Synthesis of glycoprotein A, the major surfactant-associated protein, was demonstrated in Type II epithelial cells isolated from rat lung. Predominant, secreted forms migrated as glycoproteins with asparagine-linked, complex-type oligosaccharides (32,000-36,000 daltons, pl 4.2-4.8). Primary in vitro translation products of the glycoprotein migrated as five distinct proteins of approximately 26,000 daltons which were processed by pancreatic microsomal membranes in vitro to 30,000-34,000-dalton, endoglycosidase F-sensitive forms. These in vitro processed forms of glycoprotein A co-migrated with intracellular forms immunoprecipitated from [35S]methionine-labeled, Type II cells. Pulse-chase experiments with [35S]methionine-labeled cells demonstrated rapid synthesis of endoglycosidase H-sensitive precursors of 34,000 daltons, pl 4.7-4.8, which were neither secreted from Type II cells nor detected in surfactant from alveolar lavage. These high-mannose forms were slowly processed to more acidic, endoglycosidase H-resistant, neuraminidase-sensitive forms. At between 10 and 180 min, fully sialylated or other endoglycosidase H-resistant forms were a minor fraction of intracellular glycoprotein A. After 16 h, intracellular glycoproteins A were primarily present as endoglycosidase H-resistant forms. Secretion of mature, sialylated, glycoprotein A was first detected 1 h after labeling, and was also readily detected after 16-24 h chase period.

Tunicamycin, which blocks N-linked protein glycosylation, resulted in synthesis of three major 26,000-dalton proteins which co-migrated with the nonglycosylated, surfactant-associated proteins A1 present in surfactant from alveolar lavage and with the major in vitro translation products of rat lung poly(A)* mRNA. Tunicamycin inhibited secretion of glycoprotein A. Swainsonine, which inhibits Golgi α-mannosidase II, completely inhibited synthesis of the fully sialylated molecule. Swainsonine produced forms of glycoprotein A which were both neuraminidase- and endoglycosidase H-sensitive and were readily secreted. Monensin, an ionophore that alters protein transport, markedly inhibited intracellular sialylation and secretion. These studies demonstrate that pulmonary Type II cells rapidly synthesize and process surfactant-associated glycoprotein A precursors to endoglycosidase H-sensitive forms, which are slowly sialylated prior to secretion.

Surfactant-associated proteins, identified in lung lavage material from a variety of species (1), appear to be an essential component of pulmonary surfactant, apparently binding to surfactant phospholipids and enhancing the adsorption of surfactant to an air-liquid interface (2, 3). Although a number of surfactant-associated proteins have been reported, the major forms are oligomers of proteins of 30,000-40,000 daltons, first described by King and Clements (4). In lung lavage material isolated from rat, these proteins (herein called surfactant-associated glycoproteins A to distinguish them from serum apolipoproteins) migrate as a group of related proteins (pl 4.2-4.8), referred to as protein A1 (26,000 daltons), glycoprotein A2 (32,000 daltons), and glycoprotein A3 (36,000 daltons) charge trains. Protein A1 appears to be a nonglycosylated form of the molecule(s); size and charge heterogeneity among the forms are related to the relative amounts of carbohydrate, including significant amounts of sialic acid (5, 6).

Synthesis of N-linked, complex-carbohydrate-containing proteins has been extensively characterized (for review, see Refs. 7, 8). The lipid Glc3Man5(GlcNAc)2 is transferred to the protein and trimmed by glucosidases and mannosidases prior to addition of a variety of sugars including fucose, galactose, N-acetylgalcosamine, and sialic acid. Synthesis of the high-mannose forms appears to occur in the rough endoplasmic reticulum; glucosidase and α-mannosidase activity are localized primarily in Golgi (9, 10). Although strong evidence exists that surfactant-associated glycoproteins A are asparagine-linked, complex glycoproteins, the biosynthesis and processing of the molecule by alveolar Type II epithelial cells has not been clarified. The present study was conducted to determine the time course of synthesis, storage, and secretion of various forms of surfactant-associated glycoprotein A.

