JOURNAL OF VIROLOGY, Feb. 1973, p. 243-249

Vol. 11, No. 2
Printed in U.S.A.

Stimulation of Sendai Virion Transcriptase by Polyanions

HENRY O. STONE AND D. W. KINGSBURY

Laboratories of Virology and Immunology, St. Jude Children's Research Hospital, and The University of Tennessee Medical Units, Memphis, Tennessee 38101

Received for publication 5 September 1972

Exogenous polyribonucleotides stimulated the ribonucleic acid (RNA) transcriptase in Sendai virions. Added yeast RNA, polyadenylic acid, or polycytidylic acid increased incorporation of ^{3}H-guanosine monophosphate as much as fivefold. The products of stimulated reactions were virus-specific as determined by hybridization with Sendai virion RNA, but they sedimented more slowly (13*s*) than the product of an unstimulated reaction (16*s*). The stimulating activity was nondialyzable and heat stable, but was abolished by alkaline hydrolysis. Nucleoside monophosphates, individually or in combination, were ineffective, confirming the requirement for a polymer. Among other substances tested for effects on Sendai virion transcriptase, polyaspartic acid and polyglutamic acid stimulated the enzyme; polyinosinic acid, polyuridylic acid, and polyamines had no effect; and dextran sulfate and polyvinyl sulfate were inhibitory.

In attempting to purify the ribonucleic acid (RNA) transcriptase in Sendai virions (20, 23), we found that addition of certain polyribonucleotides and polyamino acids stimulated the crude enzyme. This phenomenon seemed worthy of further study, since it might help explain why the Sendai virion transcriptase is less active than Newcastle disease virus or vesicular stomatitis virus virion transcriptases (1, 14), and a more active enzyme might be easier to study. Our interest was further aroused by the finding that other polyribonucleotides were inactive, indicating a structural specificity which might have biological significance.

MATERIAL AND METHODS

Virus. The Sendai virus used in these experiments was plaque purified from the Enders strain and was free from incomplete virions (16). Virus was grown in embryonated eggs at 30 C, and was purified by sedimentation in sucrose gradients (23). Only virions sedimenting at 1,000*s* were used. Viral protein was measured by the Lowry (18) method, with bovine serum albumin as standard.

RNA extraction, rate zonal centrifugation, RNA hybridization, and radioactivity determination. These methods have all been described before (15, 16).

Assay of virion RNA polymerase. Sendai virion RNA polymerase was assayed as described previously (23) except that the temperature was changed to 24 C. In our earlier study (23), a temperature optimum of 28 C was determined after 90 min of incubation. When reactions were run for 4 hr or longer, more extensive RNA synthesis was observed at 24 C than at 28 C.

Materials. All materials for the RNA polymerase assay have been reported (23). ^{32}P-α-guanosine-5'-triphosphate (GTP; 3.9 Ci/mmole) was purchased from International Chemical and Nuclear Corp. Deuterium oxide (99 mole %) was obtained from BioRad Laboratories. Yeast RNA (A grade) and salmon sperm deoxyribonucleic acid (DNA) (A grade) were products of Calbiochem. Polyadenylic acid (poly A), polycytidylic acid (poly C), polyguanylic acid (poly G), polyinosinic acid (poly I), and polyuridylic acid (poly U) were obtained from Miles Laboratories. According to the manufacturer, these ribopolymers had molecular weights greater than 10^5. Sodium dextran sulfate "500" was supplied by Pharmacia. Adenosine-5'-monophosphate (AMP), cytidine-5'-monophosphate (CMP), guanosine-5'-monophosphate (GMP), uridine-5'-monophosphate (UMP), aspartic acid, glutamic acid, poly-L-aspartic acid (type I, molecular weight about 27,000), and poly-L-glutamic acid (grade III, molecular weight about 103,000) were purchased from Sigma Chemical Co. All compounds were prepared at 3 mg per ml of distilled water except when the *p*H fell outside the 7 to 8 range, in which case it was adjusted to 7.5 with tris(hydroxymethyl)aminomethane (Tris)-hydrochloride buffer.

