イネ萎縮病に対する化学療法剤のスクリーニングII

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Screening of Chemotherapeutants for Rice Dwarf Virus
II. Inhibition of Virion-Associated RNA Transcriptase

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中田昌伸*・鈴木直治*: イネ萎縮病に対する化学療法剤のスクリーニング
II. ウイルス粒子内RNA転写酵素の阻害

Summary

For the purpose of screening some antiviral agents which do not affect the function of cell DNA but inhibit the viral RNA synthesis, the *in vitro* systems for assaying RDV-RNA transcriptase and *E. coli* DNA-dependent RNA polymerase were employed and several dyes and drugs were tested for their inhibitory effects on both enzyme activities in darkness and under illumination. Acridine orange, ethidium bromide, methylene blue, azure B, azure A, azure C and thionine inhibit both *E. coli* DNA transcription and RDV-RNA transcription at P/D ratios (molecular ratios of P in RDV-RNA to dye) of 10/2 and 10/4 to almost the same extents, 40 to 70%, whereas chloroquine, quinacrine, luteoskyrin, actinomycin D and rifamycin SV inhibit only *E. coli* DNA transcription but do not RDV-RNA transcription. Since some of the dyes belonging to the first group have been known to catalyze the photodynamic reaction of DNA and RNA, azure B was tested for its catalytic effect on the same reaction of RDV-RNA. Actually, azure B inhibits RDV-RNA transcription by about 50% at a P/D ratio as low as 10/0.06 when the reaction mixture is illuminated at 20,000 lux. When RDV-RNA is illuminated in the presence of azure B, only guanine is lost at P/D ratios above 10/0.12. When RDV virions are pretreated with azure B under illumination and then added to the reaction mixture, 50% inhibition of RDV-RNA transcription occurs after 30 min. illumination at P/D ratio of 10/0.015. When ^{32}P-labeled RDV virions are illuminated in the presence of azure B and RNA is extracted by phenol method, loss of guanine in the extracted RNA can not be detected but the yields of RNA are decreased, conversely ^{32}P bound to protein is increased with the increasing dose of azure B. From these results it is concluded that the inhibition caused by azure B in darkness is due to the intercalation of dye into the double-stranded RNA, but the inhibition occurring under illumination is due to the photodynamic reaction catalyzed by the dye and is much more effective than that occurring in darkness. Since plants are exposed to sunlight during the daytime, the latter mechanism may predominate in the azure B-treated RDV-infected rice plants.

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I. Introduction

Antiviral agents should not inhibit the function of host cell DNA but inhibit only the function of viral RNA. So far as plant viruses are concerned, all viruses are RNA viruses with only the exception of cauliflower virus which has been reported to be a DNA virus[28], consequently, if we could select such agents which inhibit the replication of viral RNA or the transcription of double-stranded viral RNA, but do not inhibit the syntheses of cell nucleic acids, such agents may possibly be applicable as antiviral agents.

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Rice dwarf virus (RDV) particles contain double-stranded (ds-) RNA[23] and also RNA polymerase which transcribes ds-RNA into single-stranded (ss-) RNA[16]. ds-RNA can be separated quantitatively from host cell nucleic acids of RDV-infected rice leaves by using methylated albumin-kieselguhr[14] or cellulose column chromatography[7], and this fact enabled us to develop a method for screening such agents which inhibit ^{32}P-incorporation into ds-RDV-RNA without inhibiting ^{32}P-incorporation into ribosomal RNA[15]. Results, however, were unsatisfactory because cycloheximide, though inhibited ^{32}P-incorporation into viral RNA, inhibited the incorporation into ribosomal RNA to almost the equal extent suggesting that the antibiotic might be phytotoxic and this was true when it was administered to rice plants through culture solution at 1 and 2 ppm[37]. Blasticidin S at 3 μg and kasugamycin at 160 μg/g fresh weight of leaves were not enough to inhibit ^{32}P-incorporation into viral RNA.

