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Polyadenylate Sequences of Human Rhinovirus and Poliovirus RNA and Cordycepin Sensitivity of Virus Replication

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The polyadenylate [poly(A)] content of the genome RNA of human rhinovirus type 14 (HRV-14) is nearly twice as large as that of the genome RNA of poliovirus type 2. The poly(A) content of viral RNA was determined to be the RNase-resistant fraction of ^{32}P-labeled viral RNA extracted from purified virions. Polyacrylamide gel electrophoresis indicated that the poly(A) sequences of HRV-14 are more heterogenous and on an average larger than those of poliovirus RNA. On the basis of susceptibility to micrococcal polynucleotide phosphorylase the rhinovirus genome terminates in poly(A). Replication of both viruses is almost totally inhibited by cordycepin at 50 μg/ml. At lower concentrations, rhinovirus replication is more sensitive to cordycepin than poliovirus replication. Addition of cordycepin (75 μg/ml) to infected culture prior to or during viral RNA replication results in more or less complete inhibition of virus-specific RNA synthesis. The results do not indicate that cordycepin sensitivity of either virus is due to preferential inhibition of viral poly(A) synthesis by this antibiotic.

It is now well established that many mammalian cellular and viral mRNA's terminate in 50 to 250 nucleotides long polyadenylic acid [poly(A)] sequences. Poly(A) sequences have also been detected in togavirus and picornavirus genomes (1, 4) which serve as mRNA in the host cell. Among picornaviruses poliovirus (1), human rhinovirus types 2 and 14 (HRV-14) (8, 11), Columbia-SK virus (4), and mengovirus (18) are known to have polyadenylated genomes, whereas the RNA genome of encephalomyocarditis virus probably terminates in oligo[A] sequences (Fellner et al., Int. Congr. Virol., 3rd, Madrid, p. 161, 1975).

It has been reported that the adenosine analogue cordycepin inhibits replication of HRV-14, (11) Newcastle disease virus, Sendai virus (9), RNA tumor viruses (16), and vaccinia virus (12) but not replication of influenza virus (9) or vesicular stomatitis virus (3). In view of the evidence that cordycepin preferentially inhibits nontranscriptive poly(A) synthesis in animal cells (2), it has been suggested that resistance of viral replication to cordycepin may indicate a transcriptive mode of polyadenylation of viral mRNA (22). Limited evidence in support of this suggestion is available. For instance, the polyadenylation of vesicular stomatitis virus mRNA is transcription associated (3), whereas that of vaccinia virus mRNA (17) appears to be dissociated from transcription. Similarly, evidence indicating that nearly 50% of the poly(A) sequences of HRV-14 RNA is synthesized earlier than the remaining sequences in viral RNA has been obtained (11). On the other hand, evidence for transcriptive mechanism of polyadenylation of poliovirus RNA exists (3, 25), and poliovirus replication has been variously reported as being resistant or moderately sensitive to cordycepin (7, 14).

In this paper we compare HRV-14 and poliovirus type 2 with regard to poly(A) in viral RNA and cordycepin sensitivity of virus replication. It is shown that the poly(A) content of the genome RNA of HRV-14 is nearly twice as large as that of poliovirus genome, that the poly(A) sequences of rhinovirus RNA are more heterogenous but larger on an average than the poly(A) sequences of poliovirus genome, that the rhinovirus genome-like poliovirus genome terminates in poly(A) sequences, that the replication of poliovirus type 2 is inhibited by cordycepin, albeit less, than that of HRV-14, and that cordycepin sensitivity of both viruses is due to inhibition of viral RNA synthesis by the antibiotic.

