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UDP-GALACTOSE: GLYCOPROTEIN GALACTOSYLTRANSFERASE ACTIVITY IN A CLONAL LINE OF RAT GLIAL TUMOR CELLS AND IN RAT BRAIN

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Summary

1. UDPgalactose:glycoprotein galactosyltransferase (EC 2.4.1.-) activity was demonstrated in homogenates from whole rat brain, isolated neuronal perikarya, enriched glial cell fractions, and cultured rat glial tumor cells (clone C_6).

2. Galactosyltransferase activity was enriched 3—9-fold in neuronal perikarya and 1.4—1.8-fold in the glial cell fraction over the activity in whole brains from 19- and 40-day-old rats. The activity of galactosyltransferase in neuronal perikarya decreased with age. Extensive contamination of the glial cell fraction with membranous fragments appeared to obscure the precise specific activity of this fraction.

3. The specific activity of the enzyme in glial tumor cells was 4—8-fold higher than in brain tissue when the enzyme was assayed under identical conditions using endogenous and different exogenous acceptors.

4. Galactosyltransferase activities from adult brain and glial tumor cells had similar properties. They both required Mn^{2+} and Triton, and exhibited pH optima between 5 and 7. The apparent K_m of the enzyme for UDPgalactose was $1.3 \cdot 10^{-4}$ M for brain tissue and $2.2 \cdot 10^{-4}$ M for glial tumor cells.

5. The high galactosyltransferase activity in glial tumor cells and in neuronal perikarya of younger rats is compatible with the possibility of a role of this enzyme in developing brain.

Introduction

Some of the cerebral glycoproteins and glycosyltransferases may have a

Abbreviations: MES-buffer, 2-(*N*-morpholino)ethanesulfonic acid; DSG-fetuin, fetuin treated to remove sialic acid and galactose.

role in intercellular recognition [1—3] and glial cell function during myelination [4]. It is of interest, therefore, to study the enzymes for glycoprotein synthesis in brain tissue. A number of reports have appeared on the biosynthesis of brain glycoproteins and on the properties of some of the glycosyltransferases involved in brain glycoprotein synthesis [5,6]. The cerebral glycoproteins are associated with the membranes of synaptosomes, microsomes and glial processes and comprise about 5—12% of all cerebral proteins [7]. In this communication we have examined the cellular localization and some of the properties of UDPgalactose:glycoprotein galactosyltransferase in normal rat brain and in cultured rat glial tumor cells.

Materials and Methods

Growth of glial tumor cells

Glial tumor cells (clone C_6) from a rat astrocytoma [8] were grown in spinner cultures at 36.5° C to saturation densities of about $5 \cdot 10^5$ cells per ml. Cells were grown in Eagles' minimal essential medium modified for spinner culture [9] and supplemented with heat-inactivated horse serum (15%), heat-inactivated fetal calf serum (2.5%), penicillin (200 units per ml) and streptomycin (200 μg per ml). Cultures were screened for aerobes, anaerobes and mycoplasma to ensure that they were free of contamination.

Preparation of tissue homogenates

Adult male rats were anesthetised with sodium pentobarbital, the brains were removed and homogenized in 2.5 vol. of 0.35 M sucrose with 5—10 strokes in a Potter-Elvehjem homogenizer equipped with a teflon pestle. The homogenate was filtered through two layers of cheese cloth before use. Trial experiments showed that brain homogenates prepared in either distilled water or in sucrose had almost identical enzyme activity. Glial tumor cells (10^8) were harvested by centrifugation at 600 × *g*, washed with buffered saline and homogenized in 1 ml distilled water using a Dounce homogenizer with tightly fitting pestle. Glial tumor cells were treated in distilled water to affect uniform homogenization. Freshly prepared homogenates were used for the enzyme assays.

Preparation of nerve cell bodies and glial cells of rat brain

Nerve cell bodies and glial cells were prepared from rat brains by the procedure of Selinger et al [10]. Briefly, the minced cerebral cortices (7—8 g in 15 ml of ice-cold solution of 7.5% polyvinylpyrrolidone (w/v), 1% bovine serum albumin (w/v) and 10 mM $CaCl_2$ were passed through one thickness of nylon bolting cloth of 333 μm pore size stretched over the open end of a truncated disposable syringe. The tissue was sieved further through two layers of nylon cloth, 333 μm and 110 or 73 μm pore sizes. The filtrate was layered on a two-step gradient (6 ml of 1.0 M sucrose and 5 ml of 1.75 M sucrose, prepared in 1% bovine serum albumin) and centrifuged at 20 000 rev./min for 30 min in a Spinco SW 25.1 rotor. Neuronal perikarya were obtained after the first centrifugation. The bands of impure glial cells were diluted and layered on a gradient of 3 ml of 30% Ficoll (w/v), 6 ml of 1.2 M sucrose and 5 ml of 1.65 M sucrose, all prepared in 1% bovine serum albumin, and the tubes were