RESULTS

Stimulation of RNA polymerase activity. A detergent is required for Sendai virion RNA polymerase activity (20, 23). However, the reaction is inhibited by detergent concentrations

greater than 0.08% (H. O. Stone and D. W. Kingsbury, *unpublished data*). This inhibition could reflect dissociation of the enzyme from the template. In fact, even at optimal detergent concentration, much of the enzyme might be dissociated from the template, accounting for the low activity of Sendai virion transcriptase (20, 23).

To test for unbound RNA polymerase, 3 μg of purified 50*s* Sendai virion RNA per ml was added to reaction mixtures containing Sendai virions at the optimal detergent concentration of 0.08%. Addition of the RNA resulted in a twofold increase in the amount of ^{3}H-GMP incorporated. The increased incorporation was into virus-specific RNA as determined by hybridization of the product with Sendai virion RNA. However, the addition of 30 μg of yeast RNA per ml to the reaction (a control in the above experiment) increased incorporation of ^{3}H-GMP fivefold (Table 1). No incorporation resulted when yeast RNA was incubated in a reaction mixture without virions, showing that the RNA was not contaminated with a polymerase.

The stimulation was dependent on the concentration of yeast RNA, but not beyond 30 μg/ml (Table 1). Poly A and poly C also stimulated the reaction at concentrations effective for yeast RNA (Table 1).

TABLE 1. *Stimulation of Sendai virion polymerase by polyribonucleotides*[a]

Polynucleotide additions (μg/ml)	^{3}H-GMP incorporated (counts/min)		
	Yeast RNA	Poly A	Poly C
0.3[b]	445	437	395
3.0	549	766	943
30	2,100	1,580	1,554
300	2,012	1,500	1,905
300, No virus	5	12	8

[a] Reaction mixtures (23) of 0.1 ml final volume containing 24 μg of Sendai protein, 0.54 nmole of ^{3}H-GTP (1.4 Ci/mmole), and the indicated additions were incubated at 24 C for 5 hr. The reactions were terminated, and ^{3}H-GMP incorporated into acid-insoluble product was measured. A complete mixture with virions which was incubated at 4 C contained 41 counts/min, and this value was subtracted from each experimental value.

[b] A reaction mixture which received no polyribonucleotide incorporated 437 counts/min over background. This represents about 0.1% of the labeled precursor. Assuming all four nucleotides were present equally in the product, this amount of product was about 5% of the mass of template RNA.

The stimulating activity of yeast RNA was not lost upon dialysis or heat denaturation, indicating that it was not a contaminating low-molecular-weight component and that a double-helical structure in the RNA was not involved. When individual mononucleotides were added to a reaction, either individually or together, no increased incorporation was observed, suggesting that an intact RNA molecule is required (Table 2). This was confirmed when the stimulating activity was progressively abolished as the RNA was depolymerized by alkaline hydrolysis (Table 2). We calculate that there is one phosphodiester bond scission per 6×10^3 nucleotides every 10 sec in 0.3 N KOH at 23 C (6). Therefore, 6 min of hydrolysis should reduce an RNA of molecular weight 3×10^5 to about 1/8 its original size, and 1 hr of hydrolysis should reduce it to about 1/64, while hydrolysis for 16 hr will produce mononucleotides. Hydrolysis did not produce inhibitors which masked the stimulating activity, since RNA hydrolyzed for 1 hr did not interfere with stimulation by 30 μg of yeast RNA per ml. Similar results were obtained with poly A. We conclude that a ribopolymer is the source of the stimulating activity.

