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GLYCOSYLTRANSFERASE ACTIVITIES AND THE DIFFERENTIATION OF HUMAN PROMYELOCYTIC (HL60) CELLS BY RETINOIC ACID AND A PHORBOL ESTER

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The activities of five glycosyltransferases were measured following the induction of HL60 cells to differentiate to mature myeloid forms or to macrophages by the addition of retinoic acid or a phorbol ester, respectively. Gal-T-II, Fuc-T-I and (NeuAc-T-I) are all increased and Fuc-T-II decreased in activity upon treatment with RA. Gal-T-I and Fuc-T-II are decreased and Gal-T-II increased in activity upon with TPA treatment. The increases in enzyme activities with RA are measurable as early as 1 day but while Fuc-T-I and NeuAc-T-I are fully elevated at 2 days, Gal-T-II shows a biphasic rise with initial elevation by day 2 and a further rise at days 3 to 5. The rises in Gal-T-II are due to increases in the enzyme form present in uninduced cells.

Studies on the reversion of the malignant myeloid leukemic phenotype by both physiologic and non-physiologic inducers can provide insight into both the normal mechanism of differentiation and the origin and development of tumors whilst continuously proliferating cell lines provide a reproducible source of material which accurately reflects many aspects of normal hemopoiesis (1). A cell line (HL60) established from a patient with acute promyelocytic leukemia (2) can be induced to differentiate to more mature myeloid cells with retinoic acid (RA) (3) and to macrophages with phorbol esters (4,5).

Cell surface glycoproteins may change and play important roles in differentiation. However relatively little is known about alterations in the cell surface during differentiation in human myeloid and monocytic precursor cells. HL60 differentiation to mature myeloid cells or macrophages leads to the appearance of antigens reacting with monoclonal antisera to peripheral blood granulocytes (6) or to heterologus monocyte antiserum (7),

*Abbreviations used are: RA, retinoic acid; TPA, 12-O-tetradecanoylphorbol 13-acetate; CT, cholera toxin; OSM, ovine submaxillary gland mucin; Gal-T-I & II, galactosyltransferases I & II; Fuc I & II, fucosyltransferases I & II; NeuAc-T-I, sialyltransferase I; MEL-179, tetraploid mouse erythroleukemia cells.

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respectively. A 130 kilodalton glycoprotein appears upon myeloid differentiation and several major glycoproteins are lost (8,9) while there is a decreased synthesis of high molecular weight glycopeptides upon TPA treatment (10). Retinoic acid is known to alter cell surface glycoprotein metabolism (11,12) and in some cases this alteration appears to be a specific effect upon the addition of distal sugars (13). In HL60 cells, RA increases sialidase activity (14) but there have been no turnover studies on the sugar chains of surface glycoproteins. Sugar moieties are added to the incomplete glycoprotein acceptor by glycosyltransferase enzymes utilizing nucleotide sugars as the donor molecules (15) and in most instances there appear to be individual enzymes for each specific linkage formed with each sugar. Changes in the activity of glycosyltransferases have been described upon the transformation, altered proliferation and differentiation of cells (16). In view of the potential importance of glycosyltransferases we have studied the activity of five of these enzymes in HL60 cells induced to differentiate along different pathways by RA and TPA.

MATERIALS AND METHODS

Materials. Ovine submaxillary glands were obtained from Pel-Freez (Rogers, AZ). Fetuin and fetal bovine serum were products of GIBCO (Grand Island, NY). UDP-[6-^{3}H]Gal (16.3 Ci/mmol); GDP[U-^{14}C]Fuc (157 mCi/mmol)and GDP-[1-^{14}C]Fuc (16.6 mCi/mmol) were purchased from Amersham (Arlington Heights, IL) and CMP[9-^{3}H]NeuAc (18.9 Ci/mmol) purchased from New England Nuclear (Boston, MA). All other chemicals were obtained from Sigma Chemical Co. (St. Louis, MO). Asialofetuin (fetuin, - NeuAc) and asialo-agalacto-fetuin (fetuin, - NeuAc- Gal) were prepared as described by Spiro (17). OSM was purified as described by Hill et al.(18) and desialated by acid hydrolysis. CMP-NeuAc was prepared as previously described (19). Tetraploid mouse erythroleukemia cells, line 179 (MEL179) were a generous gift of Dr. A. Deisseroth. Difluoromethylornithine was donated by Merrell International Labs.

