Identification of Distinct Endoglycosidase (Endo) Activities in Flavobacterium meningosepticum: Endo F₁, Endo F₂, and Endo F₃

ENDO F₁ AND ENDÒ H HYDROLYZE ONLY HIGH MANNOSE AND HYBRID GLYCANs*

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Flavobacterium meningosepticum endo-β-N-acetylglucosaminidase F preparations have been resolved by hydrophobic interaction chromatography on TSK-buty]l resin into at least three activities designated endo F₁, endo F₂, and endo F₃ each with a unique substrate specificity. The 32-kDa endo F₁ protein is the principle component representing in excess of 95% of most earlier and currently available commercial endoglycosidase preparations, the remainder being a mixture of five proteins from 32 to 43 kDa. Substrate specificity studies reveal endo F₁ and endo H from Streptomyces plicatus to have nearly identical capacities to hydrolyze high-mannose oligosaccharides with a minimum Man₁6Man₁6Man₁3GlcNAc₁@4GlcNAc₁@4GlcNAc structure. Although endo H will hydrolyze fucose-containing hybrid oligosaccharides at rates approaching comparable high-mannose forms, core-linked fucose reduces the hydrolysis rate of endo F₁ by over 50-fold relative to high-mannose structures. Neither homogeneous endo F₁ nor endo H hydrolyze complex multi-antennary glycans. The biantennary cleaving activity previously reported for endo F prepararions (Tarentino, A. L., Gomez, C. M., and Plummer, T. H. Jr. (1985) Biochemistry 24, 4665-4671) is a characteristic of the contaminating endo F₂ activity.

The application of endoglycosidases in biological research has evolved significantly over the last 10 years. Earlier biochemical studies took advantage of the enzymatic potential of endoglycosidases to obtain separate protein and carbohydrate moieties from isolated asparagine-linked glycoproteins in order to determine oligosaccharide structures as well as to assess the role of carbohydrate in glycoprotein function. With available chemical means only the protein or the carbohydrate could be obtained intact, the other being destroyed during the selective isolation. Although endoglycosidases are still widely applied to the biochemical characterization of purified glycoproteins, these enzymes are being applied increasingly by cell biologists to examine aspects of protein sorting, targeting, and compartmentalization. In such studies modifications to the carbohydrate moieties of selected reporter molecules are inferred from their changing temporal sensitivity to hydrolysis by endoglycosidases of known specificity. As more processing glycosidases and sugar transferases are localized in vivo, endoglycosidases gain importance in characterizing the oligosaccharide modifications associated with a glycoprotein's inter-compartmental transport.

Given the diversity of potential applications, it is not surprising that the discovery of a new endoglycosidase is met with great interest by the scientific community. In 1982, a novel glycosidase preparation from Flavobacterium meningosepticum, designated endo F was described (1) that released intact oligosaccharides from asparagine-linked glycoproteins. This activity was later fractionated into two distinct oligosaccharide chain-cleaving enzymes (2, 3). One was an amidase designated PNGase F, that hydrolyzed the amide nitrogen of glycosylated asparagines generating a peptide, in which the previously glycosylated asparagine was converted to an aspartic acid residue, and a free oligosaccharide with 1-amino-N-acetylglicosamine on the reducing end. The 1-amino oligosaccharide hydrolyzes nonenzymatically to NH₃ and the corresponding oligosaccharide with a reducing terminal GlcNAc. PNGase F has become an important biological reagent, because it can hydrolyze essentially all naturally occurring asparagine-linked glycans so long as the N- and C-terminal ends of asparagine are in peptide bonds (4).

Endo F, the second enzyme from F. meningosepticum (3), was found to be a true endoglycosidase because, like endo H from Streptomyces plicatus, it cleaved the β1,4-linked di-N-acetyldihitobiose core of N-linked glycans. Oligosaccharides with a single reducing end GlcNAc are released, while the core-proximal GlcNAc remains attached to the originally glycosylated asparagine. These residual GlcNAc residues can serve as a valuable reporter group to determine the extent of site-specific glycosylation on defined glycoproteins (3).