Association of stimulated activity with Sendai virions. Isopycnic centrifugation in sucrose gradients was used to examine the association of the stimulated polymerase activity with Sendai virions. The distribution of purified virus in the gradient was determined by ultraviolet absorption (Fig. 1). Each gradient frac-

TABLE 2. *Dependence of stimulation on a polymer*[a]

Additions	Counts/min	Enhancement over control
None	329	
Yeast RNA	1,910	5.8
AMP, CMP, GMP, UMP[b]	310	0.9
Dialyzed yeast RNA	1,759	5.3
Heated/quick cooled yeast RNA	1,298	3.9
Yeast RNA, hydrolyzed[c] 10 min	1,545	4.7
Yeast RNA, hydrolyzed[c] 1 hr	266	0.8
Yeast RNA, hydrolyzed[c] 16 hr	170	0.5
Yeast RNA, hydrolyzed[c] 1 hr + unhydrolyzed yeast RNA	1,423	4.3

[a] Reaction mixtures of 0.1 ml final volume containing 18 μg of Sendai protein and 30 μg of the indicated addition per ml were incubated at 24 C for 5 hr. Complete mixtures with virions but no other additions which had been incubated at 4 C contained 68 counts/min, and this value was subtracted from all the experimental values.

[b] 7.5 μg each of AMP, CMP, GMP, and UMP were added per ml.

[c] RNA was hydrolyzed for the indicated times in 0.3 N KOH at 37 C. The hydrolyzed samples were neutralized by passage through a Dowex 50 column in the H^+ form.

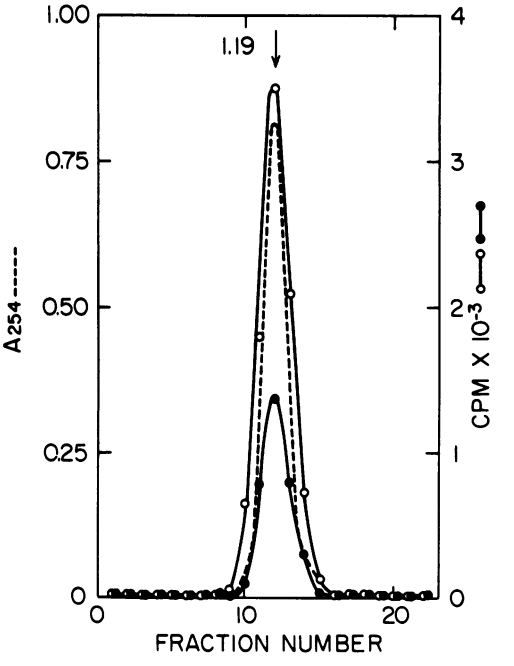

FIG. 1. *Association of stimulated activity with Sendai virions. One milligram of Sendai virus in 2 ml of 0.01 M Tris-hydrochloride, 0.03 M NaCl (pH 8.0) (TN buffer) was layered on a 35-ml linear D_2O sucrose gradient (1.15 g/cm³ to 1.30 g/cm³) in the same buffer. Centrifugation was for 16 hr at 21,000 rev/min at 4 C in a Spinco SW27 swinging-bucket rotor. Absorbance at 254 nm was monitored continuously as the gradient was fractionated. Fractions were diluted fourfold with TN buffer and centrifuged at 80,000 × g for 45 min. Pellets were resuspended in 0.1 ml of TN buffer, and 0.025-ml portions were assayed for RNA polymerase at 24 C for 5 hr. Additional 0.025-ml portions were assayed in reactions containing 30 μg of yeast RNA per ml. Symbols: ●——●, ³H-GMP incorporated into RNA in the standard reaction; ○——○, ³H-GMP incorporated into RNA in the stimulated reaction; -----, optical density at 254 nm.*

tion was assayed for RNA polymerase activity in a standard reaction and in a reaction containing 30 μg of yeast RNA per ml. There was no evidence of polymerase activity in fractions which lacked virus (Fig. 1); all the enzyme activity was virion-associated, indicating that the stimulated and unstimulated activities were the same enzyme.

Effect of poly A on the kinetics of RNA synthesis. The incorporation of ³H-GMP into an acid-insoluble product increased with time in both standard and stimulated reactions (Fig. 2). Poly A stimulated the overall rate of the reaction. Reactions stimulated by yeast RNA yielded similar data (not shown). The kinetics of RNA synthesis rule out the possibility that the effect of RNA was to stabilize a reaction which normally terminated. Poly A stimulated a standard reaction at any time after RNA synthesis started (Fig. 2); stimulation was not limited to the initiation of RNA synthesis immediately after disruption of virions. The enzyme was stimulated even after 4 hr of RNA synthesis (Fig. 2), indicating that poly A does not function by stabilizing the enzyme-template complex.