RDV particles contain RNA-transcriptase just as all other ds-RNA viruses[4,20,27,32], and the method for assay of the enzyme activity *in vitro* has been reported[16]. By using this method, it is possible to screen some agents which inhibit viral RNA-transcriptase, however, the agents should be checked not to be inhibitory on DNA-dependent RNA synthesis before being selected as antiviral agents.

In the present study, several agents, which had been reported to be antiviral or to interact with ss-/ds RNA's and DNA, were chosen and their inhibitory effects on RDV virion-associated RNA-transcriptase and also on *E. coli* DNA-dependent RNA polymerase *in vitro* were examined. Among the dyes which were found to be inhibitory to both enzymes, azure B was selected for its growth-stimulating effect on infected plants and catalytic effect on the photodynamic inactivation of RDV-RNA was examined.

II. Materials and methods

Host plant: Rice seedlings, cultivar. Norin-8, were inoculated at the 3-leaf stage with viruliferous leafhoppers, *Nephotettix cincticeps* Uhl., which had been reared on RDV-infected rice plants.

Preparation of RDV: The purified RDV was prepared by the method as described in the previous paper[16].

Preparation of RDV-RNA: double-stranded RDV-RNA was prepared from the purified RDV preparation by the cold-phenol method described by Miura *et al.*[23] The RDV-RNA was usally stored in 67% ethanol at −20 C until immediately before use.

Preparation of *E. coli* DNA-dependent RNA polymerase: The enzyme was prepared from *E. coli* (kindly provided by Dr. G. Danno, Laboratory of Food and Nutritional Chemistry, Kobe University) by the method of Kameyama *et al.*[13] except that the cells were disrupted with glass beads and the amount of 2% protamine sulfate to be added to precipitate the enzyme was determined by the titration method proposed by Ishihama[9]. The DEAE-cellulose eluate of 35–55% saturated ammonium sulfate precipitate was used as enzyme solution. *E. coli* DNA was prepared by Marmur's method[43].

Chemicals: Tetrasodium uridine-5-^{3}H-5′-triphosphate (^{3}H-UTP, 22 mCi/mmole) was obtained from New England Nuclear, U.S.A., and ammonium salt of uridine-4-^{14}C-5′-triphosphate (^{14}C-UTP, 46 mCi/mmole) from The Radiochemical Center, Amersham, England. ATP, GTP, CTP, UTP, phosphoneol pyruvate, pyruvate kinase and α-chymotrypsine were products of C. F. Boeringer and Soehne GmbH, Germany. Actinomycin D

was a gift from Merck, Sharp and Rohme Research Laboratory, Rathway, N. J., U.S.A., ethidium bromide from Boots Pure Drug Co., Nottingham, England, rifamycin SV from Hokko Chem. Co., and chloroquine from Takeda Chem. Co. Luteoskyrin was kindly furnished from Dr. H. Kurata, National Hygenic Institute. Azure A and azure B were purchased from Tokyo Kasei Co., azure C from Chroma Geselschaft, Schmidt & Co., Germany, methylene blue from Wako Pure Chem. Industry, thionine and acridine orange from Merck, Germany, and quinacrine hydrochloride from Sigma Chem. Co., U.S.A.

Assay of RDV virion-associated RNA transcriptase activity *in vitro*: RDV virion-associated RNA transcriptase activity was assayed by the method as decribed in the previous paper[16]. 0.7 OD_{260} unit, or approximately 200 μg, of purified RDV contains 2.33 μg (0.752 μmole) of phosphorus. This amount of RDV was added to each 0.25 ml of incubation mixture. Each dye or drug was added to the incubation mixture at P/D ratio (molecular ratio of P in RDV-RNA to dye) specified in each table on the basis of P-content of RDV added, excluding the P-content of XTP in the incubation mixture.

