Effects of bleomycin and methylprednisolone on the biosynthesis of oligosaccharide–lipids and glycoproteins in lung

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Tissues from rabbit lung were found to incorporate radioactivity from [^3H]mannose into the oligosaccharide of a polar lipid soluble in chloroform/methanol/water (10:10:3, by vol.). Only one oligosaccharide–lipid was formed and the composition of the radiolabelled carbohydrate moiety was $\text{Glc}_3\text{Man}_9(\text{GlcNAc})_2$. An antitumour antibiotic, bleomycin, and an anti-inflammatory steroid, methylprednisolone, partially inhibited the incorporation of [^3H]mannose into oligosaccharide–lipids and in addition resulted in the production of two new components, $\text{Man}_5(\text{GlcNAc})_2$ and $\text{Man}_2(\text{GlcNAc})_2$. Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis of the glycoproteins synthesized from the oligosaccharide–lipid intermediates showed labelled components with $M_r > 200\,000$, 130\,000, 80\,000 and 62\,000. The addition of the drugs resulted in the same pattern with the addition of a new component of M_r 36\,000. Endoglucosaminidase H-treatment of the glycopeptides isolated from the Pronase-digested glycoproteins indicated that the oligosaccharide units in the glycoproteins were of the high-mannose-type.

It is now well established that the biosynthesis of asparagine-linked glycoproteins involves the transfer and processing of an oligosaccharide from a dolichol-linked oligosaccharide to an asparagine residue of the polypeptide chain (Struck & Lennarz, 1981). Oligosaccharide–lipids are formed by a sequence of reactions in which *N*-acetylglucosamine, mannose and glucose are transferred from sugar nucleotides to dolichol to form a large oligosaccharide–lipid, the composition of the carbohydrate moiety of which has been determined to be $\text{Glc}_3\text{Man}_9(\text{GlcNAc})_2$ (Spiro *et al.*, 1976; Li *et al.*, 1978; Hubbard & Robbins, 1980; Pan & Elbein, 1982; Liu *et al.*, 1979). Processing of this oligosaccharide unit results in the formation of high-mannose- and complex-type oligosaccharides.

Glycoproteins containing asparagine-linked complex oligosaccharides have been found to be present in lavage material isolated from lungs of patients with alveolar proteinosis (Bhattacharyya & Lynn, 1977*a,b*) as well as from lung lavage of normal animals (Bhattacharyya & Lynn, 1980*b*). The biosynthesis of these glycoproteins has not been explored yet. It has been reported (Passero *et al.*, 1981; Le Vrat & Louisot, 1982) that oligosaccharide–lipids are involved in the biosynthesis of glycoproteins in lung, but the mechanism of this process

as well as the nature of the oligosaccharide–lipids have not been thoroughly investigated.

Several antibiotics, such as tunicamycin, tsuchimycin and flavomycin, and agents like glucosamine, have been found to be inhibitors of glycosylation and used to study the control of the formation of oligosaccharide–lipids (Spiro *et al.*, 1976; Kang & Elbein, 1979; Struck & Lennarz, 1981; Elbein, 1981; Bause & Legler, 1982). We report here the effects of an antitumour antibiotic (Umezawa, 1974), bleomycin, and an anti-inflammatory steroid (Phan *et al.*, 1981), methylprednisolone, on the biosynthesis of oligosaccharide–lipids as well as of glycoproteins in rabbit lung.

Materials and methods

All chemicals were of reagent grade. Eagle's amino acid mixtures were purchased from Grand Island Biological Co., New York, NY, U.S.A.

Aquasol-2, [$2\text{-}^3\text{H}$]mannose (sp. radioactivity 14 Ci/mmol), D-[U- ^{14}C]glucose (sp. radioactivity 360 Ci/mol) and D-[6(n)- ^3H]glucosamine hydrochloride (sp. radioactivity 19 Ci/mmol) were obtained from New England Nuclear, Boston, MA, U.S.A. Bleomycin sulphate (15 units) was obtained

from Bristol Laboratories, Syracuse, NY, U.S.A. Methylprednisolone was purchased from Abbott Laboratories, North Chicago, IL, U.S.A. Bio-Gel P-4 was obtained from Bio-Rad Laboratories, New York, NY, U.S.A. Endoglycosidases D and H were purchased from Miles Laboratories, Elkhart, IN, U.S.A. Standards of $\text{Man}_5(\text{GlcNAc})_2$ and $\text{Glc}_3\text{Man}_9(\text{GlcNAc})_2$ were gifts from Dr. S. C. Hubbard and Dr. P. W. Robbins, Massachusetts Institute of Technology, Boston, MA, U.S.A.