MATERIALS AND METHODS

Surfactant-associated proteins were isolated from lung lavage material obtained from Sprague-Dawley rats (200-250 g) as previously described. Protein concentration was determined in lung lavage preparations solubilized in 1% SDS, by the method of Lowry (11) using bovine serum albumin in 1% SDS as standard. Antisera were prepared in New Zealand rabbits by injection of partially purified lung material in Freund's adjuvant. Extensive adsorption with rat red blood cells and a rat serum-Sepharose affinity column produced an antisemur that selectively stained rat Type II cells (5). In immunoblots of lung lavage proteins treated with β-mercaptoethanol, this antisemur detected only proteins of 36,000, 32,000, and 26,000 daltons, pl 4.2-4.8.

Type II Cell Preparation—Type II cells were isolated from pathogen-free Sprague-Dawley male rats (200-250 g) as described by Brown and Longmore (12), a modification of the method of Dobbs and Mason (13), and were plated in Dulbecco's modified Eagle's medium.

The abbreviations used are: SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; IEF, isoelectric focusing.

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1 The abbreviations used are: SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; IEF, isoelectric focusing.
Autoradiograms were prepared after fixation and treatment of stained cultures. Lysates-Total RNA was isolated from adult rat lungs by the method of Chirgwin et al. (19). Polyadenylated mRNA was selected from total RNA using oligo(dT)-cellulose (Collaborative Research, Waltham, MA), as described by Aviv and Leder (20); and translated with nucleoside-treated rabbit reticulocyte lysate (Promega Biotec, Madison, WI) in the presence of 40-50 μCi of [35S]methionine, specific activity = 1000 Ci/mmol (New England Nuclear) as previously described (5, 6). Repeated immunoprecipitation of the lysates resulted in recovery of less than 10% of initial immunoprecipitate.

**Treatment of Immunoprecipitates with Endoglycosidase H, Endoglycosidase F, and Neuraminidase—Endo-β-acetylglucosaminidase F (endoglycosidase F), from Flavobacterium meningoepitpticum, and endoglycosidase H, from Neisseria meningitidis, were purchased from New England Nuclear. Neuraminidase, from Vibrio cholerae, was purchased from Gibco, Inc. Immunoprecipitates of cell lysates and translation products, adsorbed with protein A-Sepharose beads, were washed and pelleted as described above. For endoglycosidase F treatment, the protein pellets were treated essentially as described by Edel, and Alexander (21). For endoglycosidase H treatment, the pellets were resuspended into 250 μl of 50 mM sodium acetate, pH 5.5. Two and one-half microliters of 10% Nonidet P-40 (LBK) were added to each sample. Endoglycosidase H was added to a final concentration of 1 μg/ml. Proteins or immunoprecipitates were also incubated with neuraminidase (0.5 units/ml) in 0.2 mM Tris-Cl, pH 7.4, 0.1% Nonidet P-40. All digests were incubated at 37°C with constant shaking for 18 h.

**Effects of Tunicamycin, Swainsonine, and Monensin on Glycoprotein A Synthesis and Secretion—Freshly isolated Type II cells were preincubated for 1 h with 1 μg/ml of tunicamycin (Calbiochem-Behring), 1-50 μg/ml of swainsonine (the kind gift of Dr. R. P. TuliSanian, Vanderbilt University) or 1 μM monensin (Sigma). Type II cells were then labeled in the presence of the agent for specific times in methionine-deficient (1 mg/liter), Dulbecco's modified Eagle's medium containing 0.1% bovine serumalbumin. At this concentration (1 μg/ml) tunicamycin inhibited protein syntheses 20-25%, as well as trichloracetic acid-precipitable protein. Inhibition of protein secretion was reduced proportionally to [35S]methionine labeling. Swainsonine (1-50 μg/ml) and monensin (1 μM) did not significantly alter [35S]methionine labeling. For comparison of treatment effects, control experiments utilizing equal numbers of cells were analyzed in parallel from the same Type II preparation.

**RESULTS**

**In Vitro Translation and Processing of Glycoprotein A Precursors—Immunoprecipitation of the primary translation products of [35S]methionine-labeled adult rat lung poly(A)+ mRNA, with anti-rat surfactant antisera identified five labeled proteins (26,000 daltons, pl 4.6-4.8, Fig. 1a). The protein with intermediate isoelectric point (of the three major acidic forms) predominated in these experiments. The proteins were processed, in part, to larger forms of 30,000-34,000 daltons (pl 4.7-4.8) by canine pancreatic microsomal membranes (Fig. 1b), similar in size to the major intracellular forms observed in Type II epithelial cells after 10-min labeling with [35S]methionine (5). Endoglycosidase F reduced the in vitro processed forms to 26,000 daltons (not shown), similar in size to both the in vitro translation product and the nonglycosylated forms of the protein from surfactant (6).

