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On the inhibition of plant virus multiplication by ribavirin

Burkhard Lerch

Institut für Biochemie, Biologische Bundesanstalt für Land- und Forstwirtschaft, Messeweg 11, Braunschweig, F.R.G.

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Summary

The inhibition of the replication of potato virus X (PVX), belladonna mottle virus, tobacco mosaic virus, potato virus Y (PVY), and tobacco necrosis virus by ribavirin and pyrazofurin is described with emphasis on the inhibition of PVX by ribavirin. Ribavirin inhibits an early step of PVX replication. The inhibition is reversed to different degrees by all ribo- and deoxyribonucleosides, most strongly by thymidine. In tobacco leaves, nucleosides compete with ribavirin for phosphorylation to monophosphate by a nucleoside phosphotransferase. However, the final and main phosphorylation product of ribavirin is triphosphate. It is suggested that ribavirin triphosphate is the antiviral form and that it acts by inhibiting the capping of viral RNAs. This mode of action cannot be applied to the inhibition of PVY, the RNA of which is probably covalently linked to a protein at the 5′-terminus.

Plant virus; Ribavirin; Pyrazofurin; Reversal of inhibition; Nucleoside phosphotransferase; Mode of action

Introduction

Since its synthesis about fifteen years ago [38], ribavirin (1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide) has proved to be one of the most prominent antiviral substances. It inhibits the replication of a great number of animal RNA- and DNA-viruses [18], and since the first report on its action against plant (RNA) viruses [34] many more reports have appeared [5,7,9,15,16,19,23,25,31,32,34,35,39]

Correspondence to: B. Lerch, Institut für Biochemie, Biologische Bundesanstalt für Land- und Forstwirtschaft, Messeweg 11, D-3300 Braunschweig, F.R.G.

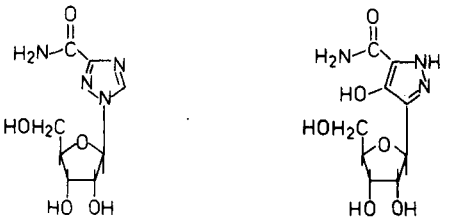

Fig. 1. Structural formulas of ribavirin (left) and pyrazofurin (right).

and are already too numerous to be cited here completely. The application of ribavirin to eliminate viruses from in vitro plants has recently been reviewed [22]. Further references can be found in this review.

There are many studies on the metabolism of ribavirin and the mode of its antiviral action in animal cells [40,43] which, however, has not yet been established unequivocally. In fact, it may have more than one mode of action. Less work has been done on the mechanism of its action against plant viruses [3,7], and to my knowledge there is no report on its metabolic fate in plants.

In the present work, the inhibition of the replication of potato virus X (PVX), belladonna mottle virus (BdMV), tobacco mosaic virus (TMV), potato virus Y (PVY) and tobacco necrosis virus (TNV) by ribavirin has been studied and compared to the inhibition by the structurally related nucleoside pyrazofurin (3-β-D-ribofuranosyl-4-hydroxypyrazole-5-carboxamide (Fig. 1). Each of these five viruses belongs to a different virus group. The goal of the research here reported was to establish a connection between the 5′-terminal structure of the RNAs of these five viruses and their response to ribavirin.

During the course of this work unexpected reversal of virus inhibition by nucleosides led to experiments on the course of the phosphorylation of ribavirin in tobacco leaves.

Materials and Methods

Substances

Ribavirin (M_r 244,21) was bought from ICN Pharmaceuticals Inc., Cleveland, Ohio. Pyrazofurin (M_r 259,22) was kindly supplied by Dr. R.L. Hamill, Lilly Research Laboratories, Indianapolis, Indiana. Carbenicillin was from Sigma Chemical Co., St. Louis, Missouri.

