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Properties of a β-*N*-Acetylgalactosaminyl Transferase and Its Mobilization from an Endogenous Pool to the Cell Surface of Embryonic Chick Neural Retina Cells†

Janne Balsamo and Jack Lilien*

ABSTRACT: We have analyzed the incorporation of [^{14}C]-GalNAc from UDP-[^{14}C]GalNAc among whole cells and homogenates of embryonic chick neural retinas. Single cells prepared by trypsinization incorporate [^{14}C]GalNAc from UDP-[^{14}C]GalNAc into a product released from the intact cells into the reaction medium. Little or no incorporation into cell-associated products is observed. The possibility of misleading results due to sugar–nucleotide breakdown or cell lysis has been excluded. The reaction is dependent on Mn^{2+} and is stimulated by Ca^{2+}. Colchicine added at zero time completely blocks incorporation. Cells which have been "repaired" in culture in the presence of cycloheximide also incorporate [^{14}C]GalNAc from UDP-[^{14}C]GalNAc into a cell-free product, but the reaction is neither stimulated by Ca^{2+} nor blocked by colchicine. Incorporation of [^{14}C]GalNAc from UDP-[^{14}C]GalNAc in whole tissue homogenates prepared in 1% Triton X-100 is linear for at least 1 h and is unaffected by cycloheximide, Ca^{2+}, or colchicine. Homogenates of trypsin-dispersed cells show a 30% reduction in activity when compared to homogenates of whole tissues. Transferase activity is further reduced to ~10% when single cells are allowed to repair in cycloheximide and then are retrypsinized and assayed as homogenates. The product of the reaction in both homogenates and intact cells is a high molecular weight glycoprotein containing terminal GalNAc residues. The results suggest that a β-*N*-acetylgalactosaminyl transferase is present in both a cell surface associated and an endogenous form. Following tissue trypsinization, the surface-associated activity is destroyed. The endogenous complement of the enzyme acceptor is then mobilized to the cell surface by a colchicine-sensitive process where transfer of [^{14}C]GalNAc occurs followed by release of the glycosylated acceptor into the reaction medium.

The presence of glycosyl transferases at the cell surface has been indicated in a variety of different systems [see Shur & Roth (1975) for a review]; however, it is still a matter of controversy whether these enzymes play a specific role at the surface or represent the passive result of fusion of Golgi vesicles with the plasma membrane [see Cook (1977)]. Cell surface glycosyl transferases have been implicated in such physiological roles as homeostasis (Jamieson, 1978; Bosman, 1972), biosynthesis of complex polysaccharides (Pat & Grimes, 1975), and cell–cell adhesion [see Shur & Roth (1975) for a review].

Previous work in this laboratory (McDonough & Lilien, 1978) has provided evidence that a β-*N*-acetylgalactosaminyl transferase is involved in the turnover of a tissue-specific ligand from the surface of embryonic chick neural retina cells. Release of the ligand into the extracellular fluid is stimulated by Mn^{2+} and inhibited by EDTA,[1] UDP (but not other nucleotides), hydroxyurea, and cytosine arabinoside. Soluble ligand preparations treated with β-*N*-acetylhexosaminidase, which liberates GalNAc and inactivates ligand (McDonough & Lilien, 1975, 1978), are reactivated upon incubation with intact cells. Like release, reactivation is Mn^{2+} dependent and is inhibited by EDTA and UDP. It was suggested that ligand exists as an endogenous pool; treatment of the cell surface with trypsin triggers the mobilization of this pool to the cell surface, where release of the ligand molecule into the medium is accomplished via a glycosylation reaction.

In this paper we demonstrate biochemically the existence of a β-*N*-acetylgalactosaminyl transferase active at the surface of embryonic chick neural retina cells and identify the products

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[1] Abbreviations used: GalNAc, *N*-acetyl-D-galactosamine; EDTA, ethylenediaminetetraacetic acid (tetrasodium salt); GalNAc-P, *N*-acetyl-D-galactosamine 1-phosphate; LDH, lactic dehydrogenase; PMSF, phenylmethanesulfonyl fluoride; Gal, galactose; $GalNH_2$, galactosamine; GluNAc, *N*-acetylglucosamine; PTA, phosphotungstic acid; Cl_3AcOH, trichloroacetic acid; BSA, bovine serum albumin; DNase, deoxyribonuclease.

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of the glycosylation reaction. In addition, we analyze the dynamics of the appearance of acceptor–enzyme at the cell surface following tissue trypsinization.