Initial specificity studies suggested that, unlike endo H, endo F hydrolyzed biantennary oligosaccharides, although at a lower rate than high-mannose glycans (3). However, during more extensive studies to compare the relative specificities of endo H and F, it was observed recently that endo F, when isolated by a new purification procedure employing Toyopearl TSK-buty]l resin, no longer hydrolyzed a defined complex biantennary IgM hexaglycopeptide (compound 8, Table I). Further investigation of this anomaly revealed that endo F had been resolved into at least two activities. One, now designated endo F₁, had a substrate specificity very similar to

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1 The abbreviations used are: endo F, endo-β-N-acetylglucosaminidase F; PNGase, peptide-N4-(N-acetyl-β-glucosaminyl)asparagine amidase; dansyl, 5-dimethylamino-naphthalene sulfonic; GlcNAc[¹H] -ol, reduced alditol form of GlcNAc; Fuc, fucose.

2 T. H. Plummer, and A. L. Tarentino, manuscript in preparation.
endoglycosidases. In this communication we examine in detail the substrate specificity of endo F₁ in comparison to that of S. plicatus endo H. Characterization of the endo F₂ and endo F₃ activities and their structural relationship to endo F₁ is in progress and will be the subject of subsequent articles from this laboratory.

**EXPERIMENTAL PROCEDURES**

**Materials**

Enzymes—Cloned endo H (EC 3.2.1.96) was purified from P.cepacia-transformed Escherichia coli (6). Endo F₁ and endo F₂ (EC 3.2.1.96) and PNGase F (EC 3.5.1.72) were isolated from F. meningosepticum by a novel purification procedure. Diplococcus pneumoniae N-acetyl-β-D-glucosaminidase (EC 3.2.1.30) and β-galactosidase (EC 3.2.1.23) were purchased from Boehringer Mannheim. Jack bean mannosidase (EC 3.2.1.24) was purified according to a modification of the method of Li and Li (7). The final product had a specific activity of 34 IU/mg with p-nitrophenyl-α-D-mannoside as substrate.

Oligosaccharides—Compounds 6 and 7 in Table I were isolated from ovumblain and represent chromatographic peaks AC-D and AC-E, respectively (8). Compounds 4 and 6 were prepared by limited α-mannosidase digestion of compound 6, and their structural configurations have been confirmed (9, 10). Compound 8 is the C₁ complex biantennary glycophosphate isolated from a thermolysin digest of IgM from a Waldenstoms patient (Ga) (11). Compound 9 is a complex biantennary tetracyclicoseotide (Aα→3Manα→6) isolated from a Pronase digest of human serum transferrin. The structure of compounds 8 and 9 were confirmed by 500 MHz NMR spectroscopy. Compound 2 was derived from compound 3 by limited α-mannosidase digestion to remove a single mannose or by periodate oxidation of compound 7 (9). Compound 6 was obtained by exhaustive α-mannosidase digestion of compound 6. The presence of the Manβ3GlcNAc linkage in the high-mannose oligosaccharide cores was confirmed using Polysulfure sulfatase β-mannosidase (9). Compound 10 was isolated from a proteolytic digest of fetuin followed by mild acid hydrolysis to remove the sialic acids (2). The monosaccharide composition of all oligosaccharides in Table I was confirmed by neutral and amino sugar analyses as described earlier (12).

Compounds 11–15 in Table II were prepared by PNGase F digestion of log-phase yeast microsomal proteins that had been Folch-extracted for isolation of GDP-[14C]Man-labeled lipid mono- and lipid oligosaccharide fractions (13). A pool of residual pellets from one-hundred 0.1 ml charging reactions was solubilized by incubation at 65 °C for 18 h in 1% sodium deoxy sulfate. Excess detergent was removed by dialysis against 0.05 M sodium phosphate buffer, pH 8.7, and the crude, 80-ml preparation was supplemented with 0.5% Triton X-100 followed by addition of 0.4 IU of PNGase F. After digestion at 25 °C for 18 h an additional 0.1 IU of PNGase F was added and incubation continued for 30 h. Hydrolysis of N-linked glycans was monitored by release of soluble 14C label into the supernatant of 50-μl aliquots of the digestion mixture precipitated by addition of 0.1 ml of ice-cold acetone. After all N-linked glycans were released, the residual protein was precipitated with 2 volumes of cold acetone at −20 °C for 18 h. The precipitate was centrifuged, resuspended in 20 ml of cold 70% acetone, and centrifuged again, and the combined supernatants were flash-evaporated to 15 ml. Oligosaccharides were desalted on a 1-cm diameter column containing 5 cm of Dowex 1-formate over 5 cm of Dowex 50-H. Residual Triton X-100 was removed from the mixed-bed eluant with Extractigel D, and the oligosaccharide preparation was reduced to 1 ml by flash evaporation.

