Journal of Cellular Biochemistry 50:13–20 (1992)

ARTICLES

# Modification of Blood Group A Antigen Expression in a Pancreatic Cancer Cell Line (PC-1) by Inhibitors of N-Glycan Processing

**Masahiko Hirota, Masatoshi Mogaki, Parviz M. Pour, and William G. Chaney**

Eppley Institute for Research in Cancer and Allied Diseases (M.H., M.M., P.M.P., W.G.C.) and Department of Biochemistry and Molecular Biology (W.G.C.), University of Nebraska Medical Center, Omaha, Nebraska 68198-4525

**Abstract** Pancreatic adenocarcinomas induced in Syrian hamsters by treatment with N-nitrosobis(2-oxopropyl) amine express blood group A antigen, which is absent in normal pancreatic cells. On membrane glycoproteins purified from tumors, blood group A antigen has been found to be expressed on multiantennary Asn-linked complex glycans. In this study, we investigated the effect of inhibitors of Asn-glycan processing on blood group A antigen bearing glycan structures in a cell line (PC-1) established from a primary induced pancreatic cancer. Expression of blood group A antigen on cells and in membrane preparations was blocked by treatment with 1-deoxymannojirimycin, an inhibitor of mannosidase I, but was retained after treatment with swainsonine, an inhibitor of mannosidase II. However, swainsonine treatment altered the glycan structure associated with blood group A antigen from an endoglycosidase H resistant type to a sensitive type, indicating that the blood group A structure might shift from a complex type to a hybrid type glycan by this treatment. These results demonstrate that Asn-linked glycans carry the major blood group A antigens in PC-1 cells. 

**Key words:** pancreatic cancer cell line, blood group A antigen, Asn-linked glycan, swainsonine, 1-deoxymannojirimycin

We have shown that pancreatic adenocarcinomas induced by N-nitrosobis(2-oxopropyl)amine (BOP) in Syrian hamsters produce blood group A (BG-A) antigen [Pour et al., 1986; Egami et al., 1990]. BG-A antigen consists of GalNAc residue joined by α1-3 linkage to Gal residue of blood group H antigen structure. Because this antigen is not present in the normal hamster pancreas, the acquisition of BG-A antigen is the neoexpression of a cancer-associated antigen [Pour et al., 1986; Egami et al., 1990; Hirota et al., 1992a]. The PC-1 cell line established from a primary induced cancer also expresses BG-A antigen [Egami et al., 1989]. Our studies have also shown that BG-A antigen on membrane glycoproteins purified from primary pancreatic cancers is found mainly on Asn-linked (N-linked) multiantennary complex type glycans [Hirota et al., 1992b].

N-linked glycosylation is initiated by the transfer of a preformed oligosaccharide from an oligosaccharide-diphosphodolichol intermediate to an Asn residue of the nascent peptide chain [Kornfeld and Kornfeld, 1985]. Oligosaccharides are then subjected to a series of processing steps involving both glycosidases and glycosyltransferases leading to the formation of mature complex oligosaccharides in glycoproteins.

Several inhibitors of specific reactions in the N-linked glycoprotein processing pathway have recently been described [Elbein, 1987]. Two such compounds are 1-deoxymannojirimycin (dMM), an inhibitor of mannosidase I, and swainsonine (SW), an inhibitor of mannosidase II. In comparison with the glycosylation inhibitor tunicamy-

Abbreviations used: A-transferase, α1–3 GalNAc transferase; BG, blood group; BOP, N-nitrosobis(2-oxopropyl)amine; Con A, concanavalin A; dMM, 1-deoxymannojirimycin; Endo H, endoglycosidase H; L-PHA, *Phaseolus vulgaris* leucoagglutinin; PMSF, phenylmethylsulfonyl fluoride; PNGase, peptide-N-glycosidase F; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; SW, swainsonine; TL, tomato lectin.

Received February 10, 1992; accepted February 12, 1992.

Address reprint requests to William G. Chaney, Department of Biochemistry and Molecular Biology, University of Nebraska Medical Center, 600 So. 42nd Street, Omaha, NE 68198-4525.

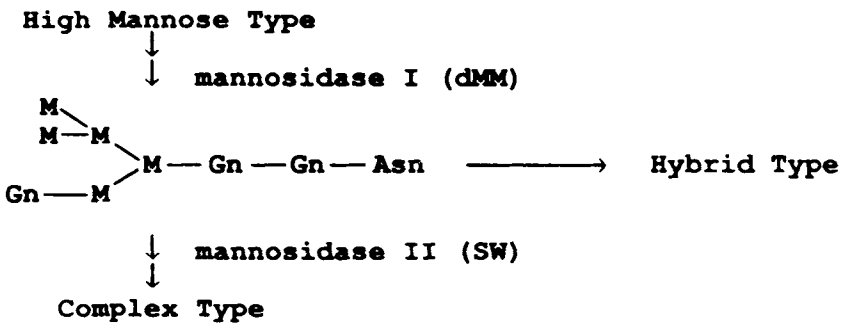


Fig. 1. Processing of N-linked glycan on glycoproteins. Inhibitors for corresponding mannosidases are indicated in parentheses. M, mannose; Gn, N-acetylglucosamine; Asn, asparagine.

cin, SW and dMM are relatively nontoxic and do not adversely effect the cell growth and viability [Humphries et al., 1986; Hughes et al., 1987].

