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Effect of Phosphorylated Ribavirin on Vesicular Stomatitis Virus Transcription

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The effect of phosphorylated ribavirin on a vesicular stomatitis virus (VSV) in vitro transcription reaction was examined. Viral mRNA synthesized in the presence of the 5′ mono-, di-, and triphosphorylated forms of the drug translated with equal efficiencies under the test conditions. However, all three phosphorylated species inhibited VSV transcription. The mono- and diphosphorylated forms of the drug possessed approximately two to three times the inhibitory activity as the triphosphorylated form. Transcripts synthesized in the presence of drug were full length and were absent of incorporated drug. Inhibition by ribavirin 5′-diphosphate could be reversed by the addition of UTP, CTP, and GTP, while the addition of GDP to the reaction did not reverse inhibition. Ribavirin diphosphate was added to a La Crosse virus in vitro transcription assay to determine whether an inhibitory effect could be established in a viral system that was more sensitive to ribavirin than was VSV; it led to profound inhibition of RNA synthesis at concentrations as low as 0.1 μg/ml. These data suggest that ribavirin has an effect on the initial steps of transcription by some RNA-dependent RNA polymerases and that this effect may be mediated by several phosphorylated forms.

Ribavirin (1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide) is a broad-spectrum antiviral agent whose molecular mode of action remains controversial. The drug was approved by the Food and Drug Administration in 1986 for aerosol use in infants with serious infection due to respiratory syncytial virus. Ribavirin is currently under clinical investigation against a variety of other viral illnesses, including those due to influenza virus and human immunodeficiency virus. However, the drug possesses in vitro activity against a remarkably broad spectrum of viral pathogens, including both DNA and RNA viruses (11), suggesting that its full clinical potential has yet to be realized.

Several theories regarding the molecular mode of action of ribavirin have been proposed. One hypothesis states that the drug leads to decreased intracellular pools of GTP, indirectly suppressing viral nucleic acid synthesis (9, 15). A more recent hypothesis proposes that ribavirin therapy of viral-infected cells results in the synthesis of RNA with abnormal or absent 5′ cap structures, which in turn leads to inefficient translation of viral transcripts (6). A third hypothesis states that the drug has a direct suppressive effect on viral polymerase activity; however, such activity has been demonstrated only for influenza virus (4, 17, 18). None of these hypotheses are mutually exclusive, and indeed, they may indicate that ribavirin acts in a complex, multiple-site fashion. In previous experiments (16) in which the effect of ribavirin on vesicular stomatitis virus (VSV) grown in Chinese hamster ovary cells was examined, it was indicated that the drug leads to the synthesis of inefficiently translated viral mRNA, with little demonstrable effect on viral polymerase, lending support to the hypothesis that the drug alters RNA cap structures. However, results of the experiments reported here, in which the effects of phosphorylated ribavirin compounds on an in vitro VSV polymerase assay were examined, indicated that the drug does indeed possess a significant direct suppressive effect on viral polymerase. This effect did not involve chain termination and was mediated to different degrees by the 5′ mono-, di-, and triphosphorylated forms of the drug. In addition, inhibition was reversible with UTP, CTP, and GTP. Results of these experiments, when interpreted in the context of prior data generated by ourselves and others, suggest that at least some of the antiviral activity of ribavirin involves interference with initiating events of viral transcription.

MATERIALS AND METHODS

Virus and materials. VSV (Indiana serotype) was prepared by sucrose gradient purification after growth in Chinese hamster ovary cells as described by Stampfer et al. (14). [α-^{32}P]UTP and [α-^{32}P]ATP (approximately 600 μCi/mmol) was obtained from New England Nuclear Corp. (Boston, Mass.). In vitro translation was performed by using a commercially available rabbit reticulocyte lysate system (New England Nuclear). Thin-layer chromatography plates (CEL 300 PEI) were purchased from Brinkman Instruments, Inc., Westbury, N.Y. Ribavirin monophosphate (RMP), ribavirin diphosphate (RDP), and ribavirin triphosphate (RTP) were the kind gift of Roland K. Robins and Daniel Smith (1). Phosphorylated ribavirin compounds were stored in the lyophilized form at −20°C and periodically suspended in 0.1 M Tris hydrochloride (pH 8.0); stock solutions were also stored at −20°C and were discarded after 3 to 6 weeks.

