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EFFECT OF LYSOLECITHIN ANALOGUES ON PLANT VIRUSES

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Summary. — Newly synthesized lysolecithin analogues 2-0-hexadecyl-glycero-3-phosphocholine (1) and its methyl (2), ethyl (3) and benzyl (4) derivatives were tested with regard to the antiphytoviral effect on potato virus X (PVX), red clover mottle virus (RCMV) and tobacco mosaic virus (TMV). The first two compounds (1, 2) reduced markedly the content of PVX and RCMV in systemically infected host plants as evidenced by precipitin test and bio-assay. Whereas compounds (1), (2) and (3) did not significantly influence the local lesion formation caused by TMV, compound (4) increased the number of necrotic lesions. In the presence of all four lysolecithin analogues, especially of (1), (2) and (3) the infectivity of virus particles was reduced.

Key words: *lysolecithin analogues*; *antiviral effect*; *potato virus X*; *red clover mottle virus*; *tobacco mosaic virus*

Introduction

Lysolecithin and its synthetic analogues are compounds with a remarkable biological activity (Nuhn *et al.*, 1982). Depending on concentration they cause lysis or fusion of cells (Croce *et al.*, 1971; Gledhill *et al.*, 1972; Halfer and Petrella, 1976). Occasionally, objections have been raised to attempts to include phosphocholines into the group of fusogenic agents, since lytic and fusogenic concentrations lie rather close to each other (Poole *et al.*, 1970). Erythrocytes were frequently used for studies to elucidate cell- and membrane-changing properties. The cytolytic and membrane-perturbing properties of lysophosphatidylcholines were comprehensively dealt with by Weltzien (1979).

As follows from certain interactions occurring between viruses and membrane systems (Parkes and Fox, 1975; Banerjee *et al.*, 1981), virus replication may be influenced by membrane active compounds. Detergents such as sodium dodecyl sulphate, Triton X-100, and Tween 80 (Taniguchi, 1976) inhibit virus multiplication. Lysolecithin analogues, because of their biological properties, are of great current interest as potential inhibitors of virus mul-

tiplication. A number of new-type lysolecithin derivatives with defined cytolytic and fusogenic properties was synthesized by Nuhn *et al.* (1982). In the present paper we report of their effects on selected plant viruses.

Materials and Methods

Viruses and host plants. The virus-host combinations used in this study were: potato virus X (PVX) strain H 19 (obtained 20 years ago from Biologische Bundesanstalt, Braunschweig, F.R.G.) on *Nicotiana tabacum* L. 'Samsun' as systemic host and red clover mottle virus (RCMV) TpM 36 (Musil and Matisová, 1967) on *Pisum sativum* convar. *speciosum* (Dierb.) Alef 'Nadja'. Hypersensitive hosts were *Gomphrena globosa* L. for PVX and *Phaseolus vulgaris* L. 'Selenta' and 'Alfa' for RCMV. To determine the effect of lysolecithin derivatives on the formation of local lesions, tobacco mosaic virus (TMV) "Green strain" cultivated for more than 20 years in this Department was also included; *Nicotiana glutinosa* L. was used for eliciting local lesions by TMV.

Design of the experiments. The experiments were performed in a climatic room (20 ° ± 2 °C; 80% air humidity; 16 hr of lighting at 9 000 lux). *N. tabacum* 'Samsun' and pea plants were used for the experiments at the age of about eight weeks and within nine to ten days after seeding, respectively. Two days before and two days after inoculation the plants were sprayed three times with 1 mmol/l aqueous solutions of the easily soluble compounds. The inoculation of systemic host plants was done mechanically using virus-containing crude sap. We used carborundum powder as an abrasivum. From the tobacco 'Samsun' the two youngest fully developed leaves of each plant were inoculated (= primarily infected), in case of *P. sativum* convar. *speciosum* the two fully developed leaves were inoculated.

To determine whether the formation of necrotic lesions caused by TMV could be influenced by lysolecithin derivatives, two well developed leaves per plant of *N. glutinosa* were detached one day before inoculation. One half-leaf was rubbed with 1 mmol/l solution of the compound and the opposite one with water (control). The detached leaves were preserved with diffuse light and high humidity in Petri dishes. Both half-leaves were infected with purified virus (1 μg/ml). The half-leaves were treated again 1 day post-infection.

Lysolecithin derivatives. The rac. phosphocholine derivatives included were the following:

(1) 2-0-hexadecyl-glycero-3-phosphocholine
(2) 1-0-methyl-2-0-hexadecyl-glycero-3-phosphocholine
(3) 1-0-ethyl-2-0-hexadecyl-glycero-3-phosphocholine
(4) 1-0-benzyl-2-0-hexadecyl-glycero-3-phosphocholine

The synthesis of these synthetic lysolecithin analogues (Fig. 1) was described by Nuhn *et al.* (1982).