In the present study, we examined the effect of dMM and SW on BG-A expression of PC-1 cells and the modification of BG-A antigen-associated glycan based on the reactivities of endoglycosidases and plant lectins.

## MATERIALS AND METHODS

### Chemicals

Phenylmethylsulfonyl fluoride (PMSF), porcine thyroglobulin, 2′ fucosyllactose, Dowex-1, UDP-GalNAc, and goat antimouse IgM and alkaline-phosphatase conjugated rabbit antigoat IgG antibodies were purchased from Sigma Chemical Co. (St. Louis, MO). UDP-[$^3$H]GalNAc was from DuPont (Boston, MA). Triton X-100 and a micro-BCA protein assay kit were purchased from Pierce Chemical Co. (Rockford, IL). Biotinylated *Phaseolus vulgaris* (L-PHA), Concanavalin A (Con A), and Tomato (TL) lectins, and avidin-labeled alkaline phosphatase were purchased from Vector Laboratories (Burlingame, CA). Endoglycosidase H (Endo H), peptide-N-glycosidase F (PNGase), swainsonine (SW), and 1-deoxymannojirimycin (dMM) were purchased from Boehringer Mannheim Co. (Indianapolis, IN). Ovalbumin was purchased from U.S. Biochemical Co. (Cleveland, OH). The monoclonal antibody (MAb) against the synthetic trisaccharides of BG-A determinant was purchased from DAKO Co. (Santa Barbara, CA). Other chemicals used were of analytical grade.

### Incubation of PC-1 Cells With SW and dMM

PC-1 cells were incubated in the presence of 0.1 mM of SW or 1 mM of dMM for 4 days, which correspond to approximately two generations of PC-1 cells, with one medium change. RPMI1640 medium containing 5% fetal bovine serum (FBS) was used as control medium. The step of the oligosaccharide processing pathway inhibited by these compounds is illustrated in Figure 1.

### Preparation of Membrane Fractions

Membrane fractions from PC-1 cells were isolated by a modification of the method of Massague and Czech [1982]. Harvested cells were homogenized in 10 mM Tris (pH 7.4), 1 mM $Na_2EDTA$, 0.25 M sucrose, 1 mM PMSF (TES-P buffer) with a Dounce homogenizer on ice. The homogenate was centrifuged at 3,000*g* for 10 min at 4°C, and the resulting supernatant fractions were pooled. Rehomogenization of the pellets in TES-P buffer and collection of supernatant with the same speed of centrifugation were repeated twice more. Combined supernatant fractions were centrifuged at 30,000*g* for 45 min to give the total membrane pellet. After washing with 10 mM Tris (pH 7.4), 1 mM $Na_2EDTA$, 1 mM PMSF (TE-P buffer), the membrane pellet was resuspended in TE-P buffer. The protein content of the membrane preparation was determined using the micro BCA protein assay (Pierce Chemical Co., Rockford, IL) [Smith et al., 1985].

### Polyacrylamide Gel Electrophoresis (SDS–PAGE) and Blotting

Proteins were resolved on 5–14% (w/v) gradient polyacrylamide gels under reducing conditions, according to the method of Laemmli [1970], and electrotransferred onto a polyvinylidine difluoride (PVDF) membrane (Immobilon P, 0.45-μm pore size, Millipore Co., Bedford, MA). Immunoblotting was performed as described [Egami et al., 1990], using mouse monoclonal anti-BG-A determinant (IgM), goat antimouse IgM, and alkaline-phosphatase conjugated rabbit anti-goat IgG antibodies. For lectin blot-

**TABLE I. Reactivities of Endoglycosidases and Plant Lectins**

| Types | Endoglycosidase | | Lectin | | |
|---|---|---|---|---|---|
| | PNGase | Endo H | L-PHA | Con A | TL |
| O-linked (mucin type) | – | – | – | – | (+)[b] |
| N-linked | | | | | |
| High mannose | + | + | – | + | – |
| Hybrid | + | + | – | – | (+)[b] |
| Biantennary complex | + | – | – | + | (+)[b] |
| Multiantennary complex | + | – | (+)[a] | – | (+)[b] |

[a]Containing [GlcNAc-β1, 6-Man-α1, 6-Man] branching.
[b]Containing poly N-acetyllactosamine structure of more than three repreating units.

ting, biotinylated lectins (Con A, L-PHA, TL) and avidin-conjugated alkaline phosphatase were used. The structures to which these lectins bind are shown in Table I [Osawa and Tsuji, 1987; Yousefi et al., 1991; Dennis et al., 1987; Pierce and Arango, 1986; Yamashita et al., 1984].