VSV polymerase reaction. VSV in vitro transcription was performed as described by Banerjee (2). The reaction mixture was composed of 100 mM sodium chloride, 15 mM Tris hydrochloride (pH 8.0), 5 mM $MgCl_2$, 4 mM dithiothreitol, 0.05% Triton X-100, 10 units of RNase inhibitor (Boeringer Mannheim Biochemicals, Indianapolis, Ind.), 20 μCi of [α-^{32}P]UTP, and 25 to 50 μg of VSV virions (except when otherwise noted) in a total volume of 0.2 ml. Nucleotide triphosphate precursors were adjusted to 1 mM ATP and CTP, 500 μM UTP, and 50 μM GTP, unless noted differently. The reaction mixture was incubated at 30°C for 2 h.

La Crosse polymerase reaction. La Crosse virus in vitro transcription was performed as described by Bellocq et al. (3). Virus was grown in BHK-21 cells and purified by

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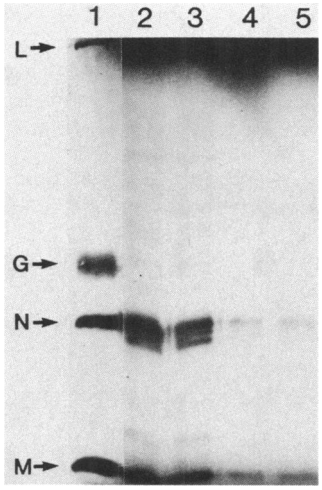

FIG. 1. VSV mRNAs were synthesized in the presence and absence of RTP (100 μg/ml), as described in the text. After 2 h of incubation, the reaction mixtures were purified by centrifugation through a 20 to 40% CsCl gradient at 200,000 × g for 16 h, and the single-stranded RNAs were collected by ethanol precipitation in 200 mM KCl. Equal quantities of RNA, as judged by trichloroacetic acid-insoluble counts, were added to a commercial rabbit reticulocyte lysate in vitro translation kit (80 mM potassium acetate, 1 mM magnesium acetate). Translation proceeded over 90 min, after which the products were analyzed by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Lane 1, VSV protein standards; lanes 2 and 3, translation of untreated and RTP-treated RNAs, respectively, at a 1:10 dilution; lanes 4 and 5, translation of untreated and RTP-treated RNAs, respectively, at a 1:50 dilution. L, G, M, and N refer to VSV structural proteins.

precipitation in polyethylene glycol and centrifuged through a 30% glycerol cushion. Virion stocks were stored in 10% glycerol–0.5% Nonidet P-40–100 mM KCl at −70°C. Polymerase reaction mixtures were composed of 30% virion stock, 40% rabbit reticulocyte lysate, and 1 mM nucleotide triphosphates. Transcription products were radiolabeled with 20 μCi of [α-^{32}P]UTP over 30 min at 30°C.

RESULTS

In previous experiments (16) in which the effect of ribavirin on VSV intracellular macromolecular synthesis was examined, it has been indicated that treatment with drug results in the production of poorly translated viral mRNAs. In an effort to extend these findings, experiments were conducted to examine the effects of ribavirin on the translation of RNA synthesized in an in vitro VSV transcription reaction. This reaction has been shown to result in the production of all five full-length, capped, and poly(A)$^+$ viral mRNAs (2). Viral transcripts were synthesized in the presence of phosphorylated ribavirin compounds and were examined for their efficiency of translation relative to those of controls synthesized in the absence of drug. When VSV transcripts were produced in the presence of RTP at 100 μg/ml, however, there was no detectable alteration in their translational efficiencies (Fig. 1). Similar results were obtained after VSV mRNAs synthesized in the presence of RDP and RMP were translated with the same concentration of drug (data not shown). Furthermore, there was no apparent disturbance of translation of VSV transcripts synthesized in the absence of ribavirin when RTP, RDP, and RMP were added directly to the in vitro translation reaction (data not shown). Therefore, the alteration of viral macromolecular synthesis observed in ribavirin-treated, infected cells could not be reproduced by using a cell-free transcription system under the test conditions.

