

Targeting of β -Glucuronidase to Lysosomes in Mannose 6-Phosphate Receptor-deficient MOPC 315 Cells

CHRISTOPHER A. GABEL and STUART KORNFELD

Departments of Internal Medicine and Biological Chemistry, Division of Hematology-Oncology, Washington University School of Medicine, St. Louis, MO 63110

ABSTRACT The murine plasma cell line MOPC 315 efficiently targets newly synthesized acid hydrolases to lysosomes in spite of a marked deficiency in the level of the mannose 6-phosphate receptor (Gabel, C., D. Goldberg, and S. Kornfeld, 1983, *Proc. Natl. Acad. Sci. USA*, 80:775–779). To better understand the routing of lysosomal enzymes in this cell line, pulse-chase experiments were performed with [2-³H]mannose and [³⁵S]methionine followed by immunoprecipitation of β -glucuronidase and IgA. By 3 h of chase, essentially all of the newly synthesized β -glucuronidase had undergone proteolytic processing, suggesting that the molecules had reached lysosomes. At this time 30% of the pulse-labeled IgA was still intracellular. The oligosaccharides on the intracellular IgA were of the high mannose-type, while the secreted IgA contained processed, complex-type oligosaccharides. This indicates that the intracellular IgA was still in the endoplasmic reticulum or an early region of the Golgi complex when all of the β -glucuronidase had reached lysosomes. Therefore, β -glucuronidase and IgA must exit from the endoplasmic reticulum or the early Golgi complex at different rates, a finding that is inconsistent with bulk phase movement of these proteins from the endoplasmic reticulum to the *trans* Golgi complex.

The addition of the ionophore monensin greatly slows the rate of IgA secretion from MOPC 315 cells and the molecules secreted have incompletely processed oligosaccharides. In contrast, monensin only slightly delays the transport of newly synthesized β -glucuronidase to lysosomes and causes no significant alteration in the extent of oligosaccharide phosphorylation, a process that appears to occur in the early (*cis*) Golgi complex. However, the labeled β -glucuronidase was deficient in sialylated, phosphorylated hybrid oligosaccharides whose biosynthesis requires the action of late stage oligosaccharide processing enzymes assumed to be localized in the *trans* Golgi complex.

Lysosomal hydrolases, like membrane and secretory proteins, are synthesized in the rough endoplasmic reticulum (ER) as preproteins (1–4). Following their biosynthesis the acid hydrolases must be sorted from other proteins present in the lumen of the endoplasmic reticulum and delivered to lysosomes. The best understood mechanism for lysosomal enzyme sorting involves the mannose 6-phosphate (Man-6-P)¹ recognition system. Newly synthesized acid hydrolases acquire Man-6-P residues by the following two step reaction. First, *N*-acetylglucosamine-1-phosphate is transferred to the C-6 position of mannose residues present on asparagine-linked high mannose oligosaccharides (5, 6). The enzyme catalyzing this

reaction, UDP-*N*-acetylglucosamine:lysosomal enzyme *N*-acetylglucosaminylphosphotransferase, is membrane-bound and is associated with the Golgi apparatus (7, 8). In the second step, the *N*-acetylglucosamine residue is removed by another Golgi-associated enzyme, *N*-acetylglucosaminylphosphoglycosidase, to generate the Man-6-P monoester (9, 10). These phosphomonoesters mediate binding of the acid hydrolase to a specific receptor (the Man-6-P receptor) (11–14), and the resulting receptor-ligand complex is translocated to lysosomes or a prelysosomal compartment where the low pH stimulates dissociation and completes the delivery process (15).

Although the Man-6-P receptor-mediated pathway has been shown to be a general mechanism by which acid hydrolases reach lysosomes, alternative pathways must also exist (16,

¹ Abbreviations used in this paper: Man-6-P, mannose 6-phosphate.

17). Of particular interest is the observation that several murine tissue culture cell lines lack (or are deficient in) the Man-6-P receptor and yet possess lysosomal organelles (18). The acid hydrolases synthesized by the Man-6-P receptor-deficient cells are phosphorylated and the oligosaccharides remain phosphorylated after the enzymes reach lysosomes; this contrasts to the situation in Man-6-P receptor-positive cells where the Man-6-P recognition signal is destroyed soon after entry of the enzymes into lysosomes (11, 19). Thus, the Man-6-P receptor-dependent and independent pathways appear to result in the formation of lysosomes possessing different enzymatic compositions.

To learn more about the pathway utilized by Man-6-P receptor-deficient cells for targeting acid hydrolases to lysosomes, we have studied the biosynthesis and processing of β -glucuronidase in MOPC 315 cells. These cells contain ~2% of the level of the Man-6-P receptor found in human diploid fibroblasts and, like the receptor-negative lines, they fail to destroy the Man-6-P recognition marker after the acid hydrolases arrive in the lysosomes (18). Since these cells also synthesize and secrete IgA, it was possible to study the synthesis and targeting of two classes of soluble glycoproteins in the same cell. The results indicate that the two proteins exit from the endoplasmic reticulum or *cis* Golgi at different rates and that monensin has a differential effect on the targeting of these proteins.

MATERIALS AND METHODS

Pulse-labeling of Cells: MOPC 315 murine myeloma cells were obtained from Dr. Scot Hickman, Washington University. The cells were maintained in α -minimal essential medium (MEM) containing 10% fetal calf serum at 37°C in a 5% CO₂ atmosphere. For labeling with [2-³H]mannose (24 Ci/mmol from New England Nuclear, Boston, MA), cells were harvested by centrifugation and resuspended to a final concentration of 6.7×10^6 cells/ml in glucose/bicarbonate-free α -minimal essential medium containing 20 mM HEPES, pH 7, 10% dialyzed horse serum, and 67 μ Ci/ml of [³H]mannose; when indicated, monensin was added from a concentrated ethanol stock solution (the final ethanol concentration never exceeded 0.1%). The cells were incubated in the labeling solution for 20 min at 37°C and the chase was initiated by diluting with 6 vol of α -minimal essential medium containing 10% fetal calf serum, 5 mM mannose, 5 mM glucose, and 20 mM HEPES, pH 7 (\pm monensin).

For experiments involving [³⁵S]methionine (1,270 Ci/mmol from Amersham Corp., Arlington Heights, IL), the cells were harvested by centrifugation, resuspended in methionine-free Earle's minimal essential medium (MFMEM) containing 10% dialyzed horse serum, 20 mM HEPES, pH 7, and recentrifuged. The washed cells were resuspended in MFMEM containing 10% dialyzed horse serum, 20 mM HEPES, pH 7, and 88 μ Ci/ml of [³⁵S]methionine (\pm monensin) to a final concentration of 5×10^6 cells/ml and incubated for 20 min at 37°C. To initiate the chase the labeling mixtures were diluted sixfold with α -minimal essential medium containing 10% fetal calf serum, 20 mM HEPES, pH 7, and 0.1 mg/ml methionine (\pm monensin).

At the end of the chase, the cells were collected by centrifugation, washed once with cold phosphate-buffered saline (PBS), and the washed cell pellet was rapidly frozen in a dry ice/ethanol bath. In some experiments, the chase medium was recovered after removal of the cells by centrifugation and filtered through a 0.2- μ M filter. The pulse-chase experiments were carried out using sterile conditions.

Immunoprecipitation of IgA and β -Glucuronidase: The frozen labeled cell pellets (containing $2.5\text{--}10 \times 10^7$ cells) were suspended in 1 ml of extraction buffer (20 mM HEPES, pH 7, containing 10 mM phosphate, 150 mM NaCl, 1% Triton X-100, 0.2 TIU/ml Trasylol, 2.5 mM mannose 6-phosphate) and either 5 mM mannose (in the case of [³H]mannose-labeled cells) or 0.7 mM methionine (for [³⁵S]methionine-labeled cells). The suspensions were sonicated for 10 s on ice at 100 Ws (Biosonic, Inc. Seattle, WA) and incubated for an additional 30 min on ice. The extracts were clarified by centrifugation (100,000 *g* for 45 min) and the pellets discarded. The supernatants at this point were divided into two fractions; 20% was diluted with 0.8 ml of extraction buffer and used for IgA immunoprecipitation. The remaining

80% of each supernatant was diluted with 0.2 ml of extraction buffer and used for β -glucuronidase immunoprecipitation.