### Immunocytochemistry

PC-1 cells were harvested by trypsinization, fixed in Bouin's solution for 10 min, washed in 70% ethanol, and embedded in paraffin. Immunoperoxidase staining was performed using mouse monoclonal anti-BG-A (IgM) and biotin labelled goat antimouse IgM antibodies, and avidin-labeled peroxidase as reported [Egami et al., 1989]. Color development was performed in the presence of 0.04% nickel chloride.

### Endoglycosidase Digestion

After boiling for 3 min, 25 μg of sample were subjected to endoglycosidase digestion according to the manufacture's instruction: 1 U of PNGase in 120 mM sodium phosphate (pH 7.5)/50 mM $Na_2EDTA$/0.15% SDS/1% 2-mercaptoethanol/1% Triton X-100; 1 mU of Endo H in 50 mM sodium citrate (pH 5.5)/50 mM $Na_2EDTA$/0.06% SDS/0.7% 2-mercaptoethanol/0.5 mM PMSF. The digestion was performed for 18 h at 37°C. The ability of the enzyme preparation to cleave N-linked oligosaccharides was confirmed using porcine thyroglobulin and ovalbumin. The reactivities of PNGase and Endo H are shown in Table I [Alexander and Elder, 1989; Tarentino et al., 1985; Trimble and Maley, 1984].

### α1–3 GalNAc Transferase (A-Transferase) Assay

The A-transferase assay used in this study was a modification of previously described methods [Itzkowitz et al., 1990; Dahiya et al., 1989]. Assays were performed in 50 μl containing 50 mM HEPES buffer (pH 7.0), 20 mM $MnCl_2$, 1% Triton X-100, 410 μM 2'fucosyllactose, 2.7 μM ($1 \times 10^5$ cpm) UDP-GalNAc, and 5 μg of cell membrane fraction as an enzyme source. Reaction mixtures were incubated at 37°C for 60 min, and were terminated by the addition of 200 μl of ice-cold water. The reaction mixtures were then applied to columns (1 ml) of Dowex-1, and the products were separated by washing each column with 2.75 ml water. Each eluate was mixed with Ecolume scintillation fluid (ICN Biomedics, Irvine, CA); radioactivity was determined by liquid scintillation spectrometry. The radioactivity released in incubations without 2' fucosyllactose (contribution of endogenous acceptors) was ~5% of total transfer and was subtracted to yield acceptor dependent activity.

## RESULTS

### Effect of SW and dMM on BG-A Antigen Expression

PC-1 cells were treated for four days with 0.1 mM SW or 1.0 mM dMM as described above. BG-A antigen was detected immunocytochemically in control and SW-treated PC-1 cells (Fig. 2a,b). However, treatment of PC-1 cells with dMM caused a near elimination of BG-A expression at the cell surface, from 95% of cells stained with anti-BG-A antibody to 5% (Fig. 2c).

In membrane preparations isolated from untreated PC-1 cells, BG-A reactivity was detected by immunoblotting as reported previously [Hirota et al., 1992b], with the strongest reactivity at a molecular weight of 135 kd (Fig. 3). In SW-treated cell membrane preparations, BG-A reactivity was retained, but the strongest reactivity was found at 125 kd. In membranes from dMM treated cells, BG-A reactivity was nearly abrogated (Fig. 3).

### Susceptibility of BG-A Antigen to Endoglycosidases

Membrane preparations from untreated and SW-treated PC-1 cells were digested with PNGase or Endo H as described above. Endo H cleaves only high mannose and hybrid N-linked glycans, while PNGase cleaves high mannose, hybrid, and all known complex oligosaccharides