MATERIALS AND METHODS

Materials. [8-^{3}H]adenosine (16 Ci/mmol), [5-^{3}H]uridine (21 Ci/mmol), carrier-free ^{32}P-labeled

phosphoric acid, and RNase-free sucrose were obtained from Schwarz/Mann, Div. of Becton, Dickinson & Co.; cordycepin, RNase A, RNase T1, agarose, RNase-free DNase, and purified yeast tRNA (type 1) were from Sigma Chemical Co. *Micrococcus lysodiecticus* polynucleotide phosphorylase was from Worthington Biochemicals Corp. Poly(U)-Sepharose 4B was from Pharmacia Fine Chemicals. Acrylamide, *N-N'*-methylenebisacrylamide, and *N-N-N'N''*-tetramethylethylenediamine (TEMED) were from Bio-Rad Laboratories, and ammonium persulfate and 30% H_2O_2 (Fisher certified, ACS) were from Fisher Scientific Co. Actinomycin D was a gift from Merck & Co.

Viruses and cells. HRV-14 poliovirus type 2 and antisera specific for these viruses were obtained from the National Institute of Allergy and Infectious Diseases, Bethesda, Md. The viruses were grown in monolayer cultures of a calf serum-adapted HeLa cell line (catalog no. 0-26340; Flow Laboratories, Inc.). Growth of cells and viruses was in Eagle minimal essential medium containing 5% calf serum, 100 μg each of streptomycin, penicillin, and neomycin per ml, and 2.5 μg of Fungizone per ml. The procedures used for harvesting and sucrose gradient purification of virus have been reported (11). Virus stocks and purified virus used in different experiments were checked for contamination by neutralization with specific antisera.

Determination of poly(A) content of viral RNA. One of a pair of HeLa monolayer cultures (~30×10^6 cells/culture) was infected with HRV-14 and the other with poliovirus type 2 at a multiplicity of infection (MOI) of ≈10. Both cultures were incubated in medium containing carrier-free ^{32}P-labeled phosphoric acid (125 μCi/ml). The poliovirus-infected culture was harvested at 9 h postinfection (p.i.) and the rhinovirus-infected culture at 16 h p.i. Details of virus purification, extraction of poly(A)-containing viral RNA, digestion of viral RNA with T1 and pancreatic RNases, and determination of acid-insoluble radioactivity were as previously described (11).

Polyacrylamide gel electrophoresis of poly(A) sequences isolated from rhinovirus RNA and poliovirus RNA. Monolayer cultures (≈30×10^6 cells/culture) were infected at high MOI with HRV-14 or poliovirus type 2. Poliovirus-infected culture was incubated with medium containing 20 μCi of [^3H]adenosine ([^3H]AR) per ml for 9 h and rhinovirus-infected culture with phosphate-free medium containing 200 μCi of ^{32}P-labeled phosphoric acid per ml for 16 h. Labeled virions were harvested and purified by sucrose gradient sedimentation (see above). Viral RNA was obtained by extracting purified virions with chloroform and phenol in the presence of 0.5% sodium dodecyl sulfate (SDS) and 0.1 M NaCl as described by Perry et al. (15). The two viral RNAs were mixed and digested with RNases (see above), and the digest was reextracted with chloroform-phenol SDS. The RNase-resistant fraction of RNA was precipitated with 2 volumes of alcohol and dissolved in electrophoresis buffer (0.05 M Tris-hydrochloride, pH 7.4). Preparation of acrylamide gels and electrophoresis of RNA were by the procedure described by Pinder and Gratzer (16). A small volume (25 μl) of the RNA solution was mixed with an equal volume of a solution containing 0.05% bromophenol blue and 50% sucrose in electrophoresis buffer and layered on a 5.5-cm cylindrical polyacrylamide gel containing 10% acrylamide and 0.1% *N-N'*-methylenebisacrylamide. The gel buffer was the same as the electrophoresis buffer. Electrophoresis was for 110 min at 5 mA/gel. The gels were fractionated into 1-mm slices with a homemade gel slicer consisting of stacked razor blades. The gel slices were solubilized by incubation with 30% H_2O_2 (0.25 ml/slice) at 80°C for 3 h, and the radioactivity of fractions was determined in a Beckman LS/230 scintillation spectrometer with a counting fluid containing 33% Triton X-100 and 66% toluene.

