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Effects of Poly(9-vinyladenine) and Poly(1-vinyluracil) on Messenger Ribonucleic Acid Template Activity

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SUMMARY

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The neutral polynucleotide analogues poly(9-vinyladenine) and poly(1-vinyluracil) were found to inhibit [^{3}H]dTTP incorporation in a system containing rabbit hemoglobin mRNA as template, oligo(*dT*) as primer, and purified avian myeloblastosis RNA-dependent DNA polymerase. The incorporation was inhibited 50% at an analogue concentration of 0.1 mM in base residues. Complexes of homopolynucleotides with vinyl polymers were tested as templates in a cell-free amino acid-incorporating system prepared from Krebs II ascites cells. Poly(9-vinyladenine) inhibited poly(U)-stimulated [^{14}C]phenylalanine incorporation, while poly(1-vinyluracil) inhibited poly(A)-stimulated [^{14}C]lysine incorporation. In neither case was the noncomplementary vinyl polymer inhibitory. Although poly(9-vinyladenine) had no effect on rabbit globin mRNA-stimulated amino acid incorporation in a cell-free system prepared from the Krebs II ascites tumor, poly(1-vinyluracil) was slightly inhibitory, with 50% inhibition occurring at a concentration of 10 mM uracil residues. However, similar inhibition occurred with a preparation of mRNA which did not contain the 3′-terminal poly(A) sequence, indicating that the inhibition occurring with high concentrations of poly(1-vinyluracil) does not

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involve the 3'-teminal poly(A) of the mRNA. The radioactive proteins produced in the cell-free system both with and without vinyl polymer coelectrophoresed with rabbit globin marker. These results suggest that the 3'-terminal poly(A) sequence of mRNA does not function in cell-free protein synthesis. Furthermore, the failure of the vinyl polymers to significantly inhibit cell-free protein synthesis suggests that the mechanism of vinyl polymer inhibition of murine leukemia virus replication in mouse cells involves inhibition of RNA-dependent DNA polymerase rather than inhibition of viral protein synthesis.

INTRODUCTION

Poly(A), poly(U), and their analogues, poly(vA)[3] and poly(vU), were potent inhibitors of murine leukemia virus RNA-dependent DNA polymerase *in vitro* (1, 2). These polymers block acute murine leukemia virus infection in mouse embryo cells, but they do not inhibit the replication of Sindbis and vesicular stomatitis virus (2, 3). Although no cellular toxicity was reported for poly(vA), high concentrations of poly(vU) inhibited growth and [^{3}H]thymidine uptake by NIH 3T3 cells (2). The inhibition of RNA-dependent DNA polymerase could be caused by interaction of the inhibitors with either the template RNA or the enzyme, while the cellular toxicity could be due to similar interactions with cellular nucleic acids and enzymes.

Most eukaryotic mRNAs and viral RNAs contain a poly(A) stretch at their 3'-terminus, and the biological function of this poly(A) is not completely understood (4). Preparations of mRNA with the poly(A) removed are functional in cell-free amino acid-incorporating systems prepared from eukaryotes (5–7). However, injection of this poly(A)-deficient globin mRNA into *Xenopus* oocytes results in globin production for only a few hours, while the poly(A)-rich mRNA stimulates globin production for 48 hr (8). Furthermore, preliminary incubation of poly(A)-containing and deadenylated globin mRNA in a Krebs II cell-free homogenate at 21° for 4 hr was recently shown to destabilize preferentially the translational activity of the poly(A)-deficient mRNA (9). The increased stability of poly(A)-rich mRNA-stimulated protein synthesis indicates that the 3'-terminal poly(A) stretch of the mRNA may be involved in the stabilization of eukaryotic protein synthesis.