**In Vitro Synthesis of Surfactant-associated Glycoprotein A by Type II Epithelial Cells—Pulse-chase experiments were performed by labeling freshly isolated Type II epithelial cells with [35S]methionine for 15 min, followed by removal of labeling media and addition of methionine-containing media. Mature glycoprotein A (charge train of 32,000-36,000 daltons, pl 4.2-4.8) was first clearly detected at 1 h of the chase period. Secreted forms (Fig. 2) co-migrated with those present in surfactant from rat alveolar lavage material, and were sensitive to digestion by neuraminidase (resulting in triplet forms of 30,000-34,000 daltons) and endoglycosidase F (resulting in triplet forms of 25,000 daltons). Re-treatment of endoglycosidase H as previously reported for the protein in surfactant (5, 6). Mature glycoprotein A was detected in media at all time points after 1 h (up to 24 h) during the chase.

**Glycosylation and Secretion of Surfactant Glycoprotein A**

(Gibco, Grand Island, NY), with 10% fetal calf serum at 37°C in 5% CO2. Cultures were generally 90% Type II epithelial cells (assessed by 3R-phosphate staining and electron microscopy of sample preparations), and approximately 95% of the cells were viable as assessed by trypsin blue exclusion after isolation. Lactate dehydrogenase was assayed using an automated media spectrophotometrically described previously (14). For [35S]methionine-labeling experiments, cells were placed in methionine-deficient (1 mg/liter of unlabeled methionine) immediately after isolation. Fetal calf serum was replaced with 0.1% bovine serum albumin. Cells were labeled at 37°C in 95% air, 5% CO2 with 67-100 μCi/ml of [35S]methionine (New England Nuclear) for 10 min to 3 h depending on the experimental protocol. Pulse-chase experiments were usually initiated by 15-min labeling with [35S]methionine. Following labeling (at 0 chase time), cells were gently centrifuged and resuspended in methionine-replete medium containing 0.1% bovine serum albumin and placed in primary culture. For protein isolation, media was carefully removed from the cells after centrifugation at 800 x g for 10 min and precipitated with iced acetone. Type II cells were washed and homogenized by three 5-s bursts with a Tekmar tissue homogenizer (Cincinnati, OH) in iced phosphate-buffered saline, pH 7.4. The homogenate was centrifuged at 1,000 x g for 3-5 min (4°C) to remove nuclear material. Proteins from the supernatants were then lyophilized. In some experiments, Type II cells were washed in phosphate-buffered 0.9% NaCl and lysed directly in immunoprecipitation buffer prior to electrophoresis. Approximately 6 x 10^9-10^10 cells were labeled with [35S]methionine and phosphatidylserine (a marker agent of the surface membrane) and separated by high speed centrifugation to separate Type II cell preparations. Trypan blue exclusion of Type II cells was 97 ± 1.2% (n = 4 determinations) after 16 h of incubation. Protein synthesis, as assessed by [35S]methionine incorporation into thionitrocellulose-precipitable protein, was linearly related to time (0-30 min) when assessed after isolation and after 16 h of culture, at which time incorporation of [35S]methionine was similar. Lactate dehydrogenase release into the media (during a 3-h incubation) was not significantly altered [35S]methionine labeling. For comparison of treatment effects, control experiments utilizing equal numbers of cells were analyzed in parallel from the same Type II preparation.
to autoradiography precipitated after pancreatic membranes. The adult rat lung poly(A+) mRNA, specifically precipitated with protein A- product and assessed surfactant glycoprotein A. Rated by IEF-SDS-PAGE (pH 3.5-6.0) migration of markers the IEF gradient. Electric points are estimated from relative migration of markers $\times 10^{-3}$ and pH of the IEF gradient.