Digestion of RNA with polynucleotide phosphorylase. [^3H]AR viral RNA prepared as described above was digested with polynucleotide phosphorylase (20). The reaction mixture contained in 1 ml of buffer (0.05 M Tris-hydrochloride [pH 8.0], 0.015 M $MgCl_2$, and 0.015 M KH_2PO_4) [^3H]AR viral RNA (≈30,000 cpm) mixed with 100 μg of purified yeast RNA and 500 μg of polynucleotide phosphorylase. Incubation was for 2 or 10 min at 37°C. The control reaction mixture from which the enzyme was omitted was incubated at 37°C for 10 min. The reaction was stopped with 0.5% SDS, and sodium chloride was added to a final concentration of 0.1 M. RNA was purified by extraction with an equal volume of a 1:1 mixture of chloroform and phenol (14) and precipitated with 2 volumes of alcohol. The precipitates were dissolved in NETS buffer (0.01 M Tris-hydrochloride [pH 7.2] 0.1 M NaCl, 0.01 M EDTA, and 0.2% SDS). The binding of RNA to and its elution from poly(U)-Sepharose were carried out by the following procedure.

Separation of poly(A)-containing RNA from poly(A)-lacking RNA. Poly(U)-Sepharose 4B (200 mg) was swollen in 5 ml of 1.0 M NaCl (pH 7.5) for 5 min. The slurry was poured into a Pasteur pipette plugged with glass wool. The column was packed by rinsing with 20 ml of 0.1 M NaCl, pH 7.5. The final rinse was with 20 ml of NETS buffer. RNA dissolved in 1 ml of NETS buffer was passed through the column. The column was then rinsed with 4 ml of the same buffer, and the effluent containing "unbound" RNA was collected. The RNA bound to poly(U)-Sepharose in the column was eluted with 5 ml of glass-distilled water. The bound and unbound RNA fractions were precipitated with alcohol, the precipitates were dissolved in NETS buffer, and the acid-insoluble radioactivity of each RNA solution was determined by scintillation counting.

Cordycepin sensitivity of virus replication. Replicate monolayer cultures containing approximately 10^6 cells/culture were infected with HRV-14 or poliovirus type 2 at an MOI of ≈10. After virus adsorption for 0.5 h at room temperature, unadsorbed virus was removed, and the monolayers were washed with 4 ml of phosphate-buffered saline without Ca^{2+} or Mg^{2+} (1 ml/wash per culture). To pairs of cultures, medium containing cordycepin at the concentrations indicated in Fig. 2 was added. Rhinovirus-infected cultures were incubated at 34°C for 10 h and polio-

virus-infected cultures at 37°C for 6 h. Virus yield was determined by the plaque assay procedure previously described (10), with the following modifications: virus dilutions and the agar overlay were made in minimal essential medium containing 5% calf serum.