The individual oligosaccharides (compounds 11–15, Table II) were separated by 2 cycles of Bio-Gel P-4 (400 mesh) chromatography as described below. Each oligosaccharide was characterized by digestion with a α-mannosidase and found to yield either heoxoGlcNAc (compound 15) or ManGlcNAc₃ (compounds 11–14). Structures shown for the yeast ManGlcNAc (14, 15), ManGlcNAc₃ (14), and ManGlcNAc₄ (14) have been independently confirmed.

Compound 18 (Table II), a hybrid oligosaccharide, was isolated from Hep G2 cells incubated with [6-3H]Fuc, exactly as described for cystic fibrotic fibroblast oligosaccharides (17). Medium containing secreted Fuc-labeled glycoproteins was reduced in volume by ultrafiltration and chromatographed on a 1.5 × 95 cm SephaGel G-50 (fine) column in 0.1 M NH₄HCO₃ as the eluant. The radioactive glycoprotein eluting in the column void was digested with PNGase F, and the soluble supernatant fraction containing the released glycans was chromatographed on Bio-Gel P-4. Approximately 60% of the [3H]Fuc label was in the hybrid oligosaccharide peak; the remainder was in peaks corresponding to bi- (10%) and triantennary (30%) structures which apparently had escaped the swaismine block of α-mannosidase II.

Compound 17 was derived from compound 18 by mild acid hydrolysis for removal of the sialic acid. Further characterization of compound 17 included sequential digestion with β-galactosidase and β-N-acetylglucosaminidase to produce compounds 18 and 19, respectively. Digestion of compound 19 with α-mannosidase yielded the predicted ManGlcNAc₃ core. Compounds 17 and 19 were confirmed (13), and compounds 21 and 22 were derived by PNGase F digestion of compounds 18 and 10, respectively, followed by conversion to their alditol form with NaB₄H₉ (18).

**Reagents and Resins**—Bio-Gel P-4 (400 mesh) and Dowex resins were from Bio-Rad and concanavalin A-Sepharose was from Pharmacia LKB Biotechnology Inc. The 3MM Chromatography paper was obtained from Whatman. GDP-[14C]Man (290 mCi/mmol) and L-[6-3H]Fuc (64 Ci/gmol) were obtained from Amersham Corp., and 5-nitro-5-[methy-3H]dansyl chloride (21.3 Ci/mmol) and NaBD₄ (500 mCi/mmol) were from Du Pont-New England Nuclear. Sodium deoxy sulfate was purchased from Eastman Kodak and Extragel D from Pierce Chemical Co. Oligosaccharide markers for P-4 column calibration were the Manα→3GlcNAc₄H₃ of yeast oligosaccharides characterized previously (18). Additional markers consisted of authentic FucGlcNAc (17) and Manα→3GlcNAc₃ (13).

**Methods**

Enzyme Assays—Preparations of homogeneous endo F, and H were standardized at about 40 IU/mg protein using 0.5 mM ovomucoid ManGlcNAc₃Asn-[3H]dansyl (peak AC-D in Ref. 8) as substrate. Hydrolysis of all substrates in Table I (compounds 1–10) was performed at 0.5 mM dansylated oligosaccharide (10 μCi/μmol) and 37 °C. The 12-μl assays contained 6 nmol of substrate, 0.6 μmol of sodium acetate, pH 5.7, 0.05% (w/v) bovine serum albumin, and an appropriate dilution of endo F or H. Reactions were started by addition of enzyme, and 2-μl aliquots were removed and spotted on 3MM paper at 0, 15, 30, 60, and 90 min. Chromatograms were developed ascendingly with butanol/ethanol/water (2/1/1, v/v), and separation of the released product, GlcNAcAsn-3H]dansyl, was monitored with a 340-nm mineral lamp. Extent of hydrolysis was determined by eluting the residual substrate and product spots from the chromatogram and counting in a liquid scintillation spectrometer (19). The level of enzyme added to the assays was empirically chosen to provide less than 30% hydrolysis over 90 min in order to maintain linear release kinetics. Values in Table I are reported as apparent enzyme specific activity (IU/mg) on the individual substrates.