Sedimentation properties of the products. The major product of the Sendai virion polymerase made in an unstimulated reaction was shown to be single-stranded RNA, complemen-

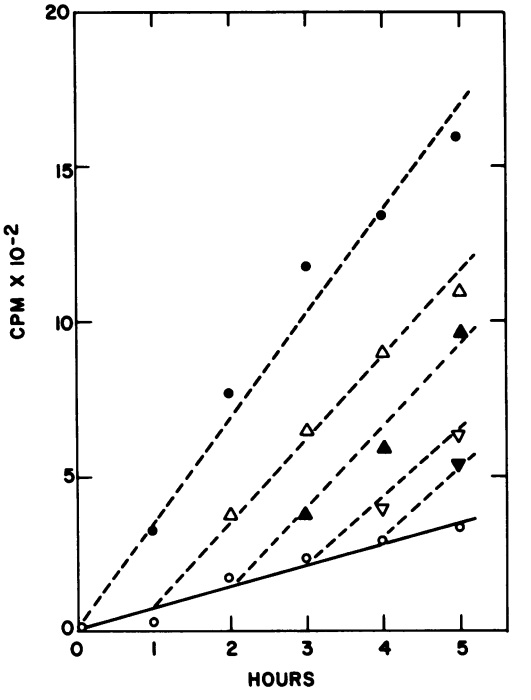

FIG. 2. *Poly A stimulation after initiation of RNA synthesis. A 5-ml reaction mixture containing 438 μg of virus protein was incubated for 5 hr at 24 C (standard reaction). A 2-ml reaction mixture containing 175 μg of virus protein and 60 μg of poly A was incubated for 5 hr at 24 C (stimulated with poly A at zero time). At hourly intervals, appropriate portions removed from the standard reaction were stimulated by the addition of 30 μg of poly A per ml and then incubated at 24 C. At hourly intervals, 0.2-ml portions were removed from all reactions, and the ³H-GMP incorporated into acid-insoluble product was measured. Symbols: ○, standard reaction; ●, stimulated with poly A at zero time; △, stimulated with poly A at 1 hr; ▲, stimulated with poly A at 2 hr; ▽, stimulated with poly A at 3 hr; ▼, stimulated with poly A at 4 hr.*

tary to 50s virion RNA, and the product sedimented at about 16s relative to 18s ribosomal RNA (reference 23 and Fig. 3, fraction 8). There was an additional peak, mostly ribonuclease-resistant, in the 50s region (reference 23 and Fig. 3, fraction 26). These results suggested that the 16s RNA was synthesized on 50s RNA template in a partially double-stranded form and then released as single-stranded RNA product (23).

The major product of a stimulated reaction sedimented at 12 to 14s (Fig. 3, fractions 6 and 7), indicating that it may be smaller than the normal product. There was an additional peak in the 50s region, about as large as the 50s peak from the unstimulated reaction, suggesting that yeast RNA did not increase the number of templates synthesizing RNA. The amount of ribonuclease-resistant radioactivity was not increased in stimulated reactions run for as little as 2 hr (data not shown).

Stimulated products were virus-specific heteropolymers. Stimulated and unstimulated enzyme products, labeled with ^{32}P-α-GTP and subjected to nearest neighbor analysis, were found to be heteropolymers (Table 3). There was close similarity, in both products, among the nearest neighbors of GMP.

To determine if the products of the stimulated reaction were complementary to viral genomes, annealing experiments were performed. Products were purified in sucrose gradients to remove 50s RNA templates from more slowly sedimenting product molecules. When labeled RNA from an unstimulated reaction was completely denatured and self-annealed, about 6% of the product became ribonuclease-resistant. When the enzyme product was completely denatured and then annealed with unlabeled Sendai virion 50s RNA, it became entirely resistant to ribonuclease (Table 4). The ribonuclease resistance of the RNA synthesized

