Vector Relations

The Enzymatic Function of Ribonuclease Determines Plant Virus Transmission by Leaf-Feeding Beetles

R. C. Gergerich and H. A. Scott

Department of Plant Pathology, University of Arkansas, Fayetteville 72701.
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ABSTRACT

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Three types of ribonucleases that differ in their modes of cleaving ribonucleic acid were found to prevent transmission of viruses not transmissible by beetles when mixtures of ribonuclease (RNase) and virus were inoculated to plants using the gross wounding technique. Mixtures of RNase and beetle-transmissible plant viruses, on the other hand, were infective when inoculated in the same manner. When pancreatic RNase, cytochrome c, and chymotrypsinogen, all of which are basic proteins, were mixed with viruses not transmissible by beetles, only pancreatic RNase inhibited virus transmission, indicating that it is not the basic protein nature of RNase, but its enzymatic activity that affects the transmissibility of plant viruses.

Plant virus transmission by beetles occurs when beetles deliver virus in regurgitant to a healthy plant during feeding. Scott and Fulton (6) found that both southern bean mosaic virus (SBMV), which is beetle-transmissible, and sunn hemp mosaic virus (SHMV), which is not, could be detected in the regurgitant of beetles that had fed on virus-infected plant tissue. Beetles deposited both viruses on the leaf surface during feeding, but only the beetle-transmissible virus caused infection in plants that were equally susceptible to both viruses when mechanically inoculated. This led to the conclusion that regurgitant was not only the source of virus for transmission, but that regurgitant also played a role in the selective transmission of plant viruses by beetles.

Both beetle-transmissible and non-beetle-transmissible viruses were equally inhibited when the effect of regurgitant on virus transmission was tested by standard mechanical assays on local lesion hosts (1). However, when virus:regurgitant mixtures were

inoculated using the gross wounding technique, which mimics the damage done by beetles during feeding, the regurgitant was shown to selectively inhibit transmission of viruses not transmissible by beetles. The factor in beetle regurgitant that prevents transmission was shown to be a heat-labile macromolecule whose effect is reversible (1,4).

The regurgitant of leaf-feeding beetles contains high levels of ribonuclease (RNase)(2). Results with various virus-host combinations demonstrated that purified pancreatic RNase is capable of reproducing the selective effect that regurgitant has on virus transmission and indicated that it is the RNase in regurgitant that prevents the transmission of most viruses by beetles (2). The work presented here compares the effects of proteins with similar properties and provides evidence that it is the enzymatic activity of RNase that is responsible for the specificity of plant virus transmission by leaf-feeding beetles.

MATERIALS AND METHODS

Viruses and gross wounding inoculation. Three beetle-transmissible viruses (SBMV, cowpea severe mosaic [CSMV], and bean pod mottle [BPMV]) and two viruses not transmissible by beetles (SHMV and tobacco ringspot [TRSV]) were used in this study. Southern bean mosaic virus, TRSV, BPMV, and SHMV were purified as described by Gergerich et al (1). Cowpea severe mosaic virus was purified as described by Monis et al (4). The buffer used to suspend virus pellets was 0.01 M phosphate buffer, pH 7.2, except for BPMV, which was suspended in 0.1 M phosphate buffer, pH 7.2. All viruses were inoculated to systemically susceptible hosts using the gross wounding technique (1). Two weeks after inoculation plants were indexed for virus infection by the Ouchterlony double diffusion test.

Ribonucleases. Three RNases that differ in their molecular weight, isoelectric point, and the manner in which they cleave RNA were tested for their ability to inhibit virus transmission using the gross wounding technique. These three RNases are all endonucleases, but they preferentially cleave RNA at different nucleotides. Bovine pancreatic RNase (Type I-A, EC 3.1.27.5; Sigma, St. Louis, MO) splits the bond between the phosphate residue at pyrimidine nucleotides; RNase T_1 (Grade V, EC 3.1.4.8; Sigma) specifically hydrolyzes the bonds of RNA at guanine sites; RNase from *Aspergillus clavatus* (Sigma) is a nonspecific RNase that cleaves RNA at all four nucleotides. RNase activity was assayed spectrophotometrically by measuring the amount of acid-soluble oligonucleotides liberated from a solution of 1% RNA (Type XI from baker's yeast; Sigma) in 0.01 M phosphate buffer, pH 7.2, at 37 C for 10 min (3).

To determine the effect of T_1 RNase and the RNase from *A. clavatus* on virus transmission the specific RNase activity of each enzyme preparation was determined as described above, and the enzymes were diluted such that the RNase activity in each inoculum mixture was equivalent to the enzyme activity of pancreatic RNase at a concentration of 100–1,000 μg/ml at pH 7.2.

