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THE ISOLATION AND CHARACTERIZATION OF PLASMA MEMBRANE FROM CULTURED CELLS

IV. THE CARBOHYDRATE COMPOSITION OF MEMBRANES ISOLATED FROM ONCOGENIC RNA VIRUS-CONVERTED CHICK EMBRYO FIBROBLASTS

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SUMMARY

1. Plasma membrane was isolated from cultures of chick embryo fibroblasts which were converted by avian sarcoma viruses, morph^{f} Fujinami virus, and a mutant of this virus, morph^{r} Fujinami virus. The morph^{f} virus-converted cells were fusiform in shape, whereas fibroblasts converted by the morph^{r} virus became round.

2. Cell membrane isolated from the morph^{r} virus-converted fibroblasts had an increase of more than 40% in neutral sugar content compared with that isolated from uninfected cells or cells converted by morph^{f} Fujinami virus.

3. The sialic acid content of the cell membrane isolated from cells converted by oncogenic viruses was decreased compared with that of plasma membrane from RAV-49-infected cells or uninfected cells. Since this decrease was found in cells converted by sarcoma-producing viruses but not in those infected with a leukemia virus, and since the decrease was not correlated with cell shape, it represents a fundamental alteration in membrane chemistry paralleling a loss of the fibroblasts' controls of growth and multiplication.

INTRODUCTION

There is currently a great deal of interest in changes in the chemistry of the cell membrane resulting from neoplastic transformation. A good experimental model for studying these changes uses cultured chicken fibroblasts and fibroblasts infected with paired avian RNA tumor viruses; for example, RBA and RAV-49. Both of these viruses replicate in the fibroblasts, but the RBA virus is the only one causing neoplastic transformation.

Plasma membranes were previously isolated by flotation equilibrium centrifugation[1] from homogenates of cultured chick embryo fibroblasts infected with the avian RNA tumor virus, RAV-49, or infected with and converted by the sarcoma-producing virus, RBA[2]. Marked differences in the carbohydrate composition of the

cell membranes were found between the untransformed cells (uninfected or RAV-49-infected) and the transformed cells (RBA-infected).

Plasma membranes isolated from uninfected and RAV-49 virus-infected cells contained about 110 nmoles of sialic acid per mg protein whereas the membranes from the RBA virus-converted cells contained 79 nmoles. The plasma membranes from the unifected and RAV-49 virus-infected cells had about 225 μg of neutral sugar per mg protein; the RBA virus-converted cells had 350 μg.

Infection of fibroblasts with oncogenic RNA viruses modifies many of the cells, controls, including controls of growth and multiplication[3], of movement[4], and of metabolite transport[5]. The viruses also influence the morphology of the cells, with various mutations determining the shape of the infected fibroblasts[6]. The oncogenic virus used in the previous study, RBA, produced round-shaped cells, whereas RAV-49 virus-infected cells were fusiform[2]. Morph^f Fujinami[7,8] and mutants of Rous Sarcoma Virus[6,9] are oncogenic avian viruses which do not cause a rounding of the converted cells. The cells are fusiform in shape. A mutant of morph^f Fujinami, morph^r, produces round cells during infection of fibroblasts[10].

The decrease in sialic acid and the increase in neutral sugar in the virus-converted cell membrane could be a manifestation of the neoplastic transformation, or it could be the result of expression of viral genes other than those responsible for the neoplastic transformation. Cultured chick embryo fibroblasts were infected with morph^f and morph^r Fujinami viruses. The plasma membrane was isolated from the converted cells to determine whether the changes in membrane carbohydrate chemistry paralleled the expression of genes producing malignancy or of genes producing other changes such as those affecting cell shape. This study established that infection and conversion by oncogenic viruses resulted in a decrease in the sialic acid content of the cell membrane. Increases in neutral sugar, however, were found only in membranes from cells which had assumed a round morphology following infection.

MATERIALS AND METHODS

Fertilized chicken eggs, culture medium, serum, and reagents were the same as used in the previous study[2]. The avian RNA sarcoma-producing virus, morph^f Fujinami virus, and a mutant of this virus, morph^r Fujinami virus, were kindly supplied by H. M. Temin[10].

Cell culturing, infection of fibroblasts with virus, plasma membrane isolation, chemical procedures, and light and electron microscopy. The methods employed in this study were the same as the ones used in previous studies[1,2,11]. Neutral sugar content of the cell membrane is given as glucose equivalents, and amino sugar as glucosamine equivalents.

RESULTS

The isolation of cell membrane

Centrifugation of homogenates of morph^f and morph^r Fujinami virus-converted cells on continuous density gradients of sucrose gave the banding pattern described in the earlier studies[1,2]. The cell membrane-containing fractions, the A and B bands, were further resolved by centrifuging them on a second continuous density gradient

of sucrose. These membranes reached equilibrium after 3 h of centrifugation and were concentrated in sucrose of a density of 1.058 ± 0.001 (S.E.) and 1.074 ± 0.004 for the A′ band of the morph^f and morph^r virus-converted cells, respectively. The membranes in the B′ band from morph^f virus-converted cells and those from morph^r virus-converted cells were both concentrated in sucrose of a density of 1.11.

