Quantification of Ganglioside GM_1 Synthetase Activity on Intact Chick Neural Retinal Cells

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ABSTRACT Neural retinal cells from 9-d-old chick embryos were assayed for uridine diphosphate (UDP)-galactose:ganglioside GM_2 galactosyltransferase, or GM_1 synthetase, activity using the oligosaccharide fragment of GM_2, oligo-GM_2, as the exogenous acceptor. The results demonstrated that this enzyme activity was present on the external surfaces of intact cells. Little difference between the specific activities of cell surface GM_1 synthetase could be detected when cells derived from dorsal and ventral segments of the neural retina were compared. These results suggested that this cell-surface enzyme was not present in a concentration gradient along the dorsoventral axis of the neural retina.

The precise projection of retinal ganglionic axons to their synaptic targets in the optic tectum during neuromorphogenesis is a principal example of the specificity of cell recognition. In the chick embryo, fibers begin to grow from the fundus of the retina on about day 6 of development and ultimately migrate to specific areas on the surfaces of the optic tectum. At about day 12, the fibers penetrate the outer layer of the tectum and then synapse with tectal neurons (3, 9). The area on the tectum to which a retinal axon migrates is highly predictable (9, 12). The retinotectal projection is inverted along both the dorsoventral and antereo-posterior axes. For example, fibers originating in the dorsal-most areas of the retina project to the ventral-most areas of the tectum.

An in vitro assay of retinotectal specificity has been developed that measures the rates of adhesion of radioactively labeled neural retinal cells dissociated from either dorsal or ventral neural retinal fragments, to dorsal and ventral tectal halves. The specificity of the retinal cell adhesion observed by this assay mimics that observed in vivo. That is, dorsal retinal cells adhere at a greater rate to ventral than to dorsal tectal halves, and ventral cells show a similar adhesive specificity for dorsal tecta. Similar results are observed when aggregates of tectal cells derived from either dorsal or ventral tectal halves are used in the adhesion assay (1, 2, 13).

Marchase (14) tested the effects of various proteases and glycosidases on the retinotectal adhesion assay. The results from these experiments suggested that a protease-resistant molecule(s) was present in greater concentration on dorsal surfaces of retina and tecta and was involved in retinotectal specific adhesion. The results also suggested that this molecule contained a terminal GalNAc (N-acetylgalactosamine) residue and, in addition, could be present in a form in which this GalNAc could be found penultimate to a terminal Gal (galactose) residue. These and other results led Marchase (14) to postulate that ganglioside GM_2, known to be present in chick neural retina, was located on the surfaces of the retinal cells and tectum and was involved in the adhesive specificities observed in the retinotectal adhesion assay.

The discovery of glycosyltransferase activities on the surfaces of intact cells led to the hypothesis that these enzymes functioned directly in intercellular recognition by binding their specific carbohydrate acceptors on adjacent cells and, possibly, modifying these acceptors by the transfer of appropriate sugars (19, 21). Since then, cell-surface glycosyltransferase activities have been localized to the cell surface by a number of methods. These include: (*a*) the assay of well-characterized plasma membrane preparations (4); (*b*) localization of cell surface products by autoradiography or an increase in lectin binding to cells after incubation with the appropriate sugar nucleotides (23); and (*c*) the transfer by intact cells of sugars to exogenous acceptors that are soluble or derivatized by insoluble supports (15, 31).

The enzyme, uridine diphosphate (UDP)-galactose:GM_2 galactosyltransferase, or GM_1 synthetase,[1] catalyzes the transfer of galactose from UDP-Gal to the terminal GalNAc of GM_2 to synthesize GM_1 (11). It is possible that GM_1 synthetase is located on the surfaces of retinal cells and can bind to GM_2 on

[1] The Svennerholm notation of ganglioside nomenclature (24). NeuNAc, N-acetylneuraminic acid. GM_2, GalNAc ($\beta 1 \rightarrow 4$)[NeuNAc($\alpha 2 \rightarrow 3$)]Gal($\beta 1 \rightarrow 4$)Glc-ceramide. GM_1, Gal($\beta 1 \rightarrow 4$)GalNAc($\beta 1 \rightarrow 4$)[NeuNAc($\alpha 2 \rightarrow 3$)]Gal($\beta 1 \rightarrow 4$)Glc-ceramide.

