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Original scientific paper

SOME CHARACTERISTICS OF CRYSTALLINE INCLUSIONS ASSOCIATED WITH HENBANE MOSAIC POTYVIRUS

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The elongated crystals provoked in the cytoplasm of *Datura stramonium* L. by a variant of henbane mosaic virus from *Physalis alkekengi* L. resemble square arrays of filaments cca 12 nm in diameter. Their proteinaceous nature was proved by digestion with pepsin.

Introduction

Infections by plant viruses accompany different virus genome coded nonstructural proteins the function of which is not always known. According to the data by Hibert and Dougherty (1988; see also Lesemann 1988a) the potyvirus genome codes for as many as seven nonstructural proteins, the best known being the one of cylindrical inclusions, structures very useful for diagnostic differentiation of potyviruses (Edwardson 1974; more recent review by Lesemann 1988b). An analysis of the variant of henbane mosaic potyvirus (HMV) isolated from *Physalis alkekengi* L. in Croatia (Mamula et al. 1988) facilitated prompt identification of the virus by detection of the HMV specifically shaped cylindrical inclusions and elongated crystalline structures of distinct appearance and high diagnostic value for HMV (Harrison and Roberts 1971, Kitajima and Lovisolo 1972). Similar elongated crystals have also been reported for plum pox virus (Bovey 1971, Van Bakel and Van Oosten 1972). Here we give evidence of the protein nature of the crystalline inclusions induced by HMV and data on their intrinsic architecture with the strain used.

Materials and Methods

Small pieces of the leaf lamina basal part of *Datura stramonium* plants infected with HMV-HZ isolate from *Physalis alkekengi* (Mamula et al. 1988) for five weeks, were fixed, postfixed, dehydrated and embedded as described previously (Mamula et al. 1988). For cytochemical studies (cf. Monneron and Bernhard 1966, Giese 1971) sections in plastic loops were pretreated in 10% hydrogen peroxide for 20 min at 37°C. After a brief rinse with water they were treated for 2–4 h with 0.5% pepsin (from porcine gastric mucosa, Boehringer, Mannheim, FRG) resolved in water containing 160 mg cystein pro 10 ml at 37°C. The sections were then rinsed with water and mounted on grids. Control sections of the same tissue were run simultaneously in the solution without enzyme or in water. All sections were stained with uranyl acetate followed by Reinhold's lead citrate and examined in a Philips CM 10 electron microscope.

Results and Discussion

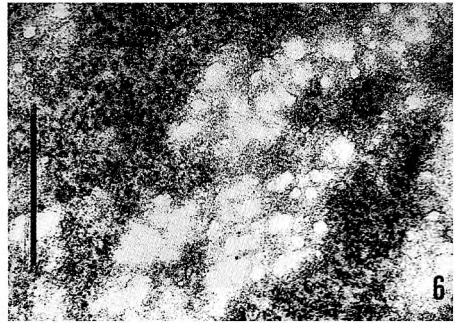
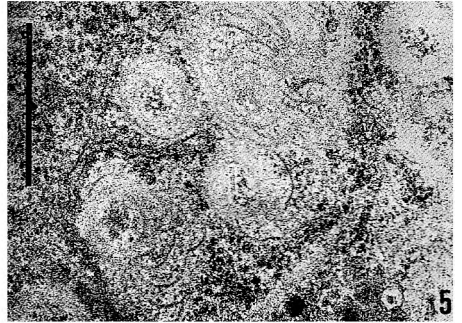
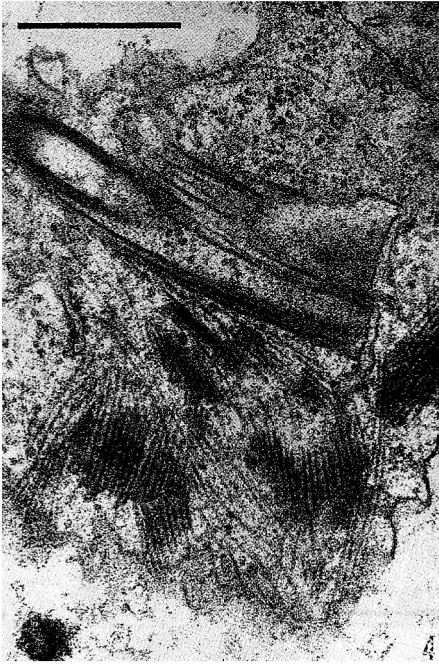
Leaf mesophyll cells of *D. stramonium* infected with HMV-HZ contained flexuous virus particles, cylindrical inclusions (pin-wheels, laminated aggregates, scrolls) and specific crystalline inclusions, all detected earlier in this particular variant of the virus (Mamula et al. 1988). The crystalline structures appeared as long needles (up to 9 $\mu\text{m} \times 400$ nm) or as clusters of several square like inclusions (Figs. 1, 1a, 2, 3). Places of defects in the crystalline structure contained cytoplasm and ribosomes.