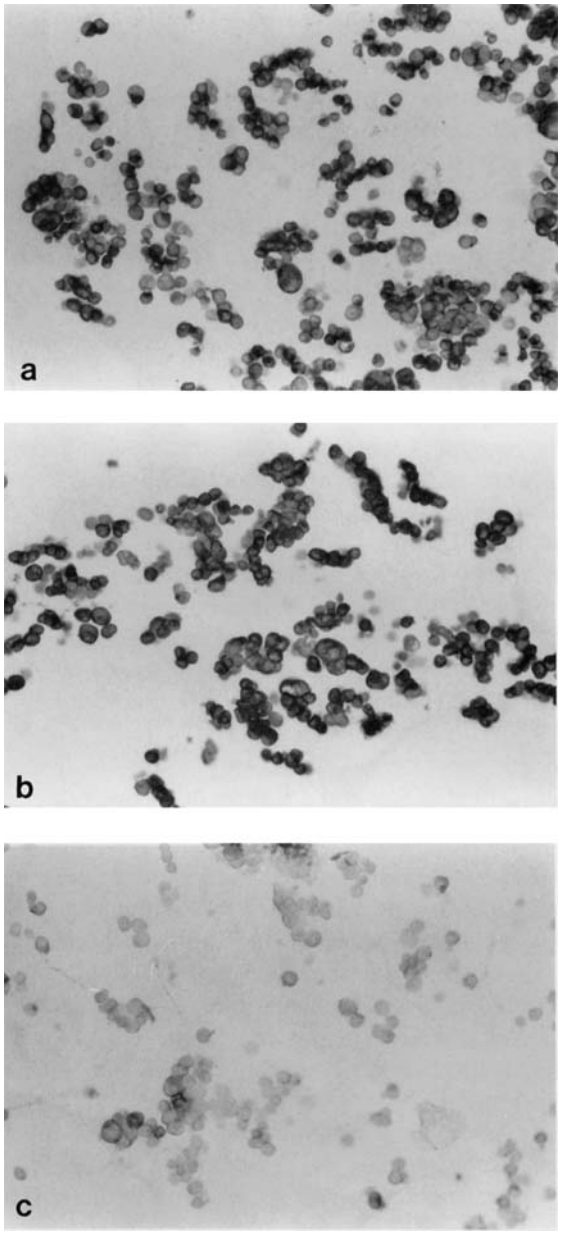


Fig. 2. Effects of SW and dMM on BG-A antigen expression in PC-1 cells. PC-1 cells were stained for BG-A antigen, which was visualized by peroxidase reaction with nickel chloride as described in Materials and Methods (×80). Based on the observation of more than 400 cells, the percentages of BG-A antigen-positive cells were as follows in the parentheses. **a:** Control PC-1 cells (94.9%). **b:** SW-treated PC-1 cells (95.5%). **c:** dMM-treated PC-1 cells (4.8%).

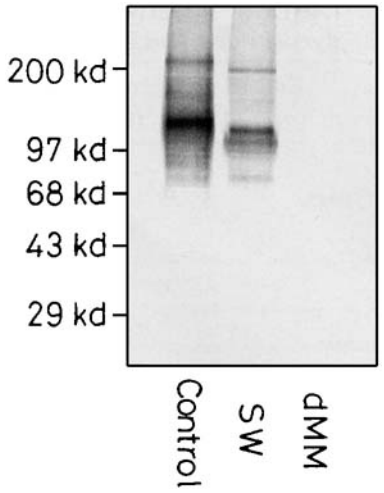


Fig. 3. Effects of SW and dMM on expression of BG-A antigen. Twenty-five μg protein of a membrane preparation were applied to each lane. Membrane glycoproteins were separated by SDS–PAGE on a 5–14% gradient gel and transferred to a PVDF membrane. BG-A antigen was visualized using a mouse monoclonal anti-BG-A antigen antibody.

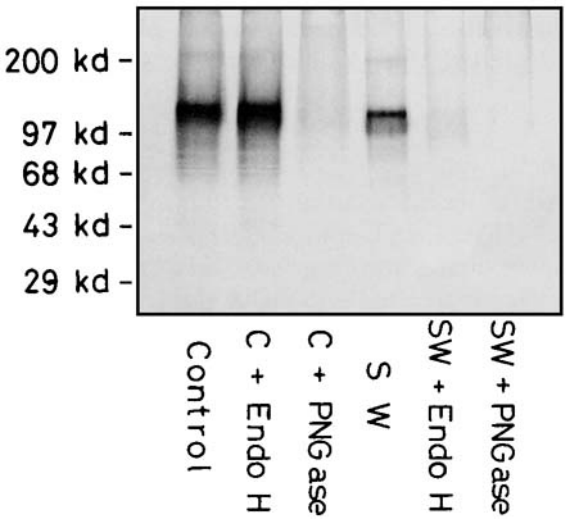


Fig. 4. Effects of endoglycosidases on BG-A antigen in PC-1 cells. Twenty-five μg protein of membrane preparations were subjected to PNGase or Endo H treatment for 18 h at 37°C. Samples were separated by SDS–PAGE on a 5–14% gradient gel and transferred to a PVDF membrane. BG-A antigen was visualized using a mouse monoclonal anti-BG-A antigen antibody.

[Alexander and Elder, 1989; Tarentino et al., 1985; Trimble and Maley, 1984]. BG-A antigen in control PC-1 cells was removed by PNGase treatment, and was resistant to Endo H treatment. BG-A antigen on SW-treated membranes was also sensitive to PNGase digestion. However, the BG-A reactivity of membrane preparation became sensitive to Endo H digestion in SW treated cells, in contrast to control cells (Fig. 4).

