Effects of Brefeldin A on oligosaccharide processing

Evidence for decreased branching of complex-type glycans and increased formation of hybrid-type glycans

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Brefeldin A (BFA), a drug that induces redistribution of Golgi-apparatus proteins into the endoplasmic reticulum, was used to determine the role of subcellular compartmentalization in the processing of asparagine-linked oligosaccharides. Baby-hamster kidney cells were pulse-labelled with [³H]mannose for 30–60 min and chased for up to several hours in the presence or in the absence of BFA or labelled continuously for several hours with and without the drug. Cellular glycoproteins were digested to glycopeptides with Pronase and either fractionated into glycan classes by lectin affinity chromatography or digested further by endoglycosidase H and endoglycosidase D. Released oligosaccharides obtained in the latter procedure were then separated from each other and from endoglycosidase-resistant glycopeptides by paper chromatography. The results show that BFA induces a very fast processing of protein-linked Glc₃Man₅GlcNAc₂ oligosaccharide down to Man₅GlcNAc₂ and conversion into complex-type and hybrid-type glycans. The major difference between untreated and BFA-treated cells is a large increase in bi-antennary and hybrid-type glycans in the latter cells. These results indicate that galactosylation of a mono-antennary GlcNAcMan₅GlcNAc₂ hybrid blocks subsequent action by mannosidase II and *N*-acetylglucosaminyl transferase II, producing galactosylated hybrid-type glycans. Similarly, galactosylation of the product of *N*-acetylglucosaminyltransferases I and II, i.e. a Man₃GlcNAc₂ core substituted with GlcNAcβ1→2 on both α1→3- and α1→6-linked mannose residues, blocks branching *N*-acetylglucosaminyltransferases IV and V, thereby causing an increase in bi-antennary glycans and a decrease in tri- and tetra-antennary glycans.

INTRODUCTION

The asparagine-linked oligosaccharides of glycoproteins are extremely heterogeneous in structure but generally fall into three classes, complex-type, hybrid-type and oligomannose-type glycans [1]. All of these classes of glycan are produced from the same Glc₃Man₅GlcNAc₂ oligosaccharide, which is transferred initially to protein and modified subsequently by a large number of enzymic steps involving processing glycosidases and glycosyltransferases. There is considerable evidence showing that processing enzymes are distributed in the intact cell in discrete organelles [2], including the endoplasmic reticulum, Golgi cisternae and the trans Golgi network. It is also known from assays *in vitro* that two or more processing enzymes may compete for a common substrate and that the prior action of one enzyme may block subsequent action by a second enzyme [1]. Consequently, the control of glycan structure represents an intricate balance between substrate-specificities of processing enzymes and their arrangement within the glycosylation machinery of the cell.

We have examined the role of subcellular compartmentalization of oligosaccharide-processing enzymes in the control of protein glycosylation using baby-hamster kidney (BHK) cells treated with Brefeldin A (BFA). Morphological studies have shown that BFA causes disassembly of the Golgi apparatus, dilation of the endoplasmic reticulum and retrograde transport of both resident and transient Golgi-apparatus proteins to the endoplasmic reticulum [3–8]. Consequently, nascent glycoproteins carrying Glc₃Man₅GlcNAc₂ oligosaccharides are subjected to a mixture of processing glycosidases and glycosyltransferases that are normally in separate compartments. The effects of BFA on

oligosaccharide processing have been examined by Perkel *et al.* [9,10], Lippincott-Schwartz *et al.* [3] and Doms *et al.* [5], with somewhat contradictory findings. Perkel *et al.* [9,10], using thyroid tissue slices, showed that the oligosaccharides of thyrotropin subunits, which are normally processed to complex-type glycans, were processed only down to Man₅₋₆GlcNAc₂ structures, suggesting limited exposure to endoplasmic-reticulum mannosidase and possibly Golgi-apparatus mannosidases. By contrast, Doms *et al.* [5] and Lippincott-Schwartz *et al.* [3] showed that the oligosaccharides of vesicular-stomatitis-virus G glycoprotein and T-cell antigen receptor subunits respectively are processed in BFA-treated cells into an endoglycosidase H (endo H)-resistant form, presumably complex-type glycans. Our results are in agreement with and extend the latter findings.