To preclear each fraction, 0.05 ml of a 10% suspension of *Staphylococcus aureus* cells (Pansorbin from Calbiochem-Behring Corp., San Diego, CA) was added and incubated for 30 min on ice. The cells were removed by centrifugation and the supernatants recovered. Goat anti- β -glucuronidase (a generous gift of Dr. Roger Ganshow, University of Cincinnati) or goat anti-mouse IgA (Gateway Laboratories, Chaokia, IL) was added to the appropriate fractions and incubated for 1.5 h at 4°C. The antigen-antibody complexes were recovered by the addition of Pansorbin (0.025 to 0.05 ml of a 10% suspension) and following a 30-min incubation on ice the bacteria were collected by centrifugation and washed (by repeated centrifugation) five times with 10 mM phosphate, pH 7, 150 mM NaCl, 1% Triton X-100, 0.5% deoxycholate, 0.05% SDS, 1 mM Man-6-P, 0.2 TIU/ml Trasylol, and once with 50 mM Tris, pH 6.8 (1 ml/wash). The final washed bacterial pellet was disaggregated in 0.1 ml of 50 mM Tris, pH 6.8, 1% SDS, 1% β -mercaptoethanol, 20% glycerol by boiling for 3 min. The suspensions were clarified by centrifugation and the supernatants analyzed by SDS PAGE (20) and fluorography using Enhance (New England Nuclear, Boston, MA). When indicated, the radioactive regions of the dried gel were excised, rehydrated in 0.2 ml of H₂O, and the gel solubilized by adding 1.3 ml of hydrogen peroxide and heating at 100°C for 2–3 h. After cooling, 15 ml of scintillation cocktail (Scintiverse I, Fisher Scientific Co., Pittsburgh, PA) were added and the radioactivity quantitated by liquid scintillation counting.

Oligosaccharide Structural Analysis: Following SDS PAGE and radioautography of the [³H]mannose-labeled immunoprecipitates, the radioactive regions of the dried gel were excised and placed in 1 ml of 0.1 M Tris, pH 8, containing 20 mM CaCl₂, 0.1 M glucose 6-phosphate, and 5 mg pronase. The digests were incubated for 15 h at 56°C, boiled for 5 min, and clarified by centrifugation. The solubilized ³H-labeled glycopeptides were desalted by Sephadex G-25 chromatography, dried by evaporation under reduced pressure, dissolved in 2 ml of 10 mM Tris, pH 8, 150 mM NaCl, 1 mM MgCl₂, 1 mM CaCl₂, 0.02% sodium azide (TBS) and applied to Concanavalin A-Sepharose (1 ml columns equilibrated in TBS). The columns were washed with TBS and eluted sequentially with 10 mM α -methylglucoside in TBS and 100 mM α -methylmannoside (56°C) in TBS (21, 22).

The high mannose-type glycopeptides (100 mM α -methylmannoside eluates) were desalted by Sephadex G-25 chromatography, dried under reduced pressure, and digested with endo- β -*N*-acetylglucosaminidase H (Endo H) as previously described (11). At the end of the digestion, the high mannose oligosaccharides from IgA were desalted by passage over a pasteur pipet column of Amberlite MB3 (Sigma Chemical Co., St. Louis, MO) and analyzed by high performance liquid chromatography (HPLC) as described previously (23). The Endo H digests (0.05 ml) of β -glucuronidase glycopeptides were diluted with 2 ml of 2 mM ammonium acetate, pH 5.3, and applied to 5-ml columns of QAE-Sephadex equilibrated in 2 mM ammonium acetate. The phosphorylated oligosaccharides were eluted using a 200 ml linear gradient of ammonium acetate, pH 5.3, from 2 to 350 mM (in acetate) as previously described (11).

The complex-type glycopeptides recovered from [³H]mannose-labeled IgA after Con A-Sepharose chromatography were desalted by Sephadex G-25 chromatography, dried by evaporation under reduced pressure, and digested with jack bean α -mannosidase (0.23 U), β -*N*-acetylglucosaminidase (0.02 U), and β -galactosidase (0.014 U) as described elsewhere (24). To remove sialic acid residues, the glycopeptides were hydrolyzed with 2 N acetic acid at 100°C for 60 min; the hydrolysates were then dried by evaporation under reduced pressure. The extent of [³H]mannose release following exoglycosidase digestion was assessed by passing the digests over a Bio-Gel P-4 column equilibrated in H₂O; free mannose is included whereas glycopeptide-associated mannose is excluded.

Subcellular Fractionation of [³H]Labeled MOPC 315 Cells: 1×10^8 cells were pulse-labeled with [2-³H]mannose and chased for 7 h as described above; 1 μ M monensin was present during both the pulse and the chase. The cells were collected by centrifugation, and washed once with cold PBS, and once with 0.25 M sucrose. The washed cells were suspended in 2 ml of 0.25 M sucrose, lysed by 10 strokes in a Dounce homogenizer, diluted with 2 ml of 0.25 M sucrose, containing 10 mM acetic acid and 10 mM triethanolamine, pH 7, and centrifuged for 10 min at 850 *g*. The supernatant was layered over 30 ml of a colloidal silica/polyvinylpyrrolidone mixture prepared as previously described (25). The lysate was centrifuged for 2.5 h in a VT150 rotor at 18,000 rpm and the resulting gradient collected in 18 fractions.

After the addition of 0.2 ml of 0.25 M HEPES, pH 7, 0.5 M NaCl, 10% Triton X-100, 50 mM mannose, 20 mM Man-6-P, and 2 TIU/ml Trasylol, each fraction was centrifuged at 100,000 *g* for 2 h to remove the colloidal silica. The supernatants were divided into two fractions (10% for IgA and 90% for β -glucuronidase) and IgA and β -glucuronidase were immunoprecipitated as described above. Before immunoprecipitating, β -glucuronidase activity was mon-

itored by assaying 0.05 ml of each solubilized fraction for 2 h at 37°C using p-NO₂-phenyl-β-glucuronide as previously described (18).

RESULTS

Pulse-chase Analysis of Intracellular Movements

β-Glucuronidase is synthesized as a proenzyme (75,000 daltons) which is proteolytically processed to a smaller molecular weight species (73,000 daltons) during its maturation (4, 26, 27). Fig. 1 shows that β-glucuronidase synthesized by MOPC 315 cells undergoes this proteolytic conversion, as evidenced by the increased mobility of the mature form of the enzyme during SDS gel electrophoresis. The cleavage is thought to occur in lysosomes (28). Using the proteolytic event as an indicator of entry into lysosomes, the kinetics of β-glucuronidase arrival in lysosomes was compared with the secretion of IgA. MOPC 315 cells were labeled for 20 min with [³⁵S]methionine and chased for various periods of time in the presence of an excess of unlabeled methionine. At the

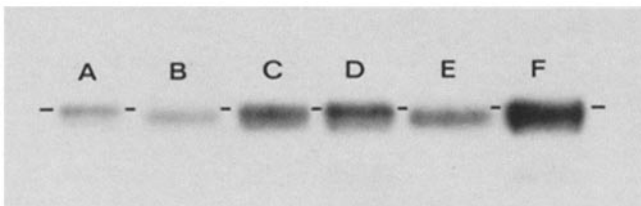


FIGURE 1 Demonstration of β-glucuronidase maturation in MOPC 315 cells. Cells were labeled for 20 min with [2-³H]mannose and then chased for either 10 or 270 min in the presence of an excess of unlabeled mannose. β-Glucuronidase was immunoprecipitated from nonionic detergent extracts of the cells and the precipitates analyzed by SDS gel electrophoresis and fluorography. The fluorograph shows: the immunoprecipitate obtained after 10 min of chase (lanes A and D); the immunoprecipitate obtained after 270 min of chase (lanes B and E); a 1:1 mixture of the 10- and 270-min immunoprecipitates (lanes C and F). Lanes D–F contain twice the amount of radioactivity as loaded in lanes A–C. The polyacrylamide concentration was 10% in the separating and 5% in the stacking gel.

end of the chase, the β-glucuronidase and IgA were immunoprecipitated and analyzed by SDS PAGE. Fig. 2 shows the radioautogram of the SDS gel. As expected, the [³⁵S]labeled β-glucuronidase recovered after 10 min of chase (lane A) migrates slightly slower than that recovered after 3–4 h of chase (lanes E and F). Because of the small difference in molecular weight, it is difficult to discern the proportions of the two forms at the intermediate times (lanes B–D); however by 3 h of chase (lane E) only the mature form of the [³⁵S] labeled β-glucuronidase is detected suggesting that the majority of the pulse-labeled molecules had reached lysosomes during this time period. The amount of IgA heavy chain (which migrates as two or three discrete bands, possibly reflecting the content of asparagine-linked oligosaccharides) recovered in the immunoprecipitates decreased with increasing time of chase (lanes G–L) as expected for a secretory product.

