Processing of MOPC 315 Immunoglobulin A Oligosaccharides: Evidence for Endoplasmic Reticulum and *Trans* Golgi α1,2-Mannosidase Activity

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ABSTRACT The processing of asparagine-linked oligosaccharides on the α-chains of an immunoglobulin A (IgA) has been investigated using MOPC 315 murine plasmacytoma cells. These cells secrete IgA containing complex-type oligosaccharides that were not sensitive to endo-β-*N*-acetylglucosaminidase H. In contrast, oligosaccharides present on the intracellular α-chain precursor were of the high mannose-type, remaining sensitive to endo-β-*N*-acetylglucosaminidase H despite a long intracellular half-life of 2–3 h. The major [^{3}H]mannose-labeled α-chain oligosaccharides identified after a 20-min pulse were $Man_8GlcNAc_2$ and $Man_9GlcNAc_2$. Following chase incubations, the major oligosaccharide accumulating intracellularly was $Man_6GlcNAc_2$, which was shown to contain a single α1,2-linked mannose residue. Conversion of $Man_6GlcNAc_2$ to complex-type oligosaccharides occurred at the time of secretion since appreciable amounts of $Man_5GlcNAc_2$ or further processed structures could not be detected intracellularly. The subcellular locations of the α1,2-mannosidase activities were studied using carbonyl cyanide m-chlorophenylhydrazone and monensin. Despite inhibiting the secretion of IgA, these inhibitors of protein migration did not effect the initial processing of $Man_9GlcNAc_2$ to $Man_6GlcNAc_2$. Furthermore, no large accumulation of $Man_5GlcNAc_2$ occurred, indicating the presence of two subcellular locations of α1,2-mannosidase activity involved in oligosaccharide processing in MOPC 315 cells. Thus, the first three α1,2-linked mannose residues were removed shortly after the α-chain was glycosylated, most likely in rough endoplasmic reticulum, since this processing occurred in the presence of carbonyl cyanide m-chlorophenylhydrazone. However, the removal of the final α1,2-linked mannose residue as well as subsequent carbohydrate processing occurred just before IgA secretion, most likely in the *trans* Golgi complex since processing of $Man_6GlcNAc_2$ to $Man_5GlcNAc_2$ was greatly inhibited in the presence of monensin.

A number of membrane, lysosomal, and secretory glycoproteins have been studied to define the subcellular location of the carbohydrate modifications required to form complex-type asparagine-linked oligosaccharide chains. It is known that the biosynthesis of the *N*-linked complex-type oligosaccharides of proteins involves the transfer of a high molecular weight oligosaccharide containing glucose, mannose, and *N*-acetylglucosamine from dolichol diphosphate to asparagine residues in the protein (1). Once transferred to protein, oligosaccharides destined to become complex-type are processed extensively. The terminal glucose residues are removed rapidly by specific membrane-bound glucosidases thought to be located in the rough and smooth endoplasmic reticulum (2–4). Subsequently, the four α1,2-linked mannose residues are removed by one or more specific α1,2-mannosidases. There is evidence indicating that this initial mannose processing could occur in the Golgi apparatus since enzymes capable of removing these α1,2-linked mannose residues have been isolated from purified rat liver Golgi membranes (5, 6). The Golgi apparatus is also the major subcellular location of the enzymes responsible for further carbohydrate modifications. These enzymes include the *N*-acetylglucosaminyltransferase,

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which attaches an *N*-acetylglucosamine residue to a $Man_5GlcNAc_2$ species (5, 7, 8), α-mannosidase II, which acts on $GlcNAcMan_5GlcNAc_2$ to remove the terminal α1,3-linked and α1,6-linked mannose residues to form $GlcNAcMan_3GlcNAc_2$ (5–8), and the glycosyltransferases that are involved in the elongation of the complex-type oligosaccharides.

Despite the isolation and partial purification of presumed processing α-mannosidases from rat Golgi membranes, others studying different cell types have suggested that the enzymatic removal of mannose residues occurs, at least in part, in the rough endoplasmic reticulum. For example, Tartakoff and Vassalli (9) have shown that trimming of mannose residues from IgM μ-chain oligosaccharides occurs in mouse plasmablasts even in the presence of carboxyl cyanide m-chlorophenylhydrazone (CCCP)[1], an uncoupler of oxidative phosphorylation known to inhibit intracellular protein migration from the rough endoplasmic reticulum (10). In contrast, Godelaine et al. (11) have suggested that only a single α1,2-linked mannose is removed in the rough endoplasmic reticulum of calf thyroid cells, since they observed the accumulation of thyroglobulin-linked $Man_8GlcNAc_2$ in thyroid slices labeled for 1 h and chased in the presence of CCCP. Hakimi and Atkinson (12) also concluded that one α1,2-linked mannose residue is removed from the Sindbis virus proteins B and PE2 in the rough endoplasmic reticulum of chick embryo fibroblasts. An α1,2-mannosidase has recently been partially purified from the rough endoplasmic reticulum of rat liver cells (13).

In view of these findings, we have examined the processing of immunoglobulin A (IgA) α-chain (heavy chain) oligosaccharides isolated from MOPC 315 plasmacytoma cells. In this study, we present evidence of at least two distinct subcellular locations of α1,2-mannosidase activity involved in the processing of IgA α-chain oligosaccharides. Processing α1,2-mannosidase activity appears to be present in the endoplasmic reticulum and the *trans* Golgi complex of these cells.

MATERIALS AND METHODS

L-[4,5-^{3}H]Leucine (130–190 Ci/mmol) was obtained from the Amersham Corp., Arlington Heights, IL. D-[2-^{3}H]Mannose (10–20 Ci/mmol) was purchased from New England Nuclear, Boston, MA. L-15 (Leibovitz) medium, L-glutamine, fetal calf serum, penicillin, and streptomycin were obtained from K. C. Biological Inc., Lenexa, KA. Leucine-deficient Eagle's minimal essential medium with Earle's base was purchased from Grand Island Biological Co., Grand Island, NY. Nonidet P-40 (NP-40) was obtained from Particle Data Laboratories, Elmhurst, IL, and phenylmethylsulfonylfluoride from the Sigma Chemical Co., St. Louis, MO. Bio-Gel P-2 and P-6 (200–400 mesh) and reagents for SDS PAGE were obtained from Bio-Rad Laboratories, Richmond, CA. Goat anti-MOPC 315 IgA was purchased from Gateway Immunosera Co., St. Louis, MO. Fixed protein A bearing *Staphylococcus aureus* Cowan strain I (SaCl) was a gift from Dr. Susan J. Cullen, Washington University School of Medicine, St. Louis, MO. [^{3}H]Mannose-labeled oligosaccharide alcohols used as standards were provided by Dr. Stuart Kornfeld (Washington University School of Medicine). Monensin, CCCP, neuraminidase (*Vibro cholerae*), and pronase were purchased from the Calbiochem-Behring Corp., La Jolla, CA. Jack bean α-mannosidase, β-galactosidase, and *N*-acetyl-β-glucosaminidase were obtained from the Sigma Chemical Co. Endo-β-*N*-acetylglucosaminidase H and endo-β-*N*-acetylglucosaminidase D were purchased from Miles Laboratories, Elkhart, IN. *Aspergillus satoi* α1,2-mannosidase was a gift from Dr. J. Baenziger (Washington University School of Medicine). 3a70 scintillation fluid and TS-1 were obtained from Research Products International Corp., Mt. Prospect, IL.

Plasmacytoma Line: The IgA-secreting MOPC 315/J cells that were established in suspension culture from MOPC 315 ascites tumor cells were provided by Dr. Richard Lynch (Department of Pathology, University of Iowa). The cells were maintained as suspension cultures in L-15 medium containing 10% fetal calf serum, 100 U/ml of penicillin, and 100 μg/ml of streptomycin. This cell line has a doubling time of ~24 h at 37°C and was kept at a density of 3×10^5 to 1.2×10^6 cells/ml by feeding and diluting every 48 h.