Preparation of [^3H]mannose-labelled oligosaccharide-lipid

Rabbits were killed by air embolus and the lungs were lavaged through trachea by 0.15 M-NaCl. After removal of trachea and bronchi, the lungs were washed twice with ice-cold incubation medium. The incubation mixture, buffered at pH 7.5, contained the following components, in milliequivalents per litre: Na^+ , 125; K^+ , 10; Ca^{2+} , 20; Mg^{2+} , 20; Cl^- , 135; HCO_3^- , 40. The medium also contained sodium pyruvate (5 mM) and Eagle's amino acid mixture (Spiro *et al.*, 1976). The lungs were minced to small pieces and 3 g (wet wt.) of minced tissues was suspended in 10 ml of incubation mixture. The samples were then incubated at 37°C for specified periods of time with 100 μCi of [^3H]mannose. CO_2 was passed over the incubation mixture to maintain adequate buffering.

Extraction of oligosaccharide-lipids were performed by the procedure of Spiro *et al.* (1976). The labelled oligosaccharide-lipids were present in a chloroform/methanol/water (10:10:3, by vol.) fraction. A portion (0.1 ml) of the fraction was suspended in 10 ml of scintillation fluid (Aquasol-2) and the radioactivity was counted in a liquid-scintillation counter (Beckman LS 7000).

Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis of radiolabelled glycoproteins

A portion of the residue left after extraction of oligosaccharide-lipids from lung tissues was dissolved in 10 mM-phosphate buffer (pH 7.5) containing 8 M-urea, 0.1% sodium dodecyl sulphate and 0.05% 2-mercaptoethanol. Sodium dodecyl sulphate/7.5% polyacrylamide-gel electrophoresis of the dissolved material was carried out by the procedure of Weber & Osborn (1969). Gels were stained with Coomassie Blue for proteins.

The unstained gels containing the radioactive proteins were sliced in a Bio-Rad gel slicer (Bio-Rad Laboratories) and each 2 mm section was placed in a scintillation vial containing 0.1 ml of 30% H_2O_2 . The vials were heated at 100°C for 45–60 min. The contents in the vials were mixed with 10 ml of scintillation-fluid (Aquasol-2) and counted for radioactivity.

Pronase digestion of radiolabelled glycoproteins

The residues left after extraction of oligosaccharide-lipids were digested for 72 h with Pronase in 0.15 M-Tris/acetate buffer (pH 7.8) containing 1.5 mM-calcium acetate at 37°C in the presence of toluene (Bhattacharyya & Lynn, 1977b).

The Pronase digest was chromatographed on a Bio-Gel P-4 column (1 cm $\times$ 87 cm), equilibrated and eluted with 50 mM-pyridine/acetate buffer (pH 5.0).

Chemical and enzymic methods

Large amounts of oligosaccharides were collected and hydrolysed in 1 M-HCl at 100°C for 6 h. Hydrolysates were diluted with water and applied to a combined column of Dowex 50 (X4; H^+ form) and Dowex 1 (X8, formate form). Water eluates from these columns contained neutral sugars and were freeze-dried. Glucosamine was retained in the column and eluted with 0.5 M-HCl. Neutral sugars along with the standards were separated by descending paper chromatography in n-butanol/ethanol/water (10:1:2, by vol.) (Bhattacharyya & Lynn, 1977b). The standard sugars on the paper were stained with silver reagent (Trevelyan *et al.*, 1950). Separated neutral sugars were extracted from the paper with water and estimated by the Nelson-Somogyi copper reduction method, as described by Spiro (1966). Glucosamine was determined by Morgan-Elson reaction as described previously (Bhattacharyya & Lynn, 1977b). For the measurement of radioactivity in individual sugars, the paper was cut at 1 cm intervals and each segment was suspended in 5 ml of water for 1 h. The extract was then counted for radioactivity.