Hydrolysis of the labeled glycans 11–19 in Table II was performed in 100-μl reactions containing 3–5 nmol of oligosaccharide (10,000–30,000 3H or 14C dpm), 7.5 μmol of sodium acetate, pH 5.7, 0.07% (w/v) bovine serum albumin (w/v), and 10 or 20 milliunits of endo F, or H based on the hydrolysis of 0.5 mM ManGlcNAc₃Asn-[3H]dansyl substrate as described above. After a suitable incubation period (Table II) assays were terminated by addition of 1 drop of glacial acetic acid followed by heating at 100 °C for 2 min. The reactions were diluted to 400 μl with 0.1 N acetic acid, 1% 1-butanol, a pair of suitable internal markers added, and the products chromatographed on Bio-Gel P-4 (400 mesh). Radioactivity in fractions was measured by treatment with 0.5 ml of scintillation fluid.
RESULTS AND DISCUSSION

Two reviews appeared recently (20, 21) which summarized the substrate specificity of endo F and endo H. Briefly, endo F and endo H readily hydrolyzed all high-mannose glycans at comparable rates, but substantial differences in specificity were observed toward hybrid-type structures, and complex biantennary oligosaccharides known to be sensitive only to endo F (3, 22, 23). The implementation of a new hydrophobic chromatography procedure for the purification of endo F and PNGase F, however, provided the first direct evidence that the specificity of endo F was a more complex issue than heretofore recognized; these new preparations of homogeneous endo F no longer hydrolyzed complex oligosaccharides. Further investigation led to the discovery that the endo F had been separated by the TSK-butyl column chromatography step into two distinct enzyme activities, designated endo F₁ and endo F₂ because of their sequential elution from this matrix.

As shown in Fig. 1, sodium dodecyl sulfate-polyacrylamide gel electrophoresis revealed endo F₁ to be the 32-kDa species that is the principal component of previous endo F preparations, while the endo F₂ activity was associated with higher molecular mass proteins distinct from either endo F₁ or the 35-kDa PNGase F.

FIG. 2. Hydrolysis of high-mannose and complex oligosaccharides by endo F₁, endo F₂ and PNGase F. Approximately 25 ng of the protein preparations shown in Fig. 1 were incubated with either Man₆GlcNAc₂Asn-[¹⁴C]dansyl (compound 6, Table I) or transferrin biantennary tetraglycopeptide-[¹⁴C]dansyl (compound 9, Table I) (BF) for 1 h at 37 °C. Paper chromatography was performed on Whatman 3MM as described under “Experimental Procedures.” The dansylated substrates, which remain at the origin, and their digestion products, which migrate ascendingly, were visualized with a longwave UV light.

TABLE I

<table>
<thead>
<tr>
<th>Compound</th>
<th>Structure</th>
<th>Hydrolysis Rate of Endo F₁, Endo F₂, and PNGase F (μmol/min·mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Nε₁(¹⁴C)GlcNAc₂-GlcNAc-[¹⁴C]-</td>
<td>&lt;0.01 0</td>
</tr>
<tr>
<td>2</td>
<td>Nε₁(¹⁴C)GlcNAc₂-GlcNAc-[¹⁴C]-</td>
<td>&lt;0.01 0</td>
</tr>
<tr>
<td>3</td>
<td>Nε₁(¹⁴C)GlcNAc₂-GlcNAc-[¹⁴C]-</td>
<td>&lt;0.01 0</td>
</tr>
<tr>
<td>4</td>
<td>Nε₁(¹⁴C)GlcNAc₂-GlcNAc-[¹⁴C]-</td>
<td>&lt;0.01 0</td>
</tr>
<tr>
<td>5</td>
<td>Nε₁(¹⁴C)GlcNAc₂-GlcNAc-[¹⁴C]-</td>
<td>19 48</td>
</tr>
<tr>
<td>6</td>
<td>Nε₁(¹⁴C)GlcNAc₂-GlcNAc-[¹⁴C]-</td>
<td>27 64</td>
</tr>
<tr>
<td>7</td>
<td>Nε₁(¹⁴C)GlcNAc₂-GlcNAc-[¹⁴C]-</td>
<td>43 42</td>
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<tr>
<td>8</td>
<td>Nε₁(¹⁴C)GlcNAc₂-GlcNAc-[¹⁴C]-</td>
<td>27 34</td>
</tr>
<tr>
<td>9</td>
<td>Nε₁(¹⁴C)GlcNAc₂-GlcNAc-[¹⁴C]-</td>
<td>0 0</td>
</tr>
<tr>
<td>10</td>
<td>Nε₁(¹⁴C)GlcNAc₂-GlcNAc-[¹⁴C]-</td>
<td>0 0</td>
</tr>
</tbody>
</table>

