*Proc. Natl. Acad. Sci. USA*
Vol. 79, pp. 4540–4544, August 1982
Biochemistry

# *endo*-β-*N*-Acetylglucosaminidase F: Endoglycosidase from *Flavobacterium meningosepticum* that cleaves both high-mannose and complex glycoproteins

(Rauscher leukemia virus gp70/glycan structure/deglycosylation/N-linked glycan)

JOHN H. ELDER AND STEPHEN ALEXANDER

Department of Immunopathology and The Committee for the Study of Molecular Genetics, Research Institute of Scripps Clinic, La Jolla, California 92037

*Communicated by Frank J. Dixon, April 5, 1982*

ABSTRACT **We have detected an endoglycosidase activity produced by *Flavobacterium meningosepticum*. This enzyme, named endo F, cleaves glycans of both the high-mannose and the complex type linked through asparagine to the protein backbone. The data indicate that cleavage occurs via hydrolysis of the glycosidic bond of the *N,N'*-diacetylchitobiose core structure adjacent to asparagine, similar to that due to endo H and endo D. Extreme variability was noted in the availability of this cleavage site among N-linked glycoproteins. Glycoproteins of retrovirus, lymphocytic choriomeningitis virus, Pichinde virus, and HLA-A and -B antigens were readily cleaved in the presence of nonionic detergent. Others, such as ovalbumin, fetuin, bromelain, ovomucoid, $\alpha_1$-acid glycoprotein, immunoglobulin G, and influenza virus hemagglutinin became susceptible only after reduction and alkylation or when cleavage was performed in the presence of 1% 2-mercaptoethanol. Endo F should prove useful in the study of glycans and protein backbones as discrete entities and for defining the nature of the glycan–protein interface.**

Specific glycosidases have proven useful in elucidation of the structures and biosynthetic pathways of biologically important glycoproteins (1–5). Two of the most useful are the endoglycosidases endo H (1) and endo D (2), which cleave high-mannose and complex glycans, respectively, from glycoproteins and glycopeptides. Cleavage by these enzymes occurs between the adjacent *N*-acetylglucosamine residues linking the glycan moiety to the asparagine of the protein backbone (1, 2). In this report, we describe an endoglycosidase that cleaves both complex and high-mannose glycans from asparagine-linked glycoproteins.

The enzymatic activity was first detected in a commercial preparation of carboxypeptidase A.* Examination of this reagent revealed considerable bacterial contamination and one of the bacterial species released endoglycosidase activity into its culture fluid. The bacteria have been typed as *Flavobacterium meningosepticum*† (6). In keeping with previous endoglycosidase nomenclature (2), we have named the enzyme endo F after its source. Our results indicate that endo F cleaves glycans from all N-linked glycoproteins tested, if conditions are used that sufficiently expose the cleavage site.

## MATERIALS AND METHODS

**Conditions of Growth of *F. Meningosepticum* and Preparation of Enzyme.** Cells were grown to stationary phase in M9 medium (7) containing Casamino acids (Difco) at 5 g/liter. The highest concentrations of endo F were obtained from cultures allowed to reach stationary phase at 22°C. Typically, 4 liters of stationary phase cells were used for subsequent treatment.

The cells were removed from the culture medium by centrifugation at 5,000 × *g* for 10 min. The culture supernatant was concentrated by addition of solid ammonium sulfate to 80% saturation, followed by centrifugation after stirring for 1 hr at 4°C. The concentrated protein was suspended in 50% ammonium sulfate. The suspension was centrifuged, and the pellet was dissolved in 0.01 M sodium phosphate, pH 7.2/0.15 M NaCl ($P_i$/NaCl)/50 mM EDTA. This material was chromatographed on a column (1.5 × 50 cm) of ACA54 (LKB, Uppsula, Sweden) at 4°C in $P_i$/NaCl/5 mM EDTA. The peak fractions were pooled and concentrated to 1/10 vol in a dialysis bag against dry Sephadex G-100. The material was made 50% glycerol and stored at −20°C. Endo F remains active under these conditions for at least 3 months.