Fig. 1. Primary translation product and in vitro processing of rat surfactant glycoprotein A. The primary translation product was immunoprecipitated after in vitro translation of adult rat lung poly(A+) mRNA, separated by IEF-SDS-PAGE (pH 3.5-6.0) and transblotted to nitrocellulose prior to autoradiography (a). b was placed on film for an identical period (24 h) and represents translation products processed in vitro by the addition of canine pancreatic membranes. The white arrow represents an unrelated protein nonspecifically precipitated with protein A-Sepharose. Molecular masses and isoelectric points are estimated from relative migration of markers $\times 10^{-3}$ and pH of the IEF gradient.

Fig. 2. Mature glycoprotein A from media of [35S]methionine-labeled Type II cells. Type II cells, approximately $10^6$ cells, were labeled after isolation and primary culture as described under "Methods." Media was collected and precipitated in acetone (−30 °C) prior to IEF-SDS-PAGE (pH 3.5-6.0) in presence of β-mercaptoethanol. Proteins were transblotted to nitrocellulose prior to autoradiography for approximately 4 days at −70 °C. Arrows mark surfactant proteins $A_1$ (26,000 daltons); $A_2$ (32,000 daltons), and $A_3$ (36,000 daltons) charge trains co-migrating with proteins identified by immunoblot analysis of rat surfactant with the antiserum used in immunoprecipitation experiments (not shown).

period. When chase media was removed from Type II cells at 16–24 h and fresh media added, labeled glycoproteins A were readily detected in media after 3 h continued incubation, demonstrating that significant labeled stores of glycoproteins A remained in spite of the prolonged chase period. Type II cells were attached, incorporated [35S]methionine into protein at rates comparable to that after isolation, and greater than 90% excluded trypan blue after the 16–24 h time period, indicating their continued viability in culture.

Intracellular glycoproteins A were identified in the pulse-chase experiments by immunoprecipitation of cell lysates with anti-rat surfactant antiserum. Major intracellular forms were detected at the earliest time points (10 min after labeling), primarily labeling triplet groups of proteins of 26,000, 30,000, and 34,000 daltons, pl approximately 4.8. Synthesis and processing of the protein with intermediate pl (of the three major charged peptides) was noted and correlated with the predominance of the middle charged protein of 26,000 daltons in proteins isolated from surfactant. The 34,000-dalton intracellular forms predominated at time points 10–180 min of the chase period (Fig. 3a). Mature sialylated forms were also clearly detectable in cell lysates 1–3 h after labeling (Fig. 3a). These sialylated forms of 32,000–36,000 daltons were resistant to endoglycosidase H (Fig. 3b), but sensitive to neuraminidase (Fig. 3c). Sialylated and nonsialylated proteins were identified by autoradiography and excised from the gels and counted; sialylated forms represented only $8.7 \pm 4\%$ ($n = 3$ determinations) of the labeled glycoprotein A at 3 h. The 34,000-dalton proteins were presumably high-mannose type glycoproteins being reduced to 26,000 daltons by endoglycosidase H (Fig. 3b). Endoglycosidase H-resistant (nonsialylated) intracellular forms of approximately 30,000 daltons were also detected (Fig. 3b), which appear to be distinct intermediate forms of the processed protein. Most of the intracellular forms present at 1–3 h migrated at 34,000 daltons and remained susceptible to endoglycosidase H. After 16 h most of the intracellular protein was endoglycosidase-resistant (sialylated), however, glycosylated (nonsialylated) 34,000-dalton high-mannose forms were still detected even after 16–24 h chase period (not shown). Approximately 50% of glycoprotein A remained within the cells after 16 h (Fig. 3d and Fig. 7), primarily as endoglycosidase H-resistant forms (not shown). Thus, sialylation and secretion rate for glycoprotein A was relatively slow compared to the rapid synthesis of endoglycosidase H-sensitive forms. The proportion of the smallest forms (26,000 daltons) decreased during the chase period (Fig. 3d).

Tunicamycin—Tunicamycin (1 μg/ml) completely blocked the synthesis of glycosylated forms resulting in accumulation of three major immunoprecipitable proteins of 26,000 daltons, pl approximately 4.8, (Fig. 4, a–c). Tunicamycin-induced forms appeared to co-migrate with the primary translation products and with the nonglycosylated forms previously identified in surfactant from lung lavage. These 26,000-dalton proteins were detected throughout the 16-h pulse-chase experiments and their rate disappearance from cell lysates was diminished; less than 20% was secreted after 16 h (Fig. 4c and Fig. 7). Mature, sialylated forms of glycoprotein A were not detected in the media from tunicamycin-treated cells (Fig. 4b). After 3 h, nonglycosylated forms were only barely detectable in the media from tunicamycin-treated cells, while fully sialylated forms were readily detected in a media from the control cells under these conditions.