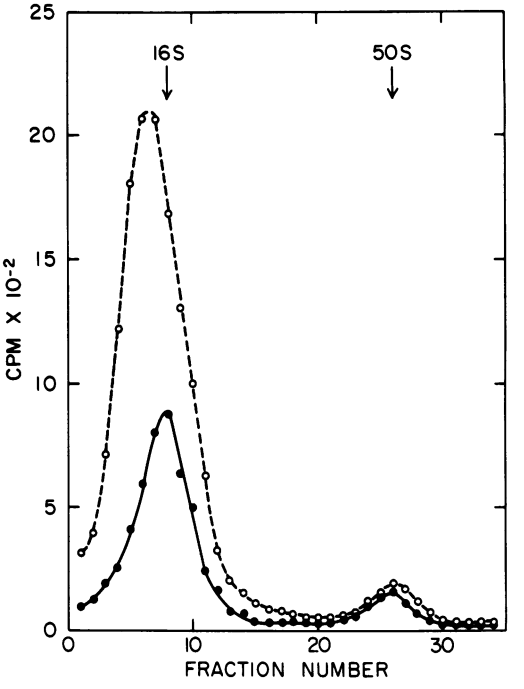

FIG. 3. *Sucrose gradient centrifugation of Sendai virion transcriptase products. Two 5-ml reaction mixtures containing 170 μg of virus protein per ml were incubated for 16 hr at 24 C. One reaction contained 30 μg of yeast RNA per ml. RNA was extracted with phenol in the presence of 0.5% sodium dodecyl sulfate (SDS) and precipitated with ethanol. The RNA extracted from each sample was dissolved in 2 ml of 0.005 M Tris-hydrochloride, 0.001 M ethylenediaminetetraacetic acid, 0.1 M NaCl, 0.5% SDS (pH 7.4) and loaded on a separate 34-ml 5 to 30% sucrose gradient in the same buffer. Centrifugation was at 18,000 rev/min for 16 hr at 20 C in a Spinco SW27 rotor. Each 1-ml fraction was precipitated by two volumes of ethanol with 100 μg of yeast RNA as carrier. Pellets were dissolved in gradient buffer lacking SDS and then acid-precipitated for counting. Symbols: O, yeast RNA-stimulated; ●, control.*

TABLE 3. *Nearest neighbors of GMP*[a]

Reaction Conditions	Mole percent[b]			
	G	U	A	C
Standard	20.8 ± 0.7	26.7 ± 0.9	38.1 ± 0.9	14.4 ± 0.7
Plus 30 μg of yeast RNA per ml	20.0 ± 1.9	22.1 ± 1.0	42.8 ± 2.0	15.1 ± 1.2

[a] Reaction mixtures contained 40 μCi of ^{32}P-α-GTP (3.9 Ci/mmole) and 390 μg of Sendai virion protein in 3 ml and were run for 24 hr. RNA was extracted with sodium dodecyl sulfate and phenol and isolated free from nucleotides by passage through a Sephadex G-50 column. RNA was hydrolyzed for 18 hr in 0.3 N KOH at 37 C, and nucleotides in the hydrolysate were separated as described elsewhere (7).

[b] Results are the means (± standard deviations) of four separate determinations. G, guanylic acid; U, uridylic acid; A, adenylic acid; C, cytidylic acid.

in a reaction stimulated by either yeast RNA or poly A was 4% when self-annealed and 90% or more when annealed with added 50*s* virion RNA (Table 4). Thus, the products in the stimulated reactions were complementary in base sequences to Sendai virion RNA, and the stimulating polyribonucleotides were not templates.

Stimulation by other substances. We tested the effects of a variety of other compounds on Sendai virion transcriptase to learn more about the chemical specificity of the stimulation. An increase in sodium chloride concentration or addition of 0.1 M ammonium sulfate inhibited the transcriptase, confirming that the stimulation did not involve ionic strength or electrostatic charge, as already indicated by results in Table 2. Three polyamines-spermine, cadaverine, and putrescine—had no effect on the enzyme at concentrations up to 30 μg/ml. Two polyamino acids, polyaspartic acid and polyglutamic acid, stimulated the Sendai virion transcriptase (Table 5), but aspartic acid or glutamic acid did not. Again, a polymer was required for stimulation. DNA was as effective as poly A and poly C in stimulating RNA synthesis. Not all polyanions stimulated the reaction, since dextran sulfate and polyvinyl sulfate were inhibitory, whereas poly I, poly G, and poly U had negligible effect (Table 5).