Unexpectedly, however, it was noted that the phosphorylated ribavirin compounds had an inhibitory effect on VSV in vitro transcription itself (Table 1). As noted by others (4), the addition of RTP at 100 μg/ml to the reaction had little effect on VSV transcription, with inhibition never exceeding 35%. Surprisingly, however, RDP and RMP were consistently more inhibitory than RTP at the same concentration. There was considerable variability over time in the degree of inhibition achievable with each ribavirin compound, and the degrees of inhibition presented in Table 1 are maximal. In spite of scrupulous storage conditions, it was noted that even lyophilized compounds gradually lost activity at an approximate rate of 50% over 12 months. In addition, the degree of inhibition was found to be sensitive to the concentrations of other components in the reaction mixture (see below). However, the effect of ribavirin on the transcription reaction was always dose dependent, and the inhibition by RMP and RDP was consistently greater than that of the triphosphorylated species. Phosphorylation of ribavirin was necessary to produce inhibition, since unphosphorylated ribavirin led to no inhibition of transcription, even at 1,000 μg/ml.

When the transcripts synthesized in the presence of ribavirin were examined by electrophoresis, it appeared that the RNAs synthesized were full length, indicating no evidence of premature chain termination. Results of agarose gel electrophoresis shown in the autoradiograph in Fig. 2A indicate that radioactive precursors incorporated in the presence of phosphorylated ribavirin compounds are found exclusively in large RNAs and are indistinguishable from controls. Transcripts were further analyzed on a polyacrylamide gel (Fig. 2B) to resolve short sequences of nucleic acid. With long exposures several stops in the transcription reaction were noted; however, there were no stops that were peculiar to ribavirin-treated mRNAs. These findings are consistent with previous data (16) that have indicated that VSV RNAs synthesized intracellularly in the presence of ribavirin are also full length. In addition, they are supported indirectly by the data presented in Fig. 1, which demonstrate that equal quantities of transcripts synthesized in the presence or absence of drug produced equal quantities of viral protein in an in vitro translation reaction.

The transcriptional products made in the presence of

TABLE 1. Effect of ribavirin compounds on VSV in vitro transcription[a]

Concn (μg/ml)	% Trichloroacetic acid-insoluble counts for[b]:			
	RTP	RDP	RMP	Nonphosphorylated ribavirin
0	100	100	100	100
10	104	64	95	ND[c]
100	69	42	33	ND
1,000	ND	ND	ND	101

[a] VSV in vitro transcription was achieved in a 200-μl reaction mixture containing 100 mM NaCl, 50 mM Tris hydrochloride (pH 8.0), 5 mM MgCl, 4 mM dithiothreitol, 0.05% Triton X-100, 1 mM ATP, 1 mM CTP, 0.5 mM UTP, 0.05 mM GTP, and 25 to 50 μg of virus. Products were radiolabeled with 20 μCi of [α-^{32}P]UTP over 2 h at 30°C.

[b] Percent trichloroacetic acid-insoluble counts in comparison with those for a control sample containing no ribavirin compound.

[c] ND, Not done.

phosphorylated ribavirin compounds were further analyzed to determine whether any of these compounds were incorporated into the growing chain. Nearest-neighbor analysis was performed by using digestion with RNase T2. The digestion products were separated by thin-layer chromatography by use of two different buffer systems (Fig. 3), to lessen the possibility that identification of a putative incorporated ribavirin compound would be obscured by comigration with other digestion products. Although migration of the resulting 3'-monophosphates was variable in the volatile isobutyric acid-ammonium hydroxide system, radioactive spots could always be indentified by comigration of standards. Unfortunately, the inability to detect RMP under UV light precluded prediction of the migration of the 3'-RMP. Nevertheless, no differences in the digestion products could be detected when they were compared with control RNA synthesized in the absence of drug. Taken together, these data suggest that phosphorylated ribavirin compounds inhibit initiation of VSV in vitro transcription to various degrees, but, once initiated, transcription proceeds without incorporation of the ribavirin compound.