centrifuged at 20 000 rev./min for 30 min. The pellets recovered after this centrifugation were mixtures of neurones and capillaries. Glial cells mixed with capillaries at the 1.2—1.65 M sucrose interface were diluted with 0.32 M sucrose and layered on a gradient of 1.3 M and 1.65 M sucrose and centrifuged at 5000 rev./min for 20 min. Finally, glial cells were collected from the 1.3—1.65 M sucrose interface. Neuronal perikarya and glial cells isolated from the brain cortex were examined by phase contrast microscopy. The perikarya cells appeared intact but the glial cells were considerably disintegrated, as was noted previously [10].

Assay of UDPgalactose:glycoprotein galactosyltransferase

Unless otherwise specified, each complete enzyme assay mixture (total volume, 100 μl) contained tissue homogenate, approximately 0.75 mg protein; 5 nmol UDP[^{14}C]galactose (0.02 μCi, 26 000 cpm); 12.5 μmol MES-buffer, pH 6.8; 1.25 μmol $MnCl_2$; 60 μmol dithiothreitol; 0.25 mg DSG-fetuin (described below) and 1% Triton X-100. The reaction was incubated for 60 min at 30° C and terminated by the addition of 1 ml of 10% trichloroacetic acid/2% phosphotungstic acid solution. The precipitate was filtered under suction through glass fiber filters (Reeve Angel 934-AH), and was washed with a large excess of cold 5% trichloroacetic acid/1% phosphotungstic acid containing 0.5% galactose, with ethanol/ether (1 : 1, v/v) and then with ether. The incorporation of radioactive galactose was measured on dried filter discs in a toluene-based scintillation solution [11]. In separate experiments, the standard assay mixture was increased 10-fold and incubated for 1 h. Acid precipitable or non-dialysable radioactive products from incubations of glial cell homogenates were hydrolysed by treating with 4 M HCl for 4 h at 100° C or by incubating for 3 h at 37° C with β-galactosidase (from *Clostridium perfringens*) in a citrate /phosphate buffer, pH 5.6, by a procedure described before [12]. The only radioactive product found in the hydrolysates after ion-exchange and paper chromatography [13], was galactose. In one experiment, when *N*-acetylglucosamine was used as acceptor for galactose, the incubation was terminated by addition of 0.010 ml 10% sodium tetraborate containing 0.25 M EDTA and aliquots were subjected to high voltage electrophoresis for the assay of radioactive *N*-acetyllactosamine [14], the product of the reaction.

Preparation of DSG-fetuin

Sialic acid was removed from fetuin by mild acid hydrolysis. Sequential degradation of the monosaccharides of fetuin was achieved by periodate oxidation followed by sodium borohydride reduction and mild acid hydrolysis as described by Spiro [15]. Using this method, only hexosamines and mannose remained attached to the peptide portion of fetuin.

Protein assays

Protein was assayed by the method of Lowry et al. [16] using bovine serum albumin as standard.

Source of materials

Culture media and sera were purchased from Grand Island Biologicals;

antibiotics from Schwarz/Mann. All nucleotides, ovalbumin, Triton X-100 and polyvinylpyrrolidone were purchased from Sigma (St. Louis, Mo.); fetuin from Calbiochem. (La Jolla, Calif.); bovine serum albumin from Armour (Chicago, Ill.); Ficoll ($M_r \approx 400\,000$, from Pharmacia (Uppsala, Sweden) and U-^{14}C-labeled UDP-galactose (254.5 Ci/mol) from New England Nuclear Co. (Dorval, P.Q.). Nylon boltings were purchased from B. and S.H. Thompson and Company, Scarborough, Ontario.

Results

Properties of UDPgalactose:glycoprotein galactosyltransferase in rat brain and glial tumor cell homogenates

Mn^{2+} was required for optimum galactosyltransferase activity in brain and tumor cell homogenates (Table I). Mg^{2+} or Ca^{2+} could not replace Mn^{2+}. Triton markedly stimulated the enzyme activity in both brain (8-fold) and glial cell (25-fold) homogenates. Dithiothreitol was required only marginally.