The appropriate radioactive regions of the dried gel were excised and the absolute amount of radioactivity determined by liquid scintillation counting after solubilizing the gel pieces. As shown in Fig. 3A, there is a slight decrease in the amount of immunoprecipitable β-glucuronidase during the 4-h chase. This loss most likely results from secretion of some of the newly synthesized molecules for the MOPC 315 cells do secrete 19% of their total β-glucuronidase activity (4). In contrast, the amount of immunoprecipitable IgA heavy chain recovered from the cells stays constant during the first 30 min of chase and then declines with a *t*_{1/2} of 87 min. After 3 h of chase, 30% of the [³⁵S]labeled IgA molecules remained intracellular.

A similar pulse-chase experiment was performed in which [2-³H]mannose was used in place of [³⁵S]methionine. As observed above, during the chase the [³H]mannose-labeled β-glucuronidase is converted to a lower molecular weight form and the amount of immunoprecipitable IgA heavy chains decreases (Fig. 4). The regions of the dried gel containing the IgA heavy chains were excised and the [³H]mannose-labeled radioactivity solubilized by pronase digestion. The resulting glycopeptides were analyzed by Con A-Sepharose chromatog-

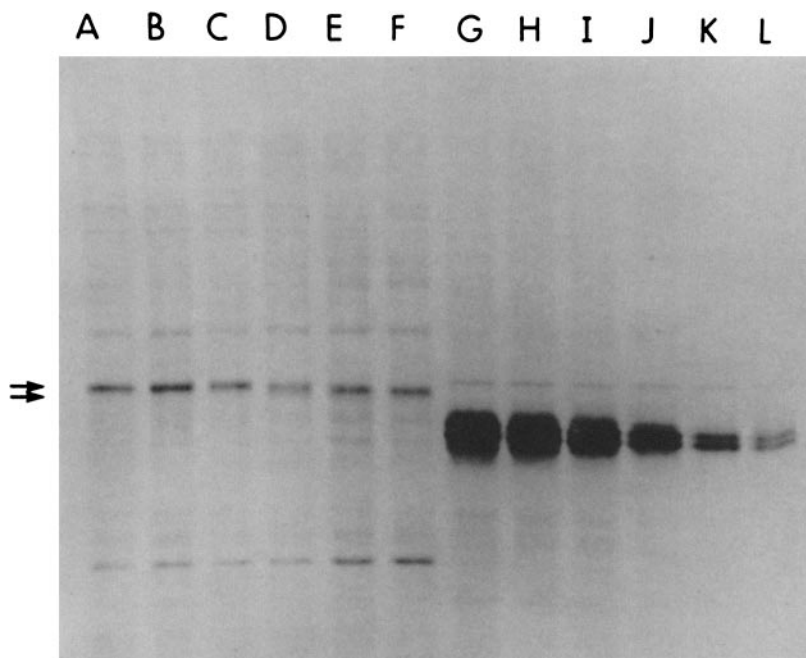


FIGURE 2 Pulse-chase analysis of the processing of β-glucuronidase and secretion of IgA by MOPC 315 cells. Cells (3×10^7 per time point) were labeled for 20 min with [³⁵S]methionine and then chased for 10 min (A and G), 30 min (B and H), 60 min (C and I), 120 min (D and J), 180 min (E and K), and 240 min (F and L). β-Glucuronidase and IgA were immunoprecipitated from detergent extracts of the cells and subjected to SDS PAGE using a 7.5% separating gel and 5% stacking gel. The fluorogram of the gel shows lanes A–F, the β-glucuronidase immunoprecipitates; lanes G–L, the IgA immunoprecipitates. The arrows indicate the position of the precursor and mature forms of β-glucuronidase.

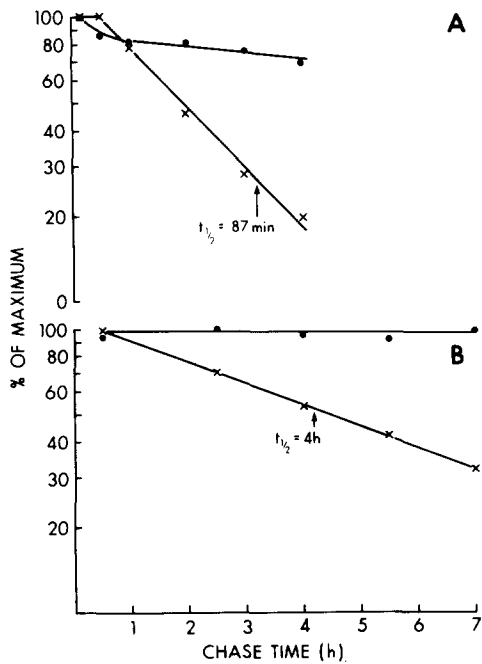


FIGURE 3 Quantitation of the amount of newly synthesized β -glucuronidase and IgA heavy chains recovered by immunoprecipitation. The appropriate regions of the dried gel shown in Fig. 2 (A) and Fig. 7 (B) were excised and the radioactivity present was determined by liquid scintillation counting. The data show the percent of the maximal amount of radioactivity recovered in β -glucuronidase (●) and IgA heavy chains (x) as a function of time of chase.

raphy. At both 30 and 240 min of chase, the intracellular forms of IgA contain predominantly high mannose-type glycopeptides that bind to Con A-Sepharose and elute with 0.1 M α -methylmannoside (Fig. 5, A and C). In contrast, the IgA heavy chains secreted into the medium contain complex-type asparagine-linked glycopeptides that pass through the Con A-Sepharose column or bind and elute with 10 mM α -methylglucoside (Fig. 6A). This indicates that the secreted IgA molecules migrate through the Golgi apparatus where the asparagine-linked oligosaccharide processing enzymes are localized (29) prior to exiting from the cell. Moreover, once processed, the molecules must rapidly exit the cell since only those forms possessing high mannose oligosaccharides are recovered intracellularly.

Fig. 5 also shows the size of the intracellular high mannose oligosaccharides recovered from the IgA heavy chains as determined by high performance liquid chromatography analysis. Following 30 min of chase, the major species contains nine mannose residues and there are smaller quantities of units containing eight, seven, and six mannose residues (Fig. 5B). However, at later times during the chase (Fig. 5D) the predominant species contains only six mannose residues.

Effect of Monensin on IgA Secretion from MOPC 315 Cells

Monensin has previously been shown to inhibit the secretion of IgA from MOPC 315 cells (30, 31). To verify that the ionophore has a similar effect in our system, MOPC 315 cells were pulse-labeled with [35 S]methionine and chased in the presence of 1 μ M monensin. At various times during the chase, the cells were solubilized and IgA recovered by im-

munoprecipitation. As expected, treatment of MOPC 315 cells with monensin decreases the amount of IgA secreted during the period of the experiment (Fig. 7); quantitation of the intracellular IgA heavy chains after SDS gel electropho-

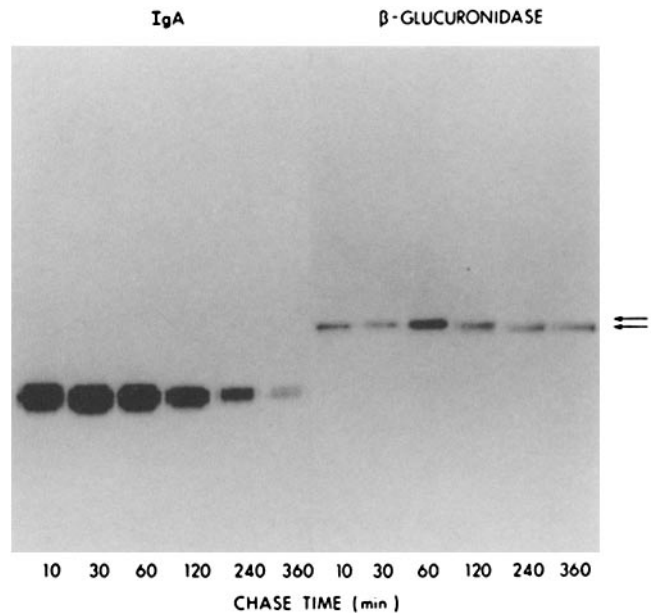


FIGURE 4 Recovery of β -glucuronidase and IgA from MOPC 315 cells pulse-labeled with [3 H]mannose. Following a 20-min pulse, the cells (5×10^7 per time point) were chased for 10, 30, 60, 120, 240, and 360 min. β -Glucuronidase and IgA were immunoprecipitated and analyzed by SDS gel electrophoresis and fluorography. The left hand lanes display the IgA immunoprecipitates (which were fractionated using a 10% separating gel) and the right hand lanes, the β -glucuronidase immunoprecipitates (which were fractionated using a 7.5% separating gel).

