MOLECULAR PHARMACOLOGY, 12, 844-853

Polyxanthylic and Polyguanylic Acid Inhibition of Murine Leukemia Virus Activities

S. K. ARYA, T. L. HELSER, AND W. A. CARTER

Department of Medical Viral Oncology, Roswell Park Memorial Institute, and Graduate Faculty in Microbiology, State University of New York at Buffalo, Roswell Park Division, Buffalo, New York 14263

P. O. P. Ts'o

Department of Biochemical and Biophysical Sciences, School of Hygiene and Public Health, The Johns Hopkins University, Baltimore, Maryland 21205

(Received March 22, 1976)

(Accepted May 7, 1976)

SUMMARY

ARYA, S. K., HELSER, T. L., CARTER, W. A. & Ts'o, P. O. P. (1976) Polyxanthylic and polyguanylic acid inhibition of murine leukemia virus activities. *Mol. Pharmacol.*, 12, 844-853.

Single-stranded polyribonucleotides inhibit the replication of murine leukemia virus (MuLV) in cultured cells. Our previous studies suggested that (a) purine polyribonucleotides may be more potent than pyrimidine polyribonucleotides, and (b) the inhibitory potency depends on the functional groups on the pyrimidine ring. In our earlier studies, a set of polyribonucleotides was not available which differed only in their purine as opposed to pyrimidine base; thus we could not clearly differentiate the effect of the base from that of the functional groups on the ring system. Poly(xanthylic acid) now provides the means to accomplish this. It is a purine polyribonucleotide with the same functional groups as poly(uridylic acid), a pyrimidine polyribonucleotide. Similarly, poly(guanylic acid) can be compared with poly(cytidylic acid), although the disposition of the functional groups is not identical in this case. We now report that poly(xanthylic acid) is, in fact, 2-3 times more potent than poly(uridylic acid) as an inhibitor of MuLV replication, and poly(guanylic acid) is similarly more potent than poly(cytidylic acid). Neither poly(xanthylic acid) nor poly(guanylic acid) significantly affects the growth rates of cultured host cells; thus their inhibition is apparently not related to any general cytotoxicity. Poly(xanthylic acid) as well as poly(guanylic acid) strongly inhibits MuLV-associated, RNA-directed DNA polymerase *in vitro*. They also inhibit host cell DNA polymerase activities *in vitro*, but analysis of inhibition kinetics shows that their inhibitor potency for viral DNA polymerase is several times greater than for cell DNA polymerases. These results substantiate our hypothesis that purine polyribonucleotides are more potent than pyrimidine polyribonucleotides. They also support the notion that inhibition of virus replication by polyribonucleotides may be related to their selective inhibition of viral RNA-directed DNA polymerase activity.

This work was supported in part by United States Public Health Service Center Grant CA-14801 in Viral Chemotherapy and Regulation.

INTRODUCTION

Single-stranded polyribonucleotides inhibit the replication of RNA tumor viruses

in cultured cells (1–5). They also reportedly suppress virus infection *in vivo* (3, 6). Tennant *et al.* (7) noted that poly(adenylic acid) and poly(2′-*O*-methyladenylic acid) have two concentration-dependent effects on the murine leukemia virus infection of cultured cells. At high concentration (100 μg/ml), poly(A) and poly(Am)[1] inhibited the uptake of MuLV by cultured Swiss mouse embryo cells; at low concentration (10 μg/ml), neither poly(A) nor poly(Am) affected uptake of the virus, and poly(Am) inhibited virus replication significantly.

We have previously reported the inhibition of progeny virus synthesis in MuLV-infected, cultured, mouse bone marrow-derived JLS-V9 cells by several polyribonucleotides at low concentrations (2–20 μg/ml) (4). These polyribonucleotides included poly(inosinic acid), poly(uridylic acid), poly(cytidylic acid), poly(A), and their 2′-*O*-alkyl derivatives. The results of these studies suggested that (a) purine polyribonucleotides may be more potent than pyrimidine polyribonucleotides, and (b) the potency of inhibition depends on the functional groups of the ring system. However, a set of polyribonucleotides was not available for these studies which differed only in their purine as opposed to pyrimidine base and also did not differ in the ring functional groups (see Fig. 1). Thus the contribution of the base to inhibitory potency could not be clearly differentiated from that of the ring functional groups. Poly(xanthylic acid) now provides the means to accomplish this. It is a purine polyribonucleotide with the same ring functional groups as poly(U), a pyrimidine polyribonucleotide. Similarly, poly(guanylic acid), a purine polyribonucleotide, can be compared with poly(C), a pyrimidine polyribonucleotide, although the disposition of the functional groups is not identical in this case (Fig. 1). Therefore we have extended our studies to poly(X) and poly(G) and report here that these polyribonucleotides show significant inhibition of virus replication in cultured cells at low concentrations (5–20 μg/ml). In view of our previously reported results (4), we find that poly(X) is, in fact, more potent than poly(U), and poly(G) is more potent than poly(C). These results substantiate our hypothesis that purine polyribonucleotides may be more potent than pyrimidine polyribonucleotides. Poly(X) and poly(G) also inhibit MuLV RNA-directed DNA polymerase *in vitro*, thus supporting the notion that inhibition of virus replication by polyribonucleotides may be related to their inhibition of virus-associated DNA polymerase activity.

