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Histone-like Proteins in Tobacco Plants Infected with Tobacco Mosaic Virus

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The histone-like protein content was determined in Xanthi-*nc* and Samsun tobacco plants two days after infection with tobacco mosaic virus (TMV). A virus-related change in the histone-like protein content was observed in a hypersensitive host-TMV combination. There were no changes in systemic host-TMV combinations (Samsun tobacco at 24°C and Xanthi-*nc* at 32°C). No qualitative changes in the histone-like protein components were detectable using gel electrophoresis. Pure histones (both plant and animal) reduced the infectivity of TMV. The increased histone-like protein content in the hypersensitive relationship suggests that proteins may play a role in the hypersensitive reaction and possibly in the curtailment of virus spread.

There is little information on the effect of plant virus infection on the histones of host plants. MISAWA and KATO (1967) found that the histone content of tobacco leaves increased two hours after infection by cucumber mosaic virus. This was also suggested in our earlier experiments when we studied the changes in the histone-like protein content of tobacco plants after TMV infection (BALÁZS, 1976). Three weeks after infection we found that the very lysine-rich histone-like protein fraction of infected plants was higher than the control. There were no differences in the H_2 fraction; however, the H_{2b} and H_3 fraction content of virus infected plants decreased, even some protein bands in the polyacrylamide gels disappeared. Some of these proteins were found in the non-analyzed soluble fraction and have characteristics of histone.

The virions of Simian virus 40 (TAN, 1977) and polyoma virus (MCMILLEN and CONSIGLI, 1974) contain host cell histones which are bound to the viral DNA. Histones are major components in the cells of higher living organisms: they are synthesized in the cytoplasm and are rapidly moved across membranes (HNILICA, 1975). GINOZA *et al.* (1954) described the differential ability of strains of tobacco mosaic virus to bind host cell nucleoprotein. Recently we confirmed that TMV strains are capable of binding host histone-like protein (BALÁZS *et al.*, 1977).

In the present study we have extended our observations and monitored the histone-like protein contents in different plants following infection by tobacco

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mosaic virus. We have also measured the histone like protein content in the hypersensitive-responding host, Xanthi-*nc*, at different temperatures including 24 °C (where hypersensitivity to virus develops) and 32 °C (when the plants develop a systemic infection). The possible role of histone-like proteins in resistance to virus disease is discussed.

Materials and Methods

Plant-virus materials

The U_1 strain of TMV was propagated in *Nicotiana tabacum* L. cv. Samsun, and cv. Xanthi-*nc* was used for local lesion assays. Plants were grown under normal greenhouse conditions and were used for the experiments at the 6–8 leaf stage. The virus was purified according to the procedure of FRAENKEL-CONRAT (1966) modified to include a precipitation with the polyethylene glycol (GOODING and HEBERT, 1967).

Histones and TMV interaction

Virus samples (100 μg/ml) in 0.06 *M* sodium phosphate buffer, pH 6.9, were mixed with different concentrations of different types of histones. Pure calf thymus histones were purchased from Sigma, St. Louis, MO USA. (Lysine-rich subgroup H_1 Type V. and arginine-rich subgroup H_3 Type VIII S). Tobacco histones were prepared as described below. Virus and histones were assayed on Xanthi-*nc*. A Latin-square arrangement of inoculated leaves minimized variation in number of local lesions due to individual plant and leaf variability.

Selective extraction of tobacco histone-like proteins

Following inoculation with buffer or TMV (0.5 mg/ml), healthy and infected Xanthi-*nc* plants were kept for two days at 32 °C in a growth chamber with constant illumination at 3600 lux. The remainder of the plants (Xanthi-*nc* and Samsun) were kept under normal greenhouse conditions of about 24 °C. Leaf samples (50 grams fresh weight) were taken from healthy and infected plants, frozen, and the proteins were extracted according to JOHNS (1964) with slight modifications: Frozen leaves were homogenized in 320 ml of 0.9% NaCl solution in a Waring Blendor at 0 °C for 5 minutes. The slurry was centrifuged, and the pellets resuspended in 200 ml of 5% perchloric acid solution. This and all other centrifugations were performed at 4 °C for 20–30 minutes at 5,500 *g*. After 2–4 minutes, the samples were centrifuged, and the supernatant solutions were collected and solid trichloracetic acid was added to 18% (w/v). The samples were stored at 4 °C overnight and the precipitates were collected by centrifugation. The pellets were dried *in vacuo*, and were washed several times with acetone. The proteins were dissolved in 1 ml of 0.06 *M* sodium phosphate buffer, pH 6.9 and were termed H_1, consistent with histone nomenclature (JOHNS,

