Purification of an Almond Emulsin Fucosidase on Cibacron Blue-Sepharose and Demonstration of Its Activity toward Fucose-containing Glycoproteins*

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The almond emulsin fucosidase that specifically hydrolyzes fucose in α(1-3) linkage to N-acetylglucosamine has been purified 1250-fold. The purification procedure includes ion exchange chromatography on sulfopropyl-Sephadex C-25, gel filtration on Sephacryl S-200, and affinity chromatography on Cibacron blue-Sepharose 4B-CL. The molecular weight of the fucosidase was estimated by gel filtration as approximately 73,000. Enzyme activity was maximal at pH 5.3 in acetate buffer and was dependent on ionic strength; at least 0.1 M NaCl was necessary for optimal activity.

The purified enzyme was free of β-galactosidase activity toward the glycoprotein substrate [3H]galactosyl-asialotransferrin and did not release fucose from substrates containing fucose in α(1-6) linkage, (bovine IgG glycopeptides) or in α(1-2) linkage, (2'-fucosyllactose). The fucosidase displayed activity toward two glycoprotein substrates known to contain fucose in α(1-3) linkage. Extensive incubations resulted in the release of 83% and 43% of the total fucose of asialoorosomucoid and lactoferin, respectively. The fucosidase did not release fucose from either the "slow" or the "fast" form of α2-macroglobulin, suggesting the absence of fucosyl α(1-3) linkages on that glycoprotein.

Fucosidases have been isolated from a number of prokaryotic and eukaryotic sources (1-6). Many of these enzymes display broad specificity or activity directed only to artificial substrates and this has limited their usefulness as structural probes for complex glycoconjugates. Kobata has recently described two fucosidase activities in almond emulsin (7, 8).

The fucosidase was estimated to be 98-fold purified and requires the complex milk oligosaccharide lacto-N-fucopentaose described two fucosidase activities in almond emulsin (7, 8). Type I is specific for Fuc α(1-3)GlcNAc and Fuc α(1-4)GlcNAc linkages, and Type II is specific for Fuc α(1-2)Gal linkages present in milk oligosaccharides. The Type I activity has been useful in a number of carbohydrate structural studies (9-12). However, the procedure used to purify the fucosidase achieved only a 98-fold purification and requires the complex milk oligosaccharide lacto-N-fucopentaose II coupled to Sepharose as an affinity medium (8).

This publication describes a rapid procedure for purifying this fucosidase 1250-fold which employs the readily available affinity medium Cibacron blue F3GA-Sepharose in the final step of the purification. Optimal conditions for enzyme activity are presented and its activity is demonstrated toward glycoprotein substrates containing Fuc α(1-3)GlcNAc linkages.

EXPERIMENTAL PROCEDURES

Materials

Almond emulsin, bovine serum albumin, transferrin, cytochrome c, myoglobin, carbonic anhydrase, immunoglobulin G, and p-nitrophenyl-glycosides were obtained from Sigma. Cibacron blue F3GA from Polysciences, Inc., Warrington, PA; GDP-['4C]fucose and NaB[3H]2H, from New England Nuclear; Sephadex and Sephadex chromatographic media from Pharmacia, Piscataway, NJ; and Dowex AG 50W-X8 resin, 100-200 mesh, from Bio-Rad. Human lactoferrin and the α(1-3) fucosyltransferase from human milk were gifts of Dr. J.-P. Prieels, Université Libre de Bruxelles, Belgium; orosomucoid was a gift of the American National Red Cross; and 2'-fucosyllactose was a gift of Dr. V. Ginsberg, National Institutes of Health. All other reagents were the highest grade commercially available.

Preparation of Asialoorosomucoid

ASOR was prepared from orosomucoid as previously described (13).

Preparation of [3H]Galactosyl-asialotransferrin

Asialotransferrin was prepared from human transferrin as previously described (13). Tritiation of terminal galactosyl residues was performed according to Morell and Ashwell (14).

Preparation of Bovine IgG Glycopeptides

Glycopeptides were prepared from 10 g of bovine IgG by pronase digestion (15) and partial purification on Sephadex G-25 SF (3.5 x 150 cm) and Sephadex G-50 (2 x 117 cm) equilibrated in 0.1 M NH4HCO3.