Several attempts by ion-exchange chromatography failed to physically separate distinct glycosidase activities. Isoelectric focusing in agarose followed by elution and assay versus $^{125}$I-labeled Rauscher murine leukemia virus (R-MuLV) glycoprotein 70 (gp70) or ovalbumin revealed that all glycosidase activity focused with a pI of 7.8. $NaDodSO_4$/polyacrylamide gel electrophoresis followed by Coomassie blue staining revealed the presence of a single band, compared with four bands in the combined ACA54 column fractions (data not shown). Under conditions tested, all glycosidic activity copurified, and purification other than by size resulted only in lower yields. All studies reported here were performed with material prepared as above, made phenotypically pure of protease by addition of EDTA (see *Results*).

**Substrates of Enzyme Assays.** R-MuLV was obtained from the Resources Branch of the National Cancer Institute. Purified gp70 was prepared as described (8). LCM and Pichinde viruses were the gift of M. Buchmeier of this institute. Influenza virus hemagglutinin was prepared from X-47 strain grown in embryonated eggs as described (9). HLA-A, -B, and -DR antigens were provided by J. Kaufman, Harvard University. Bromelain, ovomucoid, $\alpha_1$-acid glycoprotein, fetuin, and ovalbumin were obtained from Sigma. All glycoproteins were labeled by the chloramine-T procedure as described (10).

Twelve synthetic *p*-nitrophenylglycoside substrates used for

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

Abbreviations: R-MuLV, Rauscher murine leukemia virus; $P_i$/NaCl, phosphate-buffered saline; gp70, gp1, gp2, glycoproteins 70, 1, and 2, respectively; LCM virus, lymphocytic choriomeningitis virus.

* As it is not germane to the data presented here, we omit the source of this reagent. Furthermore, we have not determined the generality of the contamination.

† *F. meningosepticum* was originally isolated from hospital nurseries in association with outbreaks of meningitis in premature infants. Pathogenicity in test animals is doubtful (6) but normal precautions should be taken.

assaying exoglycosidase activity were obtained from Sigma. The reactions were carried out in 0.5 ml of 50 mM sodium acetate, pH 5.0/10 mM *p*-nitrophenylglycoside. Five microliters of enzyme was added and the reaction was stopped by addition of 1.0 ml of 1.0 M sodium bicarbonate after 40 min at 37°C. Reactions were monitored by absorbance at 400 nm.

Two glycan standards were provided by Tom Warner of the University of California at San Diego. Standard 1 is a mixture of two glycans having the structures, Gal$\beta$1→4GlcNAc$\beta$1→2Man$\alpha$1→3Man$\beta$1→4GlcNAc and Gal$\beta$1→4GlcNAc$\beta$1→2Man$\alpha$1→6Man$\beta$1→4GlcNAc. Standard 2 is a branched chain glycan of the structure Gal$\beta$1→4GlcNAc$\beta$1→2Man$\alpha$1→3(Gal$\beta$1→4GlcNAc$\beta$1→2Man$\alpha$1→6)Man$\beta$1→4GlcNAc.

**Metabolic Labeling of R-MuLV gp70.** R-MuLV was grown in the SC-1 cell line (11) in minimal essential medium/10% fetal calf serum. Cells from confluent cultures were washed with low-glucose medium (K.C. Biologicals, Lenexa, KA, 1,000 mg/liter) and incubated in low-glucose medium/10% dialyzed fetal calf serum. One millicurie of [$^3$H]glucosamine or [$^3$H]mannose (New England Nuclear; 31.1 Ci/mmol and 15.8 Ci/mmol, respectively; 1 Ci = $3.7 \times 10^{10}$ becquerels) was added to the culture, and the mixture was incubated for 18 hr at 37°C. Cells were harvested and lysed in $P_i$/NaCl/0.5% Nonidet P-40. The lysates were centrifuged at 10,000 × *g* for 15 min and the supernatant was passed through a 0.45-$\mu$m Nalgene filter. R-MuLV gp70 and its $M_r$ 85,000 precursor were purified by immunoaffinity chromatography as described (10).