*R = Asn-[¹⁴C]dansyl for compounds 1–7 and Asn-peptide-[¹⁴C]dansyl for compounds 8–10.*

As shown in Fig. 1, sodium dodecyl sulfate-polyacrylamide gel electrophoresis revealed endo F₁ to be the 32-kDa species that is the principal component of previous endo F preparations, while the endo F₂ activity was associated with higher molecular mass proteins distinct from either endo F₁ or the 35-kDa PNGase F.
Specificity of Endo F, and Endo H

Relative sensitivity of labeled oligosaccharides to hydrolysis by endo F₁ and endo H

Substrates were present at ~50 nM and hydrolysis was at 37 °C. N.D., not determined.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Structures</th>
<th>Assay n M</th>
<th>100%</th>
<th>Hvdrolvsis by Endo F₁</th>
<th>Endo H</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td>GlcNAc-Gln-Gln-Glu</td>
<td>4 200</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>12</td>
<td>GlcNAc-Gln-Gln-Glu</td>
<td>8 200</td>
<td>38</td>
<td>28</td>
<td>28</td>
</tr>
<tr>
<td>13</td>
<td>GlcNAc-Gln-Gln-Glu</td>
<td>2 50</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>14</td>
<td>GlcNAc-Gln-Gln-Glu</td>
<td>2 50</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>15</td>
<td>GlcNAc-Gln-Gln-Glu</td>
<td>2 50</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

*Activity is based on hydrolysis of Man5GlcNAcAsn-dansyl (compound 6, Table I).

A preliminary characterization of endo F₁ and endo F₂ activities was conducted using paper chromatography to qualitatively compare the hydrolysis of Man₅GlcNAc₅Asn-dansyl and dansyl-biantennary tetraglycopeptide (Fig. 2). Endo F₁ generated the expected fluorescent product, GlcNAcAsn-dansyl, from the dansylated high-mannose substrate (Fig. 2, lane 3), but there was no hydrolysis of the fluorescent complex biantennary glycan (Fig. 2, lane 4) during the 1-h incubation period, or upon prolonged incubation at 37 °C (data not shown). The endo F₂ pool hydrolyzed Man₅GlcNAc₅Asn-dansyl at a much slower rate than endo F₁, as evidenced by the intensity of the fluorescent substrate remaining at the origin (Fig. 2, lane 5). By contrast, endo F₂ completely hydrolyzed the dansylated biantennary glycopeptide to release the fluorescent GlcNAc-tetrapeptide product during the 1-h incubation (Fig. 2, lane 6).

Typical PNGase F cleavage activity is shown in Fig. 2, lanes 7 and 8. The high-mannose asparagine-oligosaccharide was not hydrolyzed due to the absence of blocked N and C termini required for activity (4), while the dansylated biantennary tetraglycopeptide was partially hydrolyzed during the 1-h incubation. The fully deglycosylated, dansylated tetrapeptide product formed by digestion with PNGase F (Fig. 2, lane 8) migrated slightly faster than the corresponding endo F₂ prod-
uct, because the residual GlcNAc residue on the latter reduced its relative chromatographic mobility.

Previously, endo F preparations were purified by gel filtration on TSK-HW55S, and enzyme activity was monitored with Man9GlcNAc2Asn-dansyl. Because endo F1 hydrolyzed this substrate much slower than endo F, (Fig. 2) it was not recognized as a distinct activity, but because of peak overlap these larger proteins were included as an estimated 2–5% contaminant in the final pool. Endo F2 was clearly responsible for the observed biantennary cleaving activity of 0.1–0.2 IU/mg protein attributed to endo F preparations (Fig. 2).