### Effect of SW and dMM on Lectin Reactivities

In the control PC-1 cells, L-PHA reactivity of membrane preparations was detected at 135 kd (Fig. 5). In membrane preparations of cells treated with SW or dMM, this L-PHA reactive

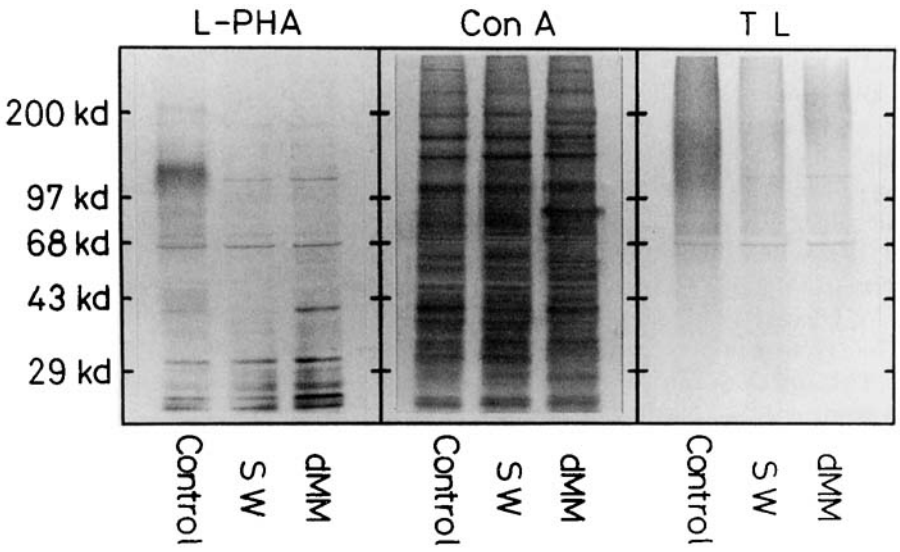


**Fig. 5.** Lectin reactivity on PC-1 cells. Twenty-five μg protein of membrane preparations from PC-1 cells (untreated control, SW-treated, and dMM-treated) were separated by a 5–14% gradient polyacrylamide gel, transferred to a PVDF membrane, and blotted with biotinylated lectins (L-PHA, Con A, TL), followed by avidin-conjugated alkaline phosphatase.

band was abrogated. Some weak stainings observed were also found by incubation with avidin–alkaline phosphatase alone (data not shown). Con A reactivity was retained during the treatment with either of the inhibitors. Con A binding was completely inhibited in the presence of 500 mM α-methylmannopyranoside (inhibitory sugar), while it was not by the same concentration of Gal, demonstrating that the Con A reactivity was dependent on oligosaccharide expression (data not shown). The reactivity with TL was also retained, but it was decreased by either SW or dMM treatment (Fig. 5). The retention of TL reactivity is probably due to the presence of Ser/Thr-linked (O-linked) glycans bearing TL reactive structures.

### Effect of SW and dMM on A-Transferase Activity

The effects of SW and dMM treatment on A-transferase activity in PC-1 cells was examined. Membrane preparations from control PC-1 cells or PC-1 cells treated for 4 days with SW or dMM were incubated with UDP-[$^3$H]-GalNAc and 2'fucosyllactose as described under Materials and Methods. A-transferase activity was not changed by treatment of PC-1 cells with SW, but dMM treatment decreased the enzymatic activity to 35% of that in untreated cells (Table II).

**TABLE II. α1,3 GalNAc Transferase (A-Transferase) Activity in PC-1 Cells***

| Cells | Activity (nmole/mg protein/hr) |
|---|---|
| Control PC-1 cells | 8.5 ± 2.6 |
| SW-treated PC-1 cells | 8.9 ± 2.8 |
| dMM-treated PC-1 cells | 3.0 ± 0.8 |

*Membrane preparations of these cells were subjected to the activity assay as described under Materials and Methods. The activity was expressed as a function of [UDP-GalNAc] transferred to exogenous acceptor/mg of membrane protein/hour. Data were shown as mean values ± SD from five experiments.

## DISCUSSION

Previous studies have demonstrated that BG-A antigen on glycoproteins purified from hamster pancreatic adenocarcinoma membranes resides predominantly on N-linked oligosaccharides [Hirota et al., 1992b]. The present study has examined the effect of two inhibitors of N-linked oligosaccharide processing on BG-A expression on whole cells and on membrane preparations, and on Con A, L-PHA, and TL reactivities in membrane preparations.

Treatment of PC-1 cells for 4 days with dMM led to a near elimination of BG-A expression at the cell surface, from 95% to 5%. However, SW treatment did not change either the number of cells expressing BG-A, or the level of expression.