DISCUSSION

We have shown that certain polyanions stimulate the synthesis of virus-specific RNA by Sendai virion RNA polymerase. The addition of polyanions to polymerase reactions may facilitate studies on other viruses with low activity, just as the addition of ammonium sulfate has aided studies on animal cell polymerases (5, 11) and on reovirus polymerase (12, 13).

Numerous compounds have been reported to stimulate nucleic acid synthesis in several different cell-free systems (2–5, 9, 10, 13, 17, 19, 21, 22). Stimulation has generally been observed only in crude enzyme preparations and not in purified preparations. Most compounds which have produced stimulation fall into the following categories: salts, polyamines, and polyanions.

Ammonium sulfate stimulated the synthesis of reovirus-specific RNA in vitro by an enzyme from infected cells (12, 13). Crude preparations of mammalian DNA-dependent RNA polym-

TABLE 4. *Hybridization of transcriptase products with unlabeled Sendai virion 50s RNA*

Labeled RNA	Treatment[a]	Ribonuclease resistance (%)
16*s* unstimulated product[b]	Self-annealed	6
	Annealed with 50*s* RNA	100
12–14*s* yeast RNA-stimulated product[b]	Self-annealed	4
	Annealed with 50*s* RNA	99
12–14*s* poly A-stimulated product[b]	Self-annealed	4
	Annealed with 50*s* RNA	90

[a] Annealing was done at 80 C for 1 hr in 0.02 ml of 0.3 M NaCl, 0.03 M sodium citrate (*p*H 7.0). Sendai virion 50*s* RNA was present at 50 μg/ml. A portion of each sample was treated with 10 μg of pancreatic ribonuclease A per ml for 30 min at 24 C before acid precipitation.

[b] Each sample contained at least 1,200 counts/min.

TABLE 5. *Effect of polyanions on Sendai virion transcriptase*[a]

Polyanion concn (μg/ml)	Poly-aspartic acid	Poly-glutamic acid	Dextran sulfate	Polyvinyl sulfate	Poly I	Poly G	Poly U	DNA
0.3	421	477	390	397	455	359	459	476
3.0	575	1,393	284	270	518	247	650	548
30	1,297	1,549	102	77	671	358	488	1,508
300	1,574	1,550	126	87	396	506	523	1,840
300, No virus	68	57	46	41	67	182	17	23

[a] A control reaction containing virus but no polyanion additions had 450 counts/min. Reaction mixtures of 0.1 ml final volume containing 23 μg of Sendai protein and the indicated additions were incubated at 24 C for 5 hr. Complete reaction mixtures incubated at 4 C contained 102 counts/min, and this value was subtracted from all the experimental values except for those in the last line of the table.

erase from rat liver were also stimulated by ammonium sulfate, but purified preparations were strongly inhibited by the salt (11). The RNA polymerase in Sendai virions was inhibited by ammonium sulfate.

High salt conditions stimulated the DNA-dependent RNA polymerase from *Escherichia coli* (22). The enzyme could not release its RNA product in vitro at low ionic strength, and elevating the salt concentration permitted the release of RNA strands in a way that enabled the enzyme to initiate the synthesis of new RNA molecules (19). Increasing the salt concentration in the Sendai virion transcriptase reaction reduced the amount of RNA synthesized. In contrast to the *E. coli* system in low salt, the normal Sendai virion reaction did not terminate in the absence of polynucleotides (Fig. 2). Thus, stimulation of Sendai virion transcriptase by polynucleotides does not appear to be a salt effect and apparently does not involve reinitiation of transcription.

Polyamines, such as cadaverine and putrescine, stimulated *E. coli* DNA polymerase when the template was pea embryo nucleohistone but inhibited DNA synthesis when purified pea embryo DNA was added as template (21). Putrescine, spermine, and spermidine stimulated the DNA-dependent RNA polymerase from *Micrococcus lysodeikticus* using T7 DNA as template (8). Spermine, cadaverine, and putrescine had no effect on the Sendai virion enzyme.

