Selective Inhibition of Viral RNA Transcription by Skyrin*

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(Received October 7, 1975)

Three structurally related pigments, rugulosin (Rug) and skyrin (Sky) which are products of *Endothia parasitica*, and luteoskyrin (Lut) which is a well-known mycotoxin produced by *Penicillium islandicum*, were tested for their inhibitory effects on rice dwarf virus (RDV) RNA transcription and *E. coli* DNA-dependent RNA polymerase activities, using *in vitro* assay systems for both enzymes. Lut inhibits only *E. coli* RNA polymerase but has no effect on RDV-RNA transcriptase. Rug strongly inhibits *E. coli* RNA polymerase but weakly RDV-RNA transcriptase. Conversely, Sky inhibits RDV-RNA transcriptase without inhibiting *E. coli* RNA polymerase. The Mg^{2+} -mediated complex formation of Lut and Rug with DNA and that of Rug with RDV-RNA may account for their inhibitory effects on both enzymes, however this is not true with Sky on DNA and Sky on RDV-RNA. Sky forms complex with Mg^{2+} because its absorption spectrum shifts to longer wave lengths with increasing amounts of Mg^{2+} added but the complex does not form floccules which can be seen in Lut- Mg^{2+} and Rug- Mg^{2+} mixtures. Sky-Mg-DNA and Sky-Mg-RDV-RNA mixtures do not form such a complex which can be precipitated by centrifugation at $59,000 \times g$ for 20 min. Sephadex G-25 column chromatography of the mixtures indicates that nucleic acids are completely separated from Sky-Mg complex. When RDV-virions are pretreated with Sky and Mg^{2+} , pelleted by centrifugation, then added to the reaction mixture, RDV-RNA transcriptase is not inhibited at all, whereas inhibited strongly by the addition of the supernatant which contains Sky. These results suggest that Sky inhibits RDV-RNA transcriptase not by its direct action to RDV-RNA present in virions but by its contact with virions at the site of transcription.

INTRODUCTION

In a series of studies on rice dwarf virus, we have attempted to find out some agents which inhibit the synthesis of viral RNA without inhibiting the syntheses of host cell nucleic acids. Rice dwarf virus (RDV) virions contain double-stranded RNA (dsRNA)¹⁾ and its transcriptase.²⁾ The dsRNA can easily be separated quantitatively from cell nucleic acids in RDV-infected leaves of rice plants by using chromatographic techniques.³⁾ This enabled us to make a screening test for some agents which inhibit ^{32}P -incorporation into viral dsRNA but not inhibit the incorpora-

tion into ribosomal RNA. The result indicated that cycloheximide inhibited ^{32}P -incorporation into both RDV-RNA and ribosomal RNA suggesting that the antibiotic might be phytotoxic and this was actually true. Blasticidin S and kasugamycin were not enough inhibitory on ^{32}P -incorporation into RDV-RNA but rather stimulating ^{32}P -incorporation into ribosomal RNA.⁴⁾ Second step of screening was directed to select some agents which inhibit RDV-RNA transcriptase without inhibiting *E. coli* DNA-dependent RNA polymerase, using *in vitro* assay systems for both enzymes. Acridine orange, ethidium bromide, methylene blue, azure B, azure A, azure C and thionine inhibited both enzymes almost equally. Chloroquine, quinacrine, luteoskyrin, actinomycin D and rifamycin SV inhibited only *E. coli* RNA polymerase but

* Screening of Chemotherapeutants for Rice Dwarf Virus (Part III)

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had no effect on RDV-RNA transcriptase.⁵⁾ Some of the agents in the first group were known to catalyze photodynamic degradation of nucleic acids,^{6,7)} and this was also the case with methylene blue and azure B on RDV-RNA, causing loss of guanine in RDV-RNA.⁵⁾ Among these dyes and drugs, only azure B stimulates the elongation of RDV-infected rice plants when applied to the culture solution at 3–6 ppm.⁸⁾ It was suggested that the safe application of azure B without causing any phytotoxicity might be due to its *in vivo* affinity particularly to inclusion bodies but not to nuclei.⁵⁾ In fact, azure B stains inclusion bodies without staining nuclei at least in living leaf tissues.

This paper reports the results of comparison of the structurally related three pigments, luteoskyrin, rugulosin and skyrin on RDV-RNA transcriptase and *E. coli* DNA-dependent RNA polymerase and the fact that skyrin inhibits RDV-RNA transcriptase without inhibiting *E. coli* RNA polymerase.