Carbohydrate composition of the cell membrane

The sialic acid content of the membranes in the A′ band from the morph^f and morph^r virus-converted cells was 75 and 64 nmoles/mg protein, respectively (Table I). The neutral sugar contents of the plasma membrane from these two morphologically distinct populations of virus-converted cells were markedly different. The morph^f virus-converted cells had levels of neutral sugar in the A′ and B′ band membranes, 235 and 152 μg/mg protein, which were the same as had been found for uninfected cells[2]. The morph^r virus-converted cells had 331 μg of neutral sugar in the A′ band membranes. This increase in glucose equivalents is about 40 % more than that found in uninfected cells and is very similar to the increase observed in the morphologically round RBA virus-converted cells[2].

An analysis of the amino sugar content of the cell membranes in the A′ and B′ bands from the uninfected fibroblasts and fibroblasts infected with leukosis virus, RAV-49, or infected and converted by sarcoma virus, RBA and morph^f and morph^r Fujinami (Table I), did not establish compositional differences which could be related to morphologecal cell shape or virus conversion. It had previously been found that the amino sugar was concentrated in membranes in the A′ and B′ bands following centrifugation of a particulate homogenate of cultured rat liver cells on a continuous density gradient of sucrose[11]. A similar concentration of amino sugar in the upper bands of the gradient was found for cultured fibroblasts. The specific concentration

TABLE I

THE CARBOHYDRATE COMPOSITION OF PLASMA MEMBRANE IN THE A′ AND B′ BANDS ISOLATED FROM UNINFECTED CHICK EMBRYO FIBROBLASTS, FIBROBLASTS INFECTED WITH LEUKOSIS VIRUS, OR FIBROBLASTS INFECTED AND CONVERTED BY THE ONCOGENIC VIRUSES RBA, MORPH^f, OR MORPH^r FUJINAMI

Values are expressed as mean ± S.E. The number in parentheses refers to the number of experiments.

Component		*Units/mg protein*		
		Homogenate	*Band A′*	*Band B′*
Sialic acid (nmoles)	morph^f-converted cells	19.8 ± 0.1 (3)	74.6 ± 0.1 (3)	76.5 ± 0.6 (3)
	morph^r-converted cells	20.4 ± 0.1 (3)	64.0 ± 5.4 (4)	67.2 ± 4.5 (3)
Glucose equivalents (μg)	morph^f-converted cells	75 ± 2 (4)	235 ± 11 (5)	152 ± 7 (5)
	morph^r-converted cells	84 ± 6 (3)	331 ± 14 (5)	157 ± 24 (5)
Glucosamine equivalents (μg)	Uninfected cells	11.7 ± 1.1 (5)	33.1 ± 2.0 (6)	30.0 ± 1.0 (8)
	morph^f-converted cells	16.2 ± 1.4 (5)	23.3 ± 1.9 (4)	32.2 ± 1.0 (6)
	morph^r-converted cells	11.2 ± 0.7 (4)	30.9 ± 1.4 (4)	31.2 ± 3.0 (4)
	RBA-converted cells	13.9 ± 0.7 (7)	34.3 ± 1.5 (7)	28.9 ± 0.9 (6)
	RAV-49-infected cells	13.4 ± 0.9 (4)	31.9 ± 1.9 (3)	30.2 ± 2.3 (4)

of amino sugar in the membranes in the A′ band was about three times greater than that of the homogenate. However, unlike what had been found for the other carbohydrate components, the specific concentration of this sugar in the B′ band membrane was about the same as that of membranes in the A′ band. In the case of morph^{f} Fujinami, the mean value for amino sugar in membranes in the B′ band was greater than those of the A′ band.

DISCUSSION

Significant biochemical differences were revealed during analysis of the plasma membrane isolated from uninfected and virus-infected fibroblasts. The decrease in the sialic acid content of this membrane from cells which had been infected with oncogenic virus was a consistent finding and was not dependent on the shape of the converted cell, because both morph^{f} Fujinami virus-converted cells and RBA virus-converted cells had similar levels of this sugar (Tables I and II). Neither could these sialic acid changes be the result of virus infection or virus production. The RAV-49-infected cells, which were releasing virus from their surface, had levels of sialic acid in their membranes which were the same as those of uninfected cells (Table II).