Polyanions, synthetic and natural, including several kinds of RNA, stimulated nucleic acid synthesis in systems with chromatin or nuclei as template (2–5, 10, 17). These stimulations seem to involve exposure of DNA template sites by complexing between polyanions and basic proteins. Two observations argue against an analagous mechanism for stimulation of Sendai virion transcriptase. (i) There was no increase in template-product complexes in a stimulated reaction (Fig. 3), indicating that polyanions did not expose additional RNA template sites. (ii) We did not detect an increase in ribonuclease sensitivity of template RNA when ^{3}H-uridine-labeled Sendai virions were stimulated by poly A (H. O. Stone and D. W. Kingsbury, *unpublished data*).

The Sendai virion transcriptase was stimulated by yeast RNA, poly A, poly C, DNA, polyaspartic acid, and polyglutamic acid. Poly U, poly G, and poly I had little, if any, effect on the reaction, and dextran sulfate and polyvinyl sulfate were inhibitory. Clearly, as in other systems (3, 4, 10, 17), there are specific stereochemical requirements for stimulation.

In some systems, polyribonucleotides inhibited nucleic acid synthesis (8, 24). This seems to involve competition for a template site on the enzyme, and the ease with which inhibition can be achieved may reflect the affinity of the enzyme for the template (8). Apparently, the reverse transcriptase of oncornaviruses is weakly bound to its RNA template, since it is readily templated by heterologous polyribonucleotides or inhibited by them (24). Failure of polyribonucleotides to inhibit the Sendai virus transcriptase thus indicates a high affinity for its template.

Besides the possibilities discussed above, two other mechanisms for polyanion stimulation of Sendai virion transcriptase are contraindicated by our data. (i) Poly A probably does not stabilize an otherwise labile enzyme-template complex, since the normal reaction did not diminish with time, and poly A stimulated substantially even after 4 hr of RNA synthesis (Fig. 2). (ii) Inhibition of a nuclease in our virion preparations by polyanions is unlikely. The lower sedimentation rate of the stimulated product is the opposite of what would be expected if a nuclease normally degraded template or product in the unstimulated reaction. In fact, we have observed no breakdown of template or product after 5 hr of RNA synthesis (H. O. Stone and D. W. Kingsbury, *unpublished data*). Moreover, stimulation by polyamino acids seems unlikely to involve nuclease inhibition, and nuclease inhibitors such as dextran sulfate and polyvinyl sulfate did not enhance transcriptase activity.

We are left with polyanions acting on the virion transcriptase itself as the most probable explanation of our findings. Since, as discussed above, there does not appear to be increased initiation, the stimulation probably represents increased rates of nucleotide polymerization.

ACKNOWLEDGMENTS

Exeen Morgan, Andrew Moseley, and Ruth Ann Scroggs provided expert technical assistance. Allen Portner contributed enlightening discussions.

This research was supported by Public Health Service research grant AI-05343 from the National Institute of Allergy and Infectious Diseases, by Childhood Cancer research grant CA-08480 from the National Cancer Institute, and by ALSAC. H.O. Stone was the recipient of Special Research Fellowship AI-47,032 from the National Institute of Allergy and Infectious Diseases, and D. W. Kingsbury received Career Development Award HD-14,491 from the National Institute of Child Health and Human Development.

LITERATURE CITED

1. Baltimore, D., A. S. Huang, and M. Stampfer. 1970. Ribonucleic acid synthesis of vesicular stomatitis virus. II. An RNA polymerase in the virion. Proc. Nat. Acad. Sci. U.S.A. **66**:572–576.

