a specific linkage of 1–2 type between the nonreducing terminal fucose residue and the adjacent α-D-galactose residue in addition to the α anomic configuration of the glycosidic linkage. Consequently, a glycopeptide, a glycoprotein, or a polysaccharide which is susceptible to the action of this enzyme most likely contains a nonreducing terminal fucose residue, linked by 1–2-α type linkage to the α-D-galactose residue in the carbohydrate chain. Such an inference would be strongly supported by the specificity properties of the enzyme summarized in Table II. Obviously, the enzyme offers a great potential in the study of fucose-containing macromolecules of the type described above.

It is quite interesting to note that the enzyme does not hydrolyze at all, even on prolonged incubation, 3-0-β, 4-0-α, and 6-0-β-L-fucopyranosyl galactoses, as well as any of the fucosyl fucoses, suggesting that 1,2-α linkage as well as both mono- saccharide units are essential for enzyme activity. Furthermore, since the configuration of the 1,2-α linked disaccharide is quite different from that of the other fucosyl galactoses having 1-3-β, 1-4-α, and 1-6-β-L-linkages, it is conceivable that probably a particular conformation is necessary for the binding of the substrate to the enzyme. The studies to delineate the hydroxyl groups which are involved in the binding and those might also be involved in the catalytic activity of enzyme are currently under investigation.

The L-fucosidases (exo), on the basis of the available information on specificity, can be broadly classified into two groups: those which are specific for the L-fucopyranosyl group and the anomic configuration of the fucosidic linkage, and those which require, in addition, the presence of a specific linkage of fucose to the next sugar residue in the carbohydrate chain. The former class of fucosidases hydrolyze alkyl or aryl fucosides and may also liberate fucose from the nonreducing ends of the oligo- or polysaccharide chains, regardless of the intersugar linkage. The mammalian fucosidases reported by Levvy and McAllan (5) and those reported by Tanaka et al. (8) belong to this class. The latter class of fucosidases will not hydrolyze alkyl or aryl fucosides, but will hydrolyze only specific fucosidic linkages in oligo- and polysaccharides. Bacterial fucosidases reported by Watkins (6) and thus reported by Aminoff (7) show this type of specificity, although, hitherto, complete characterization of the enzymes has not been reported. The A. niger enzyme, being highly specific for 1,2-α-L type linkages, falls in the second category of L-fucosidases.

**Acknowledgments**—I wish to express my gratitude to Drs. R. J. Winzler, W. Pigman, S. Roseman, and V. Ginsburg for generous gifts of canine and porcine submaxillary mucins, 2-O-α-L-fucosylactose and oligosaccharides of human milk, respectively, and to Dr. R. H. Côté and N. Sharon for their gifts of 4-O- and 2-O-α-L-fucosyl-L-fucose, respectively. Technical assistance of Mrs. Patricia Silvernail in part of the work is acknowledged.

**REFERENCES**


Glycosidases of *Aspergillus niger*: II. PURIFICATION AND GENERAL PROPERTIES OF 1,2-\(\alpha\)-L-FUCOSIDASE

Om P. Bahl


Access the most updated version of this article at [http://www.jbc.org/content/245/2/299](http://www.jbc.org/content/245/2/299)

Alerts:
- When this article is cited
- When a correction for this article is posted

[Click here](http://www.jbc.org/content/245/2/299.full.html#ref-list-1) to choose from all of JBC’s e-mail alerts

This article cites 0 references, 0 of which can be accessed free at [http://www.jbc.org/content/245/2/299.full.html#ref-list-1](http://www.jbc.org/content/245/2/299.full.html#ref-list-1)