**Endoglycosidase Assay Conditions.** Assays were carried out in 100 $\mu$l (final volume) of 0.1 M sodium phosphate, pH 6.1/50 mM EDTA/0.5% Nonidet P-40 containing substrate (e.g., 50,000 cpm of $^{125}$I-labeled R-MuLV or 10–50 $\mu$g of unlabeled protein). The amount of endo F preparation added was typically 2–5 $\mu$l of the concentrate described above. The reaction mixtures were incubated 2–18 hr. The reactions were terminated by addition of NaDodSO$_4$/polyacrylamide gel electrophoresis sample buffer and boiling. Enzyme activity was detected by autoradiography of the $^{125}$I-labeled substrates after separation by electrophoresis (12).

**Reduction and Alkylation.** Glycoproteins (2 mg/ml) were incubated for 2 hr at 37°C in 6 M guanidine·HCl/10 mM dithiothreitol/0.1 M Tris·HCl, pH 7.2. Iodacetamide (final concentration, 50 mM) was added, and this mixture was incubated for 1 hr in the dark at 22°C and then dialyzed against $P_i$/NaCl.

**Transfer of Galactose to Deglycosylated Glycoproteins.** Conditions for [$^3$H]galactose transfer to *N*-acetylglucosamine residues at the nonreducing ends of proteoglycans (deglycosylated R-MuLV gp70) by UDPgalactose:*N*-acetylglucosamine galactosyltransferase (Sigma) were those described by Schindler *et al.* (13). UDP-[1-$^3$H]galactose (11.6 Ci/mmol) was obtained from New England Nuclear.

**Analysis of Released Glycans.** Endo F cleavage products were isolated on Sephadex G-50 and labeled with [$^3$H]borohydride (Amersham) as described (2). Analysis of cleavage products was carried out using a Waters HPLC system equipped with a 0.4 × 30 cm Waters carbohydrate analysis column. Glycans were eluted by using a linear gradient of 65–35% acetonitrile as described (14).

## RESULTS

**Assay Conditions and Physical Properties of Endo F.** The standard assay for endo F was based on the decrease in the molecular weight of R-MuLV gp70 shown by NaDodSO$_4$/polyacrylamide gel electrophoresis after the enzyme was incubated with $^{125}$I-labeled virus. As shown in Fig. 1*A*, endo F activity can be detected in column fractions by this assay. The

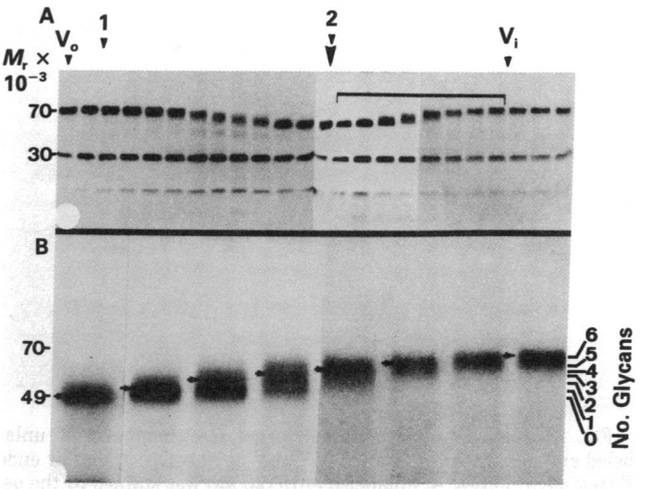


FIG. 1. Assay and cleavage characteristics of endo F. Ammonium sulfate fractions were applied to a 1.5 × 50 cm column of LKB ACA54 in $P_i$/NaCl/5 mM EDTA. The fractions were then assayed for glycosidase activity using $^{125}$I-labeled R-MuLV as substrate and NaDodSO$_4$/polyacrylamide gel electrophoresis and autoradiography to assess the extent of cleavage of the R-MuLV gp70. (*A*) Electrophoresis of column fractions from the void volume ($V_o$) to the included volume ($V_i$). Bovine serum albumin and myoglobin elute at positions 1 and 2, respectively. The peak of endo F activity (large arrow), as shown by the decrease in gp70 from $M_r$ 70,000 to $M_r$ 49,000 coincides with the elution volume of myoglobin. Nonglycosylated viral proteins p30 and p15 are unaffected by this treatment. (*B*) Enlargement of the dilution curve generated in the analysis shown in *A* (bracketed area) shows that endo F releases glycans in a series of discrete steps. A total of seven bands are generated, indicating that six glycans are released.