Incorporation of L-[^{3}H]Leucine into MOPC 315 IgA: MOPC 315 plasmacytoma cells (8.0×10^7) were washed and incubated in 20 ml of leucine-deficient Eagle's minimal essential medium containing 10% fetal calf serum, penicillin, and streptomycin. L-[^{3}H]Leucine (400 μCi) was added to the culture and the cells were incubated at 37°C in a 9% CO_2 atmosphere. After 5 h, the cells were centrifuged at 800 *g* for 5 min in a Beckman Model TJ-6 centrifuge (Beckman Instruments, Spinco Div., Palo Alto, CA). The supernatant medium was carefully removed from the cell pellet and saved. The cell pellet was washed with 20 ml of ice-cold L-15 medium and solubilized for 30 min in 0.5% NP-40 (vol/vol) containing 0.0015 M $MgCl_2$ and 0.002 M phenylmethylsulfonylfluoride. The lysate was spun for 20 min at 12,000 *g* in a Brinkmann Eppendorf centrifuge 5412 (Brinkmann Instruments, Inc., Westbury, NY) and the supernatant solution saved for subsequent analysis.

Analysis of [^{3}H]Leucine Labeled α-Chains Treated with Endo-β-N-Acetylglucosaminidase H: 0.4 ml of the cell medium containing 0.002 M phenylmethylsulfonylfluoride and 0.2 ml of the NP-40 cell lysate were adjusted to pH 5 with the addition of 0.05 ml of 1 M sodium citrate buffer. Endo-β-*N*-acetylglucosaminidase H (0.002 U) was then added to each, and the samples were incubated under a toluene atmosphere in a 37°C water bath. Control samples containing the citrate buffer but no enzyme were also incubated. After 18 h, the samples were spun at 800 *g* for 10 min.

The biosynthetically labeled IgA was isolated by immunoprecipitation using a modification of the method described previously (14). Cell lysates and medium containing cell secretions were incubated with goat anti-MOPC 315 IgA (20 μl/ml) for 75 min at 4°C. SaCl (200 μl/ml) was added and the incubation continued at 4°C for another 45 min. The SaCl was then collected by centrifugation at 12,000 *g* and washed twice with ice-cold 0.15 M NaCl, 0.01 M Tris/HCl, pH 7.4.

The immune precipitates isolated on SaCl were eluted by boiling in 40 μl of electrophoresis sample buffer consisting of 0.0625 M Tris/HCl, pH 6.8, 5% 2-mercaptoethanol, 2% SDS, 10% glycerol, and 0.0002% bromophenol blue. Analysis of [^{3}H]leucine-labeled α-chains by SDS PAGE was done using 7.5% acrylamide slab resolving gels according to the procedure of Laemmli (15). Fluorography was performed with Kodak XR-5 film following the procedure of Bonner and Laskey (16).

Pulse Labeling of MOPC 315 Cells with [^{3}H]Mannose: MOPC 315 cells ($8–12 \times 10^7$) were washed and suspended in 2–3 ml of 0.01 M sodium phosphate, 0.15 M NaCl, pH 7.4 buffer at 37°C. [^{3}H]Mannose (200–250 μCi/ml) was added and the incubation continued for 20 min at 37°C. The pulse was stopped by the addition of 20 ml of ice-cold L-15 medium. The cells were washed once, and those to be chased were suspended in L-15 medium with 10% fetal calf serum at a density of $1.5–2.5 \times 10^6$ cells/ml. At the indicated times, portions of the chase cultures were removed for the isolation of intracellular IgA by immunoprecipitation. Cells were lysed as described above, except that the NP-40 lysis buffer contained 0.1 M mannose and 0.05 M glucose-6-phosphate. Analysis of the immunoprecipitates by SDS gel electrophoresis, as described, revealed the presence of a single band or doublet of radioactivity corresponding to the α-chain of IgA.

In some [^{3}H]mannose or [^{3}H]leucine pulse-chase experiments, monensin or CCCP was added to the cells at the completion of the pulse and included in the chase incubations. The final medium concentrations of 10 μM monensin or 50 μM CCCP were obtained by adding concentrated stock solutions of these compounds freshly dissolved in ethanol. This resulted in a final medium concentration of 0.25% ethanol. Cells incubated in medium containing 0.25% ethanol were used as controls. Neither monensin or CCCP had an effect on cell viability as determined by trypan blue dye exclusion.

Preparation and Isolation of [^{3}H]Mannose-Labeled Glycopeptides and Oligosaccharides: Glycopeptides were prepared from [^{3}H]mannose-labeled IgA by incubation of the washed SaCl immunoprecipitations with 5 mg of pronase for 48 h in 500 μl of 0.1 M Tris-HCl buffer, pH 8.0 containing 0.001 M $CaCl_2$, 0.1 M mannose, and 0.05 M glucose-6-phosphate. Digestions were carried out at 37°C under toluene. Additional 5-mg amounts of pronase were added to the samples at 12, 24, and 36 h and the reactions were stopped by placing the digestion tubes in boiling water for 2 min. The pronase digests were then subjected to gel filtration on a column (1.5 × 95 cm) of Bio-Gel P-6 equilibrated and eluted with 0.1 M NH_4HCO_3, pH

[1] *Abbreviations used in this paper:* CCCP, Carbonyl cyanide m-chlorophenylhydrazone; GlcitolNAc, N-acetylglucosaminitol; HPLC, high performance liquid chromatography; IgA, immunoglobulin A; NP-40, nonidet P-40; SaCl, fixed protein A bearing *Staphlococcus aureus*.

8.0. 1 ml fractions were collected and portions of these were analyzed for radioactivity in a Tracor Mark III liquid scintillation counter (Tracor Analytic, Inc., Elk Grove Village, IL) after the addition of 3a70 scintillation fluid. Radioactive peaks were pooled as indicated, concentrated by lyophilization, treated with endo-β-*N*-acetylglucosaminidase H, and analyzed again on the Bio-Gel P-6 column.

Glycosidase Digestions: All enzymatic digestions were done at 37°C under an atmosphere of toluene. Digestions with endo-β-*N*-acetylglycosaminidase H (0.002 U) were done for 18 h in 50 μl of 0.05 M sodium citrate buffer, pH 5.5. Digestions with endo-β-*N*-acetylglucosaminidase D (0.005 U) were done for 18 h in 50 μl of 0.15 M sodium phosphate buffer, pH 6.5 containing 0.01% bovine serum albumin. Treatments of [^{3}H]mannose-labeled glycopeptides or oligosaccharides with exoglycosidases were done under the following conditions: jack bean α-mannosidase (0.08 U) in 50 μl of 0.05 M sodium citrate buffer, pH 4.5 for 18 h; *V. cholera* neuraminidase (0.2 U) in 200 μl of 0.05 M sodium acetate buffer, pH 5.6 containing 0.9% NaCl and 0.004 M $CaCl_2$ for 48 h; jack bean β-galactosidase (1.3 U) and β-*N*-acetylglucosaminidase (1.5 U) in 150 μl of 0.05 M sodium citrate buffer, pH 4.5, for 48 h; *Aspergillus satoi* α1,2-mannosidase (0.05 μg) in 30 μl of 0.01 M sodium acetate buffer, pH 5.0 for 24 h.

Analysis of Oligosaccharide Alcohols by High Performance Liquid Chromatography (HPLC): Oligosaccharides released by endo-β-*N*-acetylglucosaminidase H or endo-β-*N*-acetylglucosaminidase D were reduced in 350 μl of 0.12 M $NaBH_4$, pH 10 for 16–18 h at room temperature. The reductions were stopped by the addition of 15 μl of glacial acetic acid. 1 ml of methanol was added and methyl borate was removed by repeated methanol additions and evaporations under vacuum. The [^{3}H]mannose-labeled oligosaccharide alcohols were desalted on Bio-Gel P-2 or amberlite MB-3 and analyzed by HPLC. Methods used for HPLC were those described by Mellis and Baenziger (17) using a 4 mm × 30 cm MicroPax AX-10 column (Varian Associates, Inc., Palo Alto, CA) and a Varian Liquid Chromatography Model 5000. Columns were run at a flow rate of 1 ml/min. The initial solvent was acetonitrile/H_2O (65:35) and the oligosaccharides were eluted by increasing the H_2O content at 0.5%/min. Fractions were collected in scintillation vials at 0.3-min intervals. Radioactivity was determined after the addition of 0.15 ml of H_2O and 3.8 ml of 3a70. Elution profiles of standards were highly reproducible although they changed with repeated use of a column over a period of several months. Therefore, appropriate oligosaccharide alcohol standards were run on each day on which samples were analyzed.

