Deglycosylation of α_1-Proteinase Inhibitor by Endo-β-*N*-acetylglucosaminidase F†

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Received January 4, 1985; Revised Manuscript Received April 24, 1985

ABSTRACT: The glycosidase endo-β-*N*-acetylglucosaminidase F (endo F) from *Flavobacterium meningosepticum* was used for the deglycosylation of rat α_1-proteinase inhibitor (α_1PI). α_1PI containing three oligosaccharide side chains of the complex type was isolated from rat serum or from the medium of rat hepatocyte primary cultures. High-mannose-type α_1PI or hybrid-type α_1PI was isolated from the media of hepatocytes treated with 1-deoxymannojirimycin or swainsonine, respectively. The susceptibility of complex-type α_1PI to endo F was studied in the presence of various detergents. 3-[(3-Cholamidopropyl)dimethylammonio]-1-propanesulfonate and octyl glucopyranoside turned out to be most effective. In the absence of detergents, digestion of α_1PI with high concentrations of endo F and/or long times of incubation led to the formation of α_1PI with one and two oligosaccharide side chains. In the presence of 0.5% octyl glucopyranoside, the major cleavage products were unglycosylated α_1PI and α_1PI carrying one carbohydrate side chain. In contrast to the complex-type α_1PI, the high-mannose type can be totally deglycosylated by endo F even in the absence of detergents. The susceptibility of the hybrid-type α_1PI to endo F is between that of the complex and the high-mannose types.

In previous experiments, we examined the effect of N-glycosylation on the rates of secretion of the proteinase inhibitors α_2-macroglobulin, α_1-acute-phase globulin, and α_1-proteinase inhibitor (α_1PI)[1] (Geiger et al., 1982; Gross et al., 1982; Andus et al., 1983a) and on the biological activity of α_1PI measured by its capability to form complexes with elastase (Andus et al., 1983b). To extend these studies on the influence of the carbohydrate side chains on various properties, it is necessary to obtain the glycoproteins of interest in their unglycosylated forms and in rather large amounts.

Glycoproteins have been deglycosylated by chemical (Baker et al., 1972; Mort & Lamport, 1977; Glassman et al., 1978; Sairam, 1980; Kalyan & Bahl, 1981; Edge et al., 1981; Manjunath & Sairam, 1982; Takasaki et al., 1982; Ogata & Lloyd, 1982) as well as by enzymatic methods (Hughes & Jeanloz, 1964a,b; Koide & Muramatsu, 1974; Tarentino & Maley, 1974; Glasgow et al., 1977; Trimble & Maley, 1977a; Takahashi & Nishibe, 1981; Plummer & Tarentino, 1981; Tarentino & Plummer, 1982; Elder & Alexander, 1982; Shifrin et al., 1983; Gross et al., 1983a; Taga et al., 1984; Lienhard et al., 1984). In the former case, the deglycosylation is mostly incomplete and results in the formation of denatured proteins. In the case of enzymatic deglycosylation, the reaction conditions are milder than those used for the chemical deglycosylation, although most of the deglycosylation reactions have been carried out in the presence of detergents. Furthermore, the various glycosidases described exhibit different cleavage specificities. There are glycosidases cleaving either high-mannose glycans such as endoglucosaminidase H (Tarentino & Maley, 1974; Trimble & Maley, 1977a) or both high-mannose and complex glycoproteins (Plummer & Tarentino, 1981; Tarentino & Plummer, 1982). Recently, Elder & Alexander (1982) described a novel glycosidase from *Flavobacterium meningosepticum*, designated as endoglucosaminidase F, which cleaves both high-mannose and complex glycoproteins. In a subsequent paper (Plummer et al., 1984), it has been demonstrated that the endoglucosaminidase F preparation also contains peptide:*N*-glycosidase activity.

In the present paper, we describe the use of endo F for the deglycosylation of α_1PI. The successive removal of the three N-linked complex-type oligosaccharide side chains of α_1PI has been studied in the absence and presence of various detergents.

Materials and Methods

Chemicals. L-[^{35}S]Methionine (>1000 Ci/mmol) was purchased from Amersham-Buchler (Braunschweig). Protein A–Sepharose CL-4B was from Pharmacia (Freiburg), tunicamycin was from Calbiochem-Behring Corp. (Giessen), Protosol was from New England Nuclear Corp. (Dreieichenhain), octyl β-D-glycopyranoside, Triton X-100, cholic acid, and CHAPS were from Sigma (Taufkirchen), Nikkol was from Nikko Chemicals (Tokyo, Japan), and sodium dodecyl sulfate and Nonidet P 40 were from Roth (Karlsruhe). Phenylmethanesulfonyl fluoride was purchased from Serva (Heidelberg). Kallikrein trypsin inhibitor (Trasylol) was a generous gift of the Bayer AG (Wuppertal). Leupeptin, chymostatin, pepstatin, and antipain were obtained from the Peptide Institute Inc. (Osaka, Japan).