enzyme by this analysis comigrates with myoglobin; gp70 is reduced from $M_r$ 70,000 to $M_r$ 49,000 at the highest concentration of enzyme with no degradation of other viral proteins. These assays are carried out in the presence of EDTA. In the absence of EDTA, all viral components are degraded to polydispersed low molecular weight material, indicating the presence of an associated EDTA-inhibitable protease (not shown). An enlargement of the dilution curve for enzyme generated by this column fractionation analysis (Fig. 1*B*) shows that endo F removes carbohydrate from gp70 in discrete steps, in a manner expected of an endo- rather than an exoglycosidase. Such an analysis reveals the removal of at least six carbohydrate chains from gp70 (Fig. 1*B*, arrows). Although the amino acid sequence of R-MuLV gp70 has not been published, the sequence of the closely related Moloney leukemia virus gp70 (15) indicates seven possible sites for glycosylation. Whether R-MuLV gp70 has only six chains or we only detect six by this analysis is yet to be determined. However, previous analyses of the high-mannose precursors of other retrovirus gp70s using endo H such as those bearing the $G^+_{IX}$ marker showed the release of six chains (16).

Endo F is irreversibly inhibited by NaDodSO$_4$ but not by nonionic detergents, NaDodSO$_4$ in the presence of nonionic detergents, or 2-mercaptoethanol.

**Nature of Endo F Cleavage.** Microgram amounts of unlabeled gp70 were treated with endo F in the presence of EDTA and the products were subjected to NaDodSO$_4$/polyacrylamide gel electrophoresis. The gels were stained by the periodic acid–Schiff procedure (17) (Fig. 2). Untreated gp70 (lane A, arrow) is strongly stained while the endo F-treated sample (lane B, arrow) has no PAS-reactable material at $M_r$ 70,000 and only a little staining is observed slightly above the $M_r$ 49,000 position, primarily in an incompletely deglycosylated product. Subsequent staining with Coomassie brilliant blue revealed bands

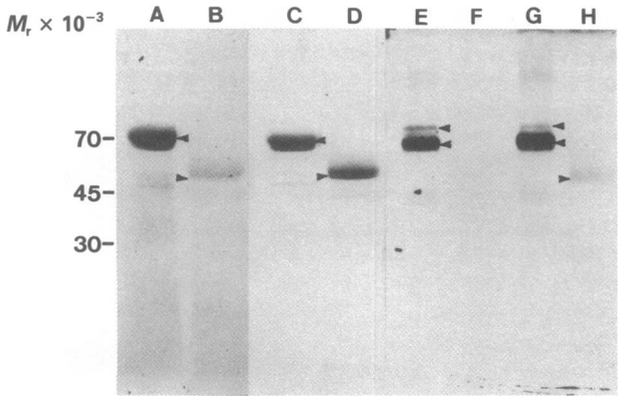


FIG. 2. NaDodSO$_4$/polyacrylamide gel electrophoresis of unlabeled and metabolically labeled R-MuLV gp70 before and after endo F treatment. Lanes: A, unlabeled gp70 (20 $\mu$g) was stained by the periodic acid–Schiff procedure (arrow); B, gp70 was treated with endo F and then treated as in lane A; C, Coomassie blue stain of unlabeled gp70 (20 $\mu$g; arrow); D, as in lane C after cleavage with endo F (arrow); E, Pr85$^{env}$ precursor (upper arrow) and gp70 (lower arrow) were metabolically labeled with [$^3$H]mannose; F, precursor and gp70 after treatment with endo F showing that all [$^3$H]mannose label is removed; G, Pr85$^{env}$ (upper arrow) and gp70 (lower arrow) were metabolically labeled with [$^3$H]glucosamine; H, precursor and gp70 after treatment with endo F showing that all but a trace (arrow) of [$^3$H]glucosamine is removed.

of equal intensity at $M_r$ 70,000 (lane C, arrow) and 49,000 (lane D, arrow) in the control and experimental gel lanes, respectively. Further indication of the loss of carbohydrate is revealed where gp70 and its $M_r$ 85,000 precursor were metabolically labeled with [$^3$H]mannose (lanes E and F) or [$^3$H]glucosamine (lanes G and H) and cleaved with endo F. All [$^3$H]mannose label (lane F) and all but a trace of [$^3$H]glucosamine label (lane H, arrow) are removed from these molecules by treatment with endo F. The envelope precursor molecule (lanes E and G, upper arrows) of murine retroviruses has been reported to be the high-mannose type (18) whereas processed gp70 molecules (lower arrows) are of the complex type (18).