Labelling oligosaccharides were liberated by hydrolysing the oligosaccharide-lipids in 0.01 M-HCl containing n-propanol for 20 min at 100°C (Hubbard & Robbins, 1980). The sample was then evaporated to dryness and dissolved in 50 mM-pyridine/acetate buffer (pH 5.0). The solution was passed through a column (1 cm $\times$ 87 cm) of Bio-Gel P-4, equilibrated and eluted with the same buffer. A portion of the fraction was counted for radioactivity.

Endoglycosidase H was used to examine the nature of the oligosaccharide moieties in the oligosaccharide-lipids or glycopeptides. For this purpose, the sample was treated with the enzyme in 50 mM-sodium acetate buffer (pH 5.5) for 15 h at 37°C. After treatment with the enzyme, the oligosaccharides were isolated by Bio-Gel P-4 column (1 cm $\times$ 87 cm) chromatography, equilibrated and eluted with 50 mM-pyridine/acetate buffer (pH 5.0). A portion of the fraction was measured for radioactivity.

Inhibition studies

To determine the effects of bleomycin and methylprednisolone, various concentrations of the drugs were added to the tissues (3g wet wt.) and after a pre-incubation of 1 h at 37°C, 100 μ Ci of [3 H]mannose was added and the tissues were allowed to incubate for 20 min to 4 h. In some experiments, [3 H]mannose was added to the tissues at the same time as the drugs and the tissues were incubated at 37°C for various periods of time. To examine whether the inhibition was reversible, the tissues (3g wet wt.) were incubated with the drugs at 37°C for 1 h, washed and incubated again with [3 H]mannose for the specified period of time. After incubation, the tissues were taken out of the media, washed and extracted for oligosaccharide-lipids. A portion of the delipidated pellets was analysed for radiolabelled glycoproteins by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis. The pellets were also digested with Pronase and the Pronase-digested glycopeptides were isolated by Bio-Gel P-4 column chromatography as described above.

Results

Incorporation of radiolabelled sugar into oligosaccharide-lipids

Incubation of tissue samples with [3 H]mannose resulted in rapid labelling of the oligosaccharide-lipids. Incorporation was linear up to 10 min and then slowed down (Fig. 1). The rate of turnover of oligosaccharide-lipids in lung was rapid and maxi-

Fig. 1. Incorporation of [3 H]mannose into oligosaccharide-lipids in lung

Tissues (3g wet wt.) were incubated at 37°C for various periods with 100 μ Ci of [3 H]mannose. Oligosaccharide-lipids were extracted from the tissues as described in the Materials and methods section. A portion of the oligosaccharide-lipid was measured for radioactivity. The arrows indicate the starts of chases with non-radioactive mannose (1 mM). Symbols: ●, [3 H]mannose; ○, tissue chased with 1 mM-mannose.

mum incorporation of [3 H]mannose was attained at 15–20 min. When mannose (1 mM) was added to the prelabelled tissues, labelled oligosaccharides were cleared from the lipid fraction. Incorporation of [3 H]mannose into oligosaccharide-lipids was linear from 20 to 100 μ Ci (results not shown).

Nature of oligosaccharide-lipids

The nature of the oligosaccharide moieties present in the chloroform/methanol/water (10:10:3, by vol.) extract was established by hydrolysing the sample in mild acid and fractionating on a Bio-Gel P-4 column. As indicated in Fig. 3(a), only one peak at fraction 42 was observed. Paper chromatography of the neutral sugar fractions of the oligosaccharide-lipids indicated the presence of only radiolabelled mannose. Glucose and *N*-acetylglucosamine were not radiolabelled (results not shown). Analysis of the individual sugar components indicated that on the basis of the presence of two residues of *N*-acetylglucosamine, the ratio of neutral sugar in this oligosaccharide unit was $2.8 \pm 0.2/8.8 \pm 0.2$ for glucose and mannose. The data suggest that the composition of the oligosaccharide moiety is $\text{Glc}_3\text{Man}_9(\text{GlcNAc})_2$.