Preparation of the Substrate of [3C]Fucosyl-ASOR

The radiolabeled substrate [3C]fucosyl-ASOR was prepared by reacting GDP-[3C]fucose and ASOR with a purified α(1-3) fucosyltransferase from human milk (16). This transferase adds fucose in α(1-3) linkage with penultimate N-acetylglucosamine residues of desialated Asn-linked oligosaccharide chains (18). 3.2 μCi of GDP-[3C]fucose was added to each reaction mixture containing 10 μg of ASOR and 4 μg of α(1-3) fucosyltransferase in 100 μl of 20 mM Tris-HCl, pH 7.5. The solution was incubated for 1 h at 37°C. The reaction mixture was chromatographed on a Sephadex G-50 column; human albumin blue F3GA equilibrated in 150 μl of NaCl. Void volume fractions containing [3C]fucosyl-ASOR were determined by liquid scintillation counter. Greater than 90% of the radiolabeled fucose was incorporated into protein. Substrate-containing fractions were pooled and stored frozen at -20°C until further use.

Descending paper chromatography in ethyl acetate/pyridine/water (5:4:3) was performed on aliquots of the substrate to verify that incubation with fucosidase-containing samples resulted in the release of 

(Received for publication, October 6, 1981)
of free fucose (17). Prior incubation of \([^{14}C]\) fucosyl-ASOR with albumin emulsin resulted in the migration of \(^{14}C\) radioactivity from the origin to a position identical with that of free fucose.

**Preparation of Human \(\alpha M\) and \(\alpha M-\text{MeNH}_2\)**

\(\alpha M\) was purified from human plasma by a modification of the method of Kurecki et al. (18), using metal chelate chromatography on zincimpregnated 4B and gel filtration on ACA-22, as previously described (19). Purified \(\alpha M\) migrated as a single band in the native "slow" state during nondenaturing polyacrylamide gel electrophoresis (20) and the trypsin-\(\alpha M\) binding stoichiometry was 1.9 as determined by the method of Ganrot (21). \(\alpha M\)-MeNH\(_2\) was prepared from purified \(\alpha M\) as previously described (19). Native polyacrylamide gel electrophoresis demonstrated the quantitative conversion from the native "slow" to the conformationally more compact "fast" state (20). \(\alpha M\)-MeNH\(_2\) was inactive when assayed for trypsin-binding activity by the method of Ganrot (21).

**Fucosidase Assay Using the Substrate \([^{14}C]\) Fucosyl-ASOR**

A substrate mixture was prepared by diluting stock \([^{14}C]\) fucosyl-ASOR 25-fold with 0.1 M NaOAc, 0.1 M NaCl, pH 5.3. Fifty \(\mu\)l of this mixture contained approximately 12,000 cpm of \([^{14}C]\) fucose. Five- to fifteen-fold aliquots of fractions to be assayed for fucosidase activity were incubated with 50-\(\mu\)l aliquots of substrate mixture at \(37^\circ C\) for times ranging from 10 min to several hours. The reaction was then quenched by the addition of 1.0 ml of 50 mM citric acid. Ion exchange chromatography of the reaction mixture was performed in Pasteur pipette columns containing 0.5 ml of Dowex AG 50W-X8 resin and equilibrated in 50 mM citric acid. The sulfonate groups of the resin retain protein-associatated radioactivity at low pH but allow free \([^{14}C]\) fucose to elute freely. The diluted reaction mixture was applied to the column and the effluent was collected directly in a mini-fraction collector. The column was then washed with 1.5 ml of 50 mM citric acid. 1.5 ml of Aquea sol II was added to the combined column effluents and the \(^{14}C\) radioactivity of the mixture was determined. Controls were determined by substituting buffer for the fucosidase activity. The assay was linear with respect to time and enzyme concentration when less than 30% of the total counts were released.

**Fucosidase Assay Using the Substrate Lactoferrin**

The fucose-containing glycoprotein lactoferrin was used as a substrate to quantitate fucosidase activity following each step of the purification. Assay mixtures contained 5 mg of lactoferrin (131 nmol substrate to quantitate fucosidase activity following each step of the assay) in a final volume of 200 \(\mu\)l. The reaction was then quenched by the addition of 1.0 ml of 0.2 M NaCO\(_3\) and the absorbance at 400 nm was determined. Controls were determined by substituting buffer for the fucosidase activity. The assay was linear with respect to time and enzyme concentration up to a release of 25 nmol of L-fucose per incubation.

