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Antagonistic Effect on TMV Infectivity Between Poly-L-lysine and Poly-L-arginine

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Polylysine pretreatment of Xanthi-nc tobacco half leaves caused a reduction of TMV local lesions compared to the control. A similar polyarginine pretreatment resulted in an increase in local lesions. A mixture of the polycations used for pretreatment did not cause reduction or increase of the number of local lesions. Using a systemic host we demonstrated the same antagonistic effect of these two polycations.

On the other hand we got similar results if we incubated the pure virus with polylysine or polyarginine. Therefore, these synthetic polycations could affected the replication of TMV in some way, perhaps through the pinocytosis mechanism, or membrane alterations are enhanced by basic polypeptides.

Reports from some laboratories have been shown that several polycations (e.g. polylysine and polyornithine) inhibit tobacco mosaic virus infectivity (STAHMANN *et al.*, 1951; STAHMANN and GOTHOSKAR, 1958; ZHURAVLEV *et al.*, 1974). STEIN and LOEBENSTEIN (1972) reported that synthetic polyanions would induce interference with the infection of tobacco mosaic virus. However, SHAW (1972) using poly-L-ornithine, has shown that this polycation produced an increase in the amount of TMV retained by inoculated tobacco leaves and in the amount of uncoating of the RNA of this retained virus. The results of these experiments with polyornithine do not agree with the conclusions from work a similar substance, polylysine. STAHMANN et al. (1951) found that mixtures of polylysine and TMV produced fewer lesions on a local lesion host than did TMV alone. The effect was attributed to the formation of ionic bonds between the ϵ-NH_2 groups of polylysine and the acidic group of the virus (BURGER and STAHMANN, 1951). These studies led to the conclusion that polybasic substances inhibit TMV by combining the virus and not by an effect on the host and that this combination prevents either the initial binding of the virus by the host cell or some other cell-virus interaction (STAHMANN and GOTHOSKAR, 1958). SHAW's (1972) experiments have shown that polyornithine treatment appeared to increase rather than inhibit the initial interaction of virus and host and, further to provide conditions which stimulates the release of the RNA of the virus. Polyornithine greatly increases for the infection of the tobacco protoplasts by TMV (TAKEBE and OTSUKI, 1969).

These contradictory data suggest that the decreased or increased infectivity

could not be attributed simply to a polyanion (virus)–polycation (polybase) complex formation. Also here we must note that there are several reports that basic polypeptides (of different molecular weights) are on or in several viruses, and this may have a role in the virus–host interaction and virus replication (GINOZA *et al.*, 1954; HERSHEY, 1955; FINE *et al.*, 1968; RUSSEL *et al.*, 1968; FLEISSNER, 1971; GIBSON and ROIZMAN, 1971; COURTNES and BENYESH-MELNICK, 1974; KRELL and LEE, 1974; BALÁZS *et al.*, 1975; POGO *et al.*, 1975). Therefore we performed experiments with polylysine and polyarginine to determine their effects on virus infectivity and virus–cell interaction.

Materials and Methods

The U_1 strain of TMV was cultured in *Nicotiana tabacum* L. cv. Samsun plants. The virus was purified according to FRAENKEL-CONRAT (1966) and GOODING and HEBERT (1967). To determine the effects of polycation induced virus susceptibility, we used *Nicotiana tabacum* L. cv. Xanthi-nc (resistant-host) and *Nicotiana tabacum* L. cv. Xanthi (susceptible-host). All tobacco plants were grown under normal greenhouse conditions and were used for the experiments at the 6 to 8 leaf stage.

Poly-L-arginine-HCl (Sigma Chemical Co. St. Louis, Mo.) type 11-B. Mol. wt. 17,000 and the degree of polymerization 81, and the polyornithine content was 3–5%. Poly-L-lysine-HCl (Sigma Chemical Co. St. Louis, Mo.) type V-A. Mol. wt. 30 000.

Polycations were applied at 10 μg/ml in 0.1 *M* phosphate buffer pH 6.9 by hypodermic syringe infiltration of one half of attached leaves. The control half leaves were infiltrated with 0.1 *M* phosphate buffer pH 6.9 containing 10 μg/ml L-lysine-HCl (Calbiochem La Jolla, Cal.) or 10 μg/ml L-arginine-HCl (BDH Poole, England) while in some experiments we used buffer infiltrated half leaves as control.

The purified virus at 100 μg/ml in 0.1 *M* phosphate buffer pH 6.9 was mixed with each the above mentioned polycations separately or with the amino acids so that the purified virus solution contained 10 μg/ml from each polycation or each amino acid. The solution after mixing was incubated for 10 min and used for inoculation of half leaves (0.1 ml/half leaf). No abrasive was added to the inoculum and leaves were washed with tap water after inoculation (cf. TAKEBE and OTSUKI, 1969).

A Latin-square arrangement of inoculated leaves minimized variation in number of lesions due to individual plant and leaf variability. Local lesions were counted 2–4 days after inoculation, depending on greenhouse conditions.

Virus multiplication in leaves of Xanthi tobacco was determined by local lesion assay. Six leaf disks (about 600 mg) were used for inoculation of *Nicotiana tabacum* L. cv. Xanthi-nc, and local lesions were counted.

