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Disaccharide uptake and priming in animal cells: Inhibition of sialyl Lewis X by acetylated Galβ1→4GlcNAcβ-*O*-naphthalenemethanol

(glycosides/glycosaminoglycans/glycoproteins/oligosaccharides/inhibitors)

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ABSTRACT **Inhibitors of glycosylation provide a tool for studying the biology of glycoconjugates. One class of inhibitors consists of glycosides that block glycoconjugate synthesis by acting as primers of free oligosaccharide chains. A typical primer contains one sugar linked to a hydrophobic aglycone. In this report, we describe a way to use disaccharides as primers. Chinese hamster ovary cells readily take up glycosides containing a pentose linked to naphthol, but they take up hexosides less efficiently and disaccharides not at all. Linking phenanthrol to a hexose improves its uptake dramatically but has no effect on disaccharides. To circumvent this problem, analogs of Xylβ1→6Galβ-*O*-2-naphthol were tested as primers of glycosaminoglycan chains. The unmodified disaccharide did not prime, but methylated derivatives had activity in the order Xylβ1→6Gal(Me)$_3$-β-*O*-2-naphthol > Xylβ1→6Gal(Me)$_2$β-*O*-2-naphthol >> Xylβ1→6Gal(Me)β-*O*-2-naphthol. Acetylated Xylβ1→6Galβ-*O*-2-naphthol also primed glycosaminoglycans efficiently, suggesting that the terminal xylose residue was exposed by removing the acetyl groups. The general utility of using acetyl groups to create disaccharide primers was shown by the priming of oligosaccharides on peracetylated Galβ1→4GlcNAcβ-*O*-naphthalenemethanol. This disaccharide inhibited sialyl Lewis X expression on HL-60 cells.**

Glycosylation inhibitors provide a powerful way to study the biological function of glycoconjugates in animal cells. Plant alkaloids such as swainsonine and deoxynojiromycin derivatives block glycoprotein biosynthesis *in vivo* by inhibiting glycosidases involved in the maturation of asparagine-linked oligosaccharides (1, 2). Recently, an exocyclic epoxide derivative of glucosylceramide was shown to inhibit glycosphingolipid biosynthesis in cells (3), presumably by making a covalent adduct to a galactosyltransferase that acts on the ceramide intermediate. A number of other substrate-based inhibitors have been described that block glycosyltransferases *in vitro* (4–6), but poor uptake prevents them from inhibiting glycosylation *in vivo*.

Another class of inhibitors consists of glycosides that resemble biosynthetic intermediates involved in glycoconjugate assembly. These compounds act as substrates and produce free oligosaccharides, diverting the assembly of chains from glycoconjugates to the added acceptors. The first type of inhibitor in this class was described >20 years ago by Okayama *et al.* (7). They showed that β-D-xylosides stimulate the synthesis of free glycosaminoglycan (GAG) chains and competitively inhibit GAG formation on proteoglycan core proteins (7). The free GAG chains can have desirable biological properties as well. For example, heparan sulfate chains produced on Xylβ-*O*-2-naphthol (naphthol-β-D-xyloside)† will bind to basic fibroblast growth factor, facilitating its interaction with high-affinity receptors (8, 9). Recent studies have shown that β-D-xylosides will prime ganglioside GM3-like compounds and partially inhibit glycolipid biosynthesis (10). In a similar way, GalNAcα-*O*-benzyl stimulates mucin oligosaccharide synthesis and inhibits O-linked glycoprotein synthesis (11, 12). Altering glycoprotein synthesis in HL-60 cells in this way inhibits the expression of sialyl Lewis X [sLe^x; NeuAcα2→3Galβ1→4(Fucα1→3)GlcNAc-] ligands and adhesion to activated endothelial cells (13).

Acceptors consisting of two or more sugars would make this strategy more useful and selective since many glycosyltransferases prefer disaccharides or larger oligosaccharides as substrates (4–6). However, poor transfer of disaccharides across cell membranes severely limits this approach. In this report, we show that decreasing the number of free hydroxyl groups to ≤5 solves the uptake problem for disaccharides linked to 2-naphthol. Acetylation of the sugars also allows disaccharides to enter the Golgi and prime oligosaccharide chains.

