GLYCOPROTEIN BIOSYNTHESIS. SUBCELLULAR LOCALIZATION AND ACTIVITY IN 3T3 AND SV-3T3 FIBROBLASTS OF GLYCOPROTEIN. *N*-ACETYLGLUCOSAMINYL TRANSFERASES*

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1. Introduction

Previous work has indicated that certain enzymes, membrane glycoprotein glycosyl transferases, which are responsible for the addition of carbohydrate residues onto membrane glycoproteins were present to a greater extent in oncogenic virus transformed fibroblasts than in the normal cells from which they were derived [1]. Furthermore these enzymes were thought to be located within the cell in the smooth endoplasmic reticulum or Golgi region of the cell [2]. Recently a highly specific glycoprotein: *N*-Acetylglucosaminyl transferase has been described [3] which catalyses the addition of *N*-acetylglucosamine from UDP-*N*-acetyl glucosamine onto a defined macromolecular acceptor prepared from fetuin. This enzyme is of particular importance because of the implication of abnormal *N*-acetylglucosamine residues on the surface of polyoma virus transformed BHK cells (PY-BHK) as opposed to normal BHK cells [4]. Recent studies have indicated that the glycoprotein: *N*-Acetylglucosaminyl transferase levels are elevated in the PY-BHK cell line compared to the BHK cell line [5]. This communication demonstrates that the glycoprotein: *N*-Acetylglucosaminyl transferase levels are elevated in Simian virus-transformed fibroblasts compared to normal fibroblasts and that the enzyme is distributed primarily in the smooth endoplasmic reticulum of HeLa cells.

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2. Methods

UDP-*N*-Acetylglucosamine-^{14}C (sp. ac. 40 Ci/Mole) was purchased from New England Nuclear Corporation. The highly specific acceptor for the assay of the fetuin: *N*-Acetylglucosaminyl transferase was prepared from fetuin [6] as previously described [3]. The acceptor was formed by acid desialization, removal of galactose with β-galactosidase, and removal of *N*-acetylglucosamine by treatment with either epididymal or jack bean meal hexosaminidase or by periodate oxidation and hydrolysis. It was demonstrated [3] that with this acceptor, under the assay conditions described below, a specific *N*-acetylglucosamine-(mannose) glycosidic linkage was formed. In every instance endogenous (without acceptor) and exogenous (with the fetuin acceptor) glycoprotein: *N*-Acetylglucosaminyl transferase activity was measured. In all instances any endogenous activity has been substracted from the presented exogenous activity; thus the exogenous activity represents fetuin: *N*-Acetylglucosaminyl transferase activity. The complete assay for endogenous activity contained: 100 μl of the 0.1% Triton X-100 extract or HeLa membrane fraction (enzyme source), 10 μl of 0.1 M $MnCl_2$, 50 μl of glass distilled water, and 10 μl of the UDP-*N*-acetylglucosamine-^{14}C (2 nCi, approx. 60 pmoles) to a final volume of 0.170 ml. pH adjustments to 5.6 were made with tris buffer. The

incubation was 37° for 1 hr, after which radioactivity was determined by methods previously described [20], precipitating the protein-bound radioactivity with 1% phosphotungstic acid in 0.5 N HCl. The pellets after centrifugation at 2500 *g* for 2 min, were washed three times with 10% trichloroacetic acid, extracted once with diethyl ether: ethanol (1:2), dried, dissolved in 1.0 N NaOH, plated onto glass filters, and the radioactivity measured in a liquid scintillation counter. The complete assay system for exogenous activity contained to a final volume of 0.170: 100 μl of the 0.1% Triton X-100 extract or HeLa membrane fraction (enzyme source), 10 μl of 0.1 M $MnCl_2$, 50 μl of the fetuin acceptor and 10 μl of UDP-*N*-acetylglucosamine-^{14}C (25 nCi, approximately 60 pmoles). pH Adustments were made with tris buffer. The incubation was 37° for 1 hr. Radioactivity was determined as given above. The HeLa cells for the membrane prepartions were grown in spinner culture in Eagle's spinner medium supplemented with 10% calf serum. All other cultures were grown in monolayer in the Dulbecco-Vogt modification of Eagle's medium supplemented with 10% calf serum. All cells were harvested in the logarithmic phase of growth by conventional procedures. Cell number was determined in a Coulter counter. Two lines of fibroblasts were investigated: 3T3, an established cell line of embryonic mouse fibroblasts and SV-3T3, an established line of sismian virus SV-40 transformed fibroblasts.

Membrane preparations of HeLa cells were prepared on discontinuous sucrose gradients as previously described [7]. In this procedure the smooth internal membranes of the HeLa cell are found in S-1 fraction of the gradient and the plasma membranes in the S-7 fraction. Rough membranes fractionate in the S3-S4 fraction while soluble proteins and other material are found in fractions S5 and S6. Smooth internal membranes (fraction S1) and plasma membranes (fraction S7) were purified by repeating the gradient centrifugation; the purified material was designated S1-1 and S7-7, respectively [7]. The data presented herein are means from 4 separate experiments; ranges between experiments amounted to 10%.