Materials and Methods

Cell Preparation and Repair. Trypsin-dispersed 10-day-old embryonic chick neural retina cells were prepared as described previously (McDonough et al., 1977). After incubation in trypsin, the tissues were washed 3 times in cold Tyrode's solution containing 2 mM phenylmethanesulfonyl fluoride (PMSF) and 50 μg/mL DNase. The cells were pelleted by centrifugation in the cold at 500*g* and resuspended in cold Tris-buffered NaCl, pH 7.2 (50 mM Tris-HCl and 150 mM NaCl), and the cell concentration was determined by using a particle counter (Coulter Electronics). The cells were pelleted again, resuspended at the desired concentration in ice-cold Tris-buffered NaCl with 2 mM Mn^{2+} and 25 μg/mL antipain (a generous gift from the U.S.–Japan cooperative cancer research program), and assayed for transferase activity. For repair, the cells were resuspended in complete medium [Eagle's basal medium supplemented with 2 mg/mL glucose, 2% nonessential amino acids (100×, Gibco), and 1 mM glutamine]. Cells (10×10^7) were allowed to repair in still cultures, at 37 °C, in 100-mm Falcon petri dishes in 6 mL of complete medium containing 5 μg/mL cycloheximide under an atmosphere of 10% CO_2–90% air. After the desired time, cultures were chilled on ice and the cells were collected by gentle flushing with a Pasteur pipet, pelleted in the cold, and resuspended in Tris-buffered NaCl containing 2 mM Mn^{2+} and 25 μg/mL antipain.

Assay for Transferase Activity in Intact Single Cells. One hundred microliters of cell suspension (20×10^6 cells) was incubated at 37 °C with 7 μM UDP-[^{14}C]GalNAc (27.5 mCi/mmol; ICN Pharmaceuticals). The reaction was terminated by chilling the cells on ice, and 1 mg of bovine serum albumin (BSA, Sigma) in 1 mL of ice-cold Tris-buffered NaCl was added. The cells were pelleted at 5000*g*, for 10 min, in the cold, and the supernatant was collected. Acid-insoluble radioactivity was determined by adding an equal volume of cold 1% phosphotungstic acid in 6% trichloroacetic acid (PTA–Cl_3AcOH) to the reaction medium. The mixture was filtered through a Whatman GF/F glass fiber filter and washed 3 times with 3 mL of PTA–Cl_3AcOH and once with 1 mL of ethanol–ether (2:1). The filters were placed in scintillation vials and counted in 5 mL of Aquasol (New England Nuclear) in a liquid scintillation spectrometer. For determination of radioactivity incorporated into the cells, the cell pellet was resuspended in Tris-buffered NaCl and dispersed by sonication. An equal volume of PTA–Cl_3AcOH was added, and the precipitate was collected on filters, washed, and counted as described above.

Preparation and Assay of Homogenates. Whole retinas or trypsin-dispersed cells were suspended in 50 mM Tris-HCl, pH 7.2, containing 2 mM Mn^{2+}, 1 mM PMSF, and 1% Triton at a ratio of 2–4 retinas [$(16–32) \times 10^7$ cells] per mL. The tissues were homogenized by 20 strokes in a Potter-Elvejhem homogenizer and allowed to remain on ice for 30 min before centrifugation at 8000*g* for 15 min. The pellet was discarded and the clear supernatant analyzed for activity. The standard assay system consisted of 100 μL of homogenates incubated at 37 °C with 7 μM UDP-[^{14}C]GalNAc in the presence of 100 μM AMP. The reaction was terminated by chilling the samples and adding 1 mL of ice-cold Tris-buffered NaCl and 1 mL of PTA–Cl_3AcOH. The precipitate was collected on GF/F filters, washed, and counted as described for intact single cells.

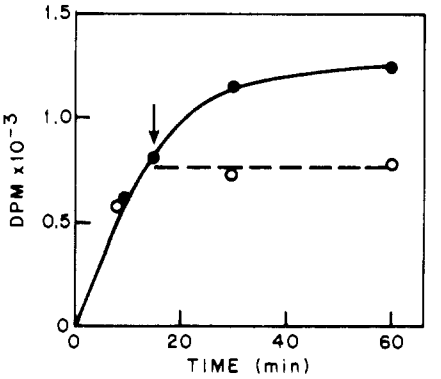

FIGURE 1: Time course of the transfer reaction among intact cells. Trypsin-dispersed cells were incubated with 7 μM UDP-[^{14}C]GalNAc in 100 μL of Tris-buffered NaCl, pH 7.2, and 2 mM Mn^{2+} for the times indicated (●—●). After 15 min of incubation, 1 μg of colchicine was added to one set of tubes and the reaction was continued for additional periods of time (○– – –○).