Virus assay. The virus content was determined serologically and by bio-assay. PVX was tested in the upper of two primarily infected tobacco leaves 6 days ± 1 day p.i. In the case of the secondarily infected leaves the next above the upper inoculated leaf was taken 12 days ±1 day p.i. Pea plants were harvested 9 days p.i. The serological methods used for testing PVX and RCMV were the drop precipitin test (PVX) and the ring precipitin test (RCMV), respectively. These serological tests were described in detail (Kluge *et al.*, 1978; Kluge and Marcinka, 1979). The results were based on the average values from about 12 plants. Each experiment was twice repeated. At the same time when the virus was detected serologically, PVX-containing crude sap diluted at a ratio of 1 : 4 was applied to equally developed leaves of *G. globosa*, while diluted RCMV-containing crude sap was applied to half-leaves of *P. vulgaris* 'Selenta' or 'Alfa'. Sap of the untreated plants (control) was applied to equally positioned leaves and opposite half-leaves, respectively. The number of local lesions was counted after three to five days. The results are average numbers of lesions from at least ten half-leaves.

Immediate contact of lysolecithin analogues with virus particles in vitro. In order to test the influence of the lysolecithin analogues on the infectivity of virus particles in vitro we put the substances in contact with purified virus. We used standard preparations of 1 mg virus/ml which were diluted 1 : 100 (PVX with aqua dest.) or 1 : 1000 (RCMV with 0.1 mol/l phosphate

buffer, pH 8.0; TMV with 0.05 mol/l borate-HCl buffer, pH 8.2). The virus preparation diluted in this way was mixed with the lysolecithin compound in a final concentration of 1 mmol/l. The inoculum without adding the substance (control) or after adding the substance and allowing to react for 15 min (variant) was applied to the half-leaves of the hypersensitive host plant.

Results and Discussion

As shown by previous investigations (Nuhn *et al.*, 1982), the synthetic compounds (1) and (4) have a lytic activity on plant protoplasts when used in a concentration of 0.1 mmol/l. In lower concentrations (0.05, 0.025 and

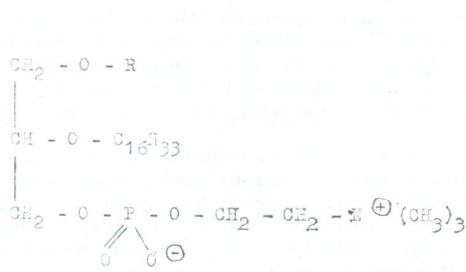

Fig 1.
Structure of lysolecithin and its synthetic analogues

0.01 mmol/l), they tend to cause protoplast fusion. In a concentration of 1 mmol/l the lysolecithin derivatives had no phytotoxic or other visible effects on intact plants. The effects of these compounds in lytic concentrations of 1 mmol/l on the virus contents in tobacco and pea plants, as determined by the precipitin test and by bioassay, are shown in Fig. 2. A marked reduction of virus content by compounds (1) and (2) is apparent in contrast to a weak inhibition and even an occasional increase in virus content after application of compounds (3) and (4), respectively. Testing of other compounds confirmed that primarily infected leaves of the systemic host are, as a rule, poor indicators of antiviral activity, notwithstanding the fact that active substances may become directly effective. This is also reflected in the inhibitory effect of compounds (1) and (2). However, in secondarily PVX-infected tobacco leaves as well as in RCMV-infected pea plants more than 50% reduction in virus contents was observed. In extreme cases, the reduction in virus content of PVX-infected leaves was as high as 88.2%. Although PVX and RCMV differ entirely in their structure and genome, their reaction to the treatment with the two compounds was of a similar nature and intensity. In the case of compounds (1) and (2), the results obtained with the two detection methods of virus content were virtually similar; only gradual differences being observed. Based on our experiments, we cannot decide whether the diminished virus content is the result of an impaired virus multiplication or its altered distribution. The effects of increasing or decreasing content of PVX and RCMV after application of com-

pounds (3) and (4) never exceeded 50%. Moreover, the serological tests and bioassay showed opposite effects for PVX. One cannot exclude the existence of various modes of action of the compounds depending perhaps on their distribution ad/or actual concentration in leaf tissue.

