DISTRIBUTION OF GANGLIOSIDES AMONG SUBCELLULAR FRACTIONS FROM RAT LIVER AND BOVINE MAMMARY GLAND

T.W. KEENAN, D. James MORRÉ and C.M. HUANG
Departments of Animal Sciences, Botany and Plant Pathology and Biological Sciences, Purdue University, Lafayette, Indiana 47907, USA

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1. Introduction

That gangliosides are specifically localized in plasma membranes of extraneural cells has been assumed [1] or suggested based on their enrichment in isolated plasma membrane fractions [2, 3]. However, we recently demonstrated that gangliosides are not specifically localized in the surface membranes of either rat liver or bovine mammary gland; only 10 to 25% of the cellular gangliosides were recovered in these membrane [4]. These sialic acid containing glycolipids are ubiquitous among mammalian tissues (e.g. [5]) and have been implicated as having immunological activity, and as functioning in cellular recognition [6] and in cell–cell adhesion [7]. More recently abnormal ganglioside metabolism has been implicated as a possible causal agent in tumorigenesis [8–10]. In view of their physiological significance, we examined the subcellular distribution of gangliosides.

2. Methods

All homogenizations were performed with a Polytron 20ST homogenizer. Crude subcellular fractions were obtained from rat liver and lactating bovine mammary tissue samples which were minced, rinsed and pressed under cold running tap water and filtered through cheesecloth after homogenization in 4 vol of 0.25 M sucrose. Crude subcellular fractions were obtained by centrifugation at 2,000 *g* for 10 min (2K pellet), 12,000 *g* for 20 min (12K pellet) and 176,000 *g* for 60 min (176K pellet). After each step the supernatant and loose, fluffy portions of the pellet were subjected to centrifugation at the next higher speed while the tightly packed portion of the pellet was retained. Final supernatant fractions were also retained for analysis. The floating lipid layers formed with mammary homogenates at each centrifugation step were combined and analyzed separately; only small quantities of floating lipids were observed in liver homogenates and these were dispersed into the final supernatant fraction. Purified membrane fractions obtained from rat liver were analyzed also. Methods for isolation of plasma membranes [11], Golgi apparatus [12] and rough endoplasmic reticulum (RER) [11] fractions were those used previously. Total microsomes, rough microsomes and smooth microsomes were prepared according to Dallner et al. [13]. Purified mitochondria were obtained by extensive washing of crude fractions prepared by differential centrifugation in the usual manner. Nuclei were purified by sedimentation through heavy sucrose according to Berezney et al. [14]. Fraction purity for the cytologically defined fractions (all except microsomes) was judged both by assay for marker enzymes and by electron microscopic examination to be at least 90%.

Gangliosides were recovered by extraction of fractions with chloroform–methanol and partitioning as described previously [4] according to standard procedures which extract gangliosides quantitatively [15]. Gangliosides were determined by assay for sialic acid with the specific periodate–resorcinol procedure of Jourdian et al. [16]. Methods for protein and lipid phosphorus determination were those used previously [4].

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Table 1
Distribution of gangliosides among crude cell fractions from rat liver and bovine mammary gland.

Fraction	Rat liver		Bovine mammary gland	
	Ganglioside sialic acid (%) *	Lipid phosphorus (%) *	Ganglioside sialic acid (%) *	Lipid phosphorus (%) *
2K pellet	17.7	33.9	6.1	5.8
12K pellet	39.4	29.9	18.2	21.3
176K pellet	39.7	28.3	53.1	49.2
Supernatant	7.6	1.7	8.4	7.7
Floating lipid			18.4	6.7
Recovery	102.4	93.8	104.2	90.7

* Percentages based on amounts recovered from portions of total homogenates.

