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The Lipid Composition of Rabies Virus

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The lipid composition of the Flury HEP strain of rabies virus grown in BHK 21/C 13 cells was determined. The dried purified virus preparation contains 5.5 % neutral and 19.5 % polar lipids. Cholesterol was found to be the major neutral lipid. Phopholipids constitute 11.2 %; and glycolipids represent 4.6 % of the virus mass. The residual 3.7 % of extracted polar lipid material could not be accounted for by any known lipids. Phosphatidylethanolamine, phosphatidylcholine and sphingomyelin are the main constituents of the viral phospholipids. Hematoside is the only ganglioside and the main glycolipid present in the virus. The glycolipids of the host cell and the virus are identical. The molar ratio of sphingolipids to glycerophospholipids is 0.8.

Introduction

Electronmicrographs of rabies virus show bullet-shaped particles of complex morphology [1]. Treatment of rabies virus with ionic detergents results in inactivation of infectivity, in the release of the ribonucleocapsid and the solubilization of viral lipids and envelope proteins [2]. The sensitivity of virus infectivity to organic solvents [3] and to phospholipase C [4] provides strong evndence for the presence of lipids as essential components of the virus structure.

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The knowledge of the quality and quantity of the viral constituents is a prerequisite for the understanding of their cooperative interactions which stabilize the physical structure of the virus particle, and of their biological functions. The objective of the present communication is to describe the lipid composition of of the Flury HEP strain of rabies virus.

Materials and Methods

Virus: The Flury HEP strain of rabies virus was propagated in BHK 21/C 13 cells and extracellular virus was purified as described recently [5].

Lipid extraction: Lipids were extracted at 20 °C from the dialyzed, lyophilized virus with chloroform:

methanol:water = 40:30:3 (6 ml/40 mg of virus material). Sonic treatment at 10 kilocycles for 3 min (Branson Sonifier, Branson Sonic Power Comp., Danbury, Conn. USA) was applied to ensure complete dispersion of the virus during extraction. The insoluble material was sedimented and reextracted with chloroform:methanol:water = 20:40:3. Further treatment with organic solvents did not result in the extraction of additional lipids. The two extracts were combined and dried in a rotation evaporator under reduced pressure.

Separation of neutral from polar lipids: The lipid extract was dissolved in chloroform : methanol : water = 40:20:3 and fractionated into neutral and polar lipids on a silica gel column packed in chloroform (Bio-Sil HA, minus 325 mesh, Bio-Rad, Richmond Calif., USA: 1 g of gel per 25 mg of lipid). The neutral lipids were eluted with 30 ml of chloroform per g of gel. Subsequently the polar lipids were eluted with the same volume of methanol. Since the lipids were applied onto the column in a chloroform/methanol/water-mixture some polar lipids were also eluted by chloroform together with the neutral lipids. This fraction was dried, dissolved in chloroform and rechromatographed as described. The methanol-eluted fractions comprising the polar lipids were combined, dried and weighed.

Separation and identification of neutral lipids: The neutral lipids eluted by chloroform were separated on a Florisil column [6]. The purity and identity of the fractions were controlled by thin layer chromatography (silica gel plates, Merck AG, Darmstadt, Germany; solvent: petrolether: diethylether:acetic = 80: 20:1). The quantitative determination of glycerides was performed by the method of VAN HANDEL and ZILVERSMIT [7]. Cholesterol and cholesterolesters were determined by the method of ZLATKIS and ZACK [8]. Free fatty acids were determined according to LAUWERY [9].

Separation and identification of phospholipids: The polar lipids were dissolved in chloroform : methanol : water = 40:20:3. Aliquots were taken for the determination of the total phospholipid content and for the quantitative determination of the phospholipid composition. The various phospholipids were separated by two-dimensional thin layer chromatography [10]. The spots were visualized by iodine vapor and identified by reference substances. The lipid spots were scraped off and their phosphorus content was determined according to BARTLETT [11]. The recoveries of the applied total phospholipids ranged between 85 % and 90 %.

Sepatration and identification of glycolipids: The glycolipids were separated from the phospholipids by Florisil chromatography [12] and identified by thin layer chromatography on silica plates with chloroform:methanol:water:ammonia 65:25:4:0.5. Hematoside was identified by treatment with neuramidase as described by KLENK and CHOPPIN [13]. The released neuraminic acid was identified by chromatography according to GRANZER [14].