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opposing cell surfaces. Preliminary evidence suggested that when the activity of GM_1 synthetase was measured in sonicates of neural retinal cells using GM_2 as the acceptor, higher specific activity was detected in ventral areas compared to dorsal areas (14). This result was particularly interesting since earlier experiments had suggested that a protease-sensitive molecule involved in retinotectal specific adhesion was present in greater concentration on ventral retinal cell and tectal surfaces.

This study was designed to test whether GM_1 synthetase is present on neural retinal cell surfaces. The oligosaccharide fragment from GM_2, oligo-GM_2, was prepared and used as an exogenous acceptor with intact cells. Under optimal conditions, the specific activity of cell-surface GM_1 synthetase was determined and compared to that of sonicated cells in the presence and absence of detergents. Over 25% of total sonicate activity toward oligo-GM_2 in the absence of detergents was detected on the surfaces of 8.5 day neural retinal cells. Control experiments demonstrated that only small amounts of activity were released from intact cells into incubation supernatants under standard assay conditions. Little asymmetry of enzyme specific activity could be detected between intact dorsal and ventral cells.

MATERIALS AND METHODS

Materials

Dulbecco modified Eagle's medium with calf serum buffered with HEPES and Silica Gel G thin-layer chromatography plates were purchased from Merck (Merck Chemical Div., Merck & (Co., Rahway, NJ). UDP-galactose, GlcNAc, adenosine 5'-monophosphate, neuraminyl-lactose, Triton CF-54, Tween 80, and Triton X-100 were obtained from Sigma Chemical Co. (St. Louis, MO). UDP-[^{3}H]galactose, 13 Ci/mmol, and NaB[^{3}H]$_4$, 42 Ci/mmol, were obtained from Amersham Searle.

Media

(HEPES-DECS) and dissociating medium were prepared as described by Barbera (1). HEPES-HBSS consisted of Hanks' basic salt solution (HBSS) buffered with 25 mM HEPES, pH 7.2.

Thin-layer Chromatography

Merck Silica Gel 60 thin-layer plates were activated by heating to 120° for 2 h before use. Solvent systems used for this study and their designations were: solvent A, chloroform:methanol:2.5 N NH_4OH (60:40:9); solvent B, chloroform: methanol:water (60:30:8); solvent C, 1-propanol:0.2% $CaCl_2$ (80:20); solvent D, 1-propanol:acetic acid:water (30:30:20); solvent E, 1-propanol:methanol:0.2% $CaCl_2$ (40:40:20).

Measurement of Radioactivity on Thin-layer Plates

Silica gel sections were scraped into 7.5-ml scintillation vials. A number of solvents were tested for their abilities to solubilize completely the radioactivity associated with the silica gel, yet have no quenching effects on counting efficiency. Using radioactively labeled gangliosides, a mixture of 0.2 ml of tetrahydrofuran and 5 ml of toluene-based PPO-POPOP scintillation fluor gave complete recovery of radioactivity from silica gel sections and had no effect on counting efficiency. Tetrahydrofuran was added to the scintillation vial containing a section of silica gel and the vial incubated at room temperature for 10 min. Scintillation fluor was then added, the contents were mixed by vigorous shaking, and the radioactivity was measured in a Searle Mark III scintillation counter.