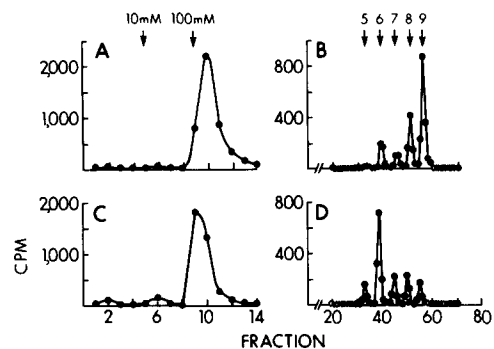


FIGURE 5 Characterization of the asparagine-linked oligosaccharides present on the intracellular IgA heavy chains. The appropriate regions of the dried gel shown in Fig. 4 were excised and the radioactivity solubilized by pronase digestion. Aliquots of the resulting glycopeptides were applied to Con A-Sepharose and the columns were eluted with 10 mM α -methylglucoside (10 mM) and 100 mM α -methylmannoside (100 mM); the elution profiles of the [3 H]labeled glycopeptides recovered after 30 and 240 min of chase are shown in A and C, respectively. The glycopeptides eluted with 100 mM α -methylmannoside were desalted, digested with endo H, and the released oligosaccharides analyzed by high performance liquid chromatography. The profiles are shown for the oligosaccharides recovered after 30 (B) and 240 min (D) of chase. The arrows indicate the elution position of the following high mannose oligosaccharide standards: 9, Mannose₉GlcNAc, 8, Mannose₈GlcNAc, 7, Mannose₇GlcNAc, 6, Mannose₆GlcNAc, and 5, Mannose₅GlcNAc.

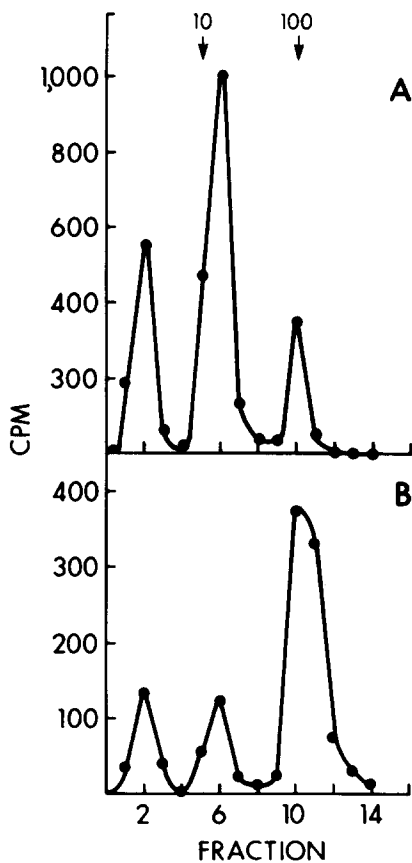


FIGURE 6 Con A-Sepharose chromatography of [³H]mannose-labeled IgA secreted from MOPC 315 cells. The appropriate regions of the dried gel shown in Fig. 8 were excised, the radioactivity solubilized by pronase digestion, and the resulting glycopeptides applied to Con A-Sepharose. The columns were subsequently eluted with 10 mM α -methylglucoside (10) and 100 mM α -methylmannoside (100). The profiles are of the glycopeptides derived from IgA secreted from control cells (A) and from cells treated with 1 μ M monensin (B).

resis of the immunoprecipitates (Fig. 3B) indicates that secretion occurs with a $t_{1/2}$ of 4 h from the ionophore-treated cells. Not only is secretion slowed but, as shown in Fig. 8, the IgA heavy chains secreted in the presence of monensin from [²⁻³H]mannose-labeled cells migrate abnormally during SDS gel electrophoresis. The regions of the dried gel containing the [³H]mannose-labeled heavy chains were excised and the radioactivity solubilized by pronase digestion; the resulting glycopeptides were fractionated by Con A-Sepharose chromatography (21, 22). As shown in Fig. 6A, IgA heavy chains secreted from control cells contain mannose-labeled glycopeptides that run through Con A-Sepharose (28%; tri- and tetraantennary complex-type), that bind and elute with 10 mM α -methylglucoside (58%; biantennary complex-type), and that bind and elute with 0.1 M α -methylmannoside (14%; high mannose-type). The IgA heavy chains secreted from the monensin-treated cells (Fig. 6B) contain the same three species of glycopeptides but the percentage of radioactivity recovered in complex-type units is decreased (14% as tri- and tetraantennary, 19% as biantennary, and 67% as high mannose).

The complex-type glycopeptides were pooled and analyzed by mixed exoglycosidase digestion to determine whether the oligosaccharide chains secreted by the monensin-treated cells were altered. As shown in Table I, [³H]mannose was released

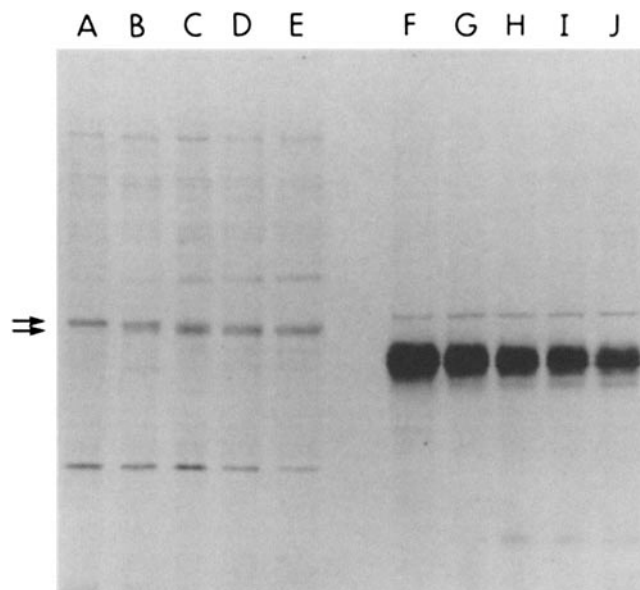


FIGURE 7 Effect of monensin of the processing of β -glucuronidase and IgA. MOPC 315 cells (2.5×10^7 /time point) were labeled for 20 min with [³⁵S]methionine and chased for 30 min (A and F), 150 min (B and G), 240 min (C and H), 330 min (D and I), and 420 min (E and J); 1 μ M monensin was present during both the pulse and chase. β -Glucuronidase and IgA were immunoprecipitated from detergent extracts of the cells and analyzed by SDS gel electrophoresis and fluorography. Lanes A–E of the fluorogram display the β -glucuronidase immunoprecipitates and lanes F–J the IgA immunoprecipitates.

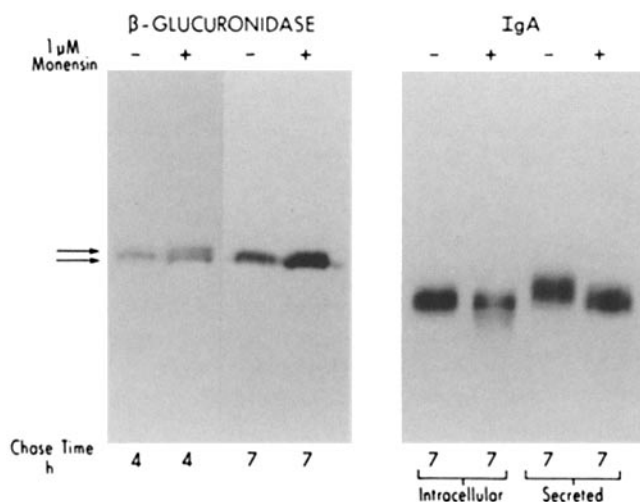


FIGURE 8 Comparison of IgA and β -glucuronidase recovered from control and monensin-treated MOPC 315 cells. Cells were labeled for 20 min with [²⁻³H]mannose and then chased for either 4 or 7 h; the monensin-treated cells were exposed to the ionophore (1 μ M) during both the pulse and chase. IgA was immunoprecipitated from the chase media and the cell extracts while β -glucuronidase was immunoprecipitated only from the cell extracts.

from the control glycopeptides only when these glycopeptides were first desialylated and then treated with the combination of β -galactosidase, β -N-acetylglucosaminidase, and α -mannosidase.² This demonstrates that each molecule contains the

² The theoretical release of radioactivity by this treatment is 50%. This is because each glycopeptide contains four labeled residues: two α -linked mannoses, one β -linked mannose and one fucose. The enzyme digestion will only release the two α -linked mannoses.