Cell Culture. HL60 and MEL179 cells were grown in RPMI 1640 supplemented with 10% fetal bovine serum after subculturing at initial densities of 1 x 10^{5} and 5 x 10^{4} cells/ml, respectively. After 2-3 generations induction of the cells was performed by adding RA(1 μm) or TPA (64 nM) to HL60 cells and continuing culture for a further 4 or 2 days, respectively and adding DMF(120 mM) to MEL-179 cells and culturing for a further 5 days. The proportions of HL60 cells differentiated to mature myeloid cells and macrophages were monitored by determining cells with nitroblue tetrazolium reducing and non-specific esterase activities, respectively (5). Differentiation of MEL-179 to hemoglobin containing cells was quantitated using a benzidine peroxide stain [20].

Enzyme Assays. The cells were harvested, washed and broken open as previously detailed (20) except that the buffer used was MOPS [3-(N-Morpholino)-propanesulfonic acid],pH 7. Enzyme activity was determined in the whole homogenate after quantitating protein by the method of Bradford (21) and preincubating the homogenate with Triton X-100 at the concentrations indicated in Table I for 1 h at 4°C. The transfer of radioactively-labelled sugar to glycoprotein acceptor was measured using a paper disc assay (19, 22). All assays were carried out in duplicate in a volume of 120 μl with all assays containing 40 mM MOPS and incubation at 37° following initiation of the reaction by adding enzyme (25-150 μg protein).

RESULTS AND DISCUSSION

With only a very few exceptions it appears that individual glycosyltransferases are able to form only a single specific linkage between two specific sugars (15). By the combined use of glycoprotein acceptors chemically modified to reveal internal sugars moieties and assay conditions optimal for a single enzyme activity it is possible to devise assays in which one is looking at individual enzyme activities. The conditions employed to assay five glycosyltransferases are detailed in Table I and the enzymes assayed are: two forms of UDP-galactose: glycoprotein galactosyltransferase catalyzing the formation of Gal β(1->3)GalNAc [Gal-T-1] and Gal β(1->4)GlcNAc [Gal-T-II]; two forms of GDP-fucose; glycoprotein fucosyltransferase synthesizing Fuc α(1->2)Gal [Fuc-T-I] and Fuc α(1->3)GlcNAc [Fuc-T-II]; and CMP-N-acetylneuraminic acid; glycoprotein sialyltransferase producing the linkage NeuAc α(2->6)Gal [NeuAc-T-I]. In order to allow a better differentiation between Fuc-T-I and Fuc-T-II use was also made of the differential sulfhydryl group requirement of these enzymes as decribed by Chou et al.(23). Fuc-T-I is insensitive to N-ethylmaleimide (3mM) while Fuc-T-II is strongly activated by dithiothreitrol (1mM) and inactivated by N-ethylmaleimide (3mM); therefore Fuc-T-II activity is taken as that activity inhibitable by 3mM N-ethylmaleimide.

HL60 cells contain high levels of galactosyltransferase activities, especially Gal-T-II and considerably lower levels of the other transferases assayed (Table II). NeuAc α (2->6)Gal and NeuAc α (2->6)GalNac sialyltransferase activities were also measured using the assay conditions of Sadler et al. (24) and found to be less than 10% of the activity of NeuAc-T-I.

TABLE I. Conditions used for the assay of glycosyltransferase activities.

Transferase	Nucleotide Sugar	Glycoprotein Acceptor	Pyrophos-phatase Inhibitor	Divalent cation	pH	Triton X-100	Assay Time
	μM	μg	mM	mM		%	min
Gal-T-I	UDP-Gal [250]	OSM(-NeuAc) [150]	NAD [1]	Mn^{2+} [7.5]	6.5	0.5	60
Gal-T-II	UDP-Gal [50]	Fetuin (-NeuAc, -Gal)[400]	ATP [1]	Mn^{2+} [15]	6	0.75	30
Fuc-T-I	GDP-Fuc [20]	Fetuin (-NeuAc, -Gal)[400]	NAD [3]	Mg^{2+} [2]	7	0.2	20
Fuc-T-II	GDP-Fuc [10]	Fetuin (-NeuAc) [300]	NAD [1]	Mn^{2+} [7.5]	7.5	0.2	20
NeuAc-T-I	CMP-NeuAc [50]	Fetuin (-NeuAc) [400]	--	Mn^{2+} [6]	6	0.5	60

TABLE II. Glycosyltransferase Activities in HL60 and MEL 179 cells

TRANSFERASE	CELL TYPE	
	HL60	MEL-179
	pmol glycose transferred/min/mg protein	
Gal-T-I	22.8 ± 1.8	72.5 ± 0.5
Gal-T-II	120 ± 19	457 ± 56
Fuc-T-I	6.2 ± 0.9	36.9 ± 3.7
Fuc-T-II	3.2 ± 0.1	<0.2
NeuAc-T-I	2.6 ± 0.6	0.6 ± 0.2

Results are the average of 4 (HL60) or 3 (MEL-170) expts ± S.E.M., for exponentially growing cells.