Ganglioside Purification

Ganglioside GM_2 was purified from samples of Tay-Sachs brains that had been preserved in formalin and given to us by Drs. G. Kolodny and S. Elsas. Greater than 70% of the crude ganglioside fractions from these brains was GM_2 as judged by resorcinol assays on thin-layer chromatograms (24). Ganglioside GM_1 was purified from calf brain. The method of Tettamanti et al. (28) was used to isolate the ganglioside fraction, and columns (1.5 × 70 cm) using 1-propanol: 2-propanol:30% ammonium hydroxide (35:35:30) as the solvent and a flow rate of ~10 ml/h were used to separate the gangliosides. Aliquots of fractions were chromatographed on thin-layer plates in either solvent A or B. Gangliosides were detected by staining with iodine vapors and by the modified resorcinol reagent. Fractions containing GM_2 or GM_1 were pooled, dialyzed against deionized water, concentrated by lyophilization, and rechromatographed on a second silica gel column. Purity of the gangliosides was judged by chromatography in at least three solvent systems. Gangliosides GM_1 and GM_2 were radioactively labeled using galactose oxidase in detergent and reduction with sodium borotritide (25). Typically, the specific activity of these labeled gangliosides was 1–2 × 10^6 cpm/nmol. When 2 nmol of labeled GM_2 was chromatographed on thin-layer plates using two different solvent systems, radioactivity corresponding only to GM_2 could be detected.

Preparation of Oligosaccharide Fragments from GM_2 and GM_1

The oligosaccharide fragment of GM_2 (oligo-GM_2) was prepared by the method of Wiegandt and Bucking (32). Samples of GM_2 (0.5–5 mg) were dissolved in 0.5 ml of chloroform:methanol (2:1) and the solution was saturated with ozone for 2.5 min using a micro-ozonizer (Supelco, Inc., Bellefonte, PA). After evaporating the sample under N_2 at room temperature, 0.5 ml of 0.1 N NaOH was added and the solution was then incubated for 16 h, also at room temperature. Sodium ions were removed by adding 0.1 ml of Dowex 50 resin, shaking the mixture, centrifuging to pellet the resin, and removing the supernatant. An additional 1.0 ml of water was added, the tube was shaken and centrifuged, and the supernatants were then pooled. The solution was lyophilized, and an aliquot was chromatographed on a Merck (Merck Chemical Div.) silica gel G plate. The products were visualized by staining with iodine vapors and resorcinol reagent. Oligo-GM_2 migrated with an R_f of 0.25 using solvent C. This product was further purified on thin-layer plates using the system of Holmes and O'Brien in solvent D (10). Oligo-GM_2 migrated with a rate of 0.64 cm/h. Areas of the chromatogram that corresponded to oligo-GM_2 were scraped and eluted in ethanol:acetic acid: water (30:30:20). Sugar analysis of this purified sample, performed by Dr. Christopher Stowell in the laboratory of Dr. Y. C. Lee (Johns Hopkins University), demonstrated the presence of Glc (glucose), Gal, GalNac, and NeuNAc (N-acetylneuraminic acid) in the molar ratio of 0.8:0.9:1.0:1.0. Within experimental error, this ratio is the same as that found in native GM_2. Purity of oligo-GM_2 preparations was also demonstrated by radioactively labeling the purified sample by reaction with sodium borotritide according to the method of Takasaki et al. (27) and subjecting aliquots to thin-layer chromatography. Using high-voltage paper electrophoresis on borate-impregnated paper (21), oligo-GM_2 migrated with a rate of 0.36 relative to phenol red. Typical yields of oligo-GM_2 were 55–70%.

Oligo-GM_1 was prepared from GM_1 by the same procedure. Using the Holmes and O'Brien chromatography system (10), oligo-GM_1 migrated with a rate of 0.54 cm/h. Oligo-GM_1 migrated with a rate of 0.07 relative to phenol red when a sample was subjected to borate electrophoresis.

Measurement of Galactosyltransferase Activities in Sonicates

Neural retinal fragments were dissected and placed in ice-cold phosphate-buffered saline (PBS). The tissue was pelleted by centrifugation, suspended in 100 mM cacodylate buffer, pH 6.8, and then disrupted by sonication. Assay tubes contained a solution of acceptor, 0.20 mM UDP-galactose, 0.766 μCi UDP-[^{3}H]galactose, and 3 mM 5'-AMP, all of which were evaporated to dryness under reduced pressure. 10 μl of a 4.5 mg/ml solution of a 2:1 mixture of Triton CF-54 and Tween 80 plus 50 mM $MnCl_2$ was added to the tubes to dissolve the dried components. To begin the reaction, 0.04 ml of the retinal sonicate was added and the tube's contents were mixed thoroughly and incubated at 37°C. After 1 h, the reaction was stopped by the addition of 0.01 ml of 0.25 M EDTA and chilling on ice (15).

