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Inhibitory Activity of Surfactants against Tobacco Mosaic Virus Infection

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계면활성제의 TMV 감염저지 효과

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초 록

AOS(α-olefin), LAS(linear alkyl benzene), OSS(dioctyl sulfosuccinate) 및 SAS (dodecyl benzene sulfonic acid)등 4종 계면활성제의 TMV 감염저지 효과를 잎담배 품종 Xanthi-nc 및 NC 82를 이용하여 조사하였다.

각 계면활성제를 2,500ppm 농도로 담배잎에 처리한 경우 바이러스(TMV) 또는 TMV RNA를 접종했을 때 모두 98% 이상의 감염저지 효과를 나타냈다. 그러나 이들을 담배 근권토양에 처리 3일후 잎에 Virus를 접종하여 병징 및 엽중 Virus 농도를 조사한 결과 무처리구와 차이가 없어 침투이행에 의한 TMV 감염저지 효과는 인정되지 않았다. 순화된 바이러스를 각 계면활성제 2,500ppm 용액과 혼합한 후 초고속원심분리에 의해 다시 Virus를 회수하여 활성을 조사한 결과 LAS는 96% 이상 Virus를 불활성화시켰으며, 이 외는 바이러스 활성에 영향을 미치지 못했다. 또 각 계면활성제가 처리된 Virus 입자의 형태는 무처리입자와 차이가 없었다.

이같은 결과들로 미루어 4종의 계면활성제가 나타낸 TMV 감염저지 효과는 바이러스 입자의 파괴 또는 감염초기 바이러스 입자의 외피 단백질 탈피억제에 의한 것이 아닌 것으로 판단된다.

Abstract

Inhibition of tobacco mosaic virus (TMV) infection by 4 surfactants, sodium salts of alpha olefin (AOS), linear alkyl benzene (LAS), dioctyl sulfosuccinate (OSS), and dodecyl benzene sulfonic acid (SAS), was examined on tobacco cv. Xanthi-nc and NC 82. Infection of virions or TMV RNA was inhibited over 98% by the surfactants (2500 ppm). However, symptom development and viral concentration in tobacco plants treated with the surfactants into the rhizosphere soil 3 days before inoculation with TMV on leaves were not different from those in untreated tobacco plants. This indicates no significant systemic effects of the surfactants on the inhibition of TMV infection. The surfactants, except LAS, had no effect on the inhibition of viral infection when purified virions mixed with each surfactant and ultracentrifuged were inoculated on the tobacco plants. The virus was almost inactivated by LAS, showing that the viral infection was reduced more than 96%. The virus particles treated with the surfactants were not distinguishable in size and demension from untreated normal particles, suggesting that the inhibitory action of the surfactants to TMV infection may not involve disintergration or uncoating of the virus at the early stage of infection.

Introduction

The most important viral disease on tobacco in Korea is mosaic (TMV) because of its high incidence, wide distribution, and great reduction in yield and quality of leaf tobacco. Sanitary and cultural practices, elemination of inoculum sources and cultivation of resistant cultivars, are the recommendable ways to control the viral disease in the farmer's field. But the control practices are often impracticable to farmers. Use of resistant cultivars also has some limitations due to the innate poor yield and quality. Control of viral disease with chemicals may provide a valuable additive way for existing control strategies. Numerous chemical compounds have been identified, which inhibit the infection or multiplication of plant viruses (3,6,10, 14,15), although no one identified so far is selective enough to be useful against virus disease of crops.

Surfactants have diverse physico-chemical properties, showing various kinds of effects on organisms and their constituents, such as anti-microbial effects, denaturation of nucleic acid, protein and enzymes, and interaction with the biological membrane. Sodium lauryl sulfate (SDS) and Triton X100 (polyethylene nonylphenyl ether) are well known materials effective in seperation, purification and analysis of biological substances, and the surfactants were revealed to have inhibitory activity against plant viral infection (13). Nonionic surfactants, alpha olefin sulfonate and dodecylbenzene sulfonate, also inibited the infection of tobacco mosaic virus to French bean and tobacco(8,14). However, little is known concerning detail action properties of the surfactants to plant viral infection.

This paper deals with the inhibitory action properties of 4 surfactants on TMV infection of tobacco plants cv.Xanthi-nc as a local lesion host, and cv. NC 82.

Materials and Methods

Surfactants

Sodium salts of alpha olefin(AOS ; 35%), linear alkyl benzene (LAS ; 96%), dioctyl sulfosuccinate(OSS ; 70%), and dodecyl benzene sulfonic acid(SAS ; 50%) were used.