Effects of bleomycin and methylprednisolone on the biosynthesis of oligosaccharide-lipids in lung

Lung tissues were incubated with various concentrations of bleomycin and methylprednisolone at 37°C for 1 h. After 1 h, [3 H]mannose was added to the tissues and the incubations were continued for 20 min. Fig. 2 indicates the effects of various concentrations of bleomycin and methylprednisolone on the incorporation of [3 H]mannose into oligosaccharide-lipids and into glycoproteins. Bleomycin (0.006 unit) caused about 50% inhibition, whereas 50% inhibition of methylprednisolone required about 50 μ g. Also, bleomycin and methylprednisolone caused substantial inhibition of mannose incorporation into glycopeptide fractions (Fig. 2).

To examine the effects of different concentrations of bleomycin on the nature of oligosaccharides in oligosaccharide-lipids, the lung tissues were pre-incubated for 1 h with 0.002 and 0.006 unit of bleomycin. Similarly, the lung tissues were pre-incubated for 1 h with 25 and 50 μ g of methylprednisolone. After 1 h, the tissues were incubated with [3 H]mannose for an additional 20 min. At the end of incubation, oligosaccharide-lipids were extracted from the tissues, partially hydrolysed with mild acid and then released oligosaccharides were separated by Bio-Gel P-4 column chromatography. In the absence of the inhibitors, only one peak at fraction 42, corresponding to a composition of $\text{Glc}_3\text{Man}_9(\text{GlcNAc})_2$, was observed (Figs. 3a and

Fig. 2. Effects of different concentrations of bleomycin and methylprednisolone on the incorporation of $[^3\text{H}]$ -mannose into oligosaccharide-lipids in lung

Tissues (3 g wet wt.) were incubated at 37°C with different concentrations of the drugs. After 1 h, $100\ \mu\text{Ci}$ of $[^3\text{H}]$ mannose was added to the tissues and the incubations were continued for 20 min. Oligosaccharide-lipids were extracted from the tissues. A portion of the oligosaccharide-lipid was measured for radioactivity. The delipidated pellets were digested with 1% (w/w) Pronase at 37°C for 72 h. After digestion, glycopeptide fractions containing radioactive mannose were isolated by Bio-Gel P-4 column (1 cm $\times$ 87 cm) chromatography equilibrated and eluted with 50 mM-pyridine/acetate buffer (pH 5.0). A portion of the glycopeptide fraction was measured for radioactivity.

3d). This peak is referred to as peak 1. At concentrations of 0.002 unit of bleomycin and $25\ \mu\text{g}$ of methylprednisolone, peak 1 was reduced and a second peak, peak 2, appeared at around fraction 50 (Figs. 3b and 3e). Standard $\text{Man}_5(\text{GlcNAc})_2$ appeared in the same position of the second peak. At concentrations of 0.006 unit of bleomycin and $50\ \mu\text{g}$ of methylprednisolone, a third peak (peak 3), corresponding to a composition of $\text{Man}_2(\text{GlcNAc})_2$, appeared at around fraction 65 (Figs. 3c and 3f). Thus, the changes in the nature of oligosaccharides

Fig. 3. Effects of bleomycin and methylprednisolone on the oligosaccharide-lipids formed by lung

Tissues (3 g wet wt.) were pre-incubated with either bleomycin or methylprednisolone at 37°C for 1 h before addition of $100\ \mu\text{Ci}$ of $[^3\text{H}]$ mannose. After incubation for 20 min with the radiolabelled sugar, oligosaccharide-lipids were extracted from the tissues. Oligosaccharide-lipids were hydrolysed with 0.01 M-HCl containing n-propanol for 20 min at 100°C and then fractionated on a Bio-Gel P-4 column (1 cm $\times$ 87 cm), equilibrated and eluted with 50 mM-pyridine/acetate buffer (pH 5.0). (a), Control; (b), bleomycin (0.002 unit); (c), bleomycin (0.006 unit); (d), control; (e), methylprednisolone ($25\ \mu\text{g}$); (f), methylprednisolone ($50\ \mu\text{g}$). Abbreviations used: B.D., Blue Dextran; G, $\text{Glc}_3\text{Man}_9(\text{GlcNAc})_2$; M, $\text{Man}_5(\text{GlcNAc})_2$; S, stachyose; M₁, mannose.

present in the oligosaccharide-lipids were dependent on the concentration of the drugs.

