Antiviral Effects of Tea Catechins and Black Tea Theaflavins on Plant Viruses

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Having noticed that the tea infusion exerts an antiviral effect on tobacco mosaic virus (TMV), and that the inhibition of TMV infectivity by tannic acid was reported by Thornberry[1], the present study was initiated aiming at the pesticidal utilization of water-soluble substances of tea.

Tannins contained in tea have a property to make a bond with water-soluble protein as tannic acid does. The author found out that tea tannins have an inhibitory effect on virus, and among catechins isolated from the tannins the ester type catechin (galloyl catechin) shows a remarkable inhibition on TMV and cucumber mosaic virus (CMV)[2]. Further, it was revealed that theaflavins, an enzymatically oxidized product of catechins, inhibit virus activity equally as catechins but with less injury to host plants, and also they have an inhibitory effect on infectious TMV-RNA[3]. It was presumed that, unlike the catechins which exert their effect by making a bond with viral protein[4], the theaflavins combine with viral protein as well as viral nucleic acids, and the bond with nucleic acids occurs at bases of nucleic acids[3].

Results of these studies will be presented below.

Antiviral effects of tea catechins

The fact that tea tannins inhibit the infection of TMV and CMV is considered probably based on the property of tannins to make bonds with proteins. Therefore, four major kinds of catechin [(−) epicatechin: EC, (−) epigalocatechin: EGC, (−) epicatechin gallate: ECg and (−) epigalocatechin gallate: EGCg], isolated from tea infusion and crystallized, were used for testing their inhibitory effect on the systemic infection of TMV and CMV with the following method.

Tomato seedlings and tobacco KY-57 seedlings planted in pots were used as the assay host for TMV and CMV respectively. TMV and CMV solutions, to which each of the four major catechins prepared as above was added at a 0.5% concentration, were inoculated to

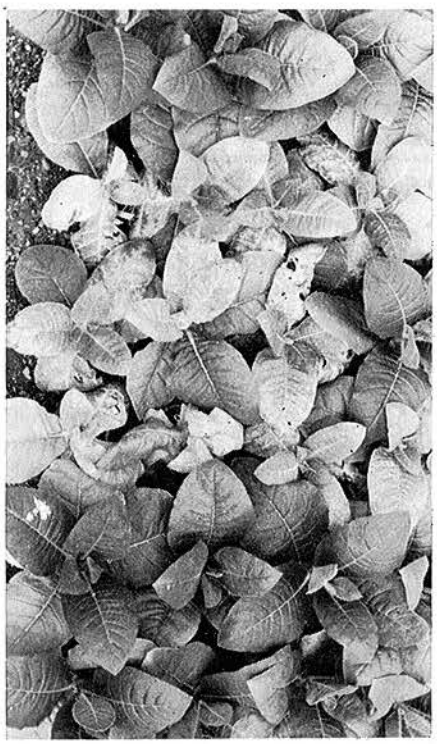

Plate 1. Inhibitory effect of 4 kinds of catechins on TMV

the entire surface of a lower healthy leaf of the seedlings. Observation was continued for 30 days. For either TMV or CMV, the inhibitory effect of catechins on the systemic infection was greater with galloyl catechins, (−) ECg and (−) EGCg, than free catechins, (−) EC and (−) EGC (Plate 1).

Then, the effect of catechins on the formation of local lesions of TMV was examined using galloyl catechin, (−) ECg and (−) EGCg, added to the inoculum at 7 different levels of concentration, 0.02, 0.05, 0.1, 0.2, 0.3, 0.4, and 0.5% (Fig. 1). The result showed that in the control plot without catechin added 260–800 local lesions were produced per leaf whereas the number of lesions was apparently decreased with the catechin addition, resulting in no lesion at all at 0.5% catechins. This result is well consistent with that of the systemic infection, and it is considered that galloyl catechins, which are more liable to combine with water-soluble protein, exert an inhibitory effect on TMV and CMV infection.