In this study we investigated the effects of poly(vA) and poly(vU) on rabbit globin mRNA as template for RNA-dependent DNA polymerase and protein synthesis. Vinyl polymers are synthetic analogues of homopolynucleotides with hydrocarbon chains substituting for the ribose phosphate backbone, and they form complementary base-paired complexes with polynucleotides (10–12). Vinyl polymers inhibit both complementary homopolynucleotide-stimulated binding of aminoacyl-tRNA to ribosomes and polypeptide synthesis in cell-free systems prepared from *Escherichia coli* by forming complementary base-paired complexes with the template polynucleotide (13). We have now studied vinyl polymer complexes of homopolynucleotides and hemoglobin mRNA as templates for cell-free protein synthesis and DNA synthesis catalyzed by purified RNA-dependent DNA polymerase.

MATERIALS AND METHODS

[^{14}C]Phenylalanine and [^{14}C]lysine with specific activities of 455 and 342 mCi/mmole were obtained from Schwarz-Mann. [^{3}H]Leucine and [^{3}H]dTTP with specific activities of 33 and 20 Ci/mmole were obtained from New England Nuclear. Poly(A) (s_{20} of 7–11 S), poly(U) (s_{20} of 3–6 S), and oligo(dT) were obtained from Miles Laboratories. Creatine phosphate, creatine phosphokinase, dithiothreitol, nucleotide triphosphates, and Sigmacell type 38 cellulose were purchased from Sigma Chemical Company. The chemical syntheses of vinyl polymers were described

[3] The abbreviations used are; poly(vA), poly(9-vinyladenine); poly(vU), poly(1-vinyluracil); TCA, trichloracetic acid; S-30, a protein synthesis system prepared by homogenization and centrifugation at 30,000 × g.

previously (10, 11). Vinyl polymers are linear, without branching, with s_{20} values of approximately 5 S, but with a wide distribution of molecular weights (12). Concentrations of polynucleotides and vinyl polymers are expressed as A_{260} units or as moles of base residues. One A_{260} unit is the quantity of material in 1 ml which will yield an absorbance of 1.0 at 260 nm. The extinction coefficients used were: for poly(A), 10,100 at 259 nm, pH 7.0 (14); for poly(U), 9430 at 261 nm, pH 7.5 (15); for poly(vA), 9000 at 256 nm, pH 7.0 (10); for poly(vU), 6000 at 264 nm, pH 7.0 (11).

Extraction and purification of messenger RNA. Reticulocyte polysomes, prepared from phenylhydrazine-treated rabbits, were dissolved at a concentration of 100–200 A_{260} units/ml in Tris-HCl buffer, pH 9.0, and 10 mM EDTA and made 0.5% in sodium dodecyl sulfate (16, 17). The polysomes were extracted five times with an equal volume of buffer-saturated phenol–chloroform, 1:1 (v/v), and the RNA was precipitated with ethanol. Poly(A)-rich RNA was purified by either oligo(*dT*)-cellulose (Collaborative Research, Inc.) or Sigmacell type 38 cellulose chromatography, and these preparations consisted of approximately 50% 10 S globin mRNA (18, 19). Poly(A)-deficient globin mRNA of mouse spleen erythroblasts was prepared as described previously, and only the fraction not retained by oligo(*dT*)-cellulose in 0.5 M NaCl was employed in these studies (5). The preparation consisted of approximately 10% 8.5 S poly(A)-deficient globin mRNA.

RNA-dependent DNA polymerase reactions. Avian myeloblastosis viral polymerase was purified as described previously, and the enzyme was stored in 1 mM dithiothreitol and 50% (v/v) glycerol at −20° (20). The assay mixture (0.1 ml) contained 50 mM Tris-HCl (pH 8.3), 6 mM $MgCl_2$, 50 mM KCl, 0.2 mM unlabeled dATP, dCTP, and dGTP, and 40 μM [^{3}H]dTTP. Template mRNA (3 μg/reaction) was incubated for 10 min with vinyl polymers before oligo(*dT*) (0.2 μg/reaction) was added. The reactions were started by the addition of enzyme and were incubated at 37° for 45 min, precipitated by the addition of cold 5% TCA, filtered onto Millipore membranes, and quantitated by liquid scintillation counting.