[^{3}H]Ribavirin (12 Ci/mmol) was from ICN Pharmaceuticals Inc., Chemical & Radioisotope Division, Irvine, California. [*methyl*-^{3}H]Thymidine (45 Ci/mmol), [6-^{3}H]uridine (15 Ci/mmol), and [8-^{3}H]guanosine (5 Ci/mmol) were from Amersham-Buchler, Braunschweig. Radioactivity was counted in the scintillation cocktail PICO-FLUOR 15 from Packard. Unlabeled nucleosides were from Pharma-Waldhof GmbH, Düsseldorf. DEAE-Sephadex A-25 was from Pharmacia, cellulose powder MN 300 UV 254 from Macherey and Nagel, Düren. Antisera (rabbit)

against PVX, BdMV, and PVY were a gift from Dr. R. Koenig, antiserum against TMV from Dr. H.L. Paul, Braunschweig. The γ-globulins were conjugated with alkaline phosphatase from Boehringer Mannheim. The other substances used were of analytical grade.

Plants

Nicotiana tabacum L. cv. 'Samsun' was grown in a greenhouse at 20–22°C. Daylight was supplemented with light from red fluorescent tubes (16 h day, 3000 lux). About 7 wk after seeding, when the plants were in the eight-leaf stage, the top of the plant and all leaves except for the 5th and the 6th ones from the bottom were cut off with scissors. After 4–5 days, those two remaining leaves had grown to an abnormal size and were used for inoculations.

Viruses

Unspecified strains of PVX, TMV, and TNV were used. PVY was strain GO 16, belonging to the Y° group [8]. BdMV was the strain Jankulowa [30]. All viruses were maintained in *N. tabacum* L. cv. 'Samsun'.

PVX was isolated and purified by a procedure using $AgNO_3$ and polyethylene glycol 6000 as described for helenium virus S [21]. The purified virus preparation had an A_{260}/A_{280} of 1.18 (not corrected for light scattering). A concentration of 1 mg PVX/ml was assumed for $A_{260} = 2.97$ [29].

Infection and virus propagation

Leaves were dusted with carborundum powder and inoculated with the supernatant of an aqueous homogenate of infected leaves (1/20, w/v). 2–3 h after inoculation (unless indicated otherwise), disks of 10 mm diameter were punched out with a cork borer, avoiding larger veins, and were randomized and vacuum-infiltrated (oil pump, 2 min) with either a solution of carbenicillin (100 μg/ml) in water (controls) or with a solution containing in addition ribavirin or pyrazofurin (10^{-5} M). In reversal experiments nucleosides were added in a tenfold higher concentration (10^{-4} M) than the inhibitor. All solutions were adjusted to pH 6.5 with NaOH. Carbenicillin was added to suppress growth of microbes.

The disks were floated, upside down, in 9 cm diameter Petri dishes on 40 ml of the solutions and illuminated continuously by red fluorescent tubes (3000 lux) at 21–22°C. Samples of 50 disks were taken out at indicated times and stored at −30°C until the end of the experiment.

Assay of viruses

Virus concentrations were assayed by double antibody sandwich ELISA [6]. The samples of leaf disks were homogenized in a mortar and the homogenate taken up in 50 ml PBS-Tween 20. Six wells of a microtiter plate were filled with each solution and the mean of the readings at 405 nm was taken as the absorbance value for that sample.

The concentration of PVX could be determined in a range of about 1–5000 ng PVX/ml by using a standard curve prepared with purified PVX. The relative con-

centrations of BdMV, TMV, and PVY were calculated by means of dilution curves. The replication of TNV was estimated by counting the local lesions [20] on detached and infiltrated leaves of *N. tabacum* L. cv. 'Samsun'.

Thin-layer chromatography

A layer (0.25 mm) of cellulose was spread on glass plates (20 × 20 cm). Chromatograms were developed twice with 2-propanol/25%NH_3/H_20 (7/1/2) at 4°C. Before use, the plates were pre-run with this solvent.