Preparation of Rat Hepatocyte Monolayers and Their Radioactive Labeling. Suspensions of rat hepatocytes were prepared as described by Bischoff et al. (1976) and other publications from this laboratory (Gross et al., 1982, 1983b,c). Labeling of hepatocytes was carried out as detailed in these papers (Gross et al., 1982, 1983b,c).

† This work was supported by grants from the Deutsche Forschungsgemeinschaft, Bonn, and the Fonds der Chemischen Industrie, Frankfurt.

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[1] Abbreviations: α_1PI, α_1-proteinase inhibitor; CHAPS, 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate; endo D, endo-β-*N*-acetylglucosaminidase D; endo F, endoglycosidase F (endo-β-*N*-acetylglucosaminidase F); endo H, endo-β-*N*-acetylglucosaminidase H; Nikkol, *n*-dodecyl octaethylene glycol monoether; NP 40, Nonidet P 40; SDS–PAA, sodium dodecyl sulfate–polyacrylamide; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; EDTA, ethylenediaminetetraacetic acid; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride.

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Purification of Rat α_1-Proteinase Inhibitor from Serum and Preparation of a Specific Antiserum. α_1PI was purified from rat serum to homogeneity by ammonium sulfate precipitation (50% and a subsequent 80% saturation with ammonium sulfate) followed by affinity chromatography on activated thiol–Sepharose 4B as described by Laurell et al. (1975) and on concanavalin A–Sepharose according to Saklatvala et al. (1976) and by gel filtration on Sephacryl S-200. The protocol for the immunization of rabbits was as described for α_2-macroglobulin (Andus et al., 1983a).

Immunoprecipitation and Electrophoretic Separation of α_1-Proteinase Inhibitor. Immunoprecipitations were carried out as previously described (Gross et al., 1982, 1983b; Andus et al., 1983a). For the quantification of the radioactivity incorporated into α_1PI carrying three, two, one, and zero carbohydrate side chain(s), the respective bands identified by fluorography (Bonner & Laskey, 1974) were cut from the SDS–PAA gels (King & Laemmli, 1979), solubilized with protosol/water (9:1 v/v) at 45 °C overnight, and counted in a liquid scintillation spectrometer.

Preparation of Endoglucosaminidase F. A culture of *Flavobacterium meningosepticum* was a generous gift from Drs. S. Alexander and J. H. Elder, La Jolla, CA. The bacteria were grown, and the partial purification of endo F was carried out according to a protocol of the authors mentioned above.

Cells grown to stationary phase were removed from the culture medium by centrifugation at 5000*g* for 15 min. The supernatant was concentrated by 80% saturation with solid ammonium sulfate overnight, followed by centrifugation at 27000*g* for 30 min. The precipitate was dissolved in 50 mM EDTA and the solution centrifuged at 6000*g* for 15 min to remove insoluble material. In subsequent steps, the protein was concentrated by addition of solid ammonium sulfate to 30%, 50%, and 80% saturation and centrifugation, respectively. The sediments of the three ammonium sulfate fractions were dissolved in 50 mM EDTA and analyzed for enzymatic activity. The main endo F activity was found in the 30–50% fraction. This partially purified enzyme preparation was used for the deglycosylation experiments.

Conditions for Deglycosylation of α_1-Proteinase Inhibitor. Except when otherwise stated, the enzyme reactions were carried out in 50 mM sodium phosphate buffer, pH 6.1, and 35 mM EDTA at 37 °C. Endo F, dissolved in the same buffer, was added to the incubation mixtures to start the reactions. To prevent evaporation, a layer of paraffin oil was placed on top of the incubation mixture. Incubation times and concentrations of detergents and endo F were varied as mentioned in the legends to the figures. The reactions were terminated by the addition of sample buffer used for SDS–PAGE and heat treatment at 95 °C for 5 min.