Protein synthesis by a Krebs II cell-free system. A previously incubated cell-free protein synthesis system was prepared from Krebs II ascites cells, and the supernatant following 30,000 × *g* centrifugation (S-30) was employed in all protein synthesis experiments (21). It was enriched with a partially purified rabbit reticulocyte initiation factor preparation (22). Incubations of 50 μl contained 50 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid buffer (pH 7.5), 100 mM potassium chloride, 3 mM magnesium acetate, 1 mM dithiothreitol, 1 mM ATP, 0.1 mM GTP, 0.6 mM CTP, 10 mM phosphocreatine, 1 enzyme unit of creatine phosphokinase, 40 μM each of 19 [^{12}C]L-amino acids (minus the radioactive one), 2.5 or 0.25 μCi of [^{3}H]- or [^{14}C]amino acids, respectively, 0.1 A_{260} unit of Krebs II S-30, and 0.1 mg of protein of reticulocyte initiation factors. Vinyl polymers, mRNA preparations, and homopolynucleotides were as described in the figure legends, and they were always annealed at 24° for 10 min before the addition of the Krebs II S-30 and initiation factors to the reactions. Samples (10–40 μl) of each reaction were spotted on filters (Whatman No. 3, 1.6-cm diameter), which were placed in 10% TCA with 1% casein hydrolysate, heated to 100° for 5 min, and further processed as described by Bollum (23). Radioactivity was measured by liquid scintillation counting. Reaction samples for electrophoretic analysis were processed by the addition of EDTA to 10 mM and a mixture of 10 enzyme units of T_1 ribonuclease and 20 μg of ribonuclease A (Sigma), with incubation for 20 min at 37°.

Electrophoretic characterization of proteins synthesized in the cell-free system. Reaction mixtures were diluted to 100 μl with water and centrifuged at 50,000 rpm in a type 65 rotor (Beckman) for 60 min in polycarbonate screw-cap tubes. Postribosomal supernatants were precipitated with 10% TCA, heated to 90° for 20 min, cooled, and centrifuged. The precipitates were then washed and centrifuged successively five times with 5% TCA, and once each

with ethanol, an ethanol–diethyl ether mixture (1:1, v/v), and diethyl ether, with drying under vacuum. The samples were then dissolved in 10 mM sodium phosphate buffer (pH 7.0), 1% sodium dodecyl sulfate, and 1% 2-mercaptoethanol, and subjected to electrophoresis as described (24). Gels were sliced into 2-mm portions, which were digested with 0.2 ml of 30% hydrogen peroxide in scintillation vials overnight at 50°. Radioactivity was measured in Aquasol (New England Nuclear).

RESULTS

Effects of vinyl polymers on RNA-dependent DNA polymerase using rabbit globin mRNA as template and oligo(dT) as primer. Because previous experiments which demonstrated vinyl polymer inhibition of RNA-dependent DNA polymerase used detergent-disrupted virus and polynucleotide template, experiments were designed employing purified hemoglobin mRNA as template and purified avian myeloblastosis virus RNA-dependent DNA polymerase. Hemoglobin mRNA did not stimulate [^{3}H]dTTP incorporation in the absence of oligo(*dT*), and with oligo(*dT*) primer added, the incorporation was 34.0 pmoles/0.1 ml of reaction mixture. Omission of the nonradioactive dGTP, dATP, or dCTP, either individually or in combination, resulted in a decrease in the [^{3}H]dTTP incorporation to 3.0 pmoles/0.1 ml. In experiments not shown, the DNA product hybridized to globin mRNA under conditions which minimized the formation of poly(rA)·poly(dT). These results suggest that copying of the main portion of the globin mRNA had occurred, as was previously reported for this system (25–27). The incorporation was 50% inhibited by either vinyl polymer at a concentration of 0.1 mM, with almost total inhibition occurring at 1 mM (Fig. 1).