Phosphorylation of ribavirin and nucleosides in extracts of tobacco leaves

To determine the pH optimum of the phosphorylation reaction, extracts of mature tobacco leaves with 0.1 M Soerensen's buffer (1/1, w/v) of various pH, containing 0.2% mercaptoethanol and 0.14 mg/ml bovine serum albumin, were filtered through rhovyl (polyvinylchloride) paper and centrifuged at 4°C for 10 min at 12000 × *g*. The supernatants were dialyzed for 2 h against the respective buffer and centrifuged again. To 50 µl of the supernatant were added 25 nmol dThd, 250 nmol AMP, and 1 µCi [^{3}H]dThd in 50 µl of buffer. After 30 min at 37°C the reaction was stopped by immersing the tube in a boiling water bath for 90 s. Ten µl of the solution was streaked on a thin-layer plate. After drying, 10 µl of 10^{-2} M unlabeled dTMP was applied on the same spot. The spot of dTMP, visible under UV light, was scratched off the developed plate, the cellulose suspended in 1.2 ml 0.1 M HCl, and, after 1 h standing with occassional shaking, centrifuged for 10 min at 18000 × *g*. 0.8 ml of the supernatant was mixed with 5.0 ml PICO-FLUOR 15 and counted in a Packard Tri-Carb 3380.

To determine the phosphorylation of nucleosides and ribavirin, reactions were performed in 0.05 M imidazole-HCl, pH 7.5, instead of Soerensen's buffer. The extract was prepared with and dialyzed over-night against the same buffer. The reactions were stopped by addition of 5 µl 4 M $HClO_4$ and the precipitated proteins centrifuged down. The concentrations of AMP and nucleosides were as stated in Table 3. Ten µl of a 10^{-2} M solution of the respective unlabeled nucleotides was applied as marker on the starting spot. Blind reactions were stopped with $HClO_4$ immediately before adding the radioactive nucleoside. The cpm of corresponding regions of blind reactions on the plate were subtracted as background. Other conditions were the same as above.

Phosphorylation of ribavirin in tobacco leaves

Two young detached tobacco leaves (length 11.5 cm) were allowed to take up through the petiole 0.2 ml of a solution containing (a) 4 nmol ribavirin + 5 µCi [^{3}H]ribavirin, and (b) 4 nmol ribavirin + 5 µCi [^{3}H]ribavirin + 40 nmol dThd, followed in both cases by 0.1 ml water. Taking up the liquid was accelerated by blowing a stream of cold air over the leaves and was completed after about 90 min. The leaves were then floated in Petri dishes on water and illuminated by red fluorescent tubes (3000 lux) for 90 min. The leaf laminas (1.0 g) were homogenized at 4°C with 2.0 ml 0.5 M $HClO_4$ in a mortar, the homogenate centrifuged for 5 min at 18000 × *g* and the 1.9 ml supernatant vortexed with 2.3 ml of 0.5 M trioctylam-

TABLE 1

Inhibition of PVX, BdMV, TMV, and PVY in disks of infected tobacco leaves, infiltrated 2 – 3 h after infection with and floated on solutions of the inhibitors.

Inhibitor (10^{-5} M)	% Inhibition of			
	PVX[a]	BdMV[a]	TMV[a]	PVY[b]
Ribavirin	100.0	100.0	23.5	68.2
Pyrazofurin	83.4	79.3	82.3	100.0

[a] 120 h after infection.
[b] 144 h after infection.

ine in trichlorofluoromethane. The aqueous phase (1.8 ml) was applied to a column (1 × 27 cm) of DEAE-Sephadex A-25, equilibrated with 0.05 M Tris-HCl, pH 8.2. The column was first washed with 36 ml of this buffer and then eluted with a linear gradient of 0.05–0.25 M NaCl (210 ml each) in this buffer at a flow rate of 36 ml/h. Fractions of 7.2 ml were collected, 0.8 ml of each fraction mixed with 5.0 ml PICO-FLUOR 15 and counted.

Results

Inhibition of PVX, BdMV, TMV, PVY, and TNV by ribavirin and by pyrazofurin

Under the experimental conditions, the replication curves (not shown) of PVX, BdMV, and TMV had a similar shape, apart from slight differences in the replication rate. The concentration of these viruses in the controls reached a plateau 72–96 h after infection. PVY multiplied more slowly; its concentration had not yet reached a plateau 144 h after infection.