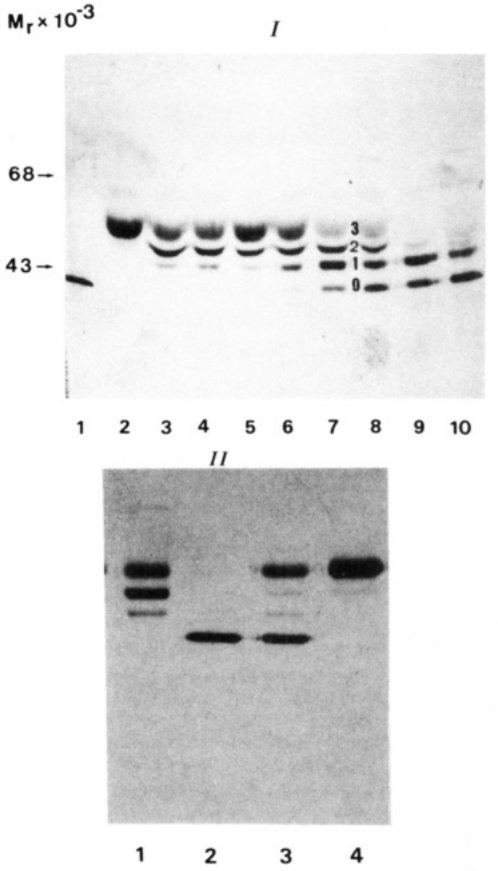

FIGURE 1: Cleavage pattern of rat serum α_1-proteinase inhibitor by endoglucosaminidase F in the absence and presence of various detergents. Rat hepatocyte primary cultures (7.8×10^6 cells per dish) were incubated with 100 μCi of [^{35}S]methionine for 4.5 h as previously described (Gross et al., 1982; Andus et al., 1983a). 100-μL aliquots of centrifuged (10000*g*, 15 min) culture medium containing [^{35}S]-methionine-labeled α_1PI were added to a total volume of 300 μL of 50 mM phosphate buffer, pH 6.1, containing 2 or 4.2 μg of endo F and 35 mM EDTA, 20 μg/mL each of leupeptin, pepstatin, chymostatin, antipain, and kallikrein trypsin inhibitor, and 1 mM phenylmethanesulfonyl fluoride without and with various detergents in final concentrations of 0.5% at 37 °C for 2.5 h. After incubation, the reaction products were immunoprecipitated and subjected to SDS–PAGE as described under Materials and Methods. (I) Unglycosylated α_1PI from tunicamycin-treated (3 μg/mL) hepatocytes (lane 1); untreated α_1PI (lane 2); α_1PI plus endo F, no detergent (lane 3); α_1PI plus endo F in the presence of 0.5% Nikkol (lane 4) or in the presence of 0.5% SDS (lane 5); NP 40 (lane 6); Triton X-100 (lane 7); cholic acid (lane 8); CHAPS (lane 9); octyl glucopyranoside (lane 10). Bovine serum albumin (68 000) and ovalbumin (43 000) were used as molecular weight markers. (II) α_1PI plus 2 μg of endo F without detergent (lane 1); unglycosylated α_1PI from hepatocytes treated with 0.1 (lane 2), 0.01 (lane 3), and 0.001 μg/mL (lane 4) tunicamycin.

RESULTS

When hepatocyte primary cultures were incubated with [^{35}S]methionine for 4.5 h, radioactively labeled α_1PI was secreted into the medium. The secreted α_1PI was immunoprecipitated and subjected to SDS–PAGE and fluorography. A single radioactive band with an apparent molecular weight of 54 000 was detected on the fluorogram (Figure 1I, lane 2). It has been shown by several authors that human α_1PI contains three N-linked complex-type oligosaccharide side chains (Mega et al., 1980a,b; Carrell et al., 1981; Hodges & Chan, 1982). Similarly, three carbohydrate side chains are present in rat α_1PI. This has been demonstrated by incomplete inhibition of glycosylation with tunicamycin (Gross et al., 1982), by incomplete digestions of high-mannose-type α_1PI by endo H (Carlson & Stenflo, 1982), and by analysis of the carbohydrate composition of rat serum α_1PI (Ikehara et al., 1981). In previous work, we have identified unglycosylated α_1PI by immunoprecipitation from tunicamycin-treated hepatocytes as well as from the hepatocyte medium (Geiger et al., 1982). An apparent molecular weight of 41 000 was estimated for the unglycosylated form of α_1PI (Figure 1I, lane 1).