The inhibitory effect of RDP was of particular interest, since mediation of antipolymerase activity by a diphosphorylated nucleotide analog has not been well described. Further efforts to characterize the effect of ribavirin on VSV transcription, therefore, concentrated on the effect of this compound. The inhibition of RDP could be reversed by increasing the concentration of GTP in the reaction mixture (Table 2). This finding was not unexpected, as ribavirin has been shown to be structurally similar to guanine (10), and other investigators (7, 8, 15) have demonstrated that drug-related effects are reversed with guanine species. Surprisingly, however, the inhibitory effects of RDP on VSV transcription could be reversed with other nucleotide triphosphates as well (Table 2). The inhibitory effect of RDP in reactions composed of low, but not rate-limiting, concentrations (50 μM) of CTP and UTP (2) was less than that noted when the GTP concentration was similarly adjusted; the percent inhibition under these circumstances resulted in approximately one-half the inhibition seen with GTP. Nevertheless, readjusting the concentration of the respective nucleotides to 500 μM eliminated any inhibition. In contrast, inhibition by RDP could not be similarly achieved with ATP at concentrations that were not rate limiting. The addition of GDP to the reaction mixture, on the other hand, did not result in any apparent reversal of inhibition by RDP (Table 3). At 50 μM the addition of GDP had no effect on the transcription reaction at all; at a 10-fold higher concentration, the presence of GDP itself, similar to the results obtained with RDP, appeared to be inhibitory to the reaction. These data suggest that ribavirin interacts with a site(s) on the polymerase that normally recognizes at least three of

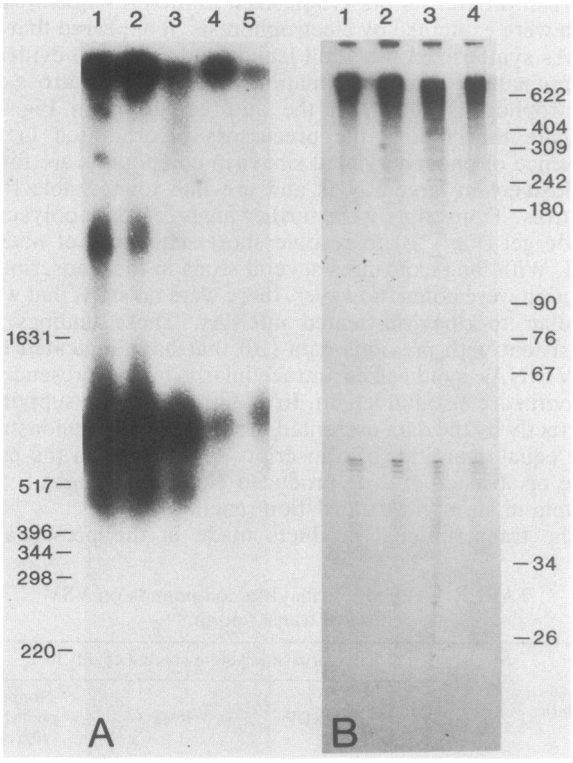

FIG. 2. (A) VSV transcription was performed under the conditions described in footnote *a* to Table 1. The products were purified by phenol extraction and repeated ethanol precipitations and were electrophoresed through a denaturing 1% agarose gel. Lanes 1 through 4, mRNA synthesized in the presence of 100 μg of RMP, RDP, RTP, and nonphosphorylated ribavirin per ml, respectively; lane 5, untreated mRNA. (B) VSV RNAs were synthesized and purified as described above and analyzed on a 12.5% denaturing polyacrylamide gel. Lane 1, RNA synthesized in the absence of ribavirin; lanes 2 through 4, RNA synthesized in the presence of 100 μg of RMP, RDP, and RTP per ml, respectively. Numbers represent nucleic acid markers.