**Determination of Other Glycosidase Activities**

Activity toward the \(p\)-nitrophenyl-glycoside substrates \(p\)-nitrophenyl-\(\beta\)-galactoside, \(\beta\)-N-acetylgalcosaminide, \(\alpha\)-galactoside, and \(\alpha\)-mannoside was determined on the solution of alond emulsin and final fraction of purified fucosidase. Assay mixtures contained 3 \(\mu\)M \(p\)-nitrophenyl-glycoside substrates, 0.05 M citrate, 0.1 M NaCl, pH 5.0, and were incubated with aliquots of enzyme activity in a final volume of 70 \(\mu\)l at 37 \(^\circ\)C for 1 h. The reaction was quenched by the addition of 1.0 ml of 0.2 M NaCO\(_3\) and the absorbance at 400 nm was determined. Background incubations were performed on assay buffers alone.

**Beta-Galactosidase Activity toward an Asialoglycoprotein Substrate**

Activity toward the \(p\)-nitrophenyl-glycoside substrates \(p\)-nitrophenyl-\(\beta\)-galactoside, \(\beta\)-N-acetylgalcosaminide, \(\alpha\)-galactoside, and \(\alpha\)-mannoside was determined on the solution of alond emulsin and final fraction of purified fucosidase. Assay mixtures contained 3 \(\mu\)M \(p\)-nitrophenyl-glycoside substrates, 0.05 M citrate, 0.1 M NaCl, pH 5.3, and were incubated with aliquots of enzyme activity in a final volume of 70 \(\mu\)l at 37 \(^\circ\)C for 1 h. The reaction was quenched by the addition of 1.0 ml of 0.2 M NaCO\(_3\) and the absorbance at 400 nm was determined. Background incubations were performed on assay buffers alone.

**Molecular Weight Estimation by Gel Filtration on Sephacryl S-200**

The method of Andrews (24) was used to estimate the molecular weight of the native, affinity-purified fucosidase. Gel filtration was performed on a column of Sephacryl S-200 (1.5 X 149 cm) equilibrated in 0.05 M NaOAc, 0.15 M NaCl, pH 5.0, at 4 \(^\circ\)C. Blue dextran was used to determine \(V_s\), and the following globular proteins were used to calibrate the column: cytochrome \(c\) (Mr = 11,700), myoglobin (Mr = 16,500), carbonic anhydrase (Mr = 29,000), ovalbumin (Mr = 43,000), and horse serum albumin (Mr = 68,000). Each of these proteins was dissolved in equilibration buffer (5-10 mg/ml) and applied to the column. Two-ml fractions were collected at a flow rate of 20 ml/h. Elution positions were determined spectrophotometrically. The purified fucosidase (200 \(\mu\)l) was likewise applied to the Sephacryl column. The elution position of the fucosidase activity was determined by measurement of the column. The column was washed with 1.0 liter of equilibration buffer. A linear gradient of increasing ionic strength was applied to elute the fucosidase activity from the ion exchange column. The buffer reservoirs of the gradient apparatus contained 400 ml of 0.05 M NaOAc, 0.1 M NaCl, pH 5.0. Fractions (12.2 ml) were collected at a flow rate of 15 ml/h. The column was washed with 1.0 liter of elution buffer. A linear gradient of increasing ionic strength was applied to elute the fucosidase activity from the ion exchange column. The buffer reservoirs of the gradient apparatus contained 400 ml of 0.05 M NaOAc, 0.1 M NaCl, pH 5.0, and 400 ml of 0.05 M NaOAc, 0.4 M NaCl, pH 5.0, respectively. Fractions (5 ml) were collected at a flow rate of 15 ml/h. Absorbing at 280 nm and conductivity determinations were made on fractions equilibrated at room temperature. Fucosidase activity was determined by measuring \([^{14}C]\) fucose release from \([^{14}C]\) fucosyl-ASOR, as described above. Fractions 66-83 were pooled (86 ml) and concentrated to 7 ml with a Millipore CX-30 concentrator.