[1] The abbreviations used are: poly(Am), poly(2′-*O*-methyladenylic acid): MuLV, Moloney murine leukemia virus; PFU, plaque-forming units; PBS, 0.02 M sodium phosphate (pH 7.4)–0.15 M NaCl.

MATERIALS AND METHODS

Polynucleotides. Poly(X) (4.7 S), poly(G) (13.3 S), poly(A) (8.1 S), poly[d(A–T)] (15.1 S), and oligo(dT)$_{12-18}$ were obtained from Miles Laboratories. The concentration of polynucleotide in 0.01 M NaCl–0.01 M Tris-HCl (pH 7.2) was determined spectrophotometrically by using the following absorbtivities for a 1 mg/ml solution: poly(X), 23.4 at 260 nm; poly(G), 24.5 at 260 nm; poly(A), 28.5 at 258 nm; poly[d(A–T)], 20.8 at 260 nm. Tritium-labeled thymidine triphosphate (50 Ci/mmole) was obtained from New England Nuclear Corporation, and unlabeled nucleoside triphosphates, from P-L Biochemicals.

Cells and virus. Mouse bone marrow-derived JLS-V9 cells (8) were grown as monolayers in plastic flasks, using RPMI-1640 culture medium containing 10% fetal calf serum (Grand Island Biological Company). The Moloney strain of murine leukemia virus was obtained from cultured JLS-V9 cells chronically infected with this virus. The culture medium from such cells, after being clarified by slow centrifugation, was used as a virus inoculum for uninfected cells. The preparations of virus used in this study contained 8–12 × 10^6 PFU/ml of virus when measured by the XC plaque assay (9).

Purified virions were obtained from the medium of the infected cells harvested on a continuous basis as described before (10). This involved rate-zonal sucrose gradient centrifugation of the virus preparation, followed by banding to equilibrium in sucrose gradients.

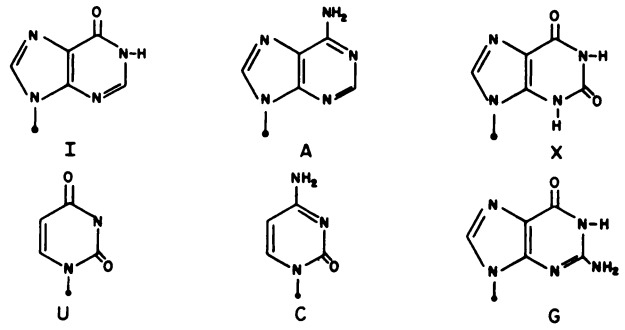

FIG. 1. *Structures of constituent bases of polyribonucleotides*
I, inosine; A, adenine; X, xanthine; U, uracil; C, cytosine; G, guanine.

Polyribonucleotide treatment and virus infection. Duplicate or triplicate monolayer cultures of JLS-V9 cells ($1.6 \pm 0.2 \times 10^6$ cells/25-cm^2 plastic flask) were treated for 30 min with 2 ml of DEAE-dextran (10 μg/ml) in RPMI-1640 medium, washed with PBS, and incubated for 2 hr with 2 ml of polyribonucleotide solution in RPMI-1640 medium. The control cultures received medium alone. Cultures were washed with PBS and infected with MuLV by incubating them for 1 hr with 2 ml of virus inoculum (multiplicity of infection = 5–6 PFU/cell). Cultures were washed again with PBS and incubated for 20 hr with 5 ml of polyribonucleotide solution in RPMI-1640 medium plus 10% fetal calf serum; the control cultures received medium without polyribonucleotide. Subsequently cultures were washed with PBS and reincubated with fresh RPMI-1640 medium plus 10% fetal calf serum but without polyribonucleotide. Twenty hours later the culture medium was harvested and the titer of progeny virus in the harvested medium was determined by XC plaque assay (9) and by measuring the DNA polymerase activity of the partially purified virions in the harvested medium as described before (4).

For determining the effect of polyribonucleotides on the growth rates of cultured cells, eight replicate monolayer cultures of infected JLS-V9 cells (1.6×10^4 cells/16-mm plate) and triplicate monolayer cultures of MuLV-infected JLS-V9 cells (2.8×10^4 cells/16-mm plate) were treated for 30 min with 1 ml of DEAE-dextran (10 μg/ml) as described above. They were then grown in the continuous presence of 1 ml of polyribonucleotide in RPMI-1640 plus 10% fetal calf serum. Cultures were replenished every 24 hr with fresh medium containing polyribonucleotide. The control cultures received medium without polyribonucleotide. At specified times the cells were detached from the surface by mild trypsinization and counted. In some experiments, monolayers were treated in a manner identical with that described above for virus infection but were mock-infected, and the cells were counted at intervals. All operations were carried out at 37° in a humidified CO_2 incubator.