Concerning the effect of lysolecithin analogues upon the formation of necroses after TMV infection (Table 1), the observation was made that compounds (1), (2) and (3) in general did not affect significantly the formation of local lesions. A significant, though small increase in the number of local lesions has been observed after application of compound (4), which

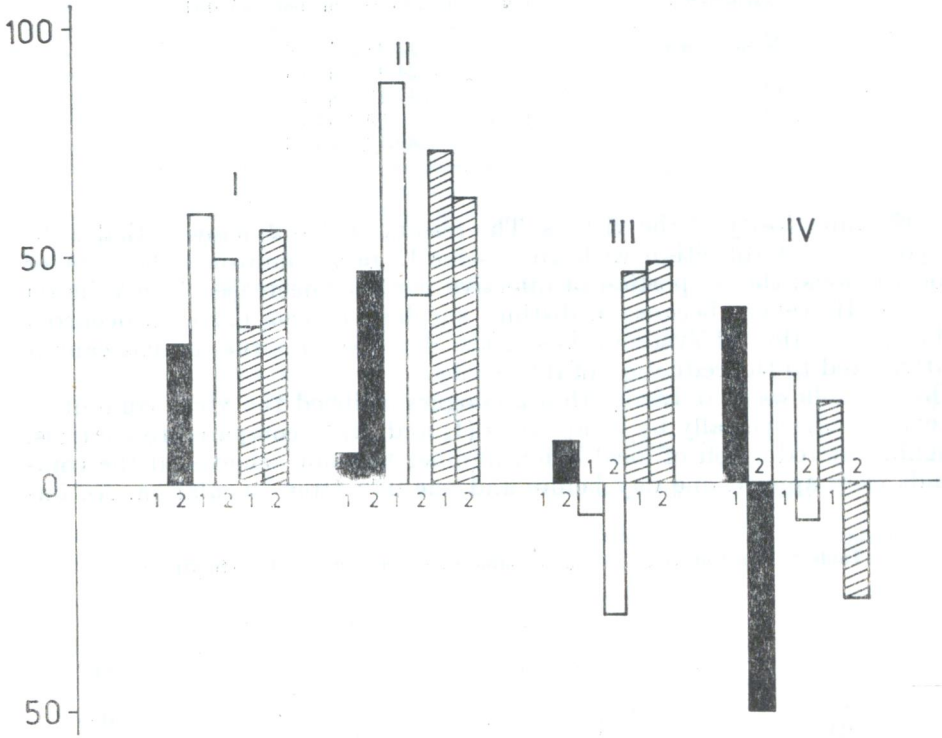

Fig. 2.

Inhibition of virus multiplication by lysolecithin analogues
black columns: tobacco leaves primarily infected with PVX
white columns: tobacco leaves secondarily infected with PVX
dashed columns: RCMV-infected pea plants
1 — virus detected by precipitin test
2 — virus detected by bioassay
I — after application of compound (1)
II — after application of compound (2)
III — after application of compound (3)
IV — after application of compound (4)
Ordinate: per cent of inhibition (positive or negative).

tends to initiate promotion of viral multiplication as detected by bioassay even in the case of systemic infection with PVX and RCMV. Since we could not rule out the possibility that infection of the systemic host plants or of the host hypersensitive to TMV is affected by interactions of the virions with the lysolecithin analogues, we examined the influence of the compounds

Table 1. Effect of lysolecithin analogues on the number of local lesions caused by TMV on Nicotiana glutinosa L.

Treatment	Number of local lesions per half-leaf
None (control)	14.2 ± 4.2
(1)	24.9 ± 5.9
(2)	17.1 ± 5.0
(3)	20.3 ± 2.2
(4)	30.1 ± 4.9

upon the infectiosity of the virions. The results (Table 2) showed that a direct contact of lysolecithin with virions for 15 min diminished the number of local lesions; the proportion of infective particles decreased to maximum of 4.2%. However, because a distinct surplus of virus particles occurred during inoculation of systemic hosts, the decrease of virus content cannot be attributed to the reduction of infectivity.

Thus, it follows that lysolecithin analogues reduced the virus content in systemic hosts especially by compounds (1) and (2) as compared to controls. A significant reduction of local lesion number was not achieved if the compounds were applied one day before and one day after inoculation. An im-

Table 2. Effect of some lysolecithin analogues on virus particles in vitro

Virus	Local lesions (%)				
	control	(1)	(2)	(3)	(4)
PVX	100	22.7	32.7	29.4	40.5
RCMV	100	4.2	5.3	25.2	19.2
TMV	100	44.8	22.7	68.8	75.4

mediate contact of each of the tested compounds with free virus particles led to a striking inhibition of their infectivity if the chemical compounds were present in the course of inoculation. These results are in some aspects similar to the inhibitory activity of mineral oil (Loebenstein *et al.*, 1964; Peters and Lebbink, 1975), which is active nearly in the same concentration. Further investigations are necessary to characterize comprehensively the conditions of the inhibitory action of lysolecithin derivatives, these interesting members of a biologically active group of compounds.

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