TABLE I
Exoglycosidase Digestion of the Complex-Type Glycopeptides
Isolated from Secreted IgA

Treatment	Total radioactivity released	
	Control %	Monensin-treated %
α -Mannosidase + β -N-Acetylglucosaminidase + β -galactosidase	5	20
Mild acid hydrolysis followed by:		
(a) α -Mannosidase	6	7
(b) α -Mannosidase + β -N-Acetylglucosaminidase	7	15
(c) α -Mannosidase + β -N-acetylglucosaminidase + β -Galactosidase	44	41

Following Con A-Sepharose chromatography, the [3 H]mannose-labeled complex-type glycopeptides isolated from the IgA secreted from control and monensin-treated MOPC 315 cells were desalted and digested with the indicated exoglycosidases. In some cases, the glycopeptides were pretreated with mild acid (2N acetic acid for 1 h at 100°C) to remove sialic acid residues prior to adding the enzymes. The amount of radioactivity released was determined by applying the digests (sequentially) to a Bio-Gel P-4 column.

expected sequence sialic acid→galactose→N-acetylglucosamine→mannose. In the case of the glycopeptides from monensin-treated cells, 15–19% [3 H]mannose release was observed following digestion with only β -N-acetylglucosaminidase and α -mannosidase (not shown). The addition of β -galactosidase (in combination with N-acetylglucosaminidase and α -mannosidase) resulted in a small increment in [3 H]mannose release (not shown). However, desialylation and treatment with all three exoglycosidases was needed for maximal [3 H]mannose release. These results indicate that some, but not all, of the complex-type units from monensin-treated cells are deficient in both sialic acid and galactose residues. Therefore, treatment of MOPC 315 cells with 1 μ M monensin slows the rate of IgA secretion, and impairs the processing of the asparagine-linked oligosaccharides on the molecules that are secreted.

Effect of Monensin on β -Glucuronidase Processing

β -Glucuronidase was immunoprecipitated from the same [35 S]methionine-labeled, monensin-treated MOPC 315 cell extracts as used above for the IgA analysis. As shown in Fig. 7, monensin does not inhibit the conversion of the precursor to mature form of β -glucuronidase, although the time required for complete conversion is increased relative to the control cells (Fig. 2). Quantitation of the radiolabeled β -glucuronidase recovered by immunoprecipitation during the chase indicates that there is no significant loss of the hydrolase from the ionophore-treated cells during the 7-h chase (Fig. 3B).

To determine whether monensin affects the phosphorylation of the high mannose oligosaccharides on β -glucuronidase, MOPC 315 cells were pulse-labeled for 20 min with [2 - 3 H]mannose and chased for 4 or 7 h in the presence and absence of 1 μ M monensin. At the conclusion of the chase, the β -glucuronidase was immunoprecipitated and analyzed by SDS gel electrophoresis. As shown in Fig. 8, the [3 H]mannose-labeled β -glucuronidase isolated from the control

cells at both 4 and 7 h of chase is recovered as the mature form. β -Glucuronidase recovered from the monensin-treated cells contains both precursor and mature forms after 4 h of chase and predominantly the mature form following the 7-h chase; these results agree with the [35 S]methionine data presented above. The regions of the dried gel containing β -glucuronidase were excised, and the high mannose oligosaccharides isolated and analyzed by QAE-Sephadex chromatography. The elution profiles are shown in Fig. 9. The enzyme recovered from both the control and the monensin-treated cells contains ~40% of the label in the form of phosphorylated high mannose oligosaccharides. Thus, monensin does not inhibit the overall extent of phosphorylation of β -glucuronidase by the Golgi-associated N-acetylglucosaminylphosphotransferase. A comparison of the profiles shows that after 4 h of chase both the control and monensin-treated β -glucuronidase contain oligosaccharides possessing phosphomonoesters and phosphodiester. The monensin-treated β -glucuronidase contains a somewhat higher proportion of oligosaccharides possessing phosphodiester at the 4-h point (compare 9, A and B). By 7 h of chase the QAE-Sephadex profiles of the phosphorylated oligosaccharides recovered from the control and monensin-treated β -glucuronidase are similar in that the primary species in both fractions corresponds to oligosaccharides containing one or two phosphomonoesters (Fig. 9, C and D). However, one significant difference was noted. The profile of the control material shows two additional peaks between fractions 25–45 that are missing in the profile of the monensin-treated material (compare Fig. 9, C to D). These peaks represent sialylated, phosphorylated hybrid oligosaccharides (e.g., one sialic acid and one phosphodiester or one sialic acid and one phosphomonoester) (13).

Thus, 1 μ M monensin delays both proteolysis of newly synthesized β -glucuronidase precursor and the conversion of the phosphorylated oligosaccharides from phosphodiester to phosphomonoesters. The extent of phosphorylation of β -glucuronidase is very similar in both the control and monensin-treated cells, but the enzyme from the monensin-treated cells is missing the sialylated, phosphorylated species.

Segregation of IgA and β -Glucuronidase in the Presence of Monensin

Although the proteolytic processing of the precursor form of β -glucuronidase in the presence of monensin suggests that the newly synthesized molecules are reaching lysosomes, additional experiments were performed to show that the intracellular forms of IgA and β -glucuronidase are actually segregated in the presence of the ionophore. MOPC 315 cells were labeled for 20 min with [2 - 3 H]mannose and chased for 7 h in the presence of 1 μ M monensin. At the end of the chase, cell lysates were prepared and subjected to centrifugation on a colloidal silica gradient. As shown in Fig. 10A, β -glucuronidase activity distributes into both a light and heavy fraction as has previously been observed (18). In human fibroblasts the lighter peak has been shown to contain many subcellular organelles, including endoplasmic reticulum and Golgi vesicles, whereas the denser peak is greatly enriched in lysosomal structures (25). The difference in the nature of lysosomal structures between the two peaks is unknown. The distribution of total radioactivity is shown in Fig. 10B; the majority of the radioactivity co-sediments with the lighter peak of β -glucuronidase activity or remains at the top of the gradient.

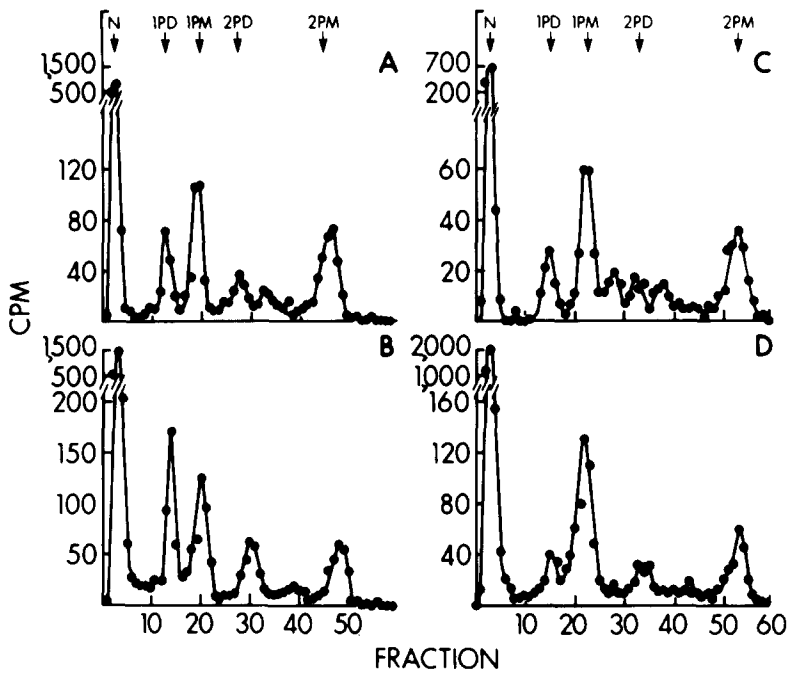


FIGURE 9 QAE-Sephadex chromatography of β -glucuronidase high mannose oligosaccharides. MOPC 315 cells ($0.7-1 \times 10^8$ /time point) were labeled for 20 min with [^3H]mannose and chased for either 4 or 7 h ($\pm 1 \mu\text{M}$ monensin). Intracellular β -glucuronidase was immunoprecipitated and analyzed by SDS gel electrophoresis and fluorography. The regions of the gel containing β -glucuronidase were excised, the radioactivity solubilized by pronase digestion, and the resulting glycopeptides fractionated on Con A-Sepharose and eluted with α -methylmannoside (not shown). The high mannose-type glycopeptides were desalted, digested with endo H, and each digest was applied to a QAE-Sephadex columns which was subsequently eluted with a linear gradient of ammonium acetate from 2 to 350 mM (100 ml each); 2-ml fractions were collected and the radioactivity determined by liquid scintillation counting. The QAE-Sephadex profiles are of the high mannose oligosaccharides recovered in the immunoprecipitate from: (A) control cells, 4-h chase; (B) monensin-treated cells, 4 h chase; (C) control cells, 7-h chase; (D) monensin-treated cells, 7-h chase. The elution position of standard high mannose oligosaccharides containing 1 phosphodiester (1 PD), 1 phosphomonoester (1 PM), 2 phosphodiesters (2 PD), and 2 phosphomonoesters (2 PM) are indicated. Neutral oligosaccharides (N) do not bind to QAE-Sephadex.