Basic proteins. The unusually high isoelectric point of 9.45 for pancreatic RNase (5) suggested that this property of the protein, rather than its enzymatic activity, might be responsible for the effect on virus transmission. To test this hypothesis, chymotrypsinogen and cytochrome c, two proteins with isoelectric points of 9.5 and 10.6, respectively, were assayed for their effect on virus transmission. Cytochrome c (Type VI from horseheart; Sigma), chymotrypsinogen A (Type II from bovine pancreas; Sigma), and pancreatic RNase, each at 0.01 mM in 0.01 M phosphate buffer, pH 7.2, were mixed with purified SHMV and TRSV (viruses not transmissible by beetles) and SBMV and BPMV (beetle-transmissible viruses) and inoculated to Black Valentine bean using the gross wounding technique.

RESULTS AND DISCUSSION

Three types of RNase that digest RNA by attacking at different sites within a polynucleotide chain exhibited the same selective inhibition of plant virus infection as beetle regurgitant. When inocula containing these enzymes were applied to plants using the gross wounding method of inoculation, the beetle-transmissible viruses were infective, but viruses not transmissible by beetles were not (Table 1). This provides evidence that the enzymatic digestion of RNA results in prevention of virus infection, regardless of the type of RNase used.

Initial studies that demonstrated the selective effect of purified RNase on the infection of plant viruses (2) used pancreatic RNase in the inoculum mixtures that were applied to plants by the gross wounding technique of inoculation. As mentioned above, the basic nature of this protein rather than its enzymatic function could be responsible for the selective effect on plant virus transmission. The results in Table 2 show that the basic proteins cytochrome c and chymotrypsinogen have no effect on the transmissibility of plant viruses, whereas pancreatic RNase at the same molarity selectively inhibits the transmission of viruses not transmissible by beetles.

We conclude that the ability of RNase to digest RNA probably is responsible for its effect on transmission when viruses are inoculated by gross wounding or by beetle feeding, although the data to support this conclusion are only correlative. Ribonuclease may block some early event in virus infection of plant cells by digesting RNA. The fact that some viruses, namely those transmissible by beetles, are not affected by RNase suggests that the manner in which these viruses infect plants differs in some key way from other plant viruses.

TABLE 1. Effects of different types of ribonuclease (RNase) on virus transmission when inoculated by the gross wounding technique

Type of RNase	RNase enzyme activity (units/ml)[a]	Proportion of plants infected by inocula[b] containing SHMV (50 mg/ml)	TRSV (21 mg/ml)	CSMV (33 mg/ml)	SBMV (25 mg/ml)
Pancreatic	90,000	0/37	0/36	27/34	19/31
	9,000	2/37	0/37	25/34	31/31
Aspergillus oryzae, T_1	90,000	2/37	0/37	24/34	15/31
	9,000	1/37	2/37	31/34	24/31
Aspergillus clavatus	33,000	3/35	0/35	33/35	35/35
Buffer	0	28/35	26/35	33/35	35/35

[a] One milligram of pancreatic RNase contained 90,000 units of enzyme activity as determined under the defined assay conditions. All enzyme preparations were diluted to contain between 9,000 to 90,000 units/ml, equivalent to 100–1,000 μg/ml of pancreatic RNase.

[b] The viruses were suspended in 0.01 M phosphate buffer, pH 7.2. Virus suspensions were mixed with an equal volume of this buffer or with RNase in this buffer. Viruses not transmissible by beetles: sunn hemp mosaic (SHMV), tobacco ringspot (TRSV). Viruses transmissible by beetles: southern bean mosaic (SBMV), cowpea severe mosaic (CSMV). Hosts were Black Valentine bean for SHMV, TRSV, and SBMV, and Monarch cowpea for CSMV.

TABLE 2. Effects of basic proteins on transmission of viruses to Black Valentine bean when inoculated by the gross wounding technique

Protein (0.01 mM)[b]	Proportion of plants infected when inoculated with[a] SHMV (52 mg/ml)	TRSV (20 mg/ml)	SBMV (25 mg/ml)	BPMV (23 mg/ml)
Pancreatic RNase	0/30	0/30	30/30	10/30
Cytochrome c	24/30	27/30	30/30	21/30
Chymotrypsinogen	15/30	20/30	30/30	15/30
Buffer	23/30	28/30	30/30	23/30

[a] Viruses not transmissible by beetles: sunn hemp mosaic (SHMV), tobacco ringspot (TRSV). Viruses transmissible by beetles: southern bean mosaic (SBMV), bean pod mottle (BPMV).

[b] The viruses and proteins were suspended in 0.01 M phosphate buffer, pH 7.2, except for BPMV, which was suspended in 0.1 M phosphate buffer, pH 7.2. Virus suspensions were mixed with an equal volume of the protein solutions or the appropriate buffer.

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