Hepatocyte medium containing ^{35}S-labeled α_1PI was incubated with endo F in the absence and presence of a series of detergents in final concentrations of 0.5%. Figure 1I shows that endo F removes the carbohydrate side chains of α_1PI, resulting in differently glycosylated forms, which can be separated electrophoretically. α_1PI carrying three, two, one, and zero oligosaccharide side chain(s) can be visualized on the gel as indicated by the corresponding numbers between lane 7 and 8. It is demonstrated in Figure 1 that the extent

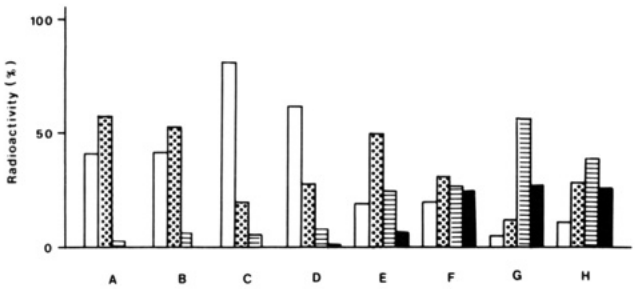

FIGURE 2: Distribution of α_1-proteinase inhibitor deglycosylation products after endoglucosaminidase F treatment in the absence and presence of various detergents. The radioactively labeled bands of the various α_1PI forms of Figure 1 were cut out from the gels, and the radioactivity was determined. The radioactivity of all bands in each lane of Figure 1 was set at 100%, and the radioactivities of the individual bands were related to it. (A) to (H) correspond to lanes 3–10 of Figure 1. (□) Fully glycosylated α_1PI carrying three oligosaccharide side chains; (⊡) α_1PI with two side chains; (⊟) α_1PI with one side chain; (■) completely deglycosylated α_1PI.

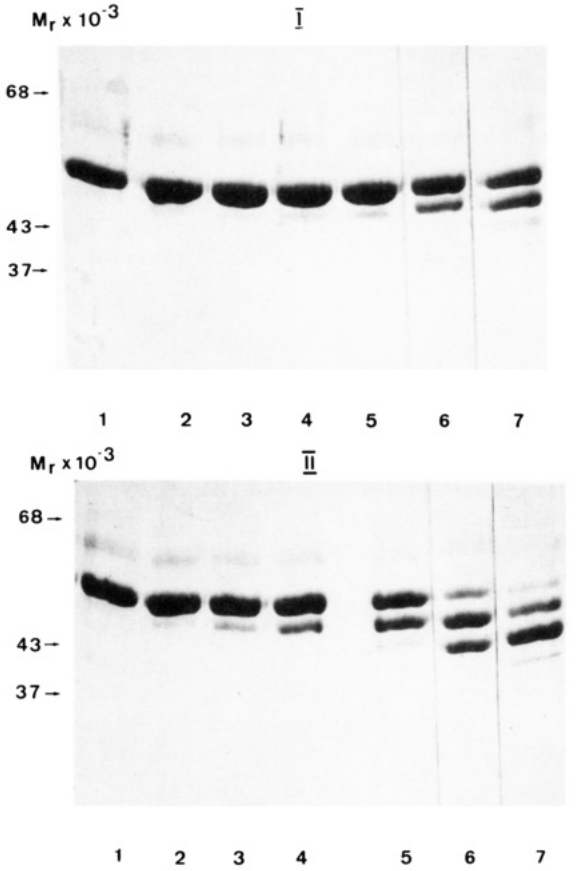

FIGURE 3: Time course of deglycosylation of α_1-proteinase inhibitor with endoglucosaminidase F in the absence or presence of octyl glucopyranoside. In a total volume of 40 μL, 6 μg of α_1PI isolated from rat serum was incubated with 0.14 μg of endo F in 75 mM phosphate buffer, pH 6.1, and 50 mM EDTA without (I) or with (II) octyl glucopyranoside in a final concentration of 0.5% at 37 °C for 1 (lane 2), 5 (lane 3), 15 (lane 4), 30 (lane 5), or 120 min (lane 6) and overnight (lane 7). Incubation of α_1PI overnight, but no endo F addition (lane 1). The reaction was stopped by heat treatment at 95 °C for 5 min. The lyophilized samples were subjected to SDS–PAGE and subsequently stained with Coomassie Blue R-250.

of deglycosylation differs for the various detergents used. The comparison between α_1PI partially deglycosylated by endo F with α_1PI synthesized and secreted by hepatocytes in the presence of low concentrations of tunicamycin (Figure 1II) shows identical electrophoretic mobilities of the different α_1PI forms after tunicamycin and endo F treatment. To compare the effect of the different detergents on the deglycosylation of α_1PI, the radioactive bands of α_1PI corresponding to the three, two, one, and zero carbohydrate side-chain forms were excised from the SDS–PAA gel, and their radioactivity was determined (Figure 2). It is evident that in the absence of detergents (A) mainly one carbohydrate side chain is removed by the action of endo F. The addition of 0.5% Nikkol (B) results in the same pattern; SDS (C) and NP 40 (D) are less effective. In the presence of 0.5% Triton X-100 (E) or cholic acid (F), all four possible α_1PI forms are detected. The most effective detergents in terms of complete deglycosylation of α_1PI by endo F are CHAPS (G) and octyl glucopyranoside (H), where α_1PI with one and zero carbohydrate side chain represent the major deglycosylation products.