Paper Chromatography Analyses: Descending paper chromatography was done with Whatman No. 1 paper (Whatman Laboratory Products, Inc., Clifton, NJ). To establish that the radioactivity incorporated into the labeled compounds was mannose, we hydrolyzed portions of various oligosaccharides in 2 N H_2SO_4 for 5 h at 100°C, desalted, and subjected them to descending paper chromatography using solvent A, 1-butanol/ethanol/H_2O (10:1:2). Jack bean α-mannosidase digestions were analyzed using solvent B consisting of ethyl acetate/pyridine/H_2O/acetic acid (5:5:3:1). 1 cm strips of the dried chromatograms were soaked in 0.15 M of H_2O in scintillation vials and analyzed for radioactivity after the addition of 3.8 ml of 3a70 scintillation fluid.

RESULTS

Effects of Endo-β-N-Acetylglucosaminidase H Treatment on the Electrophoresis of Intracellular and Secreted [^{3}H]Leucine-labeled IgA α-Chains

The IgA-secreting MOPC 315 cell line was chosen for study since it is known to secrete large amounts of a single IgA glycoprotein. Previous studies using pulse-chase experiments have shown that the secretion of newly synthesized IgA from these cells is relatively slow, with ~50% of radiolabeled MOPC 315 IgA being secreted during the 2–3 h after pulse labeling (10, 14). Experiments therefore were designed to define the time course of oligosaccharide processing. The enzyme endo-β-*N*-acetylglucosaminidase H was used to determine whether a significant pool of processed α-chains accumulated intracellularly before secretion.

Fig. 1 shows the SDS PAGE migration of the [^{3}H]leucine-labeled intracellular and secreted α-chains before and after enzymatic treatment. The analysis of intracellular α-chains by SDS PAGE often revealed two proteins of very similar

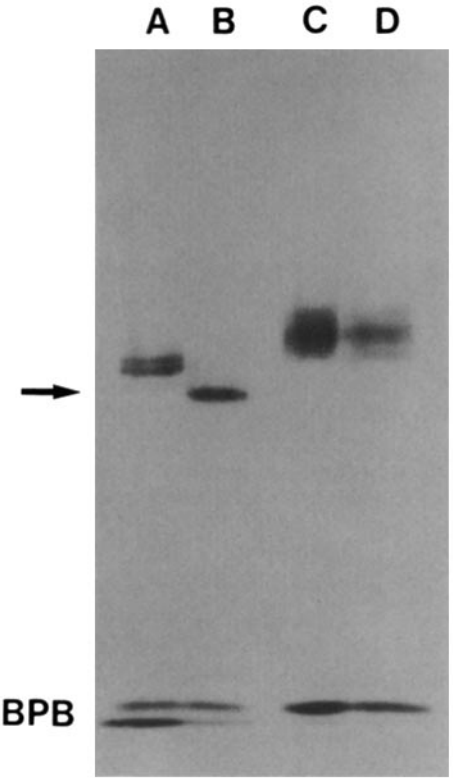

FIGURE 1 Effect of endo-β-*N*-acetylglucosaminidase H on the electrophoretic migration of intracellular and secreted MOPC 315 α-chains. MOPC 315 cells were incubated with [^{3}H]leucine for 5 h. Portions of NP-40 cell lysates and secretions were incubated with endo-β-*N*-acetylglucosaminidase H before isolation of [^{3}H]leucine-labeled IgA by immunoprecipitation, as described in Materials and Methods. A 7.5% SDS polyacrylamide slab gel was run in the presence of 2-mercaptoethanol. Light chains are not resolved on 7.5% gels. The labeled low molecular weight material migrating with the tracking dye bromophenol blue (*BPB*) was not observed in [^{3}H]mannose-labeled samples. After electrophoresis, the gel was prepared for fluorography by the procedure of Bonner and Laskey (16). (*A*) α-chains isolated from a NP-40 cell lysate; (*B*) α-chains isolated from a cell lysate after treatment with endo-β-*N*-acetylglucosaminidase H; (C) secreted α-chains isolated from the labeling medium; (*D*) secreted α-chains isolated from the labeling medium after treatment with endo-β-*N*-acetylglucosaminidase H. The arrow indicates the migration of nonglycosylated α-chains synthesized in the presence of tunicamycin.

apparent molecular weights (Fig. 1*a*). This electrophoretic pattern of murine α-chains has been described previously (18). The heterogeneity appears to be the result of carbohydrate content since subsequent analysis after the treatment with endo-β-*N*-acetylglucosaminidase H resulted in the appearance of a single band of radioactivity (Fig. 1*b*). The treated intracellular α-chains have a more rapid electrophoretic migration identical to that of nonglycosylated α-chains synthesized in the presence of tunicamycin. The fact that the entire intracellular pool of α-chains was susceptible to the activity of endo-β-*N*-acetylglucosaminidase H indicates that oligosaccharide processing is not complete until the time of secretion. This intracellular susceptibility is not complete until the time of secretion. This susceptibility of the intracellular α-chains to endo-β-*N*-acetylglucosaminidase H is like that described for murine IgM μ-chains (9) and human chorionic gonadotropin (19).

In contrast to intracellular α-chains, the SDS PAGE migra-

tion of secreted IgA α-chains was not affected by treatment with endo-β-*N*-acetylglucosaminidase H, consistent with the presence of predominantly complex-type oligosaccharide units.

Analysis of Intracellular [^{3}H]Mannose-labeled α-Chain Glycopeptides

Further experiments with endo-β-*N*-acetylglucosaminidase H were performed to confirm that the changes in electrophoretic migration of the intact intracellular α-chains were in fact the result of the removal of high mannose-type oligosaccharide units. Fig. 2 shows the Bio-Gel P-6 elution profile of [^{3}H]mannose pulse-labeled α-chain glycopeptides immunoprecipitated immediately at the conclusion of the pulse and after 5½ h of chase incubation. Two major peaks of radioactivity were observed at both time points (Fig. 2, *a* and *c*), each of which was susceptible to endo-β-*N*-acetylglucosaminidase H treatment as indicated by the change in gel filtration elution shown in Fig. 2, *b* and *d*. Thus, glycopeptides derived from pulse-labeled α-chains chased for 5½ h remained susceptible to endo-β-*N*-acetylglucosaminidase H, a result consistent with the data shown in Fig. 1. This demonstrates that the carbohydrate processing of intracellular α-chains is not complete even after prolonged periods of time and that the final processing events must occur rapidly at the time of secretion, since no intracellular α-chain glycopeptides resistant to endo-β-*N*-acetylglucosaminidase H could be identified. The two major glycopeptide peaks identified by gel filtration contain oligosaccharides of similar size as determined by gel filtration (Fig. 2, *b* and *d*) and HPLC (Fig. 3) of the endo-β-*N*-acetylglucosaminidase H released structures.

