MOLECULAR PHARMACOLOGY, 16, 297-305

Polyribonucleotide Inhibition of Ribonucleic Acid Directed Deoxyribonucleic Acid Polymerase of Mouse Mammary Tumor (Type B) Virus and Simian Sarcoma (Type C) Virus

S. K. ARYA,[1] B. GORDON, AND N. A. YOUNG

Viral Oncology and Molecular Pathology Section, Laboratory of Pathology, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20014

(Received December 11, 1978)
(Accepted February 26, 1979)

SUMMARY

ARYA, S. K., B. GORDON, AND N. A. YOUNG: Polyribonucleotide inhibition of ribonucleic acid directed deoxyribonucleic acid polymerase of mouse mammary tumor (type B) virus and simian sarcoma (type C) virus. *Mol. Pharmacol.* **16**, 297-305, (1979).

The response of the RNA-directed DNA polymerases of mouse mammary tumor virus (MMTV) and simian sarcoma virus (SiSV) to synthetic polynucleotides is investigated in this study. Detergent-disrupted, virus-associated—as well as partially purified DNA polymerase—activities were analyzed. DNA polymerase activities from both viruses were inhibited by single-stranded polyribonucleotides. While no major qualitative differences were found, the quantitative responses of the DNA polymerases of the two viruses were different. Analysis of inihibition kinetics revealed the following order of inhibitory potency: Poly(U) > poly(I) ≫ poly(A) for MMTV DNA polymerase and poly(I) > poly(U) ≫ poly(A) for SiSV DNA polymerase. Poly(A) was less and poly(U) was more inhibitory for SiSV DNA polymerase when compared with MMTV DNA polymerase. In addition, MMTV DNA polymerase displayed roughly the same affinity for the template: primers poly(A):oligo(dT) and poly(C):oligo(dG). In contrast, SiSV DNA polymerase preferred poly(C):oligo(dG) over poly(A):oligo(dT).

INTRODUCTION

Single-stranded polyribonucleotides inhibit the functions of RNA tumor viruses *in vitro* (1-10) and *in vivo* (11-13). We have previously reported the effect of several polyribonucleotides on the replication of Moloney murine leukemia virus (MuLV)[2] in cultured murine cells (8-10). We noted that poly(I) specifically inhibits the synthesis of MuLV proviral DNA in cultured cells (10), suggesting that polyribonucleotides affect intracellular viral RNA-directed DNA synthesis. This was in accord with our previous findings that these polymers inhibit MuLV RNA-directed DNA polymerase *in vitro* (4, 7, 9). For several polyribonucleotides studied, a good correlation was observed between their effect on virus replication in cell culture and viral DNA

[1] An IPA research scientist at the National Cancer Institute, on leave of absence from Roswell Park Memorial Institute, Buffalo, New York.

[2] The abbreviations used are: MuLV, murine leukemia virus; MMTV, mouse mammary tumor virus; SiSV, simian sarcoma virus; AMV, avian myeloblastosis virus; poly(A), poly(riboadenylic acid); poly(C), poly(ribocytidylic acid); poly(I), poly(riboinosinic acid); poly(U), poly(ribouridylic acid); oligo(dT), oligo(deoxythymidylic acid); oligo(dG), oligo(deoxyguanylic acid).

0026-895X/79/040297-09$02.00/0

polymerase *in vitro* (7–10). Similar effects of some polyribonucleotides have been reported for murine leukemia-sarcoma viruses (1, 3), and avian and feline leukemia-sarcoma viruses (5, 6).

Studies reported thus far have been largely confined to type C RNA tumor viruses, which differ from type B RNA tumor viruses morphologically as well as biochemically. For example, the genomic RNA of type B viruses does not display significant sequence homology with that of type C viruses (14).[3] Similarly, the RNA-directed DNA polymerases of these two virus types are dissimilar immunologically (15) and prefer different cation cofactors (15, 16). Biologically, type C viruses generally cause leukemias, lymphomas and sarcomas, and type B (and type D) viruses are associated with mammary carcinomas (17).