When N-acetylglucosamine (GlcNAc) was the acceptor, synthesis of N-acetyllactosamine was determined after separating the products of the reaction by borate electrophoresis. When GM_2 was the acceptor, GM_1 synthesis was measured either by borate electrophoresis or gel chromatography on a Sephadex G-25 column eluted with chloroform:methanol:water (60:30:4.5) (7). GM_1 was identified by chromatography of the void volume fractions using thin-layer plates and solvent A or C. When oligo-GM_2 was the acceptor, the product, oligo-GM_1, was identified by borate electrophoresis and further characterized by thin-layer chromatography. The material was eluted from minced areas of electrophoresis papers by ten successive rinsings with 2 N acetic acid, and sodium ions were removed with Dowex 50. Vacuum evaporation after the addition of methanol, repeated five times, removed borate ions. The samples were concentrated and applied to silica gel plates with purified standards in adjacent lanes. Plates were

chromatographed in solvent E and dried. The standards were detected using resorcinol reagent.

Optimal activity for the three acceptors was observed with 10 mM $MnCl_2$ and 0.20 mM UDP-galactose. Optimal concentrations of the acceptors, GM_2, oligo-GM_2, and GlcNAc were 0.20 mM, 1.0 mM, and 10 mM, respectively. Under these conditions, transfer to all three acceptors was linear for at least 90 min and linear with increasing protein concentration up to 0.40 mg/assay.

Measurement of Galactosyltransferase Activities on Intact Retinal Cells

Whole neural retinas, or fragments sectioned into dorsal, middle, and ventral thirds were dissected, and single-cell suspensions prepared from them by trypsinization according to methods described previously (1). The cells were then preincubated in HEPES-DECS at 37°C on a gyratory shaker (120 rpm). During the preincubation, cells were gently triturated at ½-h intervals to inhibit formation of large aggregates. Cells were then collected by centrifugation, washed with HBSS, and counted in a hemacytometer. Assay tubes contained 0.20 mM UDP-Gal, 14.6 μCi UDP-[^{3}H]Gal, and, if acceptor was present, either 10 mM GlcNAc or 1 mM oligo-GM_2. This mixture was then evaporated to dryness. Before addition of the retinal cells, 0.02 ml of HEPES-HBSS that contained 50 mM $MnCl_2$ and 15 mM 5′-AMP were added to the assay tubes to dissolve the dried components. Aliquots of the retinal cell suspension (0.08 ml) were then added, the tubes gently agitated, and the mixture was incubated at 37°C for up to 3 h. To stop the reaction, cells were pelleted by centrifugation (170 *g* for 4 min) and the supernatant was transferred to another tube that contained 0.01 ml of 250 mM EDTA. After mixing, the contents of the tube were applied to Whatmann 3 MM paper and the products of the reaction separated by borate electrophoresis. The cell pellets were washed twice with HEPES-HBSS, the supernatants discarded, and the pellets resuspended in a mixture of 0.04 ml of the same solution plus 0.01 ml of 250 mM EDTA. Aliquots of this suspension were then subjected to borate electrophoresis, the origins excised, and the radioactivity was determined in a scintillation counter. Viability of the cells before and after the assay was measured by trypan blue exclusion (21).

When enzyme activity of assay supernatants was to be measured, an aliquot of intact cells was added to a standard assay tube that contained only unlabeled UDP-gal. After incubating for 90 min at 37°C, the cells were gently pelleted by centrifugation at 100 *g* for 2 min. The supernatant fraction was removed, adjusted to 0.10 ml with HEPES-HBSS, and added to an incubation tube containing 14.6 μCi UDP-[^{3}H]Gal. After a 90-min incubation, the reaction was stopped by removing 0.08 ml and adding 0.02 ml of a 250 mM EDTA solution. Synthesis of oligo-GM_1 was determined by subjecting the entire contents of this assay tube to borate electrophoresis.

