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Short communication

Antiviral activity of alginate against infection by tobacco mosaic virus

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Abstract

In the course of searching for antiviral substances to tobacco mosaic virus (TMV), it was found that sodium alginate (Alg) had a high inhibitory activity against TMV infection. The addition of Alg to the inoculum solution greatly reduced the number of local lesions formed on Xanthi NN tobacco leaves. The degree of inhibition increased with Alg concentration and was higher in Alg polymer of lower composition of mannuronate to guluronate ratio (M/G ratio) than higher Alg, which suggests that the strength of inhibition relates to the stiffness of polymer chain of Alg. This behavior was similar to chondroitin sulfate (Chs), However, in contrast to Chs, the infectivity of TMV in the case of Alg was enhanced at very low concentrations of Alg. The range of maximum infectivity shifted to the higher Alg concentration with increasing M/G ratios. The degree of inhibition increased with the molecular weight of Alg (M/G ratio at 0.8). The electron micrograph showed that the TMV suspension itself was almost monodisperse and that the addition of Alg caused TMV to form large raft-like aggregates. The antiviral activity of Alg on infectivity of TMV may be caused by blocking the decapsulation process of TMV protein on the cell membrane surface.

Keywords: Tobacco mosaic virus; Alginate; Antiviral activity

1. Introduction

Tobacco mosaic virus (TMV) is a rod-shaped virus composed of single-stranded RNA encapsulated in a coat protein capsid. For the infection of TMV to take place in leaves, it is necessary that TMV penetrates directly into the cell and makes contact with materials in the plasma membrane or with intracellular organelles.

The TMV coat protein is present in a large number of association sates, A-protein (4-7S), double disk (20S) and helical rods ($\gg$ 20S). The helical rod aggregates can be formed by hydrophobic interaction (Butler, 1984; Sano et al., 1996). The extremity of the cylindrical rod therefore has the hydrophobic sites exposed and these regions can penetrate extensively into the hydrophobic membrane lipid layer. In addition the A-protein formed after decapsulation from the extremity of the cylindrical rod had interacted with the hydrophobic region of the lipid molecule (Sano, 1989).

The substances inhibiting the process of viral infection are classified according to their antiviral actions, as (i) inhibitors of infection and (ii) inhibitors of virus multiplication. The inhibitors of infection are the substances that prevent infection from occuring, when inoculated onto leaves simultaneously with the virus. The inhibitors of virus multiplication are the substances that retard the rate of multiplication of the infecting virus. Cationic and anionic polyelectrolytes of both natural and synthetic origin such as heparin and hyaluronic acid (Cohen, 1942), polylysine and polyvinylamine (Burger and Stahmann, 1951), polyglutamte, polyacrylate and polypectate polymers (Stahmann and Gothoskar, 1958), polyacrylic acid (Gianinazzi and Kassanis, 1974), polycarboxylate (Stein and Loebenstein, 1972) and chondroitin sulfate (Chs) (Sano, 1995; Sano, 1997) have been shown to inhibit TMV. It is also known that sulfated polysaccharides are potent and selective inhibitors of human immunodeficiency virus and that the mecanism of action is also attributed to inhibition of virus adsorption onto the cell membrane.

The mechanism for the inhibitory activity of polysaccharides on TMV is not yet clear. When mixed solution of TMV and Chs were centrifuged, most infectivity was found in the precipitate (Sano, 1995; Sano, 1997). This indicates that the aggregation of TMV formed in the presence of Chs greatly affects the infectivity of TMV. Like Chs, alginate (Alg) is an anionic polysaccharide that can be extracted from different marine algae, and is of considerable technological importance both for its solution properties, and as a widely used gelling agent in the food industry. In the course of searching for antiviral substances to TMV, we examined the antiviral activity of Alg.

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Table 1
Physical properties of sodium alginate samples

Type	500M	500	500G
Viscosity(cp)	568	500	550
pH	6.6	6.6	6.7
M/G ratio	1.05	0.80	0.41

2. Materials and methods

2.1. Materials

TMV, Japanese common strain OM, was separated from systemically infected leaves of Nicotiana tabacum L. var Bright Yellow and purified by polyethylene glycol precipitation and differential centrifugation. The sodium alginates (Bioreactor grade) were purchased from Kibun Food Chem. Ltd. (Tokyo, Japan). The ratio of mannuronic acid to guluronic acid residues (M/G) was determined using the method of Haug et al. (1974). The results are shown in Table 1. The heavy metal was below 20 μg/g in each sample. All other reagents were of the purest grade available.