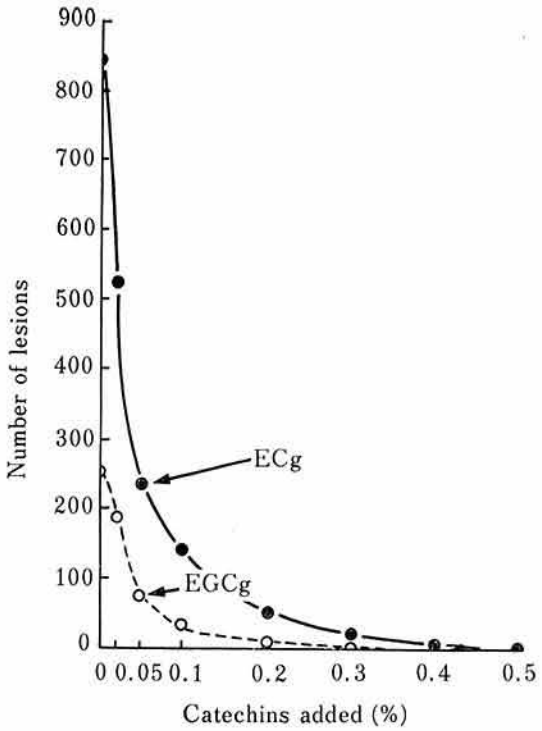

Fig. 1. Addition of galloyl catechins and number of lesions developed

Recovery of virus activity by gelatin

To make clear whether the inhibited infection of TMV and CMV by catechins is caused by the bonding of catechins with viral protein or not, the following experiment was carried out.

To TMV solutions, (−) ECg or (−) EGCg was added at a 0.5% concentration, and further gelatin solution was added to make about 1% concentration. The solutions were then filtered and inoculated to seedlings of *Nicotiana glutinosa*. For the control plot, water was used in place of gelatin. Mode of disease occurrence by TMV inoculum contain-

Table 1. Recovery of viral activities by gelation suspension

Kinds of catechin	Period of reaction to gelatin min	Total number of lesions		Percent recovery %
		Control	Treated	
(−) ECg	immediately after	4476	6	0.1
	10	4216	133	2.9
	30	4277	239	5.6
	60	2725	1458	53.5
(−) EGCg	immediately after	1456	1	0
	10	2343	106	4.5
	30	1156	38	3.3
	60	1047	47	4.5

Number of lesions is an average of 5 leaves

ing gelatin was observed to be different between (−) ECg and (−) EGCg added. In case of (−) ECg, the longer the period of reaction with gelatin, the more was the recovery of virus activity, showing a 5% recovery with 30 min of reaction, and about 53% recovery with 60 min of reaction. On the contrary, the effect of reaction period was small with EGCg, showing a 3.3% recovery with 30 min, and only a 4.5% recovery even with 60 min of reaction. Judging from these results, it seems reasonable to assume that the inhibition of virus infection by catechins is caused by an inactivation of virus due to the bonding of catechins with viral protein, and that the inhibitory effect is greater with (−) EGCg than (−) ECg.

Quantity of catechins required for inhibiting infection

To know the quantity of catechins required for inhibiting the viral infection, (−) ECg or (−) EGCg was added to the purified TMV inoculum at varying concentrations, and the development of lesions was examined in relation to the quantiative ratio of added catechins to TMV protein. The protein quantity was determined as N (by Nessler reagent) ×6.25.

As shown in Table 2, the required quantity of catechins differed slightly with kinds of catechins, but 90% or more than 98% inhibition was observed at the ratio of 31.

Development of lesions in tobacco seedlings which absorbed catechins

Seedlings of *N. glutinosa* were allowed to absorb (−) ECg or (−) EGCg solutions of 1,000 and 2,000 ppm for 2 days, and then inoculated with TMV at an entire leaf surface. Amount of catechins absorbed was different among individual seedlings even though the absorption was made under a same condition. With the 1,000 ppm solutions, the more the absorption of catechins, the less was the local lesions, and a correlation of $r=-0.887^{**}$ was recognized between amounts of absorbed (−) ECg or (−) EGCg and the number of lesions developed. On the other hand, with the 2,000 ppm solutions, the number of lesions was less as a whole. Thus, it was made clear that the virus activity (in terms of local lesion development) can be inhibited by the catechins absorbed by plants.