Hematoside was quantitated with thiobarbituric acid directly from the polar fraction by its neuraminic acid content as described recently [15], multiplying the results with a factor of 1.25. This factor is derived from comparative analysis of pure hematoside for neuraminic acid content by the resorcinol [16] and the thiobarbituric acid method. The incomplete recovery of neuraminic acid from gangliosides by the thiobarbituric acid assay is well known [17]. After chromatography of the polar lipids according to VANCE and SWEEIEY [18] the neutral glycolipids were quantitated by weighing.

Glycolipids of the BHK cells were obtained after extraction of the lipids with chloroform/methanol according to FOLCH *et al.* [19] and subsequent Florisil chromatography [12].

Reference substances: Galactosylceramide was isolated from a cerebroside mixture obtained from Applied Science Laboratories, Glucosylceramide, dihexosyl ceramide and *N*-glycolylneuraminosyllactosyl ceramide (*N*-Glycolylhematoside) were prepared from bovine spleen by a modification of the method of HAKOMORI and STRYCHARZ [20]. *N*-acetyl hematoside was prepared from 3T3 cells [12]. Digalactosylglycosyl ceramide was kindly supplied by Dr. S. HAKOMORI, University of Seattle, USA. Tetrahexosyl ceramide (a cleavage product of gangliosides) was a gift from Dr. STOFFEL, University of Köln, Germany. Neuraminidase from *Vibrio cholerae* was obtained from Behringwerke, Marburg, Germany. *N*-acetylneuraminic acid was purchased from Serva, Heidelberg, Germany. *N*-glycolylneuraminic acid was prepared from ox spleen hematoside by treatment with neuraminidase [13].

Weight determinations: For all weight determinations of lipid fractions aliquots of the lipid solution were placed in aluminium trays. The solvents were evaporated in a stream of nitrogen. The lipid samples were dried over P_2O_5 under reduced pressure and weighed with an electric microbalance (Cahn G-2 mount, Calif., USA).

Results

Tle lipid composition of the Flury HEP strain of *rabies* virus grown in BHK 21/C 13 cells was determined in four independent experiments (Table I). The dried Flury HEP virus contains 25 % of chloroform/methanol soluble material. The lipids were separated into neutral and polar constituents. The polar lipids constitute 19.5 % of the dry virus mass. Approximately 5.5 % of the dry virus mass are neutral lipids. The neutral lipids were fractionated by Florisil chromatography into their different compounds and quantitated (Table II). Cholesterol represents 80 % of the neutral lipids. Each of the other neutral lipids accounts for less than 5 %. About 95 % of the compounds of the neutral lipid fraction of HEP virus could be chemically identified.

The components of the polar lipid fraction were separated by two-dimensional thin layer chromatogra-

Table I. Lipid content of Flury HEP rabies virus.

ipid fraction	% (w/w) of dry virus mass	
total lipids (chloroform/methanol soluble material)	25.3 ± 0.3	
netutral lipids	5.5 ± 0.2	
polar lipids	19.5 ± 0.4	
phospholipid and glycofractio of polar lipids	15.8 ± 0.4	11.2 ± 0.5 phospholipids 3.1 ± 0.2 hematoside 1.5 glucosyl+ lactosyl ceramide

Table II. The lipid composition of Flury HEP rabies virus.

	% (w/w) of lipid fraction	% (w/w) of dry virus mass*
neutral lipids		
cholestrol	80.0 ± 1.0	4.40
cholesterolesters	1.9 ± 0.4	0.10
triglycerides	4.5 ± 1.0	0.25
diglycerides	5.0 ± 0.3	0.28
monoglycerides	1.6 ± 0.8	0.09
free fatty acids	2.4 ± 0.8	0.13
total	95.4 ± 2.2	5.25
pohspholipids		
phosphatidylcholine	25.2 ± 1.0	2.82
phosphatidylethanolamine	27.0 ± 1.2	3.02
lysophosphatidylethanolamine	6.5 ± 2.0	0.73
pohsphatidylserine	8.4 ± 0.9	0.94
phosphatidylinositol	3.5 ± 0.3	0.39
sphingomeyelin	26.8 ± 0.5	3.00
total	95.5 ± 1.5	10.90
glycolipids		
hematoside	~66	3.04
glucosyl + lactosyleramide	~33	1.52
total		4.56
molar ratio of cholesterol to phospholipid		0.87 ± 0.02