Both DSG-fetuin and the low molecular weight acceptor *N*-acetylglucosamine showed excellent acceptor properties for galactosyltransferase (Table II). Native fetuin and ovalbumin were less active. Consequently, DSG-fetuin was used for subsequent experiments. The enzyme activity was found to be 4- to 8-fold higher in glial tumor cells than in brain tissue when their activities were measured with endogenous and different exogenous acceptors.

In both brain and glial tumor homogenates, enzyme activity increased with time for 60 min (Fig. 1a) and with enzyme protein concentration up to about 400—500 μg (Fig. 1b). The optimum pH for both brain and glial tissue enzymes, using three different buffer systems for assay, appeared to be within a pH range of 5 to 7 (Fig. 2). Galactosyltransferase activities in brain and glial cell homogenates were saturated at concentrations of 350—450 μM of UDP-galactose. The apparent K_m values for substrate were calculated to be $1.3 \cdot 10^{-4}$ M and $2.2 \cdot 10^{-4}$ M for glial tissue and brain homogenates, respectively (Fig. 3). The difference in the K_m values is quite small considering the crude enzyme preparations used for the assays.

TABLE I

REQUIREMENTS FOR UDP-GALACTOSE : GLYCOPROTEIN GALACTOSYLTRANSFERASE ACTIVITY IN RAT BRAIN AND GLIAL TUMOR CELL HOMOGENATES

Enzyme activity was assayed as described in the text.

Reaction mixture	Enzyme activity (pmol [^{14}C] galactose/mg protein/h)	
	Brain	Glial tumor cell
Complete	588	3132
−Dithiothreitol	540	2597
$-Mn^{2+}$	32	72
$-Mn^{2+} + Mg^{2+}$	38	42
$-Mn^{2+} + Ca^{2+}$	22	47
−Triton	40	123

TABLE II

GALACTOSYLTRANSFERASE ACTIVITY IN RAT BRAIN AND GLIAL TUMOR CELL HOMOGENATES: EFFECT OF DIFFERENT EXOGENOUS ACCEPTORS

Enzyme assay procedures are described in the text.

Acceptor	Enzyme activity (pmol/mg protein/h)		Ratio of enzyme activity (glial cell/brain)
	Brain	Glial cell	
Endogenous	34	162	4.76
Fetuin (0.5 mg)	250	975	3.90
DSG-fetuin (0.25 mg)	407	2516	6.18
DSG-fetuin (0.5 mg)	541	2901	5.36
Ovalbumin (0.5 mg)	206	1752	8.50
Ovalbumin (1 mg)	268	1743	6.50
N-Acetylglucosamine (10 mM)	600	3450	5.76

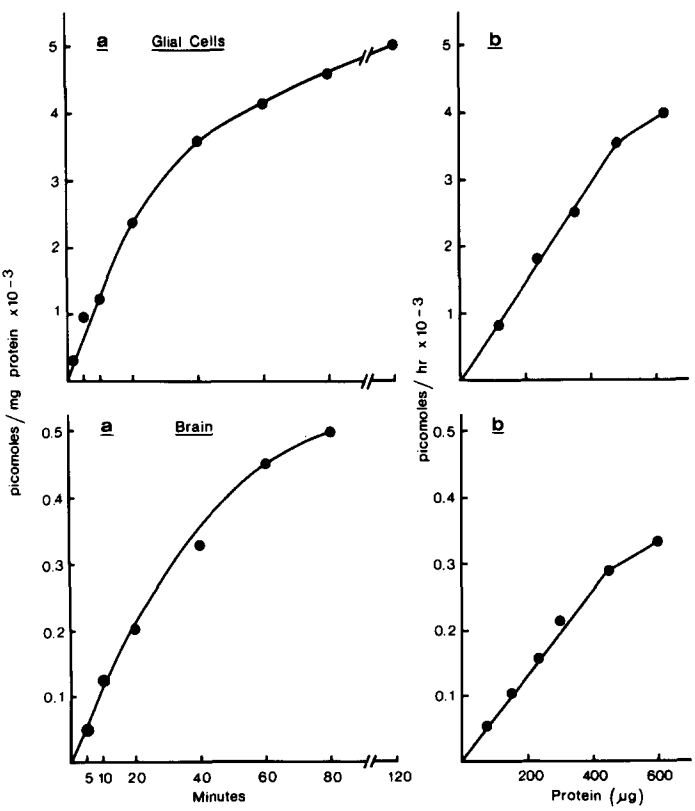

Fig. 1. Galactosyltransferase activity in glial cell (upper panel) and brain (lower panel) homogenates assayed with DSG-fetuin as exogenous acceptor. Dependence of time (a) and enzyme protein (b) concentrations are shown.