Effect of cordycepin on virus-specific RNA synthesis. Replicate monolayer cultures in 75-cm² plastic flasks (Corning Glass Works) containing approximately 20 × 10⁶ cells/flask were infected with purified virus at an MOI of ≃10. After virus adsorption and removal of unadsorbed virus (see above), the cultures were incubated with growth medium. Cordycepin was added to give a final concentration of 75 μg/ml to all but one culture infected with each virus at the times indicated in Fig. 3. Actinomycin D was added to a final concentration of 5 μg/ml to all cultures 1 h before labeling with [³H]AR. Poliovirus-infected cultures were labeled from 3.5 to 4.0 h p.i. and rhinovirus-infected cultures from 8 to 10 h p.i. with [³H]AR (20 μCi/ml). After labeling, the radioactive medium was removed, and the cultures were washed twice with phosphate-buffered saline (5 ml/wash). The cells were lysed with acetate-SDS buffer (0.01 M sodium acetate [pH 6.0], 0.1 M NaCl, 0.001 M EDTA, and 0.5% SDS), and the lysate was extracted with an equal volume of a 1:1 mixture of chloroform and phenol (14). The aqueous phase was removed and reextracted two more times. RNA was precipitated twice from the aqueous phase with alcohol, dissolved in Tris-hydrochloride buffer (0.01 M Tris-hydrochloride [pH 7.5], 0.1 M NaCl, and 0.01 M $MgCl_2$), incubated with DNase-1 (10 μg/ml) at 37°C for 0.5 h, reprecipitated with alcohol, and dissolved in Loening electrophoresis buffer (0.036 M Tris-hydrochloride [pH 7.8], 0.03 M NaH_2PO_4, 0.001 M EDTA, and 0.2% SDS). A sample of the RNA solution (25 μl) was mixed with an equal volume of a solution of 50% sucrose and 0.05% bromophenol blue and layered on a 2% polyacrylamide gel containing 0.5% agarose. Details of gel polymerization and electrophoresis have been described (5). After electrophoresis for 2.75 h at 5 mA/gel, the gels were fractionated, and the radioactivity in fractions was determined as described above.

RESULTS

Poly(A) content of rhinovirus RNA and poliovirus RNA. The content of poly(A) sequences in poliovirus RNA has been reported to be 1.14% of the genome (21). This figure is in good agreement with our estimate of 1.16% for the poly(A) content of poliovirus RNA (Table 1). In comparison, the poly(A) content of purified rhinovirus RNA is 2.12% (Table 1) or nearly twice as much as that of poliovirus RNA. On the assumption that the genomes of HRV-14 and poliovirus type 2 have molecular weights of 2.4 × 10⁶ and 2.6 × 10⁶, respectively (10), and because the fraction of polyadenylated molecules in both RNAs is the same (unpublished observation), the poly(A) content of HRV-14 RNA would correspond to an average chain length of 150 nucleotides.

Heterogeneity and size of poly(A) sequences of rhinovirus RNA. In keeping with the above expectation, a significant fraction of poly(A) isolated from rhinovirus RNA migrated more slowly through a 10% polyacrylamide gel than poly(A) isolated from poliovirus RNA (Fig. 1). It will be noted, however, that over

TABLE 1. *Poly(A) content of viral RNA*

Virus	³²P-labeled viral RNA (cpm/ml)		
	Acid insoluble	RNase resistant	% RNase resistant
HRV-14	211,130	4,480	2.12
Poliovirus type 2	155,160	1,800	1.16

[a] The counts-per-minute data represent averages of triplicate determinations.

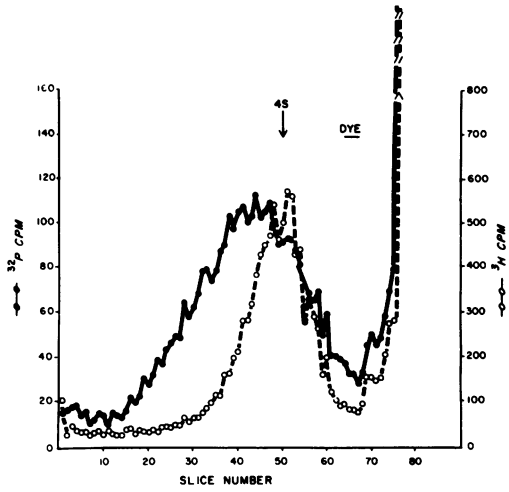

FIG. 1. *Polyacrylamide gel electrophoresis of poly(A) sequences isolated from rhinovirus and poliovirus RNA. ³²P-labeled rhinovirus RNA and [³H]AR poliovirus RNA prepared as described in Materials and Methods were mixed, digested with RNases, extracted with chloroform-phenol-SDS, and precipitated with alcohol in the presence of carrier yeast tRNA. The precipitate was dissolved in electrophoresis buffer, and 25 μl of the solution was subjected to electrophoresis on a cylindrical 10% polyacrylamide gel. The position of yeast tRNA marker was obtained by scanning the gel at 254 nm in an ISCO gel scanner. The gel was fractionated, and the fractions were solubilized and assayed for radioactivity by scintillation counting. (See text for details of procedure.) Symbols: ●, ³²P-labeled poly(A) isolated from rhinovirus RNA; ○, ³H-labeled poly(A) isolated from poliovirus RNA. The arrow indicates the position of the absorbance peak of yeast tRNA.*