MATERIALS AND METHODS

Host plant, insect vector and virus isolate: Seedlings of rice plant (Norin-8) were inoculated at 3-leaf stage by viruliferous leafhoppers, *Nephotettix cincticeps* Uhl., which had been reared on RDV-infected rice plants. The virus isolate and the insect colony used in this study are the same as previously reported.²⁾

Preparation of RDV: The method of purification was essentially as described by Kimura.⁹⁾ Hundred g of leaves of infected rice plants were cut into small pieces, homogenized in 300 ml of 0.1M phosphate buffer (PB), pH 6.8, and 100 ml of carbontetrachloride using a Vir-Tis homogenizer under ice-cooling. The homogenate was centrifuged at 3,000 rpm for 10 min. The supernatant was mixed with polyethylene glycol (6,500) and NaCl to final concentrations of 6% and 0.3M respectively and incubated at 4°C for 3 hr. The mixture was centrifuged at 9,500 × g for 1 hr and the precipitate was suspended in 20 ml of PB. The suspension was added with half volume of fluorocarbon (Daifron S3), stirred vigorously and centrifuged at 3,000 rpm for 10 min. The supernatant was

layered on a sucrose density gradient consisting of 10 ml each of 20 and 30% solutions, and centrifuged at 24,000 rpm for 1 hr using Hitachi RPS-25 rotor. The bottom band was separated, layered on a sucrose density gradient consisting of 10 ml each of 30 and 40% solutions, then centrifuged at 24,000 rpm for 1 hr. The bottom band was separated, dialyzed against 0.1M acetate buffer, pH 6.0, overnight, then centrifuged at 24,000 rpm for 1 hr. The pellet was resuspended in a small amount of acetate buffer and used as purified RDV preparation.

Preparation of RDV-RNA: dsRDV-RNA was prepared from the purified RDV preparation by the cold-phenol method as described by Miura *et al.*¹⁾ The RNA was usually stored in 67% ethanol at -20°C until use.

Preparation of *E. coli* DNA: *E. coli* DNA was prepared by Marmur's method.¹⁰⁾

Assay of RDV-RNA transcriptase activity: The standard reaction mixture for assay of RDV-RNA transcriptase activity contained in a final volume of 0.25 ml:

ATP, GTP, CTP, UTP	0.2 μmoles each
³ H-UTP	2.0 μCi
MgCl ₂	2.0 μmoles
phosphoenol pyruvate	1.0 μmoles
pyruvate kinase	25 μg
chymotrypsin	50 μg
Tris-HCl, pH 8.5	25 μmoles
RDV	200 μg
	(0.08 μmoles of P)

After incubation at 35°C for 60 min the reaction was stopped by adding 3 ml of ice-cold 5% trichloroacetic acid (TCA) containing 0.02M sodium pyrophosphate (NaPP). The precipitates were collected on Schleicher and Schuell membrane filters (0.45 μm pore-size) and washed with 20 ml of 5% TCA containing NaPP, and then with 95% ethyl alcohol. The filters were placed in scintillation vials, dried, and the radioactivity was determined by liquid scintillation spectrometry.

Assay of *E. coli* DNA-dependent RNA polymerase activity: The standard reaction mixture for assay of *E. coli* RNA polymerase contained in a final volume of 0.25 ml:

ATP, GTP, CTP, UTP	0.1 μmoles each
³ H-UTP	2.0 μCi

Fig. 1 Absorption spectra of skyrin (—) and rugulosin (-----) in ethanol.

Fig. 2 Shift of absorption spectrum skyrin with varying pH's.

magnesium acetate 1.25 μ moles
 manganese sulfate 0.5 μ moles
 mercaptoethanol 1.25 μ moles
 Tris-HCl, pH 7.8 30 μ moles
E. coli DNA 24 μ g
 (0.08 μ moles of P)
E. coli RNA polymerase 0.5 unit
 (Boeringer-Mannheim Co.)

After incubating at 37°C for 60 min, the reaction was stopped by adding 3 ml of cold TCA containing 0.02M NaPP. The procedure for counting radioactivity was as described above.