Analysis of the Product. For analysis of the product by affinity chromatography or molecular sieve chromatography, the reaction supernatant was dialyzed in the cold twice against 10^3 volumes of 0.1 M NH_4HCO_3 and 0.15 M NaCl, pH 8, and once against 0.1 M NH_4HCO_3 over a period of 2 days. The dialyzed reaction product was made 1% in Triton X-100, and a 1-mL sample was applied to a Sephadex G-150 column (1.0×7.5 cm). The column was eluted with 0.1 M NH_4HCO_3, pH 8, containing 1% Triton, and 0.6-mL samples were collected and counted. The void volume and the salt volume were determined by using blue dextran and sodium chromate, respectively.

Lectin affinity columns were prepared by covalently binding concanavalin A (Con A) or *Sophora japonica* lectin to polyacrylic hydrazide Sepharose (Miles-Yeda Ltd.) as described by Lotan et al. (1977). Con A was purchased from Calbiochem as a twice crystallized product, and *S. japonica* lectin was prepared in the laboratory by Dr. Peter Lipke by using the procedure described by Allen & Johnson (1976).

Paper chromatography of the reaction supernatant was carried out on Whatman No. 3 MM paper in both 95% ethanol–1 M ammonium acetate, pH 3.8 (5:2), for 25 h and butanol–pyridine–H_2O (6:4:3) for 15 h. Treatment with purified β-*N*-acetylhexosaminidase (Miles, *Turbo cornutus*) was carried out for 30 min at room temperature at a concentration of 0.01 unit of enzyme per 100 μL of reaction volume adjusted to pH 5.0 with sodium acetate. Radioactivity peaks were determined by cutting the paper into 1-cm strips which were counted in Aquasol. [^{3}H]GalNAc was included as a standard.

Results

β-N-Acetylgalactosaminyl Transferase Activity in Intact Cells. When trypsin-dissociated neural retina cells are incubated in suspension with UDP-[^{14}C]GalNAc, ^{14}C-labeled acid-insoluble molecules accumulate in the medium, while little, if any, radioactivity remains associated with the cells. Incorporation increases for ~30 min, reaching a plateau, in the presence or absence of a protein synthesis inhibitor (Figure 1). Treatment of the product with purified β-*N*-acetylhexosaminidase results in the release of over 90% of the acid-insoluble radioactivity as a ^{14}C-labeled product which migrates identically with [^{3}H]GalNAc on paper chromatography.

Transfer of [^{14}C]GalNAc from UDP-[^{14}C]GalNAc to endogenous cell acceptors is optimal at pH 7.2. The reaction is stimulated by manganese, with an optimum at 2 mM (Figure 2). In the presence of 2 mM Mn^{2+}, the transfer

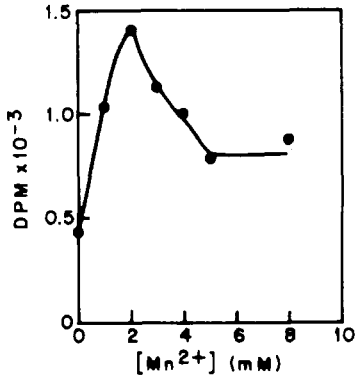

FIGURE 2: Effect of Mn^{2+} concentration on the transfer reaction catalyzed by single cells. 20×10^6 freshly prepared cells were incubated for 15 min, at 37 °C, in 100 μL of Tris-buffered NaCl, pH 7.2, with 7 μM UDP-[^{14}C]GalNAc and the indicated concentrations of Mn^{2+}.

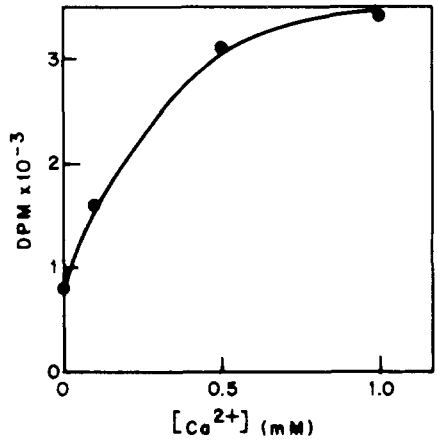

FIGURE 3: Effect of Ca^{2+} concentration on the transfer reaction catalyzed by freshly prepared single cells. 20×10^6 freshly prepared cells were incubated for 10 min in 100 μL of Tris-buffered NaCl with 7 μCi of UDP-[^{14}C]GalNAc and 2 mM Mn^{2+}.

reaction among freshly prepared single cells is strongly stimulated by calcium ions, showing an optimum at ~1 mM (Figure 3).