Determination of Lactate Dehydrogenase Activities

This assay, based on the method of Schnaar et al. (22), was used to estimate cell breakage and release of soluble enzymes into assay supernatants.

RESULTS

To quantify the specific activity of GM_1 synthetase, measurements must be made at saturating levels of the substrates, GM_2 and UDP-gal. When intact cells are used as the enzyme source, however, GM_2 cannot be used as an exogenous acceptor because gangliosides in solution become incorporated into cells (29). Because of this association, no assumptions can be made concerning the amounts of acceptor or product gangliosides free in solution. To circumvent this difficulty, the oligosaccharide portion of GM_2, oligo-GM_2, was prepared from GM_2 and tested for its activity as an acceptor for galactose using neural retinal cell sonicates and intact cells.

Optimal conditions had previously been determined for the measurement of GM_1 synthetase activity in neural retinal cell sonicates (15). Oligo-GM_2 was substituted for GM_2 in these sonicate assays and the kinetics of the transfer of Gal from UDP-Gal to product were determined. Product formation was measured by the amounts of radioactivity remaining at the origins of borate electrophoretograms. Oligo-GM_1, the presumed product of the enzymatic reaction, was prepared from radioactively labeled GM_1 and shown to be immobile in this electrophoretic system. The transfer of Gal to oligo-GM_2 was linear when the time of incubation was <90 min and when less than 0.40 mg of protein per assay was used. The apparent K_m with respect to oligo-GM_2 concentration was calculated to be 92 μM. (M. Pierce, data not shown).

The product from these reactions was recovered from the origins of electrophoretograms and, after desalting, applied to a thin-layer plate. After chromatography, >90% of the radioactivity applied to the plate comigrated with the oligo-GM_1 standard, shown in Fig. 1, demonstrating that oligo-GM_1 was the major product of the enzymatic reaction using oligo-GM_2 as the acceptor.

Since there are a number of galactosyltransferases that can use glycolipids as acceptors (5), it is possible that production of oligo-GM_1 was not catalyzed by GM_1 synthetase. To determine whether the same enzyme catalyzed both reactions, a competition experiment was performed in which activities with GM_2 and oligo-GM_2 were first determined separately and then in incubations containing both acceptors at saturating concentrations. If the activities were competitive, the initial rates would be less than additive and be determined by the relative affinities of the active site for the acceptors and by the acceptor concentrations (6).

As a point of comparison, GlcNAc, whose activity as a Gal acceptor is known not to be competitive with GM_2, was mixed with GM_2, and the acceptor activity of the mixture was determined. The K_m of GM_1 synthetase for GM_2 was calculated from previous experiments (15). The results, summarized in Table I, show that the activity toward oligo-GM_2 and GM_2 together was less than additive and that the observed activity was identical to the value predicted by assuming competition between acceptors. By contrast, the activities toward GlcNAc