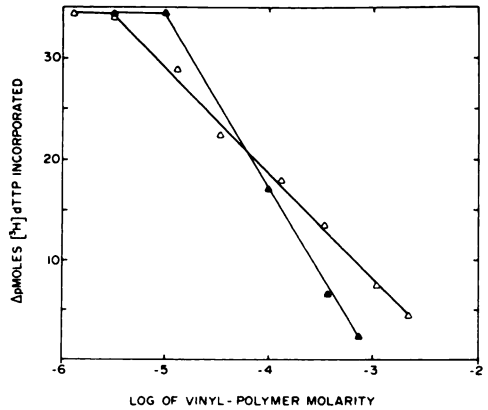

FIG. 1. *Effects of vinyl polymers on RNA-dependent DNA polymerase, using rabbit globin mRNA as template and oligo(dT) as primer*

The incubation conditions are described under MATERIALS AND METHODS, and the vinyl polymers were incubated with mRNA (3 μg) for 10 min at 24° before the addition of oligo(*dT*) (0.2 μg) and the enzyme. Reaction mixtures were then incubated at 37° for 45 min. An incorporation of 0.38 pmole of [^{3}H]dTTP in the absence of oligo(*dT*) was subtracted from all values shown, and mRNA with oligo(*dT*)-stimulated incorporation in the absence of vinyl polymer was 34.0 pmoles. Neither vinyl polymer stimulated [^{3}H]dTTP incorporation in the absence of globin mRNA. The values shown are incorporation in the presence of poly(vU) (△——△) and poly(vA) (▲——▲).

Vinyl polymer inhibition of homopolynucleotide-stimulated incorporation of amino acids by a Krebs II ribosomal system. The vinyl polymers were first tested for inhibition of homopolynucleotide-stimulated amino acid incorporation in a Krebs II cell-free system. Increasing amounts of poly(U) were annealed at 24° for 10 min with 125 nmoles (2.5 mM) of poly(vA) or poly(vU). Addition of Krebs II S-30 and incubation revealed that [^{14}C]-phenylalanine incorporation was inhibited by poly(vA) but not by poly(vU). Furthermore, this inhibition was reversible by the addition of more poly(U) (Fig. 2). In a similar experiment 60 nmoles (1.2 mM) of poly(vU) completely inhibited poly(A)-stimulated [^{14}C]lysine incorporation at low concentrations of poly(A), with recovery of the stimulation by the addition of more poly(A) (Fig. 3). On the other hand, 60 nmoles of poly(vA) increased the poly(A)-stimulated incorporation. As in the bacterial cell-free system, vinyl polymer inhibition of complementary polynucleotide-stimulated amino acid incorporation in Krebs II S-30 is dependent on the ratio of vinyl polymer to polynucleotide. If the vinyl polymer is in excess, the reaction is inhibited; however, if the polynucleotide is in excess,

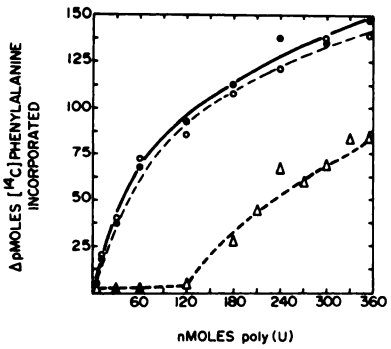

FIG. 2. *Poly(vA) inhibition of poly(U)-stimulated [14C]phenylalanine incorporation by a Krebs II ribosomal system*

The incubation conditions are described under MATERIALS AND METHODS, except that the magnesium acetate was increased to 5 mM. Poly(U) was annealed with the vinyl polymers for 10 min at 24° before the addition of Krebs II S-30 and incubation at 37° for 1 hr. Neither vinyl polymer stimulated [14C]phenylalanine incorporation into polyphenylalanine in the absence of poly(U). An endogenous incorporation of 9 pmoles of [14C]phenylalanine per 50 μl of reaction mixture was subtracted from the values shown: poly(U) (●——●), poly(U) plus 125 nmoles of poly(vU) (○- - -○), and poly(U) plus 125 nmoles of poly(vA) (Δ- - -Δ).