MATERIALS AND METHODS

Synthesis of Glycosides. The syntheses of Xylβ-*O*-2-naphthol and L-Araα-*O*-2-naphthol have been described (8). Galβ-*O*-9-phenanthrol, Galβ1→3Galβ-*O*-9-phenanthrol, Galβ1→3Galβ-*O*-2-naphthol, and Galβ1→4Xylβ-*O*-2-naphthol were prepared by reacting the bromo sugar with the sodium salt of the alcohol (A.K.S. and J.D.E., unpublished results). Xylβ1→6Galβ-*O*-2-naphthol was obtained by reacting acetobromoxylose with Galβ-*O*-2-naphthol (Sigma) in the presence of silver carbonate (8). The disaccharide intermediate Xyl(Ac)$_3$β1→6Galβ-*O*-naphthol was partially methylated by reaction with trimethyloxonium tetrafluoroborate in the presence of 2,6-di(*tert*-butyl)trimethyl pyridine and the acetyl groups were subsequently removed with sodium methoxide (A.K.S. and J.D.E., unpublished results). Galβ1→4GlcNAcβ-*O*-naphthalenemethanol was made by coupling acetylated Galβ-S-C_2H_5 and 3,6-di-*O*-benzoylGlcNAcβ-*O*-naphthalenemethanol followed by deacetylation with sodium methoxide (15). Acetylation of sugars was performed with acetic anhydride in pyridine. All compounds were purified by silicic acid chromatography and their structures were confirmed by both ^{1}H NMR and ^{13}C NMR. They were ≥95% pure by NMR and by sulfuric acid charring on thin-layer plates.

Cell Culture. Chinese hamster ovary cells (CHO-K1, CCL 61), mouse embryonal carcinoma cells (F9, CRL 1720), and human promyelocytic leukemia cells (HL-60, CCL 240) were

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Abbreviations: GAG, glycosaminoglycan; NDV, Newcastle disease virus; sLe^x, sialyl Lewis X; mAb, monoclonal antibody.

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†All glycosides are written as "sugar-linkage-aglycone" to draw attention to the exposed sugar on the nonreducing terminus. Unless otherwise noted, all sugars have the D-configuration and are in the pyranose form.

obtained from the American Type Culture Collection. Wild-type CHO cells and the xylosyltransferase-deficient mutant pgsA-745 were maintained in Ham's F-12 medium containing 7.5% (vol/vol) fetal bovine serum (HyClone), 100 units of penicillin per ml, and 100 μg of streptomycin sulfate per ml as described (16). F9 cells were grown in DMEM containing 12.5% fetal bovine serum and antibiotics on plates coated with a solution of 0.1% (wt/vol) gelatin as described (17). HL-60 cells were maintained in suspension in RPMI 1640 medium (18).

Uptake Studies. Compounds were dissolved in dimethyl sulfoxide and added to growth medium so that the final concentration of vehicle was 0.5%. Confluent cells were incubated with supplemented growth medium at 37°C for the times indicated in the figure and table legends. The cultures were placed on ice, the medium was aspirated, and the cells were washed three times with cold PBS (19) containing 1 mg of bovine serum albumin per ml. Cells were solubilized in a small volume of 0.1 M NaOH, and the extracts were neutralized with 1 mol equivalent of acetic acid. After the samples were centrifuged, the supernatants were applied to Sep-Pak Plus C_{18} cartridges (Waters) and washed with water (10 ml) and 20% methanol in water (5 ml). Bound material was eluted with 5 ml of 40% methanol in water and concentrated to dryness. The residue was dissolved in methanol and its fluorescence was measured (SLM-Aminco, Urbana, IL). The excitation and emission wavelengths for 2-naphthol derivatives were 293 and 355 nm, respectively. Phenanthrol derivatives were excited at 310 nm and the emission was measured at 365 nm. The fluorescence for 9-phenanthrol derivatives was corrected for a 2.6-fold greater intensity compared to comparable naphthol derivatives.

Uptake of Galβ1→4GlcNAcβ-*O*-2-naphthalenemethanol derivatives was measured in the same way except that cells were scraped from the plate and protein and nucleic acid were precipitated with 10% (wt/vol) trichloroacetic acid in the cold. The sample was centrifuged and the supernatant was applied to a Sep-Pak C_{18} cartridge. The cartridge was washed with water (10 ml), 5% methanol in water (5 ml), and, finally, 60% methanol in water (5 ml). The latter was concentrated to dryness and the residue was dissolved in methanol for measuring its fluorescence.