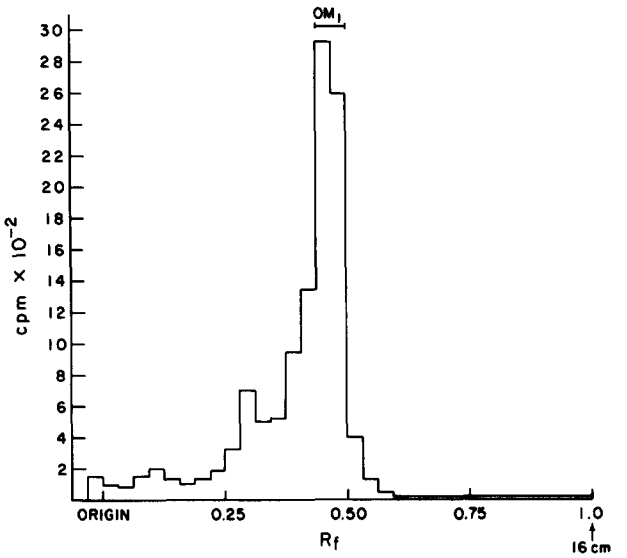

FIGURE 1 Characterization of oligo-GM_1 product. Sonicates of 9-d-old neural retina were incubated with oligo-GM_2 under standard incubation conditions for 2 h, except that the volume of the incubation was increased to 0.20 ml and the specific activity of the UDP-Gal was 80 cpm/pmol. The reaction was stopped by adding EDTA and the contents of the tube were subjected to borate electrophoresis. The origin of the electrophoretogram, which contained the reaction product, was extracted, and the extract was desalted and concentrated by evaporation. The residue was dissolved in ethanol and applied to a thin-layer plate. After development in solvent E, the plate was sectioned and the radioactivity in each slice measured. OM_1, oligo-GM_1 standard.

TABLE I

Galactosyltransferase Acceptor Competition between GM_2 and Oligo-GM_2 or N-Acetylglucosamine

Acceptor(s)	Specific activity*
	nmol galactose transferred/mg · h
GM_2	2.74
Oligo-GM_2	2.27
GlcNAc	1.67
GM_2 + Oligo-GM_2	2.35 (5.01; 2.32)‡
GM_2 + GlcNAc	4.38 (4.41; ND§)

* 8.5-d retinal sonicates were used as enzyme source. Each incubation contained 10 mM $MnCl_2$, 0.200 mM UDP-galactose, 0.766 nCi UDP-galactose and 0.01 ml of a 4.5 mg/ml solution of a 2:1 mixture of Triton CF-54, and Tween 80 in a final volume of 0.050 ml of 100 mM cacodylate buffer, pH 6.8. Saturating acceptor concentrations were 0.200 mM, 1.03 mM, and 5.0 mM for GM_2, oligo-GM_2, and GlcNAc, respectively. Transfer of radioactivity to acceptors was determined by subjecting reaction solution to borate electrophoresis and measuring radioactivity remaining at the origin.

‡ The total activity of incubations that contained two acceptors, assuming that they did not compete for the same enzyme, was predicted by simple addition. The second value represents the predicted activity, if the acceptors were assumed to compete with one another, and was calculated by the method of Dixon and Webb (7).

§ Not determined.

and GM_2 as acceptors were not competitive. Thus, the same enzyme most likely catalyzed the transfer of Gal to both GM_2 and oligo-GM_2, and the latter may therefore be used as the exogenous acceptor for cell surface GM_1 synthetase assays.

Single-cell suspensions were prepared from 9-d neural retina and preincubated exactly as those cells that are used in the retinotectal adhesion assay. Intact cells catalyzed the production of oligo-GM_1 from oligo-GM_2 and UDP-gal, as measured by the kinetics of the formation of product remaining in incubation supernatants only in the presence of oligo-GM_2. A typical specific activity determination for the enzyme on intact cells using optimal incubation conditions is shown in Table II.

The presence of oligo-GM_1 in incubation supernatants could, however, result not from cell-surface enzyme activity but from enzyme released into the supernatant from partly or completely lysed cells. To test whether GM_1 synthetase activity itself was present in supernatants during the incubation period, the supernatant of a typical incubation of intact cells was measured for activity, and, in addition, an aliquot of the same cell suspension was sonicated and used as enzyme source in a standard incubation to determine what percentage of activity not stimulated by detergent was present on intact cells. These results are summarized in Table II. Less than 15% of total activity was present in the incubation supernatant following centrifugation. This centrifugation is not normally part of the assay protocol and is, itself, most probably responsible for release of some activity into the supernatant. Moreover, 25% of oligo-GM_1 synthetase activity present in sonicates not stimulated by detergents was detectable on intact cells.