Conversion of fibroblasts by oncogenic virus, which causes the cells to assume a round shape, is accompanied by a 40 % increase (up to a maximum of 350 μg per mg protein) in the neutral sugar content of their isolated plasma membranes. This observation was reported previously for the RBA virus-converted cells[2] and also applies to fibroblasts converted by the morph^{r} Fujinami virus (Tables I and II). Cells which were spindle shaped – even those which were infected with the leukosis

TABLE II

THE CARBOHYDRATE COMPOSITION OF PLASMA MEMBRANE IN THE A′ BAND ISOLATED FROM UNINFECTED CHICK EMBRYO FIBROBLASTS AND FROM FIBROBLASTS INFECTED WITH LEUKOSIS VIRUS OR INFECTED AND CONVERTED BY ONCOGENIC VIRUS

Virus	*Oncogenic*	*Cell morphology*	*μmoles carbohydrate per mg protein*		
			Neutral sugar	*Amino sugar*	*Sialic acid*
Uninfected	–		1.34 ± 0.04 *	0.18 ± 0.01	0.106 ± 0.003 *
RAV-49	–		1.09 ± 0.10 *	0.18 ± 0.01	0.114 ± 0.003 *
morph^{f} Fujinami	+		1.29 ± 0.06	0.13 ± 0.01	0.077 ± 0.006
morph^{t} Fujinami	+		1.82 ± 0.08	0.17 ± 0.01	0.067 ± 0.005
RBA	+		1.97 ± 0.07 *	0.19 ± 0.01	0.079 ± 0.003 *

* Data from Perdue *et al.* (ref. 2, Tables I and II).

virus, RAV-49, or with the sarcoma-producing virus, morph^{f} Fujinami – contained about 230 μg of neutral sugar (Table II).

The amino sugar content of the membrane in the A′ band was about the same for all the cell preparations with the exception of morph^{f} Fujinami (Table I). This membrane had significantly less amino sugar, a decrease which paralleled the decrease in sialic acid.

A molar ratio of 11:2:1 for neutral sugar, amino sugar, and sialic acid has been calculated from the data in Table II for the isolated plasma membrane from uninfected chick embryo fibroblasts. Plasma membrane isolated from RAV-49-infected cells had a molar ratio of 9:2:1. The ratio for the carbohydrate in the membrane isolated from cells converted by morph^{r} virus was increased by a factor of 2 in its neutral sugar content owing to the concomitant loss of sialic acid and marked increase in hexose. A molar ratio of 10:2:1 for these saccharides is different from that generally observed for glycoproteins and isolated glycopeptides. Carbohydrate analyses of receptor site proteins[12], blood group substances, serum proteins, and glycolipids[13,14] generally establish ratios of neutral sugar to amino sugar and sialic acid of the order of 6–4:2:1. Plasma membrane preparations isolated from blood platelets[15] had a molar ratio for the above sugars of 16:6:1 (calculated from the authors' data); that isolated from the BRL cells[11] was 17:2:1. The molar ratio of total cell hexose to sialic acid as a function of the cell cycle was determined by Glick *et al.*[16] for KB cells. At 0 and 16 h after a thymidine block these ratios were about 10:1. The amino sugar content of the KB cells was not determined.

The quantity of carbohydrate in the plasma membrane isolated from the cultured chick fibroblasts is also high when compared with previously published values. The level of 100–110 nmoles of sialic acid per mg protein is the highest reported for isolated cell membranes. Emmelot and Benedetti[17] isolated plasma membranes from hepatoma and rat liver that contain about 44 nmoles of this sugar per mg protein but Evans[18] isolated liver plasma membrane which had 75 nmoles. The plasma membrane obtained from the cultured BRL cells contained 75 nmoles of sialic acid[11]. The cell surface membranes from these BRL cells and from the cultured chick fibroblasts also had large quantities of neutral sugar – 1.2 and 1.3 μmoles per mg protein, respectively. A value of 0.8 μmole has been reported for plasma membrane isolated from blood platelets[15] whereas plasma membrane isolated from rat liver had 65 nmoles (ref. 17).

The content of carbohydrate in plasma membranes of cultured chick embryo fibroblasts is not so unique as we had at first believed. Cell membranes have been isolated from chicken sarcomas produced from morph^{r} Fujinami- and RBA-converted fibroblasts by the procedures reported in this study. The sialic acid and neutral sugar content of these membranes was similar to that of the membranes isolated from the *in vitro*-converted fibroblasts.

Our observations of a decrease in cell membrane sialic acid are in agreement with numerous observations on the change in the content of this sugar in the glycoproteins and glycolipids of virus-converted cells[2,19,20]. Since this decrease was found in cells converted by oncogenic, sarcoma-producing viruses but not in those infected with leukosis virus, and since the decrease was not correlated with cell shape, it represents a fundamental alteration in membrane chemistry which paralleled a loss of the fibroblasts' controls of growth and multiplication. The increase in neutral

sugar content of plasma membrane from virus-converted cells that assumed a round morphology is also a virus-directed process. However, these carbohydrate changes are not required to maintain the malignant state, since there was no discernible increase of hexose in the morph[f] Fujinami virus-converted cells.

At this time it is not known if the decreases in sialic acid are in both glycolipids and glycoproteins. Neither do we know the mechanism of neutral sugar increase. Is it the result of the synthesis of new types of macromolecules, or is it owing to the synthesis of additional copies of existing carbohydrate-containing components? Quantitative chemistry of the glycolipids and glycoproteins in isolated plasma membranes is under investigation.

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