Priming of Oligosaccharides. Priming of GAG chains was measured in pgsA-745 cells deficient in xylosyltransferase (16). The cells were grown to confluence in 96-well microtiter plates (Falcon). Fresh medium (0.2 ml) containing 50 μCi of $H_2{}^{35}SO_4$ per ml (25–40 Ci/mg; 1 Ci = 37 GBq; Amersham) and the test compounds were added to the wells. The top row of wells contained 500 μM glycoside and each subsequent row contained a serial dilution (1:3^n; vol/vol). Cells were incubated for 5 h at 37°C and solubilized by adding 20 μl of 0.5 M NaOH to the medium. The contents of each well were adjusted to 0.2% Zwittergent 3-12, 25 μg of chondroitin sulfate per ml, 0.2 M acetic acid, and 10 mM Na_2SO_4. The samples were transferred to a GeneScreen*Plus* membrane (DuPont/NEN) placed on top of a piece of 1Chr paper (Whatman) on a vacuum Minifold (Schleicher & Schuell). A wash solution of 0.2% Zwittergent 3-12, 0.2 M acetic acid, and 10 mM Na_2SO_4 (0.5 ml) was passed through each well. The membranes were removed from the Minifold, soaked for 5 min in 20 ml of wash solution, air-dried, and exposed to RX x-ray film (Fuji) for 10–18 h.

Priming of oligosaccharides on Galβ1→4GlcNAcβ-*O*-2-naphthalenemethanol was measured in F9 cells grown on gelatin-coated six-well plates (17). The medium was replaced with low-glucose (4.5 g/liter) DMEM containing 15% fetal bovine serum, antibiotics, test compounds, and 10 μCi of [6-^{3}H]glucosamine HCl per ml (33.3 Ci/mmol; DuPont/NEN). After 24 h at 37°C, the plate was chilled on ice, the medium was separated from the cells, and the monolayer was washed with buffer (2 ml) containing 140 mM NaCl, 4 mM KCl, and 20 mM Hepes (pH 7.2). The medium and wash solution were centrifuged to remove cell debris and the supernatant was applied to a Sep-Pak C_{18} cartridge. The cartridge was washed with 0.5 M NaCl (10 ml) in water (50 ml) and then with 40% methanol in water (5 ml). The latter fraction was dried, dissolved in water, and counted by liquid scintillation spectrometry. A portion of the material was analyzed by anion-exchange chromatography using QAE-Sephadex (20) before and after treating with Newcastle disease virus (NDV) α2→3 sialidase or *Clostridium perfringens* α2→3/α2→6 sialidase (ref. 21; Oxford Glycosystems, Abingdon, U.K.). Another portion was analyzed by gel filtration chromatography using P2 resin (Bio-Rad).

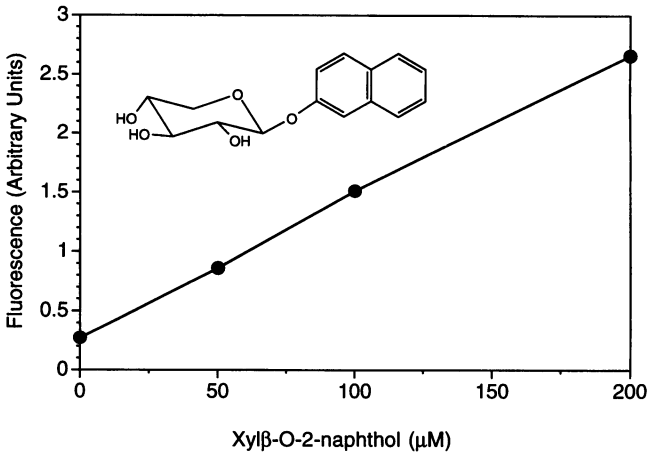

FIG. 1. Uptake of Xylβ-*O*-2-naphthol by CHO cells occurs by diffusion. Confluent monolayers of CHO cells were incubated with the indicated concentration of Xylβ-*O*-2-naphthol for 30 min at 37°C. The amount of glycoside taken up was determined by fluorescence assay.

Cell Sorting. HL-60 cells were incubated with or without 200 μM peracetylated Galβ1→4GlcNAcβ-*O*-2-naphthalenemethanol for 40 h in RPMI 1640 medium supplemented with 10% fetal bovine serum. Cells were centrifuged, washed with PBS containing 2% serum and 0.5% sodium azide, and then incubated for 30 min at 4°C with anti-sLe^x monoclonal antibody (mAb) CSLEX-1 (ref. 22; Becton Dickinson). The cells were washed twice and then incubated with a fluorescein isothiocyanate-conjugated goat anti-mouse IgM for 30 min at 4°C. Cells were washed again, resuspended in PBS containing 1% paraformaldehyde, and analyzed by cell sorting (FACStar; Becton Dickinson). Human IgG (Sigma) was present in all incubations (2 mg/ml). A mouse nonspecific IgM (Sigma) was used as a control antibody. In one experiment, the cells were treated with NDV sialidase (10 milliunits; 1 h; 37°C) in 50 mM sodium acetate buffer (pH 5.5) before reaction of the cells with CSLEX-1.