The existence of these and other distinctions between type B and C viruses suggested that their DNA polymerases might also have important functional differences such as dissimilar polynucleotide binding sites. These possibilities were explored in the present investigation by examining the effects of synthetic polynucleotides on the DNA polymerases of MMTV (a prototype B virus) and SiSV (a prototype C virus).

MATERIALS AND METHODS

Polynucleotides and viruses. Poly(A) (9.6S), poly(C) (8.9S), poly(I) (12.6S), poly(U) (6.0S), and oligo(dT)$_{12-18}$ were obtained from Miles Laboratories. Polynucleotides were extracted with phenol:CHCl$_3$:isoamyl alcohol (48:48:1, v/v) in the presence of 0.1–0.5% sodium dodecyl sulfate and were then ethanol precipitated. Oligo(dG)$_{12-18}$ and unlabeled deoxyribonucleoside triphosphates were obtained from P. L. Biochemicals. Tritium labeled thymidine triphosphate (59.5 Ci/mmole) and deoxyguanosine triphosphate (34.4 Ci/mmole) were purchased from New England Nuclear Corporation. The concentration of polynucleotides was determined spectrophotometrically as described before (4, 7).

Purified MMTV and SiSV were supplied by Dr. Charles Benton (Frederick Cancer Research Center) through the courtesy of Dr. Jack Gruber, Office of Program Resources and Logistics, National Cancer Institute. MMTV was obtained from virus-infected cultures of C3H mouse derived Mm5mt/c$_1$ cells. SiSV was from virus-infected human NC-37 cells. These viruses had been purified by double equilibrium sucrose density gradient centrifugation. MMTV was obtained as a fresh preparation and SiSV was rebanded to equilibrium in a 15–60% (w/w) sucrose density gradient (4, 10). The virus preparations displayed expected protein profiles when analyzed by SDS-polyacrylamide gel electrophoresis. Cross hybridization experiments with RNAs and complementary DNAs of the two viruses and of AKR murine leukemia virus did not reveal any cross contamination.

Purification of viral RNA-directed DNA polymerase. Partially purified preparations of MMTV and SiSV DNA polymerase were obtained by affinity chromatography on poly(C)-agarose (P. L. Biochemicals, type 6). The procedure was essentially that described by Marcus *et al.* (18). Briefly described, the procedure involved disrupting the virus and solubilizing polymerase activity by incubating the virus suspension at 0° for 15 min in 50 mM Tris-HCl (pH 7.9), 10 mM dithiothreitol, 0.4 M KCl, 1% NP-40 (Bethesda Research Laboratories), and 0.5% sodium deoxycholate. The suspension was diluted ten fold with 50 mM Tris-HCl (pH 7.9)-1 mM dithiothreitol-10% (v/v) glycerol (TDG buffer) and applied to a column of poly(C)-agarose (3 ml bed in a 9 mm diameter column) at 4°. The column had been prewashed with 0.1 M glycine (to block unoccupied activated residues on the agarose matrix) and pre-equilibrated with TDG buffer. It was washed with at least ten bed volumes of TDG buffer and eluted with 0.4 M KCl in TDG buffer. The fractions were assayed for DNA polymerase activity using poly(A):oligo(dT) as a template:primer (Fig. 1). Almost all of the bound activity eluted as a sharp band (one bed volume) with 0.4 M KCl.

RNA-directed DNA polymerase assays. DNA synthesis catalyzed by detergent-disrupted virus-associated DNA polymerase

[3] S. K. Arya and N. A. Young, unpublished results.

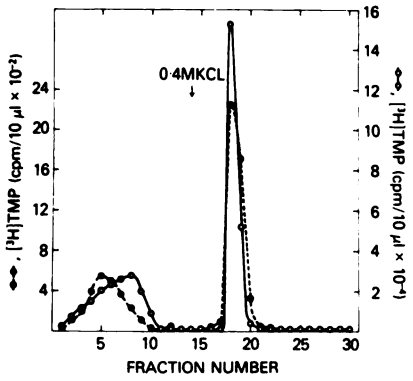

FIG. 1. *Purification of MMTV and SiSV DNA polymerase by affinity chromatography on poly(C)-agarose.*