When the incubations are carried out for 10 min, an increase in incorporation is seen as a function of cell concentration, up to $\sim 20 \times 10^6$ cells/100-μL reaction volume. Although acceptors for the GalNAc transferase are endogenous, activity is saturable by UDP-[^{14}C]GalNAc at a concentration of ~14 μM.

A variety of criteria were used to attest to the surface localization of the GalNAc transferase activity. (1) After a 10-min standard incubation, chromatographic analysis of the reaction mixture showed no detectable radioactivity comigrating with GalNAc and no increase in the level of GalNAc-P already present in the UDP-[^{14}C]GalNAc preparation used. (2) Intact single cells incubated under standard assay conditions but with [^{3}H]GalNAc at concentrations ranging from 10 μM to 1 mM showed no detectable incorporation of radioactivity into acid-insoluble molecules either in the cell pellet or in the reaction supernatant. (3) No extensive cell lysis was observed under standard assay conditions for as long as 60 min, as determined by measuring lactic dehydrogenase (LDH) activity. Values for LDH in the medium ranged from 0.5 to 2% of the activity of a lysate of comparable cell concentration. The possibility of transferase leakage into the extracellular medium was analyzed by incubating cells under standard assay conditions but without the sugar donor. The supernatant was collected and incubated with UDP-[^{14}C]GalNAc for an additional 10-min period. No incorporation of label into acid-insoluble molecules was observed. (4) Transferase activity is sensitive to residual trypsin left at the cell surface following cell preparation. Inclusion of the covalent trypsin inhibitor phenylmethanesulfonyl fluoride (PMSF) in the cell preparation results in significantly higher levels of incorporation.

Table I: Effect of Colchicine on Transferase Activity in Intact Trypsin-Dispersed Cells and in Whole Tissue Homogenates[a]

inhibitor	% act. in intact cells	% of act. in whole tissue homogenate
colchicine		
0.1 μg/mL	40	
1.0 μg/mL	20	94
10.0 μg/mL	2	90
20.0 μg/mL	4	
lumicolchicine[b]		
10 μg/mL	100	
20 μg/mL	94	93

[a] The assays were carried out for 10 min under the conditions described in the text. Results are expressed as the percent of controls (no drugs added). [b] Lumicolchicine is an ultraviolet irradiation product of colchicine which does not disrupt microtubules (Wilson & Friedkin, 1967).

β-N-Acetylgalactosaminyl Transferase in Cell Homogenates. Following homogenization in 1% Triton, all transferase activity is recovered in the supernatant after a 30-min centrifugation at 10000*g*. Incorporation of [^{14}C]GalNAc into acid-insoluble molecules catalyzed by whole tissue homogenates is linear with time for at least 60 min. As observed with intact cells, the reaction is saturable by UDP-[^{14}C]GalNAc, with an apparent K_m of ~4 μM. Incorporation increases with protein concentration up to 150 μg of homogenate protein per reaction volume (equivalent to $\sim 6 \times 10^6$ 10-day-old neural retina cells). Activity is stimulated by addition of manganese ions, reaching a plateau at 2 mM. In contrast to what is observed for freshly dispersed intact cells, no inhibition is observed at higher concentrations of manganese, nor do calcium ions have an effect on the reaction.

Treatment of the homogenate product with β-*N*-acetylhexosaminidase releases radioactive GalNAc and no GluNAc. In addition, increasing concentrations of unlabeled UDP-GluNAc (up to 10000 times the concentration of UDP-GalNAc) have no effect on the incorporation of radioactive GalNAc from UDP-GalNAc. These results indicate that UDP-GalNAc is also the substrate for the homogenate transferase reaction.

Evidence for an Intracellular Pool of Transferase–Acceptor. The time course of transferase activity observed for intact trypsin-dispersed cells (Figure 1) may reflect both the rate of enzyme catalysis and the rate at which transferase–acceptor becomes available at the cell surface following trypsin treatment. To distinguish these possibilities, we analyzed the effect of colchicine, an inhibitor of ligand pool mobilization (McDonough et al., 1977), on the transferase reaction among freshly dispersed cells. Colchicine completely blocks transfer of [^{14}C]GalNAc from UDP-[^{14}C]GalNAc catalyzed by intact single cells when added to the assay medium at zero time (Table I). Furthermore, if colchicine is included in the assay medium after 15 min of incubation, no further accumulation of acid-insoluble radioactivity is observed (Figure 1). However, colchicine has no effect on activity in cell homogenates (Table I).