RESULTS

Animal cells take up β-D-xylosides and prime GAG chains with great efficiency (7–9). For priming to occur, the glycoside must pass through the plasma membrane and enter the Golgi, where the glycosyltransferases reside. To study this process in more detail, we measured the uptake of Xylβ-*O*-2-naphthol in CHO cells by a fluorescence assay. Uptake depended on concentration and did not saturate, suggesting that it was limited by diffusion (Fig. 1).‡ Uptake also occurred rapidly and the amount associated with cells reached a plateau in <1 min (Fig. 2). In contrast to the behavior of Xylβ-*O*-2-naphthol, the uptake of Galβ-*O*-2-naphthol was slow and reached a lower

‡In contrast to uptake, priming of GAG chains saturates at ≈10 M, suggesting that a step in chain formation limits priming.

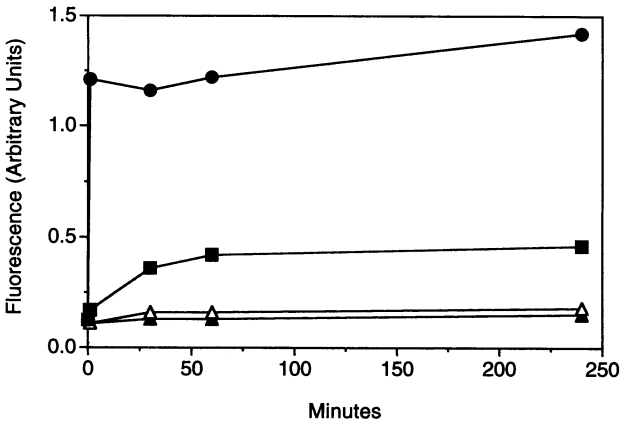

FIG. 2. Uptake of glycosides by CHO cells varies with the size of the glycan. The various glycosides were added (100 μM) to confluent CHO cell monolayers and at the times indicated the amount of glycoside taken up was quantitated by fluorescence assay. ●, Xylβ-*O*-2-naphthol; ■, Galβ-*O*-2-naphthol; △, Galβ1→4Xylβ-*O*-2-naphthol; ▲, Galβ1→3Galβ-*O*-2-naphthol.

steady-state level. Xylβ-*O*-2-naphthol has three hydroxyl the rate and extent of uptake. Disaccharides such as Galβ1→4Xylβ-*O*-2-naphthol (six hydroxyls) and Galβ1→3Galβ-*O*-2-naphthol (seven hydroxyls) were not taken up at all (Fig. 2).

Experiments with other glycosides showed that L-Araα-*O*-2-naphthol was taken up nearly as well as Xylβ-*O*-2-naphthol (0.8 vs. 1.0 unit of fluorescence). Changing the aglycone from 2-naphthol to 9-phenanthrol stimulated the uptake of galactoside (from 0.3 to 4.2 units) to a level greater than observed for Xylβ-*O*-2-naphthol (1.0 unit). However, Galβ1→3Galβ-*O*-2-phenanthrol was not taken up (<0.1 unit). Thus, the addition of the third aromatic ring to the aglycone was sufficient to overcome the extra hydroxyl group in a hexoside (four vs. three hydroxyls) but not the multiple hydroxyl groups in a disaccharide (seven hydroxyls).

To determine the maximum number of hydroxyl groups that a naphthol glycoside can have, we synthesized the disaccharide Xylβ1→6Galβ-*O*-2-naphthol (six hydroxyls). Since β-D-xylosides prime GAG chains regardless of the aglycone (8), we reasoned that the disaccharide would behave similarly if the galactose residue was treated as part of the aglycone. Therefore, uptake of the disaccharide was measured indirectly by determining the incorporation of $^{35}SO_4$ into GAG chains. To distinguish GAGs produced on the glycoside from those made on endogenous proteoglycans, we used a CHO cell mutant deficient in xylosyltransferase (16). This enzymatic deficiency blocks GAG synthesis on endogenous core proteins but does not prevent chain synthesis on synthetic β-D-xylosides (8). [^{35}S]GAG synthesis was measured by incubating mutant cells in a 96-well plate with glycoside derivatives at different doses. The cells and spent media were then transferred to a positively charged nylon membrane to detect newly made chains by autoradiography.