**RESULTS**

**Fucosidase Purification**

The elution profiles for each of the three chromatographic steps in the purification are presented in Figs. 1-3, and Table I summarizes the purification at each stage. An overall 1250-fold purification and 23% yield of fucosidase was obtained from commercially available almond emulsin. The major loss of activity occurred during the first step, ion exchange chromatography on SP-Sephadex. The 10% loss determined after gel filtration on Sephacryl S-200 occurred during the concentration step prior to chromatography. The blue-Sepharose column elution was attempted with a linear salt gradient, but major activity losses resulted from enzyme dilution. Pulse gradient elution, however, provided a high yield and a concentrated stock of purified enzyme.
Almond emulsin contains a glycopeptidase which cleaves $\beta$-asparagine-glycosamine linkages (30). This enzyme is also absent from the final fucosidase preparation by virtue of the same reasoning. The final preparation of purified fucosidase was not free of contaminating proteins. Native gel electrophoresis at pH 3.8 and 9.5 revealed multiple discrete protein bands still present.

Fucosidase activity was remarkably stable in the final purified and concentrated state. Greater than 90% of the activity in freshly purified fucosidase remained following refrigeration at 4°C for 6 months with added 0.02% NaN₃. However, freezing and thawing resulted in a one-third loss of initial activity.

Molecular Weight Estimation by Gel Filtration—The elution position of affinity-purified fucosidase was determined on a column of Sephacryl S-200 calibrated by well characterized globular proteins (Fig. 4). Assuming that the fucosidase is a globular protein, the observed elution position is consistent with $M_r = 73,000$.

Other buffer systems were decidedly less desirable as assay media. Fucosidase activity increased with increasing ionic strength until reaching a maximum level at 80 mM NaCl in the presence of malonate buffer (Fig. 6A). Further salt addition actually inhibited activity. Increasing concentrations of malonate itself above 75 mM markedly inhibited activity (Fig. 6B). This same pattern of salt and buffer concentration dependence was also observed at pH 5.3 (Fig. 5A) and variations in acetate concentration between 25 and 100 mM did not greatly affect activity (Fig. 5C).

The final pool of purified fucosidase was assayed for the presence of contaminating exoglycosidase and protease activities. The purified fucosidase was assayed for $\beta$-galactosidase, $\beta$-hexosaminidase, $\beta$-galactosidase, and $\alpha$-mannosidase activity toward synthetic $p$-nitrophenyl-glycoside substrates. The color yield from these experiments was essentially at background levels which indicates that these activities are present at less than a few thousandths of a per cent of the activity originally present in the almond emulsion. More importantly, there was no remaining $\beta$-galactosidase activity toward the radiolabeled asialo-glycoprotein, $[^3H]$galactosyl-asialotransferase, nor was there detectable proteolytic activity toward radiolabeled casein. These data also demonstrate that GlcNAc and mannose were not released by the fucosidase preparation since these residues cannot be released without concomitant loss of the terminal $[^3H]$galactose residues from the glycopeptide which has the carbohydrate structure:

$$[^3H]Gal\beta1,4GlcNAc\beta1,2Man\beta1,4GlcNAc-\text{Asn}$$

$$[^3H]Gal\beta1,4GlcNAc\beta1,2Man\beta1,4GlcNAc-\text{Asn}$$
Purification of an Almond Emulsin Fucosidase

Summary of steps leading to the purification of almond emulsin fucosidase

<table>
<thead>
<tr>
<th>Details regarding each step are provided under &quot;Experimental Procedures.&quot;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Table I</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Volume (ml)</th>
<th>Milligram</th>
<th>Total units</th>
<th>Specific activity</th>
<th>Yield</th>
<th>Purification</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Almond emulsin</td>
<td>200</td>
<td>2400</td>
<td>124</td>
<td>0.052</td>
<td>100</td>
<td>1</td>
</tr>
<tr>
<td>2. SP-Sephadex pool</td>
<td>86</td>
<td>148</td>
<td>47.8</td>
<td>0.32</td>
<td>39</td>
<td>6.3</td>
</tr>
<tr>
<td>4. Sephacryl S-200 pool</td>
<td>104</td>
<td>9.7</td>
<td>35.8</td>
<td>3.68</td>
<td>75</td>
<td>11.4</td>
</tr>
<tr>
<td>4. Blue-Sepharose pulse</td>
<td>3.6</td>
<td>0.44</td>
<td>28.4</td>
<td>64.50</td>
<td>79</td>
<td>17.5</td>
</tr>
</tbody>
</table>

* Aliquot from each step were assayed for their ability to release fucose from human lactoferrin as described under "Experimental Procedures." Total units are given as nanomoles of fucose released/min; specific activity is given as nanomoles of fucose released/min/mg of protein.