To characterize the cleavage site of endo F, we examined whether R-MuLV gp70 that had been deglycosylated by endo F could act as an acceptor for the transfer of galactose from UDP-galactose by galactosyltransferase. This enzyme has previously been shown to transfer galactose to nonreducing *N*-acetylglucosamine residues of glycoproteins (19). Thus, if endo F cleaved the *N*,*N*′-diacetylchitobiose core (like endo H and endo D), a nonreducing *N*-acetylglucosamine would be exposed and galactose transfer would occur. Fig. 3 shows that galactose is transferred to deglycosylated gp70 in a substrate-dependent manner. Neither R-MuLV gp70 that has not been treated with endo F nor endo F alone acts as an acceptor.

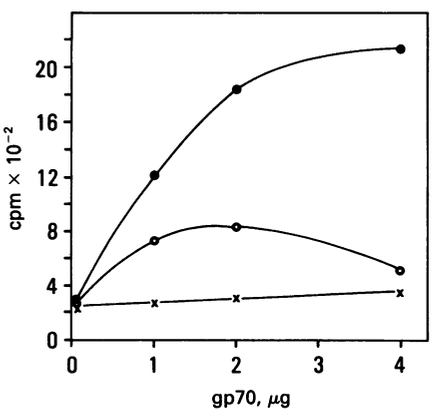


FIG. 3. Transfer of [$^3$H]galactose to R-MuLV before and after treatment with endo F using UDPgalactose:*N*-acetylglucosamine galactosyltransferase, which transfers galactose from UDP-galactose to *N*-acetylglucosamine at the nonreducing ends of proteoglycans (13). Untreated R-MuLV (○), R-MuLV treated with endo F (●), and endo F alone (×) were used as substrates for the transferase in the presence of [$^3$H]UDP-galactose. The concentration of gp70 was determined by radioimmunoassay.

**Tests for the Presence of Exoglycosidase Activity.** To determine whether exoglycosidase activity was also present, we assayed endo F preparations against 12 different *p*-nitrophenylglycosides and detected no activity. Under the same conditions, extracts of *Dictyostelium discoideum*, which are known to possess exoglycosidase activities (20), rapidly hydrolyzed the substrates. Moreover, we attempted to cleave two purified and characterized glycan moieties (see *Materials and Methods* for structures), and neither was degraded as judged by HPLC. These data indicated that endo F has no detectable exoglycosidase activity.

**Activity of Endo F on Diverse Glycoprotein Substrates.** The NaDodSO$_4$/polyacrylamide gel electrophoresis analysis of various substrates susceptible to endo F is shown in Fig. 4. As shown above, R-MuLV gp70 is degraded from $M_r$ 70,000 (lane A, arrow) to $M_r$ 49,000 (lane B, arrow) by endo F. The major glycoproteins of both Pichinde virus (lanes C and D) and lymphocytic choriomeningitis (LCM) virus (lanes E and F) are also susceptible. The major glycoprotein of Pichinde virus is degraded from $M_r$ 33,000 (lane C, arrow) to $M_r$ 25,000 (lane D, arrow) by endo F. LCM virus has two glycoproteins, gp1 and gp2 (lane E, arrows) of $M_r$ 45,000 and $M_r$ 35,000, respectively (21). After endo F treatment, both gp1 and gp2 are degraded to $M_r$ 26,000–29,000 (lane F, arrow). In experiments in which these two glycoproteins were separately recovered after electrophoresis separation and then cleaved, gp1 was partially re-