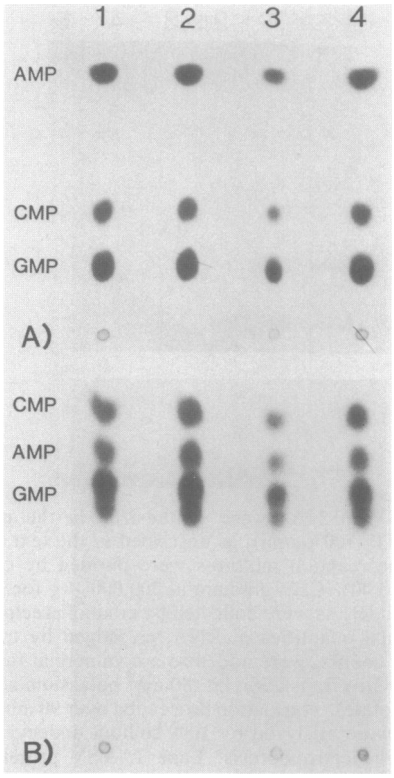

FIG. 3. VSV RNA was synthesized and purified as described in the legend to Fig. 2. Samples were heat denatured and subjected to digestion with repeated additions of 10 units of RNase T2 over 4 h. Samples were spotted onto thin-layer plates and chromatographed in two buffer systems. Species were identified by 3'-monophosphate markers. (A) Chromatography achieved with isobutyric acid–concentrated NH_4OH–water (66:1:33). (B) Chromatography achieved with 1 M LiCl.

TABLE 2. Reversal of inhibition of RDP on VSV transcription with GTP, CTP, and UTP[a]

Nucleotide and concn (μM)	RDP concn (μg/ml)	% Control
GTP[b]		
50	0	100
50	100	26
500	100	96
CTP[c]		
50	0	100
50	100	63
500	100	102
UTP[d]		
50	0	100
50	100	63
500	100	103

[a] VSV transcription was performed in a manner identical to that described in the text, except that nucleotide concentrations were adjusted as noted. Trichloroacetic acid-insoluble counts were compared with those for a sample containing no RDP.

[b] Reaction mixture contained 20 μCi of [α-^{32}P]UTP, 0.5 mM UTP, 1.0 mM CTP, and 1.0 mM ATP.

[c] Reaction mixture contained 20 μCi of [α-^{32}P]UTP, 0.5 mM UTP, 1.0 mM GTP, and 1.0 mM ATP.

[d] Reaction mixture contained 20 μCi of [α-^{32}P]ATP, 0.5 mM ATP, 1.0 mM CTP, and 1.0 mM GTP.

the triphosphorylated precursors of RNA synthesis and that the drug does not irreversibly bind to the enzyme.

Unexpectedly, the inhibitory activity of RDP was also dependent on the amount of virion added to the reaction mixture (Table 4). The addition of enzyme and template led to the expected increase in the velocity of the reaction. However, at higher concentrations of virion, the degree of inhibition of RDP was substantially less. Although the virion component responsible for this observation has not been identified, these results emphasize the highly reversible nature of ribavirin in this system.

In an attempt to both substantiate and generalize the finding that RDP is inhibitory to viral transcription, the effect of RDP on a second in vitro viral transcription system, that of La Crosse virus, was examined. Like VSV, La Crosse virus is a negative-stranded RNA virus; however, La Crosse virus is much more sensitive in vitro to ribavirin than VSV (P. Canonico, personal communication). The addition of RDP to the La Crosse virus transcription reaction led to profound inhibition of polymerase activity (Table 5). No RNA synthesis was detectable at concentrations as low as 1 μg/ml; there was still 50% inhibition at 0.1 μg/ml. Results of this experiment support the conclusion that in at least some viral systems, the antiviral effect of ribavirin may be mediated through phosphorylated analogs other than the triphosphate form and that the effect is directed against viral polymerase activity.

TABLE 3. Effect of GDP on inhibition of VSV transcription by RDP[a]

RDP concn (μg/ml)	GDP concn (μM)	% Control
0	0	100
100	0	57
100	50	55
100	500	26

[a] VSV transcription was performed in the presence or absence of GDP, as noted.

TABLE 4. Reversal of inhibition of RDP on VSV transcription with virion[a]

Virion (μg)	RDP concn (μg/ml)	cpm (10^3)	% Inhibition
5	0	50	47
5	150	27	
15	0	129	28
15	150	93	
30	0	211	7
30	150	197	

[a] VSV transcription was performed as described in the text with various concentrations of virion and RDP, as noted.

