SUBCELLULAR DISTRIBUTION OF GLYCOLIPIDS IN A HAMSTER CELL LINE

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Received 7 March 1973

1. Introduction

We have previously reported that incorporation of [1-^{14}C]palmitate into ceramide tri, tetra, and pentahexoside of NIL2 hamster cells increases with increasing cell culture density, although incorporation into the other major glycolipid, hematoside, is relatively unaffected [1, 2]. Density dependent glycolipids have been found in some other cell lines [3–6] but are consistently absent in the transformed derivatives [1–6]. The possibility that these molecules are important determinants of cellular interaction has led us to study their location in the cell. We have found that the density dependent glycolipids of NIL2 hamster cells are present at, but not confined to, the cell surface.

2. Methods

NIL2 Syrian hamster cells and those transformed by hamster sarcoma virus NIL2/HSV [7] were labelled while still sparse with 0.25 μCi/ml [1-^{14}C]palmitate, (55 mCi/mmole) and harvested by scraping 48 hr later when confluent. The cells were washed in cold phosphate buffered saline and nuclear, mitochondrial, endoplasmic reticulum and plasma membrane fractions isolated by the method of Graham [8]. Plasma membrane and nuclear fractions were also prepared by the method of Warren et al. [9]. After estimation of total incorporation/mg protein, the subcellular fractions were dialysed against water at 4° for 48 hr, freeze dried and lipid extracted with 2:1 then 1:2 chloroform–methanol. To check the stability of lipids during the procedure, fractions were extracted directly with 20 vol of 2:1 chloroform–methanol followed by Folch partition. Lipids were separated by two dimensional thin-layer chromatography detected by autoradiography, and quantitated by scintillation counting [2].

3. Results and discussion

Enzymatic characterization of the subcellular fractions isolated by the method of Graham [8] is shown in table 1. The plasma membrane fractions from both NIL2 and NIL2/HSV cells demonstrate a 17–20-fold enrichment in Na^+/K^+—Mg^{2+} ATPase over the cell homogenate. Levels of this enzyme in the endoplasmic reticulum fraction from NIL2 and NIL2/HSV suggest a contamination with plasma membrane of the order of 5 and 12% respectively. Because of the high levels of Mg^{2+} ATPase in mitochondria it is difficult to assay the Na^+/K^+ stimulated -Mg^{2+} ATPase, and 5′-nucleotidase provides a better estimation of plasma membrane contamination. This was found to be less than 2% for both cell types. Contamination of the nuclear fraction by plasma membrane was at a similar low level. Activities of NADH diaphorase indicate that contamination of plasma membrane by endoplasmic reticulum is about 7% for NIL2 and 18% for NIL2/HSV. The assay method for this enzyme also detects the NADH oxidase of mitochondria, but the lack of significant succinate-cytochrome *c* reductase activity in any fraction except for mitochondria indicates that oxidation of NADH by endoplasmic reticulum was not due to mitochondrial contamination.

The glycolipids of the plasma membrane fraction of NIL2 cells reproducibly showed the greatest incorporation of [1-^{14}C]palmitate label per mg protein,

Table 1
Enzymatic characterization of subcellular fractions from normal (N) and transformed (T) NIL2 hamster cells.

Fraction	Na^+/K^+ stimulated Mg^{2+}-ATPase		5'-Nucleotidase		NADH diaphorase		Succinate Cyt *c* reductase	
	N	T	N	T	N	T	N	T
Plasma membrane	8.6	2.5	14.0	4.1	4.7	45.7	0.2	0.2
Endoplasmic reticulum	0.4	0.3	2.9	0.8	72.0	252.0	0.2	0.2
Mitochondria	0.05	0.05	0.3	0.05	40.0	154.5	3.1	2.4
Nuclei	0.1	0.05	0.2	0.05	ND	ND	ND	ND
Homogenate	0.5	0.1	0.2	0.05	8.4	38.2	0.2	0.2

From 1.6–5 $\times 10^8$ cells were disrupted by nitrogen cavitation in 0.25 M sucrose, 5 mM Tris, 0.2 mM Mg^{2+}, pH 7.4. After chelation of Mg^{2+} by 1 mM EDTA nuclei were sedimented at 1,000 *g* for 10 min, and the post nuclear supernatant separated into mitochondrial, microsomal and soluble fractions on a discontinuous sucrose zonal gradient. Plasma membrane and endoplasmic reticulum were isolated from the total microsomal fraction on a Mg^{2+}-Dextran gradient [8]. Enzymatic activities represent μmoles substrate utilised/hr/mg protein. ND = Not determined.

Table 2
Distribution of [1-^{14}C]palmitate in subcellular fractions from dense NIL2 hamster cells.

Incorporation per mg protein	Cell homogenate	Nuclei	Mitochondria	Plasma membrane	Endoplasmic reticulum
Total lipid	0.71*	0.46	0.56	3.25	1.1
Phospholipid	0.56	0.33	0.45	2.53	0.86
Glycolipid	0.05	0.03	0.05	0.39	0.12
Neutral lipid	0.10	0.09	0.05	0.33	0.13
Phospholipid/ glycolipid	11.5	12.8	10.0	6.6	7.3
% Distribution of label in glycolipid					
CmonoH	8.3	9.4	5.9	9.7	5.3
CdiH	5.4	4.8	6.1	5.9	7.5
CtriH	19.0	19.8	17.9	21.0	21.5
CtetraH	34.4	32.8	31.0	30.6	35.2
CpentaH	16.3	18.5	17.9	18.3	19.8
Hematoside	17.0	14.8	21.0	14.3	10.7

Subcellular fractions were isolated by the method of Graham [8]. CmonoH to CpentaH denotes neutral glycosphingolipids containing from one to five sugar residues.