Assay of *E. coli* DNA-dependent RNA polymerase activity: The standard reaction mixture for assay of *E. coli* DNA-dependent RNA polymerase activity *in vitro* contained in a final volume of 0.25 ml: 30 μmoles Tris-HCl, pH 7.8, 1.25 μmoles magnesium acetate, 0.5 μmoles manganese sulfate, 1.25 μmoles β-mercaptoethanol, 0.1 μmole each of ATP, GTP, CTP, and UTP which was ^{14}C-labeled at a final specific activity of 1 μCi/μmole, 24 μg DNA and 5.7 unit of RNA polymerase. After incubation at 37 C for 60 min., the reaction was terminated by adding 3 ml of ice-cold 5% trichloroacetic acid (TCA), and the mixture was kept in ice cold water for 15 min. The precipitate was collected on a glass fiber filter (Toyo-roshi No. GB-100) and washed with 20 ml of 5% TCA, followed by 5 ml of 95% cold ethanol. The filters were placed in scintilation vials, dried and the radioactivity was counted in a toluene-based scintilation fluid (0.4% POP and 0.01% POPOP in toluene) using a Packard liquid scintilation spectrometer.

Dye-catalyzed photoinactivation of RDV-RNA transcription: The standard reaction mixtures for assay of RNA synthesis containing azure B or methylene blue at definite P/D ratios were placed in small test tubes and irradiated at 20,000 lux with a 150 W Sun Light Lamp (Toshiba). To remove the light below 380 nm wave length a Bonset Film (IC Kasei Co.) was interposed between the lamp and the test tubes. The mixtures were incubated at 35 C for 60 min.

Analysis of base composition of azure B-treated RDV-RNA: 0.25 ml of 0.1 M Tris-HCl, pH 8.5, containing 200 μg of RDV-RNA and azure B at different P/D ratios were incubated at 35 C for 60 min. under light and dark conditions. RNA was precipitated with 2 volumes of ethanol. The precipitate was hydrolyzed with 0.3 N KOH at 37 C for 18 hr. The composition of resulting mixture of nucleotides was determined by two-dimensional paper chromatographic method as described by Miura[23].

Recovery, by phenol method, of RNA from azure B-treated RDV-virion and determination of RNA bound to protein: 1 mg of ^{32}P-labeled RDV-virions were suspended in 1.25 ml of 0.1 M Tris-HCl, pH 8.5, and treated with azure B under light and dark conditions at 35 C for 60 min. The sample was added to an equal volume of 80% phenol and the mixture was shaken vigorously for 20 min. Centrifugation at 3,000 rpm for 10 min. separated three layers. The top water layer was removed with a pipette.

One ml of 1 M KCl solution was added to the remaining protein and phenol layers and the tube was centrifuged at 3,000 rpm for 10 min. The resulting top layer was removed with a pipette and combined with the first water layer. The water layer was washed with ethyl ether to remove phenol, filled up to 2 ml and 1 ml aliquot was taken in a planchet to measure the radioactivity using a gas flow counter. To the denatured protein and phenol layer were added equal volumes of 1 M KCl and ethanol. The denatured protein was collected on a glass fiber filter, washed with 1 M KCl, and the radioactivity of ^{32}P bound to the protein was counted.

III. Results

A. *Comparison of inhibitory effects of several dyes and drugs on RDV virion-associated RNA transcriptase and on E. coli DNA-dependent RNA polymerase.*

Several dyes and drugs were tested for their inhibitory effects on RDV virion-associated RNA transcriptase and *E. coli* DNA-dependent RNA polymerase by adding each at P/D ratios 10/4 and 10/2 to the standard reaction mixtures for assay of both enzymes as described in Materials and Methods. Results are shown Table 1.

Results indicate that acridine orange, ethidium bromide, methylene blue, azure B, azure A, azure C, and thionine inhibit both enzymes at P/D ratios 10/2 and 10/4 almost equally, whereas chloroquine, quinacrine, and actinomycin D inhibit only *E. coli* DNA-dependent RNA polymerase but do not inhibit RDV-RNA transcriptase, and luteoskyrin and rifamycin SV inhibit markedly *E. coli* DNA-dependent RNA polymerase but only slightly RDV-RNA transcriptase.