Viral and cellular DNA polymerases. The MuLV DNA polymerase was partially purified according to Wu and Gallo (11). Briefly, a suspension of purified virions was disrupted with Triton X-100 in the presence of a high salt concentration, and the solubilized preparation was chromatographed on a column containing phosphocellulose which was eluted with a linear KCl gradient. The fractions containing poly(A):oligo(dT)-directed DNA polymerase activity were pooled and used as a source of partially purified MuLV DNA polymerase.

The JLS-V9 cellular DNA polymerases were partially purified by a procedure similar to that described by Spadari and Weissbach (12). The pooled cytoplasmic and nuclear extracts, solubilized with Triton X-100, were applied to a column containing DEAE-cellulose. The column was eluted with a linear KCl gradient, and

fractions containing DNA polymerase activity were pooled and applied to phosphocellulose. The column was developed with a linear KCl gradient, and fractions were assayed for DNA polymerase activity using activated DNA, poly[d(A-T)], and poly(A):oligo(dT) as templates. Three peaks of DNA polymerase activity, eluted at 0.16 M, 0.24 M, and 0.34 M, were detected. We have tentatively termed these activities polymerases I, II, and III, respectively. These activities apparently show differential preferences for various templates. For example, polymerase I utilizes activated DNA as a template more efficiently than other templates, and polymerase III shows some preference for poly(A):oligo(dT) over poly[d(A-T)]. These activities are being further characterized, and the details of purification and characterization will form the subject of a separate report.

Polyribonucleotide inhibition of DNA polymerase activities. The DNA polymerase assay with MuLV DNA polymerase was performed in a reaction mixture (50 or 100 μl) containing 0.05 M Tris-HCl (pH 7.9), 0.06 M NaCl, 0.001 M $MnCl_2$, 0.02 M dithiothreitol, 50 μM [^{3}H]dTTP (2600 cpm/pmole), 5–100:0.5–10 μM poly(A):oligo(dT) (molar ratio, 10:1), various concentrations of inhibitor polyribonucleotide, and partially purified MuLV DNA polymerase or purified MuLV virions plus 0.05% NP-40. The cellular DNA polymerase assays utilizing poly(A):oligo(dT) as a template were performed in a similar reaction mixture, except that KCl replaced NaCl. The reaction mixture for cellular DNA polymerase utilizing poly[d(A-T)] as a template contained 0.05 M Tris-HCl (pH 7.9), 0.06 M KCl, 0.006 M magnesium diacetate, 0.02 M dithiothreitol, 50 μM [^{3}H]dTTP (2600 cpm/pmole), 100 μM dATP, 10–100 μM poly[d(A-T)], various concentrations of inhibitor polyribonucleotide, and the enzyme preparation. The reaction mixture was incubated at 37°, and the radioactivity incorporated into acid-insoluble material was determined by trichloracetic acid precipitation of aliquots (10 or 20 μl) withdrawn at specified times as described before (13). Under these conditions the reaction mixture contained a limiting concentration of the enzyme and saturating concentrations of deoxyribonucleoside triphosphates.

The kinetic data were treated according to Lineweaver and Burk by constructing $1/v$ vs. $1/S$ plots (14). The concentration of the template was used as the substrate concentration (S), and the incorporation of the precursor deoxynucleoside triphosphate was taken as a measure of template utilization, or velocity of the reaction (v). The Michaelis constant K_m was estimated from the intercept on the $1/S$ axis, and the inhibition constant K_i, from the slopes of the lines representing the uninhibited and inhibited reaction (14).

RESULTS

Polyribonucleotide effects on virus replication. The effects of poly(X) and poly(G) on progeny virus synthesis in cultured JLS-V9 cells infected with MuLV are summarized Table 1. For these studies, mono-

TABLE 1

Polyguanylic and polyxanthylic acid inhibition of Moloney murine leukemia virus infection of cultured JLS-V9 cells

Duplicate or triplicate monolayers of cultured cells were treated with DEAE-dextran (10 μg/ml, 30 min), washed with PBS, and incubated for 2 hr in RPMI-1640 culture medium with or without polynucleotide (10 μg/ml). They were washed with PBS and infected with MuLV (multiplicity of infection = 5–6 PFU/cell) for 60 min. The cultures were washed with PBS and incubated for 20 hr in culture medium plus 10% fetal calf serum with or without polynucleotide (10 μg/ml). They were washed again with PBS and reincubated with fresh culture medium plus 10% fetal calf serum lacking polynucleotide. Twenty hours later the culture medium was harvested and the titer of progeny virus was estimated by XC plaque assay and by determining the DNA polymerase activity of partially purified virions in the harvested medium.