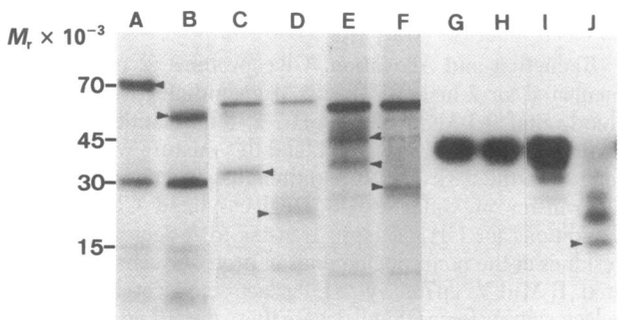


FIG. 4. NaDodSO$_4$/polyacrylamide gel electrophoresis of various $^{125}$I-labeled substrates before and after treatment with endo F. Lanes: A, R-MuLV before treatment with endo F showing gp70 at $M_r$ 70,000 (arrow); B, R-MuLV after treatment with endo F showing the decrease of gp70 to $M_r$ 49,000 (arrow) with no cleavage of other viral constituents; C, Pichinde virus before treatment with endo F showing the major glycoprotein (arrow) of $M_r$ 33,000; D, Pichinde virus after treatment with endo F showing the decrease to $M_r$ 25,000 of the major glycoprotein (arrow); E, LCM virus before treatment with endo F showing the major glycoproteins gp1 (upper arrow) and gp2 (lower arrow); F, LCM virus after cleavage with endo F (the arrow indicates primarily gp2 after cleavage to $M_r$ 28,000; gp1 is partially resistant to cleavage in the presence of nonionic detergents and appears as a series of partial cleavage products of $M_r$ 44,000–26,000); G, untreated $\alpha_1$-acid glycoprotein; H, $\alpha_1$-acid glycoprotein after treatment for 2 hr with endo F in the presence of Nonidet P-40; I, $\alpha_1$-acid glycoprotein after treatment for 2 hr with endo F in the presence of Nonidet P-40 and 2-mercaptoethanol (1%); J, as in I, but reaction was carried out for 16 hr. Six bands are observed, indicating removal of five glycan chains and culminating in a final protein backbone (arrow) of $M_r$ 20,000.

sistant to cleavage and the majority of material remained as a series of partial cleavage products (data not shown). gp2, however, was readily cleaved in the presence of nonionic detergents and represents the majority of the material at the arrow in Fig. 4, lane F. The glycan structures of Pichinde and LCM viruses are unknown, although the relative labeling patterns suggest that they are of the complex type (M. Buchmeier, personal communication). HLA-A and -B antigens, which contain a single complex glycan (22), are completely deglycosylated by endo F whereas the HLA-DR antigen, which contains both complex and high-mannose structures (23), was unaffected (data not shown).

Subsequent tests of endo F activity against various other substrates, including the high-mannose glycoprotein ovalbumin (3), the complex glycoproteins fetuin (24), immunoglobulin G (25), and $\alpha_1$-acid glycoprotein (26), and the hybrid glycoproteins ovomucoid (27) and stem bromelain (28), suggested that many were only partially susceptible or totally resistant to cleavage by endo F in the presence of nonionic detergent. Serendipitously, we found that, although influenza hemagglutinin was only slightly susceptible, after it had been reduced and alkylated, its carbohydrate side chains were rapidly removed. Thus, we reduced and alkylated some of the substrates previously found to be resistant, subsequently tested them, and found that they had become susceptible to deglycosylation by endo F. Bromelain, which is susceptible to neither endo H nor endo D (29), is deglycosylated by endo F under these conditions. The denaturing conditions (i.e., 6 M guanidine·HCl) alone were not sufficient to render these glycoproteins susceptible. However, high concentrations of 2-mercaptoethanol (1.0%) in the presence of Nonidet P-40 could mimic the effect of reduction and alkylation. Similar findings with endo H have been reported previously (4). Untreated $\alpha_1$-acid glycoprotein (Fig. 4, lane G) has a $M_r$ of 44,000; treatment with endo F in the presence of Nonidet P-40 has little effect and only a slight reduction in molecular weight is observed. However, after treatment with endo F in the presence of Nonidet P-40 and 2-mercaptoethanol (Fig. 4, lanes I and J), $\alpha_1$-acid glycoprotein becomes susceptible and carbohydrate chains are removed in discrete steps. This analysis (Fig. 4, lane J) indicates that five chains are removed, yielding a final protein backbone of $M_r$ 20,000. These results are in agreement with values previously reported for this glycoprotein (26).