B. *Azure B-catalyzed photodynamic inactivation of RDV-RNA transcription.*

Dye-catalyzed photo-oxidation of nucleic acids, including DNA and viral RNA, has been reported by many workers[10,25,29~31,42]. In our preliminary test on the therapeutic effect of the dyes listed in Table 1, only azure B stimulated leaf elongation of RDV-

Table 1. Inhibitory effects of several dyes and drugs on RDV virion-associated RNA transcriptase and *E. coli* DNA-dependent RNA polymerase

	% Inhibition at P/D ratios			
	10 : 2		10 : 4	
	RDV ds-RNA→RNA	*E. coli* DNA→RNA	RDV ds-RNA→RNA	*E. coli* DNA→RNA
Acridine orange	64	31	87	67
Ethidium bromide	33	49	68	72
Methylene blue	62	40	64	44
Azure B	47	42	68	54
Azure A	57	54	61	58
Azure C	43	36	44	50
Thionine	43	38	44	40
Chloroquine	0	31	2	52
Quinacrine	0	44	0	57
Luteoskyrin	0	54	24	85
Actinomycin D	0	100	0	100
Rifamycin SV	14	100	27	100

infected rice seedlings when the seedlings were grown in a nutrient solution containing the dye at 3 to 6 ppm[36]. The tests were performed in a greenhouse under sunlight. It seemed likely that azure B would catalyze the photo-oxidation of RDV-RNA just as methylene blue does.

The reaction mixtures containing 200 μg of RDV and azure B at varying P/D ratios were incubated at 30 C for for 60 min. under 20,000 lux illumination with Toshiba Sun light Lamp and inhibitory effects on RDV-RNA transcription were examined by measuring the radioactivity of resulting TCA-insoluble polynucleotide. Results are shown in Fig. 1.

As clearly shown in the figure, azure B caused 50% inhibition of the transcription at a P/D ratio of 10/0.06 when illuminated at 20,000 lux, the ratio being as low as 1/32 that causing 50% inhibition in darkness (see Table 1).

The inhibition proceeds exponentially with the increasing P/D ratio according to one hit inactivation model as decribed with the action of thiopyronine, proflavine[31], and acridine orange[10,25] on TMV-RNA.

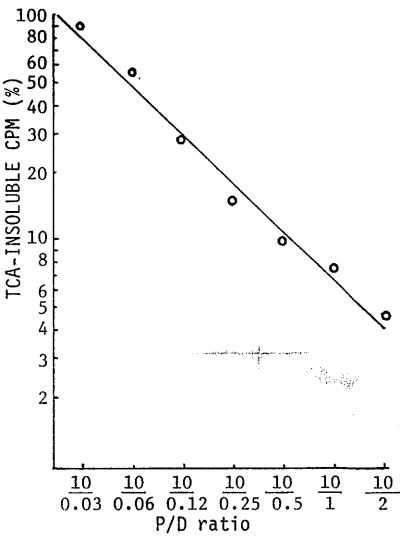

Fig. 1. Azure B-catalyzed photodynamic inactivation of RDV-RNA transcription.
Irradiation for 60 minutes at 20,000 lux. Light was filtered through a Bonse Film to remove the light of wave lengths below 380 nm.

C. *Decrease of guanine-content in RDV-RNA after treatment with azure B under light and dark conditions.*

It is presumable that the inhibition of RDV-RNA transcription by azure B under illumination might be caused by the photodynamic action of azure B not on the transcriptase itself but on RDV-RNA as already reported by Simon *et al.*[29,30] and Singer *et al.*[31] Different amounts of azure B were added to each 200 μg of RDV-RNA dissolved in 2.5 ml of 0.1 M Tris-HCl, pH 8.5, and the mixtures were incubated at 35 C for 60 min. under light and dark conditions, or at 0 C under the light condition and base compositions were determined. Results are shown in Table 2.

Results indicate that at 0 C guanine-content decreases almost negligibly at P/D ratios 10/0.5 to 10/2.0 and very slightly at 10/8.0 even under illumination, whereas at 35 C G-content decreases markedly in the light as the amount of dye increases and also in darkness though to less extents.