The disaccharide (Xylβ1→6Galβ-*O*-2-naphthol; six hydroxyls) did not prime [^{35}S]GAGs even at 500 μM, the highest concentration tested (Fig. 3, lane 2). In contrast, Xylβ-*O*-2-naphthol primed chains efficiently (lane 1). Next, a series of methylated derivatives of the disaccharide was synthesized in which the internal Gal residue contained one, two, or three methyl groups attached randomly to the 2-OH, 3-OH, or 4-OH position. Blocking one of the hydroxyls with a methyl group [Xylβ1→6Gal(Me)β-*O*-2-naphthol; five hydroxyls] stimulated [^{35}S]GAG synthesis, with a maximum effect achieved at ≈60 μM (lane 3). Higher concentrations were less effective possibly because of the detergent properties of the glycoside. The dimethylated derivative [Xylβ1→6Gal(Me)$_2$β-*O*-2-naphthol;

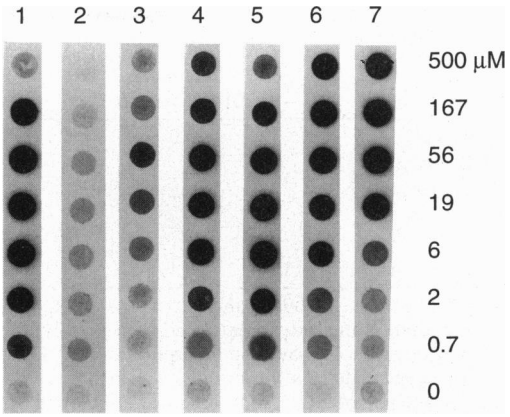

FIG. 3. Priming of GAGs by acetylated glycosides. Various compounds at the indicated concentrations were provided to confluent CHO pgsA-745 cells in a 96-well plate. GAG synthesis was measured by autoradiography of [^{35}S]GAG collected on a positively charged nylon membrane. Lanes: 1, Xylβ-*O*-2-naphthol; 2, Xylβ1→6Gal-*O*-2-naphthol; 3, Xylβ1→6Gal(Me)-*O*-2-naphthol; 4, Xylβ1→6Gal(Me)$_2$-*O*-2-naphthol; 5, Xylβ1→6Gal(Me)$_3$-*O*-2-naphthol; 6, Xyl(Ac)$_3$β-*O*-2-naphthol; 7, Xyl(Ac)$_3$β1→6Gal(Ac)$_3$-*O*-2-naphthol.

four hydroxyls] primed at a much lower concentration (2–6 μM; lane 4). The trimethylated derivative [Xylβ1→6Gal(Me)$_3$β-*O*-2-naphthol; three hydroxyls] primed at <1 μM (lane 5), nearly as well as Xylβ-*O*-2-naphthol (three hydroxyls; lane 1). Thus, five or more free hydroxyl groups limit uptake and utilization of naphthol disaccharides under these conditions.

Acetylation provides another way to block hydroxyl groups and renders sugars more hydrophobic. As shown in Fig. 3, Xyl(Ac)$_3$β-*O*-2-naphthol primed GAGs in CHO cells, although somewhat less effectively than the nonacetylated compound (compare lanes 1 and 6). An earlier study showed that acetylated *N*-xylosides will prime GAG chains in cells as well (23). Similarly, peracetylated Xylβ1→6Galβ-*O*-2-naphthol primed GAG chains, even at a concentration as low as ≈6 μM (lane 7).

We also tested whether acetylation of Galβ1→4GlcNAcβ-*O*-2-naphthalenemethanol would result in priming of oligosaccharides in F9 embryonal carcinoma cells. These cells make polylactosaminoglycan chains and sLe^x antigens that contain the type II core Galβ1→4GlcNAc unit (24, 25). Uptake of the unmodified disaccharide was very limited as expected (six hydroxyls), whereas uptake of the peracetylated derivative was nearly as efficient as Xylβ-*O*-naphthol (Table 1). Priming was measured by incubating cells with the disaccharides and [^{3}H]GlcN, a precursor of amino sugars (GlcNAc, GalNAc, and NeuAc). A large amount of labeled material was generated on the peracetylated derivative (Table 1) in comparison to the nonacetylated disaccharide. Separating the material by anion-exchange chromatography (20) showed that ≈25% of the ^{3}H oligosaccharides were charged. About 50% of this material was sensitive to NDV α2→3 sialidase and ≈70% was sensitive to *C. perfringens* α2→3/α2→6 sialidase, indicating that the material contained mostly α2→3-linked sialic acid and a small amount of α2→6-linked sialic acid (21). Gel filtration chromatography showed that ≈35% of the charged material eluted in the void volume of a P2 column, suggesting that more than one sugar had been added to some of the primer as well.