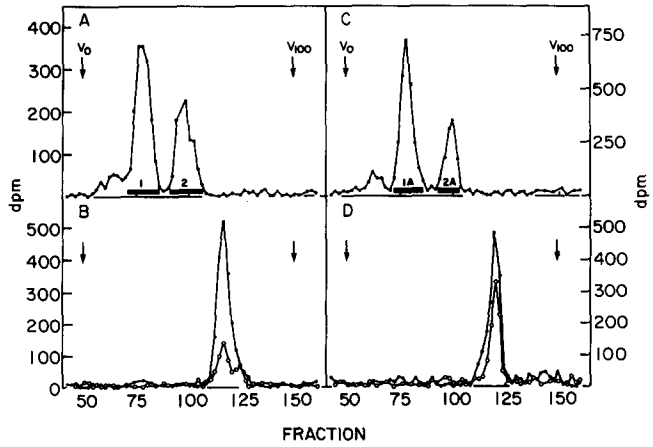

FIGURE 2 Gel filtration of intracellular [^{3}H]mannose-labeled α-chain glycopeptides and oligosaccharides. Samples were applied to a 1.5 cm × 95 cm column of Bio-Gel P-6 (200–400 mesh) equilibrated and eluted with 0.1 M NH_4HCO_3. 1-ml fractions were collected, and portions of every other fraction analyzed for radioactivity. (*A*) the pronase digest of [^{3}H]mannose-labeled α-chains isolated from 20-min pulse-labeled cells. (*B*) consecutive analyses of pooled fractions of peaks 1 and 2 indicated in panel A after treatment with endo-β-*N*-acetylglucosaminidase H. ●, peak 1; ○, peak 2. (C) the pronase digest of [^{3}H]mannose-labeled α-chains isolated from cells pulsed for 20 min and chased for 5½ h; (*D*) consecutive analyses of pooled fractions of peaks *1A* and *2A* indicated in panel C after treatment with endo-β-*N*-acetylglucosaminidase H. ●, peak *1A*; ○, peak *2A*. V_0 and V_{100} indicate the elution positions of bovine serum albumin and mannose, respectively, determined in a separate experiment.

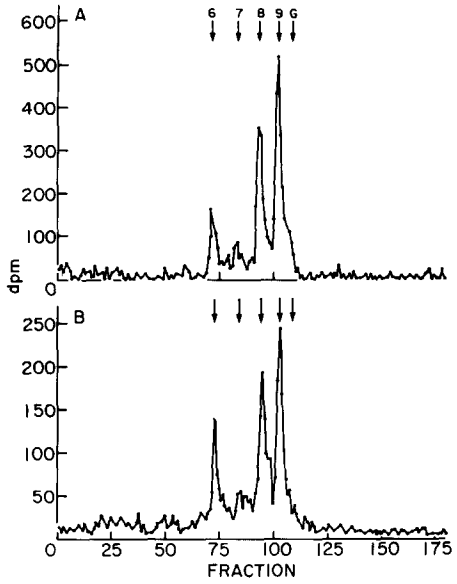

FIGURE 3 HPLC analyses of endo-β-*N*-acetylglucosaminidase H-released oligosaccharides from pulse-labeled α-chains. MOPC 315 cells were labeled for 20 min with [^{3}H]mannose. Following immunoprecipitation and pronase digestion of the intracellular α-chains, the labeled oligosaccharides were released from glycopeptides with endo-β-*N*-acetylglucosaminidase H, characterized by gel filtration, and reduced with sodium borohydride as described in Materials and Methods. Portions of the labeled oligosaccharide alcohols were then injected on a Micro Pak AX-10 column in the initial solvent of acetonitrile/H_2O (65:35). The H_2O content was increased to 65% over the following 60 min. The flow rate was 1 ml/min and fractions were collected directly into scintillation vials at 0.3-min intervals. *A*, oligosaccharide alcohols obtained from peak 1 glycopeptides shown in Fig. 2*A*; *B*, oligosaccharide alcohols obtained from peak 2 glycopeptides, Fig. 2*A*. The arrows labeled 6 through 9 refer to the elution of known [^{3}H]mannose-labeled standards $Man_6GlcitolNAc$, $Man_7GlcitolNAc$, $Man_8GlcitolNAc$, and $Man_9GlcitolNAc$, respectively. The arrow labeled G indicates the elution of $Glc_1Man_9GlcitolNAc$.

Characterization of Endo-β-*N*-Acetylglucosaminidase H Released α-Chain Oligosaccharides

Fig. 3 shows the elution pattern of pulse-labeled intracellular α-chain oligosaccharide alcohols. The two major glycopeptide peaks isolated by gel filtration (Fig. 2*a*) had identical oligosaccharide structures that eluted from the HPLC column in fractions identical to the known standards $Man_6GlcitolNAc$, $Man_7GlcitolNAc$, $Man_8GlcitolNAc$, and $Man_9GlcitolNAc$. Since we have observed identical structures in both glycopeptide peaks in all experiments and at all time points, subsequent data has been derived from the larger of the two peaks corresponding to peaks *1* and *1A* of Fig. 2. Acid hydrolysis of intracellular oligosaccharides and subsequent paper chromatography in solvent A resulted in a single peak of radioactivity corresponding to mannose (data not shown).

At the conclusion of the 20-min pulse, few oligosaccharides containing glucose could be identified. A small amount of radioactivity that eluted with the standard $Glc_1Man_9GlcitolNAc$ was seen as a shoulder present on the large $Man_9GlcitolNAc$ peak (Fig. 3). An even larger percentage (~50%) of glucosylated oligosaccharides was observed in a short 3-min pulse experiment (data not shown). This indicates

that glucose-containing oligosaccharides are transferred to α-chains and is consistent with previous findings that the terminal glucose residues are rapidly removed following transfer of the oligosaccharides from lipid to protein (1). Further characterization of the 20-min pulse-labeled α-chain oligosaccharides was obtained by measuring the susceptibility of these structures to the exoglycosidase jack bean α-mannosidase. Fig. 4 shows the analysis by paper chromatography in solvent B of a jack bean α-mannosidase digestion revealing a large peak of radioactivity that co-migrated with free mannose and a smaller peak of radioactivity with a migration similar to that of Manβ GlcitolNAc. A very small peak of radioactivity was observed near the origin of the chromatogram shown in Fig. 4. This α-mannosidase-resistant material was tentatively identified as Glc_1Man_4GlcitolNAc by comparison to the migration of known standards and most likely was derived from the small shoulder of glucosylated oligosaccharides detected by HPLC (Fig. 3).

Fig. 3 also shows the presence of pulse-labeled α-chain oligosaccharides containing less than nine mannose residues. A sizable portion of the HPLC-characterized oligosaccharides obtained at the conclusion of a 20-min pulse eluted in a position similar to that of the standard Man_8GlcitolNAc. The proportion of Man_8GlcitolNAc relative to Man_9GlcitolNAc varied from 38 to 70% in several 15- to 20-min pulse-labeling experiments (data not shown). In addition, lesser amounts of labeled structures judged by HPLC to be Man_7GlcitolNAc and Man_6GlcitolNAc were also identified. We cannot exclude the possibility that small amounts of these structures are transferred directly to α-chains. However, the presence of the much higher percentage of glucosylated structures identified after a shorter 3-min pulse indicates that most of these oligosaccharides are derived from a larger glucosylated precursor. Thus, the removal of some α1,2-linked mannose residues begins soon after glycosylation of α-chains despite the relatively long intracellular half-life of this glycoprotein.

The intracellular oligosaccharides present after 1, 3, and 5½ h of chase incubation of pulse-labeled α-chains are shown in Fig. 5. By 1 h, the predominant peak of radioactivity identified by HPLC was Man_6GlcitolNAc, although >50% of the radioactivity was still present in peaks identified as Man_8GlcitolNAc and Man_9GlcitolNAc. No glucosylated oligosaccharides were identified by HPLC, and no jack bean α-mannosidase-resistant structures were observed by paper chromatography analyses (data not shown). After 3 h of chase incubation, at a time when ~50% of the pulse-labeled IgA has been secreted, there was a relative increase of $Man_6GlcNAc_2$ on the remaining intracellular α-chains. At 5½ h, the major oligosaccharide alcohol identified by HPLC was Man_6GlcitolNAc. Only small amounts of Man_7GlcitolNAc, Man_8GlcitolNAc, and Man_9GlcitolNAc could be identified. No further processing of the $Man_6GlcNAc_2$ could be detected even after this long chase incubation. No pools of smaller oligosaccharides were found, suggesting that the subsequent processing of $Man_6GlcNAc_2$ must be closely coupled to secretion of the glycoprotein.