![Fig. 4. Plot of V̇/V₀ against log molecular weight for protein standards and fucosidase on a Sephacryl S-200 column. Details are provided under "Experimental Procedures." Protein standards (●) used were: 1. cytochrome c (M_r = 11,700); 2. myoglobin (M_r = 17,800); 3. carbonic anhydrase (M_r = 29,000); 4. ovalbumin (M_r = 43,000); and 5. bovine serum albumin (BSA) (M_r = 68,000). The V̇/V₀ value obtained for affinity-purified fucosidase activity is indicated by the arrow.]

![Fig. 5. Effects of pH and concentrations of NaCl and NaOAc on fucosidase activity. Assay buffers were prepared at various combinations of pH, [NaCl], and [NaOAc]. For each series of assays, either [NaCl] or [malonic acid] was held constant (as shown in each panel), while the other was varied: A, [NaCl] and B, [malonic acid]. Fucosidase activity assays were performed as described in the legend to Fig. 5 and under "Experimental Procedures." The presence of 10 mM Hg²⁺ reduced activity by almost 80%, perhaps due to its binding surface sulphydryl groups. Activity of the purified Fucosidase toward Fucose-containing Glycoconjugates—The purified enzyme was specific for fucose in α(1-3) linkage with N-acetylglucosamine residues of Asn-linked oligosaccharide chain branches. Enzyme incubations were performed with glycoprotein, glycopeptide, and oligosaccharide substrates which contain fucose in a number of different glycosyl linkages, in order to verify this specificity (Table III). The glycoprotein asialoorosomucoid (M_r = 40,000) contains 0.7% fucose or 1.7 mol of fucose/mol (31, 32). Structural studies indicate that all fucose in ASOR is present in the glycosyl linkage Fuc(α(1-3))GlcNAc (33, 34). Extensive incubations with increasing amounts of fucosidase resulted in the release of at least 83% of the fucose calculated to be present on ASOR (Table III) and this result was consistent with the presumed specificity of the enzyme. The iron-binding protein lactoferrin (M_r = 76,500) contains 2 mol of fucose/mol (35). The fucoside, however, is present in α(1-6) linkage to the core N-acetylglucosamine as well as in
TABLE II
Effects of fucose, divalent metal ions, and EDTA on fucosidase activity

<table>
<thead>
<tr>
<th>Additions</th>
<th>Concentration</th>
<th>[14C]Fucose released</th>
<th>Relative activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. No addition</td>
<td>220</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>2. L-Fucose</td>
<td>2.5</td>
<td>212</td>
<td>0.96</td>
</tr>
<tr>
<td>10</td>
<td>118</td>
<td>0.54</td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>69</td>
<td>0.31</td>
<td></td>
</tr>
<tr>
<td>3. Ca2+</td>
<td>10</td>
<td>249</td>
<td>1.13</td>
</tr>
<tr>
<td>4. Mn2+</td>
<td>10</td>
<td>225</td>
<td>1.02</td>
</tr>
<tr>
<td>5. Mg2+</td>
<td>10</td>
<td>246</td>
<td>1.11</td>
</tr>
<tr>
<td>6. Hg2+</td>
<td>10</td>
<td>246</td>
<td>0.23</td>
</tr>
<tr>
<td>7. EDTA</td>
<td>10</td>
<td>237</td>
<td>1.08</td>
</tr>
</tbody>
</table>

TABLE III
Fucosidase activity toward fucose-containing glycoconjugates

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Milligrams</th>
<th>Total fucose</th>
<th>Fucosidase</th>
<th>Δf (65°C)</th>
<th>L-Fucose released</th>
<th>Relative activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. ASOR</td>
<td>1.0</td>
<td>77</td>
<td>5</td>
<td>35</td>
<td>18</td>
<td>23</td>
</tr>
<tr>
<td>2. Lactoferrin</td>
<td>3.6</td>
<td>94</td>
<td>5</td>
<td>35</td>
<td>23</td>
<td>52</td>
</tr>
<tr>
<td>3. AS</td>
<td>2.0</td>
<td>85</td>
<td>50</td>
<td>24</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4. AS-MeNH2</td>
<td>1.8</td>
<td>77</td>
<td>50</td>
<td>24</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5. IgG glycopeptides</td>
<td>90</td>
<td>15</td>
<td>35</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>6. α-D-Fucosylactose</td>
<td>170</td>
<td>20</td>
<td>18</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

α(1–3) linkage to GlcNAc, as in ASOR (36, 37). The glycopeptides of lactoferrin are heterogeneous with regard to their content of Fuc α(1–3)GlcNAc (36). The quantity of Fuc released from lactoferrin by the purified fucosidase (Table III) is in good agreement with the estimated Fuc in α(1–3) linkage to GlcNAc based on the proposed glycopeptide structures (36).