Polynucleotide	XC plaque-forming assay		DNA polymerase assay	
	Activity	Inhibition	Activity	Inhibition
	PFU/ml $\times 10^{-4}$	%	*pmoles/ml*	%
None	87.5 ± 2.5		95.0 ± 6.8	
Poly(X)	42.5 ± 2.5	51.4	47.8 ± 1.4	49.7
Poly(G)	62.5 ± 7.5	28.5	67.5 ± 4.0	28.8
Uninfected	0		0.4 ± 0.01	

layer cultures of JLS-V9 cells were treated with a 10 μg/ml solution of polyribonucleotide and infected with MuLV as described in MATERIALS AND METHODS. The titer of progeny virus in the medium harvested 40 hr after infection was determined by XC plaque assay as well as by measuring the poly(A):oligo(dT)-directed DNA polymerase activity of the partially purified virions in the harvested medium (4). The estimates of virus titer and of the extent of inhibition scored by these two assays appear to be in good agreement. A similar correlation between these two assays was reported by Stephenson *et al.* (15) and Arya *et al.* (4). At a concentration of 10 μg/ml, poly(X) and poly(G), respectively, yielded about 50% and 28% inhibition of progeny virus synthesis relative to the control cultures, which did not receive polyribonucleotide.

The dependence of inhibition of progeny virus synthesis on the concentration of these polyribonucleotides is depicted in Fig. 2, which shows the log dose-response curves for poly(X) and poly(G). For this set of experiments, the titer of progeny virus in the medium harvested from control and polyribonucleotide-treated cultures was estimated by the virion-associated, RNA-directed DNA polymerase assay, and the results are presented as percentage inhibition relative to the controls. As expected, the degree of inhibition increases with increasing concentration of polyribonucleotide. The concentration of poly(X) yielding 50% inhibition of progeny virus synthesis is about 9 μg/ml, and that of poly(G) is about 18.5 μg/ml.

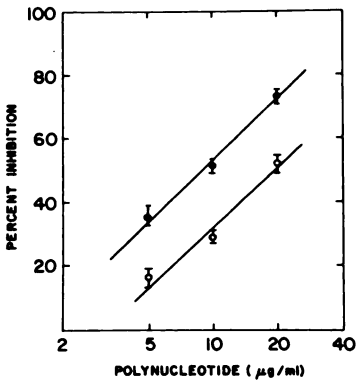

FIG. 2. *Concentration dependence of polyxanthylic and polyguanylic acid inhibition of murine leukemia virus synthesis in cultured JLS-V9 cells*

The protocol of polyribonucleotide treatment and virus infection was the same as described in the legend to Table 1. The titer of progeny virus in the harvested medium was assayed by determining the DNA polymerase activity of partially purified virions in the harvested medium. ●——●, poly(X), ○——○, poly(G).

Since it is known that inhibitors of cell division also inhibit the synthesis of RNA tumor viruses (for recent reviews, see refs. 16 and 17), it was important to determine whether the inhibition of virus synthesis by poly(X) and poly(G) might not be due to their cytotoxicity for the cultured cells used in this study. Tennant *et al.* (1) reported that poly(G) is cytotoxic for cultured Swiss mouse embryo cells, albeit at the relatively high concentration of 100 μg/ml.

Therefore we investigated the effects of poly(G) and poly(X) on the growth rates of cultured JLS-V9 cells at concentrations used for their effect on virus replication. Figure 3 shows the effects of poly(G) and poly(X) on the population growth rates of uninfected and MuLV-infected cultured JLS-V9 cells. For these studies, monolayer cultures of cells were treated with DEAE-dextran, grown in the continuous presence of polyribonucleotide, and replenished every 24 hr with fresh medium containing polyribonucleotide. It is apparent that at 10 μg/ml, neither poly(G) nor poly(X) has any marked effect on the population growth rates of cultured JLS-V9 cells (Fig. 3a). Similarly, these polyribonucleotides do not appear to affect significantly the growth rates of MuLV-infected JLS-V9 cells (Fig. 3b). When the viability of the cultured cells was scored by the trypan blue exclusion test (18), no difference between the control and polyribonucleotide-treated cultures was observed. Since the cells were grown in the continuous presence of the polynucleotides, these results diminish the possibility that polymer degradation products significantly affect cell growth. In addition, the cells in monolayer cultures used for the study of polyribonucleotide inhibition of progeny virus synthesis were routinely counted at the end of the experiment. No appreciable differ-

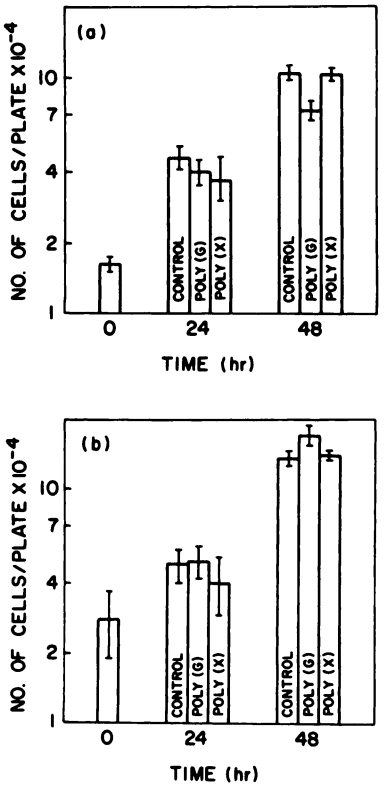

FIG. 3. *Effect of polyxanthylic and polyguanylic acid on growth of uninfected (a) and MuLV-infected (b) JLS-V9 cells*

Replicate monolayers of cultured cells in 16-mm plastic plates were treated with DEAE-dextran (10 μg/ml, 30 min), washed with PBS, and incubated with RPMI-1640 medium containing polyribonucleotide (10 μg/ml). Control cultures received medium alone. At specified times monolayers were trypsinized gently and the number of cells in the culture was counted.