EXPERIMENTAL

Materials

Brefeldin A was obtained from Epicentre Technologies (Madison, WI, U.S.A.) and stock solutions (10 mg/ml) in ethanol were stored in small portions at –70 °C. D-[2-³H]Mannose (18.5 Ci/mmol) and L-[5,6-³H]fucose (45 Ci/mmol) were from Amersham International (Amersham, Bucks., U.K.). Endoglycosidases H and D were from Boehringer Mannheim. 1-Deoxymannojirimycin (dMM) was from Sigma Chemical Co. (Poole, Dorset, U.K.). Concanavalin A (Con A)–Sepharose 4B (8 mg/ml) and lentil lectin–Sepharose 4B (2 mg/ml) were from LKB Pharmacia (Milton Keynes, Bucks., U.K.). Columns of *Tetracarpidium conophorum* lectin (TCA)–Sepharose 4B (8 mg/ml) were kindly supplied by T. Animashaun and S. Sato, National Institute for Medical Research [11]. Jack-bean α-

Abbreviations used: BFA, Brefeldin A; Con A, concanavalin A; TCA, *Tetracarpidium conophorum* lectin; dMM, 1-deoxymannojirimycin; PBS, phosphate-buffered saline; BHK, baby-hamster kidney cells; endo H, endoglycosidase H; endo D, endoglycosidase D.

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mannosidase was from Sigma Chemical Co. Standard [^3H]mannose-labelled oligosaccharides were obtained as described previously [12,13]. Phosphate-buffered saline (PBS) contained NaCl (8.0 g) KCl (0.29 g), Na_2HPO_4 (1.159 g), KH_2PO_4 (0.29 g), CaCl_2 (0.19 g) and MgCl_2 (0.19 g) diluted to 1 litre with water, pH 7.04.

Metabolic labelling

The baby-hamster kidney cell line BHK21 C13 was grown at 37 °C in 60 mm-diameter dishes as monolayers in modified Eagle's medium (GIBCO BRL, Paisley, Scotland, U.K.) supplemented with 10 % foetal-calf serum, penicillin and streptomycin. For metabolic labelling, nearly confluent monolayers were rinsed twice with Eagle's medium containing one-tenth (2 mM) the usual concentration of glucose and 10 % (v/v) dialysed serum and incubated for various periods at 37 °C with the same medium (2 ml) containing 100–500 μCi of [^3H]mannose or [^3H]fucose. In pulse-chase-labelling experiments, the monolayers, after labelling usually for 30–60 min, were rinsed twice with 2 ml of Eagle's medium containing 20 mM-glucose and 10 % serum plus 5 mM unlabelled mannose and chased for various times in 2 ml of this medium. As indicated, the growth medium (2 ml) contained either or both BFA (5 $\mu\text{g}/\text{ml}$) and 2.5 mM-dMM (250 $\mu\text{g}/\text{ml}$). After being labelled the cells were rinsed twice with PBS on ice and then flooded with 2 ml of ice-cold methanol [14]. The cells were scraped into tubes immersed in ice and the dishes were rinsed with 1 ml of methanol. The cell suspensions (3 ml) were thoroughly mixed with chloroform (4.5 ml) and water (1.5 ml) and centrifuged at 3000 g for 10 min to stabilize phase separation with a protein residue at the interface. After removal of the top aqueous layer, water (1.5 ml) and methanol (5 ml) were added and, after thorough mixing to produce a single phase, the lipid-free protein residue was recovered by centrifugation as before, washed twice with chloroform/methanol/water (10:10:3, by vol.) and air-dried.

Enzyme digests

Protein pellets were suspended in 50 mM-Tris/HCl buffer (pH 7.5)/20 mM- CaCl_2 /0.02 % NaN_3 (0.3 ml), heated at 90 °C for 5 min, cooled and treated with 100 μl of Pronase (0.4 % in the above buffer) at 37 °C for 2 days. The digests were reheated, centrifuged at 10000 g for 10 min and the supernatants either were used directly or were chromatographed on a column (2 cm $\times$ 27 cm) of Bio-Gel P-2 run in water. Fractions (2 ml) were collected. The column fractions 17–24 containing radioactively labelled glycopeptides were freeze-dried and redissolved in water (0.5 ml). Endoglycosidase digestion of glycopeptides was performed at 37 °C for 1–2 days in 20 μl of 0.1 M-sodium acetate buffer, pH 6.0, containing endo H (2.5 μl , 2.5 munits) or endo D (2.5 μl , 2.5 munits) or a mixture of both enzymes. Jack-bean α -mannosidase treatment of oligosaccharides or glycopeptides was carried out in 40 μl of 0.1 M-sodium acetate buffer (pH 5.0)/0.3 mM- ZnSO_4 and 10 μl (0.1 unit) of enzyme solution. After incubation the digests were usually diluted with water and heated at 90 °C for 5 min to inactivate the enzymes.