Solution of TMV and Alg dissolved in double-distilled water was dialyzed against distilled water in a cold room at 4°C. Fresh solutions were prepared for each experiment and were clarified by centrifugation at 13,000 rpm for 35 min, before use. Concentration of TMV was determined spectrophotometrically.

2.2. Electron microscopy

A drop of TMV alone and the mixed soltion of TMV and Alg in 0.1 M sodium phosphate buffer (pH 7.0) was placed on a carbon-coated grid, and negatively stained with a few drops of 3% of uranyl formate. After excess fluid had been absorbed on filter paper, the specimen was observed with an electron microscope type H-500 (Hitachi, Co. Ltd.).

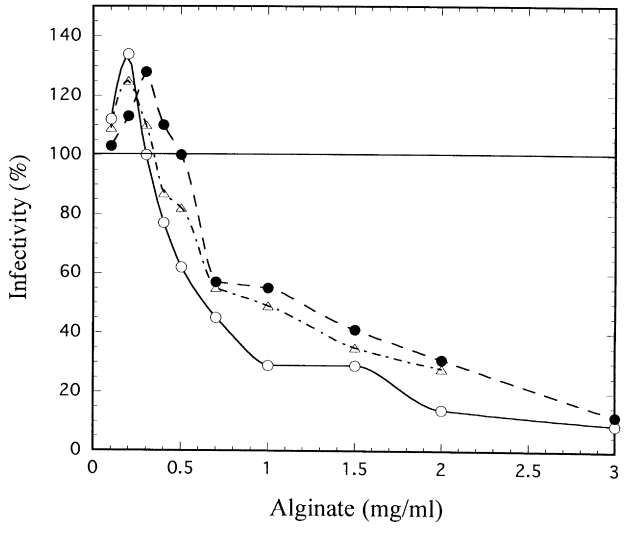

Fig. 1. Infectivity of Alg 500G (M/G = 0.41) (solid line), Alg 500 (M/G = 0.8) (chain line) and Alg 500M (M/G = 1.05) (dotted line).

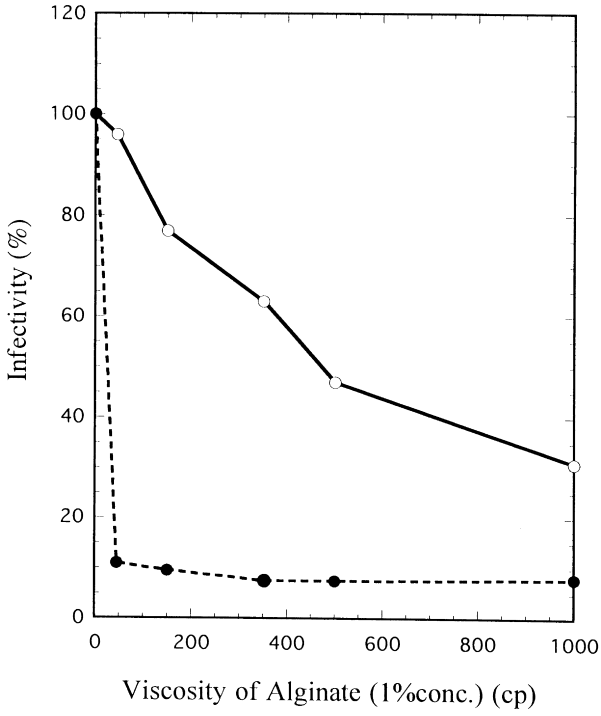

Fig. 2. Infectivity of Alg (M/G = 0.8) having different molecular weight. Alg concentrations are 1% (closed circles) and 0.1% (open circles).

2.3. Inhibition tests

The inhibitory effect of Alg on the infectivity of TMV was assayed on Nicotiana tabacum var. Xanthi NN by inoculating opposite halves of a leaf (half-leaves method) with TMV (0.05 μg TMV/ml in 0.1 M sodium phosphate buffer, pH 7.0) alone and with TMV and Alg mixtures. Xanthi plants were grown in a glasshouse and selected for uniformity in each experiment. After inoculating and then washing with water, half-leaves were put on wet paper in flat boxes and kept at 23°C.