The square outline of the crystals is considered as transections of long needles and it showed a square lattice of points at an angle of 45 degrees to the surface of the crystal (Figs. 1, 2); the point to point distance was about 23 nm, the point diameter was about 12 nm. Randomly but mainly longitudinally oriented sections of the crystalline inclusions showed different patterns: rectangular structures (profiles) contained lines with a distance of about 14 nm parallelly oriented to one edge (Fig. 3), or broader lines with a distance of about 23 nm at an angle of 45 degrees to the surface (Fig. 3), or rhombic shaped structures showing several strips of short parallelly arranged lines (Figs. 1, 1a, 2). The lines showed a minimum width of about 12 nm, and broader ones could be interpreted as projections of two elements lying almost one upon the other. Consequently, we suggest that the crystalline inclusions consist of parallelly oriented filaments (12 nm) producing a point pattern in cross section.

Figs 1, 1a. Parts of mesophyll cells of a HMV—HZ systemically infected leaf of *D. stramonium*. Fig. 1. Crystalline structures in transverse (CT) and almost longitudinal (CL) sections close to cylindrical inclusions. Fig. 1a. Elongated crystalline structure contiguous to the tonoplast in a cytoplasmic strand. Bars represent 0.5 μm .



Fig. 1, 1a



Figs. 2—6.

Judging from the above cited measurement data of the crystals provoked by our variant of H MV and data published by Kitajima and Lovisololo (1972) for the Rothamsted type (H MV-H) and 'alke-kengi' (H MV-A) strains, it follows that the elongated crystals related to infections by each of the three isolates consist of long filaments. However, the crystals of a similar external appearance and size described by Harrison and Roberts (1971; cf. Govier and Plumb 1972, Edwardson 1974) in cells infected with their *Atropa* isolate of H MV show a honeycomb-like-arrangement of hexagonal units. Since most sections of the crystalline structures presented by Harrison and Roberts (1971) are oblique, we suggest that the sections of their crystals and of the long crystalline structures presented by Kitajima and Lovisololo (1972) coincide with the structures presented here. Different measurement data of distances between filaments (points) could be explained by different methods used.

We also noticed that the surface of crystals appeared to be in close contact with virus-like particles which seemed to be in end-to-end union with elements of the crystalline inclusion (Fig. 4).

After pretreatment with hydrogen peroxide, scrolls and lamellar inclusions, but not pinwheels (Fig. 5) and crystals, were unspecifically hydrolysed. Successful digestion of crystals and pinwheels was obtained after a 150 min treatment with pepsin which indicated their proteinaceous nature (Fig. 6). Virus particles were not affected by the enzyme digestion procedure.

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Fig 2—6. Parts of mesophyll cells of a H MV—HZ systemically infected leaf of *D. stramonium*. Fig. 2. Transections of crystals are square, oblique sections are rhombic. Fig. 3. Randomly oriented sections of crystals with lines spaced 14 or 23 nm. Fig. 4. The crystals in close contact with virus particles; cylindrical inclusion in longitudinal section is present. Fig. 5. Lamellar inclusion and scrolls hydrolysed in hydrogen peroxide. Fig. 6. Completely digested crystals after treatment with pepsin for 150 min. Bars represent 0.5 μ m.

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SAŽETAK

NEKA SVOJSTVA KRISTALIČNIH UKLOPINA KOJE PRATE ZARAZU VIRUSOM
MOZAIKA BUNIKE

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Produženi citoplazmatski kristali u listovima biljke *Datura stramonium* L., zaražene jednom varijantom virusa mozaika bunike iz biljke *Physalis alkekengi* L., imaju strukturu koja nalikuje kvadratičnom poretku vlaknastih elemenata promjera oko 12 nm. Njihova proteinska priroda dokazana je otapanjem s pomoću pepsina.

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