DISCUSSION

VSV is a prototypic negative-stranded RNA virus with a molecular biology that closely resembles that of several important human pathogens, such as respiratory syncytial virus, parainfluenza virus, and measles virus. Unlike these viruses, VSV can be manipulated in the laboratory with relative ease. In particular, its in vitro transcription reaction is well defined, and the messages synthesized from this reaction can be readily and efficiently translated in vitro. Although it is less sensitive to ribavirin than other viruses, it has nevertheless served as a convenient model system with which to probe the molecular activity of this drug, with the anticipation that the results will be applicable toward the treatment of the more fastidious pathogens that are responsible for human disease.

Our attempts to synthesize poorly translatable VSV mRNAs in the presence of ribavirin in the cell-free transcription reaction were not successful. The efficiency of translation of uncapped mRNAs in in vitro translation systems is influenced by several factors, including salt and mRNA concentrations and temperature of reaction (1a). Adjustment of some or all of these factors may be required to demonstrate a difference in translational efficiency between ribavirin-treated and untreated viral mRNAs. We used a rabbit reticulocyte lysate translation system to ensure comparability to previous experiments in which similar conditions were used (16). However, uncapped messages translate more inefficiently in wheat germ extracts than in reticulocyte lysates (1a), suggesting that the former system may be the better one with which to test the translational efficiency of

TABLE 5. Effect of RDP on La Crosse virus in vitro transcription[a]

RDP concn (μg/ml)	Trichloroacetic acid-insoluble cpm
0	18,023
10^{-4}	13,667
10^{-3}	15,814
10^{-2}	6,160
10^{-1}	1,501

[a] La Crosse virus in vitro transcription reactions were performed as described in the text in the presence of various concentrations of RDP. Trichloroacetic acid-insoluble radioactivity was assayed after a 30-min incubation.

viral mRNAs synthesized in the presence of ribavirin. Finally, transcription was achieved in the absence of a methyl donor; consequently, alterations of cap methylation by ribavirin would not be demonstrable under the conditions used in this study.

Nevertheless, several aspects of the molecular activity of ribavirin on viral RNA synthesis emerged from examining its effects on the VSV transcription reaction. First, an inhibitory effect of ribavirin on this viral polymerase activity was documented. Such inhibition has been described for influenza virus in experiments involving RTP. In most other reports (4, 12, 13), including our own, in which the effect of ribavirin on VSV grown intracellularly (16) has been examined, it has been indicated that there is little or no inhibitory effect on a wide variety of viral and nonviral polymerase systems. Eriksson et al. (4) found only minor inhibition by RTP on cell-free VSV transcription, similar to our results; however, the inhibitory activity of the other phosphorylated ribavirin species against VSV polymerase was not examined. The experiments described in the current report confirm that ribavirin can have a direct effect on an RNA-dependent RNA polymerase. It may be argued that the observed inhibition of VSV polymerase activity seen here could actually reflect the instability of mRNA, which was quickly degraded. However, sizing of the transcripts by gel electrophoresis suggested no fragmentation of VSV mRNAs synthesized in the presence of drug. In addition, in experiments (e.g., see Fig. 1) in which ribavirin-treated transcripts were translated in solution at 37°C for 90 min, there was no inherent instability of mRNAs in comparison with controls based on the amount of viral protein that was successfully synthesized.

There was a considerable decrease in inhibitory activity of the ribavirin compounds on VSV transcription over the time course of these experiments. The compounds were noted to be very hygroscopic and became moist in spite of careful storage. In addition to adding irrelevant weight, moisture is known to lead to rapid dephosphorylation of at least the triphosphate, although it is thought that the other compounds are more stable (R. K. Robins, personal communication). Consequently, strict definition of inhibitory activity for a given concentration of drug was impossible. Nevertheless, the inhibitory effects were always dose dependent and were reproducible in experiments performed within short time frames. In addition, the profound activity of RDP against La Crosse virus transcription provided support that the inhibition noted for VSV was both real and relevant.