In order to extract the enzymes from the transformed and non-transformed fibroblasts a given harvest of cells was homogenized at the temperature of melting ice with 30 volumes of 0.1% Triton X-100 by 12 strokes in a Dounce homogenizer. This mixture was extracted for 16 hr in the cold and then centrifuged at 30,000 *g* for one hr. The supernatant fluid was lyophilized and either made up to suitable volume with distilled water and used for assay. Protein was determined by the method of Lowry et al. [8] with bovine serum albumin as standard.

3. Results

The results in table 1 indicate that both the endogenous and exogenous glycoprotein: *N*-Acetylglucosaminyl transferase activity was found primarily in the smooth endoplasmic reticulum fraction of HeLa cells. A 37-fold purification of the fetuin: *N*-Acetylglucosaminyl occurred in the S1-1 purified smooth membrane fraction with a 47% yield. There was no activity in the rough endoplasmic reticulum fraction but 1% of the activity was associated with the S7 and S7-7 plasma membrane fractions (table 1).

The data presented in table 2 indicate that both the endogenous and exogenous glycoprotein: *N*-Acetylglucosaminyl transferases were present to a lesser extent in the normal fibroblast cell line, 3T3, than in the simian virus transformed cell line, SV-3T3. The difference in the fetuin: *N*-Acetylglucosaminyl activity was most marked: the activity in the SV-3T3 was four times that in the 3T3 with added acceptor.

4. Discussion

The results demonstrate that the glycoprotein: *N*-Acetylglucosaminyl transferase is located in the smooth endoplasmic reticulum and as such functions as part of the multienzyme group of glycosyl transferases necessary for the synthesis of membrane glycoproteins [1, 9]. Coincident with this the fetuin: *N*-Acetylglucosaminyl transferase upholds the proposed "one enzyme-one linkage" hypothesis which states that membrane glycoprotein synthesis proceeds by the sequential addition of monosaccharides from nucleoside diphosphate monosaccharide precursors onto completed protein acceptors released from ribosomes catalyzed by distinct glycosyl transferases (specific for linkage, substrate and monosaccharide) at the smooth membrane level. Furthermore the results demonstrate that upon transformation by an oncogenic

Table 1

Distribution of glycoprotein: *N*-Acetylglucosaminyl transferase in fractions obtained from HeLa cells. In each instance the fractions were assayed with the complete system as given in the Methods.

Fraction	Cells (cpm/10^9)		Protein (cpm/mg)		Fetuin: *N*-acetylglucosaminyl	
	Endogenous acceptor	Acceptor added*	Endogenous acceptor	Acceptor added*	Purification factor	% Yield
Cell homogenate (Dounce)	420,000	3,160,000	1,800	13,500	1.0	100
Supernatant fluid (4S)	290,000	2,110,000	2,420	17,500	1.3	67
Pellet (4P)	100,000	1,000,000	910	9,100	0.7	31
S1	180,000	1,600,000	45,000	400,000	29.6	51
S2	10,000	200,000	2,500	50,000	3.7	6
S3	10,000	0	2,500	0	0	0
S4	0	0	0	0	0	0
S5	0	0	0	0	0	0
S6	0	0	0	0	0	0
S7	2,000	32,000	15,400	24,600	1.8	1
S1-1	170,000	1,500,000	56,600	500,000	37.0	47
S7-7	1,800	30,000	21,100	35,000	2.6	1

* Acceptor added refers to cpm attached onto the fetuin acceptor, endogenous acceptors are not characterized.

Table 2

Glycoprotein: *N*-Acetylglucosaminyl activity in normal and viral-transformed fibroblasts.

Cell Line	Cells (cpm/10^6)		Protein (cpm/mg)	
	Endogenous acceptor	Acceptor added	Endogenous acceptor	Acceptor added
3T3	509 ± 36	189 ± 16	472 ± 16	182 ± 19
SV-3T3	1011 ± 33	781 ± 19	989 ± 37	739 ± 80

* Each value is derived from assays performed on four or more independently cultured cell populations and is expressed as mean ± S.E. In each instance the complete assay system as given in Methods was present with the extract from the indicated cell line. Endogenous acceptor activity was determined with tris buffer substituted for the acceptor in the assay.

virus and thus a change toward a neoplastic state the firbroblast has greatly elevated levels of the glycoprotein *N*-acetylglycosaminyl transferases. These elevated levels may affect membrane glycoprotein carbohydrate content [5] and thereby affect cellular membrane properties such as contact inhibition, invasiveness, communication and transport.

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