Extraction of rugulosin and skyrin from mycelial mat of *Endothia parasitica*: Luteoskyrin was kindly furnished by Dr. H. Kurata, National Hygienic Institute. Methods for the extraction and purification of rugulosin and skyrin from mycelial mats of *Endothia parasitica* were the same as described by Shibata

*et al.*¹¹⁻¹⁴⁾ Absorption spectra of rugulosin and skyrin were shown in Fig. 1. Shift of absorption spectrum of skyrin with varying pH's is shown in Fig. 2.

RESULTS

1. Comparison of Inhibitory Effects of Three Pigments on *E. coli* RNA Polymerase and RDV-RNA Transcriptase

Luteoskyrin (Lut), rugulosin (Lut) and skyrin (Sky) were dissolved in minimum amounts of 0.2N Na₂CO₃, diluted with water and the pH was adjusted to 7.8 for *E. coli* RNA polymerase assay system and to 8.5 for RDV-RNA transcriptase assay system. These were added to both assay systems at *P/D* ratios of 10/2 and 10/4, where *P* is moles of phosphorus in nucleic acids, 0.08 μ moles in each 0.25 ml of reaction mixture, and *D* is moles of pigments, 0.016 and 0.032 μ moles for 10/2 and 10/4 ratios respectively. Inhibitory effects of three pigments are shown in Table 1.

Results indicate that Lut inhibits *E. coli* RNA polymerase only and Rug inhibits both *E. coli* RNA polymerase and RDV-RNA transcriptase, whereas Sky inhibits RDV-RNA transcriptase without in-

Table 1 Comparison of inhibitory effects of luteoskyrin, rugulosin and skyrin on *E. coli* DNA-dependent RNA polymerase and RDV-RNA transcriptase.

Inhibitor	<i>P/D</i> ratio	Percent inhibition Transcription of	
		<i>E. coli</i> DNA	RDV-RNA
Luteoskyrin	10 : 2	64	0
	10 : 4	85	0
Rugulosin	10 : 2	62	14
	10 : 4	66	27
Skyrin	10 : 2	0	36
	10 : 4	4	61

Numbers in the table are the average of four repetitions.

Table 2 Effect of Mg^{2+} concentrations on RDV-RNA transcriptase activity.

MgCl ₂ (μ mole*)	³ H-UMP incorporated into TCA-insol. RNA (CPM)	%
0	691	3
0.5	9,328	40
1.0	22,274	97
2.0	23,081	100
3.0	15,629	68
4.0	9,073	39
6.0	3,646	16
9.0	1,250	5
12.0	869	4

* μ moles in 0.25 ml of standard reaction mixture.

hibiting *E. coli* RNA polymerase.

2. Complex Formation of *E. coli* DNA and RDV-RNA with Pigments in the Presence of Mg^{2+}

Mg^{2+} is essential for the *in vitro* syntheses of RNA directed by *E. coli* DNA and RDV-RNA. Optimum concentration of Mg^{2+} in the reaction mixture for RDV-RNA transcriptase lies in the range from 1.0 to 2.0 μ moles/0.25 ml (4–8 mM) (Table 2). In the ranges below 0.5 and above 4.0 μ moles the rate of transcription is markedly suppressed. This fact means that Mg^{2+} is always present in the reaction mixtures where pigments interact with DNA or RDV-RNA. It has been reported that Lut forms complex with DNA in the presence of Mg^{2+} , the molar ratio of DNA/Mg/Lut being 4–6:1:1 and that Lut forms complexes with poly-U and poly-C in the presence of Mg^{2+} with 1:1:1 ratio.^{15–17)} Questions arise as to whether or not other two pigments form complexes with DNA and RDV-RNA in the presence of Mg^{2+} .

The lowest lines in Table 3 and 4 show that OD₂₆₀ values of *E. coli* DNA and RDV-RNA dissolved in 0.1M Tris-HCl, pH 8.5, containing Mg^{2+} , decrease by about 10% after 20 hr incubation at room temperature and subsequent centrifugation at 59,000×*g* for 20 min. This 10% decrease of OD₂₆₀ value was taken into account for reading the data shown in Tables 3 and 4.

Table 3 indicates that Lut and Rug produce floccules immediately after addition of

Table 3 Effects of Mg^{2+} on the sedimentation of DNA-luteoskyrin, DNA-rugulosin and DNA-skyrin mixtures.