It was of interest to examine whether the yield of unglycosylated α_1PI could be increased either by longer times of incubation or by the use of larger amounts of endo F. α_1PI purified from rat serum was used for these experiments. The time course of the deglycosylation of α_1PI by endo F was studied in the absence (Figure 3I) and presence of octyl glucopyranoside (Figure 3II). The deglycosylation of α_1PI occurs at a faster rate in the presence of octyl glucopyranoside. As early as 1 min after endo F addition, α_1PI with two side chains is formed (Figure 3II, lane 2). In the absence of detergent, there is hardly any α_1PI with one or zero carbohydrate side chain detectable. On the other hand, when the deglycosylation is carried out in the presence of 0.5% octyl glucopyranoside for 2 h or overnight, formation of a large amount of α_1PI with one carbohydrate side chain is observed (Figure 3II, lanes 6 and 7).

When the effect of increasing concentrations of endo F on the deglycosylation of α_1PI was studied in the absence (Figure 4I) and presence of 0.5% octyl glucopyranoside (Figure 4II), a steady decrease of α_1PI with three carbohydrate side chains (open circles) was observed in both cases. In parallel, the α_1PI forms with one (squares) and zero (closed circles) carbohydrate side chain increase. Simultaneously, α_1PI with two carbohydrate side chains (triangles) increases and then decreases at the expense of the formation of α_1PI with one and zero carbohydrate side chain. Comparison of the deglycosylated α_1PI carried out without and with octyl glucopyranoside in the incubation mixture shows that the deglycosylation is more effective when octyl glucopyranoside is present, where the deglycosylation of α_1PI was almost complete at the highest endo F concentration used.

Furthermore, we examined the dependence of the octyl glucopyranoside concentration on the deglycosylation of α_1PI, since this detergent was highly effective (Figure 5). Octyl glucopyranoside concentrations of up to 0.25% led mainly to a two-carbohydrate side-chain form of α_1PI. The use of concentrations of 0.25–0.5% results in an almost complete disappearance of α_1PI with three and two carbohydrate side chains and the formation of α_1PI species with one and zero carbohydrate side chain. A further increase in octyl glucopyranoside concentration did not increase the amount of unglycosylated α_1PI.

In further experiments, we studied the endo F susceptibility of hybrid-type and high-mannose-type α_1PI obtained from the media of swainsonine- (Gross et al., 1983b) or 1-deoxymannojirimycin-treated rat hepatocytes (Gross et al., 1985), respectively. Figure 6 shows that besides the complex-type carbohydrate side chains of α_1PI also the hybrid-type and high-mannose-type oligosaccharide side chains were cleaved. After incubation with endo F in the absence of detergent, the majority of complex-type α_1PI still carries two carbohydrate side chains (lane 2), whereas the majority of the hybrid-type