To observe the effect of time of incubation on the changes in oligosaccharides, a similar experiment to that shown in Fig. 3 was done except that in this case the tissues were pre-incubated with the drugs for 4 h before addition of $[^3\text{H}]$ mannose. After incubation with $[^3\text{H}]$ mannose for 20 min, the oligosaccharides were isolated from oligosaccharide-lipids. As shown in Figs. 4(b) and 4(e), bleomycin (0.002 unit) and methylprednisolone ($25\ \mu\text{g}$) produced three peaks (peaks 1, 2, and 3) instead of two peaks (peaks 1 and 2) observed before (Figs. 3b and 3e). As the concentrations of bleomycin and methylprednisolone were increased (0.006 unit and $50\ \mu\text{g}$ respectively), peaks 1 and 2 were reduced, whereas peak 3 was increased considerably (Figs. 4c and 4f). In the absence of the drugs, only peak 1 was observed when the tissues were incubated with $[^3\text{H}]$ mannose (Figs. 4a and 4d). Thus, the effects of the drugs appeared to be dependent both on concentrations and time of incubation.

Fig. 4. Effects of bleomycin and methylprednisolone on the incorporation $[^3\text{H}]$ mannose into oligosaccharide-lipids in lung

The protocol for this experiment was the same as for Fig. 3 except that the tissues (3 g wet. wt.) were incubated with bleomycin and methylprednisolone at 37°C for 4 h before addition of $100\ \mu\text{Ci}$ of $[^3\text{H}]$ mannose. After incubation for 20 min with $[^3\text{H}]$ mannose, oligosaccharide-lipids and labelled oligosaccharides were isolated as described in the text. (a) Control; (b), bleomycin (0.002 unit); (c), bleomycin (0.006 unit); (d), control; (e), methylprednisolone (25 μg); (f), methylprednisolone (50 μg). Other abbreviations are the same as in Figure 3.

Fig. 5. Reversibility of the changes in oligosaccharide profiles in lung induced by bleomycin and methylprednisolone

Tissues (3 g wet wt.) were incubated with either 0.006 unit of bleomycin or 50 μg of methylprednisolone at 37°C for 1 h. After 1 h, one batch of tissues was washed with the incubation medium, $100\ \mu\text{Ci}$ of $[^3\text{H}]$ mannose was added to all the tissues and incubation was continued for 20 min or 4 h. Oligosaccharide-lipids and oligosaccharides were isolated from the tissues. (a), Bleomycin and $[^3\text{H}]$ mannose for 20 min; (b), washed tissue (bleomycin) and $[^3\text{H}]$ mannose for 20 min; (c), washed tissue (bleomycin) and $[^3\text{H}]$ mannose for 4 h; (d), methylprednisolone and $[^3\text{H}]$ mannose for 20 min; (e), washed tissue (methylprednisolone) and $[^3\text{H}]$ mannose for 20 min; (f), washed tissue (methylprednisolone) and $[^3\text{H}]$ mannose for 4 h. For definition of abbreviations, see the legend to Fig. 3.

Reversibility of inhibition of biosynthesis of oligosaccharide-lipids in lung by bleomycin and methylprednisolone

In order to examine whether the inhibition of the biosynthesis of oligosaccharide-lipids by the drugs was reversible, the lung tissues were incubated with either 0.006 unit of bleomycin or 50 μg of methylprednisolone for 1 h and then one batch of tissues was washed with the incubation medium. $[^3\text{H}]$ Mannose was then added to all the tissues and incubation was continued for various periods of time. Oligosaccharides were isolated from oligosaccharide-lipids. As shown in Fig. 5, all three peaks (peaks 1, 2, and 3) were present when the tissues were incubated with $[^3\text{H}]$ mannose for 20 min (Figs. 5a and 5d). When the drugs were removed from the medium and the tissues were incubated with $[^3\text{H}]$ mannose for 20 min, the three peaks were still present (Figs. 5b and 5e). The three oligosaccharides were found to be present even after the washed tissues were incubated with $[^3\text{H}]$ mannose for 4 h (Figs. 5c and 5f). In the control experiment, only peak 1 was observed. The results indicate that under the conditions of the present experiment, the effects of the drugs on the biosynthesis of oligosaccharide-lipids were not reversible.