The viruses responded quite differently to the inhibitors (Table 1). PVX and BdMV were inhibited completely by ribavirin; they did not attain a detectable concentration. TMV and PVY were inhibited partially. Only PVY was inhibited completely by pyrazofurin, but TMV was more strongly inhibited by pyrazofurin than by ribavirin.

Of 10 tobacco leaves infected with TNV, 394 lesions were counted 96 h after

TABLE 2

Inhibition of PVX in disks of infected tobacco leaves punched out and infiltrated at different times after infection and floated on 10^{-5} M ribavirin solution.

Time interval[a] (h)	% Inhibition 120 h after infection
2	100.0
12	98.5
24	78.4
36	17.5

[a] Time of infiltration after infection.

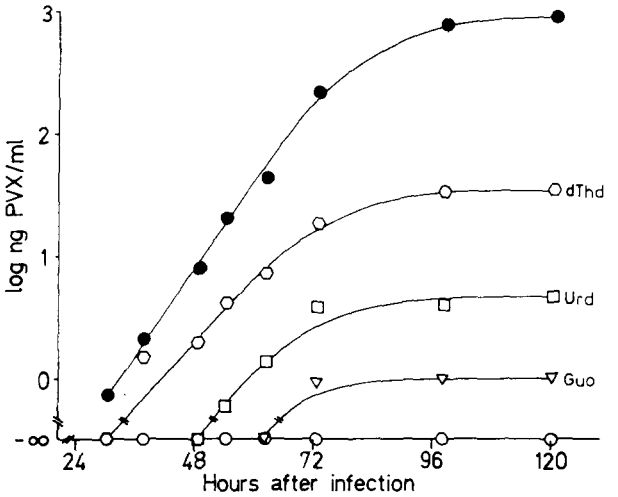

Fig. 2. Growth curves of PVX in leaf disks infiltrated with water (controls, •), 10^{-5} M ribavirin (○), 10^{-5} M ribavirin + 10^{-4} M dThd (○), 10^{-5} M ribavirin + 10^{-4} M Urd (□), or 10^{-5} M ribavirin + 10^{-4} M Guo (▽) 2–3 h after infection. In the series of ribavirin + dThd, + Urd, and + Guo only the last readings with zero extinction are drawn on the time axis to avoid excessive superimposition of the symbols.

infection on half-leaves infiltrated with water 2 h after infection and 413 lesions on half-leaves infiltrated with 10^{-5} M ribavirin. Thus, TNV is not inhibited by ribavirin.

The inhibition of PVX replication depended on the time interval between infection and the infiltration with ribavirin (Table 2). The degree of inhibition decreased with increasing time interval. If ribavirin was infiltrated as late as 36 h after infection, only little inhibition occurred.

Reversal of the inhibition of PVX replication by nucleosides

All dexoyribo- and ribonucleosides reversed the inhibition of PVX replication by ribavirin, though to different degreees. dThd gave the strongest reversal of all nucleosides, closely followed by dCyd. dGuo was the least reversing of the deoxyribonucleosides.

Urd was the most and Guo was the least reversing ribonucleoside. For simplicity, the reversal of PVX inhibition by nucleosides is shown with dThd, Urd, and Guo only (Fig. 2).

The inhibition of PVX replication by pyrazofurin was also reversed by dThd, and more strongly than by Urd (Fig. 3).