3. Results and discussion

Gangliosides were found in all crude particulate fractions from both bovine mammary gland and rat liver (table 1). While the bulk of the gangliosides were contained in the 176K pellet from mammary gland, liver gangliosides were more evenly distributed among the particulate fractions. The more provocative finding was that gangliosides were present in the particulate-free supernatant fractions from both liver and mammary gland and in the floating lipid fraction from mammary gland. In view of the gentle nature of homogenization with the Polytron [12] and the centrifical force applied, it is reasonable to assume that these supernatant fractions were free of membranous material. However, nearly 8% of the cellular gangliosides were present in the supernatant fractions from both tissues. Gangliosides are water soluble and could exist free in the cytoplasm or, alternatively, might be associated with lipoproteins in the cytoplasm. The parallelism between the percentages of gangliosides and lipid phosphorus suggests an association with lipoprotein for mammary gland. This argument does not apply to rat liver, where more than a 4-fold greater percentage of gangliosides than phospholipid was present in the supernatant. A relatively large percentage of the total cellular gangliosides was present in the floating lipids from mammary gland (table 1). This floating lipid fraction is believed to represent milk fat globules in process of formation [17]. Forming milk fat globules occur free in the cytoplasm of mammary secretory cells and are not surrounded by a typical bilayer-type membrane. These forming globules are composed primarily of triglycerides but do contain some phospholipids, primarily choline phosphatides, which have been suggested to function in stabilization of the lipid globule [18, 19]. It is quite possible that the highly surface active gangliosides, which were found in high concentrations relative to phospholipids, also serve to stabilize these forming fat globules. The excellent recoveries obtained confirm the reliability of the methods used (table 1).

Thin-layer chromatographic analysis revealed qualitatively similar patterns for all crude fractions from mammary gland (fig. 1). While the same six ganglioside components were present in all fractions, differences in their distribution were evident. Since glycoproteins would have low mobilities in the solvent system used, the fact that resorcinol-positive material was absent in lower regions of the chromatogram confirms that the sialic acid measured in our assay was bound to lipids and not proteins. Similar results were obtained with liver fractions (not shown).

All purified subcellular fractions from liver contained gangliosides (table 2). Plasma membranes contained the largest amount of ganglioside sialic acid on both a protein and lipid phosphorus basis and showed the highest enrichment relative to total homogenates. Golgi apparatus was intermediate between the plasma membrane and the RER and microsomal fractions with respect to levels of ganglio-