* 5.5 % neutral lipids + 15.8 % determined polar lipids (see Table I).

phy. Phospholipids were identified by reference substances and their amount determined by their phosphorus content (Table II). The three main phospholipids present in the virus are phosphatidylethanolamine, phosphatidylcholine and sphingomyelin, each representing 25 to 27 % of the total phospholipids. Phosphatidylserine and phosphatidylinositol constitute only 8.4 % and 3.5 % of the phospholipids, respectively. In all preparations lyso-phosphatidylethanolamine was detected which accounts for 6.5 % of the total phospholipids. In some cases, however, a much higher content of this lyso-phospholipid was found with a concomittant decrease of the phosphatiylethanolamine content.

Decomposition of phosphatidylethanolamine has never been observed when this lipid was prepared from other sources than Flury HEP virus by the same method. The decomposition of phsphatidylethanolamine from Flury HEP virus may indicate the presence of a specific phospholipase present in the viral envelope.

The phospholipids account for 11 % of the dry virus mass. The molar ratio of cholesterol to phospholipid was found to be 0.87.

The glycolipids of the rabies virus were compared by thin layer chromatography with those of BHK 21-C 13 cells (Fig. 1*). In both cases three glycolipids, glucosylceramide, lactosylceramide and hematoside were detected. Hematoside, the only ganglioside found, was shown to be predominantly the *N*-acetyl derivate in both, the cells and the virus (Fig.2). Hematoside constitutes 3.1 % of the dry virus mass as determined by its sialic acid content. Glucosylceramide and lactosylceramide constitute 1.5 % of the dry virus mass. Phospholipids and glycolipids account together for 15.8 % of the dry virus mass. The remaining 3.7 % of the polar lipids could not be identified.

Discussion

The analysis of the lipid composition of purified Flury HEP strain of rabies virus grown in BHK 21/C 13 cells reveals two interesting features which appear to apply generally for lipid containing viruses as well as cellular plasma membranes [21–35]:

1. A high content of cholesterol corresponding to a high cholesterol/phospholipid ratio of 0.87.
2. A high content of sphingolipids such as sphingomyelin, hematoside, glucosyl- and lactosylceramide.

The glycolipids incorporated into the viral envelope of Flury HEP virus were found to be identical with those found in the host cells. Similar results were reported for the glycolipids of *vesicular stomatitis* virus (VSV) [36], grown in BHK 21 cells and for the glycolipids of *simian* virus (SV 5) except the lack of sialic acids in the glycolipids of the latter virus which are split off by the virus associated neuraminidase [13].

Similarities of the lipid composition of plasma membranes of the host cells and the envelope of viruses

* Figs 1 and 2 see Table page 92a.

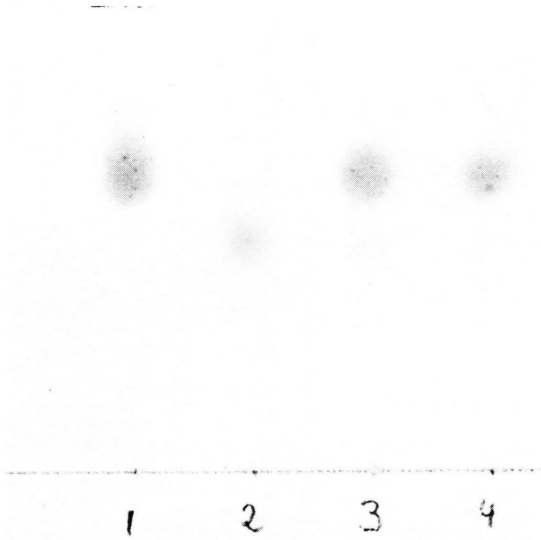

Fig. 1. Thin layer chromatography of glycolipids from BHK 21/C 13 cells (8) and Flury HEP rabies virus (9) on silica gel. Solvent: Chloroform : methanol : water : 25% ammonia = 65 : 25 : 4 : 0.5. The spots were visualized after spraying with 50-perc. sulfuric acid.
Reference substances: (1) Galactosylceramide, (2) glucosylceramide, (3) lactosylceramide, (4) digalactosyl-glucosylceramide, (5) *N*-acetyl hematoside, (6) *N*-glycolyl hematoside, (7) tetrahexosylceramide.