Phosphorylation of ribavirin and nucleosides in extracts of tobacco leaves

Extracts of tobacco leaves contain a nucleoside phosphotransferase with an optimum at pH 7.5 (Fig. 4). Although of all nucleotides dTMP proved to be the most efficient phosphate donor, AMP was used because in tobacco leaves it is the 5′-nucleotide present in the highest concentration [26]. The reactivity of dThd, Urd,

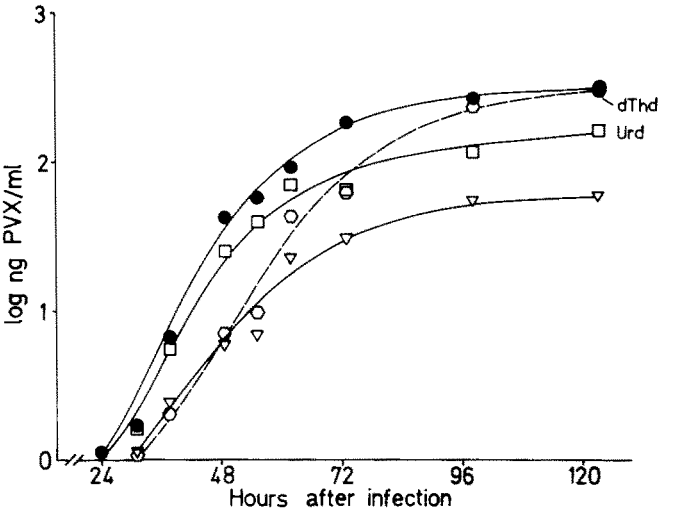

Fig. 3. Growth curves of PVX in leaf disks infiltrated with water (controls, •), 10^{-5} M pyrazofurin (▽), 10^{-5} M pyrazofurin + 10^{-4} M dThd (○), and 10^{-5} M pyrazofurin + 10^{-4} M Urd (□) 2–3 h after infection.

Guo, and ribavirin as acceptors of the phosphate group decreased in this order. As expected, the ability of dThd, Urd, and Guo to decrease the rate of phosphorylation of ribavirin also decreased in the same order (Table 3).

Phosphorylation of ribavirin in tobacco leaves

The phosphorylation of ribavirin in a whole detached tobacco leaf was delayed by dThd, as demonstrated by the increase from 85375 cpm in the peak of un-

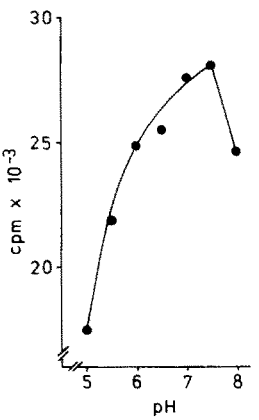

Fig. 4. pH profile of nucleoside phosphotransferase activity in a tobacco leaf extract. Enzyme activity is expressed as cpm of [^{3}H]dTMP.

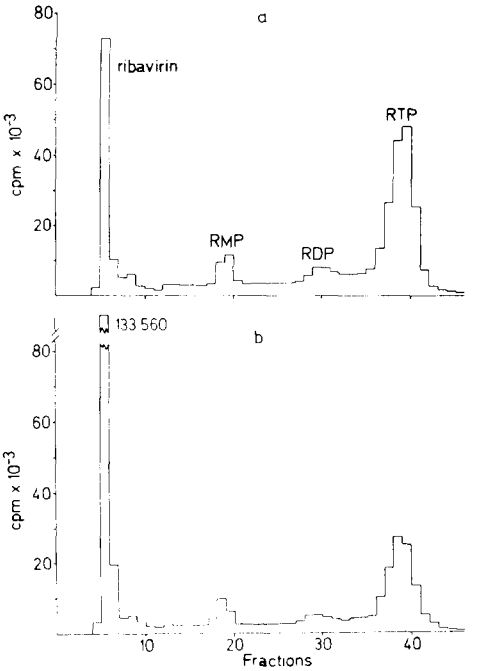

Fig. 5. Chromatography of [^{3}H]ribavirin and its phosphorylation products on DEAE Sephadex A-25 after absorption by a tobacco leaf. The leaves (1.0 g) had taken up (a) 4 nmol ribavirin + 5 μCi [^{3}H]ribavirin, (b) 4 nmol ribavirin + 5 μCi [^{3}H]ribavirin + 40 nmol dThd and were then incubated for 90 min under illumination (3000 lux). The extract of nucleosides and nucleotides was applied to a column (1 × 27 cm), equilibrated with 0.05 M Tris-HCl, pH 8.2. After washing with 36 ml the column was eluted with a linear gradient of 0.05–0.25 M NaCl (210 ml each) in this buffer at a flow rate of 36 ml/h. Of each fraction (7.2 ml) 0.8 ml was mixed with 5.0 ml PICO-FLUOR 15 and counted.