Chromatography

Samples for paper chromatography were applied in 20 μl or less to Whatman no. 1 paper and separated over 3–7 days by development with ethyl acetate/pyridine/acetic acid/water (5:5:1:3, by vol.). After chromatography each track was cut into 1 cm segments. Each segment was immersed in 1 ml of water to elute oligosaccharides, and portions of the eluates were counted for radioactivity. Lectin affinity chromatography on Con A-Sepharose or lentil lectin-Sepharose columns (1.5 ml bed volume) was carried out as described previously [15,16]. Samples

were dissolved in 10 mM-Tris/HCl buffer (pH 7.5)/1 mM- CaCl_2 /1 mM- MgCl_2 /1 mM- MnCl_2 /0.02 % NaN_3 , applied to the lectin columns and eluted with hapten sugars dissolved in the same buffer. Chromatography on TCA-Sepharose was carried out as follows. The glycopeptide fraction was applied in 1 ml or less of the above buffer to TCA-Sepharose columns (1.5 ml bed volume). After incubation for 1 h at room temperature (approx. 20 °C), the columns were washed at 20 °C with 10 ml of buffer followed by 10 mM-lactose and 100 mM-lactose [11]. Fractions (1 ml) were collected and samples were assayed for radioactivity. Appropriate peak radioactive fractions were pooled, desalted by Bio-Gel P-2 chromatography, freeze-dried and reconstituted in water (0.5–1 ml). For quantification, the radioactivity recovered under each peak after paper chromatography or lectin affinity chromatography was calculated and expressed as a percentage of total recovered radioactivity. In some cases the radioactivity recovered in the peak fractions was corrected for the number of mannose residues in each product: three mannose residues for complex-type glycans and nine to five residues for $\text{Man}_6\text{GlcNAc}$ to $\text{Man}_5\text{GlcNAc}$ species respectively. The relative amount of each species was then calculated as a percentage of total products. Ion-exchange chromatography on a column (1 cm $\times$ 30 cm) of DEAE-Sepharose was carried out as described previously [15]. The glycopeptide sample in 0.05 M-acetic acid (1 ml) was applied at 2 °C to the column, which was then washed with 0.05 M-acetic acid (10 ml) followed by a salt gradient (40 ml of 0.5 M-NaCl in 0.05 M-acetic acid and 40 ml of 0.05 M-acetic acid). Fractions (1 ml) were collected.

RESULTS

Pulse-labelling of protein-bound oligosaccharides in control and BFA-treated cells

Nearly confluent BHK cell monolayers were labelled with

Fig. 1. Metabolic labelling of BHK cells with [^3H]mannose in the presence or in the absence of BFA

Monolayer cultures were labelled for 4 h at 37 °C without (a) or with (b) BFA (5 $\mu\text{g}/\text{ml}$). Labelled glycoproteins were digested with Pronase, and oligosaccharides released from the glycopeptide fraction with endo H were analysed by paper chromatography as described in the Experimental section. Arrows indicate the migration of $\text{Man}_6\text{GlcNAc}$ (9), $\text{Man}_5\text{GlcNAc}$ (8), $\text{Man}_4\text{GlcNAc}$ (7), $\text{Man}_3\text{GlcNAc}$ (6) and $\text{Man}_2\text{GlcNAc}$ (5) standards. Horizontal bars indicate radioactive components remaining at or close to the origin of the chromatograms.