The plasma protease inhibitor, α2-macroglobulin (Mr = 726,000), contains 31 mol of fucose/mol (38, 39). No report of the nature and/or distribution of all of the fucosyl linkages present in α2M has yet been made. The fucosidase was incubated with the native, electrophoretic “slow” form of α2M, and with the conformationally compact, electrophoretic “fast” form, generated by exposure to methylamine (40). However, no release of fucose was observed with either form of α2M (Table III). Fuc α(1–3)GlcNAc linkages are presumably absent or enzymatically inaccessible on this glycoprotein.

Finally, no fucose release occurred from bovine IgG glycopeptides, known to contain Fuc α(1–6)GlcNAc linkages in the core region (41, 42), or from the milk oligosaccharide 2-fucosyllactose, which contains fucose in α(1–2) linkage with galactose (42).

**DISCUSSION**

Cibacron blue-Sepharose has been used successfully to purify many enzymes requiring NAD or ATP as a cofactor (43-46). It was therefore surprising to find that blue-Sepharose was an excellent affinity medium for the almond emulsin fucosidase I, especially since a number of fucose-containing media have not proved very useful. Yoshima et al. (8), reported only an additional 3-fold purification of fucosidase I activity on lacto-N-fucopentaose II coupled to Sepharose, with a 70% loss of activity. Initial attempts in this laboratory to purify the enzyme on agarose-c-aminoacaproylfucosamine (47), and on fucosylxyrane-Sepharose (48), were unsuccessful.

The fucosidase bound very tightly to blue-Sepharose at low ionic strength, but eluted readily in the presence of 0.1 M NaCl. Since the enzyme activity in acetate buffer displays a parallel dependence on ionic strength (Fig. 5), it is possible that the fucosidase undergoes ionic strength-dependent conformational changes. At very low ionic strengths (g < 2.0 mmho), the enzyme may exist in an inactive state which has a high affinity for the Cibacron blue dye. In the presence of increasing salt, the fucosidase may assume an active conformation which does not bind to Cibacron blue.

The final preparation of purified enzyme is of sufficient enzymatic homogeneity to be useful as a carbohydrate structural tool. Only trace levels of glycosidase activities toward synthetic p-nitrophenyl-glycoside substrates were detected and there was no β-galactosidase activity toward asialoligoprotein substrates in the purified fucosidase. The final preparation was also free of contaminating protease activity. The specificity of the final preparation for Fuc α(1–3)GlcNAc linkages was supported by its lack of activity toward substrates containing Fuc α(1–2)Gal and Fuc α(1–6)GlcNAc linkages (Table III).

The purified fucosidase was active toward glycoprotein substrates containing Fuc α(1–3)GlcNAc linkages (Table III). The present results are consistent with recent structural studies of oligosaccharides obtained from orosomucoid (33, 34) and lactoferrin (36, 37). Almost all of the fucose present on ASOR was removed following extensive incubations, in agreement with reports that all fucose present in orosomucoid exists in α(1–3) linkage (33, 34). Almost half of the lactoferrin fucose was removed, in agreement with the anticipated content of α(1–3)-linked fucose (30, 37).

Finally, the fucosidase was used to investigate the fucose-rich (31/mol), plasma protease inhibitor α2M (49). The native form of this glycoprotein is a tetramer of four identical (Mr = 185,000) subunits which undergo a conformational rearrangement when α2M binds a protease or is exposed to methylamine (38, 40). Studies were directed toward whether the exposure of Fuc α(1–3)GlcNAc linkages on α2M, if present, might be dependent on the conformational state. The fucosidase, however, released no fucose from either form of the glycoprotein (Table III) suggesting that Fuc α(1–3) linkages are either missing altogether or are buried within the quaternary structure of α2M.

**Acknowledgment**—We wish to thank Dr. Robert L. Hill for his review and helpful criticism of this manuscript.

**REFERENCES**

Purification of an Almond Emulsin Fucosidase