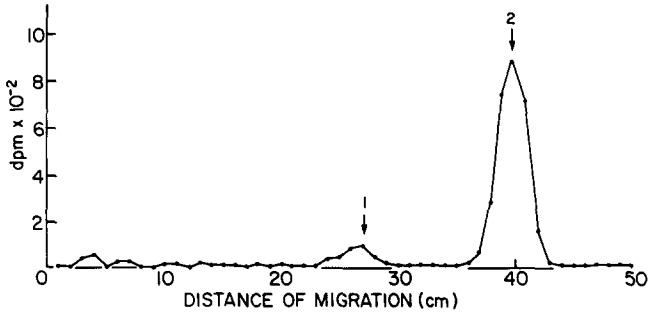

FIGURE 4 Paper chromatography of jack bean α-mannosidase-digested, pulse-labeled α-chain oligosaccharide alcohols [³H]Mannose-labeled α-chain oligosaccharide alcohols isolated from MOPC 315 cells pulsed for 20 min with [³H]mannose were incubated with jack bean α-mannosidase for 18 h. The digestion mixture was then subjected to descending paper chromatography in solvent B for 18 h. The arrows labeled 1 and 2 indicate the migration of Manβ GlcitolNAc and mannose standards, respectively.

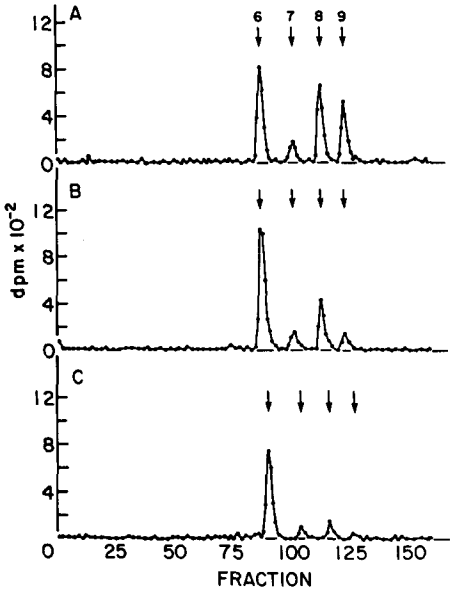

FIGURE 5 HPLC analyses of endo-β-*N*-acetylglucosaminidase H-released oligosaccharides from pulse-labeled α-chains chased in nonradioactive medium. MOPC 315 cells were labeled for 20 min with [³H]mannose and chased in nonradioactive medium. At the indicated times, IgA was immunoprecipitated from NP-40 cell lysates and digested with pronase. The labeled α-chain oligosaccharides were released with endo-β-*N*-acetylglucosaminidase H, isolated by Bio-Gel P-6 gel filtration, reduced with sodium borohydride, and analyzed by HPLC as described in Materials and Methods and in the legend to Fig. 3. *A*, elution profile of oligosaccharide alcohols obtained from cells chased for 1 h; *B*, elution profile of oligosaccharide alcohols from cells chased for 3 h; *C*, elution profile of oligosaccharide alcohols from cells chased for 5½ h. The arrows labeled 6 through 9 indicated the elution with known [³H]mannose-labeled standards Man_6GlcitolNAc, Man_7GlcitolNAc, Man_8GlcitolNAc, and Man_9GlcitolNAc, respectively.

Effect of CCCP and Monensin on the Processing of IgA α-Chain Oligosaccharides

The above experiments are consistent with the presence of glycoprotein processing α1,2-mannosidase activity in two different subcellular locations since the removal of the final α1,2-linked mannose residue was closely associated with late processing enzymes such as *N*-acetylglucosaminyl transferase I, α-mannosidase II, and other glycosyltransferases thought to be located in the *trans* Golgi complex (20, 21). CCCP and monensin were used, therefore, in an attempt to define further the subcellular sites of α1,2-mannosidase activity. CCCP has been shown to block the intracellular migration of immunoglobulins at the level of the rough endoplasmic reticulum,

whereas monensin blocks within the Golgi apparatus (10). Initially, pulse-chase experiments were performed to determine whether these agents blocked the secretion of the MOPC 315 IgA. As shown in Fig. 6, the presence of either CCCP or monensin in the chase medium inhibited the secretion of 315 IgA during the 5 h after pulse labeling with [^{3}H]leucine. This inhibition of secretion was not the result of increased intracellular degradation, as determined by immunoprecipitation of the labeled IgA in NP-40 cell lysates at each time point (data not shown). A small amount of [^{3}H]leucine-labeled IgA was secreted in the presence of monensin (25% of control at 5 h), whereas secretion in the presence of CCCP was inhibited by >85% at all time points analyzed. Similar results have been reported previously for MOPC 315 IgA secretion (10).

Analyses of the oligosaccharides obtained from α-chains that were pulse labeled with [^{3}H]mannose for 20 min and then blocked intracellularly with CCCP or monensin are shown in Fig. 7. Despite the presence of these inhibitors of secretion, no gross alterations of intracellular carbohydrate processing could be detected. The major oligosaccharide present after 3 h of chase eluted with the $Man_6GlcitolNAc$ standard regardless of whether the intracellular block occurred at the level of the rough endoplasmic reticulum (CCCP) or within the Golgi apparatus (monensin). Thus, the removal of the first three α1,2-linked mannose residues occurs in the endoplasmic reticulum, since the time course for their removal is not affected by a CCCP-induced block of protein migration. In addition, the fact that the major oligosaccharide that accumulated in monensin-treated cells also has six mannose residues indicates that the final α1,2-linked mannose residue is removed distal to the monensin block, and therefore in the *trans* Golgi complex. A small peak of radioactivity was identified in both CCCP- and monensin-treated cells that eluted with the standard $Man_5GlcitolNAc$. However, no large accumulation of $Man_5GlcNAc_2$ or smaller structures was ever observed. The major peak of radioactivity was identified as $Man_6GlcitolNAc$ even with chase periods of 5–6 h (data not shown).

Characterization of the Oligosaccharides Containing Six Mannose Residues

Although the above data are compatible with two subcellular locations of α1,2-mannosidase activity involved in the processing of IgA secreted from MOPC 315 plasmacytoma

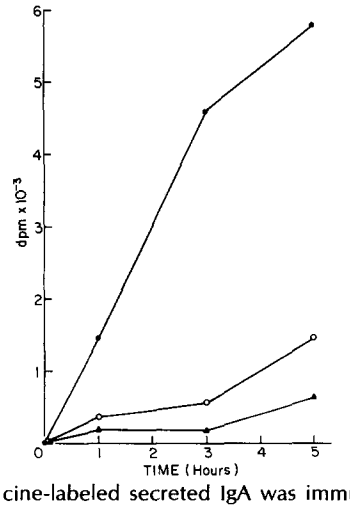

FIGURE 6 Effect of CCCP and monensin on the secretion of pulse-labeled IgA. MOPC 315 cells (7.5 × 10^7) were pulsed with 400 μCi of [^{3}H]leucine for 20 min. At the completion of the pulse, the cells were divided equally into three flasks of L-15 medium containing either ethanol 0.25% (control), CCCP 50 μM, or monensin 10 μM. Portions of the cell suspensions were removed at the indicated times. After the cells were removed by centrifugation, the [^{3}H]leucine-labeled secreted IgA was immunoprecipitated from the medium, dissolved in TS-1, and counted as described in Materials and Methods. ●, control; ○, monensin; ▲, CCCP.