TABLE 3

Phosphorylation of nucleosides by nucleoside phosphotransferase[a].

A[b]		B[c]	
Nucleoside	cpm[d] of Nucleoside-MP	Nucleosides	cpm[d] of Ribavirin-MP
dThd	7489	Ribavirin + dThd	615
Urd	3734	Ribavirin + Urd	702
Guo	2869	Ribavirin + Guo	814
Ribavirin	843		

[a] In dialyzed tobacco leaf extract with 0.05 M imidazole-HCl, pH 7.5 (1/1, w/v), 30 min. 37°C.
[b] Reaction volume 100 μl, containing 50 μl extract, 250 nmol AMP, and 1 nmol ^{3}H-nucleoside (1 μCi) in buffer.
[c] As in [b], but 1 nmol [^{3}H]ribavirin (1 μCi) + 10 nmol nucleoside.
[d] In 0.8 ml of 1.2 ml extract of spot from thin-layer plate.

reacted ribavirin of the leaf with ribavirin only (Fig. 5a, fractions 4–6) to 155885 cpm in the corresponding fractions of the leaf that took up ribavirin + dThd (Fig. 5b). This indicates that in the leaf as well as in the extract the first and rate-limiting step is the phosphorylation to monophosphate by reaction with the phosphotransferase. In the leaf, however, the phosphorylation does not stop at the stage of the monophosphate, but goes on, probably by means of a nucleotide kinase and ATP, to the triphosphate, which is the main reaction product of ribavirin without and with added dThd.

The total yield of radioactivity counts of the leaf with ribavirin + dThd was only 91.5% of that of the leaf with ribavirin only. This should be taken into account in comparing the two graphs.

The amount of unphosphorylated ribavirin (fractions 4–6) in the leaf with ribavirin + dThd was about twice (183%) of that in the leaf with ribavirin only, whilst the peak of monophosphate (fractions 17–20) was 80% and that of the triphosphate (fractions 35–44) was 60%. Ribavirin diphosphate was present as a low peak only in both cases. Thus, the main differences noted were between the amounts of unreacted ribavirin and its triphosphate.

Discussion

From the numerous studies on the mode of action of ribavirin against animal viruses two main theories have emerged. There is general agreement that ribavirin acts in a phosphorylated form, but the question is whether as 5′-mono- or triphosphate. According to the first theory [43], monophosphate is the antiviral form. Ribavirin-5′-phosphate is a potent inhibitor of the enzyme inosine-5′-phosphate dehydrogenase, thus inhibiting the penultimate step in the biosynthesis of guanylic acid and depleting its pool needed for the synthesis of viral RNA or DNA. One important argument for this theory is that the inhibition of measles virus replication by ribavirin is reversed by guanosine or xanthosine (by inosine to a lesser degree) and not by other nucleosides.

However, this theory does not explain the specificity of the antiviral action of ribavirin, because all viruses need guanylic acid for the synthesis of their RNA or DNA.

The second theory assumes ribavirin triphosphate to be the antiviral form [13,40]. It has been shown that the capping of vaccinia virus, which codes for its own guanylyl transferase, is inhibited by ribavirin triphosphate and not by its monophosphate [13]. This mechanism can explain why viruses with an uncapped RNA are not inhibited.

