SHORT COMMUNICATIONS

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Carbohydrate composition of the plasma membranes of rat ascites hepatoma

It is believed that carbohydrates on cell surfaces may play important roles in many biological functions of the plasma membranes[1]. In this respect, studies on cancer cells are of great importance since it has been predicted that malignant transformation of normal cells may be accompanied by changes in the carbohydrate composition of the plasma membranes[2,3]. Actually, EMMELOT *et al.*[4] and BENEDETTI AND EMMELOT[5] found larger amounts of neutral sugars and sialic acid in the plasma membranes of rat hepatoma-484 than in those of normal liver cells, whereas hexosamine contents were similar. The analysis was, however, not complete in that neither neutral sugars nor hexosamines were determined individually.

We have prepared the plasma membranes from a strain of rat ascites hepatoma and precisely determined their carbohydrate composition. The carbohydrates appeared to be from both glycoproteins and glycolipids.

Ascites hepatoma, AH 7974F, which is a free cell tumor, was transplanted intraperitoneally to inbred rats of Moriyama strain weighing 150 g ($1 \cdot 10^7$ cells per rat), and the cells were harvested after 3 days. The cells were washed with 0.9 % NaCl by centrifugation at 150 $\times$ *g* for 1 min. To prepare the plasma membranes, the method of WARREN *et al.*[6] was adopted with some modifications which had been used for the preparation of the plasma membranes from L cells. Thus, 3 vol. of a solution of fluorescein mercuric acetate (1 mg/ml of 20 mM Tris–HCl buffer, pH 8.0) were added to the washed cell suspension (1 g of wet cells per 2 ml of 0.9 % NaCl). After standing for 5 min at room temperature, the suspension was chilled in ice and homogenized in a tightly fitting Dounce homogenizer. 25–30 strokes of the homogenizer were found to be optimal for obtaining large cell ghosts, checked with a phase-contrast microscope. The homogenates were then mixed with an equal volume of 60 % sucrose and centrifuged at 400 $\times$ *g* for 30 min to remove nuclei. The supernatant was further centrifuged at 2000 $\times$ *g* for 60 min to spin down the plasma membranes. The sediment was suspended in 60 % sucrose containing 10 mM Tris–HCl buffer, pH 8.0, and 15 mM NaCl, the volume being equal to that of the starting cell suspension. Three ml of the suspension were placed at the bottom of a centrifuge tube and thereupon 1 ml each of 45 and 30 % sucrose was layered successively. After centrifugation at 100000 $\times$ *g* for 60 min in an RPS-40 rotor of a Hitachi ultracentrifuge 55P-2, the plasma membranes were isolated as bands at the interphase between 30 and 45 % sucrose. The membranes were collected using a J-type pipette, washed 7–8 times with 10 mM Tris–HCl buffer, pH 8.0, containing 15 mM NaCl by centrifugation at 2000 $\times$ *g* for 30 min, then 2–3 times in a similar manner after the suspension stood overnight in the cold and finally suspended in water. This way of washing was necessary to ensure the removal of sucrose. The yield of plasma membranes was about 1 mg of protein from 1 g of packed cells. Purity of the membranes thus obtained was examined by electron microscopy.

The electron micrograph (Fig. 1) shows the plasma membranes to be large ghosts, as originally shown by WARREN *et al.*[6]. Some fuzzy material was attached at the inner surface of the membranes, but contamination by other cell structures such as mitochondria or endoplasmic reticulum appeared to be insignificant.