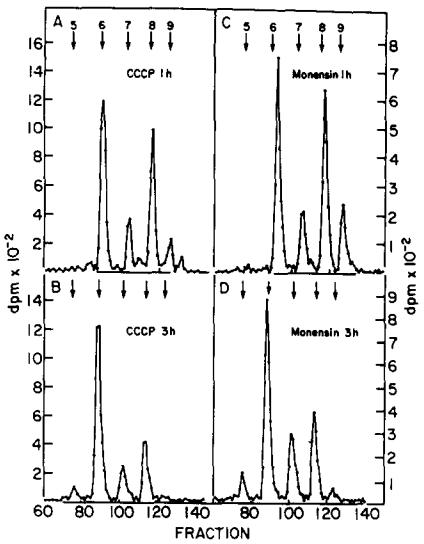

FIGURE 7 HPLC analyses of endo-β-*N*-acetylglucosaminidase H-released oligosaccharides from pulse-labeled α-chains chased in nonradioactive medium containing either CCCP or monensin. MOPC 315 cells were labeled for 20 min with [^{3}H]mannose and chased in nonradioactive medium containing either CCCP (50 μM) or monensin (10 μM). At the indicated times, IgA was immunoprecipitated from NP-40 cell lysates. The labeled oligosaccharides were obtained and prepared prior to HPLC analyses as described in Materials and Methods and the legend to Fig. 3. *A*, α-chain oligosaccharide alcohols from cells chased in the presence of CCCP for 1 h; *B*, α-chain oligosaccharide alcohols from cells chased in the presence of CCCP for 3 h; *C*, α-chain oligosaccharide alcohols from cells chased in the presence of monensin for 1 h; *D*, α-chain oligosaccharide alcohols from cells chased in the presence of monensin for 3 h. The arrows indicate the elution of $Man_5GlcitolNAc$, $Man_6GlcitolNAc$, $Man_7GlcitolNAc$, $Man_8GlcitolNAc$, and $Man_9GlcitolNAc$ standards.

cells, this conclusion can only be made if the structures identified as $Man_6GlcitolNAc$ contain an α1,2-linked mannose residue and if the mature, secreted oligosaccharides have been further processed to complex type structures. Purified *Aspergillus satoi* α1,2-mannosidase therefore was used to characterize the $Man_6GlcitolNAc$ structures. This α-mannosidase releases only terminal α1,2-linked mannose residues from carbohydrate structures (22, 23). As seen in Fig. 8*a*, HPLC analysis shows that the major intracellular [^{3}H]mannose-labeled oligosaccharide alcohol structure from pulse-labeled α-chains chased for 5½ h was $Man_6GlcitolNAc$. Treatment of this sample with *Aspergillus satoi* α1,2-mannosidase before HPLC resulted in the formation of two different peaks of radioactivity, shown in Fig. 8*b*. The major peak had an elution profile that corresponded to that of the standard $Man_5GlcitolNAc$, whereas the smaller peak eluted much earlier in a position that corresponded to mannose. The relative sizes of the radioactive peaks are consistent with the release of a single α1,2-linked mannose residue. Thus, the structure identified as $Man_6GlcitolNAc$ does contain an α1,2-linked mannose residue which, if further processing occurs, is removed at the time of secretion.

Determination of the Number of Mannose Residues in Secreted IgA α-Chain Oligosaccharides

The exact structures of the fully processed IgA carbohydrate chains are not known, but the secreted protein appears to

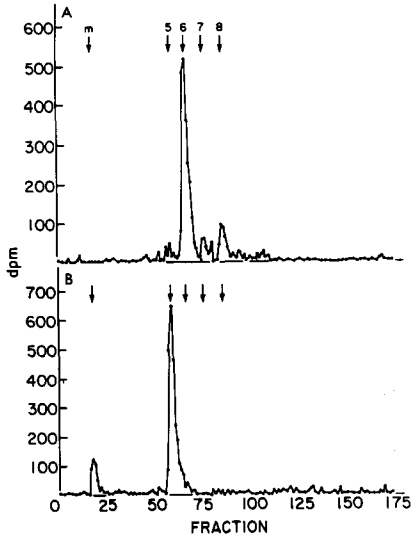

FIGURE 8 HPLC analysis of partially processed MOPC 315 α-chain oligosaccharides digested with *Aspergillus satoi* α1,2-mannosidase. α-Chain oligosaccharides were isolated from MOPC 315 cells that had been labeled with [^{3}H]mannose for 20 min and chased in nonradioactive medium for 5½ h. Following reduction with sodium borohydride, portions of the labeled oligosaccharide alcohols were incubated at 37°C for 24 h with or without *Aspergillus satoi* α1,2-mannosidase as described in Materials and Methods prior to analyses by HPLC. *A*, 5½-h chase α-chain oligosaccharide alcohols; *B*, 5½ chase α-chain oligosaccharide alcohols treated with α1,2-mannosidase before HPLC. The arrows indicate the elution of known radioactive mannose, $Man_5GlcitolNAc$, $Man_6GlcitolNAc$, $Man_7GlcitolNAc$, and $Man_8GlcitolNAc$ standards.

have mainly complex-type oligosaccharides as determined by resistance to endo-β-*N*-acetylglucosaminidase H (Fig. 1). Typically, processing of complex-type oligosaccharides results in core structures containing only three mannose residues linked to a chitobiose unit (24). To determine the number of mannose residues present in the fully processed secreted molecule, we isolated and characterized [^{3}H]mannose-labeled 315 IgA from the medium. Fig. 9*a* shows the gel filtration profile of the glycopeptides derived from secreted IgA. In contrast to their intracellular precursors, these structures have a higher apparent molecular weight, eluting near the void volume of the P-6 column. Treatment of these glycopeptides with endo-β-*N*-acetylglucosaminidase H released only a small amount of radioactivity (~10%, data not shown). The exact origin of these high-mannose structures is unknown. Nevertheless, most of the [^{3}H]mannose-labeled, secreted IgA glycopeptides were resistant to endo-β-*N*-acetylglucosaminidase H.

Fig. 9*b* shows the P-6 gel filtration profile of these glycopeptides following sequential treatment with neuraminidase, β-galactosidase, and endo-β-*N*-acetylglucosaminidase. Two new peaks of radioactivity with lower apparent molecular weights could be identified suggesting that the secreted α-chains contain terminal monosaccharides other than mannose. Following exoglycosidase treatment, the oligosaccharides could be released from peptides by treatment with endo-β-*N*-acetylglucosaminidase D as judged by gel filtration on the P-6 column (Fig. 9*c*). This endoglycosidase will cleave the chitobiose unit of complex-type oligosaccharides once the outer chain sugars are removed from the core structure Manα1 → 3 (Manα1 → 6)-Manβ1 → 4GlcNAcβ1 → 4GlcNAc → Asn. The α-mannose residue linked to the innermost β-linked mannose residue by a 1–3 linkage must be unsubstituted for enzymatic activity (25, 26). Fig. 10 shows the HPLC analysis of the oligosaccharide alcohol derived from the major peak of endo-β-*N*-acetylglucosaminidase D-released radioactivity. A large peak of radioactivity could be identified that had an elution profile identical to that of the standard $Man_3GlcitolNAc$, indicating that complete processing to typical complex-type core structures does occur in the majority of IgA-associated oligosaccharides. Thus, the final α1,2-linked mannose residue is removed from the intracellular $Man_6GlcNAc_2$ α-chain oligosaccharides as well as further processing to complex-type structures. As shown, these events occur rapidly at the time of secretion, since no large pools of partially processed oligosaccharides could be identified.