Priming of charged oligosaccharides on acetylated Galβ1→4GlcNAcβ-*O*-2-naphthalenemethanol suggested that it might inhibit expression of sLe^x [NeuAcα2→3Galβ1→4(Fucα1→3)GlcNAc-] on membrane glycoconjugates. To test this possibility, we incubated HL-60 cells in the presence of 200 μM acetylated disaccharide and the expression of sLe^x on the cell surface was measured by cell sorting with mAb CSLEX-1

Table 1. Uptake and priming of Galβ1→4GlcNAcβ-*O*-naphthalenemethanol and its acetylated derivative in F9 cells

Compound	Uptake, arbitrary units	Priming, ^{3}H cpm/μg
Xylβ-*O*-naphthol	9.0	ND
Galβ1→4GlcNAcβ-*O*-naphthalenemethanol	0.5	20
Acetylated Galβ1→4GlcNAcβ-*O*-naphthalenemethanol	6.4	250

The uptake of Xylβ-*O*-2-naphthol, Galβ1→4GlcNAcβ-*O*-naphthalenemethanol, and the peracetylated derivative by F9 cells was measured by fluorescence assay. The incorporation of [^{3}H]GlcN into oligosaccharides generated on the primers was measured by absorption to Sep-Pak C_{18} cartridges. Control incubations without added glycoside yielded fluorescence values of 1.0 and 30 ^{3}H cpm per μg of cell protein. These values were subtracted from the data obtained for cells treated with compounds. ND, not determined.

(22) and fluorescein isothiocyanate-labeled goat anti-mouse IgM. About 60% of untreated HL-60 cells exhibited strong expression of sLe^x (fluorescence, $>10^3$) compared to cells treated with a nonspecific IgM (compare Fig. 4 *A* and *B*). Treating the cells with NDV sialidase shifted the entire population to lower fluorescence (Fig. 4*C*). The density of cells after 2 days of incubation with acetylated disaccharide was reduced ≈40% compared to untreated cells, suggesting that a minor decrease in growth rate had occurred. About 95% of the cells were viable by exclusion of trypan blue. Cells grown with acetylated Galβ1→4GlcNAcβ-*O*-2-naphthalenemethanol shifted to a lower mean fluorescence (Fig. 4*D*), suggesting that the disaccharide inhibited sLe^x formation on the cell surface.

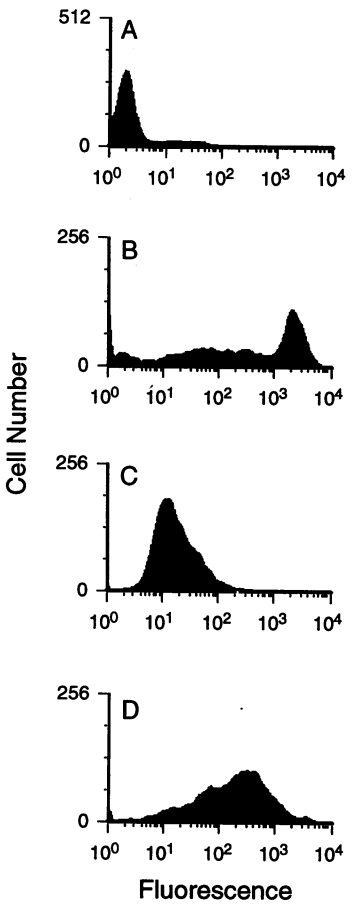

FIG. 4. Cell sorting of HL-60 cells reacted with mAb CSLEX-1. HL-60 cells were grown in the presence or absence of 200 μM acetylated Galβ1→4GlcNAcβ-*O*-naphthalenemethanol for 40 h. They were then treated with CSLEX-1 and subjected to cell sorting as described. (*A*) Cells were treated with a nonspecific mouse IgM. (*B*) Cells were treated with mAb CSLEX-1. (*C*) Cells were treated with NDV α2→3 sialidase before reaction with CSLEX-1. (*D*) Cells were grown in medium containing 200 μM acetylated Galβ1→4GlcNAcβ-*O*-naphthalenemethanol before reaction with mAb CSLEX-1.