		Before centrif.	After centrif.
Luteoskyrin	OD ₄₅₀ OD ₂₆₀	0.22 0.32	0.20 0.30
Lut.+MgCl ₂	OD ₄₅₀ OD ₂₆₀	floccul.	0.05 0.10
Lut.+DNA	OD ₄₅₀ OD ₂₆₀	0.24 1.30	0.10 1.00
Lut.+MgCl ₂ +DNA	OD ₄₅₀ OD ₂₆₀	0.24 1.25	0.09 0.89
Rugulosin	OD ₄₂₀ OD ₂₆₀	0.37 0.40	0.37 0.40
Rug.+MgCl ₂	OD ₄₂₀ OD ₂₆₀	floccul.	0.10 0.15
Rug.+DNA	OD ₄₂₀ OD ₂₆₀	0.37 1.45	0.36 1.35
Rug.+MgCl ₂ +DNA	OD ₄₂₀ OD ₂₆₀	0.43 1.40	0.20 0.69
Skyrin	OD ₅₃₀ OD ₂₆₀	0.12 0.29	0.12 0.28
Sky.+MgCl ₂	OD ₅₃₀ OD ₂₆₀	0.12 0.30	0.12 0.28
Sky.+DNA	OD ₅₃₀ OD ₂₆₀	0.11 1.35	0.10 1.20
Sky.+MgCl ₂ +DNA	OD ₅₃₀ OD ₂₆₀	0.13 1.30	0.12 1.15
<i>E. coli</i> DNA+MgCl ₂	OD ₂₆₀	1.06	0.95

Each mixture contains 0.53 μ mol. of *E. coli* DNA (P), one of the pigments (0.07 μ mol. of luteoskyrin, 0.074 μ mol. of rugulosin or 0.073 μ mol. of skyrin) and 1.0 μ mol. of MgCl₂ in 3.5 ml of 0.1M Tris-HCl, pH 8.5. After 20 hr incubation at room temperature, the mixtures were centrifuged for 20 min at 30,000 rpm in Hitachi RP-40 rotor.

Mg^{2+} , whereas Sky does not. The production of floccules of Lut-Mg and Rug-Mg complexes is inhibited by the presence of *E. coli* DNA. After 20 hr-incubation at room temperature, Lut-Mg-DNA and Rug-Mg-DNA complexes are precipitated by centrifugation at 59,000×*g* for 20 min, resulting in the decrease of OD₂₆₀ values of the supernatants. Nothing is precipitated from the mixture of Sky-Mg-DNA.

Table 4 indicates that the presence of RDV-RNA also inhibits that production of floccules which are to be produced in Lut-Mg and Rug-Mg mixtures. Precipitation of pigment-Mg-RDV-RNA complex after 20 hr-incubation and subsequent centrifugation oc-

Table 4 Effects of Mg^{2+} on the sedimentation of RDV-RNA-luteoskyrin, RDV-RNA-rugulosin and RDV-RNA-skyrin mixtures.

		Before centrif.	After centrif.
Luteoskyrin	OD ₄₅₀ OD ₂₆₀	0.22 0.32	0.20 0.30
Lut. + $MgCl_2$	OD ₄₅₀ OD ₂₆₀	floccul.	0.05 0.10
Lut. + RDV-RNA	OD ₄₅₀ OD ₂₆₀	0.24 1.24	0.22 1.05
Lut. + $MgCl_2$ + RDV-RNA	OD ₄₅₀ OD ₂₆₀	0.23 1.17	0.10 1.05
Rugulosin	OD ₄₂₀ OD ₂₆₀	0.37 0.40	0.37 0.40
Rug. + $MgCl_2$	OD ₄₂₀ OD ₂₆₀	floccul.	0.10 0.15
Rug. + RDV-RNA	OD ₄₂₀ OD ₂₆₀	0.36 1.33	0.36 1.33
Rug. + $MgCl_2$ + RDV-RNA	OD ₄₂₀ OD ₂₆₀	0.40 1.29	0.10 1.00
Skyrin	OD ₅₃₀ OD ₂₆₀	0.12 0.29	0.12 0.28
Sky. + $MgCl_2$	OD ₅₃₀ OD ₂₆₀	0.12 0.30	0.12 0.28
Sky. + RDV-RNA	OD ₅₃₀ OD ₂₆₀	0.11 1.19	0.11 1.07
Sky. + $MgCl_2$ + RDV-RNA	OD ₅₃₀ OD ₂₆₀	0.11 1.18	0.11 1.05
RDV-RNA + $MgCl_2$	OD ₂₆₀	1.03	0.93