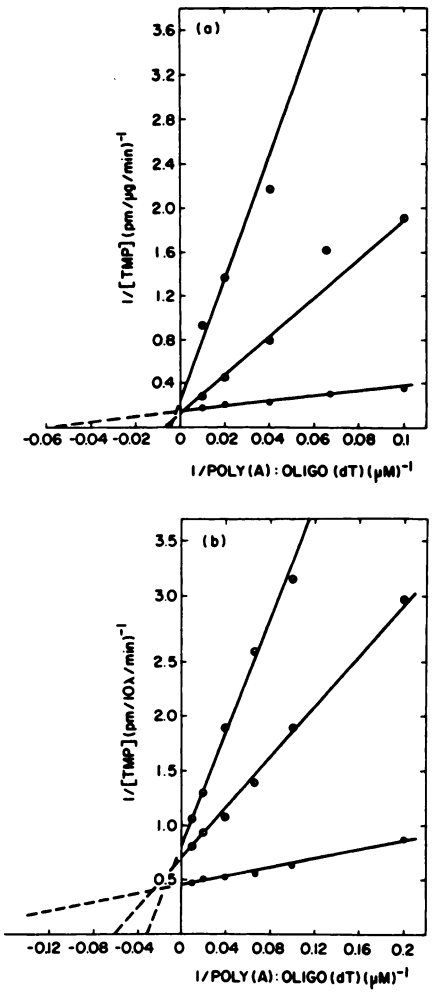

FIG. 4. *Kinetics of poly(X) inhibition of detergent-disrupted (a) and partially purified (b) MuLV DNA polymerase*

The reaction mixture (50 or 100 μl) contained 0.05 M Tris-HCl (pH 7.9), 0.06 M NaCl, 0.02 M dithiothreitol, 0.001 M $MnCl_2$, 50 μM [^{3}H]dTTP (2600 cpm/pmole), 5–100:0.5–10 μM poly(A):oligo(dT) (molar ratio, 10:1), 2 or 10 μM poly(X), and purified MuLV preparation plus 0.05% NP-40 or partially purified MuLV DNA polymerase. The reaction mixture was incubated at 37° for 10 min, and aliquots (10 or 20 μl) were precipitated with trichloroacetic acid. The radioactivity incorporated into acid-insoluble material was counted. ●——●, incorporation in the absence of poly(X); ◐——◐, in the presence of 2 μM poly(X); ⊖——⊖, 10 μM poly(X).

ences between the population densities in control and polyribonucleotide-treated cultures were noted.

Polyribonucleotide effects on DNA polymerases. The effects of poly(X) and poly(G) on detergent-disrupted and partially purified MuLV DNA polymerase as well as on host cell (JLS-V9) DNA polymerases were also investigated. Poly(X) and poly(G) strongly inhibit MuLV DNA polymerase activity. Figure 4 shows the kinetics of poly(X) inhibition of detergent-disrupted, virion-associated, and partially purified MuLV RNA-dependent DNA polymerase directed by poly(A):oligo(dT). Similar data for poly(G) inhibition of MuLV DNA polymerase are shown in Fig. 5. Judging from these Lineweaver-Burk plots, the kinetics of inhibition apparently

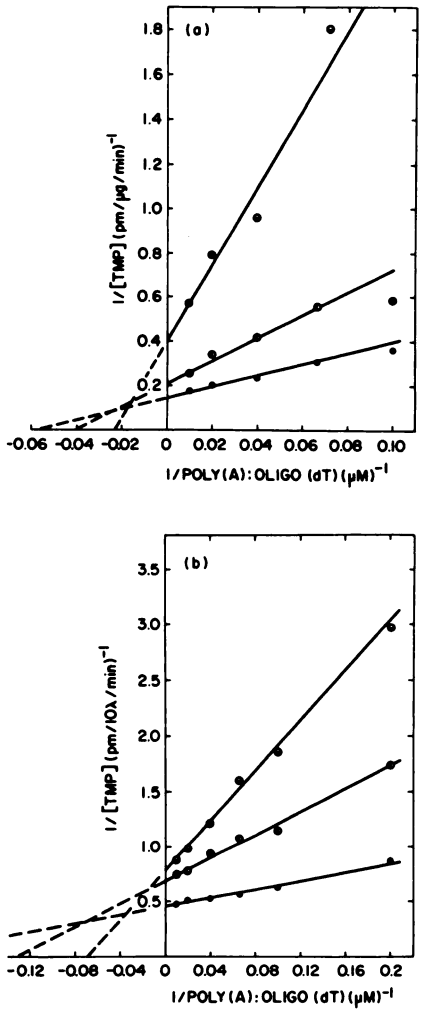

FIG. 5. *Kinetics of poly(G) inhibition of detergent-disrupted (a) and partially purified (b) MuLV DNA polymerase*

The reaction conditions were the same as described for Fig. 4. ●——●, incorporation in the absence of poly(G); ◐——◐, in the presence of 2 μM poly(G); ⊖——⊖, 10 μM poly(G).

is consistent with neither simple competitive nor noncompetitive inhibition. It appears to be a mixed type of inhibition, possibly partially competitive (14); both the K_m and V_{max} of the reaction are affected by the inhibitor polyribonucleotide. The kinetic constants can be obtained from these plots regardless of the type of inhibition. The Michaelis constant K_m is estimated from the intercept on the $1/S$ axis of the line representing the reaction in the absence of inhibitor. The inhibition constant K_i is estimated from the relative slopes of lines representing the uninhibited and inhibited reaction. These constants are listed in Table 2.