DISCUSSION

Over 20 years ago, Okayama *et al.* (7) showed that cells will take up β-D-xylosides and prime GAG chains. These compounds are now widely used to study GAG biosynthesis and to inhibit proteoglycan assembly. At high concentrations, they also inhibit glycolipid biosynthesis (10). In a similar way, α-*N*-acetylgalactosaminides will prime mucin-like oligosaccharides and alter the assembly of O-linked oligosaccharides on glycoproteins (11–13). The studies reported here show that uptake of these compounds occurs by diffusion. Their ability to produce oligosaccharide chains implies that the compounds diffuse across the plasma membrane and into the endoplasmic reticulum/Golgi network. They may diffuse into all cell compartments equally well, or some preferential sorting may occur if the glycosides flow along pathways used by lipids (26).

More complex saccharides are not taken up and the experimental evidence suggests that the number of hydroxyl groups in the glycan determines the rate and extent of uptake. Thus, pentosides (L-Araα-*O*-2-naphthol and Xylβ-*O*-2-naphthol) diffuse more readily than hexosides (Galβ-*O*-naphthol) containing the same aglycone, showing that only one additional hydroxyl group poses a major obstacle to uptake. Presumably this difference reflects the difficulty of passing the polar hydroxyl groups through the interior of a membrane, which resembles a low dielectric solvent (27). The multiple hydroxyl groups present in a disaccharide and larger oligosaccharides provide an even larger energy barrier to surpass.

A strong correlation exists between membrane permeability and partitioning of solutes into an organic solvent (27). Thus, increasing the hydrophobicity of the aglycone should increase uptake. Although this was true for galactose, attaching phenanthrol to a disaccharide did not improve its uptake. Increasing the size and hydrophobicity of the aglycone further might enhance uptake, but strongly amphipathic compounds act like detergents. Thus, pyrene derivatives (T.A.F. and J.D.E., unpublished results) (four fused aromatic rings) and glycosides containing alkyl chains of ≥8 carbons cause cell lysis (8).

To circumvent this problem, we tested whether modifying the sugar residues would facilitate uptake. Acylation has been used to improve bioavailability of drugs (e.g., acetylsalicylic acid), second messengers (e.g., dibutyryl cAMP), and glycosidase inhibitors (e.g., carbonoyloxy analog of swainsonine) (28–30). Cells have numerous esterases that can remove the acyl groups and convert these compounds into their active forms. Our studies support the idea that one or more of these enzymes reside in the lumen of the endoplasmic reticulum/Golgi network (31) since the disaccharides presumably had to retain the acetyl groups for entry into this compartment. Priming of oligosaccharide chains on Galβ1→4GlcNAcβ-*O*-naphthalenemethanol increases with time and does not saturate for several hours (A.K.S. and J.D.E., unpublished results). Thus, the rates of both diffusion and deacetylation will affect the efficiency of priming. The affinity of the disaccharide substrate for its target glycosyltransferase also plays a role (4–6).

The usefulness of acetylated disaccharides was shown by the inhibition of sLe^x expression on the surface of HL-60 cells by acetylated Galβ1→4GlcNAcβ-*O*-naphthalenemethanol. Inhibition probably occurred by blocking the formation of sLe^x on O-linked oligosaccharides of glycoproteins (13). The decrease

in sLe^x expression has a corresponding effect on cell adhesion mediated through sLe^x–selectin interactions (ref. 32; A.K.S. and J.D.E., unpublished results).

Other disaccharides may prove useful, such as primers that resemble other parts of asparagine-linked and O-linked oligosaccharides of glycoproteins, glycosaminoglycan chains of proteoglycans, and the oligosaccharides of glycolipids. Larger oligosaccharides have not yet been modified and tested as primers. These compounds would be especially useful since larger oligosaccharides are recognized more specifically by glycosyltransferases. Analogs of acetylated disaccharides attached to an appropriate aglycone also may provide a new source of inhibitors for studying the biology of glycoconjugates (14).