2. Bannai, S., and H. Terayama. 1967. Regulation of RNA synthesis in chromatin (aggregate enzyme) from rat liver. Biochim. Biophys. Acta **142**:410-418.
3. Brown, D. G., and D. S. Coffey. 1971. Release of nuclear DNA template restrictions by specific polyribonucleotides. Science **171**:176-178.
4. Brown, D. G., and D. S. Coffey. 1971. The release of chromatin template restrictions by natural polyribonucleotides. Biochem. Biophys. Res. Commun. **42**: 326-333.
5. Chambon. P., M. Ramuz, P. Mandel, and J. Doly. 1968. The influence of ionic strength and a polyanion on transcription in vitro. I. Stimulation of the aggregate RNA polymerase from rat liver nuclei. Biochim. Biophys. Acta. **157**:504-519.
6. Dahlberg, J. E., and R. Haselkorn. 1966. The ribosome-binding sites in turnip yellow mosaic virus RNA. Proc. Nat. Acad. Sci. U.S.A. **55**:1269-1276.
7. East, J. L. 1968. Nucleotide composition of ribonucleic acid by spectral analysis and thin-layer chromatography. Anal. Biochem. **24**:409-418.
8. Fox, C. F., R. I. Gumport, and S. B. Weiss. 1965. The enzymatic synthesis of ribonucleic acid. V. The interaction of ribonucleic acid polymerase with nucleic acids. J. Biol. Chem. **240**:2101-2109.
9. Fox, C. F., and S. B. Weiss. 1964. Enzymatic synthesis of ribonucleic acid. II. Properties of the deoxyribonucleic acid-primed reaction with *Micrococcus lysodeikticus* ribonucleic acid polymerase. J. Biol. Chem. **239**:175-185.
10. Frenster, J. H. 1965. Nuclear polyanions as de-repressors of synthesis of ribonucleic acid. Nature (London) **206**:680-683.
11. Goldberg, M. L., H. D. Moon, and W. Rosenau. 1969. Partial purification of RNA polymerase from rat liver nuclei. Biochim. Biophys. Acta **171**:192-194.
12. Gomatos, P. J. 1968. Reovirus-specific, single-stranded RNA's synthesized in vitro with enzyme purified from reovirus-infected cells. J. Mol. Biol. **37**:423-439.
13. Gomatos, P. J. 1970. Comparison of the virion polymerase of reovirus with the enzyme purified from reovirus-infected cells. J. Virol. **6**:610-620.
14. Huang, A. S., D. Baltimore, and M. A. Bratt. 1971. An RNA polymerase in virions of Newcastle disease virus: comparison with the vesicular stomatitis virus polymerase. J. Virol. **7**:389-394.
15. Kingsbury, D. W. 1966. Newcastle disease virus RNA. II. Preferential synthesis of RNA complementary to parental viral RNA by chick embryo cells. J. Mol. Biol. **18**:204-214.
16. Kingsbury, D. W., A. Portner, and R. W. Darlington. 1970. Properties of incomplete Sendai virions and subgenomic viral RNAs. Virology **42**:857-871.
17. Kraemer, R. J., and D. S. Coffey. 1970. The interaction of natural and synthetic polyanions with mammalian nuclei. I. DNA synthesis. Biochim. Biophys. Acta **224**: 553-567.
18. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265-275.
19. Richardson, J. P. 1969. RNA polymerase and the control of RNA synthesis. Progr. Nucl. Acid Res.**9**:75-116.
20. Robinson, W. S. 1971. Ribonucleic acid polymerase activity in Sendai virions and nucleocapsid. J. Virol. **8**:81-86.
21. Schwimmer, S. 1968. Differential effects of putrescine, cadaverine, and glyoxal-bis-(guanylhydrazone) on DNA- and nucleohistone-supported DNA synthesis. Biochim. Biophys. Acta **166**:225-254.
22. So, A. G., E. W. Davie, R. Epstein, and A. Tissieres. 1967. Effects of cations on DNA-dependent RNA polymerase. Proc. Nat. Acad. Sci. U.S.A. **58**:1739-1746.
23. Stone, H. O., A. Portner, and D. W. Kingsbury. 1971. Ribonucleic acid transcriptases in Sendai virions and infected cells. J. Virol. **8**:174-180.
24. Tuominen, F. W., and F. T. Kenney. 1971. Inhibition of the DNA polymerase of Rauscher leukemia virus by single-stranded polyribonucleotides. Proc. Nat. Acad. Sci. U.S.A. **68**:2198-2202.