The K_m of poly(A):oligo(dT) for detergent-disrupted, virion-associated DNA polymerase is about 18 μM, and that for partially purified viral DNA polymerase is about 4 μM. The K_i values of poly(X) for detergent-disrupted and partially purified viral DNA polymerase activities are about 0.35 μM and 0.13 μM, respectively. The K_i values of poly(G) for these two viral DNA polymerase activities are 1.8 μM and 0.34 μM, respectively (Table 2). The reasons for the differences in the K_m of the template and the K_i values of the inhibitors between the detergent-disrupted and the partially purified viral DNA polymerase activities are not clear; they may be related to interaction of the template and inhibitors with extraneous proteins and other macromolecules present in the detergent-disrupted virion preparation. Similar differences in the K_m of the templates for detergent-disrupted and partially purified avian myeloblastosis virus DNA polymerase activities have been reported recently by Erickson and Grosch (19).

Poly(X) and poly(G) also inhibit the partially purified host cell DNA polymerase activities. We have isolated three apparent DNA polymerase activities from JLS-V9 cells and have tested two of these activities for polyribonucleotide inhibition. These two activities, termed DNA polymerases I and III, show some preference for DNA and RNA templates, respectively (see MATERIALS AND METHODS). Figure 6 depicts the effect of poly(X) on the kinetics of polymerization by partially purified JLS-V9 DNA polymerases I and III. Figure 6a shows the kinetics of poly(X) inhibition of the poly[d(A-T)]-directed reaction of DNA polymerase I, and Fig. 6b presents the kinetic data for poly(A):oligo(dT)-directed reaction of DNA polymerase III. Similar kinetic plots were obtained for poly(G) inhibition of these cellular DNA polymerase activities (data not shown). As with viral DNA polymerase (Figs. 4 and 5), the kinetics of inhibition is consistent with neither simple competitive nor non-

TABLE 2

Polyguanylic and polyxanthylic acid inhibition of Moloney murine leukemia virus and JLS-V9 cell DNA polymerases

K_m and K_i values were estimated from Lineweaver-Burk ($1/v$ vs. $1/S$) plots. At least five concentrations of the template:primer and two concentrations of the inhibitor polynucleotide were used. The concentrations are expressed as molar nucleotide residues in the single strand.

DNA polymerase	K_m of template	Inhibition constant, K_i	
		Poly(X)	Poly(G)
	μM	μM	μM
Virion-associated MuLV DNA polymerase	18.2[a]	0.35 ± 0.01	1.8 ± 0.2
Partially purified MuLV DNA polymerase	4.0[a]	0.13 ± 0.03	0.34 ± 0.06
Partially purified JLS-V9 DNA polymerase I	9.4[b]	5.2 ± 0.4	17.8 ± 4.0
Partially purified JLS-V9 DNA polymerase III	8.3[a]	4.5 ± 0.2	7.0 ± 2.5

[a] The template:primer was poly(A):oligo(dT) (molar ratio, 10:1).

[b] The template:primer was poly[d(A-T)]n

competitive inhibition. It appears to be a mixed type of inhibition; both the K_m and V_{max} of the reaction are affected (14). The kinetic constants estimated from these plots for poly(X) and similar plots for poly(G) are listed in Table 2. The K_i values of poly(X) for DNA polymerases I and III are 5.2 μM and 4.5 μM, respectively, and those of poly(G) are 17.8 μM and 7.0 μM. It is notable that the RNA-directed DNA polymerase activity of cellular DNA polymerases is affected by these polyribonucleotides more than the DNA-directed DNA polymerase activity.

DISCUSSION

Our previous studies of polyribonucleotide inhibition of murine leukemia virus replication suggested that (a) purine polyribonucleotides may be more potent than pyrimidine polyribonucleotides, and (b) inhibitory potency depends on the functional groups of the ring system (4). However, for these studies, a set of polyribonucleotides was not available which differed only in their purine as opposed to pyrimidine base. Thus a clear distinction between the contribution of the base and of functional groups to the inhibitory potency could not be made. Poly(X) now provides the means to accomplish this. It is a purine polyribonucleotide with the same functional groups as poly(U), a pyrimidine polyribonucleotide (see Fig. 1). Similarly, poly(G), a purine polyribonucleotide, can be compared with poly(C), a pyrimidine polyribonucleotide, although the disposition of the ring functional groups is not identical in this case (see Fig. 1).