Detergent solubilized polymerase activity was applied to poly(C)-agarose contained in column at 4°. It was washed with 50 mM Tris-HCl (pH 7.9)-1 mM dithiothreitol-10% (v/v) glycerol. The column was then eluted with the above buffer containing 0.4 M KCl. Aliquots of fractions were assayed for DNA polymerase activity using poly(A):oligo(dT) as a template:primer.

was measured in a reaction mixture (50 or 100 µl) containing 50 mM Tris-HCl (pH 7.9), 50 mM NaCl, 30 mM dithiothreitol, 0.08% NP-40, 50 µM [^{3}H]TTP (700 cpm/pmole) or [^{3}H]dGTP (700 cpm/pmole), various amounts of poly(A):oligo(dT) (molar ratio, 5:1) or poly(C):oligo(dG); (molar ratio, 5:1), 20 mM magnesium chloride for MMTV and 1 mM manganese chloride for SiSV, and virus preparation (20 µg viral protein/ml). The virus preparation was preincubated at 0° for 15 min with 100 mM dithiothreitol and 0.26% NP-40. The reaction mixture for DNA polymerase assay with purified enzyme contained 50 mM Tris-HCl (pH 7.9), 80 mM KCl, 10 mM dithiothreitol, 25 µM [^{3}H]TTP (1,400 cpm/pmole) or [^{3}H]dGPT (1,400 cpm/pmole), various amounts of poly(A):oligo(dT) (molar ratio, 5:1) or poly(C):oligo(dG) (molar ratio, 5:1), 20 mM magnesium chloride for MMTV DNA polymerase and 1 mM manganese chloride for SiSV DNA polymerase, and an aliquot of purified enzyme. The reaction was initiated by the addition of detergent-disrupted virus or purified DNA polymerase to the reaction mixture containing all the components. It was incubated at 37° for 20 min or for specified times, aliquots were precipitated with cold trichloroacetic acid, and radioactivity incorporated into acid-insoluble material was determined as described before (4, 7).

Under these assay conditions, the concentration of enzyme was in the linear range of response and that of deoxyribonucleoside triphosphate was in excess. There was negligible incorporation of precursors directed by the endogenous template RNA in the case of detergent-disrupted virus preparation. Given the obvious complexity of the enzyme system, certain assumptions were made to facilitate analysis of the kinetic data. At least three binding sites—template, primer and precursor—are conceived. Since no adequate kinetic model for enzyme-template:primer-precursor system is available, a simplified model was used. The template:primer was employed as a substrate (in the presence of excess precursor) and the incorporation of precursor was taken as a measure of template:primer utilization. To obtain kinetic parameters, double-reciprocal (Lineweaver-Burk) plots were constructed by least-squares fitting of the experimental data. The apparent K_s was determined by the intercept and apparent K_i from the relative slopes of the least-squares fitted lines representing the uninhibited and inhibited reaction (19).

RESULTS

The kinetics of DNA synthesis catalyzed by virus-associated MMTV DNA polymerase directed by poly(A):oligo(dT) and poly(C):oligo(dG) is shown in Figure 2. The kinetics of inhibition caused by polyribonucleotides [poly(I), poly(A) and poly(U)] is also represented. The rate of DNA synthesis was nearly linear for 20 min or more. This time period was chosen for subsequent experiments that analyzed inhibition kinetics. Figure 2 also shows that polyribonucleotides, particularly poly(I) and poly(U), markedly inhibited MMTV DNA polymerase activity. Sets of template:primer and inhibitor polynucleotides were chosen such that the inhibitor polynucleotide would not form base-pairs with the template or primer strand under the assay conditions. Thus, the inhibition kinetics should not be complicated by substrate-inactivation kinetics.

