LIPID IN POTATO YELLOW DWARF VIRUS

Mahmoud E. Ahmed and L. M. Black

Department of Botany, University of Illinois, Urbana, Illinois

E. G. Perkins, B. L. Walker and F. A. Kummerow

The Burnsides Research Laboratory, University of Illinois, Urbana, Illinois

Received July 6, 1964

INTRODUCTION

Nucleic acid and protein are two essential constituents of all complete virus particles. The only additional substance reported in plant viruses is a polyamine (Johnson and Markham, 1962). We present evidence for the presence of lipid as an intrinsic part of potato yellow dwarf virus particles.

MATERIALS AND METHODS

Virus purification - New York potato yellow dwarf virus (PYDV) was purified from infected leaves and stems of Nicotiana rustica L. After preliminary treatment of extracts to eliminate chlorophyll and remove coarser particles the virus was pelleted in a Servall SS1 centrifuge and then re-suspended in a small volume of a solution containing 0.1M glycine and 0.01M $MgCl_2$ adjusted to pH 7.0 (Brakke, 1956). This concentrated suspension was then purified by zonal density-gradient centrifugations (Brakke, 1951, 1953, 1955), specifically by two "rate" followed by two "quasi-equilibrium" centrifugations in a Spinco SW 25.1 rotor through sucrose gradients prepared in the glycine-$MgCl_2$ solution. All purification procedures were carried out at 2-4°C.

Judging by examination of similar preparations by electron microscopy,

analytical ultracentrifugation, zonal density-gradient centrifugation and zonal density-gradient electrophoresis the virus preparations were virtually free of contaminating host components, so that such components could not account for the more than 20% lipid in the virus preparations described below.

Extraction and estimation of the virus lipid.

Pellets of purified virus were suspended in a minimum amount of water, transferred to a preweighed tube, lyophylized and weighed. Lipid was extracted from the dry virus by shaking with 20 volumes of a mixture of chloroform-methanol (2:1, v/v) for one hour. The solution was filtered through a Whatman filter paper pre-extracted with chloroform-methanol. The filtrate was evaporated to dryness in vacuo using a rotary evaporator and the residue re-extracted with chloroform and filtered. The filtrate containing the lipid was washed with three volumes of distilled water, transferred to a preweighed flask, dried and weighed. The virus was found to contain over 20% lipid.

Because of the difficulties involved in obtaining a large amount of highly purified virus, exact results as to the percentage of lipid in the virus could not be obtained. The dried lipid was redissolved in chloroform and was stored at -25°C under nitrogen gas until required. Precautions to minimize contamination of our samples were taken.

Extraction of the healthy plant lipid.

Combined samples of leaves, stems, and flowers were taken from healthy N. rustica plants and extracted with chloroform in a soxhlet apparatus; after 24 hours extraction the chloroform solution was replaced by diethyl-ether and the extraction continued for another 12 hrs. The organic layers were combined and evaporated to dryness in vacuo. The residue was saponified by refluxing for 2 hrs. in 100 ml. of 1N sodium hydroxide in 95% ethanol. The unsaponifiable material was separated and the saponified material,

after conversion to the free fatty acids was washed, dried and stored at -25°C under N_2 until required.

Preparation of methyl esters and gas-liquid chromatography.

Methyl esters were prepared by refluxing a sample of the lipid with 1% H_2SO_4 in anhydrous CH_3OH. The methyl esters were subjected either directly to gas-liquid chromatography or were purified by thin layer chromatography before analysis. Gas chromatographic analyses of the methyl esters were carried out on a Barber Coleman Model 10 instrument employing a B-ionization detection system (tritium triode).

Thin-layer chromatography (TLC).

Samples of virus lipid (50-100 μg) were chromatographed on glass plates coated with silica gel G. Polar lipids were separated with chloroform-methanol-water, 65:25:3.5, as eluant, and the non-polar lipids were fractionated on a separate plate with hexane-diethylether-acetic acid, 90:10:1, as the eluting solvent. The component spots were visualized by spraying with one of the following reagents: 50% aqueous sulfuric acid saturated with potassium dichromate (Kirchner, et. al. 1951); 0.025% Rhodamine 6-G in 95% ethanol (Kaufmann et. al. 1961); ninhydrin in butanol (Schlemmer, 1961); Dragendorff reagent (Schlemmer, 1961); or diphenylamine (Wagner, 1960). Standard compounds were run on the appropriate plate when available. The components of the virus lipid were identified by comparison of their Rf values with those of the standards, and by reaction with the specific reagents listed above. Additional tentative identifications were made by comparison with results in the literature.