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1. Elbein, A. D. (1991) *FASEB J.* **5,** 3055–3063.
2. Winchester, B. & Fleet, G. W. (1992) *Glycobiology* **2,** 199–210.
3. Zacharias, C., van Echten-Deckert, G., Plewe, M., Schmidt, R. R. & Sandhoff, K. (1994) *J. Biol. Chem.* **269,** 13313–13317.
4. Hindsgaul, O., Kaur, K. J., Srivastava, G., Blaszczyk-Thurin, M., Crawley, S. C., Heerze, L. D. & Palcic, M. M. (1991) *J. Biol. Chem.* **266,** 17858–17862.
5. Khan, S. H., Crawley, S. C., Kanie, O. & Hindsgaul, O. (1993) *J. Biol. Chem.* **268,** 2468–2473.
6. Lowary, T. L. & Hindsgaul, O. (1994) *Carbohydr. Res.* **251,** 33–67.
7. Okayama, M., Kimata, K. & Suzuki, K. (1973) *Biochem. J. (Tokyo)* **74,** 1069–1073.
8. Fritz, T. A., Lugemwa, F. N., Sarkar, A. K. & Esko, J. D. (1994) *J. Biol. Chem.* **269,** 300–307.
9. Hua-Quan, M., Fritz, T. A., Esko, J. D., Yayon, A. & Vlodavsky, I. (1995) *J. Cell. Biochem.* **57,** 173–184.
10. Freeze, H. H., Sampath, D. & Varki, A. (1993) *J. Biol. Chem.* **268,** 1618–1627.
11. Kuan, S.-F., Byrd, J. C., Babaum, C. & Kim, Y. S. (1989) *J. Biol. Chem.* **264,** 19271–19277.
12. Zhuang, D., Grey, A., Harris-Brandts, M., Higgins, E., Kashem, M. A. & Dennis, J. W. (1991) *Glycobiology* **1,** 425–433.
13. Kojima, N., Handa, K., Newman, W. & Hakomori, S. (1992) *Biochem. Biophys. Res. Commun.* **182,** 1288–1295.
14. Varki, A. (1993) *Glycobiology* **2,** 97–130.
15. Kihlberg, J. O., Leigh, D. A. & Bundle, D. R. (1990) *J. Org. Chem.* **55,** 2860–2863.
16. Esko, J. D., Stewart, T. E. & Taylor, W. H. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 3197–3201.
17. Strickland, S. & Mahdavi, V. (1978) *Cell* **15,** 393–403.
18. Collins, S. J., Gallo, R. C. & Gallagher, R. E. (1977) *Nature (London)* **270,** 347–349.
19. Dulbecco, R. & Vogt, M. (1954) *J. Exp. Med.* **99,** 167–182.
20. Roux, L., Holojda, S., Sundblad, G., Freeze, H. H. & Varki, A. (1988) *J. Biol. Chem.* **263,** 8879–8889.
21. Paulson, J. C., Weinstein, J., Dorland, L., Van Halbeek, H. & Vliegenthart, J. F. G. (1982) *J. Biol. Chem.* **257,** 12734–12738.
22. Fukushima, K., Hirota, M., Terasaki, P. I., Wakisaka, A., Togashi, H., Chia, D., Suyama, N., Fukushi, Y., Nudelman, E. & Hakomori, S. (1984) *Cancer Res.* **44,** 5279–5285.
23. Wang, L., Maniglia, C. A., Mella, S. L. & Sartorelli, A. C. (1983) *J. Med. Chem.* **26,** 629–632.
24. Muramatsu, T., Gachelin, G., Damonneville, M., Delarbre, C. & Jacob, F. (1979) *Cell* **18,** 183–191.
25. Heffernan, M., Lotan, R., Amos, B., Palcic, M., Takano, R. & Dennis, J. W. (1993) *J. Biol. Chem.* **268,** 1242–1251.
26. Pagano, R. E. (1990) *Curr. Opin. Cell Biol.* **2,** 652–663.
27. Gennis, R. B. (1989) *Biomembranes, Molecular Structure and Function* (Springer, New York), pp. 235–269.
28. Silverman, R. B. (1992) *The Organic Chemistry of Drug Design and Drug Action* (Academic, New York), pp. 352–422.
29. Bundgaard, H. (1985) *Design of Prodrugs* (Elsevier, Amsterdam).
30. Dennis, J. W., White, S. L., Freer, A. M. & Dime, D. (1993) *Biochem. Pharmacol.* **46,** 1459–1466.
31. Harano, T., Miyata, T., Lee, S., Aoyagi, H. & Omura, T. (1988) *J. Biochem. (Tokyo)* **103,** 149–155.
32. Rosen, S. D. & Bertozzi, C. R. (1994) *Curr. Opin. Cell Biol.* **6,** 663–673.