Characterization of Oligosaccharides on IgA α-Chains Secreted in the Presence of Monensin

As shown in Fig. 6, monensin inhibited the secretion of IgA from MOPC 315 cells. However, 25% of the [^{3}H]leucine pulse-labeled IgA was secreted after 5 h of chase. This incubation time was used to obtain sufficient amounts of secreted [^{3}H]mannose-labeled IgA so that the oligosaccharides could be characterized. A higher percentage (~40%) of [^{3}H]man-

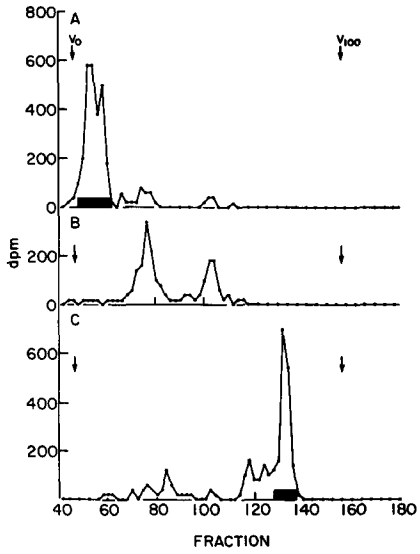

FIGURE 9 Gel filtration of secreted [^{3}H]mannose-labeled α-chain glycopeptides and oligosaccharides. MOPC 315 cells were pulsed for 20 min with [^{3}H]mannose and chased in nonradioactive medium. After 5½ h of chase, the secreted labeled IgA was immunoprecipitated from the medium. Following treatment with the indicated enzymes, the [^{3}H]mannose-labeled α-chain glycopeptides and oligosaccharides were analyzed by gel filtration on a Bio-Gel P-6 column as described in Fig. 2. (*A*) the immunoprecipitated IgA containing [^{3}H]mannose-labeled α-chains was treated with pronase for 48 h and applied to the P-6 column; (*B*) the major peak of radioactivity, indicated by the bar in *A*, was concentrated and treated sequentially with neuraminidase, β-galactosidase, and β-*N*-acetylglucosaminidase. A portion of the final digestion was applied directly to the P-6 column; (C) following sequential treatment with exoglycosidases, the [^{3}H]mannose-labeled glycopeptides were desalted and treated with endo-β-*N*-acetylglucosaminidase D before applying to the P-6 column. The major peak of radioactivity was pooled as indicated by the bar, concentrated, reduced, and analyzed by HPLC. V_0 and V_{100} indicate the elution positions of bovine serum albumin and mannose, respectively, determined in a separate experiment.

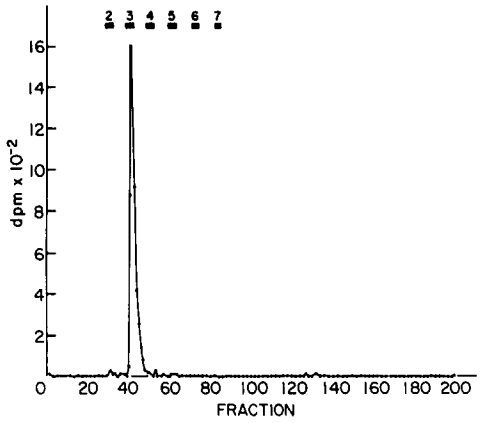

FIGURE 10 HPLC analysis of secreted α-chain core oligosaccharides released by endo-β-*N*-acetylglucosaminidase D. [³H]Mannose-labeled secreted IgA was treated with pronase and glycosidases as indicated in Fig. 9. The major peak of radioactivity released by endo-β-*N*-acetylglucosaminidase D, shown in Fig. 9, panel *C*, was concentrated, reduced with sodium borohydride, and analyzed by HPLC as described in Fig. 3. The bars labeled *2* through *7* indicate the elution volumes of the known [³H]mannose-labeled standards Man_{2-7}GlcitolNAc.

nose-labeled IgA was secreted in the presence of monensin relative to [³H]leucine-labeled IgA. As shown below, this is a result of incomplete oligosaccharide processing. Fig. 11*a* shows a Bio-Gel P-6 gel filtration profile of the glycopeptides obtained after extensive pronase digestion. The elution profile was considerably different from that of IgA glycopeptides isolated from control cell secretions depicted in Fig. 9*a*. In fact, the gel filtration elution profile was similar to that of glycopeptides derived from intracellular IgA (Fig. 2*c*). Furthermore, a large proportion of the glycopeptides derived from IgA secreted in the presence of monensin were susceptible to endo-β-*N*-acetylglucosaminidase H treatment, as determined by gel filtration (Fig. 11*b*). Analysis by HPLC of the endo-β-*N*-acetylglucosaminidase H–released, reduced oligosaccharides revealed that they were similar in size to intracellular oligosaccharides present after several hours of chase incubation. Fig. 12 shows that the major oligosaccharide had an elution identical to that of the known standard Man_6GlcitolNAc. Smaller amounts of Man_5GlcitolNAc, Man_7GlcitolNAc, and Man_8GlcitolNAc were also identified. Free mannose and ManβGlcitolNAc were identified when these oligosaccharides were treated with jack bean α-mannosidase and analyzed by paper chromatography in solvent A, further showing that only mannose and GlcitolNAc were present (data not shown).

DISCUSSION

Recent investigations have elucidated the steps involved in the formation of complex-type *N*-linked asparagine oligosaccharides (1). Following the transfer of $Glc_3Man_9GlcNAc_2$ from dolichol diphosphate to proteins, the glucose residues are rapidly removed by glucosidases located in the rough and smooth endoplasmic reticulum. This investigation has focused on the subsequent removal of the four α1,2-linked mannose residues, which is the next major stage of processing involved in the formation of complex-type *N*-linked oligosaccharides. Enzymes capable of specifically cleaving α1,2-linked mannose residues from high mannose substrates have been partially purified from mammalian tissues (5, 6, 13, 27). Since several of these have been isolated from rat Golgi membrane preparations, it has been speculated that the Golgi complex is the subcellular site for this processing step (5, 6). However, others have presented evidence to indicate that the rough

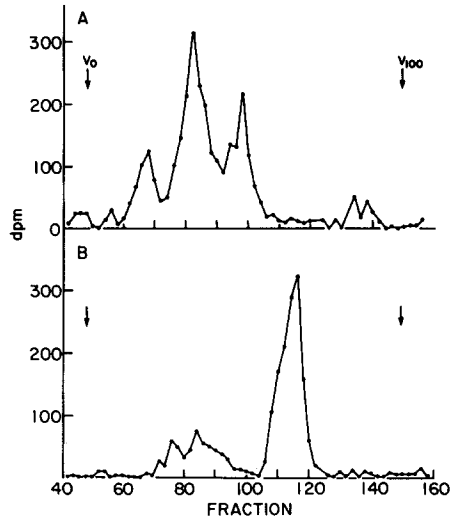

FIGURE 11 Gel filtration of [³H]mannose-labeled α-chain glycopeptides and oligosaccharides secreted in the presence of monensin. MOPC 315 cells were pulse-labeled for 20 min with [³H]-mannose and chased in nonradioactive medium containing monensin (10 μM). After 5½ h, the secreted IgA was immunoprecipitated from the medium. Following the enzyme treatments indicated, the [³H]mannose-labeled α-chain glycopeptides and oligosaccharides were analyzed by gel filtration on a Bio-Gel P-6 column as described in Fig. 2. *A*, the immunoprecipitated IgA containing [³H]mannose-labeled α-chains was treated with pronase for 48 h. A portion of the digestion was applied to the P-6 column. *B*, pronase digested glycopeptides were treated with endo-β-*N*-acetylglucosaminidase H for 18 h and applied to the P-6 column. Fractions 105 to 122 containing released oligosaccharides were pooled, concentrated, reduced with sodium borohydride, and analyzed by HPLC. V_0 and V_{100} indicate the elution of bovine serum albumin and mannose, respectively, determined in a separate experiment.

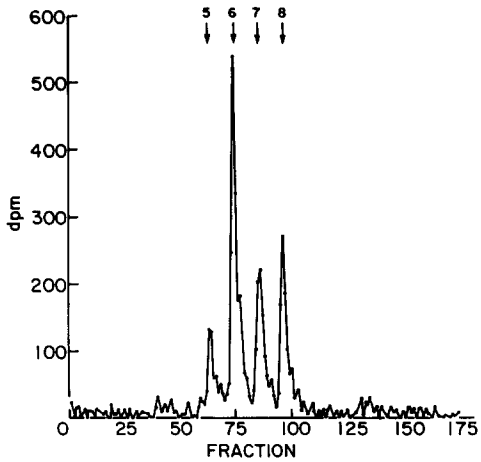

FIGURE 12 HPLC analysis of endo-β-*N*-acetylglucosaminidase H released oligosaccharides from pulse-labeled α-chains secreted in the presence of monensin. Bio-Gel P-6 fractions (Fig. 11*b*) containing oligosaccharides released from α-chains secreted in the presence of monensin (10 μM) were reduced with sodium borohydride and analyzed by HPLC as described in Fig. 3. Arrows refer to the elution of Man_{5-8}GlcitolNAc standards.

endoplasmic reticulum is a major site of α1,2-mannosidase activity (9, 11–13).