Virus

A common strain of TMV was propagated in tobacco cv. NC 82 for three weeks. Leaf-juice

inoculum was prepared by squeezing the tobacco leaves and diluted to 3 x 10^{-3} with distilled water, and was purified by the method of Gooding(5). Viral concentration of the purified virus for local lesion assay was adjusted with distilled water to 10 μg /ml, unless otherwise noted.

TMV RNA

TMV RNA was prepared by the method of Ralph and Berquist(9) using bentonite to minimize nuclease activity during RNA extraction. The bentonite was prepared by the method of Fraenkel-Conrat et al.(4). The RNA prepared with 260/280-nm absorbance ratio being 2.5 was used in the experiment. For inoculation test concentration of the RNA was adjusted to be 2 μg/ml of 0.1 M phosphate(Na_2HPO_4-KH_2PO_4) buffer (pH 7.2).

Estimation of inhibitory effect

Each of the surfactants was diluted with distilled water to 5000 ppm, 1250 ppm, 625 ppm, and 313 ppm, and was sprayed on the upper surfaces of 6 half-leaves of tobacco cv. Xanthi-nc 1-2 hours before inoculation with TMV-infected tobacco sap. Inoculations were done by rubbing carborandum-dusted leaves with cotton swab dipped in the inoculum. The plants inoculated were kept in a temperature-controlled greenhouse(24$\pm$ 2°C) for 4 days. Inhibitory activities(%) of the surfactants were estimated by comparing number of local lesions produced on surfactant-treated half leaf with that on the other untreated half leaf.

Effects of surfactants on the formation of local lesions at different inoculum concentration of the purified virus ranging 0.5 to 50 μg/ml were evaluated by the same manner as described above. The activities of the surfactants against infection of TMV RNA were also assayed on tobacco cv. Xanthi-nc. The RNA, 2 μg/ml of 0.1 M phosphate buffer(pH 7.2), was inoculated with glass spatula 1 to 1.5 hr after spraying the surfactants. Immediately after rubbing, the leaf surface was washed gently with running tap water.

To investigate infectivities of the virus treated with surfactants, each of the surfactants(2500 ppm) containing 3 mg of the purified TMV was kept at 20°C for 0-4 hours, and the virus was then precipitate by ultracentrifugation at 105,000 g for 120 min. The pellet was resuspended in distilled water to make 10 μg/ml virus concentration. The relative infectivities were assayed on tobacco cv. Xanthi-nc.

Systemic inhibitory effect of the surfactants was examined by drenching the surfactants to the rhizosphere soil of tobacco plants cv. NC 82 grown in 11 cm-diameter pots in a greenhouse. For each plant 50 ml surfactant(2500 ppm) was drenched into the rhizosphere soil 3 days before inoculation of two largest leaves of the tobacco plant with TMV-infected crude sap. Visual symptom severity and viral concentration by ELISA were observed 4 weeks after inoculation. Precedures for double sandwich ELISA technique followed those described by Clark and Adams(2).

Electron microscopy of the TMV particle treated with surfactant

The purified virus(TMV) mixed with each of 1250 ppm surfactants was incubated for 90 min. at room temperature, and was observed under an electron microscope after negative staining with 2% potassium phosphotungstate.

Result and Disscussion

Concentration of surfactants

Effect of the surfactants with different concentrations on viral infection were examined by spraying the diluted (from 5000 ppm to 313 ppm) surfactants on tobacco leaves 1 to 2 hours before inoculation. Based on local lesion formation, more than 95% inhibitions were obtained by 1250 ppm

Table 1. Inhibitory activities of different concentrations of surfactant against TMV infection on tobacco cv. Xanthi-nc[1]

Surfactant[2]	*Inhibitory activity (%)*[3]				
	5000 ppm	2500 ppm	1250 ppm	625 ppm	313 ppm
AOS	99.7	99.0	81.9	72.7	61.0
LAS	100.0	98.7	97.2	88.4	69.8
SAS	100.0	98.8	94.6	75.5	68.7
OSS	100.0	98.2	92.3	74.1	—

[1] Each of the surfactants was sprayed on 6 half-leaves of tobacco Xanthi-nc 1-2 hr before inoculation.
[2] AOS : alpha olefin sodium salt, LAS : linear alkyl benzene sodium salt, OSS : dioctyl sulfosuccinate sodium salt, SAS : dodecyl benezene sulfonic acid sodium salt.
[3] Inhibitory activity (%)=(1-x/y) x 100, where the x and Y are no. of lesions produced on surfactant-sprayed half-leaf and on unsprayed opposit half-leaf, respectively.

for LAS, and 2500 ppm for other 3 surfactants tested (Table 1). The surfactants at higher than 2500 ppm almost completely inhibited the viral infection, but phytotoxicity was noted, showing slight rolling-up and mild necrosis of the leaves.