Effects of bleomycin and methylprednisolone on the incorporation of $[^3\text{H}]$ mannose into protein products

In order to study the effects of the drugs on the incorporation of $[^3\text{H}]$ mannose into proteins, the lung tissues were pre-incubated with 0.006 unit of bleomycin and 50 μg of methylprednisolone for 1 h before addition of $[^3\text{H}]$ mannose. After incubation of 20 min with $[^3\text{H}]$ mannose, oligosaccharide-lipids were extracted from the tissues. The delipidated residues were solubilized in sodium dodecyl sulphate/urea buffer containing 2-mercaptoethanol and subjected to polyacrylamide-gel electrophoresis. Some gels were stained with Coomassie Blue for proteins. Other gels were sliced and counted for radioactivity. As shown in Fig. 6, incorporation of $[^3\text{H}]$ mannose into lung tissues resulted in incorporation into four proteins of $M_r > 200\,000$, 130 000, 80 000 and 62 000 (Fig. 6a). Photographs of Coomassie-stained gels are shown in Fig. 6. When the tissues were pre-incubated with the drugs before addition of $[^3\text{H}]$ mannose, a fourth peak, corres-

Fig. 6. Distribution of radioactivity after sodium dodecyl sulphate/polyacrylamide-gel electrophoresis of protein products formed upon incubation of lung tissues with bleomycin and methylprednisolone

Tissues (3 g wet wt.) were incubated with either 0.006 unit of bleomycin or 50 μ g of methylprednisolone at 37°C for 1 h. After further incubation for 20 min with 100 μ Ci of [3 H]mannose, the tissues were delipidated as described in the text. The residues were then subjected to sodium dodecyl sulphate/polyacrylamide-gel electrophoresis. (a), [3 H]Mannose; (b), bleomycin and [3 H]mannose; (c), methylprednisolone and [3 H]mannose. The profiles on the left show radioactivity in slices of the gels whose photographs are shown on the right (after staining with Coomassie Blue). The migration of standard proteins (myosin H-chain, phosphorylase b, bovine serum albumin, ovalbumin, α -chymotrypsinogen, β -lactoglobulin and cytochrome c) and alveolar glycoprotein (M_r 80 000, 62 000 and 36 000; Bhattacharyya *et al.*, 1976; Bhattacharyya & Lynn, 1977a, 1979) are indicated by arrows. Abbreviation used: B.B., Bromophenol Blue.

ponding to a protein of M_r 36 000, appeared (Figs. 6b and 6c). As judged by the polyacrylamide-gel profile, the major labelled protein in the tissues was the protein of M_r 62 000. However, incorporation of [3 H]mannose was highest in the protein of M_r > 200 000.

Effects of bleomycin and methylprednisolone on the nature of oligosaccharides in glycoproteins

In order to examine the effects of the drugs on the nature of oligosaccharides in glycoproteins, the lung tissues were incubated with 0.006 unit of bleo-

Fig. 7. Nature of oligosaccharides present in the glycopeptides from lung labelled with [3 H]mannose in the presence and in the absence of either bleomycin or methylprednisolone

Protocol for this experiment was the same as that described in the legend to Fig. 6 except that the delipidated residues were digested with Pronase and the glycopeptides were isolated by Bio-Gel P-4 column (1 cm $\times$ 87 cm) chromatography as described. The glycopeptides were treated with endoglucosaminidase H and the labelled oligosaccharides were separated on the same Bio-Gel P-4 column (1 cm $\times$ 87 cm). (a), Glycopeptides; (b), glycopeptides and endoglucosaminidase H; (c), glycopeptides (bleomycin) and endoglucosaminidase H; (d), glycopeptides (methylprednisolone) and endoglucosaminidase H. Abbreviations used: B.D., Blue Dextran; G, $\text{Glc}_3\text{Man}_9(\text{GlcNAc})_2$; M_1 , mannose.

mycin and 50 μ g of methylprednisolone for 1 h before addition of [3 H]mannose. After 20 min of incubation with the radiolabelled sugar, oligosaccharide-lipids were extracted from the tissues. The delipidated residues were digested with Pronase and the glycopeptides were isolated by Bio-Gel P-4 column chromatography. The entire glycopeptides from Pronase-digested tissues were treated with endoglucosaminidase H, the enzyme known to cleave only high-mannose glycoproteins (Elbein, 1981).