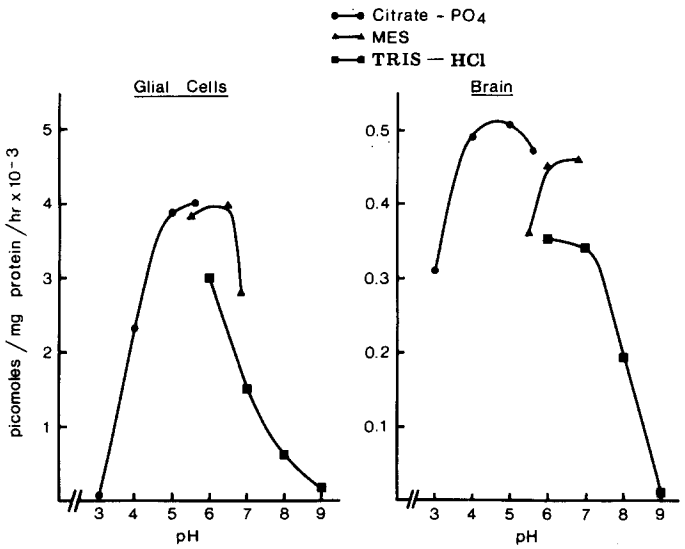

Fig. 2. Effect of different buffers and pH on the galactosyltransferase activity in glial cells (left panel) and brain (right panel), assayed with DSG-fetuin as exogenous acceptor.

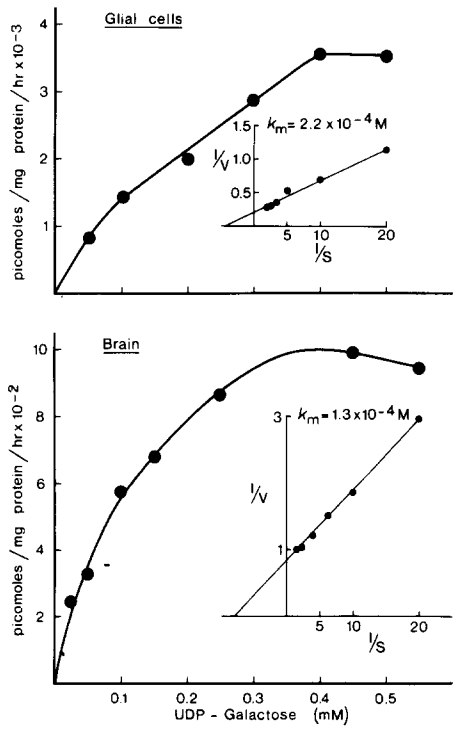

Fig. 3. Effect of varying concentrations of UDPgalactose on the galactosyltransferase activity in glial tumor cells (upper panel) and brain (lower panel) homogenates, assayed with DSG-fetuin as exogenous acceptor. The insets show the data plotted by the method of Lineweaver and Burk [25].

Effect of nucleotides on galactosyltransferase activity in glial tumor cell and brain homogenates

The galactosyltransferase activity in glial cells was slightly stimulated (10—60%) by small doses of nucleotides (Table III). CTP appeared to be the most effective nucleotide examined. In contrast, the enzyme activity in brain tissue homogenates was not stimulated by these nucleotides. In higher doses (1—2 mM), some of the nucleotides inhibited the enzyme activity. Using enzyme assay conditions similar to those used here, nucleotides have been shown to stimulate galactosyltransferase activity (3—8-fold) in homogenates and microsomes of rat liver [11,17,18]. Such stimulation of the enzyme activity by some of the nucleotides have been shown to be mainly due to an inhibition of UDPgalactose pyrophosphatase activity thereby increasing the amount of substrate available for glycoprotion synthesis [18]. Lack of any marked stimulation of the enzyme by nucleotides in the brain and glial tumor cells suggest that UDPgalactose pyrophosphatase activity may be present only in small amounts in these tissues.