It is a well-known fact that in the presence of methylene blue or thiopyronine and light, guanine in nucleic acids is specifically decomposed[29~31], and this is also the case with azure B vs RDV-RNA under illumination.

As shown in Fig. 1, the inhibition of RDV-RNA transcription occurs even at P/D ratio of 10/0.06, however, no significant decrease of G-content can be seen by the chemical analysis of azure B-treated RDV-RNA under illumination. This fact suggests that the inhibition may occur even by a slight change in RDV-RNA which can not be detected by chemical analysis.

Table 2. Decrease of G-content in RDV-RNA after treatment with azure B under light and dark conditions

[pH 8.5, 35 C, 60 min.]

		P/D ratio	G	A	C	U
Light	Control (Base composition)	10/0	100 (21.5)	100 (27.5)	100 (21.0)	100 (30.0)
	Azure B	10/0.12	96	106	100	99
		10/0.5	88	100	93	100
		10/2.0	51	100	99	96
		10/8.0	17	99	96	96
Dark	Azure B	10/0.12	—	—	—	—
		10/0.5	99	103	98	97
		10/2.0	89	99	98	96
		10/8.0	23	99	96	96
[pH 8.5, 0 C, 60 min.]						
Light	Control	10/0.0	100 (21.4)	100 (28.0)	100 (22.0)	100 (28.5)
	Azure B	10/0.5	95	97	94	94
		10/2.0	96	106	96	105
		10/8.0	88	98	104	102

Table 3. Inhibitory effects of pretreatment of RDV virions with Azure B under illumination on RDV virion associated RNA transcriptase

[20,000 lux, 35 C, 30 min.]

	P/D ratio					
	10/0.0017	10/0.0033	10/0.007	10/0.015	10/0.03	10/0.06
% Inhibition	0	6.4	11.1	46.7	70.3	95.2

Table 4. Inhibitory effects of pretreatment of RDV virion with Azure B under illumination at 20,000 and in darkness on RDV-RNA transcriptase

Pretreatment in minutes	% Inhibition		
	under illumination (35 C) at P/D ratio		in darkness (35 C) at P/D ratio
	10 : 0.06	10 : 0.12	10 : 2
0	0	0	53
10	62.5	95	93
20	88.7	—	96
30	97.4	100	98
40	99.3	—	100
50	100	—	100
60	100	—	100

D. *Inhibition of RDV-RNA transcription by pretreatment of virons with azure B under illumination*

In the experiment shown in Table 1, P/D ratios were expressed only on the basis of P-content of RDV and the P-contents of XTP in the reaction mixture were not taken into account. In the present experiment, RDV virions suspended in Tris buffer were treated with azure B at varying P/D ratios for 30 minutes (Table 3) or for different periods from 10 to 60 minutes (Table 4) under illumination and then added to the reaction mixture, consequently the confusion in P/D ratios due to XTP in the reaction mixture will be avoided.

95% inhibition can be seen at P/D ratio of 10/0.06, at which less than 50% inhibition could be seen when RDV and the dye were directly added to the reaction mixture (See Fig. 1).

Table 4 shows that maximum inhibition can be seen after 30 minutes illumination at 20,000 lux and more than 50% inhibition after 10 minutes at P/D ratio of 10/0.06. When the amount of dye is much larger, or the P/D ratio is 10/2, the inhibition occurs by the pretreatment with azure B even in darkness. This may be explained by the fact that guanine-content is decreased by the treatment in darkness when the temperature is 35 C (Table 2).

E. *Recovery of RNA from RDV virions after treatment with azure B and light.*

When bacterial cells are hit by ultra-violet light (UV), yields of DNA from the cells decrease with increasing doses of UV irradiated to the cells[33,34], possibly due to the occurrence of protein-DNA cross-linkage.

Table 5. Decrease in recoveries of RNA, by phenol method, from RDV virions* after treatment with Azure B under light and dark conditions [35 C, 60 min.]