Effects of Swainsonine and Monensin on Glycoprotein A
Fig. 3. Intracellular surfactant-associated glycoproteins A. Cell lysates were immunoprecipitated from [35S]methionine-labeled Type II cells after 3 h of labeling (a). The more acidic intracellular forms present at 3 h were resistant to endoglycosidase H (b) and sensitive to neuraminidase (c). b represents endoglycosidase H treatment of the immunoprecipitate 3 h after labeling. Control experiments with equal amounts of label from swainsonine-treated cells demonstrated nearly complete digestion of the larger forms to 26,000 daltons under these conditions (see Fig. 5c). d represents the immunoprecipitation of glycoprotein A from pulse-chase experiments with control Type II cells after one-dimensional SDS-PAGE using 3-20% polyacrylamide gradient gels. Molecular mass marker 30,000 daltons is seen in the far left lane. Cells were labeled for 15 min and immunoprecipitates prepared at 0, 10, and 30 min and 1, 3, and 16 h thereafter. Two-dimensional gel analysis confirmed the forms identified in the pulse-chase experiments.

Fig. 4. Effect of tunicamycin on glycoprotein A synthesis and secretion. Intracellular forms synthesized in the presence of 1 µg/ml tunicamycin were immunoprecipitated 3 h after labeling and subjected to IEF-SDS-PAGE and autoradiography. Three major proteins were detected in cell lysates at all time points from time 0 to 16 h (a). Media from these cells was precipitated with acetone at -30 °C. Tunicamycin markedly inhibited appearance of the 26,000-dalton proteins in the media, and these forms were barely detectable in the media after 3 h (b). In the presence of tunicamycin, approximately 80% of the nonglycosylated intracellular forms remained after 16 h of the pulse-chase experiment (c and Fig. 7). Proteins were subjected to SDS-PAGE (3-20% polyacrylamide), and the autoradiography for pulse-chase experiments is represented by c. Molecular mass markers shown in far left lane are 30,000 and 14,000 daltons.

Synthesis and Secretion—Swainsonine (1–50 µg/ml) or monensin (1 µM) did not alter [35S]methionine incorporation or secretion of total labeled proteins into the media. Treatment with swainsonine blocked synthesis of fully sialylated glycoproteins A, and resulted in secretion of partially sialylated forms which were both neuraminidase- and endoglycosidase H-sensitive (Fig. 5, a–c). Intracellular 30,000-dalton forms synthesized in the presence of swainsonine were nearly completely endoglycosidase H-sensitive (Fig. 5c). In contrast, significant amounts of 30,000-dalton, endoglycosidase H-resistant proteins were seen in control cells (Fig. 3b). Such nonsialylated, endoglycosidase H-sensitive, intracellular forms were not detected in the media in either control or swainsonine experiments. In the presence of swainsonine, the amount of partially glycosylated glycoprotein A appearing in the media was similar to that in control experiments at 3 and 16 h. In the presence of swainsonine, secreted proteins remained sensitive to both neuraminidase and endoglycosidase H (not shown). Experiments with 1, 5, 20, and 50 µg/ml swainsonine produced identical results.

In the presence of monensin, synthesis and secretion of fully glycosylated glycoprotein A was markedly inhibited, and was barely detectable after 3 h incubation (Fig. 6, a–c). Most of the labeled protein remained in the cells after 16 h incubation during the pulse-chase experiments (Fig. 6c and Fig. 7). After treatment with monensin, completely processed forms of the protein were detectable only after prolonged exposure of the autoradiograms from cell lysates or media.

DISCUSSION

The present study describes the synthesis and secretion of the major pulmonary surfactant-associated glycoprotein by Type II epithelial cells isolated from rat lung. Present work confirms previous work which supports the premise that the presence of complex carbohydrates contributes to the multiple forms of this protein observed in mammalian surfactants (5, 6, 27–29). We have demonstrated that these forms result from asparagine-linked glycosylation of heterogeneous precursors of 26,000 daltons to high-mannose endoglycosidase H-sensitive forms. Their subsequent sialylation results in complex charge trains of 32,000–36,000 daltons, pl 4.2–4.6. Complex, sialylated forms from control cells and sialylated, endoglycosidase H-sensitive forms from swainsonine-treated cells were readily secreted. Inhibition of protein glycosylation by tunicamycin, and of intracellular transport by monensin, markedly inhibited glycoprotein A secretion.