The ability of pure endo F1, and endo H to hydrolyze 22 defined glycopeptides and oligosaccharides is summarized in Table I and II. Table I shows the actual rates of hydrolysis in IU/mg protein for 10 representative glycopeptides, and Table II lists the percent hydrolysis of radiolabeled substrates at selected enzyme concentrations (with units based on hydrolysis of compound 6). Both endo F1 and H require the linear trimannosyl residues Man1α3Man1α6Man1β4 for appreciable activity (Table I, compounds 4–7). This is also the case for oligosaccharides with either di-N-acetylchitobiose or di-N-acetylectitobitol on their reducing ends (Table II, compounds 13–20). Neither endo F1 nor H hydrolyzed biantennary (±Fuc) or nonfucosylated triantennary glycopeptides (Table I, compounds 8–10) or oligosaccharides (Table II, compounds 21 and 22), even though these reactions were driven with enzyme as high as 2–3 IU/ml (based on hydrolysis of compound 6) for 24 h at 37 °C. In all cases, endo H and F1 were confirmed to be fully active at the end of the incubation period by assay with Man9GlcNAc2Asn-[3H]dansyl as substrate.

While revealing only minor differences in hydrolysis of high mannosyl glycans, endo F1, and endo H do reveal major rate differences when fucose is present in the core of hybrid oligosaccharides (compare compounds 16–19 in Table II). It has been known for some time that endo H hydrolyzes hybrid oligosaccharides (24), but to our knowledge rates of cleavage relative to comparable non-fucosylated high-mannose forms have not been established. The data in Table I, compound 6, and Table II compounds 13–19, show that neither fucose on the reducing end, nor the complex chain consisting of sialic oligosaccharides (24), but to our knowledge rates of cleavage the absence of the linear Manα3Manα4GManβ4+C mixture of glycopeptides (3, 22, 23), the activity of endo F1, unlike that of endo H, appears to be limited by the presence of a triantennary-type β4-linked residue on the lower arm GlcNAcβ14Manα3Manβ4→ in non-fucosylated hybrid-type molecules. These subtle substrate specificity differences may be exploited under judiciously chosen conditions of time and enzyme millimolar to distinguish high-mannose from hybrid core hybrid glycans by sequential hydrolysis of glycoproteins of interest. Initial digestion with endo F1 will remove high mannose forms, then following isolation of the released oligosaccharides, a second digestion with endo H will release hybrid forms.

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REFERENCES


These results appear to explain the pattern of hydrolysis observed earlier by endo F and H on PNGase F-released [3H] Fuc-labeled oligosaccharides from normal and swainsonine-treated fibroblasts (17). Although the endo F preparation released nearly all the fucose label in normal oligosaccharides as [3H]FucGlcNAc-ol, release from swainsonine-induced hybrids was minimal. It would appear that the endo F2 contaminant in the earlier endo F preparations released FucGlcNAc-ol from biantennary forms present in the normal oligosaccharide pools, because endo H treatment of these glycans confirmed the absence of appreciable hybrid component. The low level of FucGlcNAc-ol released from the swainsonine hybrids by the endo F preparation compared to endo H is a consequence of the slow rate at which endo F1 and F2 hydrolyze hybrid glycan forms relative to endo H.

In summary, endo F1 from F. meningosepticum and endo H from S. pilaticus have very similar substrate specificities that are restricted to high-mannose and hybrid-type oligosaccharides. This specificity is determined by a requirement of both enzymes for the linear sequence Manα3Manα6Manβ4→. While addition of fucose to the core of such oligosaccharides has little effect on the hydrolysis rate by endo H, this substrate decreases the activity of endo F1 in excess of 50-fold (Table II). Also, based on the cleavage of ovalbumin peak AC-C mixture of glycopeptides (3, 22, 23), the activity of endo F1, unlike that of endo H, appears to be limited by the presence of a triantennary-type β4-linked residue on the lower arm GlcNAcβ14Manα3Manβ4→ in non-fucosylated hybrid-type molecules. These subtle substrate specificity differences may be exploited under judiciously chosen conditions of time and enzyme millimolar to distinguish high-mannose from hybrid core hybrid glycans by sequential hydrolysis of glycoproteins of interest. Initial digestion with endo F1 will remove high mannose forms, then following isolation of the released oligosaccharides, a second digestion with endo H will release hybrid forms.
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