P/D ratio	Recovery of RNA				^{32}P-CPM bound to protein	
	Light		Dark			
	CPM	%	CPM	%	Light	Dark
10/8	1221	77.3	1504	95.2	1158	209
10/4	1223	77.4	1517	96.0	1065	198
10/2	1214	76.8	1532	97.0	1106	157
10/1	1326	78.2	1550	98.1	971	137
10/0.5	1245	78.8	1552	98.2	720	94
10/0.25	1247	78.9	1585	100.3	697	107
10/0.12	1328	84.1	1554	98.4	472	85
10/0.06	1388	87.9	1560	98.7	297	87
10/0.03	1395	88.3	1604	101.5	259	93
Control**			1580	100		88
10/8, after Pronase E treatment	1047					

* 2 mCi of ^{32}P was administered to detached RDV-infected rice leaves (2 g) and after 24 hr ^{32}P-labeled RDV was extracted. Labeled RDV was diluted with cold carrier RDV to final specific activity of 4112 cpm/mg.

** RDV was incubated at 4 C for 60 min. in darkness.

Parallel to the experiment shown in Table 2, in which RDV-RNA was treated with azure B under illumination, RDV virions were treated in the same way, however no decrease of guanine-content was found but only the decrease in the yield of RDV-RNA from treated virions when extracted by phenol method.

In order to examine whether or not the decrease of RNA yield is due to the occurrence of RNA-protein linkage, ^{32}P-labeled RDV virions were treated with azure B under illumination and the radioactivities of RDV-RNA and protein fractions resulted from phenol method were determined. Results are shown in Table 5.

Results indicate that the yield of RNA from treated virions decreases with increasing doses of azure B, conversely the amount of RNA bound to protein increases by the treatment.

IV. Discussion

The purpose of this study is to screen such agents which specifically inhibit the viral RNA transcription but do not the DNA transcription, by using the *in vitro* systems for RDV-RNA transcription and *E. coli* DNA transcription. Results indicate that the dyes and drugs tested are divided into two groups, one inhibits both RDV-RNA and *E. coli* DNA transcriptions to almost the same extent, and the other inhibits only DNA transcription without inhibiting RDV-RNA transcription. The first group involves acridine orange, ethidium bromide, methylene blue, azurer B, azure A, azure C, and thionine and the second group chloroquine, quinacrine, luteoskyrin, actinomycin D and rifamycin SV. The second group of agents seems to be of no use as antiviral agents.

Above experiments were conducted in darkness. Since the intercalation model for the binding of diaminoacridine to DNA was first proposed by Lerman[21] in 1963, many drugs and dyes, including proflavine, acridine orange, ethidium bromide, chloroquine, quinacrine, actinomycin D, etc., have been found to intercalate between adjacent base-pairs of DNA and affect its function[5,6,11,17,22,24,35], i. e., some of them are mutagenic and others inhibit replication or transcription of DNA.

Double-stranded RNA possesses a double-helical structure similar to that of DNA with slight differences in the length of a pitch, number of base pairs per pitch, tilting of base pairs against RNA-axis, and orientation of ribose against base-pairs[1~3,8,18,26,38]. This suggests that the above described dyes may intercalate between adjacent base-pairs of ds-RNA. Our unpublished data[12] revealed that acridine orange, ethidium bromide and azure B form complexes with *E. coli* DNA just as already described by Waring[39~41], and LePecq *et al.*[19], the P/D ratio at the maximum binding is 10 : 2; very similarly these dyes form complexes with RDV-RNA which are stable in 1.0 M NaCl, cause increase in flurescence intensities (ethidium bromide and acridine orange), shift of absorption spectra, hypo- (ethidium bromide and acridine orange) and hyperchromicities (azure B); and P/D ratio at maximal binding is 10 : 1 as against 10 : 2 in DNA. These results suggest that the mechanism of inhibition in the experiment conducted in darkness lies in the intercalation of dyes between base-pairs of DNA and ds-RNA.