The mixture contains 0.51 μ mol. of RDV-RNA (P) in 3.5 ml.

curred only in Rug-Mg-RDV-RNA mixture, though the amount of sediment was much less than that of Rug-Mg-DNA mixture. In the case of Lut-Mg-RDV-RNA mixture, OD₄₅₀ of the supernatant was decreased conspicuously, whereas OD₂₆₀ was decreased not more than 10% of the original value, indicating that only Lu-Mg complex, but not Lut-Mg-RDV-RNA, was precipitated by the centrifugation. No sediment was obtained from Sky-Mg-RDV-RNA mixture.

3. Binding of Mg^{2+} to Sky

Although Sky does not produce floccules with Mg^{2+} , it is presumable

Table 5 Effect of pretreatment of RDV-virions with skyrin on RDV-RNA transcriptase.

Treatment	CPM	% Loss	% Inhibition
(1) Preincubation of virions at pH 8.5, 35°C, 60 min	25,379	38	—
(2) Preincubation of virions with Sky, pH 8.5, 35°C, 60 min	30,356		0
(3) Virions in (1) + Supernatant (2)	458		88
(4) Control (virions are added to reaction mixture without preincubation)	41,113		—
(5) (4) + Sky at <i>P/D</i> ratio of 10 : 4	1,979		95
(6) (4) + Sky at <i>P/D</i> ratio of 10 : 2	6,519		84
(7) (4) + Sky at <i>P/D</i> ratio of 10 : 1	34,364		16

- (1) Virions (2mg) were incubated in incubation mixture (2.5 ml) containing 250 μ moles of Tris-HCl (pH 8.5) and 20 μ moles of $MgCl_2$. After incubation 200 μ g of virions were taken into reaction mixture.
- (2) Virions (2mg) were incubated in incubation mixture (2.5 ml) containing 250 μ moles of Tris-HCl (pH 8.5), 20 μ moles of $MgCl_2$, and 0.48 μ moles of Sky. After incubation 200 μ g of virions were taken into reaction mixture.
- (4)-(7) Virions (200 μ g) were added to reaction mixture without preincubation of virions.

Fig. 3 Shift of absorption spectrum of skyrin with the addition of Mg^{2+} .

Fig. 4 Gel filtration of the components of Sky-Mg-DNA and Sky-Mg-RDV-RNA mixtures on Sephadex G-25. Eluted with 0.1M Tris-HCl, pH 8.5, containing 12mM MgCl_2 .

from its chemical structure that Sky forms complex with Mg^{2+} , and this is true because the absorption spectrum of Sky shifts to longer wave length with the increasing amounts of Mg^{2+} added (Fig. 3).

4. Chromatographic Separation of Components in Sky-Mg-DNA and Sky-Mg-RDV-RNA Mixtures

Sky-Mg-DNA and Sky-Mg-RDV-RNA mixtures in Tris-HCl (pH 8.5) were incubated for 20 hr at room temperature, then subjected to Sephadex G-25 column chromatography. OD_{260} and OD_{530} were measured for each 4ml eluent. Figure 4 indicates that DNA and RDV-RNA are completely separated from Sky-Mg complex, suggesting that Sky-Mg does not bind to DNA or RDV-RNA in the mixture.

5. Effect of Pretreatment of RDV-virions with Sky on RDV-RNA Transcriptase Activity

The reaction mixture for assay of RDV-RNA transcriptase activity contains RDV-virions as sources of dsRNA and transcriptase, i.e., the template RNA is not directly exposed to the reaction mixture but is enclosed in capsids. When virions are pre-treated with Sky dissolved in 0.1M Tris-HCl (pH 8.5) containing Mg^{2+} , precipitated by centrifugation, and the precipitates are added to the reaction mixture, RDV-RNA transcriptase activity is not inhibited at all,

whereas the addition of supernatant, which contains Sky, inhibits the activity conspicuously. Table 5 shows that the mere incubation of virions in Tris buffer, containing Mg^{2+} , causes loss of activity by 38%, possibly because of the release of RNA from virions. When this loss of activity by preincubation is taken into account, the Sky-treated virions clearly retain their activity. The result suggests that Sky inhibits the transcriptase not by binding to RDV-RNA but by contacting virions at the site of RNA synthesis.