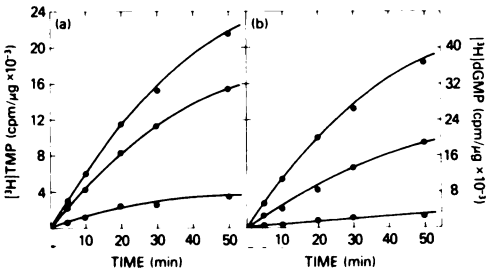

FIG. 2. *Kinetics of DNA synthesis catalyzed by virus-associated MMTV DNA polymerase.*

The reaction mixture contained 50 mM Tris-HCl (pH 7.9), 50 mM NaCl, 20 mM magnesium chloride, 30 mM dithiothreitol, 0.08% NP-40, 50 μM [³H]TTP (700 cpm/pmole) or [³H]dGTP (700 cpm/pmole), 5:1 μM poly(A):oligo(dT) or 10:2 μM poly(C):oligo(dG), and detergent-disrupted virus preparation (1 μg viral protein/50 μl). The reaction mixture was incubated at 37° for specified times and 50 μl aliquots were precipitated with trichloroacetic acid. (a) Poly(A):oligo(dT)-directed reaction in the absence (-●-●-) and presence of poly(I) (-⊖-⊖-, 1 μM; -◐-◐-, 5 μM). (b) Poly(C):oligo(dG)-directed reaction in the absence (-●-●-) and presence of 100 μM poly(A) (-⊖-⊖-) and 20 μM poly(U) (-◐-◐-).

Kinetics of polyribonucleotide inhibition of MMTV DNA polymerase. A double-reciprocal plot of the kinetics of poly(I) inhibition of poly(A):oligo(dT) directed DNA synthesis catalyzed by virus-associated MMTV DNA polymerase is presented in Figure 3. A complex inhibition kinetics pattern was apparent, one consistent neither with simple competitive inhibition nor with noncompetitive inhibition; both the apparent K_s and V_m were affected by the inhibitor (19). The apparent K_s for poly(A):oligo(dT) in the absence of poly(I) was 3.6 ± 0.4 μM. The apparent K_i for poly(I), estimated from the relative slopes of least-squares fitted lines, was 1.6 ± 0.2 μM. These data, along with data for other sets of template:primer and inhibitor polynucleotides, are listed in Table 1. Figure 4 shows representative data for poly(U) inhibition of poly(C):oligo(dG)-directed DNA synthesis by virus-associated MMTV DNA polymerase. The apparent K_s and K_i were estimated to be 3.8 ± 0.1 μM and 0.5 ± 0.08 μM, respectively. Much higher concentrations of poly(A) were required to obtain significant inhibition of poly(C):oligo(dG)-directed virus-associated DNA polymerase; the apparent K_i, estimated from double-reciprocal plots (data not shown), was 42.8 ± 1.2 μM (Table 1).

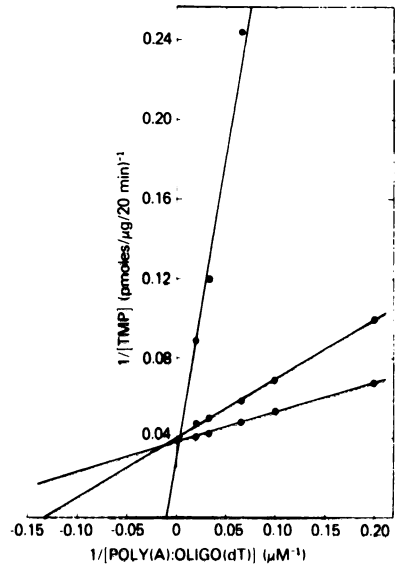

FIG. 3. *Kinetics of poly(I) inhibition of poly(A):oligo(dT)-directed virus-associated MMTV DNA polymerase.*

The reaction mixture (50 μl) contained 50 mM Tris-HCl (pH 7.9), 50 mM NaCl, 20 mM magnesium chloride, 30 mM dithiothreitol, 0.08% NP-40, 50 μM [³H]TTP (700 cpm/pmole), various concentrations of poly(A):oligo(dT), 2 or 30 μM poly(I) and detergent-disrupted virus preparation (1 μg viral protein/50 μl). The reaction mixture was incubated at 37° for 20 min and precipitated with trichloroacetic acid. Poly(T) synthesis in the absence (-●-●-) and presence of poly(I) (-⊖-⊖-, 2 μM; -◐-◐-, 30 μM).