As a further estimate of leakage of enzymes from lysed cells into cell incubation supernatants, lactate dehydrogenase activities of cell pellets and supernatants of incubations were determined. This soluble enzyme can presumably diffuse readily and should provide a maximum estimate of cell breakage. After a standard incubation of intact cells with oligo-GM_2, but without radioactive UDP-gal, cells were pelleted and the supernatant was removed. Cells were resuspended in PBS and aliquots from cells and supernatants were assayed for lactate dehydrogenase activity in the presence of 10% Triton X-100. Table II shows that ~10% of total cell pellet activity was present in incubation supernatants. This estimate of cell breakage agrees closely with that obtained by direct assay of incubation supernatants for oligo-GM_1 synthetase activity. After all cell-surface enzyme assays, >90% of the cells excluded trypan blue. These findings also suggest that only small amounts of oligo-GM_1 present in assay supernatants result from the action of internal GM_1 synthetase exposed by cell lysis.

If cell-surface GM_1 synthetase is involved in retinotectal adhesive specificity, its specific activity may vary along the dorsoventral axis of the retina (14). Therefore, the specific activity of this enzyme was measured on intact cells derived from either dorsal or ventral neural retinal fragments that had been prepared exactly as those cells that are used in the adhesion assay. During preincubation, the specific activity of the enzyme on cells from whole neural retina increased about threefold. (M. Pierce, data not shown). In addition, as a point of comparison, GlcNAc was used as an acceptor for Gal using aliquots from the same cell suspensions. The enzyme that transfers Gal to this acceptor and synthesizes N-acetyllactosamine has been shown to be present on neural retinal cell surfaces (17, 21). The results of a typical experiment are shown in Table III. Although the specific activity of GM_1 synthetase was some 1.4-fold higher than that of the enzyme that utilizes GlcNAc as acceptor, the specific activity of the ganglioside synthetase was greater on ventral than dorsal cells by <15%. This difference is near the limits of the accuracy of the assay itself. A large asymmetry in the specific activity of this cell-surface enzyme was not detected in cells from dorsal areas of the retina compared to those from ventral ones.

DISCUSSION

This study has demonstrated that intact chick neural retinal cells transferred Gal from UDP-gal to the oligosaccharide fragment from GM_2 and synthesized GM_1 oligosaccharide. The enzyme that catalyzed this transfer was most likely GM_1 synthetase since competition experiments using cell sonicates as the enzyme source suggested that the same enzyme transferred Gal to oligo-GM_2 and native GM_2. The results of Marchase (14) suggested that the oligosaccharide found in

TABLE II

Enzymatic Specific Activities of Intact Neural Retinal Cells, Supernatants of Intact Cell Incubations, and Disrupted Cells

	Source of activity		
Enzyme activity	Intact cells	Disrupted cells	Incubation supernatant
GM_1 synthetase* (pmol Gal transferred/2.0 × 10^7 cells · h)	84.0	316	11.6
Lactate dehydrogenase‡ (Δ absorbance units/2.0 × 10^7 cells · h)	ND§	44.3	3.95

* Single-cell suspensions of 8.5-d neural retinal cells were prepared by trypsinization and preincubated for 90 min. An aliquot of cells was then sonicated, and synthesis of oligo-GM_1 by both intact cells and sonicates measured in duplicate incubations after 90 min by borate electrophoresis. Another aliquot of single cells was incubated identically but without radioactive UDP-galactose, the cells were pelleted by centrifugation, and the supernatant was analyzed for synthesis of oligo-GM_1.

‡ An assay identical to that described for GM_1 synthetase activity on intact cells, but without radioactive UDP-galactose, was centrifuged after 90 min. The supernatant was removed, aliquots were diluted, and their LDH activities were determined by measuring the decrease of NADH absorbance at 340 nm. Cell pellets were resuspended in PBS and the activities of aliquots determined similarly. Triton X-100 (10%) was present in both LDH assays.

§ Not determined.

TABLE III

Galactosyltransferase Activities of Intact Cells from Dorsal and Ventral Neural Retinal Fragments

Acceptor	Specific activity*	
	Dorsal cells	Ventral cells*
	pmol Gal transferred 2.0×10^7 *cells* · *h*	
N-Acetylglucosamine	68	85
Oligo-GM_2	64	94

* Assays were conducted as described in Table II and contained either 5.0 mM N-acetylglucosamine or 1.0 mM oligo-GM_2. Cell number was determined by sonicating an aliquot of cell suspension, measuring the amount of protein present, and normalizing by use of a reference series of measurements that expressed amount of protein as a function of cell number.

ganglioside GM_2 functions in retinotectal adhesive specificity. In addition, experiments using antisera specific for GM_2 have shown the presence of GM_2 oligosaccharide on the surfaces of embryonic chick neural retinal cells (M. Pierce, manuscript submitted for publication).