DISCUSSION

Lut is a well-known mycotoxin produced by *Penicillium islandicum*. On the basis of the spectral change of Lut,¹⁸⁾ it was presumed that Lut bound *in vitro* with thymus DNA in the presence of Mg^{2+} to form a complex (DNA-Mg-Lut) with a molecular ratio of 4-6 : 1 : 1. Subsequently, the DNA-Mg-Lut complex was isolated by gel filtration of SE Sephadex C-25 and the measurement of flow dichroism demonstrated that the orientation of the planar chromophore of Lut was parallel with the axis of DNA double-helix. The inhibitory effect of Lut on DNA-dependent RNA polymerase activity was demonstrated by Ueno *et al.*¹⁹⁾ and Senetac *et al.*²⁰⁾ Lut also inhibits the DNA synthesis *in vitro*. Detailed review on the mode of action of Lut on nucleic acid metabolism is

given by Enomoto and Ueno.²¹⁾

Regulosin is a kind of toxin produced by *Endothia parasitica* and has a biological activity similar to lut.²²⁾ Sky is another pigment produced by the same fungus but is not toxic to mammals.²³⁾

The purpose of this study is to seek some agents which inhibit the synthesis of viral RNA without inhibiting the functions of host cell DNA. From this view point, Lut and Rug are useless for the present purpose but are used to compare their modes of action on DNA and RDV-RNA with that of Sky because these are closely related with each other in their chemical structures.

The test for inhibitory effects of three pigments on RNA syntheses directed by *E. coli* DNA and RDV-RNA revealed that only Sky was satisfactory for the present purpose because this pigment inhibited only RDV-RNA transcriptase but had no effect on *E. coli* RNA polymerase. Conversely, Lut and Rug inhibited *E. coli* RNA polymerase but inhibited not at all or only very slightly RDV-RNA transcriptase.

Above described effects appear to be correlated with the formation of pigment-Mg-nucleic acid complexes which can be precipitated by centrifugation at $59,000 \times g$ for 20 min in cases of Lut and Rug, but are not in the case of Sky. The relationship can be seen in the following:

	Inhibition of RNA synthesis directed by		Formation of precipitable complex with	
	<i>E. coli</i> DNA	RDV-RNA	<i>E. coli</i> DNA	RDV-RNA
Lut+Mg ²⁺	++	—	++	—
Rug+Mg ²⁺	++	+	++	+
Sky+Mg ²⁺	—	++	—	—

Lut and Rug produce floccules immediately after the addition of Mg²⁺, whereas Sky does not. It is presumable that Sky forms complex with Mg²⁺ because of the spectral change. Results of chromatographic separation, using Sephadex G-25, of the components of Sky-Mg-DNA and Sky-Mg-RDV-RNA mixtures revealed that DNA and RDV-RNA are not bound to Sky in the presence of Mg²⁺.

RDV-virions pretreated with Sky in the presence of Mg²⁺ retained the transcriptase activity, whereas this was markedly inhibited by the addition of supernatant, which contains Sky. This suggests that the inhibition by Sky occurs not by its binding to RDV-RNA but by contacting the virions at the site of RNA synthesis. Precise mechanism of action remains to be solved in future. It is not known why *E. coli* RNA polymerase is not inhibited by Sky in the presence of Mg²⁺.

In the case of reovirus, dsRNA is synthesized via single-stranded RNA which is produced by the transcription of dsRNA (see the scheme presented by Silverstein *et al.*²⁴⁾ If this is also the case with RDV, it is presumable that the synthesis of dsRDV-RNA will be inhibited by inhibiting the transcription of dsRNA with some agent. Our unpublished data revealed that when Sky, at 20–40 $\mu\text{g/g}$ fresh weight, was administered to rice leaves detached from RDV-infected plants, together with ³²P and incubated at 25°C for 24 hr, ³²P-incorporation into dsRDV-RNA was apparently decreased without decrease of ³²P-incorporation into cell ribosomal RNA, whereas Rug at the same dosage decreased ³²P-incorporation into both RNA's. Tests on the preventive effect of Sky on RDV-infection to rice plants and its therapeutic effect on RDV-infected plants are now in progress. Since Sky is an acidic pigment and hardly soluble in water, a problem remains to be solved as to how to stimulate its penetration and translocation to target tissues when applied to the leaf surface.