one-half of rhinovirus poly(A) consists of sequences similar in size to poliovirus poly(A). Therefore, it appears that the poly(A) sequences present in the virion RNA of HRV-14 are more heterogenous than such sequences contained in the virion RNA of poliovirus type 2.

Evidence for 3'-terminal location of poly(A) in rhinovirus RNA. Controlled digestion with the enzyme polynucleotide phosphorylase is known to remove 3'-terminal poly(A) from mRNA (20). This procedure was used to determine whether the poly(A) sequences were located at the 3'-terminal of rhinovirus RNA. Viral RNA extracted from purified virions was digested with *Micrococcus* polynucleotide phosphorylase as described under Materials and Methods. Loss of poly(A) was monitored by the inability of RNA lacking poly(A) to bind to poly(U)-Sepharose. The results shown in Table 2 indicate that digestion with the enzyme for 10 min significantly reduced the fraction of RNA molecules binding to poly(U)-Sepharose. Additional control experiments showed that: (i) unbound RNA when reapplied to poly(U)-Sepharose did not become bound, (ii) ^{3}H-labeled poly(A) in NETS buffer was completely bound to poly(U)-Sepharose and could be quantitatively eluted with water, and (iii) incubation of viral RNA in 2× standard saline citrate buffer for 10 min with or without polynucleotide phosphorylase did not appreciably alter the extent of binding to poly(U)-Sepharose. In view of these observations, it is unlikely that the observed loss of binding of viral RNA to poly(U)-Sepharose upon digestion with polynucleotide phosphorylase was unrelated to the poly(A) content of viral RNA or that it was due to degradation by an endonuclease which may have been present in the enzyme preparation. Therefore, the results are interpreted to mean that rhinovirus RNA terminates in poly(A) sequences.

TABLE 2. *Removal of poly(A) from rhinovirus RNA with polynucleotide phosphorylase*

Length of incubation with PP[a]	Counts per minute			
	Unbound[b]	Bound[c]	Bound/unbound	Total bound (%)
Control[d]	11,241	14,973	1.35	57
2 min	15,867	12,092	0.76	43
10 min	18,678	7,431	0.40	29

[a] Micrococcal polynucleotide phosphorylase (PP) at 500 μg/100 μg of RNA per ml.

[b] Counts per minute not retained by polyuridylic acid-Sepharose column.

[c] Counts per minute retained by polyuridylic acid-Sepharose column.

[d] Sample of RNA incubated for 10 min without enzyme.

Cordycepin sensitivity of virus replication. Replication of HRV-14 was previously shown to be inhibited by cordycepin (11). Poliovirus replication, on the other hand, has been reported to be resistant or moderately sensitive to cordycepin inhibition (6). We have repeatedly observed poliovirus replication to be highly sensitive to inhibition by cordycepin. In an attempt to understand the underlying mechanism(s), the effect of exposing infected cultures to various concentrations of the antibiotic, on production of infectious virus progeny and the effect of a single inhibitory concentration on the synthesis of virus-specific RNAs were determined. The results are presented in Fig. 2 and 3. Whereas growth of HRV-14 was completely inhibited by cordycepin at 25 μg/ml, higher concentrations were required to comparably inhibit poliovirus replication (Fig. 2). Cordycepin