Our data indicate that there is α1,2-mannosidase activity in the rough endoplasmic reticulum of MOPC 315 cells. The pulse-chase experiments show that, despite the long intracellular half-life of IgA α-chains, processing of the first three α1,2-linked mannose residues occurs soon after the high-mannose oligosaccharide is transferred to this protein. The use of CCCP, an inhibitor of protein migration in the rough endoplasmic reticulum, provided further evidence to define the subcellular location of processing α1,2-mannosidase activity. The time course for the removal of the first three α1,2-linked mannose residues was not affected by a CCCP-induced block, consistent with mannosidase activity in the rough endoplasmic reticulum.

The extent of endoplasmic reticulum α1,2-mannosidase processing of α-chains in MOPC 315 cells is somewhat different from that observe for other proteins in other cell types. Tartakoff and Vassalli (9) suggested that the mannose trimming of newly synthesized μ-chains in murine plasmablasts occurred in the rough endoplasmic reticulum. However, structural analyses were not performed to document the exact extent of processing. Godaleine et al. (11) have reported processing α1,2-mannosidase activity in both the endoplasmic reticulum and Golgi apparatus of calf thyroid cells. They found that a single α1,2-linked mannose residue was removed from thyroglobulin in the endoplasmic reticulum. Likewise, Hakimi and Atkinson (12) concluded that one mannose residue was removed from Sindbis virus proteins B and PE2 in the rough endoplasmic reticulum of chick embryo fibroblasts. Our evidence that α1,2-mannosidase activity is in the rough endoplasmic reticulum is consistent with those described above. However, three α1,2-linked mannose residues of newly synthesized MOPC 315 α-chain oligosaccharides are removed in the endoplasmic reticulum. Compared with the other studies, this difference may be a result of the particular glycoprotein studied since our investigation does not exclude the presence of α1,2-mannosidase activity in the early or *cis* Golgi apparatus of MOPC 315 plasmacytoma cells. Indeed, the extent of α1,2-mannosidase processing in any subcellular location may depend on the individual protein. It may be that the relatively slow secretory rate of the α-chains lengthens the time of their exposure to endoplasmic reticulum α1,2-mannosidase, resulting in the formation of $Man_6GlcNAc_2$ oligosaccharides.

Of interest was the time course for the removal of the final α1,2-linked mannose residue from the IgA α-chains. Processing of the oligosaccharides containing six mannose residues to complex-type structures occurred rapidly at the time of secretion, since no oligosaccharides containing five or less mannose residues could be isolated from cells during long chase incubations. Thus, this α1,2-mannosidase activity is associated with the subsequent processing enzymes known to be located in the *trans* Golgi apparatus (20, 21). The results obtained from the experiments using monensin are consistent with the presence of α1,2-mannosidase in this subcellular location. Recent studies have indicated that the site of action of monensin is between the *cis* and *trans* Golgi elements (20, 21, 28), although evidence for a block beyond the Golgi apparatus has also been proposed (29). In the presence of monensin, the major α-chain oligosaccharide isolated from the cells and medium contained six mannose residues. Therefore, α1,2-mannosidase activity that can remove the final α1,2-linked mannose residue is located beyond the monensin-induced block. We cannot exclude the possibility that some α1,2-mannosidase activity responsible for the removal of the final α1,2-linked mannose exists before the monensin block since very small, but measurable, amounts of $Man_5GlcNAc$ were isolated from cells treated with either monensin or CCCP (Fig. 7) and from the medium of cells treated with monensin (Fig. 12). Nevertheless, appreciable amounts of $Man_5GlcNAc_2$ α-chain oligosaccharides were not identified after long incubations in the presence of these inhibitors. This, plus the pulse-chase experiments depicting an association of the removal of the final α1,2-linked mannose residue with enzymes responsible for subsequent carbohydrate processing, provides strong evidence for the presence of α1,2-mannosidase activity in the *trans* Golgi apparatus. Processing α1,2-mannosidase in this Golgi subcompartment has not been characterized extensively. In fact, there have been conflicting reports as to whether α1,2-mannosidase activity exists in the *cis* or *trans* Golgi apparatus (30–32). These studies have involved subcellular fractionation of various cell types. However, the documentation of α1,2-mannosidase activity in various subcellular fractions may well require specific substrates. For example, we have recently determined that the structure of the intracellular IgA oligosaccharides containing six mannose residues is a single species with the α1,2-linked mannose residue present on the mannose-linked α1,3 to the innermost β-linked core mannose residue (data not shown). This suggests that the purported MOPC 315 *trans* Golgi α1,2-mannosidase is specific. If so, it would be necessary to have a Man_6-substrate containing a single specific α1,2-linked mannose residue in order to detect enzymatic activity in the *trans* Golgi apparatus of these cells. Such an oligosaccharide used as a substrate would be ideal for detecting the *trans* Golgi apparatus α1,2-mannosidase activity but may well be a poor substrate for rough endoplasmic reticulum or *cis* Golgi α1,2-mannosidases. Likewise, a substrate containing eight mannose residues might be a poor substrate for a *trans* Golgi α1,2-mannosidase. We are currently investigating this hypothesis using a number of well characterized [^{3}H]mannose-labeled substrates.

It is not know whether a *trans* Golgi apparatus α1,2 mannosidase that specifically removes the last α1,2-linked mannose residue from $Man_6GlcNAc_2$ is present in all cell types. Preliminary data have indicated the presence of such an enzyme in MOPC 460 cells, another murine IgA-secreting plasmacytoma cell line. Likewise, Ruddon et al. (19) using cultured human choriocarcinoma cells have shown that pulse-labeled α-subunits of human chorionic gonadotropin have a relatively long intracellular half-life and contain oligosaccharides that remain sensitive to endo-β-*N*-acetylglucosaminidase H until secreted. Furthermore, a major α-subunit oligosaccharide species accumulating intracellularly appeared to be $Man_6GlcNAc_2$, a result similar to our findings with the α-chain of MOPC 315 IgA. Both human chorionic gonadotropin and IgA are secretory proteins known to be secreted continuously without appreciable intracellular storage. Whether this pattern of α1,2-linked mannose processing is unique to secreted glycoproteins remains to be verified.

The removal of the final α1,2-linked mannose residue and subsequent carbohydrate modifications do not appear to be absolute requirements for the secretion of IgA since even in the presence of monensin, some α-chains with partially processed high mannose-type oligosaccharides are secreted. Recently, it has also been shown that monensin treatment of

human fibroblasts in culture results in the secretion of fibronectin with abnormal carbohydrate chains (33). Our study clearly demonstrates that the initial carbohydrate-processing events are not affected by monensin, as the glucose residues are rapidly removed and the time course for the removal of the first three α1,2-linked mannose residues is unchanged. However, α-chains secreted in the presence of monensin have carbohydrate structures similar to those accumulating intracellularly. Whether monensin directly inhibits the processing enzymes in the *trans* Golgi apparatus or in some way causes the partially processed α-chains to bypass this site leading to secretion by another subcellular route remains to be elucidated.

We conclude that the processing of MOPC 315 IgA α-chain oligosaccharides involves the activity of α1,2-mannosidase in at least two subcellular locations. This may represent the presence of several enzyme proteins as have been described in rat liver (5, 6). To date, we do not have direct evidence for the presence of multiple α1,2-mannosidase enzymes in MOPC 315 cells. It is possible that a single enzyme protein exists within the endoplasmic reticulum and Golgi apparatus. Various changes in the subcellular environment might affect substrate-enzyme interactions, resulting in preferential hydrolysis of mannose residues from a specific oligosaccharide structure. Although we believe that this is unlikely, direct evidence must await isolation and characterization of the proteins responsible for the α1,2-mannosidase activities demonstrated in this study.

We would like to thank Dr. S. Kornfeld and Dr. J. Baenziger for their gifts and helpful discussions.

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