Fig. 2. Products obtained by endo H/endo D digestion of glycopeptides from control (a) or BFA-treated (b) cells labelled with [3 H]mannose and chased for various periods (as indicated)

Monolayer cultures were labelled for 1 h with [3 H]mannose and either harvested immediately or chased in a medium containing non-radioactive mannose in the absence or in the presence of 5 μ g of BFA/ml. Glycopeptides were prepared from delipidated cell residues and the oligosaccharides released by endo H/endo D were separated by paper chromatography as described in Fig. 1. The total radioactivity recovered from individual peaks was calculated as described in the Experimental section and the amount of each product expressed as a percentage of total products. a, Origin material; b, $\text{Man}_5\text{GlcNAc}$; c, $\text{Man}_6\text{GlcNAc}$; d, $\text{Man}_7\text{GlcNAc}$; e, $\text{Man}_8\text{GlcNAc}$; f, $\text{Man}_9\text{GlcNAc}$.

[3 H]mannose for 4 h in the absence or in the presence of 5 μ g of BFA/ml. After labelling, the cells were harvested into methanol and extracted in chloroform/methanol/water (3:2:1, by vol.) to remove glycolipids. The glycoprotein fraction was digested with Pronase to give glycopeptides and these were treated with endo H. The products were analysed by paper chromatography (Fig. 1). In control cells, approx. 70% of total label was recovered as oligosaccharides migrating with $\text{Man}_9\text{GlcNAc}$ standards. The remaining radioactivity (30%) remained at the origin. The predominant oligosaccharide was $\text{Man}_5\text{GlcNAc}$, with progressively smaller amounts of $\text{Man}_6\text{GlcNAc}$ and $\text{Man}_7\text{GlcNAc}$ and a trace of $\text{Man}_8\text{GlcNAc}$; no $\text{Man}_9\text{GlcNAc}$ was detected. In BFA-treated cells, approx. 36% of total radioactivity was recovered in a broad peak at or close to the origin, the proportion of radioactivity in $\text{Man}_5\text{GlcNAc}$ was greatly diminished compared with the control cell profile and a significant amount of $\text{Man}_9\text{GlcNAc}$ was detected. In other experiments we used endo D in combination with endo H to release oligosaccharides from the glycopeptide fraction and a shorter running time for chromatography in order to detect further processing of $\text{Man}_5\text{GlcNAc}$ to smaller oligomannosidic structures. However, no $\text{Man}_4\text{GlcNAc}$ or $\text{Man}_3\text{GlcNAc}$ components were present in the digests (results not shown).

Chase of protein-bound oligosaccharides in control and BFA-treated cells

The above results were confirmed in a pulse-chase experiment (Fig. 2). In control cells after 1 h of pulse-labelling approx. 25% of total [3 H]mannose-labelled products remained at or close to the origin, and during the chase this amount increased to approx. 60% of the total. At the same time, the major oligomannosidic

species present after 1 h of pulse, $\text{Man}_5\text{GlcNAc}$, was processed extensively, so that $\text{Man}_5\text{GlcNAc}$ was the major species at the end of the chase. In BFA-treated cells the relative amount of material remaining at or close to the origin rose to approx. 85% of total products during the chase and extensive processing of $\text{Man}_5\text{GlcNAc}$ species was observed, so that after 2 h of chase the predominant oligomannosidic component was $\text{Man}_5\text{GlcNAc}$. $\text{Man}_9\text{GlcNAc}$ was barely detectable at this time in the control cells.

To determine if the enhanced processing of oligomannosidic glycans down to $\text{Man}_5\text{GlcNAc}_2$ and subsequently into complex-type or hybrid-type glycans involved Golgi-apparatus-specific enzymes, redistributed into the endoplasmic reticulum by BFA treatment, or was due to prolonged exposure of nascent glycoproteins to resident endoplasmic-reticulum mannosidase, we monitored glycan processing in the presence of dMM. This drug is a potent inhibitor of cis Golgi-apparatus mannosidases IA/IB [17] but has no effect on the endoplasmic-reticulum mannosidase described by Bischoff & Kornfeld [18,19]. In a pulse-chase experiment with control cells (Fig. 3) $\text{Man}_5\text{GlcNAc}$ was the major labelled species after a 30 min pulse, and after the chase approx. 60% of the products were present as origin material, in agreement with the previous results (Fig. 2). In the presence of dMM this value was decreased to about 25% of total products. The difference (35%) was largely accounted for by a net increase in $\text{Man}_5\text{GlcNAc}$ of 20% and in $\text{Man}_6\text{GlcNAc}$ of 15%. This suggests, in agreement with our earlier analysis [20], that the dMM-resistant endoplasmic-reticulum mannosidase is responsible for only about one-half of the initial processing step removing one mannose residue from the asparagine-linked $\text{Man}_9\text{GlcNAc}_2$ oligosaccharide and that the remainder is due to

