ムギ北地モザイクウイルスに含まれるステロール

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著者	鳥山, 重光
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Sterol Composition of Northern Cereal Mosaic Virus

Shigemitsu TORIYAMA*

鳥山重光*：ムギ北地モザイクウイルスに含まれるステロール

Northern cereal mosaic virus (NCMV) is a rhabdovirus and phospholipids and sterols have been reported as viral components[1]. Among other plant viruses, an enveloped spherical virus, tomato spotted wilt virus[2] and a rhabdovirus, potato yellow dwarf virus[3] have been also reported to contain lipids as a component of virus particles. Lipid components are more common among animal viruses and abundant analytical evidence indicate that the phospholipid composition of the viral envelope broadly resembles that of the host membrane[4-8]. However, detailed information regarding lipid composition is not available for plant viruses. This report describes the results of gas chromatographic analysis of sterols extracted from purified NCMV and its host, the barley plant. Purification of NCMV was carried out by the method previously described[1]. Lipids from purified NCMV was extracted by chloroform-methanol (2:1) for 4 hr at room temperature following washing with 0.5 % NaCl solution. The organic solvent was evaporated *in vacuo* to dryness. Extraction procedure of sterols from barley plant tissues was as follows. Healthy or infected barley plants, at the same growth stage, were chopped into sections of about 5 mm and soaked in 80 % acetone for one day. The tissue sections were then extracted by homogenizing in 80% acetone. The combined acetone fraction was condensed *in vacuo* at a temperature under 40 C and the remaining aqueous fraction was extracted three times with an equal volume of ethyl acetate. The ethyl acetate extract was dried on anhydrous Na_2SO_4 and then evaporated to dryness. The dried residue was saponified with ethanolic 10% KOH. In many cases, crude unsaponified neutral lipids were fractionated by means of silicic acid chromatography[9]. Lipids extracted from virus and plant tissues were dissolved in a small volume of chloroform and subjected to analysis by gas liquid chromatography. For analysis of sterols, a Shimazu model GC 4A chromatograph was used with N_2 as the carrier gas. Two glass columns (4 mm × 1.5 m) packed with 1.5% OV-17 coated on Gas Chrom Q were used as the stationary phase at a column temperature of 255 C. A hydrogen flame ion detecter was used. Sterols were identified by comparing retention times with those of authentic ones. Relative quantities of sterols were determined by triangulation of peak area on chromatograms, with the assumption that all sterols were

* Faculty of Agriculture, The University of Tokyo, Bunkyo-ku, Tokyo, Japan. 東京大学農学部

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eluted with equal efficiency. Isolation of intracellular organelles of barley plants was by the methods of Kemp & Mercer[10], and sterols were extracted by the same method described for plant tissues. Four sterols, i.e. β-sitosterol, stigmasterol, campesterol and cholesterol were detected in NCMV, in a ratio of 50.2%, 25.6%, 12.9 % and 10.7%, respectively (Table 1). Table 2 shows sterols and their contents in

Table 1. Sterol composition of northern cereal mosaic virus

Exp. no.	% of sterols			
	Cholesterol	Campesterol	Stigmasterol	β-Sitosterol
1	6.6	16.6	22.6	54.2
2	11.5	13.5	26.8	46.4
3	14.1	8.6	27.3	50.0
Avg.	10.7	12.9	25.6	50.2

Table 2. The composition of sterols extracted from barley plant, healthy or virus infected

Plant source	Exp. no.	% of sterols			
		Cholesterol	Campesterol	Stigmasterol	β-Sitosterol
Healthy	1	1.4	16.1	34.2	48.3
	2	0.8	21.3	35.8	42.1
	3	1.7	17.4	24.4	56.5
	Avg.	1.3	18.3	31.5	49.0
Infected	1	1.0	14.0	29.8	55.2
	2	1.7	13.7	41.0	43.6
	3	0.5	21.6	24.4	53.5
	Avg.	1.1	16.4	31.7	50.8

the extracts from healthy and infected barley plants. The content of cholesterol was about one tenth of that detected in purified viruses, and the contents of campesterol and stigmasterol were slightly higher than those of viral sterols. There was no discernible difference in β-sitosterol content between virus and plant tissues. Occasionally, a small peak appeared as a slow-moving shoulder in the chromatogram of plant tissue sterols, and was omitted from calculation of sterol composition. There was no difference in sterol composition of nuclear and microsomal fractions isolated from infected or healthy plants. Table 3 shows analytical results of sterol composition of the microsomal fraction. The sterol composition of nuclear and microsomal fractions was more similar to that of plant tissues rather than the virus. The phospholipid composition of animal viruses coincides with that of host cell membranes rather than whole cells[5,6]. For the reason, it has been believed that virus nucleocapsids are enveloped by budding through the host cell membrane. Only

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Table 3. Sterol composition of microsomal fractions obtained from barley plant, healthy or virus infected

Plant source	% of sterols			
	Cholesterol	Campesterol	Stigmasterol	β-Sitosterol
Healthy	0.6	15.4	30.0	54.0
Infected	0.7	14.0	36.8	48.5

cholesterol and cholesterol ester have been reported so far as sterols of animal viruses. Sterol composition and the relative contents of respective sterols of NCMV broadly resemble those of the host plant. However, it can be concluded only with caution that the nucleocapsids of NCMV are enveloped by budding from preexisting host membranes. Observations of NCMV *in situ* have shown that NCMV particles exist in the cisternae of endoplasmic reticulum, but budding profiles have been seen only occasionally[11]. Relatively high cholesterol content in virus particles could not simply be accounted for by the cholesterol content of the microsomal fraction (ER?), which seems to be closely associated with the assembly of virus particles of NCMV on the basis of electron microscopical observation. Lipid-containing bacteriophage PM 2 has been reported to envelope near the plasmamembrane without budding by incorporation of host phospholipids, the composition of which is altered by infection [7,12]. Cholesterol might be incorporated selectively into the envelope of NCMV. Further research is necessary for elucidating precisely the relationship between the viral envelope and host cell membrane. In this regard, studies aimed at discerning the composition of viral and host cell phospholipids, which are essential to biological membranes, are required as a subsequent avenue of investigation.

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