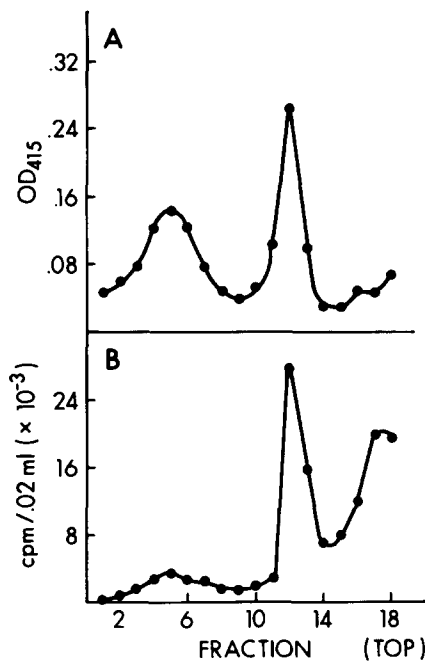


FIGURE 10 Colloidal silica density gradient centrifugation of monensin-treated MOPC 315 cells. Following a 20-min pulse with [^3H]mannose and a 7-h chase (both in the presence of $1 \mu\text{M}$ monensin), the MOPC 315 cells (1×10^8) were lysed and the postnuclear supernatant fractionated by colloidal silica density gradient centrifugation. The resulting gradient was subfractionated into 18 2-ml fractions and aliquots of each were assayed for β -glucuronidase activity (A) or for radioactivity by liquid scintillation counting (B).

β -Glucuronidase and IgA were immunoprecipitated from the gradient fractions to determine the distribution of the labeled molecules. As shown in Fig. 11 A, the [^3H]labeled IgA

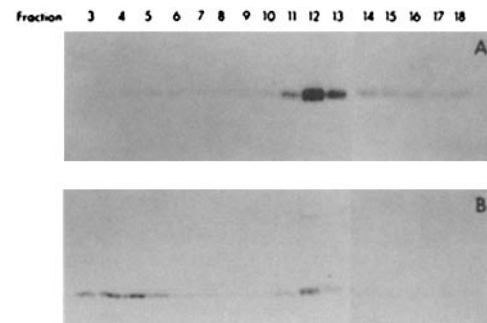


FIGURE 11 Distribution of newly synthesized IgA and β -glucuronidase in the colloidal silica gradient. The gradient fractions shown in Fig. 10 were centrifuged for 2 h at 100,000 g to remove the colloidal silica following the addition of Triton X-100. Fractions 1 and 2 at the bottom of the gradient were not worked up due to their high viscosity. β -Glucuronidase and IgA were immunoprecipitated from the resulting supernatants and analyzed by SDS gel electrophoresis and fluorography. The fluorograms show (A) the distribution of [^3H]labeled IgA heavy chains and; (B) the distribution of [^3H]labeled β -glucuronidase.

molecules are predominantly recovered in fractions 12 and 13, which correspond to the lighter peak of β -glucuronidase activity (Fig. 10). In contrast, the majority of the [^3H]labeled β -glucuronidase is recovered in the denser region of the gradient (Fig. 11 B). There is also [^3H]labeled β -glucuronidase associated with the lighter fractions. Since the distribution across the gradient of β -glucuronidase (as detected by activity) from the monensin-treated cells parallels that of untreated MOPC 315 cells (18), these results indicate that within 7 h of chase the radiolabeled β -glucuronidase molecules achieve the expected intracellular distribution. It is interesting to note that the denser fractions from the gradient contain only the mature form of β -glucuronidase while the lighter fractions contain

both the mature and precursor forms. Brown et al. (27) have previously observed a similar separation of the two forms of β -glucuronidase on colloidal silica gradients. Our ability to detect the precursor in this experiment but not from total cells most likely results from its enrichment in the lighter fractions.

DISCUSSION

These experiments demonstrate that IgA and β -glucuronidase are targeted to their respective destinations at very different rates in MOPC 315 cells. The kinetic analysis revealed that essentially all the newly synthesized β -glucuronidase molecules made during a 20 min pulse-labeling are processed to mature forms within 3 h of chase. Since the proteolytic processing is thought to occur in lysosomes (28), the data suggest that all of the newly formed β -glucuronidase has arrived in lysosomes by 3 h after synthesis. We have previously shown in this cell line that the phosphorylation of lysosomal enzyme oligosaccharide units is almost complete after 1 h of chase (18). Since the *N*-acetylglucosaminylphosphotransferase which mediates the phosphorylation appears to be localized in the early, or *cis* Golgi (10, 32), we conclude that most of the lysosomal enzymes move from the endoplasmic reticulum to the Golgi complex by 1 h of chase. The remaining 2 h of chase time required for complete maturation of β -glucuronidase presumably represents the time required for translocation of the molecules from the Golgi apparatus to lysosomes and for the proteolytic processing reaction. By contrast, ~30% of the pulse-labeled IgA molecules are recovered intracellularly after 3 h of chase and these molecules contain only high mannose-type oligosaccharides. When the intracellular IgA was examined in more detail, it was found that its oligosaccharides are processed from an initial $\text{Man}_9\text{GlcNAc}_2$ species during the chase period. This observation confirms the previous finding of Hickman et al. (31). Two enzymes have been identified that can mediate this mannose trimming. The first is an α 1,2-mannosidase that is localized in the endoplasmic reticulum (33). This enzyme appears to convert $\text{Man}_9\text{GlcNAc}_2$ oligosaccharides to $\text{Man}_8\text{GlcNAc}_2$ quite efficiently, and then slowly hydrolyzes additional mannose residues. The other enzyme is the Golgi α 1,2-mannosidase I which also can act on the $\text{Man}_9\text{GlcNAc}_2$ species but efficiently forms the $\text{Man}_5\text{GlcNAc}_2$ oligosaccharide (34–36). This mannosidase acts in concert with other late stage processing enzymes so that the $\text{Man}_5\text{GlcNAc}_2$ species is rapidly converted to complex-type oligosaccharides. Since the intracellular IgA contained very little $\text{Man}_5\text{GlcNAc}_2$ or complex-type oligosaccharides, it seems likely that the mannose trimming is mediated by the endoplasmic reticulum α -mannosidase. Based on these data, we conclude that the intracellular IgA resides within a region of the endoplasmic reticulum/Golgi complex that is located proximal to the Golgi-associated enzymes which mediate the late-stage events in oligosaccharide processing. Therefore, β -glucuronidase and IgA must exit from the endoplasmic reticulum (or early *cis* Golgi complex) at different rates.

Recently Lodish et al. (37) have demonstrated in human hepatoma HepG2 cells that five secreted proteins leave the rough endoplasmic reticulum at different rates. Similar findings have been reported by Leford and Davis (38) and by Fitting and Kabat for two viral membrane glycoproteins (39). Lodish et al. pointed out that their results are incompatible

with bulk-phase movement of the luminal contents of the endoplasmic reticulum and suggested that there is a receptor protein in the endoplasmic reticulum membrane that selectively mediates the transport of newly synthesized proteins from the rough endoplasmic reticulum to the Golgi complex. These authors postulate that proteins that bind most avidly to the receptor are transported to the Golgi complex at the fastest rates. They also point out that slow-moving proteins need not bind to the receptor, but could be transported by bulk-phase movement of the luminal contents. Our findings provide additional support for the notion that proteins exit the endoplasmic reticulum at different rates by extending the observations to a different cell type and another class of soluble proteins, the lysosomal enzymes.