Anti-viral effect of theaflavins

When tobacco (KY-57) seedlings growing by water-culture absorbed directly the (−) ECg or (−) EGCg solutions with concentrations higher than 2,000 ppm, the seedlings showed an outward curling of leaves and a browning of veins. The cause of this symptom is not clear, but from the fact that unidentified polyphenols are increased in leaves by the absorption of catechins, it may presumably be caused by a reaction of absorbed catechins

Table 2. Inhibition on infection by purified virus

Kinds of catechins	Ratio of catechins to crude TMV protein	Total number of lesions		Percent inhibition %
		Control	Treated	
(−) ECg	31	1698	164	90
	62	1510	16	99
(−) EGCg	31	3226	46	98
	62	3959	29	99

TMV protein used was 32 g/plot
Number of lesions is an average of 5 leaves

Table 3. Antiviral activity of theaflavins on TMV

Test material	Number of local lesions by TMV		Inhibition (%)
	control	Treated	
TF	320	68	78
TF-MG	331	0	100
TF-DG	459	0	100
GA	582	178	66

The concentration of theaflavins and gallic acid was 0.5 mg/ml in the inoculum.
Numbers of local lesions by TMV show the average of two experiments.

to protein or enzymes. Using three components of theaflavins extracted from black tea and separated, i.e., free theaflavin (TF-F), theaflavin monogallate (TF-MG), and theaflavin digallate (TF-DG), and also gallic acid (GA), the inhibitory effect of them on TMV infection was examined.

Table 4. Antiviral activity of theaflavins on TMV-RNA

Preincubation Period	Theaflavin	
	TF-MG	TF-DG
hr	%	%
0	97	95
2	100	100

TMV-RNA: 2μg/ml
TF-MG and TF-DG: 2.6×10^{-4}M and 3×10^{-4}M
%: Inhivitory ratio by TMV-RNA is shown by the average of three experiments

When each of them was added to TMV inoculum at a 0.5% concentration, all of them showed an inhibition to the lesion development, although TF-MG and TF-DG were more effec-

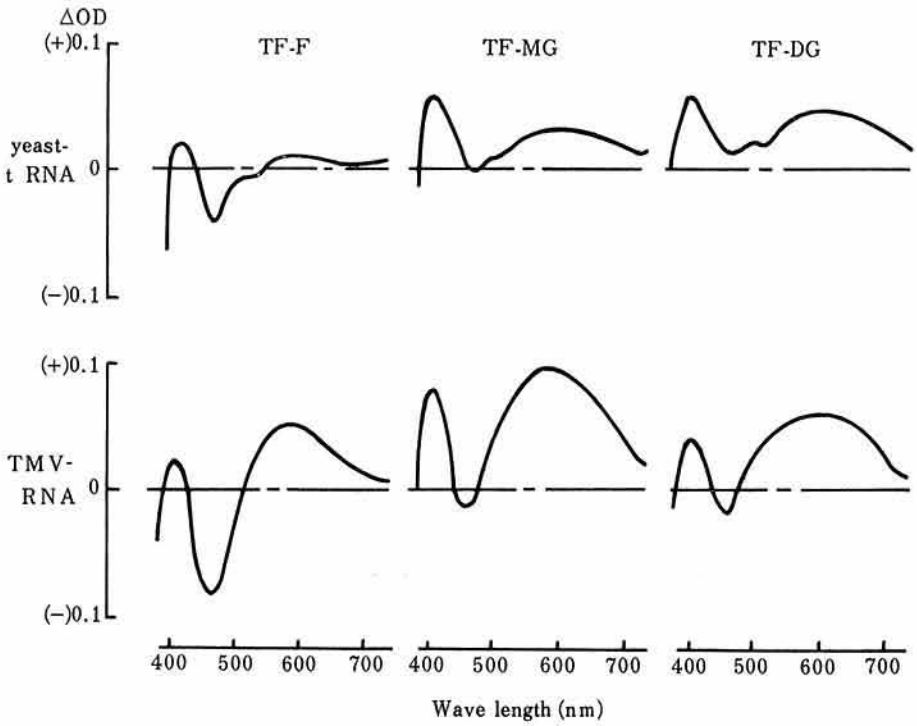

Fig. 2. Interaction of theaflavins with RNAs
Difference spectra were measured on theaflavin (0.5 mg/ml) and yeast tRNA (1×10^{-4}M, phosphorus) or TMV RNA (5μg/ml) against theaflavin (0.5 mg/ml) in 0.01M acetate buffer containing 0.01M NaCl, pH 5.5.