Fig. 3. Effect of dMM on oligosaccharide processing in control (a) and BFA-treated (b) cells

Cell monolayers were labelled with [^3H]mannose for 30 min and chased in non-radioactive medium for 18 h in the absence or in the presence of 5 μg of BFA/ml and 2.5 mM-dMM. The glycopeptides from the cells were treated with endo H/endo D and analysed by paper chromatography. See Fig. 2 legend for further experimental details.

Fig. 4. Serial lectin affinity chromatography of [^3H]mannose-labelled glycopeptides

BHK cells were labelled for 4 h in the presence of 5 μg of BFA/ml, and glycopeptides obtained by Pronase digestion were chromatographed on Con A-Sepharose (a). Selected radioactive peaks as indicated were then chromatographed on a lentil lectin-Sepharose column (b). The lectin columns were eluted first with buffer followed by: A, 10 mM-methyl α -glucoside; B, 500 mM-methyl α -mannoside; C, 200 mM-methyl α -glucoside.

dMM, but less so than in non-treated cells from about 80% to 45% of total products (Fig. 3). In addition, the oligomannosidic pool contained comparable amounts of $\text{Man}_6\text{GlcNAc}$ to $\text{Man}_6\text{GlcNAc}$ components. These results suggest that in BFA-treated cells dMM-resistant processing occurs to a significantly greater extent than in non-BFA-treated cells.

Characterization of complex-type and hybrid-type glycans

The radioactive material remaining at or near the origin (Fig. 1) presumably represents endo H/D-resistant glycopeptides and hybrid-type glycans released from endo H/D-sensitive glycopeptides. In order to distinguish these components, origin material obtained from BFA-treated BHK cells (Fig. 1b) was fractionated on Con A-Sepharose, when 18% of the total [^3H]mannose radioactivity was recovered in the unbound fraction, 46% in the weakly bound fraction and 36% in the tightly bound fraction. Tetra- and tri-antennary complex-type structures do not bind to Con A-Sepharose, bi-antennary complex-type structures bind weakly and are eluted with 10 mM-methyl α -glucoside and hybrid-type structures, except those carrying a bisecting GlcNAc residue, bind tightly and are eluted with 500 mM-methyl α -mannoside [16]. BHK cells lack *N*-acetylglucosaminyltransferase III [21] and hence the possible presence of bisected hybrid-type glycans can be excluded. The hybrid fraction obtained from Con A-Sepharose was treated with jack-bean α -mannosidase and rechromatographed on Con A-Sepharose to separate released mannose residues from residual oligosaccharide. Jack-bean α -mannosidase released 39–43% of radioactivity after mannosidase treatment in two separate experiments. Since jack-bean mannosidase can readily remove the two outer-branch mannose residues from a $\text{GlcNAcMan}_5\text{-GlcNAc}$ structure producing $\text{GlcNAc}\beta 1 \rightarrow 2\text{Man}\alpha 1 \rightarrow 3[\text{Man}\alpha 1 \rightarrow 6]\text{Man}\beta 1 \rightarrow 4\text{GlcNAc}$ [22,23], whereas the remaining terminal $\text{Man}\alpha 1 \rightarrow 6$ residue is extremely resistant to further mannosidase degradation [24], these results support a hybrid-type structure

dMM-sensitive mannosidase in the endoplasmic reticulum or to exposure to mannosidases IA/IB after transfer of nascent glycoproteins into the cis Golgi apparatus. In BFA-treated cells the amount of origin material was significantly decreased by

Fig. 5. Fractionation of $[^3\text{H}]$ fucose-labelled glycopeptides obtained from BFA-treated (a) or control (b) cells

Glycopeptides obtained by Pronase digestion from cells labelled for 4 h were fractionated on Con A-Sepharose followed by chromatography of peak fractions on lentil lectin-Sepharose as described in Fig. 4 legend. The bi-antennary complex-type glycopeptides eluted from lentil lectin-Sepharose were finally chromatographed on TCA-Sepharose before (a and b) or after (a) desialylation. Elution from TCA-Sepharose used 10 mM-lactose (D) and 100 mM-lactose (E). Fractions 1, 2 and 3 were pooled as indicated.

containing five mannose residues. Such a structure would be sensitive to endo H and presumably represents material migrating away from the origin in paper chromatography (Fig. 1b).