Primary translation products of glycoproteins A are heter-
Glycosylation and Secretion of Surfactant Glycoprotein A

FIG. 5. Effect of swainsonine on intracellular forms and secretion of glycoproteins A. Type II cells were labeled for 3 h in the presence or absence of 50 µg/ml swainsonine. a represents the major immunoprecipitable intracellular forms after 3 h of labeling which migrated with an isoelectric point of approximately 4.8, 30,000–34,000 daltons. More acidic intracellular forms (pI = 4.6 or less) were clearly detected in the presence of swainsonine (a). b represents acetone-precipitated media from labeled Type II cells after treatment with swainsonine demonstrating partially sialylated forms. c represents immunoprecipitation and endoglycosidase H treatment of swainsonine-induced forms after 3 h of labeling.

FIG. 6. Effect of monensin on synthesis and secretion of glycoproteins A. Type II cells were prepared and labeled in the presence of 1 µM monensin. Major intracellular forms were similar to control cells at early time points. Synthesis of the sialylated acidic forms, which are readily detected at 3 h in control cells, was markedly decreased by monensin (a). b represents acetone precipitated proteins from media after 3 h, demonstrating that secretion of mature protein was markedly inhibited, and was barely detectable at 3 h compared with control experiments. c represents immunoprecipitates of glycoprotein A after SDS-PAGE (3–20% polyacrylamide) of a pulse-chase experiment in the presence of monensin. Cells were lysed after 10 and 30 min, and after 1–16 h of the chase period.

FIG. 7. Surfactant-associated glycoprotein A remaining within Type II cells in the presence or absence of monensin (1 µM) and tunicamycin (1 µg/ml). The time course of the loss of the intracellular protein was estimated by laser densitometry of the autoradiograms, comparing time 0 to subsequent time points during the pulse-chase experiments. The amount of time 0, the time at which methionine containing media was added, is 100%.

A major finding in this study is that surfactant glycoprotein A polypeptide is rapidly processed to high-mannose, endoglycosidase H-sensitive proteins; however, complete processing and secretion proceeds very slowly. After 3 h of incubation, most of the protein remains endoglycosidase H-sensitive, and these precursor forms are detected even after a 16-h pulse-chase period. Approximately half of the mature protein is secreted in 16 h, a secretion rate similar to that of phospholipid secretion from Type II epithelial cells under primary culture conditions in our laboratory (14, 24). Since high-mannose forms are characteristic of proteins in rough endoplasmic reticulum, it appears that significant pools of surfactant-associated glycoproteins A precursor may be present in sites proximal to Golgi and lamellar bodies. Indeed, Williams et al. (25) have demonstrated significant labeling of endoplasmic reticulum and Golgi in Type II cells by immunostaining with anti-sera generated against surfactant-associated glycoproteins A.

ogogeneous, migrating as five distinct proteins of similar molecular mass. The proteins of intermediate isoelectric point (of the three major forms) are predominant in surfactant, in forms generated in in vitro translation assay, in the partially processed, high-mannose forms present in the cell, and after in vitro processing of the translation product by pancreatic microsomal membranes. Size and charge heterogeneity of the translation product has also been recently reported by Floros et al. (22) and Weaver et al. (23) for the human and dog surfactant-associated protein, respectively. In contrast to the findings in dog and human which identified three distinct translation products, the present findings clearly identify five proteins in the rat.
Glycosylation and Secretion of Surfactant Glycoprotein A