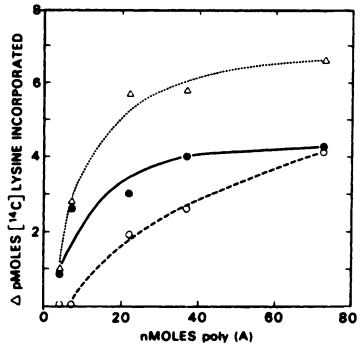

FIG. 3. *Poly(vU) inhibition of poly(A)-stimulated [14C]lysine incorporation by a Krebs II cell-free ribosomal system*

The assay was conducted as described in Fig. 2, except that the reactions were stopped with TCA–tungstate reagent (0.25% sodium tungstate in 5% TCA at pH 2.0). Neither vinyl polymer stimulated [14C]lysine incorporation into polylysine in the absence of poly(A). Endogenous incorporation of 0.14 pmole of [14C]lysine per 50 μl of reaction mixture was subtracted from each point. Incubation conditions were: ●——●, poly(A) alone; ○- - -○, poly(A) plus 60 nmoles of poly(vU); Δ····Δ, poly(A) plus 60 nmoles of poly(vA).

the normal polynucleotide-stimulated amino acid incorporation occurs. The inhibitory combining ratio for poly(vA) with poly(U) is approximately 1:1. This combining ratio is consistent with the relative molecular sizes of the two polymers and with the known physicochemical properties of the complex (12). The combining ratio shown in Fig. 3 for poly(vU) with poly(A) is approximately 9:1. This is consistent with spectroscopic and electron microscopic evidence that poly(vU) forms multiple complexes with poly(A) (12).

Effects of vinyl polymers on globin mRNA-stimulated amino acid incorporation by a Krebs II cell-free ribosomal system. Rabbit globin mRNA, containing

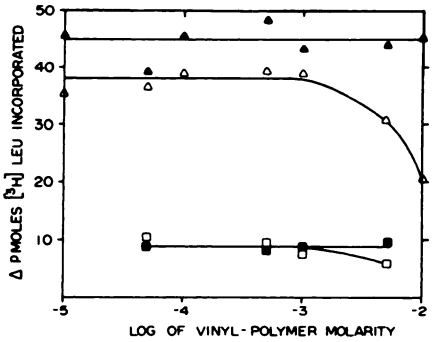

FIG. 4. *Effect of different concentrations of vinyl polymers on globin mRNA translation in a Krebs II ribosomal system*

The preparation of the Krebs II cell-free ribosomal system and the incubation conditions are described under MATERIALS AND METHODS. Globin mRNA was incubated at 24° for 10 min in the indicated concentrations of vinyl polymer. Reactions were started by the addition of Krebs II S-30 and rabbit reticulocyte initiation factor preparation, with incubation at 37° for 1 hr. Endogenous incorporation of 4 pmoles of [3H]leucine per 50 μl of reaction mixture was subtracted from each point. Poly(A) containing rabbit globin mRNA (3 μg/50 μl of reaction) stimulated [3H]leucine incorporation to 45.5 pmoles. ▲——▲, incorporation in the presence of increasing concentrations of poly(vA); Δ——Δ, in the presence of increasing concentrations of poly(vU). Poly(A)-deficient mouse globin mRNA preparation (10 μg, of which approximately 1 μg was 8.5 S mRNA) stimulated [3H]leucine incorporation to 13.4 pmoles/50 μl of reaction mixture. ■——■, results of incubations containing this mRNA plus increasing concentrations of poly(vA); □——□, mRNA plus poly(vU).

poly(A) at the 3'-terminus, stimulated [^{3}H]leucine incorporation by Krebs II S-30 10-fold (Fig. 4). This stimulation was unaffected by the addition of poly(vA) to concentrations of 10 mM. Poly(vU) inhibited this reaction in the 0.01–1 mM range approximately 15%; in the 1–10 mM range the reaction was more severely inhibited. A 1:1 complex should be formed between 0.01 mM poly(vU) and the 3'-poly(A) segment of rabbit globin mRNA, and a maximum complex of 9:1 would be formed at 0.1 mM, assuming a 10–20% 3'-terminal poly(A) content of the 3 μg/50-μl reaction mixture of rabbit globin mRNA used. Consequently 10 mM poly(vU) represents a 100-fold excess above the optimum 9:1 complex formation, and this concentration inhibits rabbit globin mRNA translation only by 50%. Although this inhibition, at very high concentrations of poly(vU), cannot be taken as evidence for involvement of the poly(A) portion of rabbit globin mRNA in protein synthesis, the slight inhibition which occurs at low concentrations of poly(vU) could possibly represent alteration of the translational process.