1964). The perchloric-acid-pellets were resuspended in 80 ml of ethanol (96%) and left 1 h at room temperature. After centrifuging the supernatants were collected, five volumes of acetone and 2 ml concentrated HCl were added. The samples were left overnight at 4 °C and the precipitated proteins were collected by centrifugation. The samples were dissolved in 5 ml distilled water plus 32 ml absolute alcohol and 2 ml 5 *N* HCl. The samples were dialysed against 600 ml 95% ethanol for 16 h and then for 4 h against the same volume of ethanol. The precipitates were collected by centrifugation, washed with ethanol, and dried *in vacuo*. The proteins were dissolved in 1 – 2 ml of 0.06 *M* sodium phosphate buffer pH 6.9 and were termed H_3. The supernatant fraction in the dialysis tube was mixed with three volumes of acetone and allowed to sit at 4 °C for 24 h. The precipitate was collected by centrifugation, washed with ethanol and dried *in vacuo*. The proteins were dissolved in 2 ml of 0.06 *M* sodium phosphate buffer pH 6.9 and termed H_2. The ethanol-treated slurry was treated for 1 h with 0.25 *N* HCl (80 ml) and centrifuged. The supernatant solution was mixed with three volumes of acetone and stored overnight at 4 °C. The precipitate was collected by centrifugation, washed with ethanol, finally dissolved in 2 ml of 0.06 *M* sodium phosphate buffer (pH 6.9) and labelled as H_{2b}.

Polyacrylamide electrophoresis of histone-like proteins

All histone samples were electrophoresed in 15% polyacrylamide gels for 2.5 hours at 130 V (PANYIM and CHALKLEY, 1969). The 0.6 × 8.5 cm gels contained 2.5 *M* urea in 0.9 *N* acetic acid. The electrophoresis buffer contained 0.9 *N* acetic acid and 2.5 *M* urea (pH 2.7). Samples of 100 – 400 μg proteins in 20% sucrose were layered onto the gels and 50 – 100 μl of 8 *M* urea was layered above each sample. The electrophoresis was performed at 4 °C. Following electrophoresis the gels were immersed for 10 min in a staining solution of 20% ethanol containing 1% amido Black 10 B in 7% acetic acid.

Protein concentrations were determined by the method of LOWRY *et al.* (1951).

Results

Histone-like protein content of plants after infection

Changes in the histone-like protein content of the healthy and virus-infected plants were observed: however, a virus-related change occurred only in the hypersensitive host-virus combination. There were no differences in the histone-like protein content of systemic infections (Xanthi-*nc* at 32 °C, when the plant gave a systemic reaction, and Samsun at 24 °C). In the hypersensitive situation, the H_2 and H_3 fractions both increased disproportionately in infected plants (Table 1). These two fractions represent the arginine-rich histone-like proteins. It was also observed that high plant growth temperature (32 °C) resulted in higher histone-like protein

Table 1

Histone-like protein content of healthy and tobacco mosaic virus-infected tobacco plants at two days after inoculation

Plant	Xanthi-*nc*				Samsun	
Temperature	24°C		32°C		24°C	
	Healthy	Infected	Healthy	Infected	Healthy	Infected
Histone fractions						
H_1	16[a]	15	21	22	16	17
H_2	19	26[b]	19	20	20	20
H_{2b}	22	22	34	33	21	22
H_3	22	51[b]	31	30	24	21

[a] Data are expressed in μg histone per gram fresh weight and are the average of five replications

[b] Significantly different at the 0.01 % level from the respective healthy control

contents but there were no differences between the healthy and virus infected plants (Table 1). Furthermore, using gel electrophoresis in no situation were able to demonstrate any qualitative changes in the histone-like protein components after two days of infection (Fig. 1).