Evidence that endo F cleaves high-mannose glycoproteins via removal of intact glycans is shown in Fig. 5. [$^3$H]Mannitol is slightly retained in this system and is not a product of endo F cleavage of ovalbumin. Discrete glycan peaks are detected as cleavage products and retention times are consistent with the range of mannose-rich oligosaccharides reported for ovalbumin (14).

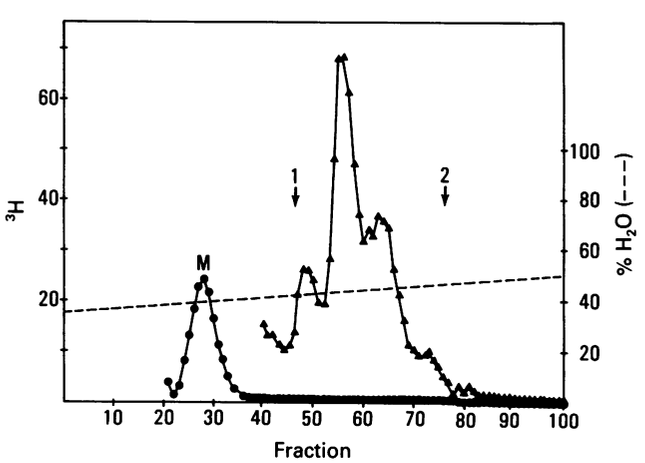


FIG. 5. HPLC of ovalbumin glycans released by endo F. Ovalbumin (10 mg) was treated with endo F for 18 hr and the products were subjected to Sephadex G-50 chromatography. Included carbohydrates, detected by the phenol-sulfuric acid assay, were pooled, lyophilized, labeled with [$^3$H]borohydride (2), and analyzed as described (14). [$^3$H]Mannitol standard (M; ●, cpm × $10^{-4}$) is slightly retained. Glycans released by endo F treatment (▲, cpm × $10^{-3}$) are resolved as heterogeneous peaks with retention times approximating those previously reported for ovalbumin using this system (14). ↓: 1 and 2, retention times of linear and branched chain, respectively, complex carbohydrates, whose structures are described in *Materials and Methods*.

## DISCUSSION

*Flavobacterium meningosepticum* has been shown to secrete an endoglycosidase (endo F) that has properties useful for the study of the structure and function of glycoproteins. As with other endoglycosidases (e.g., endo H and endo D), endo F hydrolyzes the glycosidic bond of the *N,N'*-diacetylchitobiose core structure N-linked to asparagine, thus removing the bulk of the glycan moiety *en bloc*. Unlike endo H and endo D, endo F appears to have broader specificity in as much as it can deglycosylate both the high-mannose and the complex type of glycoprotein. Similar broad specificity has been described for an endoglycosidase from almond extract (29). However, in contrast to endo F, that enzyme appears to act on glycopeptides only and is less efficient as the number of amino acid residues increases (29).

Endo F is extremely useful for preparation of intact glycans and protein chains free of their glycan moieties. We have used endo F to deglycosylate proteolytically derived fragments of R-MuLV gp70, thus allowing more accurate estimation of molecular weights. Moreover, when monoclonal antibodies against R-MuLV gp70 were tested against control and deglycosylated material, drastic differences were found: some antisera lost reactivity while others gained it. Thus, use of the enzyme allows more substantial characterization of antigenic sites (unpublished).

These studies indicate that extensive heterogeneity exists in the availability of the glycan–protein interface among N-linked glycoproteins as evidenced by differential susceptibility to endo F. The enzyme serves as a useful reagent for monitoring these differences and further studies might yield generalities that could be related to glycoprotein biosynthesis and functions.