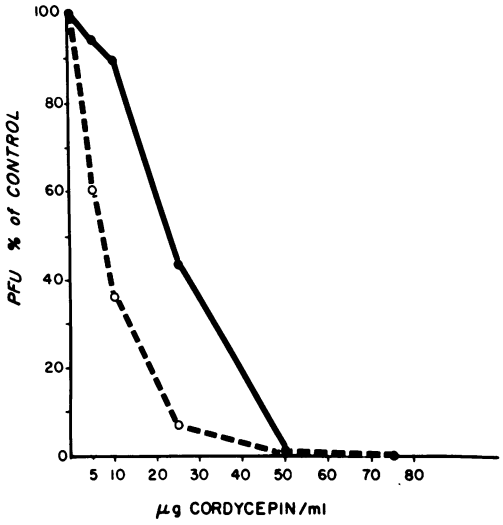

FIG. 2. *Inhibition of rhinovirus and poliovirus replication by cordycepin: effect of cordycepin concentration. Replicate monolayer cultures were infected with HRV-14 or poliovirus type 2 at an MOI ≈10. After four washes with phosphate-buffered saline, pairs of cultures were incubated with growth medium containing the indicated concentrations of cordycepin for 6 h (poliovirus-infected cultures) or 10 h (rhinovirus-infected cultures). Infected control cultures were similarly incubated but without cordycepin. Virus yield was determined by plaque assay. The average virus yield from each cordycepin-treated pair of cultures is plotted as a percentage of that from control cultures. (See text for details of procedure.) Symbols: ○, Rhinovirus-infected cultures; ●, poliovirus-infected cultures.*