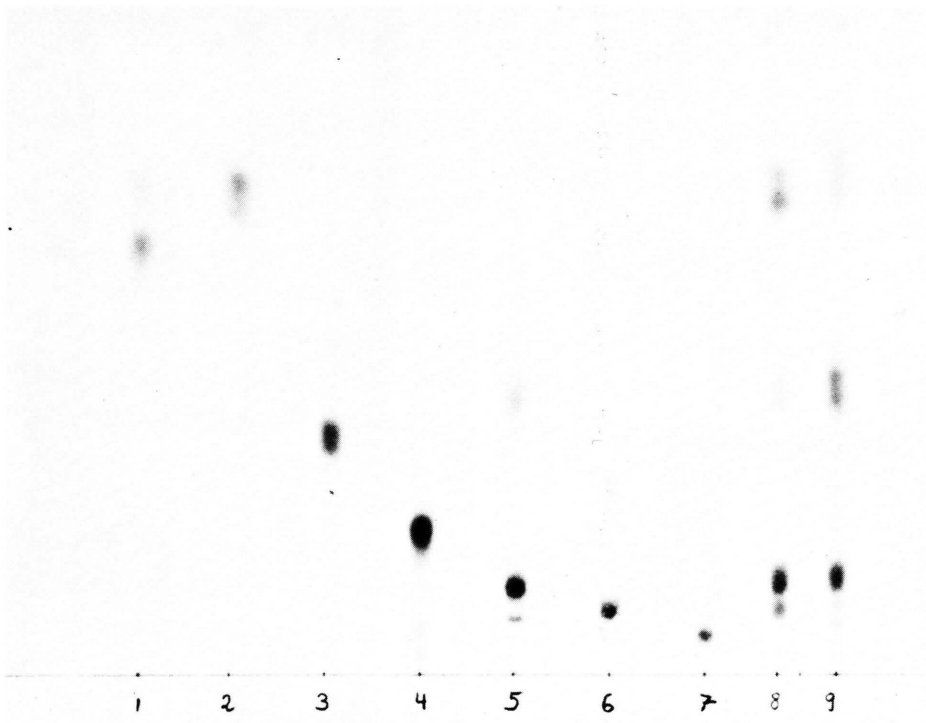

Fig. 2. Thin layer chromatography of sialic acids released from hematoside of BHK 21/C 13 cells (3) and of Flury HEP rabies virus (4) after treatment with neuraminidase. Solvent: Propanol : water = 7 : 3. The spots were developed with Bial's reagent [42]. Reference substances: (1) *N*-acetylneuraminic acid (2) *N*-glycolyl neuraminic acid.

have been reported when one and the same virus is grown in different host cells [21-23]. This is not surprising in the case of budding viruses such as myxo- and paramyxoviruses, which mature and assemble at the cellular plasma membrane and are subsequently released from the plasma membrane by a budding process [37]. In some cases significant differences of the lipid composition of virus and plasma membranes were observed and an important role of viral proteins in the selection of the lipids to be incorporated into the virus membrane has been suggested [22, 23, 38].

In the case of myxo- and paramyxoviruses mature particles are not observed within the cytoplasma of the host cell. In BHK 21 cells infected with Flury HEP virus, mature virus particles not only are released into the culture medium, but complete infectious virus particles are found abundantly in the cytoplasma [1, 4]. This indicates, that Flury HEP virus can be assembled during a budding process as well as at cytoplasmic matrices of the host cells. The analytical data about the lipid composition of Flury HEP virus in this communication refer to extracellular virus particles. The lipid composition of the extracellular Flury HEP virus is significantly different from the lipid composition of the BHK 21 plasma membrane [35]. Particularly the sphingomyelin content of the extracellular virus is markedly higher than that reported for the host cells [35] (27 % versus 17 %). This observation and the fact that Flury HEP virus can also mature remote from the plasma membrane suggest a specific function of viral proteins in selecting lipids during virus assembly. The fact that the Flury HEP virus and other viruses as well as cellular plasma membranes exhibit relative high amounts of cholesterol and sphingomyelin may reflect a similar biological function of virus envelope and cellular plasma membrane. In such membranes cholesterol could have an important stabilizing function [21, 39].

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