In tobacco leaves, ribavirin is phosphorylated first to monophosphate by a nucleoside phosphotransferase (EC 2.7.1.77). Nucleoside phosphotransferases of plants are well known, e.g. of carrots or wheat shoots [2,12]. These enzymes have their optimums at about pH 5 or below. To my knowledge, a nucleoside phosphotransferase in a higher plant with an optimum at pH 7.5 has been described extensively only in the fern *Asplenium nidus* [14], but apparently it is more widely

distributed. Both enzymes in *A. nidus* and in tobacco have the same pH optimum. For both of them dThd is the best phosphoryl acceptor and dTMP the best donor, and the activity of both enzymes is higher in mature leaves than in young ones (not shown). The sequence in reactivity as a phosphate acceptor of dThd, Urd, and Guo towards the phosphotransferase in the leaf extract is the same as their sequence in efficacy to reverse the inhibition of PVX replication by ribavirin in leaf disks (Fig. 2). This indicates that in leaf disks, too, these nucleosides compete with ribavirin for the transfer of the phosphate group and do not act by a specific antagonism. Furthermore, Guo is the least reversing ribonucleoside. Thus it is unlikely that PVX is inhibited via a depletion of the guanylic acid pool.

dThd delays the phosphorylation of ribavirin also in a whole tobacco leaf (Fig. 5). As the efficacy of the inhibition of PVX replication by ribavirin depends on the time of its application after the infection (Table 2), such a delay of phosphorylation amounts to the same as a later application.

The 5′-phosphate of pyrazofurin is an inhibitor of orotidylate decarboxylase [10] and Urd protects from its cytotoxic action [28]. A similar mode of action in leaf disks would explain the initially stronger reversal of PVX inhibition by Urd. That the inhibition of PVX replication by pyrazofurin is also reversed by dThd, and finally more strongly than by Urd (Fig. 3), suggests that pyrazofurin is also phosphorylated by the phosphotransferase and its phosphorylation is delayed by nucleosides. Thus, in plants the phosphorylation by phosphotransferase may be a general way to channel exogenous nucleosides into the nucleotide metabolism.

The final phosphorylation product of ribavirin in tobacco leaves, however, is triphosphate (Fig. 5), which surpasses in amount the monophosphate even after a short incubation time and an incomplete phosphorylation of ribavirin. Thus, it is tempting to assume the triphosphate to be its antiviral form in plants. If the foregoing asumption were true, and if ribavirin acted by inhibiting the capping of viral RNAs, this would render intelligible why the antiviral action of ribavirin depends on the time of its application, for capping is an early event in the synthesis of mRNAs [33]. TMV is also inhibited by ribavirin at an early step of its replication [7]. But how does this assumption comply with the specificity of ribavirin against different plant viruses?

One of the five viruses investigated in this study, TNV, is not at all inhibited by ribavirin. The RNA of TNV is not capped, its 5′-terminal sequence is ppApGpUp...[24].

Two of the viruses, PVX and BdMV, are efficiently inhibited by ribavirin. The lowest concentration of ribavirin to inhibit the replication of BdMV completely under the experimental conditions is 10^{-5} M, for PVX it is 5×10^{-6} M (not shown). The RNA of PVX is known to be capped [42]. The RNA of BdMV is not known to be capped, but, as the RNA of the related turnip yellow mosaic virus (TYMV) is capped [1], this may be assumed. The replication of TYMV is also efficiently inhibited by ribavirin [31]. PVX was eliminated from infected tobacco protoplasts [35] or potato plants [32] with ribavirin and the replication of TYMV in chinese cabbage plants was completely suppressed by spraying with ribavirin [19].

On the other hand, TMV has a capped RNA and is only partially inhibited. The

cap structure of a mRNA is supposed to have two functions. It protects from digestion by nucleases and it facilitates the formation of the initiation complex with ribosomes. However, the susceptibility of different RNAs without a cap to attack by nucleases may vary, and certainly the importance of the cap for protein synthesis varies. A cap seems to be obligatory for the translation of reovirus RNA and vesicular stomatitis virus RNA in wheat germ extract [27], but, depending on their concentrations, the RNAs 3 and 4 of brome mosaic virus retain 8–50% and 12–40% of their translational activity in wheat germ extract after decapping [36]. The translation of alfalfa mosaic virus (AMV) RNA 4 is almost independent of a functional cap [41], and decapped TMV RNA also retains a considerable translational activity in wheat germ extract [45]. But, because the translational efficacy of capped and decapped mRNAs in wheat germ extract depends on their concentration and on the salt concentration, no direct comparison between their performance in the extract and in the cell is possible. However, a residual translational activity of uncapped RNA of TMV may account for its partial inhibition by ribavirin.