Analysis of BG-A expression in membrane preparations by Western blotting following separation by SDS-PAGE demonstrated a difference in BG-A expression after SW treatment of

PC-1 cells. The major BG-A bearing glycoprotein migrated at a molecular weight of 125 kd, instead of 135 kd in untreated cells. The shift of molecular weight from 135 kd to 125 kd in SDS–PAGE might be due to an inability of further processing of the branches of the oligosaccharides. While remaining sensitive to PNGase digestion, this BG-A bearing glycoprotein acquired sensitivity to Endo H. These results suggest that in SW treated PC-1 cells the BG-A reactivity shifted from complex to hybrid oligosaccharides. Tulsiani and Touster [1983] reported a shift from complex carbohydrates to hybrid structures after treatment of human skin fibroblasts by SW. Although high mannose glycans are also sensitive to Endo H treatment, they are unable to express BG-A antigen because they lack processed oligosaccharide branches as sites for addition of BG-A antigen.

In contrast to extracellular proteins and intracellular nonglycosylated proteins, intracellular glycoproteins possess short half lives. The half-lives of membrane and cytoplasmic proteins were reported to be 16–99 h [Reutler and Tauber, 1983; Tanabe et al., 1979; Hong et al., 1989; Tauber et al., 1983, 1989; Chu and Doyle, 1985]. The glycan moiety of glycoproteins shows faster turnover rate. Half lives of glycan part of membrane glycoproteins were shown to be 12–66 h in rat normal liver [Reutler and Tauber, 1983; Tauber et al., 1983; Warren and Doyle, 1981]. Turnover of N-linked glycans in membrane glycoproteins was about 20 h in rat hepatoma [Tauber et al., 1983]. The incubation period used in this experiment with SW or dMM (4 days) is reasonable to see the alteration of glycan structure, even though other investigators used shorter incubation periods [Hughes et al., 1987; Montefiori et al., 1988; Myc et al., 1989; Dennis, 1986]. As a matter of fact, dMM inhibited the expression of BG-A antigen in almost all cells, and SW changed the glycan structure from Endo H resistent form to sensitive form. The small percentage of remaining BG-A positive cells in dMM-treated PC-1 cells might represent a population with a slow glycan turnover or cells that are in quiescent state. Since the glycan moieties of glycoproteins contribute to many important cellular functions, higher turnover of glycan structure might be a reflection of fine control of cell surface. Furthermore, since nearly all cells lost BG-A antigen after dMM treatment, the predominant form of BG-A bearing structure at the cell surface in hamster pancreatic cancer cells must be N-linked glycans.

Western blotting analysis showed that L-PHA reactivity was suppressed in cells treated with SW or dMM. On the other hand, Con A reactivity was retained. These results confirm that SW and dMM are acting in the expected manner, because if these inhibitors act properly, high mannose N-glycans, which can be recognized by Con A, should be retained, and multiantennary complex carbohydrates, which can be recognized by L-PHA, should be lost. TL recognizes poly N-acetyllactosamine, which is thought to be a cancer associated glycan structure [Pierce and Arango, 1986; Yamashita et al., 1984]. PC-1 cells showed TL reactivity, which was suppressed, but not eliminated, by the treatment with SW or dMM. This result may be due to poly N-acetyllactosamine structures being attached to both N- and O-linked glycans.

In recent years, the importance of glycan moieties in biological functions has been recognized. Human chorionic gonadotropin [Goverman et al., 1982; Kalyan and Bahl, 1983], immunoglobulin G [Tsuchiya et al., 1989], erythropoietin [Takeuchi et al., 1990], $Na^+/H^+$ antiporter [Yusufi et al., 1988], basic fibroblast growth factor receptor [Feige and Baird, 1988], and cholecystokinin receptor [Santer et al., 1990] were shown to require N-linked glycans for their functions. Furthermore, the metastatic potential of cancer cells was shown to be associated with N-linked glycans [Yousefi et al., 1991; Dennis et al., 1987; Pierce and Arango, 1986]. In this regard, cancer treatment with selective inhibitors of N-glycan synthesis has been advocated as future therapeutic value [Humphries et al., 1986; Dennis, 1986].

The A-transferase activity was not affected by SW treatment. Although the level of A-transferase was decreased by dMM treatment to 35% that found in control cells, the level of A-transferase activity is probably sufficient to synthesize BG-A antigen. Thus, the loss of nearly all detectable BG-A antigen both on membrane preparations and on whole cells must be due to the inhibition of N-linked oligosaccharide processing. The decrease of A-transferase activity in dMM-treated cells might be ascribed to the inappropriate glycan processing of A-transferase itself. Recently, the cDNA for human A-transferase has been cloned, and a potential site of N-linked glycosylation was identified [Clausen et al., 1990; Yamamoto et al., 1990; Paulson and Colley, 1989]. Our data would suggest that complex or hybrid type N-linked glycans may be necessary for optimal enzymatic

activity. The importance of complex and hybrid type N-linked glycan for the function of glycoproteins has been elucidated in other experimental systems [Feige and Baird, 1988; Montefiori et al., 1988; Elbein et al., 1982; Schwarz et al., 1976]. However, whether the decrease in A-transferase activity observed in dMM treated cells is due to changes in A-transferase levels or kinetic properties is not known. Further studies are under way to investigate this question.