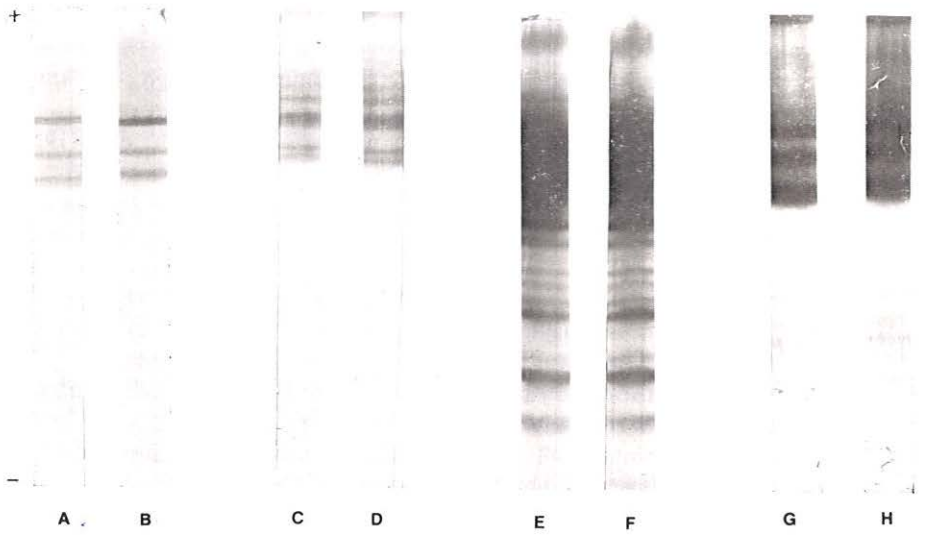

Fig. 1. Electrophoresis of histone-like protein fractions from healthy and TMV infected Xanthi-*nc* held at 32°C. A, healthy H_1; B, infected H_1; C, healthy H_2; D, infected H_2; E, healthy H_{2b}; F, infected H_{2b}; G, healthy H; H infected H_3

Histone and virus interaction

To test the effect of histones on the infectivity of TMV, we mixed calf thymus histones and tobacco histones with purified TMV and performed assays on Xanthi-*nc*. The histones reduced the infectivity of TMV (Table 2 and Fig. 2) depending on the concentration of histones.

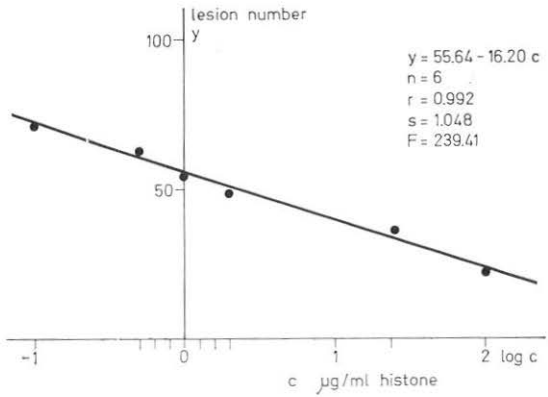

Fig. 2. Effects of pure histones on the infectivity of tobacco mosaic virus. Controll preparation of TMV (0.1 mg/ml) had an infectivity expressed in 100 local lesion per half leaves. In this experiment series included the arginine-rich histones and the lysine-rich histones at different concentrations

Table 2

Effects of histones on the infectivity of tobacco mosaic virus

Histone fraction 50 µg/ml	virus alone	virus plus histone	% reduction
Calf thymus			
arginine-rich	104[a]	43	59
lysine-rich	103	51	50
Tobacco			
H_3	107	51	52

All treatments are different from the controls at the 0.01 % level

[a] Data are expressed as the average number of local lesions per half leaf. The experiments were repeated five times