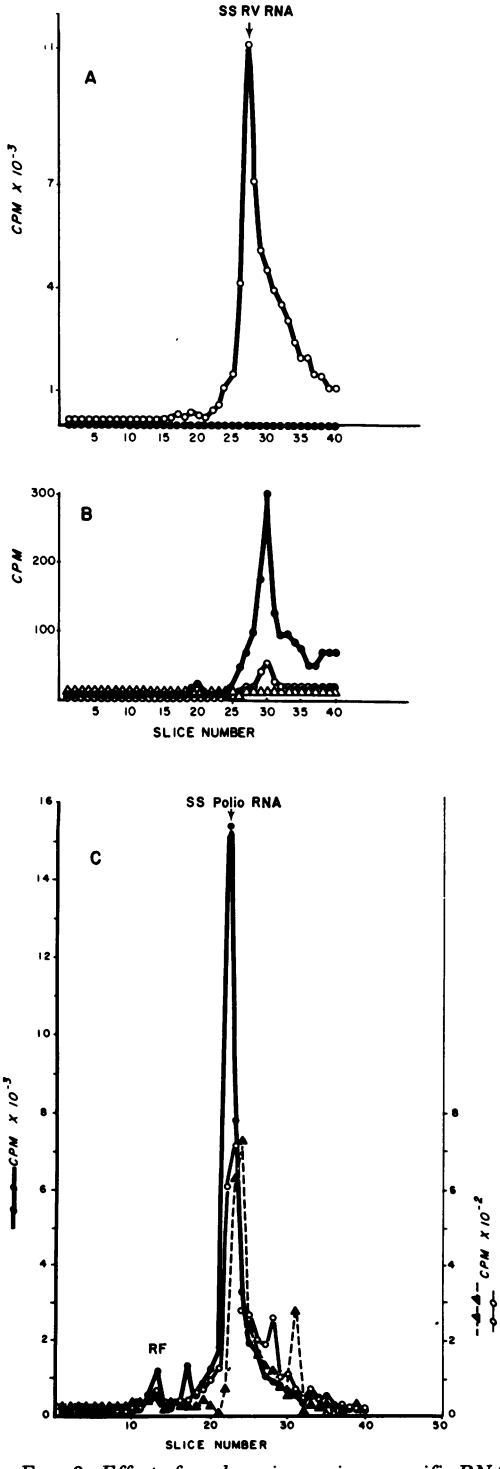

FIG. 3. *Effect of cordycepin on virus-specific RNA synthesis. Replicate HeLa monolayer cultures were infected with HRV-14 or poliovirus type 2 at an MOI of ≈10. Cordycepin was added at 0, 2, 5, or 7 h p.i. to rhinovirus-infected cultures and at 0 or 2 h p.i. to poliovirus-infected cultures. Infected control cultures did not receive any cordycepin. Rhinovirus-infected cultures were labeled between 8 and 10 h p.i. and poliovirus-infected cultures between 3.5 and 4 h p.i. with [³H]AR. After cell lysis with acetate-SDS buffer, total RNA of each culture was extracted with chloroform-phenol, precipitated from the aqueous phase with alcohol, dissolved in Tris-hydrochloride buffer, digested with DNase I, reextracted with chloroform-phenol, precipitated with alcohol, and dissolved in Leoning electrophoresis buffer. A constant fraction of each RNA preparation was subjected to electrophoresis on a separate polyacrylmide gel containing 2% acrylamide and 0.5% agarose. The gels were fractionated, and the fractions were solubilized and assayed for radioactivity (see text for details of procedure). (A) RNA from rhinovirus-infected control culture (○) and mock-infected culture (●). (B) RNA from rhinovirus-infected, cordycepin-treated cultures. Symbols: Cordycepin added at 0 (●), 2 (○), 5 (▲), and 7 h p.i. (△). (C) RNA from poliovirus-infected cultures. Symbols: RNA from infected control culture (●); cordycepin added 0 (○) and 2 h p.i. (▲).*

inhibition of virus replication was not MOI dependent, and maximum inhibition of replication of either virus required addition of cordycepin before the onset of viral RNA synthesis (unpublished observations). Addition of cordycepin prior to or during viral RNA synthesis resulted in complete inhibition of subsequent synthesis of rhinovirus-specific RNAs (Fig. 3A, B). Addition of the drug to poliovirus-infected cells at 0 or 2 h p.i. similarly inhibited all except a small fraction of poliovirus-specific RNA synthesis (Fig. 3C). Furthermore, both single-stranded and double-stranded viral RNAs were inhibited more or less equally (Fig. 3C).

DISCUSSION

The results reported in this communication confirm preliminary data that suggested that the RNA of HRV-14 is rich in poly(A) sequences (12). Evidence for a 3′ end location of poly(A) sequences is also presented. Of particular interest is the observation that the poly(A) sequences of rhinovirus genome are on the average nearly twice as large as those of poliovirus genome but more heterogenous than the latter. These differences may possibly bear on the mechanism(s) of polyadenylation of the two genomes. There is evidence that the poly(A) in poliovirus RNA is transcribed from a polyuridylic acid-containing intermediate (3, 24, 25). Limited evidence now available points to a different mechanism for the addition of poly(A) to rhinovirus RNA. First, the proportion of [³H]AMP residues in the poly(A) portion of rhi-

novirus RNA was reported to be 7.4% when the infected culture was labeled with [^{3}H]AR from 0 h p.i., but only 4.6% when labeled from 6 h p.i. (12). (On repeating this experiment we have obtained 8.6 and 4.1% for the RNase-resistant fraction of [^{3}H]AMP residues in rhinovirus RNA, corresponding to the two intervals of labeling.) This finding was interpreted to suggest possible ligation of preformed poly(A) sequences to rhinovirus RNA (12). These results could also be explained by the alternative assumption that early synthesized rhinovirus RNA has larger poly(A) sequences than late viral RNA. Approximately 90% of rhinovirus RNA is synthesized later than 5 h p.i. and contains about 4% of adenosine residues in the poly(A) portion (see above). By analogy to poliovirus RNA this would correspond to 80 to 100 adenosine residues. Therefore, for the observed doubling of label in the poly(A) portion of viral RNA (see above) to be due to the larger size of poly(A) in early viral RNA, the 10% or so of viral RNA molecules synthesized before 5 h p.i. will have to contain poly(A) sequences about 1,000 nucleotides long. It is not possible from our results to determine the exact size of the largest sequences contained in rhinovirus poly(A), even though it is clear that a substantial fraction of rhinovirus poly(A) consisted of much larger sequences than poliovirus poly(A) (Fig. 1). Second, by the technique of binding to poly(A)-Sepharose, poly(U) sequences were not detected in rhinovirus-specific intermediate RNAs isolated from infected HeLa cells (9). This finding, if confirmed by more direct procedures, would also support a nontranscriptive mechanism for polyadenylation of rhinovirus RNA, such as ligation of preformed poly(A) sequences to viral RNA as previously suggested (12) or terminal addition by a (cellular?) riboadenylate transferase (3).