As shown in Fig. 7(a), one minor and a major peak were observed when the Pronase-digested glycopeptides from the lung tissues incubated without the drugs were fractionated on a Bio-Gel P-4 column. When the glycopeptides were digested with endoglucosaminidase H, the major peak was shifted further, leaving behind a trace of the original peak (Fig. 7b). The new peak appeared at the same position as standard $\text{Glc}_3\text{Man}_9(\text{GlcNAc})_2$. When the glycopeptides isolated from the tissues treated with the drugs were digested with the enzyme and fractionated on the same column, a similar result was obtained (Figs. 7c and 7d). The results suggest that under the condition of the experiment, the oligosaccharide unit(s) in the glycoproteins was of the high-mannose-type.

Discussion

The results reported in the present paper indicate that, as for all other tissues and cells (Struck & Lennarz, 1981), the biosynthesis of oligosaccharide moieties of the asparagine-like glycoproteins in lung also involves the formation of the oligosaccharide-lipid precursor $\text{Glc}_3\text{Man}_9(\text{GlcNAc})_2$. The presence of chitobiose unit in the oligosaccharide-lipid ruled out the possibility of the absence of this unit in alveolar glycoproteins reported previously (Bhattacharyya & Lynn, 1977b).

Bleomycin and methylprednisolone partially inhibited the incorporation of [3 H]mannose into the oligosaccharide-lipid in lung. When the lung tissues were incubated with the drugs, there was a time-dependent loss of the large oligosaccharide, $\text{Glc}_3\text{Man}_9(\text{GlcNAc})_2$, with the appearance of two new oligosaccharides, $\text{Man}_5(\text{GlcNAc})_2$ and $\text{Man}_2(\text{GlcNAc})_2$. Since the mannosyl donor for the addition of the sixth mannose residue is known to be dolichyl phosphate mannose, whereas the donor for the first five mannose-residues is GDP-mannose (Schutzbach *et al.*, 1980; Struck & Lennarz, 1981; Gershman & Robbins, 1981; Rearick *et al.*, 1981; Pan & Elbein, 1982), the drugs might partially inhibit the transfer of mannose from GDP-mannose to dolichyl phosphate, thus preventing the formation of dolichyl phosphate mannose. Alternatively, the

drugs might have some effect on the transfer of mannose from dolichyl phosphate mannose to the lipid acceptor. Similar changes in the pattern of oligosaccharide profiles have been noted in virus-infected tissue culture cells inhibited by glucosamine (Pan & Elbein, 1982) and also by Gershman & Robbins (1981) in Chinese hamster ovary cells deprived of glucose.

It has been reported previously (Elbein, 1981; Bause & Legler, 1982) that flavomycin and tsushimycin inhibit the formation of dolichyl phosphate sugars, resulting in the accumulation of five to seven sugar residues. The results reported in the present paper indicate that bleomycin, a mixture of glycopeptide antibiotics (Umezawa, 1974), resulted in the formation of $\text{Glc}_3\text{Man}_9(\text{GlcNAc})_2$, $\text{Man}_5(\text{GlcNAc})_2$ and $\text{Man}_2(\text{GlcNAc})_2$ in lung. These observations suggest that antibiotics have inhibitory effects on the biosynthesis of oligosaccharide-lipids containing different sugar units.

In addition to antibiotics, the steroids have been shown to affect the growth of cells as well as the glycosylation of cell-surface glycopeptides (Muramatsu *et al.*, 1973; Ceccarani *et al.*, 1975); Sasak *et al.*, 1982). Recently, it has been reported (Ramachandran *et al.*, 1982) that dexamethasone stimulated the dolichol-mediated glycosylation of proteins in HeLa cells by increasing the rate of synthesis of oligosaccharide-lipid. However, the results reported in the present paper indicated that methylprednisolone partially inhibited the incorporation of [3 H]mannose into oligosaccharide-lipids and glycoproteins in lung. The difference in action of dexamethasone on the biosynthesis of oligosaccharide-lipids and glycoproteins in HeLa cells and methylprednisolone in lung may reflect differences in the mechanism of action of two steroids or differences in the responses of two cell types.