On the other hand, plants are always exposed to sunlight during the daytime and the dyes such as methylene blue[29,30], thiopyronine[31], and acridine orange[10,25] are known to catalyze the photodynamic reaction of nucleic acids. It is presumable that azure B,

in which only one of the four CH_3 of methylene blue is replaced by H, will catalyze the photodynamic reaction, and this is true because 50% inhibition of RDV-RNA transcription is attained at the P/D ratio of 10/0.06 under illumination as against 10/2 in darkness. The P/D ratios in above experiments were expressed only on the basis of P-content of RDV-RNA but the P-content of XTP in the reaction mixture was neglected. To avoid this confusion, RDV virions suspended in Tris-HCl were pre-treated with azure B under illumination and then added to the reaction mixture. Results revealed that 30 minutes illumination at a P/D ratio as low as 10/0.015 or 10 minutes illumination at a P/D ratio of 10/0.06 was enough to attain 50% inhibition. All these data clearly indicate that azure B catalyzes the photodynamic reaction of RDV-RNA just as methylene blue does with DNA[29].

When RDV-RNA is illuminated in the presence of azure B, only guanine is lost, however the loss of guanine is chemically detectable by treatment with azure B at P/D ratios above 10/0.12. When virions are illuminated in the presence of azure B, the loss of guanine cannot be detected but the total yield of RNA from treated virions decreases after extraction by phenol method. When ^{32}P-labeled RDV virions were illuminated in the presence of azure B, recovery of RNA from treated virions decreased with increasing doses of azure B, conversely, radioactivity in protein is increased. 10% loss in the yield of RNA can be seen even at a P/D ratio of 10/0.03.

From these results it is concluded that the inhibition of RDV-RNA transcription by azure B is mainly due to the photodynamic reaction catalyzed by the dye and that a very small change occurring in RDV-RNA, which cannot be detected by chemical analysis but is detectable by the formation of RNA-protein complex, causes the inhibition

This study was focused only on the effects of azure B on RDV-RNA transcription solely because the dye stimulated leaf elongation of rice seedlings when administered through culture solution[36]. However, it is highly possible that the dye affects the function of DNA when tested on the *in vitro* DNA transcription system. The only way to escape from the inhibitory effect of the dye on DNA function is its higher *in vivo* affinity to inclusion bodies than to nuclei in the living rice plants.

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和文摘要

イネ萎縮病に対する化学療法剤のスクリーニング
II. ウィルス粒子内 RNA 転写酵素の阻害

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イネ萎縮病ウイルス粒子内に含まれる RNA 転写酵素および大腸菌 DNA 依存 RNA ポリメラーゼの活用を測定するための *in vitro* の系を用い，数種色素および薬剤の両酵素活性に対する阻害程度を比較検討した。アクリジンオレンジ，エチジュムブロマイド，メチレン青，アズール B，アズール A，アズール C，チオニンは P/D 比 10/2～10/4 で両酵素ともほぼ同程度に 30～70% 阻害した。クロロキン，キナクリン，アクチノマノマイシン D，ルテオスカイリン，リファマイシン SV は大腸菌 DNA の転写を阻害し，RDV-RNA の転写は全く～ほとんど阻害しなかった。メチレン青などについては光化学反応を触媒することが知られているのでアズール B について同じ効果を検討した結果，20,000 ルックス照明下では P/D 比 10/0.06 で RDV-RNA 転写の 50% 阻害がおきた。反応液に含まれる XTP が P/D 比 (RNA の含む P と色素との分子比) の効果の解釈に混乱をおこすことをまぬがれるため，トリス-塩酸中に RDV と色素とを加えて照明し，のちに反応液に入れて酵素活性を測定したところ，P/D 比 10/0.015，30 分照明で 50% 阻害がみられた。RDV-RNA に色素を加えて照明するとグアニンだけが減少する。^{32}P ラベル RDV 粒子に色素を加えて照明すると，フェノール法抽出による RNA の収量が減り，その減少分はタンパク分画に結合される。以上によりアズール B は RDV-RNA の転写を，暗黒下においては塩基対間挿入により，照明下においては光化学反応を触媒することにより阻害し，後者の方がはるかに効果的である。罹病イネをアズール B で処理した場合，日中太陽光線下においては光化学反応による阻害が塩基対間挿入による阻害よりも優勢であると判断される。