The presence of hybrid-type glycans in BFA-treated cells and

the composition of the complex-type glycans was also examined by using serial lectin affinity chromatography of glycopeptides [15,16]. Three fractions were obtained after Con A-Sepharose chromatography of glycopeptides obtained from BFA-treated

Fig. 6. Ion-exchange chromatography of glycopeptides

Fractions 1, 2 and 3 from TCA-Sepharose chromatography (Fig. 5a) were applied separately to DEAE-Sepharose and eluted first with 0.05 M-acetic acid followed by an NaCl gradient (0–0.5 M) started at the arrow. Fractions (1 ml) were collected and assayed for radioactivity. Δ , Fraction 1; $\circ$, fraction 2; $\bullet$, fraction 3.

cells labelled with [^3H]mannose for 4 h (Fig. 4.). When the fraction eluted with 10 mM-methyl α -glucoside was applied to a lentil lectin-Sepharose column, over 80% of the total radioactivity was bound and eluted with 200 mM-methyl α -glucoside. A small proportion (about 15%) of the Con A-Sepharose-unretarded fraction also bound to lentil lectin-Sepharose. Lentil lectin binds with high affinity bi-antennary glycans, as well as tri-antennary glycans carrying lactosamine branches on C-2 of $\text{Man}\alpha 1 \rightarrow 3$ and C-2 and C-6 of $\text{Man}\alpha 1 \rightarrow 6$ residues of the core sequence, provided that a $\text{Fuc}\alpha 1 \rightarrow 6$ substitution is present on the core chitobiose unit [16]. Thus in BFA-treated cells most of the bi-antennary glycan fraction is core-substituted with fucose. When the fraction eluted from Con A-Sepharose with 500 mM-methyl α -mannoside was applied to lentil lectin-Sepharose, most of the radioactivity was unretarded, representing oligomannosidic glycans, but a significant fraction was eluted with 200 mM-methyl α -glucoside representing hybrid-type glycans carrying a core fucose substituent [22–25].

Results obtained with [^3H]fucose-labelled cells confirmed and extended these findings (Fig. 5). A significant peak was eluted from Con A-Sepharose by 500 mM-methyl α -mannoside with glycopeptides from BFA-treated cells (Fig. 5a), and this was much decreased in control cell glycopeptides (Fig. 5b), which are known to contain only trace amounts of hybrid-type glycans [24]. Essentially all of the [^3H]fucose label of the fraction from BFA-treated cell glycopeptides bound to lentil-Sepharose (Fig. 5a). Considering the complex-type glycopeptide fractions from Con A-Sepharose, there was an increased proportion of bi-antennary glycans relative to tetra- and tri-antennary glycans in BFA-treated cells (Fig. 5a) compared with control cells (Fig. 5b). The [^3H]fucose-labelled bi-antennary fractions from either BFA-treated or control cells bound totally to lentil lectin-Sepharose, and approx. 11% and 19% of the unretarded fraction from Con A-Sepharose bound to lentil lectin-Sepharose for BFA-treated (Fig. 5a) and control (Fig. 5b) cell glycopeptides respectively.

Sialylation of glycopeptides in BFA-treated cells

In order to examine sialylation of the complex-type glycans, we used chromatography on TCA-Sepharose [11]. TCA shows high affinity for the $\text{Gal}\beta 1 \rightarrow 4\text{GlcNAc}$ sequence of complex-