Viral concentration for inoculum

The effect of surfactants(2500 ppm) at different inoculum levels, 50 μg, 5 μg, and 0.5 μg/ml viral concentrations, on viral infectivities was evaluated by local lesion assay system. Regardless of the inoculum levels tested, the inhibitory effects of viral infection were similar, indicating that the activities of surfactants might not be dependent on the viral concentration. In all combinations of surfactant-viral concentration, more than 95% inhibitory was observed (Table 2.)

Table 2. Inhibitory activities of surfactants (2500 ppm) against inoculation with different concentrations of purified tobacco mosaic virus.

Surfactant	Inhibition (%)		
	50 μg/ml	5 μg/ml	0.5 μg/ml
AOS	95.0	96.6	99.8
LAS	98.6	96.6	99.7
OSS	98.0	96.9	99.7
SAS	97.9	97.9	99.4

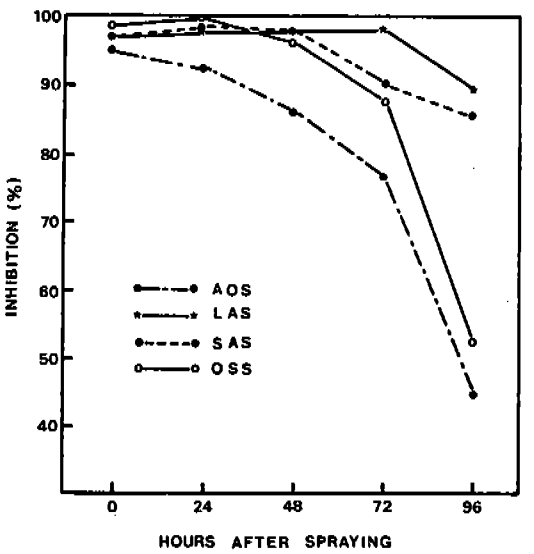

Fig. 1. Inhibitory activities of surfactants (2500 ppm) sprayed on tobacco cv. Xanthi-nc 0-96 hrs before inoculation with TMV against local lesion formation.

Application time of surfactants

The surfactants were applied at different times, i.e. before or after viral inoculation, to investigate possible mode of actions of them. When surfactants were applied 0 to 4 days before TMV inoculation, the effect (>90% inhibition) lasted for 4 days for LAS, 3 days for SAS, and 1 day for AOS (Fig. 1). On the other hand, except AOS, the local lesion formation was inhibited by more than 80% when the surfactants were applied within 8 hours after inoculation (Fig. 2). This might correspond to the time required to initiate the replication of the RNA for viral infection of the host cell(2). AOS was less effective than others.

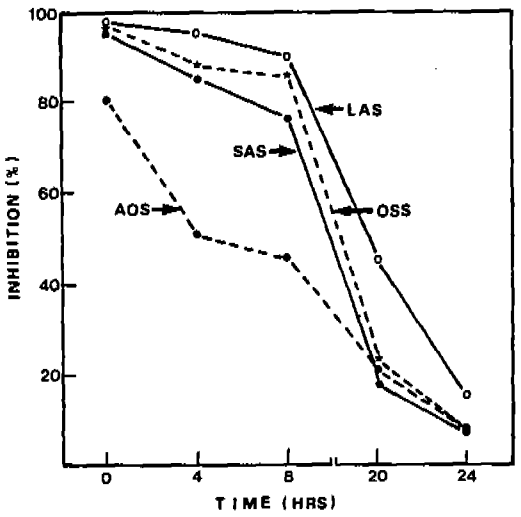

Fig. 2. Inhibitory activities of surfactants (2500 ppm) sprayed on tobacco cv. Xanthi-nc 0-24 hrs after TMV inoculation agasinst local lesion formation.

Systemic effect of surfactants

Each surfactant was drenched into rhizospheres of tobacco plants grown in 11 cm-diameter pots 3 days before inoculation of TMV to examine systemic activity of the surfactants. Based on symptom severity and antigenic viral concentration by ELISA 4 weeks after the inoculation, no antiviral effects of the surfactants were noted. Symptom severity and viral concentration were identical between the surfactant treatments and untreated check (Table 3), indicating that the surfactants had no systemic inhibitory effect.