The ionophore monensin was used to further analyze the movement of IgA and β -glucuronidase in these cells since a number of studies have suggested that it preferentially disrupts the *mid* to *trans* Golgi cisternae and thereby inhibits the normal *cis* to *trans* migration of proteins through the Golgi stacks (40–45). The major finding was that newly synthesized β -glucuronidase molecules are efficiently separated from intracellular IgA and targeted to lysosomes in the presence of monensin whereas IgA secretion is markedly slowed. These observations can be explained in two ways. The first is that movement of these proteins to the *cis* Golgi may be sufficient to allow them to be sorted. According to this interpretation, the two proteins would exit the *cis* Golgi in separate vesicles and those carrying β -glucuronidase would go specifically to lysosomes or to an intermediate compartment where lysosomes are formed. The alternate explanation is that in the presence of monensin β -glucuronidase is translocated more efficiently than IgA from the *cis* to the *trans* Golgi complex and that sorting occurs at the *trans* Golgi. Since β -glucuronidase normally exists the endoplasmic reticulum/early Golgi regions more rapidly than IgA, it may also pass through the monensin-induced block more efficiently.

In addition to the effect on the rate of protein targeting, monensin also caused several alterations in the processing of the oligosaccharide units of these two proteins. Thus, whereas IgA secreted by control cells contains predominantly completed (sialylated) complex-type oligosaccharides, IgA secreted by monensin-treated cells contains primarily high mannose-type oligosaccharides and complex-type units that are deficient in terminal sialic acid and galactose residues. The oligosaccharides present on β -glucuronidase from monensin-treated cells were phosphorylated normally, but the number of sialylated-phosphorylated hybrid species was markedly reduced. The assembly of the latter species requires the action of the same galactosyl- and sialyltransferases that are needed to synthesize the complex-type oligosaccharides of IgA. Since galactosyltransferase (and presumably sialyltransferase) is localized in the *trans* Golgi elements (46–48) these findings are consistent with the notion that monensin disrupts the normal *cis* to *trans* movement of IgA and β -glucuronidase.³ In addition, the secreted IgA that contains high mannose units might represent molecules that have bypassed the *trans* Golgi complex. Alternatively, as suggested above, these molecules may have slowly passed through the *trans* Golgi complex but not

³ Since only a portion of the oligosaccharides of β -glucuronidase are sialylated-phosphorylated hybrids, we cannot determine what percent of the β -glucuronidase molecules normally pass through the *trans* Golgi.

been processed due to an inhibition of the processing enzymes. This would also explain the lack of sialylated-phosphorylated hybrid species on β -glucuronidase. The normal phosphorylation of the β -glucuronidase oligosaccharides in the presence of monensin is consistent with the observation that the enzymes involved in the generation of the Man-6-P recognition signal appear to be localized in the *cis* Golgi complex.

The findings of Strous and co-workers (49) on the effect of monensin on the processing of transferrin and VSV G protein may be relevant to these issues. Using a hepatoma cell line, these investigators found that monensin inhibits the secretion of transferrin and the migration of the VSV G protein to the cell surface. However, the intracellular transferrin had endo H sensitive (high mannose) oligosaccharides whereas the VSV G protein contained endo H-resistant (complex-type) oligosaccharides (40). Normally the mature form of both proteins contains complex-type units. When the cells were examined by immunocytochemistry, it was found that the two proteins co-exist in the same Golgi vesicles (49). On this basis the authors suggested that monensin may not affect the routing of the glycoproteins, but rather the ionophore may have a differential effect on the way newly synthesized glycoproteins are processed by the Golgi-associated enzymes. What is unclear, however, is whether the antigens detected morphologically within the monensin-treated cells represent newly synthesized molecules or molecules that were trapped within the Golgi at the time of ionophore application.

While the exact mechanism by which monensin disrupts the processing of some glycoproteins and not others remains unresolved, it is striking that the β -glucuronidase molecules synthesized by MOPC 315 cells in the presence of ionophore achieved the same intracellular distribution as in untreated cells. Moreover, the β -glucuronidase molecules were processed proteolytically, an event that is thought to occur in lysosomes. Although we did not measure the lysosomal pH within the monensin-treated MOPC 315 cells, it has been shown that treatment of macrophages with similar ionophores, nigericin and X537A, results in an increase in the intralysosomal pH (50). The normal targeting of acid hydrolases to lysosomes observed in the presence of monensin suggests that the Man-6-P receptor-deficient MOPC 315 cells may utilize a delivery mechanism that is independent of a low lysosomal pH. This is in sharp contrast to the Man-6-P receptor mediated pathway for the delivery of acid hydrolases to lysosomes which is disrupted by treatment of cells with agents that alter lysosomal and endosomal pH (15).

The main purpose of this study was to learn more about lysosomal enzyme sorting in Man-6-P receptor-deficient cells. We utilized MOPC 315 cells since they target most of their newly synthesized lysosomal enzymes to lysosomes and also synthesize large amounts of IgA, a secretory protein. However, these cells do possess very low levels of Man-6-P receptor activity (<2% of the activity found in human diploid fibroblasts). While it seems unlikely that this small amount of Man-6-P receptor can translocate the newly synthesized lysosomal enzymes, we cannot exclude some role of the Man-6-P receptor in this cell line. Ideally these studies should be performed with a cell line that is totally deficient in the Man-6-P receptor but since the receptor-negative lines described secrete 60–70% of the newly synthesized lysosomal enzymes (18), it is not possible to selectively track a cohort of molecules to lysosomes as was done in the present studies.

The authors wish to thank Dr. Scot Hickman for his advise and for sharing his unpublished data, and Dr. Roger Ganshow for providing the anti- β -glucuronidase serum. From C. A. Gabel, a special thanks to Laurie Bourisaw for her secretarial assistance and to the past and present members of the Kornfeld laboratory family who created a very special environment through the sharing of ideas, talents, and friendships.

Received for publication 15 November 1983, and in revised form 3 April 1984.