TMV could not be eliminated from tissue cultures of *N. tabacum* cv. 'Xanthi' with ribavirin (up to 8×10^{-3} M) as the sole antiviral compound [25]. The concentration of AMV was lowered in meristematic cultures of of *N. rustica* grown on a medium with 4×10^{-4} M ribavirin, but it could not be eliminated. Cucumber mosaic virus could be eliminated under similar conditions [39] and also from explants of *N. tabacum* cv. 'Xanthi' grown on medium with 2×10^{-4} M ribavirin for 22 wk [4].

PVY, the type member of the potyvirus group, is poorly inhibited by ribavirin. The 5′-terminus of the RNAs of two viruses of this group, tobacco etch virus [17] and tobacco vein mottling virus [37], is covalently linked to a protein (VPg). The VPg of tobacco etch virus is not needed for infectivity of the RNA. Perhaps all potyviruses have this characteristic in common. Ribavirin is reportedly not active against polio virus [18], which also has a VPg linked to its RNA. However, the function of the VPg of plant viruses is not known, nor how it is bound to the RNA, nor the linking process. Thus, the lack of a cap may explain why PVY is not inhibited efficiently, but at present no mechanism can be suggested for its partial inhibition.

PVY could be eliminated from explants of tobacco with 2×10^{-4} M ribavirin [4], and under similar condiitions the elimination of PVY from potato explants, together with that of PVX, potato virus S (PVS), and potato virus M has been claimed [5]. In a recent paper, the elimination of PVY and PVS shoot-tips of potatoes growing on liquid medium with ribavirin in concentrations ranging from 5–20 mg/l has been reported [44].

Consistent with the response of TNV, PVX, BdMV, TMV, and PVY, the assumption can be made that for a plant virus a capped RNA is necessary, but not a sufficient requirement for a good inhibition by ribavirin.

Hence, knowing their capping status, one may predict which plant viruses will be readily inhibited. Such considerations may be useful prior to an attempt to eliminate a plant virus by chemotherapy with ribavirin. However, it should be kept

in mind that other factors than the minimal concentration of ribavirin required for a complete inhibition may play a role in the elimination of viruses in shoot-tip culture. Apical meristematic parts of potatoes are free of PVY, but not of PVX [11]. The uneven distribution of PVY in growing tissue may contribute to its successful elimination by chemotherapy.

Viruses like PVX and BdMV, which do not reach a detectable concentration in leaf disks with ribavirin at a concentration of the order of 10^{-5} M are regarded as easily inhibited. However, most plant viruses cannot be progagated in leaf disks (e.g. because they are not sap transmissible, or replicate too slowly, or the leaves of the host plant are not suitable to punch out disks, etc.). Furthermore, at higher concentrations of ribavirin the inhibition may be improved, either because it is dose-dependent or because other modes of action than the one proposed here may result. Thus, the differentiation between viruses readily and not readily inhibited by ribavirin is qualitative only and is based on an experimental method which is not generally applicable. The inhibition of virus replication in protoplasts might be an alternative method.

Apparently readily inhibited are members of the potex- and the tymovirus groups, which possess a capped RNA.

Plant viruses with RNAs which are capped, but which are not highly dependent on the cap for translation probably include the tobamoviruses and viruses with more than one RNA molecule, such as the bromo-, the cucumo-, and the ilarviruses. They should not be readily inhibited by ribavirin.

Moreover, viruses with RNAs linked to a protein at the 5′-terminus should not be readily inhibited by ribavirin. Viruses of this type include potyviruses, luteoviruses, comoviruses, sobamoviruses, and nepoviruses.

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