Consequently experiments were designed employing a preparation of mouse globin mRNA which is substantially deficient in the 3'-poly(A) segment (6). This mRNA stimulated [^{3}H]leucine incorporation approximately 3-fold (Fig. 4). The stimulation was only slightly inhibited by the presence of either vinyl polymer at concentrations of 1 mM or lower, while at very high concentrations poly(vA) had no effect and poly(vU) inhibited the incorporation approximately 50%. Although these data point to nonspecific inhibition of the system, it was possible that the poly(A)-rich rabbit globin mRNA inhibition by poly(vU) resulted from overlapping of large complexes of poly(vU) onto the information-containing portion of the mRNA. This would result in the production of smaller proteins.

For this reason the size of the proteins produced in rabbit globin mRNA-stimulated incorporations was determined by sodium dodecyl sulfate–polyacrylamide gel electrophoresis. The ^{3}H-proteins produced in cell-free amino acid-incorporating systems were compared with ^{14}C-proteins produced by incubating rabbit reticulocytes with [^{14}C]leucine (Fig. 5). No differences between the [^{14}C]globin or the [^{3}H]globin produced in the cell-free system could be observed, nor was there any difference in the products from incubations containing poly(vA). Products isolated from reaction mixtures containing poly(vU), although containing less total radioactivity, were identical in size with the rabbit [^{14}C] globin and the rabbit [^{3}H] globin prepared in cell-free incubation mixtures. Not only does the rabbit globin mRNA stimulate incorporation of [^{3}H]leucine into proteins

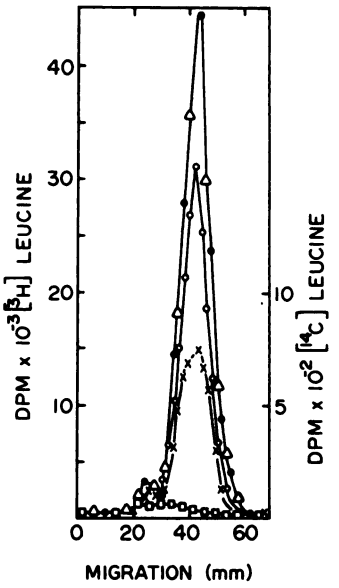

FIG. 5. *Dodecyl sulfate–polyacrylamide gel electrophoresis of [^{3}H]leucine-labeled protein from Krebs II cell-free incubations*

Incubation conditions are described under MATERIALS AND METHODS and in the legend to Fig. 4. [^{3}H]Leucine-labeled proteins were isolated and the gels were processed as described under MATERIALS AND METHODS. [^{14}C]Leucine-labeled rabbit globin marker was subjected to electrophoresis together with the [^{3}H]leucine-labeled proteins. The radioactivity was measured by liquid scintillation counting as double labels, and the 10% spill of ^{14}C counts into the tritium channel was subtracted from each point. □——□, electrophoresis of [^{3}H]leucine-labeled proteins isolated from endogenous incorporation; ●——●, mRNA alone; △——△, mRNA plus 4.7 mM poly(vA); ○——○, mRNA plus 3 mM poly(vU); ×——×, rabbit [^{14}C]globin marker.

which coelectrophorese with rabbit globin, but neither vinyl polymer induces any alteration in the electrophoretic migration of the product. This result suggests that the slight inhibition observed represents total cessation of the translation of globin mRNA species, either through a mechanism involving massive interaction with the globin mRNA or through nonspecific poisoning of the system.