type glycans and substitution with $\text{NeuNAc}\alpha 2 \rightarrow 6$ or $\text{NeuNAc}\alpha 2 \rightarrow 3$ residues inhibits binding [11]. When the [^3H]fucose-substituted bi-antennary glycopeptide fraction obtained from control cells after Con A-Sepharose and lentil lectin-Sepharose chromatography (Fig. 5b) was applied to TCA-Sepharose, most of the radioactivity was eluted with buffer in a broad retarded peak and the remainder was eluted with 10 mM-lactose (Fig. 5b). By contrast, only one-third of the radioactivity of the fraction from BFA-treated cells was eluted with buffer (Fig. 5a). After desialylation, the bi-antennary fraction from BFA-treated cells (Fig. 5a) or control cells (results not shown) bound more tightly to TCA-Sepharose and was eluted with 100 mM-lactose. Each peak fraction obtained by TCA-Sepharose chromatography (Fig. 5) was subjected to ion-exchange chromatography (Fig. 6). Subfraction 3 emerged from the column before subfraction 2, which in turn was eluted at a lower salt concentration than subfraction 1, consistent with the bi-antennary glycans present in subfractions 1, 2 and 3 having respectively two, one and no sialic acid residues per chain [15], in agreement with the known carbohydrate-binding specificity of TCA-Sepharose [11]. Ion-exchange chromatography of subfractions 1 and 2 from control cells (Fig. 5b) gave results identical with those obtained with glycopeptide fractions 1 and 2 from BFA-treated cells (results not shown). These results indicate that the bi-antennary glycans from BFA-treated cells are only partially sialylated whereas most of these glycans are sialylated in control cells.

DISCUSSION

The present results support other work [3–7] indicating that processing of oligomannosidic-type glycans into endo H-resistant glycans occurs in cells treated with BFA. The kinetic data show that mannosidase processing appears to proceed significantly faster and more extensively than in non-treated cells and is less sensitive to dMM, an inhibitor of Golgi-apparatus mannosidases. Presumably this is due to prolonged exposure of nascent glycoproteins to the dMM-insensitive endoplasmic-reticulum mannosidase, which *in vitro* has been shown to be capable of processing down to $\text{Man}_5\text{GlcNAc}_2$ [18,19]. Interestingly, smaller oligomannosidic structures were not detected in BFA-treated cells, indicating that a novel broad-specificity mannosidase present in BHK cells [12] does not act on these glycans in bulk cellular glycoproteins during their biosynthesis or that this enzyme is not redistributed into the endoplasmic reticulum by BFA.

The main finding of the present study is that BFA appears to induce an enhanced biosynthesis of bi-antennary complex-type and hybrid-type glycans compared with untreated cells. Other work using assays *in vitro* has shown that galactose addition to nascent glycans prevents further action of *N*-acetylglucosaminyltransferases II, III, IV and probably V as well as mannosidase II [1]. It is likely that normally each of the latter enzymes can act before transfer of the nascent glycoproteins into the trans Golgi compartment containing galactosyltransferase. This segregation appears to be removed in BFA-treated cells since immunofluorescence staining of BFA-treated cells with specific galactosyltransferase antibodies has shown that the enzyme is present in a reticulum that also contains mannosidase II, whereas in normal cells these enzymes are segregated in separate Golgi cisternae [3,4]. The locations of *N*-acetylglucosaminyltransferase II and other branching *N*-acetylglucosaminyltransferases have not been defined but presumably are normally also segregated from galactosyltransferase and other late-acting transferases [2]. We propose that as a consequence of the redistribution induced by BFA the assembly of galactosylated hybrid-type glycans and

bi-antennary complex-type glycans in BFA-treated cells is enhanced at the expense of more highly branched structures.

The bi-antennary glycans produced in BFA-treated BHK cells are significantly undersialylated, as shown by TCA-Sepharose and ion-exchange chromatography. The block in sialylation is not complete, unlike other studies showing no detectable sialylation of the *N*-glycans of low-density-lipoprotein receptors [26] or the mannose 6-phosphate receptor [27] expressed in Chinese-hamster ovary cells. These latter results imply that the NeuNAc α 2 $\rightarrow$ 3 sialyltransferase present in the trans Golgi network of Chinese-hamster ovary cells is not equilibrated with endoplasmic-reticulum and Golgi-apparatus enzymes by BFA. However, in murine erythroleukaemia cells BFA can induce the total disorganization of the Golgi apparatus, and sialyl-transferases apparently mix with endoplasmic-reticulum contents, resulting in the appearance of sialylated glycans in the BFA-treated cells [7]. Our finding that the complex-type and hybrid-type glycans in BFA-treated cells carry Fuc α 1 $\rightarrow$ 6 core substituents was less expected, since galactosylation is believed to inhibit the fucosyltransferase in rat liver [1,28,29]. These discrepant findings may be due to differences between BHK cells and rat liver or to a relatively strong fucosyltransferase activity in BHK cells [30] that is able to act before galactosylation occurs even under conditions where there is mixing of the two competing glycosyltransferases.