3. Results

3.1. Inhibition tests

The inhibitory effect of Alg on the infectivity of TMV was assayed by inoculating opposite halves of leaves with TMV alone and with TMV and Alg mixtures. The inhibitory activity of Al was calculated as the percentage reduction in the number of local regions produced on leaves after 3 days as compared with their controls. To test whether Alg directly inactivates TMV, the suspension of each Alg samples of 500G or 500M at a final concentration of 5 mg/ml and TMV at 0.47 mg/ml in 0.1 M phosphate buffer was incubated at room temperature. TMV was pelleted by ultracentrifugation of the solution. TMV from the pellet was

dissolved in 0.1 M phosphate buffer (0.05 μg/ml) and inoculated on leaves. Alg did not inactivate TMV, even when incubated for 3 days, suggesting that the Alg does not combine irreversibly with the TMV particle, as is the same in the case of Chs (Sano, 1997). Circular dichroism spectra of TMV in both cases showed the same pattern. Therefore, the interaction of TMV and Alg was reversible at least on dilution. As is shown in Fig. 1, the infectivity of Alg continuously decreased with increasing concentration of Alg. The degree of inhibition was higher in 500G than 500M. However, Alg did not completely prevent TMV entry into the leaves.

This behavior was the same as Chs. However, in contrast to Chs, the infectivity of TMV in the case of Alg was enhanced under the very low concentration of Al. The range of the maximum shifted to higher Alg concentrations in 500M than 500G. As is shown in Fig. 2, the degree of inhibition increased with the molecular weight of Alg of M/G ratio of 0.8.

3.2. Electron microscopy

In the absence of Alg the electron micrograph of TMV showed that the TMV suspension was almost monodisperse and was free of aggregation. The addition of Alg, however, caused TMV particle to form large raft-like aggregates. The effect of Alg on infectivity may be ascribed to the aggregation formation of TMV particle. The present results are consistent with the view that TMV was precipitated by Chs through mutual incompatibility and spatial exclusion (Sano and Inoue, 1980; Sano, 1997).

4. Discussion

TMV can be precipitated by a wide variety of reagents to form needle-like para-crystalline precipitates. As is shown in the previous papers (Sano and Inoue, 1980; Sano and Inoue, 1981; Sano, 1995; Sano, 1997), when Chs molecules were added to the TMV solutions, Chs molecules occupied part of the space previously available to TMV and compressed TMV particles into a small volume fraction. As the Chs concentration increased, the fractional volume occupied by TMV became smaller until the interparticle distance of TMV molecules was decreased sufficiently for crystallization of TMV to occur (Sano and Inoue, 1980).

Chs has three forms A-, B- and C-types. The A-type has an equatorial carboxyl and an axial sulfonic acid groups, and C-type has an euqatorial carboxyl and an equatorial sulfonic acid group. These differences in primary structure are considered to influence the behavior of the molecules in solution. The C-type has the least flexible structure of the three isomers, while the B-type is the most flexible. The strength of the spatial exclusion effect is higher in the case of stiff polymer chains such as the Chs C-type than flexible polymer chains such as the Chs A-type. The strength of interaction between TMV and Chs was related to the degree of infectivity of Cs A- and C-types. The degree of inhibition was higher in stiffer polymer chain of the Chs C-type than the flexible polymer chain of A-type (Sano, 1995; Sano, 1997).

In the case of Alg, the stiffness of the polymer chain depends on the M/G content and the polyguluronate chain is much stiffer than the polymannuronate chain (Smidsrod, 1974; Mackie et al., 1989). As is shown in Fig. 1, the degree of inhibition was higher in the stiffer 500G than in the more flexible 500M.

Like Chs, the antiviral activity of Alg on infectivity of TMV may be caused by the large aggregation formation of TMV particles on the cell surface. The large aggregates of TMV formed in the presence of Alg shown by electron micrograph prevents the penetration of TMV-RNA into the cell membrane, because of blocking the decapsulation process of TMV, whose process occurs on the cell membrane at the initial stage of TMV infection (Sano, 1989; Sano, 1997).

Alg also may affect the interaction of TMV with the cell, by the strong anionic charge of Alg. Adsorption of TMV to the cell surface is thought to be achieved by interaction between the cationic amino groups of TMV and anionic phosphate groups of the cell membrane. Polyanions such as Alg interact both with TMV and the cell surface, and suppress adsorption owing to reinforcement of the net negative charge of both cell and TMV. The polysaccharide such as Alg seems to affect the TMV at its earliest infection stage.

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