When single cells are allowed to repair in culture for 30 min and then are assayed for transferase activity in the presence of colchicine to inhibit further pool mobilization, incorporation

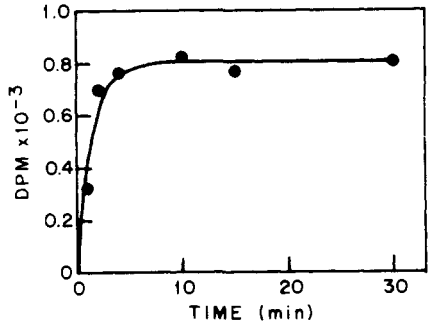

FIGURE 4: Time course of the transfer reaction catalyzed by repaired intact cells in the presence of colchicine. Single cells were allowed to repair in culture for 30 min and harvested in the cold. Aliquots containing 20 × 10^6 cells were incubated with 7 μM UDP-[^{14}C]-GalNAc in 100 μL of 10 μg/mL colchicine and 2 mM Mn^{2+}.

Table II: Comparison of Transferase Activity in Retina Cell Homogenates[a]

homogenate preparation	dpm incorpd at saturation (% of whole tissue ± SD)
whole tissues	100
single cells immediately after trypsinization	68 ± 8
single cells repaired in culture for 2 h, harvested, and retrypsinized	10 ± 5
single cells repaired in culture for 2 h with 10 μg/mL colchicine, harvested, and retrypsinized	50 ± 10

[a] Transferase activity was measured as a function of homogenate concentration, and the amount of radioactivity incorporated was compared at plateau levels. Results are the average from nine separate experiments.

of [^{14}C]GalNAc from UDP-[^{14}C]GalNAc reaches a plateau within 10 min (Figure 4). These results suggest that the time course of transferase activity among fresh trypsin-dispersed cells is largely a reflection of pool mobilization. The effect of calcium ions on the glycosylation reaction catalyzed by freshly dispersed cells (Figure 3) is also likely to be a reflection of pool mobilization, as transferase activity among cells that have been repaired in culture is not stimulated by calcium.

Evidence for an intracellular pool of transferase–acceptor is also obtained by studying total enzyme activity in cell homogenates. The effect of tissue trypsinization on total activity was estimated by comparing reactions catalyzed by homogenates of whole tissues and by homogenates of single cells at saturation levels. As illustrated in Table II, dispersion of tissues into single cells by means of trypsinization leads to a loss of 30–40% of the total enzyme activity. Similarly, homogenates of cells repaired for 2 h in the presence of cycloheximide show roughly 60% of the activity observed in whole tissues. If, however, these repaired cells are retrypsinized and then assayed as homogenates, GalNAc transferase activity is almost totally absent (Table II). This loss of activity can be prevented by repairing the cells in the presence of colchicine (Table II). These results not only provide evidence that the GalNAc transferase activity detected under our assay conditions exists in freshly prepared trypsin-dispersed cells as an endogenous pool but also strongly imply that all detectable activity eventually becomes associated with the cell surface.

As an approach to investigate the rate at which acceptor–transferase becomes available at the cell surface following trypsinization, cells were allowed to repair in culture in the presence of cycloheximide for increasing periods of time, harvested in the cold, and assayed in the presence of colchicine. Incorporation increases with time in culture, reaching a plateau

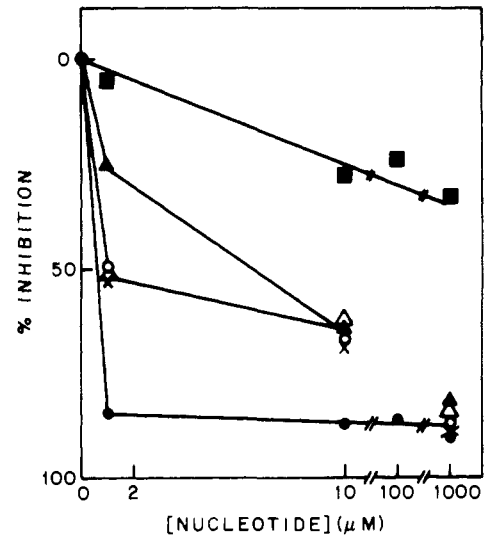

FIGURE 5: Effect of nucleotides on transferase activity among intact cells and whole tissue homogenates. Single cells were prepared and incubated for 10 min at 37 °C under standard assay conditions but with added UDP (●), UMP (○), GDP (×), CMP (△), or AMP (▲) at the indicated concentrations. (■) Whole tissue homogenates assayed in the presence of UDP.