## ACKNOWLEDGMENT

This work was supported by American Cancer Society SIG 16 and Junior Faculty Research Award 228 (W.G.C.).

## REFERENCES

Alexander S, Elder JH (1989): Endoglycosidases from *Flavobacterium meningosepticum*—Application to biological problems. Methods Enzymol 179:505–518.

Chu FF, Doyle D (1985): Turnover of plasma membrane proteins in rat hepatoma cells and primary cultures of rat hepatocytes. J Biol Chem 260:3097–3107.

Clausen H, White T, Takio K, Titani K, Stroud M, Holmes E, Karkov J, Thim L, Hakomori S (1990): Isolation to homogeneity and partial characterization of a histo-blood group A defined Fucα1 → 2Gal α1 → 3-N-acetylgalactosaminyltransferase from human lung tissue. J Biol Chem 265:1139–1145.

Dahiya R, Itzkowitz SH, Byrd JC, Kim YS (1989): Blood group antigen expression, synthesis, and degradation in human colonic adenocarcinoma cell lines. Cancer Res 49:4550–4556.

Dennis JW (1986): Effects of swainsonine and polyinosinic: Polycytidylic acid on murine tumor cell growth and metastasis. Cancer Res 46:5131–5136.

Dennis JW, Laferte S, Waghorne C, Breitman ML, Kerbel RS (1987): Beta 1–6 branching of Asn-linked oligosaccharides is directly associated with metastasis. Science 236: 582–585.

Egami H, Takiyama Y, Cano M, Houser WH, Pour PM (1989): Establishment of hamster pancreatic adenocarcinoma cell line (PC-1) producing blood group-related antigens. Carcinogenesis 10:861–869.

Egami H, Takiyama Y, Chaney WG, Cano M, Fujii H, Tomioka T, Metzgar R, Pour PM (1990): Comparative studies on expression of tumor-associated antigens in human and induced pancreatic cancer in Syrian hamsters. Int J Pancreatol 6:91–100.

Elbein AD, Dorling PR, Vosbeck K, Horisberger M (1982): Swainsonine prevents the processing of the oligosaccharide chains of influenza virus hemagglutinin. J Biol Chem 257:1573–1576.

Elbein AD (1987): Inhibition of the biosynthesis and processing of N-linked oligosaccharide chains. Annu Rev Biochem 56:497–534.

Feige JJ, Baird A (1988): Glycosylation of the basic fibroblast growth factor receptor—The contribution of carbohydrate to receptor function. J Biol Chem 263:14023–14029.

Goverman JM, Parson TF, Pierce JG (1982): Enzymatic deglycosylation of the subunits of chorionic gonadotropin. J Biol Chem 257:15059–15064.

Hirota M, Mogaki M, Pour PM, Chaney WG (1992a): Glycan structure of blood group A antigen in hamster normal tissues and pancreatic cancers. Exp Cell Res, submitted.

Hirota M, Pour PM, Tempero M, Chaney WG (1992b): Purification and analysis of glycoproteins bearing blood group A determinants from nitrosamine induced hamster pancreatic cancers. Carcinogenesis, in press.

Hong WJ, Piazza GA, Hixson DC, Doyle D (1989): Expression of enzymatically active rat dipeptidyl peptidase IV in Chinese hamster ovary cells after transfection. Biochemistry 28:8474–8479.

Hughes RC, Foddy L, Bause E (1987): Asparagine-linked oligosaccharides of BHK cells treated with inhibitors of oligosaccharide processing. Biochem J 247:537–545.

Humphries MJ, Matsumoto K, White SL, Olden K (1986): Oligosaccharide modification by swainsonine treatment inhibits pulmonary colonization by B16–F10 murine melanoma cells. Proc Natl Acad Sci USA 83:1752–1756.

Itzkowitz SH, Dahiya R, Byrd JC, Kim YS (1990): Blood group antigen synthesis and degradation in normal and cancerous colonic tissues. Gastroenterology 99:431–442.

Kalyan NK, Bahl OP (1983): Role of carbohydrate in human chorionic gonadotropin. J Biol Chem 258:67–74.

Kornfeld R, Kornfeld S (1985): Assembly of asparagine-linked oligosaccharides. Annu Rev Biochem 554:631–664.

Laemmli UK (1970): Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680–685.

Massague J, Czech MP (1982): The subunit structures of two distinct receptors for insulin-like growth factors I and II and their relationship to the insulin receptor. J Biol Chem 257:5038–5045.

Montefiori DC, Robinson WE Jr, Mitchel WM (1988): Role of protein N-glycosylation in pathogenesis of human immunodeficiency virus type 1. Proc Natl Acad Sci USA 85:9248–2952.