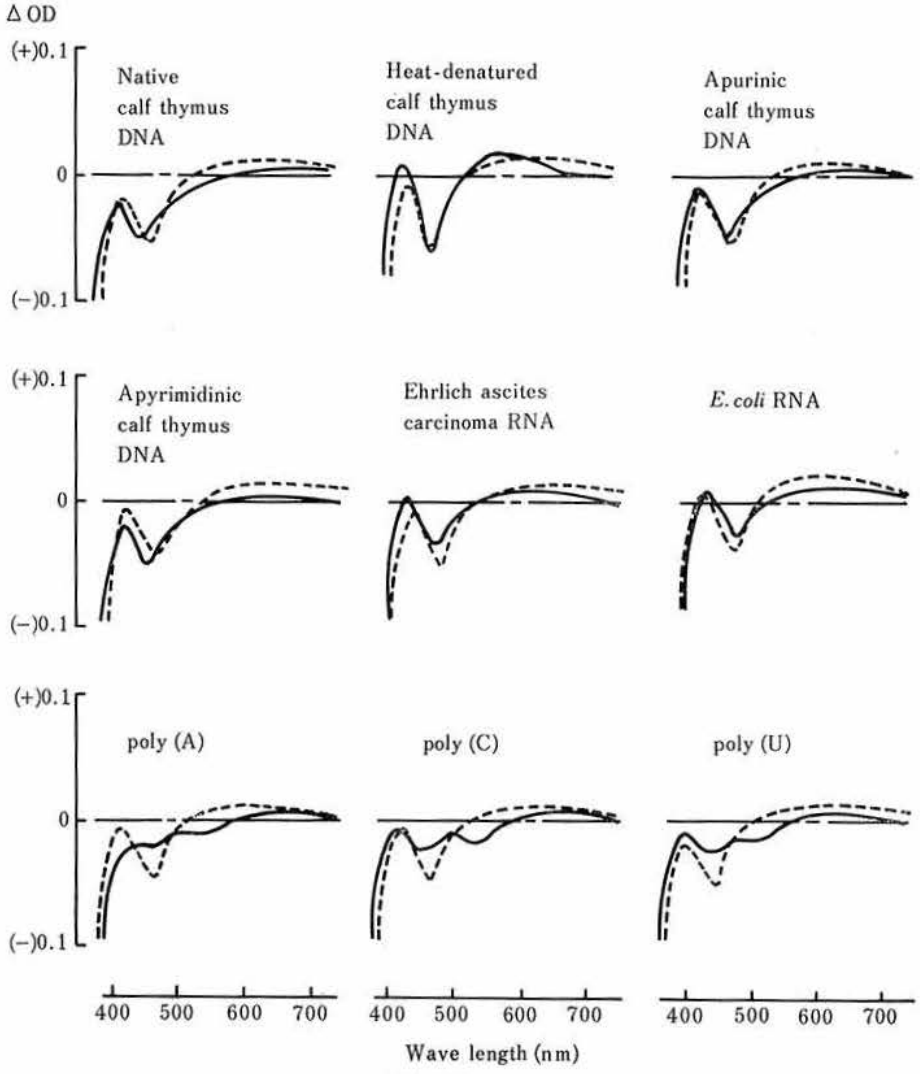

Fig. 3. Interaction of galloyl theaflavins with nucleic acids or polyribonucleotides
Difference spectra were measured on galloyl theaflavin (0.5 mg/ml) and nucleic acid (1×10^{-4}M, phosphorus) or polyribonucleotide (1×10^{-4}M, phosphorus) against galloyl theaflavin (0.5 mg/ml) in 0.01M acetate buffer containing 0.01M NaCl, pH 5.5.

tive than TF-F and GA (Table 4). The effect of these galloyl theaflavins was further examined by an inoculation test after adding 2.5×10^{-4} M of TF-MG or 3×10^{-4} M of TF-DG to the infectious TMV-RNA of 2μg/ml, and it was found that the virus activity was lost within 2 hrs.