* cpm $\times 10^6$ /mg protein.

Table 3
Distribution of [1-^{14}C]palmitate in nuclear and plasma membrane fractions of NIL2 cells.

Incorporation per mg protein	Cell homogenate	Nuclei	Microsomes	Plasma membrane	Soluble
Total lipid	0.68*	0.26	1.49	2.34	0.1
Phospholipid	0.39	0.20	0.94	1.56	0.06
Glycolipid	0.05	0.01	0.08	0.25	0.01
Neutral lipid	0.24	0.08	0.46	0.53	0.03
Phospholipid/ glycolipid	7.8	17.3	11.5	6.3	8.6

Plasma membranes were isolated by the method of Warren et al. [9]. Cells were swollen in 45 mM NaCl for 5 min, 3 vol of saturated fluorescein mercuric acetate pH 8.0 added and, after cooling to 0°, the cells disrupted in a Dounce homogeniser. Plasma membranes were isolated from nuclei on discontinuous sucrose gradients. The crude nuclear pellet was freed from intact cells and microsomes by homogenisation and extensive washing in 0.2% citric acid [10].

* cpm × 10^6/mg protein.

table 2. The specific activity of the plasma membrane glycolipid was 5-8-fold greater than the homogenate compared with enrichments of 2—4-fold for endoplasmic reticulum, 1—2-fold for mitochondrial and 0.5—1.5-fold for nuclear fractions. The ratio of incorporation into phospholipid/glycolipid was always lower in the plasma membrane and endoplasmic reticulum compared to the other fractions. Similar enrichments in incorpor-

Table 4
Incorporation of [1-^{14}C]palmitate into the subcellular fractions of dense NIL2/HSV.

Incorporation per mg protein	Cell homogenate	Nuclei	Mitochondria	Plasma membrane	Endoplasmic reticulum
Total lipid	0.4*	0.45	0.84	1.49	1.83
Phospholipid	0.35	0.40	0.72	1.15	1.56
Glycolipid	0.03	0.02	0.06	0.18	0.11
Neutral lipid	0.03	0.03	0.05	0.17	0.11
Phospholipid/ glycolipid	13.1	16.6	11.7	7.4	15.0
% Distribution of label in glycolipid					
CmonoH	11.6	13.8	11.8	16.0	9.5
CdiH	34.2	44.5	48.6	35.6	45.6
CtriH	0	0	0	0	0
CtetraH	0	0	0	0	0
CpentaH	6.3	6.6	5.2	7.1	7.0
Hematoside	48.2	34.6	30.5	41.0	37.7

Subcellular fractions were isolated by the method of Graham [8].

* cpm × 10^6/mg protein.

ation into glycolipid were found in plasma membrane fractions prepared by the method of Warren et al. [9], table 3. Nuclei prepared from the same cell homogenate and extensively purified [10] contained very little glycolipid as shown by the low specific activity and high phospholipid to glycolipid ratio. Comparable results were obtained with fractions from NIL2/HSV cells but interestingly, the phospholipid/glycolipid ratio showed a marked difference between the plasma membrane and endoplasmic reticulum which was not found in studies on the normal cell. The distribution of label in the various glycolipids of the subcellular fractions was similar to that of whole homogenate, tables 2 and 4.

Previous studies have shown a concentration of glycolipid at the cell surface [11–15] and the glycolipids of plasma membrane and endoplasmic reticulum to be similar to whole cells, [12, 13, 15] with one exception [16]. Our incorporation data show similar trends but possible differences in turnover between the subcellular structures makes detailed quantitative comparison impossible. The experiments show that the density dependent glycolipids are indeed present at the cell surface although not specifically located there. If the density dependent components were present only at the cell surface, then the distribution of label in glycolipid of endoplasmic reticulum should be different from that of plasma membrane. The fact that the profiles are very similar might be explained if none of the other intracellular organelles contain any glycolipid, and that present is derived from contamination with plasma membrane. This is considered unlikely as the endoplasmic reticulum was repeatedly enriched in incorporation into glycolipid per mg protein over the homogenate. Such an explanation may however account for the levels of glycolipid in nuclei.

The presence of the density dependent glycolipids at the cell surface agrees with the data of Hakomori and Kijimoto [17] who found that intact NIL2 cells were reactive to antibody prepared against Forssman glycolipid (ceramide pentahexoside). Insertion of a viral genome into the host cell apparently blocks synthesis of the density dependent components of all subcellular compartments and not just those of the cell surface. That transformation results in modification of complex carbohydrates other than those on the cell surface agrees with the data of Warren et al. [18], who found that fucose containing glycopeptides characteristic of transformed cells were present in mitochondrial and nuclear as well as plasma membrane fractions. The possible importance of the density dependent glycolipids at the cell surface is unknown. Roth and co-workers [19, 20] have suggested that cell surface carbohydrates may act as recognition sites by binding to specific glycosyl transferases on the surface of contiguous cells. Such a process of transglycosylation may provide an explanation for synthesis of the density dependent glycolipids although their presence in intracellular structures makes it unlikely to be the sole mechanism [21].

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