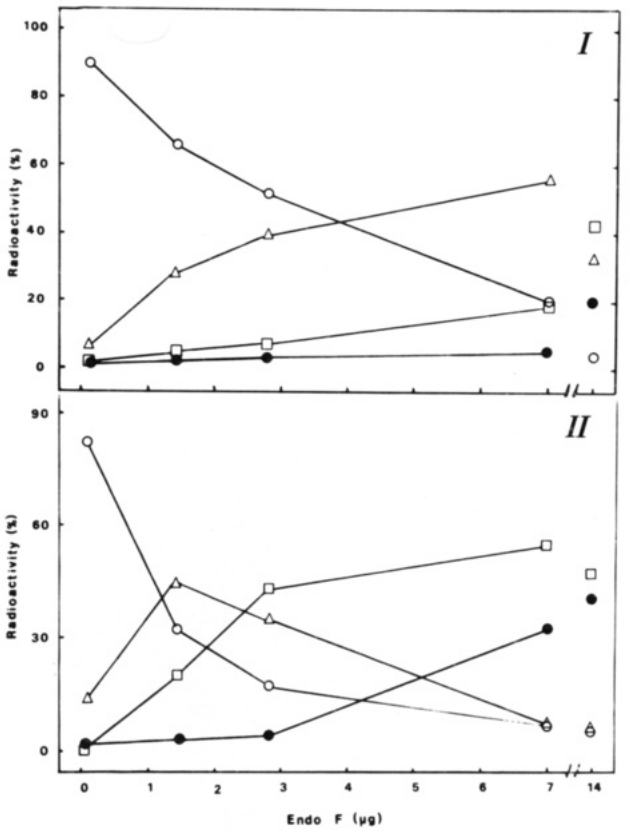

FIGURE 4: Distribution of α_1-proteinase inhibitor deglycosylation products after incubation with increasing endoglucosaminidase F concentrations in the absence and presence of octyl glucopyranoside. Rat hepatocyte primary cultures (7.8×10^6 cells per dish) were incubated with 100 μCi of [^{35}S]methionine for 4.5 h as previously described (Gross et al., 1982; Andus et al., 1983a). 100-μL aliquots of centrifuged (10000g, 15 min) culture medium containing [^{35}S]-methionine-labeled α_1PI were added to a total volume of 300 μL of 50 mM phosphate buffer, pH 6.1, containing the cocktail of proteinase inhibitors mentioned in the legend to Figure 1 and 35 mM EDTA in the absence (I) or presence of 0.5% octyl glucopyranoside (II) with increasing concentrations of endo F and incubated at 37 °C for 2.5 h. After addition of 20 mM Tris-HCl, pH 7.6, 0.14 M NaCl, 5 mM EDTA, and 1% Triton X-100, α_1PI was immunoprecipitated and subjected to SDS–PAGE and fluorography as described under Materials and Methods. The radioactively labeled bands of the various α_1PI forms were excised from the gels, and the radioactivity of the individual bands was determined. The sum of the radioactivities of all bands of each lane was set at 100%, and the radioactivities were related to it. (○) Fully glycosylated α_1PI carrying three oligosaccharide side chains; (△) α_1PI with two side chains; (□) α_1PI with one side chain; (●) completely deglycosylated α_1PI.

α_1PI (lane 5) and virtually all of the high-mannose-type α_1PI (lane 8) are totally deglycosylated.

Discussion

There does not seem to be a general rule on the function of the carbohydrate side chains on secretion, biological activity, and metabolic stability of glycoproteins (Hodges & Chan, 1982). Therefore, it is necessary to study the effect of the carbohydrate side chains on the various properties for each individual glycoprotein. A prerequisite for such studies is the availability of the glycoprotein of interest in its unglycosylated form. This can be achieved either by the inhibition of N-glycosylation in vivo by tunicamycin or by deglycosylation of the glycoprotein. Deglycosylation can be carried out by chemical or enzymatic methods. The following glycosidases have been proven useful for the deglycosylation of glycoproteins: endo H, endo D, peptide:*N*-glycosidase, and endo F. Endo H hydrolyzes the *N*,*N*′-diacetylchitobiose linkages

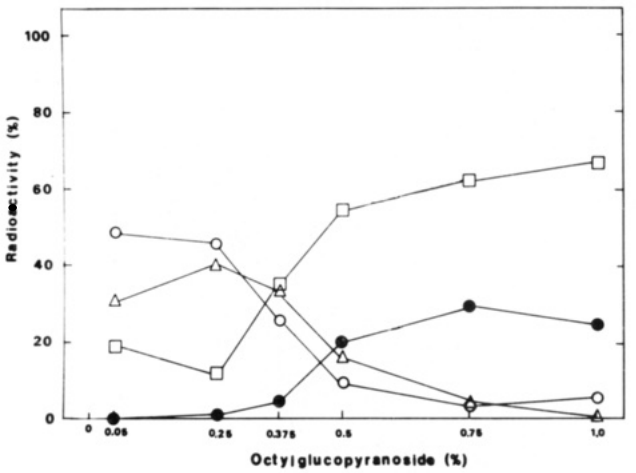

FIGURE 5: Distribution of α_1-proteinase inhibitor deglycosylation products after incubation with endoglucosaminidase F and increasing concentrations of octyl glucopyranoside. As described in the legends for Figures 1 and 4, 100-μL aliquots of centrifuged culture medium containing ^{35}S-labeled α_1PI were incubated with 4.2 μg of endo F at 37 °C for 4.5 h in the presence of increasing concentrations of octyl glucopyranoside. The radioactively labeled bands of the various α_1PI forms obtained after SDS–PAGE were excised from the gels and the radioactivities of the individual bands determined. The sum of the radioactivities of all bands of each lane was set at 100%, and the radioactivities of the individual bands were related to it. The same symbols as those in Figure 4 were used.