Table 3. Effect of soil-drenched surfactants on symptom severity and antigenic viral concentration of TMV in tobacco cv. NC82[1)]

Surfactant (2500 ppm)	Symptom[2)] severity	ELISA absorbance
AOS	5.0	1.009
LAS	5.0	1.034
SAS	5.0	1.021
OSS	5.0	1.026
Untreated control	5.0	1.039

[1)] The experiment was conducted on plants grown in 11 cm-diameter pots in a greenhouse. Five plants were used for each treatment, for which 50 ml surfactant/pot was drenched into soil 3 days before inoculation on two largest leaves of tobacco plant with TMV common strain. Disease severity and viral concentration were observed 4 weeks after inoculation.

[2)] Symptom severity was graded as 0,1,3 and 5 : no visual, mild, moderate, and severe symptom, respectively.

Infectivity of TMV treated with surfactant

Purified TMV was mixed with each of the surfactants (2500 ppm) and incubated for 0 to 4 hours at 20°C. Then the virus was recovered from the mixture by ultracentrifugation, and its infectivity was assaye on tobacco cv. Xanthi-nc. More than 97% infection occurred by AOS, OSS, and SAS, indicating that the virus was not inactivated by the 3 surfactants, while LAS inactivated the virus almost completely regardless of the duration of the treatment (Table 4). For all the surfactants duration of the treatment was not critical to the viral infectivity.

Table 4. Infectivities of tobacco masaic virus treated with surfactants for 0-4 hours[1]

Surfactant (2500 ppm)	Infectivity (%) [2]				
	0 hr	1 hr	2 hr	3 hr	4 hr
AOS	97.3	99.7	98.3	97.2	99.0
LAS	2.0	2.7	1.3	0.8	0.7
OSS	99.5	98.4	98.5	98.6	99.9
SAS	99.8	99.9	99.1	99.9	99.7

[1] Each of the surfactants containing 3 mg of purified TMV was left at 20°C for 0-4 hr and the virus was then precipitated by ultracentrifugation (105,000 g/120 min.). The pellt was resuspended in distilled water to be 10 μg/ml virus concentration.

[2] Infectivity of the surfactant-treated virus was assayed on tobacco cv. Xanthi-nc. by no. of lesions produced on 6 half-leaves compare to their opposit half-leaves inoculated with untreated virus.

Inhibitory effect against TMV RNA infection

Inoculation was made with TMV RNA on 2500 ppm surfactant-treated leaves of tobacco cv. Xanthi-nc. Percentages of inhibition were estimated based on number of local lesions produced on surfactant-applied and unapplied half leaf. All of the surfactants used in this experiment inhibit the RNA infection more than 98% (Table 5).

Electron microscopy of TMV particle treated with surfactant

The surfactants did not affect the TMV particles in demension and size. The virus treated with each of the surfactants showed straight tubular particles 300 nm long which were not distinguishable from the untreated normal particles.

The greatest effect of the surfactants on the inhibition of TMV infection could be obtained when 1250 to 2500 ppm surfactants were applied at least 1 day before the TMV inoculation. Based on the results obtained in the experiment, the surfactants

Table 5. Inhibitory activities of four surfactants against infection of TMV RNA on tobacco cv. Xanthi-nc[1]

Surfactant (2500 ppm)	No. of lesions[2] (sprayed/unsprayed)	Inhibition (%)
AOS	2 / 98	98.5
LAS	1 / 99	99.4
OSS	1 / 92	98.9
SAS	1 / 94	98.8

[1] TMV RNA, 2 μg/ml of 0.1 M Phosphate buffer (pH 7.2), was inoculated with glass spatula 1-1.5 hr after spraying the surfactants.

[2] Lesion counts are averages of 6 half-leaves.

may not be translocated from roots to the leaf tissues of the host plant. The inhibitory effect of the surfactants tested, except LAS, on TMV infection may not be due to inactivation of the virus by altering the structure of virus particles, and also may not involve uncoating of the particles at the early stage of infection.

Some studies have been performed on inhibitory actions of surfactants on TMV infection. SDS, Tween T 80 and Triton X 100 were inhibitory to TMV infection by blocking adsorption of the virus on the host cell surface or disrupting binding between virus and infective sites of the host cell (12). On the other hand, Hibi et al. (7) investigated the inhibitory action of fungicides containing dodecylbenzene sulfonate, and showed that the inhibitory action took place during the time between uncoating and initiation stage of viral RNA replication in the host cell. Other theories on the mode of action of SAS were also suggested ; disintegration of virus particles(11) and affinity of the surfactant to lipid bilayer in the cytoplasmic membrane of the host cell (14). Thus, the mode of action of the surfactants are still controversial.

The host cell metabolism and the process of

TMV replication in host cell in relation to the mechanism of inhibitory action of the surfactants remain to be studied.

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