DISCUSSION

Vinyl polymers have been shown to form complexes with complementary polynucleotides, and these complexes have been characterized by spectroscopy and electron microscopy (12). The vinyl polymer inhibition of globin mRNA-dependent DNA polymerase incorporation of [^{3}H]dTTP is consistent with the previously reported inhibition of the detergent-disrupted virus and oligo(*dT*)·poly(rA)-templated system. However, the mechanism of blockade of viral replication in mouse cells could be considerably more complex. Since poly(vU) interaction with viral mRNAs could also influence viral protein synthesis and thus viral replication, the effects of vinyl polymers on protein synthesis were investigated.

The poly(vA) inhibition of poly(U)-stimulated [^{14}C]phenylalanine incorporation in the Krebs II cell-free system was identical with that previously observed in the *E. coli* cell-free system (13). The relatively large amount of poly(vU) required to inhibit poly(A)-stimulated [^{14}C]lysine incorporation is probably due to the relative sizes of the two polymers and to the type of complex formed beteen them. The complexes formed consist of a ribbon of poly(A) with loops of poly(vU) attached (12). However, the mechanism of the vinyl polymer-mediated inhibition involves covering of the information contained in the homopolynucleotide templates, thus preventing template-stimulated amino acid incorporation. The increased observed in poly(A)-stimulated [^{14}C]lysine incorporation in the presence of poly(vA) could be due to a very weak interaction between poly(vA) and poly(A). This weak interaction could loosen the tertiary structure of the template poly(A) sufficiently to produce the stimulated [^{14}C]lysine incorporation. Although this interaction is not detectable by spectroscopic methods, it was revealed by electrophoresis of poly(A) on gels in which poly(vA) was copolymerized with acrylamide.[4]

Complex formation between poly(vU) (0.01–1 mM) and mRNA should preferentially involve the poly(A) region at the 3′-end of mRNA; yet no substantial effect of poly(vU) on mRNA-stimulated protein synthesis was observed. Nonetheless, significant inhibition of the RNA-dependent DNA polymerase reaction occurred in the 0.01 mM poly(vU) range, where no effect was observed in cell-free amino acid-incorporating systems. As both the RNA-dependent DNA polymerase reaction and the cell-free amino acid incorporation are conducted under similar conditions of salt concentrations and temperature, complex formation between poly(vU) and the poly(A) of the globin mRNA and subsequent inhibition of the oligo(*dT*) stimulation of the RNA-dependent DNA polymerase reaction indicate that complex formation should occur under the conditions of cell-free protein synthesis. At very high concentrations of poly(vU) (10 mM), when inhibition of protein synthesis occurs, short oligo(A) regions from the information portion of the mRNA could also be involved. These data, taken with the finding that preparations of mRNA with their poly(A) region complexed to poly(U) initiate protein synthesis, strongly indicate that mRNA-stimulated cell-free amino acid incorporation does not involve the poly(A) region of the mRNA (5–8, 28). The failure to detect a requirement for poly(A) in protein synthesis by cell-free systems could be due to the short-term incubation employed in these experiments. Subtle stabilization of protein synthesis might not be detectable for 24–48 hr, as with the globin mRNA in the *Xenopus* oocyte system (8).

The lack of inhibition of protein synthesis by vinyl polymers at concentrations 10-fold higher than necessary to drastically inhibit RNA-dependent DNA polymerase suggests that vinyl polymers are selective

[4] J. Pitha, personal communication.

agents. It is highly unlikely that murine leukemia virus replication is blocked by inhibition of viral protein synthesis at concentrations of vinyl polymer which do not affect protein synthesis *in vitro*. In addition, the selective action of vinyl polymers suggests that they may be useful in elucidating the biological function of other naturally occurring polynucleotides. Modification of synthetic polymers to include different backbones and specific base sequences could lead to the design of specific inhibitors of cellular or viral functions.