Discussion

There are several reports that interferon-like proteins may have a role in resistance to plant viruses, as they have in animal tissues (GIANINAZZI *et al.*, 1977, LADYGINA *et al.*, 1977). It has also been demonstrated that several polycations (i.e. polylysine, polyornithine, polyarginine) can modify plant virus infectivity (SHAW, 1972, STAHMANN *et al.*, 1951, TYIHÁK and BALÁZS, 1976, ZHURAVLEV *et al.*, 1974). Basic proteins are associated with TMV virions, too (BALÁZS *et al.*, 1977, GINOZA *et al.*, 1954). MISAWA and KATO (1967) observed that the histone content of tobacco was changed after cucumber mosaic virus infection. Our previous experiments partly confirmed this result, (BALÁZS, 1976) for in advanced systemic infections we found that the H_1 lysine-rich fraction was higher than the controls, while the H_{2b} and H_3 decreased and some protein bands disappeared from the gels. However, these proteins were found in the 0.9% NaCl soluble fraction. In that study we showed that if we used large enough samples the 0.9% NaCl solution (containing soluble proteins) had extracted large amounts of basic proteins. This fraction contained three times as much protein after extraction of virus-infected plants as from the healthy control. Gel electrophoresis of this sample (suggested name H_0) disclosed substantial H_{2b} and H_3. This soluble fraction was also analyzed by GIANINAZZI *et al.* (1977). They found considerable more of the basic proteins in the soluble fractions of homogenates of infected plants if they extracted using their low pH buffer. They also found "new" proteins in the soluble fractions after TMV infection of hypersensitive hosts; however, this conclusion regarding virus specificity was questioned by BARKER (1975) and recently by the work of VAN LOON (1977) who was able to induce these new proteins by spraying healthy plants with high concentration of Ethrel.

Basic proteins can modify the configurational state of bacteriophage DNA. This manifest in an altered thermal denaturation a change in viscosity and a change in template activity in transcription with DNA dependent RNA polymerase (BACHRACH and FRIEDMAN, 1967; CHAPRONIERE-RICKENBERG *et al.* 1964). Bacteriophages, animal viruses and plant viruses have associated basic proteins and/or polyamines (FINE *et al.*, 1968; HERSHEY, 1955; NICKERSON and LANE, 1977; POGO *et al.*, 1975). Our present results indicate that systemic infection of tobacco by TMV does not change the histone-like protein content of plants either qualitatively or quantitatively after two days of infection while in the hypersensitive host–virus relationship the histone-like protein content was increased in the H_2 and H_3 fractions. This property of Xanthi-*nc* was neglected by incubation at 32 °C, when virus localization does not occur. This suggested also that the increase in the basic protein content is associated with the appearance of necrosis. We have shown that histones from both animal and plant sources can reduce the infectivity of TMV. Since TMV is an acidic molecule (isoelectric point pH 3.5) and histones are basic molecules (isoelectric point pH 9 – 11) it is also possible that the reduction of infectivity results from a simple ionic interaction and precipitation of the virus-histone complex. This precipitation would cause a loss in infectivity as has been reported

for lectins mixed with barley stripe mosaic virus (GUMPF and SHANNON, 1977). Our preliminary experiments indicate that very low concentration calf thymus histones (0.001 μg/ml) stimulate infectivity. There have been several studies which support to connect viral resistance to a protein of host plants (GIANINAZZI *et al.*, 1977; VAN LOON, 1976). However, VAN LOON (1977) indicates that these proteins appeared not only after virus infection in the hypersensitive host but after Ethrel treatment of healthy plants, showing the lack of virus specificity of these proteins.

From this controversial result and from our results one cannot conclude that these proteins have no role in viral resistance. HADWIGER *et al.* (1977) attributed the fungal resistance of plants to histones. They were able to inhibit the fungal growth *in vitro* using pea histones. From these results and the contradictory results on plant virus resistance it is too early to conclude that histones or low molecular weight basic proteins cause a plant to be resistant. In the case of virus diseases their role in resistance is still open and their exact role remain to be determined.

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