Interaction of galloyl theaflavins with nucleic acids and their derivatives

It has been reported that RNA alone is enough for the multiplication of TMV[5], and on the other hand most substances which show

interactions with nucleic acids inhibit the biosynthesis of nucleic acids by bonding with bases of nucleic acid or forming bridges between double-strandness of nucleic acid[6,7].

As theaflavins are able to inhibit the viral activity of TMV or TMV-RNA, it was examined by the difference spectrum method using TMV-RNA and Yeast-tRNA whether the theaflavins have any interaction with nucleic acids or not. In addition, to make clear the mode of bonding with nucleic acids, interactions of TF-MG and TF-DG, which showed a marked anti-viral activity, with the native calf thymus DNA, heat-denatured calf thymus DNA, apurinic calf thymus DNA, apyrimidinic calf thymus DNA, poly A, poly C, poly U, Ehrlich ascites carcinoma RNA, and E. coli RNA were examined.

As shown in Fig. 2 and 3, the visible spectra of theaflavins were shifted at the presence of yeast-tRNA and TMV-RNA, indicating the existence of interactions. As to the effect of TF-MG and TF-DG on various nucleic acids, it was observed that these theaflavins have interactions with each of the native calf thymus DNA and its derivatives used, Ehrlich ascites carcinoma RNA, and E. coli RNA. It was also proved by these experimental results that there is a close relationship between the inhibitory effect of these theaflavins on TMV-RNA and the interaction of theaflavins with nucleic acids.

A further investigation was carried out to find out the site of bonding theaflavins in nucleic acid, and it was shown that the theaflavins react with any of the nucleic acid bases, nucleosid, or nucleotid, suggesting a direct bonding to bases. Thus, it may be concluded from the results of this investigation that theaflavins manifest the inhibitory effects on infection and multiplication of virus, by bonding themselves to nucleic acids of the virus.

Closing remark

The present study was carried out with an aim of widening the use of tea. A later half of the study concerning the interaction with nucleic acids was undertaken as a joint research with the Pharmacology Laboratory of the Shizuoka College of Pharmacy. One of the chemical components of tea, which is of our daily use, is shown to inhibit plant virus, and its inhibitory effect on multiplication and infection of virus is, unlike other substances so far known to be inhibitive, manifested by its reaction to virus itself and nucleic acids of the virus. The fact that it shows not only the inhibitory effect on TMV and CMV, but also interactions with various nucleic acids and their derivatives suggests an extremely wide range of its antiviral activities. Thus, the tea is an interesting natural substance possessing antiviral activities.

References

1) Theornberry, H. H.: Effect of tannic acid on the infectivity of tobacco-mosaic virus. *Phytopathology*, 25, 931–935 (1935).
2) Okada, F.: Inhibitory effects of tea catechine on the multiplication of plant virus (1). *Annals Phytopathological Soc. Japan*, 37, 29–33 (1971) [In Japanese with English summary].
3) Okada, F. et al.: Antiviral effect of theaflavins on tobacco mosaic virus. *Agr. Biol. Chem.*, 41, 791–794 (1977).
4) Okada, F.: Inhibitory effect of tea catechin on the local lesion formation caused by tobacco mosaic virus. *Annals of Phytopathological Soc. of Japan* [In press].
5) Miki, T.: The chemistry of virus. Asakura Shoten (Tokyo), 15–16 (1969).
6) Okada, S.: Inhibition and stimulation of the biosynthesis of protein and nucleic acid. III. Interaction of aminoquinone derivatives with nucleic acid, and their effects on the biosynthesis of nucleic acid and protein in Ehrlich mouse ascites tumor cells in vitro. *Chem. Pharm. Bull.* (Tokyo), 17, 105 (1969).
7) Tamemasu, O., Okada, S. & Mochizuki N.: Interaction of 2-amino-1.4-naphthoquinone imine-HCl (ANQI) and its related compounds with nucleic acid and their antibiological action (No. 9). Muraoka, S. & Ishida, Y.: The co-relation between chemical structure and antibiological activity on drugs action. Hirokawa Shoten (Tokyo), 236–253 (1974).