A second aspect of the antiviral activity of ribavirin which was elucidated from these experiments was that the inhibitory effects of the drug can be mediated to various degrees by all three 5′-phosphorylated compounds studied. In previous reports in which a direct inhibitory effect of the drug on enzymatic activity has been described, it has been documented that there is an effect only by the phosphorylated compounds relevant to the enzyme in question. Thus, IMPDH, which normally uses IMP as a substrate, is inhibited by RMP (15); influenza polymerase and vaccinia capping activity, which normally use triphosphorylated nucleotides as substrate, are inhibited by RTP (4, 17, 18). In the experiments with VSV, inhibitory activity has been shown with the mono- and diphosphorylated drug, as well as the triphosphate. Indeed, in this system, the triphosphate, which would most closely resemble the natural substrate of the viral polymerase and, therefore, would be expected to have the greatest potential inhibitory activity, had the least inhibitory activity. Unlike other purine analogs with antiviral activity, such as acyclovir, ribavirin's dissemblance to natural nucleotide precursors is at the base rather than the ribose. In contrast to acyclovir, ribavirin does not induce its antiviral activity by blocking chain elongation. Consequently, triphosphorylation of the ribose portion of the drug is probably not essential; rather, the degree of phosphorylation of ribavirin may be important only in the manner in which it alters the interaction between the base and the polymerase.

A third aspect of molecular activity of ribavirin observed in these experiments was that the inhibition by the diphosphate could be reversed by CTP, UTP, and GTP. The reversibility of the activity of ribavirin by guanine-related compounds has been documented repeatedly (7, 8, 15) and is an expected consequence of the structural similarity of ribavirin to guanine (10). However, reversibility of ribavirin activity by unrelated nucleotides has not been described previously. This observed reversibility suggests that ribavirin does not act specifically or exclusively as a guanine analog, as has generally been believed, but rather may interest with a site(s) that recognizes multiple RNA nucleotide precursors.

Reversibility was also observed by the addition of virion. The basis for this observation is unknown. An unlikely explanation is that ribavirin, like acyclovir, acts as a suicide inhibitor, forming a reversible complex with polymerase before becoming irreversibly bound to the enzyme (5). The addition of more enzyme would therefore be expected to replenish polymerase activity. However, suicide inhibition requires that the inhibitor serve as substrate to the enzyme in question, which is not borne out from our data. Alternatively, the addition of some virion component could interfere with the activity of ribavirin in some unknown fashion. Elucidation of this phenomenon would require enzyme kinetic studies and the addition of purified virion components to the reaction. However, a simple identification of the influence of virion concentration emphasized the influence of reaction conditions on the degree of inhibition of this drug in the VSV polymerase system.

Previous attempts to formulate a unifying hypothesis regarding the molecular action of ribavirin have been frustrated by seemingly disparate results generated from the investigation of its activity in different viral systems. Indeed, results of our experiments in which a single viral system, VSV, was used have suggested that there are different molecular targets, depending on whether the drug is examined intracellularly or in a cell-free polymerase assay (16). To date, there have been three proposed mechanisms of action for ribavirin. The first suggests that ribavirin inhibits de novo production of GMP by suppressing IMPDH activity, thereby denying virus substrate for its transcription (9, 15). A second hypothesis suggests that ribavirin has a direct effect on viral polymerase activity (4, 17), and a third states that the drug interferes with 5′ capping of viral messages, leading to inefficient translation of viral mRNA (6, 16). All previous data, as well as those presented here, support the hypothesis that ribavirin reversibly inhibits an initiating step of transcription. Interference with influenza polymerase primer generation, inhibition of capping activity by vaccinia virus, and the suppression of VSV polymerase activity without production of fragmented RNA all suggest that early, initiating events in RNA production are altered by this drug. The inhibition of IMPDH may prove important in potentiating the activity of ribavirin by decreasing the availability of a nucleotide competitor. Examination of other viral systems will help to validate this hypothesis.

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LITERATURE CITED

1. **Allen, L. B., K. H. Boswell, T. A. Khwaja, R. B. Meyer, Jr., R. W. Sidwell, J. T. Witkowski, L. F. Christensen, and R. K. Robins.** 1978. Synthesis and antiviral activity of some phosphates of the broad-spectrum antiviral nucleoside, 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide (ribavirin). J. Med. Chem. **21**:742–746.

1a. **Banerjee, A. K.** 1980. 5′-Terminal cap structure in eucaryotic messenger ribonucleic acids. Microbiol. Rev. **44**:175–205.

2. **Banerjee, A. K.** 1981. The in vitro mRNA transcription process, p. 35–50. *In* D. H. L. Bishop (ed.), Rhabdoviruses, vol. II. CRC Press, Inc., Boca Raton, Fla.