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REFERENCES

- Schachter, H. (1986) *Biochem. Cell. Biol.* **64**, 163–181
- Roth, J. (1987) *Biochim. Biophys. Acta* **906**, 405–436
- Lippincott-Schwartz, J., Yuan, L., Bonifacino, J. S. & Klausner, R. D. (1989) *Cell* **56**, 801–813
- Lippincott-Schwartz, J., Donaldson, J. G., Schweizer, A., Berger, E. A., Hauri, H.-P., Yuan, L. C. & Klausner, R. D. (1990) *Cell* **60**, 821–836
- Doms, R. W., Russ, G. & Yewdell, J. W. (1989) *J. Cell Biol.* **109**, 61–72
- Fujiwara, T., Oda, K., Yokuta, S., Takatsuki, A. & Ikehara, Y. (1988) *J. Biol. Chem.* **263**, 18545–18552
- Ulmer, J. B. & Palade, G. (1989) *Proc. Natl. Acad. Sci. U.S.A.* **6**, 6992–6996
- Nuchtern, J. G., Bonifacino, J. S., Biddison, W. E. & Klausner, R. D. (1989) *Nature (London)* **339**, 223–226
- Perkel, V. S., Miura, V. & Magner, J. A. (1989) *Proc. Soc. Exp. Biol. Med.* **190**, 286–293
- Perkel, V. S., Liu, A. Y., Miura, Y. & Magner, J. A. (1988) *Endocrinology (Baltimore)* **123**, 310–318
- Sato, S., Animashaun, T. & Hughes, R. C. (1991) *J. Biol. Chem.* **266**, 11485–11494
- Monis, E., Bonay, P. & Hughes, R. C. (1987) *Eur. J. Biochem.* **168**, 287–294
- Animashaun, T. & Hughes, R. C. (1989) *J. Biol. Chem.* **264**, 4657–4663
- Foddy, L. & Hughes, R. C. (1988) *Eur. J. Biochem.* **175**, 291–299
- Hughes, R. C. & Mills, G. (1983) *Biochem. J.* **211**, 575–587
- Merkle, R. K. & Cummings, R. D. (1987) *Methods Enzymol.* **138**, 232–259
- Fuhrmann, U., Bause, E., Langer, G. & Ploegh, H. (1984) *Nature (London)* **307**, 755–758
- Bischoff, J., Liscum, L. & Kornfeld, R. (1986) *J. Biol. Chem.* **261**, 4766–4774
- Bischoff, J. & Kornfeld, R. (1986) *J. Biol. Chem.* **261**, 4758–4765
- Hughes, R. C., Foddy, L. & Bause, E. (1987) *Biochem. J.* **247**, 537–545
- Narasimhan, S., Allen, S., Hughes, R. C. & Schachter, H. (1984) *Glycoconjugate J.* **1**, 51–61
- Foddy, L., Feeney, J. & Hughes, R. C. (1986) *Biochem. J.* **233**, 697–706
- Hughes, R. C. & Feeney, J. (1986) *Eur. J. Biochem.* **158**, 227–237
- Hughes, R. C. & Mills, G. (1985) *Biochem. J.* **226**, 487–498
- Gleeson, P. A., Feeney, J. & Hughes, R. C. (1985) *Biochemistry* **24**, 493–503
- Shite, S., Seguchi, T., Mizoguchi, H., Ono, M. & Kuwano, M. (1990) *J. Biol. Chem.* **265**, 17385–17388
- Chege, N. W. & Pfeffer, S. R. (1990) *J. Cell Biol.* **111**, 893–898
- Munro, J. R., Narashinham, S., Wetmore, S., Riordan, J. R. & Schachter, H. (1975) *Arch. Biochem. Biophys.* **164**, 269–277
- Longmore, G. D. & Schachter, H. (1982) *Carbohydr. Res.* **100**, 365–392
- Vischer, P. & Hughes, R. C. (1981) *Eur. J. Biochem.* **117**, 275–284

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