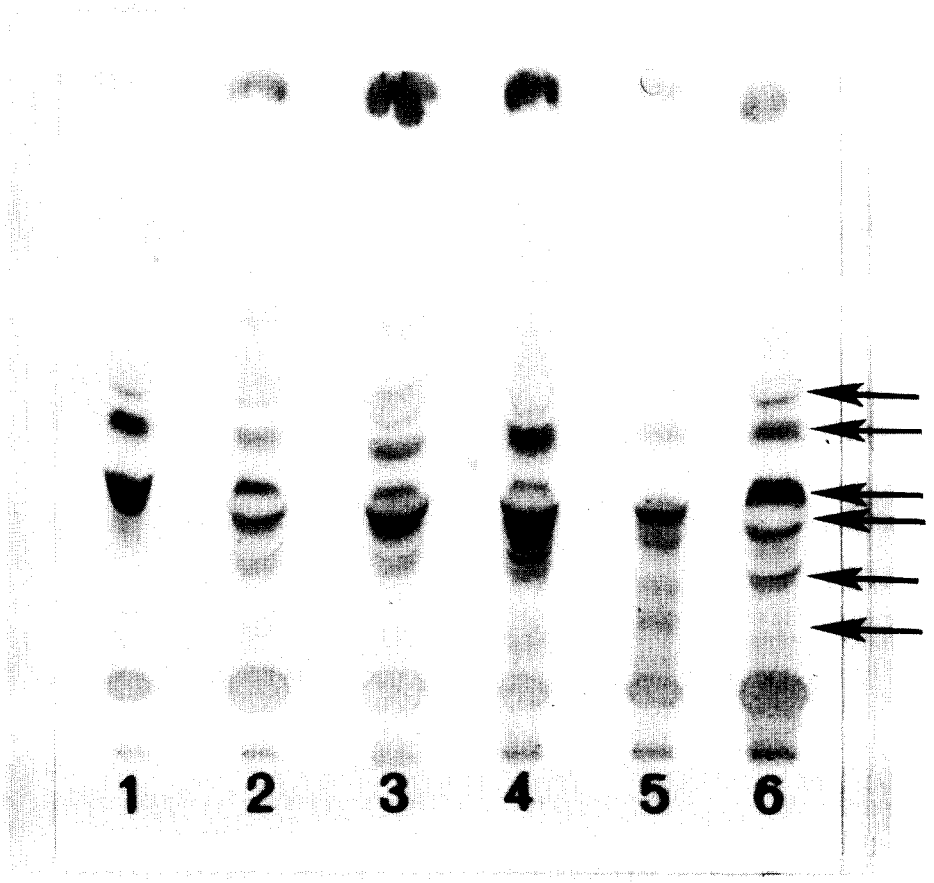

Fig. 1. Thin-layer chromatographic comparison of crude ganglioside extracts obtained from bovine mammary gland fractions. Fraction identity (nmoles sialic acid applied): 1, total homogenate (70); 2, 2K pellet (50); 3, 12L pellet (75); 4, 176K pellet (100); 5, floating lipid (60); 6, supernatant (70). Arrows denote sialic acid-positive bands. The 250 μ Silica gel G plate was developed in chloroform–methanol–28% ammonia–water (60:35:7:4, by volume) and sprayed with resorcinol reagent.

sides and in enrichment relative to the homogenates. Portions of the gangliosides in the total microsome and smooth microsome fractions might be attributed to the presence of fragments of Golgi apparatus and plasma membranes in these fractions. Rough microsomal and RER fractions were free of this contamination and thus the results show that gangliosides are indigenous to liver endoplasmic reticulum. Gangliosides were detected in nuclei but showed no enrichment relative to homogenates on a protein basis. Gangliosides were also present in the mitochondrial fraction, but in levels lower than those encountered in the homogenate.

Large variations among animals in the amount of ganglioside sialic acid present in the liver were evident. The ganglioside content of five separate liver homogenates (each homogenate was derived from the livers of 4 to 16 animals) ranged from 0.14 to 0.53 nmole sialic acid per mg protein with an average of 0.35 nmole per mg protein (table 2). To establish enrichment it is thus necessary to compare the isolated fraction with the homogenate from which it is derived as was done in the present study.

Our results reveal a heretofore unexpected complex distribution pattern for gangliosides among cellular constituents. These findings emphasize the need for

Table 2
Distribution of gangliosides among purified cellular fractions from rat liver.

Fraction	Ganglioside content [a]	Ratio [b]	Ganglioside content [c]	Ratio [b]
Plasma membrane	6.22	11.74	0.36	4.50
Homogenate	0.53		0.08	
Golgi apparatus	1.04	7.60	0.13	4.10
Total microsomes	0.52	3.80	0.04	1.19
Homogenate	0.14		0.03	
Rough microsomes	0.68	1.48	0.05	0.55
Smooth microsomes	1.08	2.36	0.08	0.94
Mitochondria	0.32	0.70	0.06	0.64
Homogenate	0.46		0.09	
RER	0.57	2.77	0.06	1.32
Homogenate	0.20		0.04	
Nuclei	0.44	1.04	0.16	2.27
Homogenate	0.42		0.07	

[a] nMoles of ganglioside sialic acid/mg protein.
[b] Ratio of content in membrane fraction to content of respective total homogenate.
Fractions were obtained from different animals and the absolute values are comparable only to other fractions from the same homogenate.
[c] nMoles of ganglioside sialic acid/μg lipid phosphorus.

examining specific cellular fractions in testing theories relating changes in gangliosides to changes in cell surface properties. While no function can as yet be ascribed to gangliosides of intracellular membranes, it is possible that they may act to stabilize membrane structure and/or participate in recognition at the intracellular level. The intermediacy of Golgi apparatus between RER and plasma membrane with respect to ganglioside sialic acid parallels results obtained on investigation of several other constituents and offers further evidence favoring the proposed functional role of Golgi apparatus in transformation of membranes from endoplasmic reticulum-like to plasma membrane-like [20].

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