The results presented in this report show that poly(X) and poly(G) can specifically inhibit the synthesis of murine leukemia virus in cultured cells. At a concentration of 10 μg/ml, poly(X) and poly(G) yield, respectively, about 50% and 28% inhibition of progeny virus synthesis relative to the controls. This is to be compared with about 20% and 10% inhibition previously observed for poly(U) and poly(C), respectively (4). The differences among the polyribonucleotides may appear to be small, but they are reproducible. Thus poly(X) is about 2.5 times more potent than poly(U), and poly(G) is about 3 times more potent than poly(C). These results therefore substantiate our hypothesis that purine polyribonucleotides may be more potent than pyrimidine polyribonucleotides.

The role of the ring functional groups in the inhibitory potency of polyribonucleotides is not yet fully defined. We suggested previously that polyribonucleotides possessing a ring ketoamino function may be more potent than polyribonucleotides lacking this function. This appears to be the

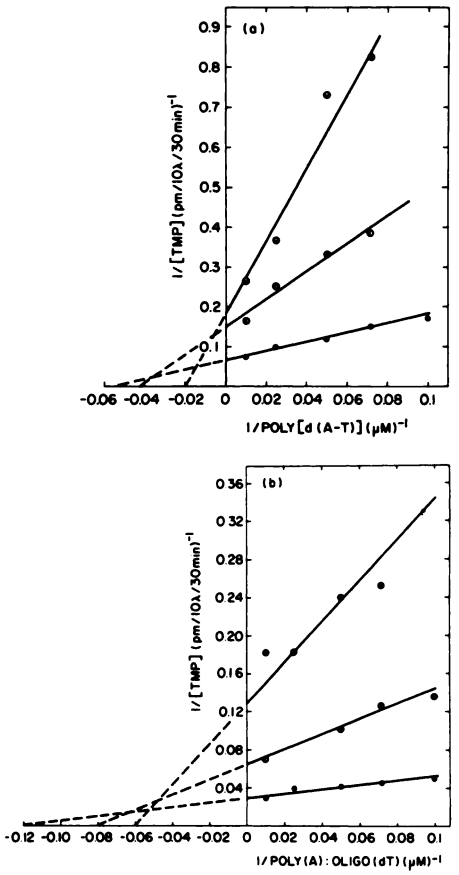

FIG. 6. *Kinetics of poly(X) inhibition of JLS-V9 DNA polymerases I (a) and III (b)*

The reaction mixture (50 or 100 μl) for polymerase I contained 0.05 M Tris-HCl (pH 7.9), 0.06 M KCl, 0.02 M dithiothreitol, 0.006 M magnesium diacetate, 50 μM [^{3}H]dTTP (2600 cpm/pmole), 100 μM dATP, 10–100 μM poly[d(A-T)], 10 or 40 μM poly(X), and polymerase preparation. The reaction mixture for polymerase III was the same as described for Fig. 4 except that NaCl was replaced by KCl. The reaction mixture was incubated at 37° for 30 min, and aliquots (20 or 40 μl) were precipitated with trichloroacetic acid. ●——●, incorporation in the absence of poly(X); ◐——◐, in the presence of 10 μM poly(X); ⊖——⊖, 40 μM poly(X).

case for polyribonucleotide inhibition of viral DNA polymerase *in vitro* (see below). Our results for polyribonucleotide inhibition of virus replication are consistent with this hypothesis, with one exception. Poly(A), which lacks the ring ketoamino function, is apparently more potent than poly(G), which possesses it. The concentration of poly(A) yielding 50% inhibition of virus replication is 12 μg/ml (4), and that of poly(G) is 18.5 μg/ml (Fig. 2). The reason(s) for this discrepancy is not clear. It is possible that poly(A) inhibits some additional intracellular activities that are not affected by poly(G) and other polyribonucleotides. Furthermore, the exact physicochemical basis of the observed structure-activity relationships is not yet clear. It should be noted that polyribonucleotides containing a ketoamino function that are more potent in our biological and biochemical assays also show a higher tendency toward self-aggregation. Poly(X) and poly(G), as well as poly(I), can form multistranded complexes in solution (20–22). This raises the possibility that the biologically active form of these polyribonucleotides may be a multistranded structure. Thus the higher potency of ketoamino-containing polyribonucleotides may not be related to the interaction of these groups with yet unidentified receptor(s) but may be due to their influence on the potential conformation of these polyribonucleotides. It is not yet known whether these polyribonucleotides do indeed exist as self-aggregated, multistranded structures intracellularly. The explanation(s) for the observed relationships obviously must reside in the solution properties (including cellular transport and half-life) of these polyribonucleotides and in their interaction with the molecular target(s) or receptor(s).