Myc A, Kunicka JE, Melamed MR (1989): Effect of swainsonine on stimulation and cell cycle progression of human lymphocytes. Cancer Res 49:2879–2883.

Osawa T, Tsuji T (1987): Fractionation and structural assessment of oligosaccharides and glycopeptides by use of immobilized lectins. Annu Rev Biochem 56:21–42.

Paulson JC, Colley KJ (1989): Glycosyltransferases. J Biol Chem 264:17615–17618.

Pierce M, Arango J (1986): Rouse sarcoma virus-transformed baby hamster kidney cells express higher levels asparagine-linked tri- and tetraantennary glycopeptides containing [GlcNAc-β(1,6)Man-α(1,6)Man] and poly-N-acetyllactosamine sequences than baby hamster kidney cells. J Biol Chem 261:10772–10777.

Pour PM, Uchida E, Burnett D, Steplewski Z (1986): Blood-group antigen expression during pancreatic cancer induction in hamsters. Int J Pancreatol 1:327–340.

Reutler W, Tauber R (1983): Turnover of plasma membrane glycoproteins from liver and hepatoma. GANN Monog Cancer Res 29:59–65.

Santer R, Leung YK, Alliet P, Lebeuthal E, Lee PC (1990): The role of carbohydrate moieties of cholecystokinin receptors in cholecystokinin octapeptide binding: Alteration of binding data by specific lectins. Biochim Biophys Acta 1051:78–83.

Schwarz RT, Rohrenschneider JM, Schmidt MFG (1976): Suppression of glycoprotein formation of Semliki forest, influenza, and avian sarcoma virus by tunicamycin. J Virol 19:782–791.

Smith PK, Krohn RI, Hermanson GT, Mallia AK, Gartner

FH, Provenzano MD, Fujimoto EK, Goeke NM, Olson BJ, Klenk DC (1985): Measurement of protein using bicinchoninic acid. Anal Biochem 150:76–85.

Takeuchi M, Takasaki S, Shimada M, Kobata A (1990): J Biol Chem 265:12127–12130.

Tanabe T, Pricer WE Jr, Ashwell G (1979): Subcellular membrane topology and turnover of a rat hepatic binding protein specific for asialoglycoproteins. J Biol Chem 254: 1038–1043.

Tarentino AL, Gomez CM, Plummer TH (1985): Deglycosylation of asparagine-linked glycans by peptide:N-glycosidase F. Biochemistry 24:4665–4671.

Tauber R, Park CS, Beutter W (1983): Intramolecular heterogeneity of degradation in plasma membrane glycoproteins. Proc Natl Acad Sci USA 80:4026–4029.

Tauber R, Park CS, Becker A, Geyer R, Reutter W (1989): Rapid intramolecular turnover of N-linked glycans in plasma membrane glycoproteins. Eur J Biochem 186:55–62.

Trimble RB, Maley F (1984): Optimizing hydrolysis of N-linked high mannose oligosaccharides by Endo-β-N-acetylglucosaminidase H. Anal Biochem 141:515–522.

Tsuchiya N, Endo T, Matsuta K, Yoshinoya S, Aikawa T, Kosuge E, Takeuchi F, Miyamoto T, Kobata A (1989): Role of sugar chains in the in vitro biological activity of human erythropoietin produced in recombinant Chinese hamster ovary cells. J Rheumatol 16:2885–2890.

Tulsiani DRP, Touster O (1983): Swainsonine causes the production of hybrid glycoproteins by human skin fibroblasts and rat liver Golgi preparations. J Biol Chem 258: 7578–7585.

Warren R, Doyle D (1981): Turnover of the surface proteins and the receptor for serum asialoglycoproteins in primary cultures of rat hepatocytes. J Biol Chem 265:1346–1355.

Yamamoto F, Marken J, Tsuji T, White T, Clausen H, Hakomori S (1990): Cloning and characterization of DNA complementary to human UDP-GalNAc: Fucα1 → 2Gal α11 → 3 GalNAc transferase (histo-blood group A transferase) mRNA. J Biol Chem 265:1146–1151.

Yamashita K, Ohkura T, Tachibana Y, Takasaki S, Kobata A (1984): Comparative study of the oligosaccharides released from baby hamster kidney cells and their polyoma transformant by hydrazinolysis. J Biol Chem 259:10834–10840.

Yusufi ANK, Szczepanska-Konkel M, Dousa TP (1988): Role of N-linked oligosaccharides in the transport activity of the $Na^+/H^+$ antiporter in rat renal brush-border membrane. J Biol Chem 263:13683–13691.

Yousefi S, Higgins E, Daoling Z, Kruger AP, Hindsgaul O, Dennis JW (1991): Increased UDP-GlcNAc:Galβ1-3Gal-NAc-R (GlcNAc to GalNAc) β-1,6-N-acetylglucosaminyl transferase activity in metastatic murine tumor cell lines. J Biol Chem 266:1772–1782.