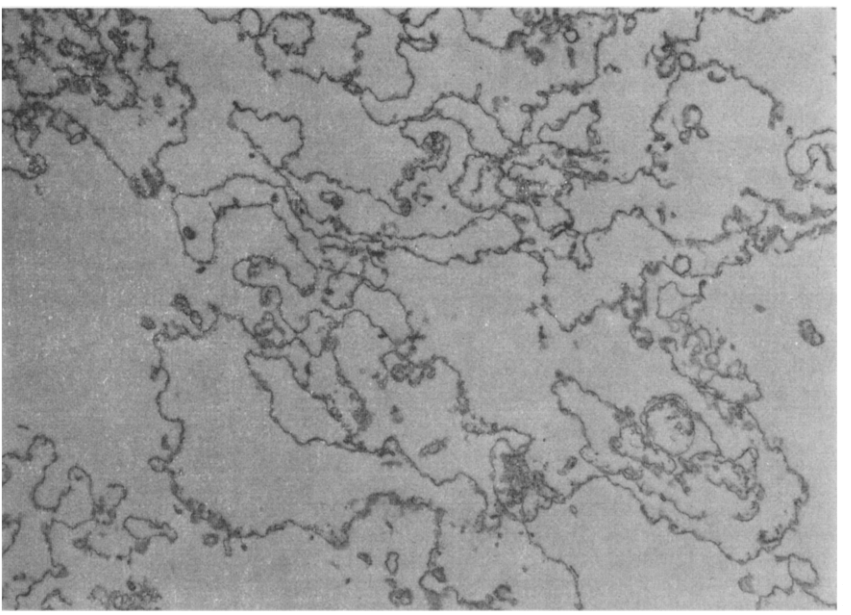

Fig. 1. Electron micrograph of isolated plasma membranes, fixed in 1% osmium tetroxide (pH 7.4), embedded in epoxy resin, and stained with uranyl acetate and lead hydroxide[8]. × 7500.

TABLE I

CARBOHYDRATE COMPOSITION OF ISOLATED PLASMA MEMBRANES OF RAT ASCITES HEPATOMA AH 7974F

Analysis of carbohydrates was carried out as follows; neutral sugars were determined by gas–liquid chromatography after hydrolysis in 1 M HCl at 100° for 8 h followed by methanolysis and trimethylsilylation*, hexosamines by a Hitachi amino acid analyzer KLA-2B after hydrolysis in 2 M HCl at 100° for 16 h and sialic acid by the method of AMINOFF[9] after hydrolysis in 0.1 M HCl at 80° for 1 h. Lipid-free membranes were prepared by extracting the dried membranes with chloroform–methanol (2:1, v/v) for 24 h at room temperature. Protein was determined according to LOWRY *et al.*[10] after solution in 0.1 M NaOH.

	Carbohydrate (nmoles/mg protein)	
	Intact membranes	*Lipid-free membranes*
Fucose	33.6	16.1
Mannose	57.2	57.2
Galactose	97.8	43.2
Glucose	56.4	11.4
Galactosamine	52.2	19.9
Glucosamine	59.2	53.7
Sialic acid	28.8	20.7

* Details of the analytical methods will be published elsewhere[11].

Table I shows the results of analyses of carbohydrates in isolated plasma membranes. Neutral sugars, hexosamines and sialic acid were detected, and their contents were 245, 111.4 and 28.8 nmoles/mg protein, respectively. Total carbohydrate content was 4% of dry weight of the membranes.

These values are considerably different from those of EMMELOT AND BENEDETTI[5] reported for hepatoma-484, *i.e.* 170–300 nmoles of neutral sugars, 57 nmoles of hexosamines and 44 nmoles of sialic acid. This suggests that carbohydrate composition could be characteristic of plasma membranes of each cell type, although a possibility that the difference might be due either to the manner of growth of the cells (solid *versus* ascites) or to the method of preparing the membranes cannot be ruled out.

Some of the carbohydrates were found to be components of glycolipids, since the greater part of glucose and about half each of fucose, galactose and galactosamine were extractable into chloroform–methanol (2:1, v/v).

The carbohydrates found in the lipid-free membranes were considered to be constituents of glycoproteins. The composition is markedly different from that of a glycopeptide isolated by LANGLEY AND AMBROSE[7] from tryptic digests of Ehrlich ascites carcinoma in that the glycopeptide contained sialic acid and galactosamine but no neutral sugars. Thus, the carbohydrate composition of glycoproteins in plasma membranes seems to differ from one type of cell to another. Preparation and structural studies of glycopeptides from the isolated plasma membranes are useful in this regard and are now in progress.

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