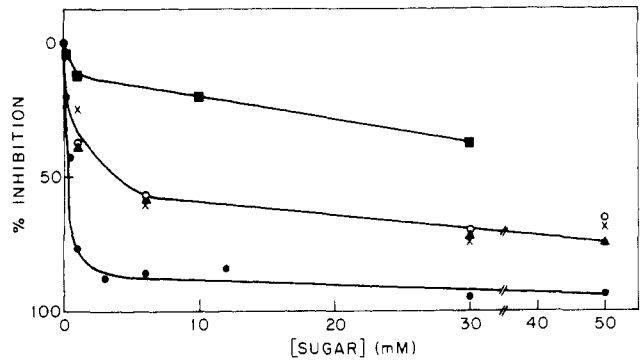

FIGURE 6: Effect of monosaccharides on transferase activity among intact cells and homogenates. Single cells were prepared and incubated for 10 min at 37 °C under standard assay conditions but with the indicated concentrations of GalNAc (●), $GalNH_2$ (▲), Gal (×), or GluNAc (○). (■) Whole tissue homogenates assayed in the presence of GalNAc.

between 1 and 2 h, presumably reflecting an increase in the transferase–acceptor available at the cell surface. The difference between the time course of the reaction observed in this protocol vs. that seen in Figure 1 may be a reflection of a difference in the rate of pool mobilization when cells repair in suspension vs. attach to a substrate.

Inhibitors of the Glycosylation Reaction. Nucleoside mono- and diphosphates have been reported to inhibit both soluble (Ko & Raghupaltry, 1973) and membrane-bound (Ishibashi et al., 1976) transferases. A number of nucleotides inhibit the transfer of [^{14}C]GalNAc catalyzed by freshly dispersed single cells, the most effective by far being UDP (Figure 5). The reaction in whole tissue homogenates is also affected by UDP, although much higher concentrations are necessary to achieve the levels of inhibition observed in intact cells (Figure 5).

Hydroxyurea and cytosine arabinoside, both reported inhibitors of glycosyl transferases (Hawtrey et al., 1974), inhibit by ~90% the transfer of [^{14}C]GalNAc catalyzed by single cells at concentrations of 10 and 1 mM, respectively.

The effect of the monosaccharides Gal, $GalNH_2$, GalNAc, and GluNAc on the transferase reaction at concentrations ranging from 0.1 to 50 mM was also analyzed. For intact cells 50% inhibition is seen at concentrations as low as 0.3 mM

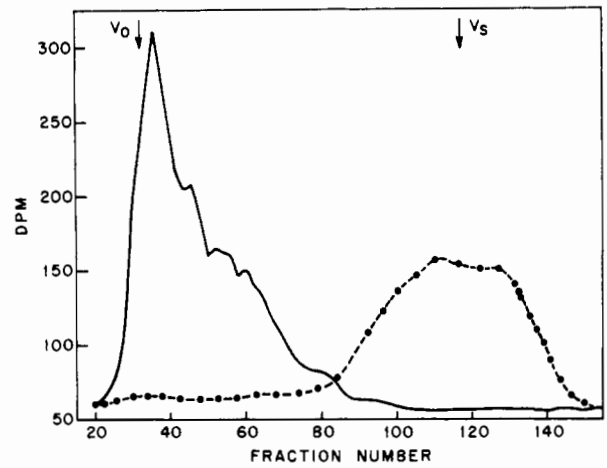

FIGURE 7: Sephadex G-150 chromatography of intact cell reaction product. Single cells were prepared and allowed to repair in culture for 30 min. 20×10^6 cells were incubated in 100 μL of Tris-buffered NaCl, pH 7.2, with 2 mM Mn^{2+}, 7 μM UDP-[^{14}C]GalNAc, 1 μg/mL colchicine, and 2.5 μg of antipain. After 10 min at 37 °C the cells were chilled and 0.5 mL of buffer containing 0.5 mg of BSA was added. The cells were pelleted, and supernatants from four separate assay tubes were pooled and dialyzed. The dialyzed sample was divided into two aliquots: one was made 1% in Triton and applied to a Sephadex G-150 column and the other was treated with 500 μg of Pronase overnight at room temperature prior to being applied to the column. (—) Control; (---) Pronase treated.