要 約

構造的に近縁な3種の糸状菌生産色素ルテオスカイリン (Lut), ルグロシン (Rug), スカイリン (Sky) について, イネ萎縮病ウイルス (RDV) RNA 転写酵素および *E. coli* RNA ポリメラーゼに対する阻害効果を, それぞれの *in vitro* 合成系を用いて比較した. Lut は *E. coli* RNA ポリメラーゼを強く阻害し, RDV-RNA 転写酵素は阻害しない. Rug は前者を強く, 後者を弱く阻害する. Sky は RDV-RNA 転写のみを阻害する. Mg²⁺ 存在下で Lut, Rug は DNA と, Rug は RDV-RNA とも, 複合体を作り, これは $59,000 \times g$ 20 分の遠心分離

で沈殿する。Sky は DNA とも RDV-RNA とも Mg^{2+} 存在下で複合体を作らない。RDV 粒子を Sky と Mg^{2+} で前処理しても粒子は転写酵素活性を保持するが、これに前処理液の上清 (Sky を含む) を加えると活性は阻害される。このことから、Sky は RDV-RNA と結合することなく、粒子の外にあって RNA 合成の場に接触して RNA 合成を阻害すると推論した。

REFERENCES

- 1) K. Mimura, I. Kimura & N. Suzuki: *Virology* **28**, 571 (1966)
- 2) T. Kodama & N. Suzuki: *Ann. Phytopath. Soc. Japan* **39**, 251 (1973)
- 3) T. Kodama, I. Kimura & N. Suzuki: "Biochemical Regulation in Diseased Plants or Injury," *Phytopath. Soc. Japan*, p. 59, 1968
- 4) N. Suzuki, I. Kimura & T. Kodama: *Ann. Phytopath. Soc. Japan* **39**, 1 (1973)
- 5) M. Nakata & N. Suzuki: *Ibid.* **41**, 345 (1975)
- 6) M.I. Simon & H. Van Vunakis: *J. Mol. Biol.* **4**, 488 (1962)
- 7) B. Singer & H. Fraenkel-Conrat: *Biochemistry* **5**, 2446 (1966)
- 8) N. Suzuki: *Botyu-Kagaku* **35**, 153 (1970)
- 9) I. Kimura: *Ann. Phytopath. Soc. Japan* **40**, 208 (1974)
- 10) J. Marmur: *J. Mol. Biol.* **3**, 208 (1961)
- 11) S. Shibata, O. Tanaka, G. Chihara & H. Mitsuhashi: *Pharm. Bull. Japan* **1**, 302 (1973)
- 12) S. Shibata, T. Murakami, O. Tanaka, G. Chihara & M. Sumimoto: *Ibid.* **3**, 274 (1955)
- 13) S. Shibata, O. Tanaka & I. Kitagawa: *Ibid.* **4**, 143 (1956)
- 14) S. Shibata, T. Murakami & M. Takido: *Ibid.* **4**, 303 (1956)
- 15) Y. Ueno, A. Platel & P. Fromageot: *Biochim. Biophys. Acta* **134**, 27 (1967)
- 16) Y. Ueno, I. Ueno, K. Mizumoto & T. Tatsuno: *J. Biochem.* **63**, 395 (1968)
- 17) Y. Ueno, I. Ueno & K. Mizumoto: *Japan J. Exptl. Med.* **38**, 47 (1968)
- 18) T. Ohba & P. Fromageot: *Eur. J. Biochem.* **1**, 147 (1967)
- 19) Y. Ueno, I. Ueno & T. Tatsuno: *Seikagaku* **38**, 687 (1966)
- 20) A. Sentenac, A. Ruet & P. Fromageot: *Bull. Soc. Chim. Biol.* **49**, 247 (1967)
- 21) M. Enomoto & I. Ueno: "Mycotoxins," ed. by I.F.H. Purchasw, Elsevier Amsterdam, p. 303, 1974
- 22) Y. Ueno, I. Ueno, N. Sato, M. Itoi, M. Enomoto & H. Tsunoda: *Japan. J. Exptl. Med.* **38**, 47 (1968)
- 23) H. Tsunoda: *J. Food Hyg. Soc. Japan* **2**, 33 (1961)
- 24) S.C. Silverstein, C. Astell, J. Christman, H. Klett & G. Acs: *J. Virology* **13**, 740 (1974)