We thank Dr. Richard Lerner for valuable discussions and use of laboratory facilities and Dr. Marsha Rosner of the Massachusetts Institute of Technology for helpful suggestions and preliminary column analyses of cleavage products. We also thank Dr. Frank Dixon and Ms. Patricia McConahey of this institute for performing radioimmunoassays and Dr. David Mirelman of the Weizmann Institute for helpful advice. The technical assistance of Mses. Paula Eisenhart and Ann Cibulsky is gratefully acknowledged. This research was supported in part by National Institutes of Health Grants CA 25803 and CA 25533 and in conjunction with the Committee for the Study of Molecular Genetics. This is publication no. 2669 from the Research Institute of Scripps Clinic.

1. Tarentino, A. L. & Maley, F. (1974) *J. Biol. Chem.* **249**, 811–817.
2. Koide, N. & Muramatsu, T. (1974) *J. Biol. Chem.* **249**, 4897–4904.
3. Tai, T., Yamashita, K., Ogata-Arakawa, M., Koide, N., Muramatsu, T., Shintaro, I., Inoue, Y. & Kobata, A. (1975) *J. Biol. Chem.* **250**, 8569–8575.
4. Tarentino, A. L., Plummer, T. & Maley, F. (1974) *J. Biol. Chem.* **249**, 818–824.
5. Hubbard, S. C. & Robbins, P. W. (1979) *J. Biol. Chem.* **254**, 4568–4576.
6. King, E. O. (1959) *Am. J. Clin. Path.* **31**, 241–247.
7. Davis, R. W., Botstein, D. & Roth, J. R. (1980) *Advanced Bacterial Genetics* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), p. 203.
8. Strand, M. & August, J. T. (1973) *J. Biol. Chem.* **248**, 5627–5633.

9. Wylie, D. E. & Klinman, N. R. (1981) *J. Immunol.* **127**, 194–198.
10. Elder, J. H., Jensen, F. C., Bryant, M. L. & Lerner, R. A. (1977) *Nature (London)* **267**, 23–28.
11. Hartley, J. W. & Rowe, W. P. (1975) *Virology* **65**, 128–134.
12. Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685.
13. Schindler, M., Mirelman, D. & Schwarz, U. (1976) *Eur. J. Biochem.* **71**, 131–134.
14. Mellis, S. J. & Baenziger, J. U. (1981) *Anal. Biochem.* **114**, 276–280.
15. Shinnick, T. M., Lerner, R. A. & Sutcliffe, J. G. (1981) *Nature (London)* **293**, 543–548.
16. Rosner, M. R., Tung, J-S., Hopkins, N. & Robbins, P. W. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 6420–6424.
17. Fairbanks, G., Steck, T. L. & Wallach, D. F. H. (1971) *Biochemistry* **10**, 2606–2617.
18. Rosner, M. R., Grinna, L. S. & Robbins, P. W. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 67–71.
19. Priels, J. P., Dolmans, M., Schindler, M. & Sharon, N. (1976) *Eur. J. Biochem.* **66**, 579–582.
20. Loomis, W. F. (1975) *Dictyostelium Discoideum: A Developmental System* (Academic, New York), p. 120.
21. Buchmeier, M. J. & Oldstone, M. B. A. (1979) *Virology* **99**, 111–120.
22. Parham, P., Alpert, B. N., Orr, H. T. & Strominger, J. L. (1977) *J. Biol. Chem.* **252**, 7555–7567.
23. Shackelford, D. A., Lampson, L. A. & Strominger, J. L. (1981) *J. Immunol.* **127**, 1403–1410.
24. Baenziger, J. U. & Fiete, D. (1979) *J. Biol. Chem.* **254**, 789–795.
25. Baenziger, J., Kornfeld, S. & Kochwa, S. (1974) *J. Biol. Chem.* **249**, 1897–1903.
26. Satake, M., Okuyama, T., Ishihara, K. & Schmid, K. (1965) *Biochem. J.* **95**, 749–757.
27. Bayard, B. & Montreuil, J. (1974) *Colloq. Int. CNRS* **221**, 209–218.
28. Ishihara, H., Takahashi, N., Oguri, S. & Tejima, S. (1979) *J. Biol. Chem.* **254**, 10715–10719.
29. Takahashi, N. & Nishibe, H. (1978) *J. Biochem.* **84**, 1467–1473.