Although sialyltransferase activities in some tissues are independent of a divalent cation (24) the activity in HL60 extracts was strongly dependent upon added Mn^{2+}. As a comparison with another cell type which can be induced to differentiate in vitro we have used tetraploid mouse erythroleukemia cells. These cells contain higher levels of galactosyltransferases and Fuc-T-I but very low levels of Fuc-T-II and NeuAc-T-I.

The differentiation of HL60 cells to more mature myeloid forms by RA results in significant increases in Gal-T-II, Fuc-T-I and NeuAc-T-I activities (Table III). These enzymes are also elevated when the cells are differentiated to macrophages by TPA but the elevations are smaller and only significant at the level P=0.5 with Gal-T-II. Fuc-T-II activity is significantly reduced by both inducers but a reduction in Gal-T-I activity is specific to differentiation along the macrophage pathway. Markers of HL60 differentiation indicated that both RA and TPA caused more than 85% differentiation in all experiements. In contrast to these results the differentiation of MEL-179 cells with DMF did not results in significant changes in any of the glycosyltransferase activities. Therefore the occurence of changes in glycosyltransferase activities is not essential in all differentiation pathways. Furthermore the different patterns of change in glycosyltransferase enzymes induced by RA and TPA suggest that in HL60 cells there are specific alterations related to the pathway of differentiation.

Differentiation is concomitant upon a cessation of proliferation. It has been shown that difluoromethylornithine causes HL60 cells to stop proliferation but neither induces differentiation nor prevents the differentiation induced by RA or TPA (25). Cells were therefore treated with 5 mM Difluoromethylornithine for 2 days to arrest proliferation and the levels

TABLE III. Effect of Inducers of Differentiation upon Glycosyltransferase Activities

TRANSFERASE	HL60				MEL 179
	RA	TPA	4-O-MeTPA	CT	DMF
	% Control Activity				
Gal-T-I	114 ± 18	62 + 1[a]	59	97	145 ± 34
Gal-T-II	356 ± 30[a]	204 ± 20[a]	78	363	89 ± 11
Fuc-T-I	195 ± 19[a]	148 ± 32	74	169	107 ± 30
Fuc-T-II	53 ± 16[a]	58 ± 6[a]	100	65	n.d
NeuAc-T-I	238 ± 58[a]	173 ± 38	102	204	92 ± 6

[a] $P<0.05$

Results are the average of 4 (HL60) or 3 (MEL-179) experiments ± S.E.M. for RA, TPA and DMF and of two experiments for 4-O-MeTPA and CT.

of glycosyltransferase enzymes compared to these of proliferating HL60 cells. There were no significant changes in glycosyltransferase activity (not shown) indicating that the alterations produced by RA and TPA are not simply the result of arrested proliferation. HL60 cells can also be converted to mature myeloid forms by compounds which raise intracellular cyclic AMP levels such as prostaglandin E and cholera toxin (26). Table III shows that CT is able to produce similar effects upon glycosyltransferase activities as those brought about by RA. Therefore the changes appear to be specific to the process of differentiation and not dependent upon the chemical used to induce differentiation. 4-O-Methyl-TPA is an analog of TPA which does not exhibit its tumor promoting activities and is unable to cause the conversion of HL60 cells to macrophages. The changes in Gal-T-II, Fuc-T-I, Fuc-T-II, and NeuAc-T-I caused by TPA are not micked by 4-O-M-TPA suggesting that these alterations are part of the differentiation process. In contrast the fall in Gal-T-I is caused by 4-OMe-TPA suggesting either that this is not a differentiation dependent alteration or that 4-O-Me-TPA could cause some of the early events of differentiation and that the change in Gal-T-I is one such early event. It has been suggested that 4-O-Me-TPA is not a satisfactory negative control for TPA (27) as it does indeed have a number of positive effects and this enzyme is currently under investigation with other TPA analogs.