Four proteins of $M_r > 200\,000$, 130\,000, 80\,000 and 62\,000 were found to be glycosylated under controlled conditions, whereas an additional protein of M_r 36\,000 was labelled in the drug-treated tissues. The oligosaccharides present in the glycoproteins were of the high-mannose-type. The molecular weights of these labelled proteins were similar to the collagen-like and complex type glycoproteins isolated from lung lavage material of patients with alveolar proteinosis (Bhattacharyya & Lynn, 1977a,b, 1979, 1980a; Bhattacharyya, 1981) as well as from lung lavage of normal rabbit (Bhattacharyya & Lynn, 1980b).

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References

- Bause, E. & Legler, G. (1982) *Biochem. J.* **210**, 481–487
- Bhattacharyya, S. N. (1981) *Biochem. J.* **193**, 447–457
- Bhattacharyya, S. N. & Lynn, W. S. (1977a) *Biochim. Biophys. Acta* **494**, 150–161
- Bhattacharyya, S. N. & Lynn, W. S. (1977b) *J. Biol. Chem.* **252**, 1172–1180
- Bhattacharyya, S. N. & Lynn, W. S. (1979) *J. Biol. Chem.* **254**, 5191–5198
- Bhattacharyya, S. N. & Lynn, W. S. (1980a) *Biochim. Biophys. Acta* **625**, 451–458
- Bhattacharyya, S. N. & Lynn, W. S. (1980b) *Biochim. Biophys. Acta* **626**, 343–355
- Bhattacharyya, S. N., Sahu, S. & Lynn, W. S. (1976) *Biochim. Biophys. Acta* **427**, 91–106
- Ceccarini, C., Muramatsu, T., Tsang, J. & Atkinson, P. H. (1975) *Proc. Natl. Acad. Sci. U.S.A.* **72**, 3139–3143
- Elbein, A. D. (1981) *Biochem. J.* **193**, 477–484
- Gershman, H. & Robbins, P. W. (1981) *J. Biol. Chem.* **256**, 7774–7780
- Hubbard, S. C. & Robbins, P. W. (1980) *J. Biol. Chem.* **255**, 11782–11793
- Kang, M. S. & Elbein, A. D. (1979) *Arch. Biochem. Biophys.* **198**, 304–313
- LeVrat, C. & Louisot, P. (1982) *Biochimie* **64**, 1–11
- Li, E., Tabas, I. & Kornfeld, S. (1978) *J. Biol. Chem.* **252**, 7762–7770
- Liu, T., Stetson, B., Turco, S. J., Hubbard, S. C. & Robbins, P. W. (1979) *J. Biol. Chem.* **254**, 4554–4559
- Muramatsu, T., Atkinson, P. H. & Nathenson, S. G. (1973) *J. Mol. Biol.* **80**, 781–799
- Pan, Y.-T. & Elbein, A. D. (1982) *J. Biol. Chem.* **257**, 2795–2801
- Passero, M. A., Pass, C. W. & Hutchinson, T. R. (1981) *Exp. Lung Res.* **2**, 17–26
- Phan, S. H., Thrall, R. S. & Williams, C. (1981) *Am. Rev. Resp. Dis.* **124**, 428–436
- Ramachandran, C. K., Gray, S. L. & Melnikovich, G. (1982) *Biochem. J.* **208**, 47–52
- Rearick, J. I., Fujimoto, K. & Kornfeld, S. (1981) *J. Biol. Chem.* **256**, 2762–2769
- Sasak, W., Herscovics, A. & Quaroni, A. (1982) *Biochem. J.* **201**, 359–366
- Schutzbach, J. S., Springfield, J. D. & Jensen, J. W. (1980) *J. Biol. Chem.* **255**, 4170–4175
- Spiro, R. G. (1966) *Methods Enzymol.* **8**, 3–26
- Spiro, M. G., Spiro, R. G. & Bhoyroo, V. D. (1976) *J. Biol. Chem.* **251**, 6400–6408
- Struck, D. K. & Lennarz, W. J. (1981) in *The Biochemistry of Glycoproteins and Proteoglycans* (Lennarz, W. J., ed.), pp. 35–73, Plenum Press, New York and London
- Trevelyan, W. E., Procter, D. P. & Harrison, J. S. (1950) *Nature (London)* **166**, 444–445
- Umezawa, H. (1974) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **33**, 2296–2302
- Weber, K. & Osborn, M. (1969) *J. Biol. Chem.* **244**, 4406–4412