Galactosyltransferase activity in nerve cell bodies and glial cells of normal rat brain

Galactosyltransferase activities were examined in brain cells isolated from normal rats of 19 and 40 days of age (Table IV). The highest relative activity was found in neuronal perikarya of 19-day-old rats (about 9-fold compared to total brain). Galactosyltransferase activity in neuronal perikarya appeared to decrease with age. Neuronal capillaries also showed high enzyme activity in both 10- and 40-day-old rats. The relative enzyme activity in glial cells was only 1.4—1.8-fold higher than in total brain homogenates.

TABLE III

EFFECT OF NUCLEOTIDES ON GALACTOSYLTRANSFERASE ACTIVITY IN GLIAL CELL AND BRAIN HOMOGENATES

Enzyme assay procedures are described in the text. DSG-fetuin was used as exogenous acceptor.

Nucleotide concentration (mM)	Enzyme activity (pmol/mg protein/h)			
	CDPcholine	CTP	ATP	GTP
Glial cells				
0	2233	2233	2233	2233
0.25	2942	3675	2568	2533
0.5	2995	3740	2767	2700
1.0	3333	3771	2891	2997
2.0	3527	3107	3564	3351
Brain				
0	556	556	556	556
0.25	596	549	572	535
0.5	546	520	591	512
1.0	529	477	475	487
2.0	500	406	420	439

TABLE IV

GALACTOSYLTRANSFERASE ACTIVITY IN NERVE CELL BODIES AND GLIAL CELLS OF RAT BRAIN

Enzyme assay* and cell preparation methods are described in the text.

Cell preparations	Relative enzyme activity**	
	19-day-old rat	40-day-old rat
Brain homogenate	1	1
Glial cells	1.8	1.38
Neuronal capillaries	6.0	5.86
Neuronal perikarya	8.57	3.37

* DSG-fetuin was used as exogenous acceptor.

** Values are the averages of three experiments. The enzyme specific activities (pmol [^{14}C]galactose/mg protein/h) of brain homogenates were 524 and 400 for 19- and 40-day-old rats, respectively.

Discussion

The occurrence of UDPgalactose:glycoprotein galactosyltransferase was shown in a clonal glial tumor cell line. The properties of galactosyltransferase from glial tumor cells were similar to those of the enzyme from normal brain tissue. The specific activity of the enzyme in glial tumor cell homogenates was, however, about 4—8 times higher than in adult rat brain when the enzymes were assayed under identical conditions. The glial cell fraction isolated from normal rat brain appeared considerably contaminated and perhaps gave an underestimated value of glial specific enzyme activity, whereas the glial tumor cells were harvested from stationary viable suspension cultures. Furthermore, though tumorogenic in nature, the clonal glial cell line appears to be reasonably well differentiated [8,19,20]. Therefore, the increased relative activity of the enzyme in glial cell rich fractions of normal rat brain and in a pure glial tumor cell line suggest that galactosyltransferase activity may have a physiological function in these cells. The glycosyltransferase enzymes are required for the biosynthesis of glycoproteins and glycolipids. The carbohydrate residues in brain glycoproteins and glycolipids have been implicated in a number of important functions, including information processing events [21], intercellular adhesion [22], and development of the embryonic nervous system [23]. Therefore, the presence of high activity of galactosyltransferase enzyme in glial cells may be compatible with a role of the glial enzyme in regulating some of these intra- and intercellular events during development.

Recent work has shown that high amounts of galactosyltransferase activity present in embryonic chicken [23] and rat [17] brains bear a relation to their development. It was also of interest that a soluble form of this enzyme is present in embryonic chicken brain [23]. In contrast, in our experiments (unpublished) when the glial tumor cell homogenates were centrifuged for 1 h at 105 000 × *g* and the galactosyltransferase activity was measured with DSG-fetuin in particulate and soluble fractions, almost 90% of the enzyme activity was recovered in particulate fraction. However, these results are in agreement

with our previous report that in developing rat brain galactosyltransferase activity is mostly membrane-bound [17].

The high activity of galactosyltransferase in neuronal perikarya and the dependence of the activity upon age (Table IV) also are interesting in relation to the role of this enzyme in brain development. Furthermore, the mass of neuronal perikarya decreases in direct proportion to the age of the animals and it has been shown that its percentage yield decreased from 42.4% at 5 days of age to 0.68% at 43 days [10]. These observations are consistent with the possibility that glycosyltransferases have a role during development of the brain in early post-natal days when both the mass of neuronal perikarya and glycosyltransferase activities are relatively high. In another study [24], CMPsialic acid: glycoprotein sialyltransferase activity in neuronal cells of rats showed a similar dependence upon age.

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