It takes at least five days for the full expression of the differentiated characteristics induced by RA although a significant proportion of cells will

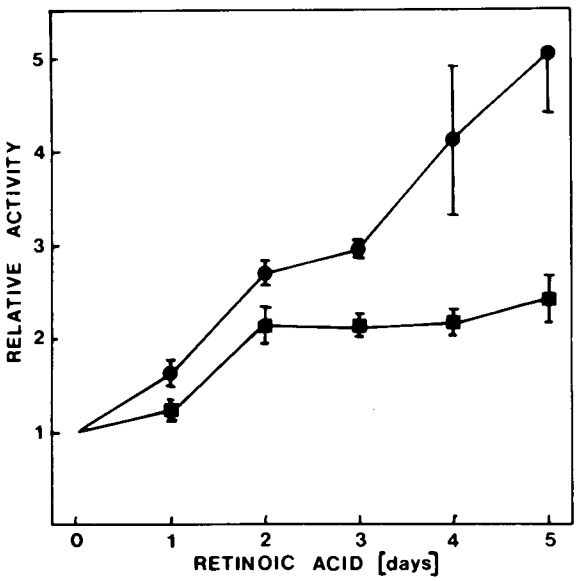

Figure 1. Time course of changes in glycosyltransferase activities in HL60 cells upon induction of differentiation with retinoic acid. ● , Gal-T-II; ■ ,NeuAc-T-I. Enzyme activity was measured in a Triton-solubilized fraction obtained from the total particulate material of an HL60 homogenate.

show nitroblue tetrazolium reduction, an early marker of myeloid differentiation, by 48 h. We therefore, studied the time course of changes in Gal-T-II and NeuAc-T-I activities (Fig. 1). NeuAc-T-I activity was maximally induced by two days as was Fuc-T-I (not shown). Gal-T-II activity also showed an initial rise of nearly 3-fold by two days and a leveling off of activity between days 2 and 3 but this was followed by a further rise in activity between days 3 and 5. It is clear from these results that the changes in glycosyltransferase activity occur very early in the sequence of events leading to differentiation.

In view of the biphasic change of Gal-T-II activity it was thought possible that a new form of this enzyme might be being induced. The effect of varying the UDP-Gal concentration upon the enzyme from control and 3 and 5 day RA-induced cells was therefore investigated (Fig. 2). All three enzymes gave an identical Km for UDP-Gal of 1.3×10^{-5}M and the increase in enzyme activity therefore seems to be likely to be due to a biphasic increase in the level of a single enzyme form.

In conclusion we have demonstrated that there are changes in the glycosyltransferase activity profile of HL60 upon RA or TPA treatment and that changes are specific to the differentiation pathway and are early events in the response to differentiation agents. We are now attempting to relate these changes to alterations in the synthesis of glycoproteins during

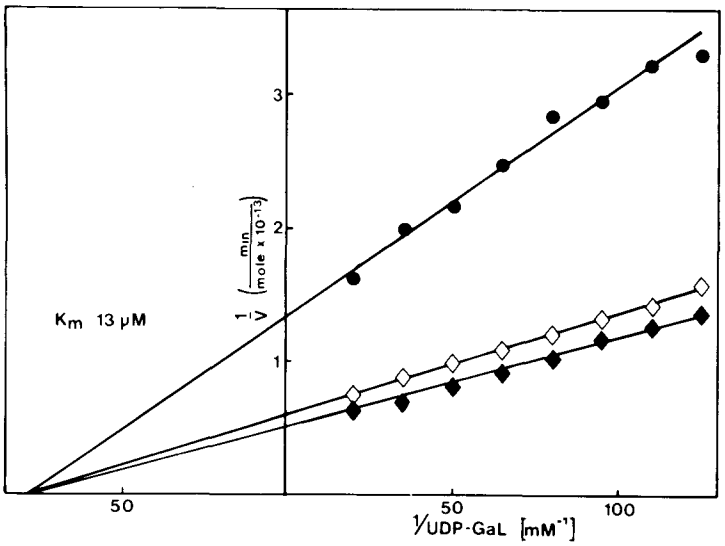

Figure 2. Kinetic analysis of the UDP-galactose requirement of Gal-T-II from control and retinoic acid induced HL60 cells. ● ,control cells; ◇ , 3 day RA treatment; ◆ ,5 day RA treatment.

differentiation and especially to investigate whether any of the alterations occur in enzymes localized at the plasma membrane.

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