Modification of surfactant glycoprotein A precursors to endoglycosidase H-resistant sialylated forms occurs very slowly, and approximately coincides with the appearance of the mature, complex-carbohydrate-containing forms in the media. Completely mature complex forms are readily secreted, and the rate-limiting step in the secretion process focuses on the conversion of endoglycosidase H-sensitive (presumably high-mannose) forms to the complex type. Endoglycosidase H-sensitive forms of glycoproteins are likely synthesized in the rough endoplasmic reticulum, and cleavage of the high-mannose forms is likely to occur distally. Glucosidase and α-mannosidase activity have been previously localized to the Golgi apparatus (7, 8). Marked differences in the secretion rates of α-anti-chymotrypsin and α- and β-C3 peptides from rat HepG2 cells have been correlated with the rate of their conversion to endoglycosidase H-resistant forms (26). The finding that partially sialylated, endoglycosidase H-sensitive forms synthesized in the presence of swainsonine are readily transported and secreted suggests that sialylation, rather than endoglycosidase H-resistance is associated with more rapid transport through the secretory pathway. Whether the rate of movement is related to bulk flow or to receptor-mediated pathways remains to be clarified, and the mechanism for the slow processing and secretion of glycoproteins A is unclear.

Proteins secreted in control and swainsonine-treated experiments were sensitive to neuraminidase (with resultant, less acidic forms, pf 4.7–4.8) and therefore contained sialic acid. This observation supports previous work by this and other laboratories demonstrating the effect of neuraminidase on the migration of this protein group in rabbit, rat, dog, and human surfactants (5, 6, 27–29). In contrast to the secreted forms in control experiments, sialylated forms secreted in the presence of swainsonine remained endoglycosidase H-sensitive. Swainsonine, an indolizidine alkaloid, inhibits α-mannosidase II activity necessary for the trimming of high-mannose containing forms that are required for subsequent sialylation to complex glycoproteins (30–32). Partially sialylated proteins were rapidly secreted in the presence of swainsonine and may result from the synthesis of hybrid, high-mannose-containing proteins which remain endoglycosidase H- as well as neuraminidase-sensitive. Tulsiani and co-workers (30, 31) have reported the synthesis of partially sialylated, endoglycosidase H-sensitive hybrid oligosaccharides by human skin fibroblasts treated with swainsonine. In addition, Yeo et al. (33) have recently reported accelerated secretion of glycoproteins containing hybrid oligosaccharides from swainsonine-treated hepatoma cells. Similar processing and secretion of surfactant-associated glycoprotein A with hybrid oligosaccharide chains may be occurring in swainsonine-treated Type II cells. It is also possible that sialylation was only partially inhibited; however, all concentrations of swainsonine employed (1–50 μg/ml) resulted in synthesis and secretion of identical, partially-sialylated forms. In addition, protein secreted in the presence of swainsonine remained endoglycosidase H-sensitive, in contrast to acidic forms secreted from control cells.

Findings with tunicamycin and monensin also support a relationship between the addition of carbohydrate and secretion of glycoproteins A. As expected, tunicamycin completely blocked addition of carbohydrate to the 26,000-dalton forms. The rate of appearance of the 26,000-dalton forms in the media was markedly inhibited by tunicamycin. Whether tunicamycin alters other aspects of protein secretory processes in Type II cells is unclear, and inhibition of glycoprotein A secretion might also be related to glycosylation-dependent effects on other proteins involved in the secretion mechanisms rather than on the lack of carbohydrate addition to glycoprotein A. Tunicamycin clearly alters the synthesis of N-linked carbohydrates in a variety of proteins, and inhibition of protein glycosylation has been associated with the decreased processing and secretion of a number of proteins (33–35).

Treatment with monensin, a dicarboxylic ionophore which selectively alters the rate of protein processing and transport through Golgi, inhibited the rate of sialylation and secretion of glycoprotein A. Monensin alters protein transport in association with marked, ultrastructural changes in the Golgi apparatus (36–38). Bartalena and Robbins (35) have recently demonstrated that tunicamycin and monensin decreased terminal sialylation in association with decreased secretion of thyroxine-binding globulin. A relationship was demonstrated between the time required for terminal glycosylation and the secretion of proteins from HepG2 cells (39).

Present studies demonstrate that tunicamycin and monensin altered glycosylation and secretion of glycoproteins A from Type II cells. Major intracellular forms of glycoproteins A occur primarily as endoglycosidase H-sensitive 34,000-dalton proteins. Endoglycosidase H-resistant and sialylated forms are detected between 30 min and 1 h in association with the secretion of the fully sialylated forms into the media. Relationships between synthesis and secretion of surfactant-associated glycoproteins A and surfactant phospholipid remain to be clarified.

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Glycosylation and Secretion of Surfactant Glycoprotein A