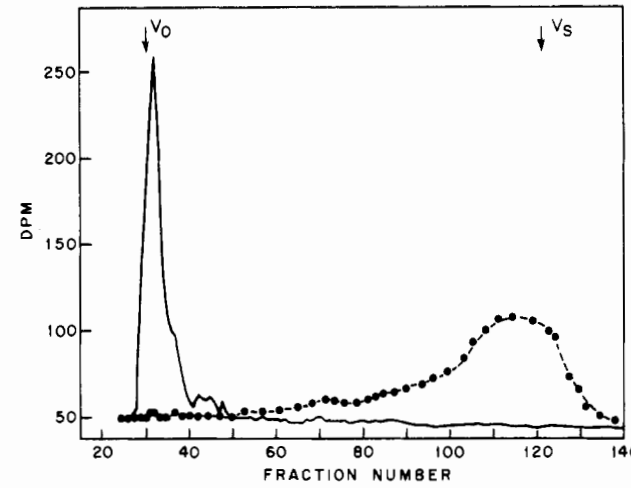

FIGURE 8: Sephadex G-150 chromatography of the homogenate reaction product. 100 μL of whole tissue homogenate in Tris buffer, pH 7.2, with 2 mM Mn^{2+}, 1% Triton, and 1 mM PMSF was incubated with 5 μM UDP-[^{14}C]GalNAc. After 30 min the reaction volume was made 1 mL with Tris-buffered NaCl and dialyzed. Half of the dialyzed product was applied to a Sephadex G-150 column, and the other half was incubated with 500 μg of Pronase overnight at room temperature prior to being applied to the column. (—) Control; (---) Pronase treated.

GalNAc, whereas all the other sugars have similar effects, inhibiting the reaction by 50% at a concentration of ~5 mM (Figure 6). Inhibition by free GalNAc of the reaction catalyzed by tissue homogenates is much less pronounced (Figure 6). Thus, the enzyme–acceptor milieu seems to be a determinant of the inhibitory effect exerted by both nucleotides and monosaccharides.

The Reaction Product. (*1*) *Intact Cells.* Between 90 and 100% of the PTA–Cl_3AcOH-insoluble counts in the supernatant of the reaction catalyzed by intact cells for a period of 10–15 min are recovered after extensive dialysis. Mild acid hydrolysis (0.1 N HCl, 20 min, 100 °C) does not render the product soluble in 10% Cl_3AcOH. Two types of experiments attest to the presence of terminal β-[^{14}C]GalNAc residues in the macromolecular product. First, when the dialyzed product is passed over an affinity column of *S. japonica* lectin, ~70% of the radioactivity is retained and can be eluted specifically by 0.3 M GalNAc in 0.5 M NaCl. It has been shown that this lectin has a binding site specific for 2-acetamido-2-deoxygalactopyranosyl groups, with a preference for the β configuration (Poretz et al., 1974). Second, an acid-soluble ^{14}C-labeled product is released by treatment of the dialyzed reaction supernatant with purified β-*N*-acetylhexosaminidase which cochromatographs with [^{3}H]GalNAc in two different paper systems.

Molecular sieve chromatography of the dialyzed reaction product on Sephadex G-150 in 1% Triton X-100 shows a high molecular weight fraction which is eluted immediately after the void volume (Figure 7). The failure to include protease inhibitors and a "carrier" protein (BSA) during dialysis and column fractionation results in a polydisperse profile, suggesting the presence of degradative enzymes in the whole cell reaction product. Treatment with Pronase converts the product into low molecular weight components, eluting near the salt volume of the column (Figure 7). The reaction product is essentially identical when prepared either from fresh trypsin-dispersed cells or from cells that have been repaired in cycloheximide and then assayed for 10 min in the presence of colchicine.

A second type of product is observed when fresh trypsin-dispersed cells are assayed for very short periods of time (2–5 min). In this case, radioactivity is incorporated into chloroform–methanol (2:1) soluble components. This glycolipid-like product is specifically retained on a Con A–Sepharose column, suggesting that it contains mannosyl-like residues. It is possible that this component is a phosphoryldolichol oligosaccharide; it is, however, unlikely that this component plays a role in the glycosylation of the high molecular weight glycoprotein product. The high molecular weight reaction product does not bind to Con A–Sepharose, suggesting that mannose residues are either absent or substituted in a manner which abolishes the affinity toward Con A. In addition, the reaction products obtained from repaired intact cells or tissue homogenates do not show the glycolipid-like product.