RESULTS

The fatty acid composition of PYDV lipid (table 1) indicated the presence of appreciable amounts of palmitic, stearic, linolenic, linoleic

and oleic acids. These fatty acids are present in appreciable amounts in the healthy plant lipid (table 1). However, the amount of palmitic acid

TABLE 1

Fatty Acid Composition of Healthy N. rustica Lipid and PYDV Lipid

Identity[1]	Healthy plant lipid[2]		Virus preparations[3]		
	A (% wt.)	B (% wt.)	A (% wt.)	B (% wt.)	C (% wt.)
12.8	----	----	----	----	0.3
13.8	0.9	----	2.3	0.1	0.2
14:0	0.7	----	1.5	----	2.3
14.5	0.3	----	0.5	1.6	0.5
15:0	0.5	0.6	0.4	3.5	----
15.3	----	----	----	0.8	0.4
15.7	----	1.6	----	0.2	----
16:0	13.1	18.3	37.7	32.6	29.3
16:1	0.7	1.1	6.2	4.8	2.2
16.9	1.5	0.6	1.2	0.6	0.7
17.3	0.6	2.7	0.9	0.3	0.4
18:0	11.1	4.7	13.6	8.8	8.9
18:1	5.7	6.1	11.7	14.9	11.9
18:2	10.7	10.4	10.6	14.1	18.4
18:3	53.7	45.0	8.9	21.7	22.4
20.2	----	----	----	0.3	----
21.0	----	----	----	5.3	1.6
22.4	----	----	4.6	2.5	----

[1] The numbers before and after the colon represent carbon chain length and number of double bonds respectively. Numbers before and after the period represent the apparent ester carbon number of unidentified components (carbon number calculated from methyl esters of n-alkanoic acids).

[2] The analysis of healthy plant lipid was made on two different samples.

[3] Results obtained upon analysis of three different samples of virus lipid is shown. Of seven different virus lipid samples analyzed, all showed closely similar results except that sample (A) gave a low value for linolenic acid. This sample is the only one from which the complete virus, instead of virus lipid, was refluxed with methanol containing 1% sulfuric acid and the methyl esters obtained further purified by TLC. The low value obtained for linolenic acid cannot be explained at this time.

in the virus lipid is appreciably more than that in the plant lipid. On the other hand, the amount of linolenic acid in the virus lipid is appreciably less than that in the plant lipid. The differences in the fatty acid composition of the virus lipid and of the healthy plant lipid also indicate that the lipid which has been isolated is an integral part of PYDV.

Thin layer chromatography of the virus lipids in the petroleum ether-diethylether-acetic acid solvent indicated the presence of sterol and possibly of free fatty acids. With the more polar solvent, several spots were obtained. With the aid of authentic standards and specific reagents, phosphatidyl ethanolamine and phosphatidyl choline were identified. In addition a number of spots were found to give a positive reaction with the diphenylamine reagent, indicating the presence in the virus lipid of several different glycolipids. Two of these glycolipids migrated to the same area of the plate to which a crude sample of kerasin migrated.

REFERENCES

Brakke, M. K., J. A. C. S. 73, 1847 (1951).
Brakke, M. K., Arch. Biochem. Biophys. 45, 275 (1953).
Brakke, M. K., Arch. Biochem. Biophys. 55, 175 (1955).
Brakke, M. K., Virology 2, 463 (1956).
Johnson, M. W., and Markham, R., Virology 17, 276 (1962).
Kirchner, J. G., Miller, J. M., and Keller, G. E., Anal. Chem., 23, 420 (1951).
Kaufmann, H. P., Makus, Z., and Deicke, F., Fette Seifen Anstrichmittel, 63, 235 (1961).
Schlemmer, W., Bull. Soc. Ital. Biol. Sper., 37, 134 (1961).
Wagner, H., Fette Seifen Anstrichmittel, 62, 1115 (1960).