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REFERENCES

1. Tuominen, F. W. & Kenney, F. T. (1971) *Proc. Natl. Acad. Sci. U. S. A.*, 68, 2198–2202.
2. Pitha, P. M., Teich, N., Lowy, D. R. & Pitha, J. (1973) *Proc. Natl. Acad. Sci. U. S. A.*, 70, 1204–1208.
3. Tennant, R. W., Farrely, J. G., Ihle, J. N., Pal, B. C., Kenney, F. T. & Brown, A. J. (1973) *J. Virol.*, 12, 1216–1225.
4. Darnell, J. E., Jelinek, W. R. & Molloy, G. R. (1974) *Science*, 181, 1215–1221.
5. Cheng, T., Polmar, S. K. & Kazazian, H. H., Jr. (1974) *J. Biol. Chem.*, 249, 1781–1786.
6. Bard, D., Efron, E., Marus, A. & Perry, R. (1974) *Cell*, 1, 101–106.
7. Williamson, R., Crossley, J. & Humphries, S. (1974) *Biochemistry*, 13, 703–707.
8. Huez, G., Marbaix, G., Hubert, E., Leclercq, M., Nudel, U., Soreq, H., Salomon, R., Lebleu, B., Revel, M. & Littaner, U. Z. (1974) *Proc. Natl. Acad. Sci. U. S. A.*, 71, 3143–3146.
9. Sippel, A. E., Stavrianopoulos, J. G., Schutz, G. & Feigelson, P. (1974) *Proc. Natl. Acad. Sci. U. S. A.*, 71, 4635–4639.
10. Pitha, P. M. & Pitha, J. (1970) *Biopolymers*, 9, 965–977.
11. Pitha, J., Pitha, P. M. & Ts'o, P. O. P. (1970) *Biochim. Biophys. Acta*, 204, 39–48.
12. Pitha, J., Pitha, P. M. & Stuart, E. (1971) *Biochemistry*, 10, 4595–4602.
13. Reynolds, F., Grunberger, D., Pitha, J. & Pitha, P. M. (1972) *Biochemistry*, 11, 3261–3266.
14. Holcomb, D. N. & Tinoco, I., Jr. (1965) *Biopolymers*, 3, 121–133.
15. Blake, R. D., Massouli, J. & Fresco, J. R. (1967) *J. Mol. Biol.*, 30, 291–308.
16. Gilbert, J. M. & Anderson, W. F. (1970) *J. Biol. Chem.*, 245, 2342–2349.
17. Brawerman, G., Mendecki, J. & Lee, S. Y. (1972) *Biochemistry*, 11, 637–641.
18. Aviv, H. & Leder, P. (1972) *Proc. Natl. Acad. Sci. U. S. A.*, 69, 1408–1412.
19. Schutz, G., Beato, M. & Feigelson, P. (1972) *Biochem. Biophys. Res. Commun.*, 49, 680–689.
20. Kacian, D. L., Watson, K. R., Burny, A. & Spiegelman, S. (1971) *Biochim. Biophys. Acta*, 246, 365–383.
21. Aviv, H., Boime, I. & Leder, P. (1971) *Proc. Natl. Acad. Sci. U. S. A.*, 68, 2303–2307.
22. Metafora, S., Terada, M., Dow, L. W., Marks, P. A. & Banks, A. (1972) *Proc. Natl. Acad. Sci. U. S. A.*, 69, 1299–1303.
23. Bollum, F. D. (1968) *Methods Enzymol.*, 12B, 169–173.
24. Weber, K. & Osborn, M. (1969) *J. Biol. Chem.*, 244, 4406–4412.
25. Kacian, D. L., Spiegelman, S., Bank, A., Terada, M., Metafora, S., Dow, L. & Marks, P. A. (1972) *Nat. New Biol.*, 235, 167–169.
26. Ross, J., Aviv, H., Scolnick, E. & Leder, P. (1972) *Proc. Natl. Acad. Sci. U. S. A.*, 69, 264–268.
27. Verma, I. M., Temple, G. F., Fan, H. & Baltimore, D, (1972) *Nat. New Biol.*, 235, 163–167.
28. Munoz, R. F. & Darnell, J. E. (1974) *Cell*, 2, 247–252.