Results and Discussion

Xanthi-nc tobacco half leaves that were infiltrated with polylysine (10 μg/ml) and after the solution evaporated were inoculated with TMV, produced

Table 1

Effect of polylysine and polyarginine on the susceptibility of Xanthi-nc tobacco leaves to TMV

Treatment	Lesion number on the half leaves
Control (buffer with lysine, 10 μg/ml)	1023
Polylysine (10 μg/ml in buffer)	767
Control (buffer with arginine, 10 μg/ml)	1240
Polyarginine (10 μg/ml in buffer)	1771
Polylysine (10 μg/ml in buffer)	880
Polyarginine (10 μg/ml in buffer)	1560

Values represents the mean of five replications. One half of the leaf was injected with polycations and the opposite half with buffer contained one of amino acid as control. The whole leaf was then inoculated after evaporation with TMV using a glass rod. Control (buffer alone) = 1150.

fewer lesions compared to the buffer or 10 μg/ml lysine in buffer infiltrated halves (Table 1). These data confirm the works of STAHMANN (1951), and of BURGER and STAHMANN (1951). However infiltration of 10 μg/ml polyarginine into the half leaves of Xanthi-nc tobacco leaves and then inoculation with TMV caused many more lesions than on the buffer treated control, or on the 10 μg/ml arginine infiltrated halves (Table 1). If we mixed the two polycations they antagonized each other.

Half leaves of the systemic host (Xanthi) infiltrated with polylysine produced fewer virus particles compared to the control half leaves, and polyarginine in the same host induced greater virus replication (Table 2). The relative virus concentration was tested on hypersensitive tobacco Xanthi-nc.

When we inoculated the tobacco leaves with a mixture of pure tobacco mosaic virus and polylysine or a mixture of pure TMV and polyarginine we got lower infectivity of TMV mixed with polylysine, and higher infectivity of TMV mixed with polyarginine (Table 3). If we mixed together the two above mentioned

Table 2

Effect of polylysine and polyarginine on the multiplication of TMV in Xanthi tobacco. Values calculated from relative virus concentration of TMV-inoculated half leaves tested on hypersensitive Xanthi-nc

Treatment	Lesion number 2 days after inoculation	Lesion number 3 days after inoculation
Control (buffer alone)	680	1358
Control (buffer with lysine 10 μg/ml)	696	1312
Polylysine (10 μg/ml in buffer)	528	920
Control (buffer with arginine 10 μg/ml)	694	1322
Polyarginine (10 μg/ml in buffer)	822	1765
Polylysine (10 μg/ml in buffer)	560	950
Polyarginine (10 μg/ml in buffer)	828	1854

Xanthi half leaves were injected with 0.1 M phosphate buffer (pH 6.9) or polycation solution in the same buffer and after evaporation were inoculated with TMV. Six leaf disks (18 mm in diameter) were extracted in 3 ml phosphate buffer at 48 h and 72 h after inoculation. Then 10 half leaves of Xanthi-nc were inoculated with the diluted extracts by using a glass rod. No abrasive was added to the inoculum. Values represent means of three replications.

polycations with the virus solutions we could not detect any changes in virus infectivity. These data were established in both host-virus systems, susceptible and resistant. The antagonism between polylysine and polyarginine may suggest a partial explanation of this effect.

Tobacco leaves retain large amounts of basic proteins, but almost none of a highly acidic protein when they are applied in the manner used for virus inoculations (SHAW, 1972). This suggests that large areas of the surface layers of tobacco are negatively charged. The TMV particles may require positively charged receptor sites for their attachment to the leaf surface. Therefore it is possible that similar to the polyornithine effect (SHAW, 1972), the effect of polyarginine on the attachment of TMV may be the result of a decrease in the electronegativity of this leaf area and the availability of a larger number of positively charged virus particles. However these ideas and results do not agree with STAHMANN's results with polylysine or with our polylysine results. The effects of polyornithine and polyarginine could also result from stimulation of a mechanism whereby virus particles either attach themselves to, or penetrate cell membranes. Pinocytosis, phagocytosis and

Table 3

Effect of polylysine and polyarginine on the TMV infectivity

Test plant — Polycation		Local lesions 10 half leaves		% Change due to polycation
		virus alone	virus plus polycation	
Xanthi-nc (A)	— polylysine	1118	552	−51%
	polyarginine	1200	1548	+29%
	polylysine + polyarginine	1190	1206	>+ 1%
Xanthi (B)	— polylysine	1095	603	−45%
	polyarginine	1140	1640	+44%
	polylysine + polyarginine	1121	1100	>− 1%

All inocula contained 100 μg/ml TMV in 0.1 M phosphate buffer pH 6.9. All polycations were used at 10 μg/ml.

Relative infectivities of TMV alone and TMV mixed with polycations are indicated by numbers of lesions on inoculated half-leaves of Xanthi-nc (A).

Relative infectivities of TMV alone and TMV mixed with polycations were also tested on Xanthi tobacco leaves. Data in table originated from six leaf disks extracted in 3 ml phosphate buffer and assayed on Xanthi-nc (B).

Absolute control of A—1169. Absolute control of B—1115.

other membrane alterations in many cells are enhanced by basic polypeptides (KATCHALSKI *et al.*, 1964). These mechanisms have been suggested as mechanisms by which viruses enter and infect plant cells (COCKING and POYNAR, 1969). It is noteworthy that several viruses have been shown to be associated with basic proteins (POGO *et al.*, 1975; BALÁZS *et al.*, 1975; FINE *et al.*, 1968; FLEISSNER, 1971; GIBSON and ROIZMAN, 1971; HERSHEY, 1955; GINOZA *et al.*, 1954).

These phenomenon suggest that these basic proteins or polypeptides have a role in virus attachment, virus-RNA uncoating and in the replication.

Our results suggest that the two type of polycations could affect the virus attachment and or the virus replication.

Probably this phenomenon depends on the specificity of the interaction of nucleotides with basic polypeptides. RIFKIND and EICHHORN (1970) established that the helical conformation of polyarginine stabilized more readily than that of polylysine. It is also possible, that the antagonistic effect could originate from the different structure of the two basic amino acids, i.e. reaction differences between ϵ-NH_2 and guanidino group (different protonation).

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