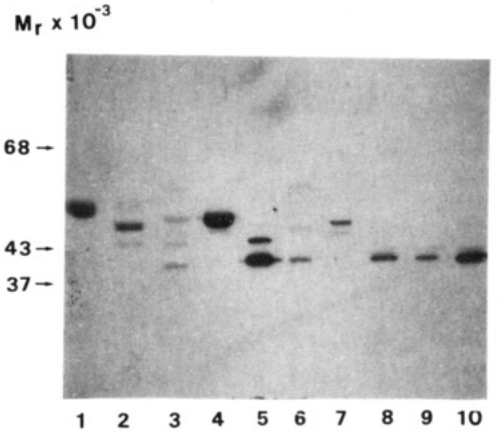

FIGURE 6: Action of endoglucosaminidase F on α_1-proteinase inhibitor with oligosaccharide side chains of complex type, hybrid type, and high-mannose type. Rat hepatocyte primary cultures were radioactively labeled with [^{35}S]methionine in the absence (lanes 1–3) or presence of 2 μg/mL swainsonine (lanes 4–6) or 100 μg/mL 1-deoxymannojirimycin (lanes 7–9) or 3 μg of tunicamycin (lane 10). 100-μL aliquots of centrifuged (10000g, 15 min) culture media containing ^{35}S-labeled α_1PI with various types of oligosaccharide side chains were incubated without (lanes 1, 4, and 7) and with 10 μg of endo F (lanes 2, 3, 5, 6, 8, and 9) in a total volume of 300 μL of 50 mM sodium phosphate buffer, pH 6.1, 35 mM EDTA, and the proteinase inhibitor cocktail described in the legend to Figure 1. Incubation with endo F was carried out at 37 °C overnight in the absence (lanes 2, 5, and 8) or presence of 0.5% octyl glucopyranoside (lanes 3, 6, and 9).

in oligosacharides and glycoproteins of the high-mannose type (Tarentino & Maley, 1974, 1975; Arakawa & Muramatsu, 1974; Kobata, 1979) and the hybrid type (Gross et al., 1983b). Modification of glycoprotein structure by alkylating agents, detergents, and thiols has been successfully employed to promote deglycosylation of high-mannose-type glycoproteins by endo H (Trimble & Maley, 1977b; Trimble et al., 1981). Endo D cleaves complex-type oligosaccharides after removal of the peripheral sugars by exoglycosidases between the two innermost GlcNAc residues (Koide & Muramatsu, 1974;

Glasgow et al., 1977; Shifrin et al., 1983). In contrast to endo H and endo D, peptide:*N*-glycosidase from almond emulsin cleaves both high-mannose- and complex-type oligosaccharides between asparagine and the first GlcNAc (Takahasi & Nishibe, 1981; Plummer & Tarentino, 1981; Tarentino & Plummer, 1982; Takahashi et al., 1982; Gross et al., 1983a). Takahashi et al. (1982) succeeded in the deglycosylation of 10% of native taka-amylase by peptide:*N*-glycosidase immobilized on Sepharose 6B. Tarentino & Plummer (1982) emphasize the importance of protein unfolding reagents such as SDS and chaotropic salts for the oligosaccharide accessibility in glycoproteins to peptide:*N*-glycosidase.

Endo F was first described by Elder & Alexander (1982). They found that the enzyme cleaves glycans of both the high-mannose and complex type linked through asparagine to the protein backbone. Their data indicated that cleavage occured 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. Elder & Alexander (1982) as well as Lienhard et al. (1984) described the necessity of denaturing conditions for the efficient deglycosylation of several glycoproteins. Different denaturing conditions such as the presence of nonionic detergents, and/or mercaptoethanol, or reduction and alkylation were found to be important for the deglycosylation of individual glycoproteins.

In a recent paper, Plummer et al. (1984) describe the presence of peptide:*N*-glycosidase F activity in their endo F preparations. The endo F preparation which has been used in our study also seems to contain a contaminating peptide: *N*-glycosidase F, since (1) the electrophoretic mobility of the α_1PI deglycosylated by endo F was identical with that of α_1PI obtained from tunicamycin-treated hepatocytes, whereas incubation of the high-mannose-type α_1PI with endo H results in an α_1PI form with a slightly lower electrophoretic mobility than the totally unglycosylated α_1PI (Gross et al., 1982), and (2) incubation of [^{3}H]fucose-labeled α_1PI synthesized by swainsoine-treated hepatocytes with endo F resulted in the removal of 90% of the [^{3}H]fucose, whereas [^{3}H]fucose could not be removed by incubation with endo H (Gross et al., 1983b).