3. **Bellocq, C., R. Raju, J. Patterson, and D. Kolakofsky.** 1987. Translational requirement of La Crosse virus S-mRNA synthesis: in vitro studies. J. Virol. **61**:87–95.

4. **Eriksson, B., E. Helgstrand, N. G. Johannson, A. Larsson, A. Misiorny, J. O. Noren, L. Philipson, K. Stenberg, G. Stening, S. Stridh, and B. Oberg.** 1977. Inhibition of influenza virus ribonucleic acid polymerase by ribavirin triphosphate. Antimicrob. Agents Chemother. **11**:946–951.

5. **Furman, P. A., M. H. St. Clair, and T. Spector.** 1984. Acyclovir triphosphate is a suicide inactivator of the herpes simplex virus DNA polymerase. J. Biol. Chem. **259**:9575–9579.

6. **Goswami, B. B., E. Borek, D. K. Sharma, J. Fujitaki, and R. A. Smith.** 1979. The broad spectrum antiviral agent ribavirin inhibits capping of mRNA. Biochem. Biophys. Res. Commun. **89**: 830–836.

7. **Larsson, A., K. Stenberg, and B. Oberg.** 1978. Reversible inhibition of cellular metabolism by ribavirin. Antimicrob. Agents Chemother. **13**:154–158.

8. **Malinoski, F., and V. Stollar.** 1981. Inhibition of Sindbis virus replication by ribavirin: influence of cultural conditions and of the host cell phenotype. Antiviral Res. **1**:287–299.

9. **Malinoski, F., and V. Stollar.** 1981. Inhibitors of IMP dehydrogenase prevent Sindbis virus replication and reduce GTP levels in Aedes albopictus cells. Virology **110**:281–291.

10. **Prusiner, P., and M. Sundaralingam.** 1973. A new class of synthetic nucleoside analogous with broad-spectrum antiviral properties. Nature (London) **244**:116–117.

11. **Sidwell, R. W., J. N. Huffman, G. P. Khare, L. B. Allen, J. T. Witkowski, and R. K. Robins.** 1972. Broad spectrum antiviral activity of virazole: 1-β-D-ribofuronasyl-1,2,4-triazole-3-carboximide. Science **177**:705–706.

12. **Smee, D. F., R. W. Sidwell, S. M. Clark, B. B. Barnett, and R. S. Spendlove.** 1982. Inhibition of rotaviruses by selected antiviral substances: mechanisms of viral inhibition and in vivo activity. Antimicrob. Agents Chemother. **21**:66–73.

13. **Smith, R. A.** 1980. Mechanism of action of ribavirin, p. 99–118. *In* R. A. Smith and W. Kirkpatrick (ed.), Ribavirin, a broad spectrum antiviral agent. Academic Press, Inc., New York.

14. **Stampfer, M., D. Baltimore, and A. S. Huang.** 1969. Ribonucleic acid synthesis of vesicular stomatitis virus. I. Species of ribonucleic acid found in Chinese hamster ovary cells infected with plaque-forming and defective particles. J. Virol. **4**:154–161.

15. **Streeter, D. G., J. T. Witkowski, G. P. Khare, R. W. Sidwell, R. J. Bauer, R. K. Robins, and L. N. Simon.** 1973. Mechanism of action of 1-β-ribofuranosyl-1,2,4-triazole-3-carboxamide, a new broad spectrum antiviral agent. Proc. Natl. Acad. Sci. USA **70**:1174–1178.

16. **Toltzis, P., and A. S. Huang.** 1986. Effect of ribavirin on macromolecular synthesis in vesicular stomatitis virus-infected cells. Antimicrob. Agents Chemother. **29**:1010–1016.

17. **Wray, S. K., B. F. Gilbert, and V. Knight.** 1985. Effect of ribavirin triphosphate on primed generation and elongation during virus transcription *in vitro*. Antiviral Res. **5**:39–48.

18. **Wray, S. K., B. F. Gilbert, M. W. Noall, and V. Knight.** 1985. Mode of action of ribavirin: effect of nucleotide pool alterations on influenza virus ribonucleoprotein synthesis. Antiviral Res. **5**:29–37.