Replication of HRV-14 was previously shown to be inhibited by cordycepin (12). The present finding that the replication of poliovirus type 2 also is cordycepin sensitive is contrary to what others have reported (7, 14). These workers studied cordycepin action on poliovirus type 1 growing in HeLa cell suspension cultures, whereas we have employed poliovirus type 2 and HeLa monolayer cultures. It is not known whether such differences would influence cordycepin action on poliovirus replication. In our experiments cordycepin inhibited virus-specific RNA synthesis in cells infected with either virus. Rhinovirus-specific RNA synthesis was completely inhibited when cordycepin was added at 2, 5, or 7 h p.i. (6, 3, or 1 h, respectively, before addition of label), but when added at 0 h p.i. (8 h before label) there was some residual synthesis (Fig. 3B). Similarly, all except a small fraction of poliovirus-specific RNA synthesis was inhibited by the addition of cordycepin at 0 or 2 h p.i. (3.5 or 1.5 h, respectively, before adding label) (Fig. 3C). We interpret these results to mean that cordycepin inhibits transcriptive synthesis of picornavirus RNA. Cordycepin inhibition of transcription by eukaryotic cellular DNA-dependent RNA polymerases (8) and by the RNA polymerase of vaccinia virion (13) has been reported. The poly(A) content of poliovirus RNA synthesized in the presence or absence of cordycepin was the same (unpublished observation). Therefore, viral poly(A) synthesis was no more sensitive to cordycepin than viral RNA synthesis. However, it is possible that inhibition of rhinovirus RNA synthesis is primarily due to inhibition of poly(A) synthesis which somehow might be necessary for viral RNA transcription. The actual mechanism of polyadenylation of rhinovirus RNA will have to be determined before this possibility can be verified.

Residual synthesis of rhinovirus RNA 8 h after addition of cordycepin probably resulted from partial metabolic inactivation of the drug during the long interval before labeling. The reason for incomplete inhibition of poliovirus-specific RNA synthesis under conditions in which rhinovirus-specific RNA synthesis was completely inhibited might be that poliovirus polymerase has less affinity for cordycepin triphosphate than rhinovirus polymerase or that the polymerase/cordycepin triphosphate ratio was different in the two virus-cell systems at the time of labeling. The above explanations assume that cordycepin triphosphate competitively inhibits incorporation of ATP into viral RNA. Recently Weiss and Bratt (21) have suggested inhibition of protein synthesis as the mechanism for the selective inhibition by cordycepin of Newcastle disease virus genome RNA synthesis. However, their data do not preclude the alternative interpretation that cordycepin (triphosphate) inhibited incorporation of ATP into Newcastle disease virus genome RNA. We have indeed observed that cordycepin triphosphate inhibits in vitro both rhinovirus-specific and poliovirus-specific RNA synthesis by crude polymerase complexes isolated from infected HeLa cells (D. L. Panicali and C. N. Nair, manuscript in preparation). Because picornavirus RNA serves as its own messenger and picornavirus-specific polymerase is extremely labile (7), inhibition of viral RNA synthesis would be followed by rapid depletion of virus-specific polymerase in the cell.

Thus, cordycepin probably acts by inhibiting directly the activity and indirectly the synthesis of picornaviral polymerase.

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