Lack of any marked effect of poly(X) and poly(G) on host cell growth would suggest that the normal cellular biosynthetic activities are not greatly affected by these polyribonucleotides. On the other hand, poly(X) and poly(G) strongly inhibit MuLV DNA polymerase *in vitro*. The relative inhibitory potency of these and other polyribonucleotide for viral DNA polymerase is generally the same as observed for viral replication. [As noted above, poly(A) is an exception; it is less potent than poly(G) *in vitro* but more potent than poly(G) in cell culture.] These observations suggest that the polyribonucleotide inhibition of virus replication may be related to, or a consequence of, their inhibition of intracellular viral RNA-directed

DNA polymerase activity. This notion is supported by the observations that (a) polyribonucleotides do not inhibit the replication of lytic RNA viruses which do not require the RNA-directed DNA polymerase activity (2, 7) and (b) they do not affect the induction of endogenous AKR virus by iododeoxyuridine from virus-negative AKR cells (7, 23). It is possible that inhibition by polyribonucleotides may also be related to their effects on virus adsorption and penetration. We have already noted that polyadenylic acids at high concentration (100 μg/ml) inhibit virus uptake, but at low concentration (10 μg/ml), such as those used in this study, they do not affect virus uptake (7). In addition, if the inhibition by polyribonucleotides is due solely to their effect on virus uptake, it is difficult to perceive why a specific set of structure-activity relationships will be obtained. Nevertheless, this aspect merits further investigation.

Poly(X) and poly(G) also inhibit partially purified host cell DNA polymerase activities *in vitro*. However, their inhibitory potency for cellular DNA polymerases is several times lower than that for viral DNA polymerase. These results are not inconsistent with the suggestion that polyribonucleotides may be relatively selective inhibitors of viral RNA-directed DNA polymerase. Since these polyribonucleotides do not affect appreciably the growth rates of host cells, their inhibition of cellular DNA polymerases *in vitro* appears not to be involved in their effect on virus replication.

ACKNOWLEDGMENTS

We thank Ms. C. Hejna and Mr. A. Cairo for technical assistance, and Drs. B. I. Milavetz and J. S. Horoszewicz for providing a partially purified preparation of murine leukemia virus DNA polymerase.

REFERENCES

1. Tennant, R. W., Kenney, F. T. & Tuominen, F. W. (1972) *Nat. New Biol.*, 238, 51-53.
2. Pitha, P. M., Teich, N. M., Lowry, D. R. & Pitha, J. (1973) *Proc. Natl. Acad. Sci. U. S. A.*, 70, 1204-1208.
3. Arya, S. K., Carter, W. A., Alderfer, J. L. & Ts'o, P. O. P. (1975) *Mol. Pharmacol.*, 11, 501-505.
4. Arya, S. K., Carter, W. A., Alderfer, J. L. & Ts'o, P. O. P. (1976) *Mol. Pharmacol.*, 12, 234-241.
5. DeClerq, E., Billiau, A., Hobbs, J., Torrence, P. F. & Witkop, B. (1975) *Proc. Natl. Acad. Sci. U. S. A.*, 72, 284-288.
6. Tennant, R. W., Hanna, M. G. & Farrelly, J. G. (1974) *Proc. Natl. Acad. Sci. U. S. A.*, 71, 3167-3171.
7. Tennant, R. W., Farrelly, J. G., Ihle, J. N., Pal, B. C., Kenney, F. T. & Brown, A. (1973) *J. Virol.*, 12, 1216-1225.
8. Wright, B. S., O'Brien, P. A., Shibley, G. P., Mayyasi, S. A. & Lasfarques, J. C. (1967) *Cancer Res.*, 27, 1672-1675.
9. Rowe, W. P., Pugh, W. E. & Hartley, J. W. (1970) *Virology*, 42, 1136-1139.
10. Arya, S. K., Carter, W. A., Alderfer, J. L. & Ts'o, P. O. P. (1974) *Biochem. Biophys. Res. Commun.*, 59, 608-615.
11. Wu, A. M. & Gallo, R. C. (1974) *Biochim. Biophys. Acta*, 340, 419-436.
12. Spadari, S. & Weissbach, A. (1974) *J. Biol. Chem.*, 249, 5809-5815.
13. Arya, S. K., Carter, W. A., Alderfer, J. L. & Ts'o, P. O. P. (1975) *Mol. Pharmacol.*, 11, 421-426.
14. Webb, J. L. (1963) *Enzyme and Metabolic Inhibitors*, Vol. 1, pp. 149-180, Academic Press, New York.
15. Stephenson, J. R., Reynolds, R. K. & Aaronson, S. A. (1972) *Virology*, 48, 749-756.
16. Temin, H. (1974) *Adv. Cancer Res.*, 19, 48-104.
17. Green, M. & Gerard, G. F. (1974) *Prog. Nucleic Acid Res. Mol. Biol.*, 14, 187-334.
18. Phillips, H. J. & Terryberry, J. E. (1957) *Exp. Cell Res.*, 13, 341-347.
19. Erickson, R. J. & Grosch, J. C. (1974) *Biochemistry*, 13, 1987-1993.
20. Michelson, A. M. & Monny, C. (1966) *Biochim. Biophys. Acta*, 129, 460-474.
21. Arnott, S., Chandrasekaran, R. & Marttila, C. M. (1974) *Biochem. J.*, 141, 537-543.
22. Zimmerman, S. B., Cohen, G. H. & Davies, D. R. (1975) *J. Mol. Biol.*, 92, 181-192.
23. Pitha, P. M., Pitha, J. & Rowe, W. P. (1975) *Virology*, 63, 568-572.