In the present study, we compared the susceptibilities of differently glycosylated forms of α_1PI to endo F. It was found that high-mannose-, hybrid-, and complex-type glycans of α_1PI could be cleaved by endo F, and the following order of susceptibilities was established: high-mannose type > hybrid type > complex type.

Since the deglycosylation of complex-type α_1PI was most difficult, we focused on the conditions for its deglycosylation. It was found that one of the three carbohydrate side chains was easily removed, even in the absence of detergents. Among a series of detergents studied, CHAPS and octyl glucopyranoside turned out to be most effective, but even in their presence, the hydrolysis of the third oligosaccharide side chain of α_1PI was not complete. Only high-mannose-type α_1PI could completely be deglycosylated by endo F without the use of detergents. Therefore, high-mannose-type α_1PI should be used for the deglycosylation of α_1PI under nondenaturing conditions.

ACKNOWLEDGMENTS

We thank Dr. T.-A. Tran-Thi for supplying us with rat hepatocytes and Prof. K. Decker for his support of this work. We are also indebted to Drs. S. Alexander and J. E. Elder, La Jolla, CA, for the culture of *Flavobacterium meningosepticum*, Dr. T. H. Plummer, Albany, NY, for a sample of peptide:*N*-glycosidase, Dr. K. Vosbeck, Ciba-Geigy, Basel, for the gift of swainsonine, and Dr. G. Kinast, Bayer AG, Wuppertal, for 1-deoxymannojirimycin. The help of H. Gottschalk in the preparation of the manuscript is gratefully acknowledged.

Registry No. α_1PI, 9041-92-3; endo F, 37278-88-9.

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Structural Determination of the Capsular Polysaccharide of *Neisseria meningitidis* Group I: A Two-Dimensional NMR Analysis†

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Received March 7, 1985

ABSTRACT: The capsular polysaccharide antigen of *Neisseria meningitidis* group I was isolated by Cetavlon precipitation and purified by ion-exchange chromatography. The structure of the I polysaccharide was determined largely by comprehensive proton and carbon-13 nuclear magnetic resonance studies in which both one-dimensional and two-dimensional experiments were carried out directly on the I polysaccharide. The I polysaccharide is composed of the repeating unit →4)α-L-GulpNAcA(1→3)[4-OAc]β-D-Man*p*NAcA(→ in which the former residue adopts the $_4C^1$ (L) conformation and the latter residue adopts the $^4C_1$ (D) conformation. The one-bond coupling between the anomeric carbon and proton ($^1J_{^{13}C,H}$) of the 2-acetamido-2-deoxy-β-D-mannuronopyranosyl residue is not consistent with its β-D configuration. This anomalous value of $^1J_{^{13}C,H}$ for this residue is due to through-space anisotropy effects on its anomeric proton, generated by the proximity of the carboxyl group of the neighboring 2-acetamido-2-deoxy-α-L-gulurono-pyranosyl residue. The *O*-acetyl substituents of the I polysaccharide are essential for its antigenicity to group I polysaccharide-specific antibodies.

Neisseria meningitidis is a Gram-negative organism that has been classified serologically into groups A, B, C, D, 29E, L, W-135, X, Y, and Z (Gotschlich et al., 1969; Jennings, 1983; Ashton et al., 1983). Except for group D, each group produces a unique capsular polysaccharide that is the group-specific antigen, and the structures of all the polysaccharides have been determined (Jennings, 1983; Jennings et al., 1983). Ding et al. (1981) described three new meningococcal groups and designated them H, I, and K. The structure of the H polysaccharide has also been recently reported (Michon et al., 1984; Van der Kaaden et al., 1984). As a continuation of our structural studies on the meningococcal polysaccharides, we now report the structure of the I polysaccharide. The structure of the K polysaccharide has been reported elsewhere (Michon et al., 1985).

Nuclear magnetic resonance spectroscopy has played an increasingly important role in the structural elucidation of the meningococcal polysaccharides (Jennings, 1983; Jennings et al., 1983; Michon et al., 1984; Van der Kaaden et al., 1984), and the utility of this technique has been further demonstrated in the structural elucidation of the K (Michon et al., 1985) and I polysaccharides, where a number of one-dimensional and

† This is NRCC Publication No. 24816.
‡ National Research Council of Canada.
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0006-2960/85/0424-5592$01.50/0