The presence of GM_1 synthetase and oligo-GM_2 on the surfaces of neural retinal cells during neuromorphogenesis suggests that this enzyme may function in intercellular adhesion by binding to its acceptor, oligo-GM_2, on opposing surfaces. When glycosyltransferase activities were initially discovered on cell surfaces, their involvement in cell recognition and adhesion was immediately postulated (19–21). No unequivocal evidence, however, suggests that any glycosyltransferase serves this function. Rauvala and Hakomori (18) have recently presented evidence that a particular cell-surface enzyme-substrate interaction may be involved in the adhesion of NIL cells to culture dishes coated with ovalbumin. These authors demonstrated the presence of a cell-surface α-mannosidase activity and suggested that this enzyme can bind to mannose residues on the oligosaccharide side chains of the ovalbumin and mediate adhesion between the cells and the derivatized dishes, since a specific inhibitor of the α-mannosidase activity also inhibited the adhesion.

Studies by Gottlieb et al. (8) have demonstrated a gradient of adhesive specificity along the dorso-ventral axis of the chick neural retina. For example, cells from the dorsal-most area of the neural retina adhered with the greatest rate to cells from the ventral-most area. Cells from areas nearer to the dorsal pole adhered more slowly than those from areas located more ventrally. No information about the kinds of cell-surface molecules that produce this adhesive specificity has been available. Recently, Trisler et al. (30) showed that a monoclonal antibody bound to neural retina such that it detected a gradient of binding sites along the axis defined by the dorsoposterior and ventroanterior poles. The antibody, generated by immunizing with fragments of the dorsoposterior segment of the chick neural retina, bound at much higher levels to cells in the dorsoposterior segment than to cells in the ventroanterior segment. Preliminary experiments suggested that the binding site on the cells was protein in nature. An attempt to inhibit the binding of the antibody to the cells with a mixture of bovine brain gangliosides was unsuccessful, but the ability of this experiment to detect inhibition by minor gangliosides in the mixture, such as GM_2, is uncertain. It is possible that the cell-surface binding site for the antibody may function in the adhesive specificities observed between neural retinal cells, between these cells and the surfaces of the optic tectum, or both.

Experiments by Marchase (14) showed that protease-sensitive molecules crucial to retinotectal specificity were present more on ventral surfaces than on dorsal surfaces of neural retinal cells and tecta. These molecules appeared ~2 h after the initial trypsinization to produce single retinal cells, and their appearance after protease treatment was inhibited by various inhibitors of protein synthesis. Cell-surface GM_1 synthetase does increase in specific activity after incubation of previously trypsinized neural retinal cells, but little difference in specific activity could be detected between cells from dorsoventral retinal fragments assayed with or without preincubation. The inability to measure an asymmetrical distribution of the activity may be due to high and equal levels of activity on cells from both areas of the retina that have little affinity or specificity for binding to tectal surfaces. This possibility seems unlikely, however, since experiments by McClay et al. (13) that measured retinotectal adhesive specificity using aggregates of tectal cells rather than tectal halves showed that the majority of the neural retinal cells used in the assay displayed adhesion preferences.

An earlier study demonstrated an age- and time-dependent asymmetry of specific activity of GM_1 synthetase when sonicates of dorsal and ventral neural retinal cells were assayed in detergent using native GM_2 as the acceptor. These results were not obtained in this study using intact cells, no detergent, and oligo-GM_2 as the acceptor.

In conclusion, although GM_1 synthetase is located on intact neural retinal cells, no evidence suggests that this enzyme is more prevalent on the surfaces of ventral cells compared to those of dorsal cells.

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