(*2*) *Homogenates.* Analysis of the dialyzed product of the reaction catalyzed by whole tissue homogenates on a Sephadex G-150 column eluted with buffer containing 1% Triton reveals one peak of radioactivity eluting in the same position as the whole cell product, immediately after the void volume (Figure 8). This macromolecular product is also converted into low molecular weight material upon digestion by Pronase (Figure 8) and labeled [^{14}C]GalNAc is liberated upon digestion by purified β-*N*-acetylhexosaminidase, as evidenced by paper chromatography.

Discussion

The data presented here demonstrate the presence of a β-*N*-acetylgalactosaminyl transferase active at the cell surface of embryonic chick neural retinal cells. This enzyme utilizes an endogenous macromolecular acceptor, and the glycosylated product is released into the medium. Glycosylation is observed immediately following cell preparation but is dependent upon mobilization of an endogenous pool of enzyme and/or acceptor to the cell surface. Two different lines of evidence substantiate this conclusion. First, experiments utilizing whole cells show that the glycosylation reaction catalyzed by freshly dispersed cells is totally blocked by colchicine. Inclusion of this drug in the assay catalyzed by cell homogenates or cells that have been allowed to repair in culture has no effect. In addition,

calcium ions have a stimulatory effect on transfer among freshly dispersed cells, but no stimulation is observed when homogenates or repaired cells are analyzed. These two effects may be interrelated, as calcium ions may interact with cytoskeletal components that participate in mobilization of newly synthesized components to the cell surface [see Allison & Davies (1974)]. The second line of evidence comes from experiments carried out with homogenate preparations. Homogenates made from trypsin-dispersed cells show 60% of the activity of homogenates prepared from whole tissues. Transferase activity in homogenates is almost completely absent when cells are allowed to repair in culture in the presence of cycloheximide, harvested, and retrypsinized. This loss can be prevented by including colchicine during the repair period. These data also suggest that enzyme–acceptor exists in the intact tissues both associated with the cell surface and as an endogenous pool. Trypsin treatment removes the surface complement, triggering mobilization of the endogenous pool to the cell surface.

The β-GalNAc transferase activities of homogenates and intact cells are both stimulated by manganese ions, with an optimum at 2 mM. However, transferase activity in intact cells is much more sensitive to inhibition by either nucleotides or monosaccharides as compared with homogenates. The extreme sensitivity to nucleotides was also observed by McDonough & Lilien (1978) when analyzing glycosylation of a cell surface ligand by intact cell preparations. This may be due to the fact that the enzyme is more susceptible to perturbation in its native configuration at the cell surface than it is in 1% Triton homogenates.

The actual mechanism of glycosylation occurring at the cell surface is not clear. It is generally accepted that sugar–nucleotides cannot pass through the permeability barrier of the cell membrane. Lennarz (1975) has developed a model involving lipid-linked oligosaccharides as substrates for plasma membrane associated glycosyl transferases. Although there seems to be a lipid-like product formed during short-term reactions using intact freshly dispersed cells, we have no indications that this product is a precursor for the glycosylated glycoprotein.

Cell surface associated glycosyl transferases were first suggested to participate in intercellular adhesion by Roseman (1970). In his model, the enzyme on the surface of one cell interacts with an acceptor on the surface of another cell, forming the adhesive bond. Adhesive specifities are accounted for by the specificity of the enzymes involved. Out data have also led us to consider the role of glycosyl transferases in cell adhesion (Lilien et al., 1978, 1979). According to our proposed model (Balsamo & Lilien, 1974; Rutz & Lilien, 1979), intercellular adhesions among embryonic chick neural retina cells are mediated by at least three components: a cell surface glycoprotein ligand, its receptor, and a third component termed "agglutinin" which interacts with ligand molecules in adjacent cells to establish the adhesive bond. McDonough & Lilien (1978) have shown that the properties of the reaction governing the turnover of ligand mobilized from an endogenous pool match those expected of a glycosyl transferase reaction. They proposed that ligand receptor is, or is closely associated with, a glycosyl transferase and that turnover is mediated by the addition of a terminal GalNAc residue at the cell surface. While we have not assayed the product of the transferase reaction directly, the properties of the reaction catalyzing its release from the cell surface imply an identity with ligand. Thus, the fine control of transferase activity may be of critical importance for the maintenance of stable adhesions via its control of ligand turnover.

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