REFERENCES

- Sly, W. S., and Fischer, H. D. 1982. The phosphorylation recognition system for intracellular and intercellular transport of lysosomal enzymes. *J. Cell Biochem.* 18:67–85.
- Neufeld, E. F., and G. Ashwell. 1980. Carbohydrate recognition systems for receptor-mediated pinocytosis. In *The Biochemistry of Glycoproteins and Proteoglycans*. W. J. Lennarz, editor. Plenum Press, Inc., NY.
- Erickson, A. H., and G. Blobel. 1979. Early events in the biosynthesis of the lysosomal enzyme cathepsin D. *J. Biol. Chem.* 254:11771–11774.
- Rosenfeld, M. G., G. Kreibich, D. Popov, K. Kato, and D. D. Sabatini. 1982. Biosynthesis of lysosomal hydrolases: their synthesis in bound polyosomes and the role of co- and post-translational processing in determining subcellular distribution. *J. Cell Biol.* 93:135–143.
- Tabas, I., and S. Kornfeld. 1980. Biosynthesis intermediates of β -glucuronidase contain high mannose oligosaccharides with blocked phosphate residues. *J. Biol. Chem.* 255:6633–6639.
- Hasilik, A. U., Klein, A., Waheed, G., Strecker, and K. von Figura. 1980. Phosphorylated oligosaccharides in lysosomal enzymes: identification of α -N-acetylglucosamine(1)-phospho(6)mannose diester groups. *Proc. Natl. Acad. Sci. USA.* 77:7074–7078.
- Reitman, M. L., and S. Kornfeld. 1981. UDP-N-acetylglucosamine:glycoprotein N-acetylglucosamine-1-phosphotransferase. *J. Biol. Chem.* 256:4275–4281.
- Waheed, A., R. Pohlmann, A. Hasilik, and K. von Figura. 1981. Subcellular location of two enzymes involved in the synthesis of phosphorylated recognition markers in lysosomal enzymes. *J. Biol. Chem.* 256:4150–4152.
- Varki, A., and Kornfeld, S. 1981. Purification and characterization of rat liver α -N-acetylglucosaminyl phosphodiesterase. *J. Biol. Chem.* 256:9937–9943.
- Waheed, A., A. Hasilik, and K. von Figura. 1981. Processing of the phosphorylated recognition marker in lysosomal enzymes. *J. Biol. Chem.* 256:5717–5721.
- Gabel, C. A., D. E. Goldberg, and S. Kornfeld. 1982. Lysosomal enzyme oligosaccharide phosphorylation in mouse lymphoma cells: specificity and kinetics of binding to the mannose 6-phosphate receptor *in vivo*. *J. Cell Biol.* 95:536–542.
- Fischer, H. D., K. E. Creek, and W. S. Sly. 1982. Binding of phosphorylated oligosaccharides to immobilized phosphomannosyl receptors. *J. Biol. Chem.* 257:9938–9943.
- Varki, A., and S. Kornfeld. 1983. The spectrum of anionic oligosaccharides released by Endo H from glycoproteins: structural studies and interactions with the phosphomannosyl receptor. *J. Biol. Chem.* 258:2808–2818.
- Natowicz, M., D. W. Hallett, C. Frier, M. Chi, P. H. Schlesinger, and J. U. Baenziger. 1983. Recognition and receptor-mediated uptake of phosphorylated high mannose-type oligosaccharides by cultured human fibroblasts. *J. Cell Biol.* 96:915–919.
- Gonzalez-Noriega, A., J. H. Grubb, V. Talkad, and W. S. Sly. 1980. Chloroquine inhibits lysosomal enzyme pinocytosis and enhances lysosomal enzyme secretion by impairing receptor recycling. *J. Cell Biol.* 85:839–852.
- Owada, M., and E. F. Neufeld. 1982. Is there a mechanism for introducing acid hydrolases into liver lysosomes that is independent of mannose 6-phosphate recognition? *Biochem. Biophys. Res. Commun.* 105:814–820.
- Waheed, A., R. Pohlmann, A. Hasilik, K. von Figura, A. van Elsen, and J. G. Leroy. 1982. Deficiency of UDP-N-acetylglucosamine:lysosomal enzyme N-acetylglucosamine 1-phosphotransferase in organs of I-cell patients. *Biochem. Biophys. Res. Commun.* 105:1052–1058.
- Gabel, C. A., D. E. Goldberg, and S. Kornfeld. 1983. Identification and characterization of cells deficient in the mannose 6-phosphate receptor: evidence for an alternate pathway for lysosomal enzyme targeting. *Proc. Natl. Acad. Sci. USA.* 80:775–779.
- Glaser, J. H., K. J. Roozen, F. E. Brot, and W. S. Sly. 1975. Multiple isoelectric and recognition forms of human β -glucuronidase activity. *Arch. Biochem. Biophys.* 166:536–542.
- Laemmli, U. K. 1970. Cleavage of structural proteins during assembly of the head of bacteriophage T4. *Nature (Lond.)* 227:680–685.
- Narasimhan, S., J. R. Wilson, E. Martin, and H. Schachter. 1979. A structural basis for four distinct elution profiles on concanavalin A-sepharose affinity chromatography of glycopeptides. *J. Biochem.* 57:83–96.
- Kornfeld, K., M. L. Reitman, and R. Kornfeld. 1981. The carbohydrate binding specificity of pea and lentil lectins. *J. Biol. Chem.* 256:6633–6640.
- Mellis, S., and J. Baenziger. 1982. Separation of neutral oligosaccharides by high performance liquid chromatography. *Anal. Biochem.* 114:276–280.
- Goldberg, D. E., C. A. Gabel, and S. Kornfeld. 1983. Studies of the biosynthesis of the mannose 6-phosphate receptor in receptor-positive and receptor-deficient cell lines. *J. Cell Biol.* 97:1700–1706.
- Rome, L. H., A. J. Garvin, M. M. Allietta, and E. F. Neufeld. 1979. Two species of lysosomal organelles in cultured human fibroblasts. *Cell.* 17:143–153.
- Skudlarek, M. D., and R. T. Swank. 1981. Turnover of two lysosomal enzymes in macrophages. *J. Biol. Chem.* 256:10137–10144.
- Brown, J. A., G. P. Jahreis, and R. T. Swank. 1981. The synthesis and processing of β -glucuronidase in normal and egasyn deficient mouse kidney. *Biochem. Biophys. Res. Commun.* 99:691–699.
- Frisch, A., and E. F. Neufeld. 1981. Limited proteolysis of the β -hexosaminidase precursor in a cell-free system. *J. Biol. Chem.* 256:8242–8246.
- Schachter, H., and S. Roseman. 1980. Mammalian glycosyltransferases: their role in the synthesis and function of complex carbohydrates and glycolipids. In *The Biochemistry of Glycoproteins and Proteoglycans*. W. J. Lennarz, editor. Plenum Press, Inc. New

- York. pp. 85-160.
30. Tartakoff, A. M., P. Vassali, and M. Detraz. 1977. Plasma cell immunoglobulin secretion. Arrest is accompanied by alterations of the Golgi complex. *J. Exp. Med.* 146:1332-1345.
 31. Hickman, S., J. L. Theodorakis, J. M. Greco, and P. H. Brown. 1984. Processing of MOPC 315 IgA oligosaccharides. Evidence for endoplasmic reticulum and trans Golgi α 1,2-mannosidase activity. *J. Cell Biol.* 98:407-416.
 32. Goldberg, D. E., and S. Kornfeld. 1983. Evidence of extensive subcellular organization of asparagine-linked oligosaccharide processing and lysosomal enzyme phosphorylation. *J. Biol. Chem.* 258:3159-3165.
 33. Bischoff, J., and R. Kornfeld. 1983. Evidence for an α -mannosidase in endoplasmic reticulum of rat liver. *J. Biol. Chem.* 258:7907-7910.
 34. Tabas, I., and S. Kornfeld. 1979. Purification and characterization of a rat liver golgi α -mannosidase capable of processing asparagine-linked oligosaccharides. *J. Biol. Chem.* 254:11655-11663.
 35. Tulsiani, D. R. P., S. C. Hubbard, P. W. Robbins, and O. Touster. 1982. α -D-Mannosidases of rat liver Golgi membranes. *J. Biol. Chem.* 257:3660-3668.
 36. Forsee, T. W., and J. S. Schutzbach. 1981. Purification and characterization of a phospholipid-dependent α -mannosidase from rabbit liver. *J. Biol. Chem.* 256:6577-6582.
 37. Lodish, H. F., N. Kong, M. Snider, and G. J. A. M. Strous. 1983. Hepatoma secretory proteins migrate from rough endoplasmic reticulum to Golgi at characteristic rates. *Nature (Lond.)*. 304:80-83.
 38. Ledford, B. E., and D. F. Davis. 1983. Kinetics of serum protein secretion by cultured hepatoma cells. *J. Biol. Chem.* 258:3304-3308.
 39. Fitting, T., and D. Kabat. 1982. Evidence for a glycoprotein "signal" involved in transport between subcellular organelles. *J. Biol. Chem.* 257:14011-14017.
 40. Strous, G. J. A. M., and H. F. Lodish. 1980. Intracellular transport of secretory and membrane proteins in hepatoma cells infected by vesicular stomatitis virus. *Cell*. 22:709-717.
 41. Nishimoto, S. K., T. Kajiwara, and M. L. Tanzer. 1982. Proteoglycan core protein is accumulated in cultured chondrocytes in the presence of the ionophore monensin. *J. Biol. Chem.* 257:10558-10561.
 42. Pesonen, M., and L. Kaariainen. 1982. Incomplete complex oligosaccharides in Semliki forest virus envelope proteins arrested within the cell in the presence of monensin. *J. Mol. Biol.* 158:213-230.
 43. Alonso-Caplen, F., and R. W. Compans. 1983. Modulation of glycosylation and transport of viral membrane glycoproteins by a sodium ionophore. *J. Cell Biol.* 97:659-668.
 44. Tartakoff, A. M. 1983. Perturbation of vesicular traffic with the carboxylic ionophore monensin. *Cell*. 32:1026-1028.
 45. Griffiths, G., P. Quinn, and G. Warren. 1983. Dissection of the Golgi complex. I. Monensin inhibits the transport of viral membrane proteins from medial to trans Golgi cisternae in baby hamster kidney cells infected with Semliki forest virus. *J. Cell Biol.* 96:835-850.
 46. Dunphy, W. G., E. Fries, L. J. Urbani, and J. E. Rothman. 1981. Early and late functions associated with the Golgi apparatus reside in distinct compartments. *Proc. Natl. Acad. Sci. USA*. 78:7453-7457.
 47. Roth, J., and E. G. Berger. 1982. Immunocytochemical localization of galactosyltransferase in HeLa cells: Co-distribution with thiamine pyrophosphatase in trans-Golgi cisternae. *J. Cell Biol.* 93:223-239.
 48. Dunphy, W. G., and J. E. Rothman. 1983. Compartments of asparagine-linked oligosaccharide processing in the Golgi apparatus. *J. Cell Biol.* 97:270-275.
 49. Strous, G. J. A. M., R. Willemsen, P. van Kerkhof, J. W. Slot, H. J. Geuze, and H. F. Lodish. 1983. Vesicular stomatitis virus glycoprotein, albumin, and transferrin are transported to the cell surface via the same Golgi vesicles. *J. Cell Biol.* 97:1815-1822.
 50. Ohkuma, S., and B. Poole. 1978. Fluorescence probe measurement of the intralysosomal pH in living cells and the perturbation of pH by various agents. *Proc. Natl. Acad. Sci. USA*. 75:3327-3331.