The reaction kinetics obtained with partially purified MMTV DNA polymerase are presented in Figures 5 and 6. Figure 5 shows the data for poly(I) inhibition of poly(A):oligo(dT)-directed DNA synthesis. While at 2 μM poly(I) the kinetics appeared to be consistent with simple competitive inhibition; at higher concentrations both apparent K_s and V_m were affected. Thus, as in the case of SiSV DNA polymerase (see below) and MuLV DNA polymerase (7, 10), poly(I) inhibition of MMTV DNA polymerase probably reflects a case of mixed type of inhibition. The apparent K_s and K_i were estimated to be 2.0 ± 0.2 μM and 1.2 ± 0.3 μM, respectively. Poly(U) inhibition of poly(C):oligo(dG)-directed MMTV DNA polymerase also displayed a complex kinetics (Fig. 6). The apparent K_s and K_i were

TABLE 1

Polyribonucleotide inhibition of MMTV RNA-directed DNA polymerase

Template:primer	Inhibitor	K_s[a]	K_i[a]
		(μM)	(μM)
(a) *Virus-associated DNA polymerase*			
Poly(A):oligo(dT)	—	4.0, 3.2[b]	—
	Poly(I)	—	1.8, 1.4
Poly(C):oligo(dG)	—	3.8, 3.7[b]	—
	Poly(A)	—	41.7, 44.0
	Poly(U)	—	0.58, 0.44
(b) *Partially purified DNA polymerase*			
Poly(A):oligo(dT)	—	2.2, 1.8[b]	—
	Poly(I)	—	1.5, 0.9
Poly(C):oligo(dG)	—	2.0, 1.8[b]	—
	Poly(A)	—	32.4, 24.6
	Poly(U)	—	0.24, 0.31

[a] The kinetic constants were estimated from Lineweaver-Burk plots of experimental data analyzed by the least squares method. The apparent K_i's were computed from the relative slopes of the lines representing uninhibited and inhibited reaction.

[b] The amount refers to the concentration of the template strand. The concentration of the primer strand is one-fifth of the template strand.

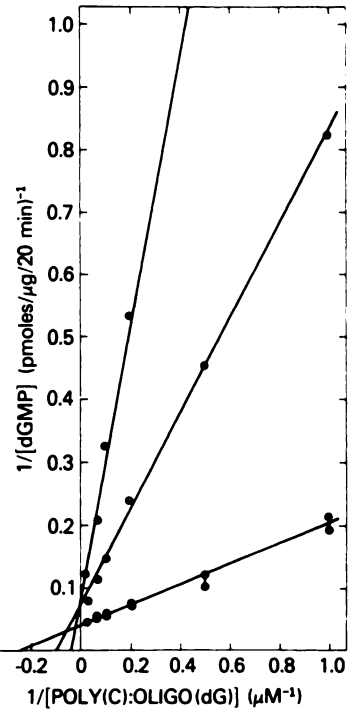

FIG. 4. *Kinetics of poly(U) inhibition of poly(C):oligo(dG)-directed virus-associated MMTV DNA polymerase.*

The reaction conditions were the same as described for Fig. 3, except poly(C):oligo(dG) replaced poly(A):oligo(dT), [^{3}H]dGTP replaced [^{3}H]TTP, and poly(U) replaced poly(I). Poly(dG) synthesis in the absence (-●-●-) and presence of poly(U) (-○-○-, 2 μM; -◐-◐-, 5 μM).

estimated to be 1.9 ± 1 μM and 0.28 ± 0.04 μM, respectively.

Kinetics of polyribonucleotide inhibition of SiSV DNA polymerase. The kinetics of inhibition of both virus-associated and partially purified DNA polymerase activities of SiSV were analyzed. The analyses included that for poly(I) inhibition of poly(A):oligo(dT)-directed, and poly(A) and poly(U) inhibition of poly(C):oligo(dG)-directed DNA polymerase reactions. Only representative graphic data are depicted; the numerical data are listed in Table 2. Figure 7 shows the kinetics of poly(U) inhibition of poly(C):oligo(dG)-directed virus-associated DNA polymerase. Similar data for partially purified DNA polymerase are depicted in Figure 8. In both cases, a complex inhibition kinetics was obtained. Similar complex inhibition kinetics was observed for all other cases examined, regardless of whether virus-associated or partially purified SiSV DNA polymerase was used.

The apparent K_s for poly(C):oligo(dG) for virus-associated SiSV DNA polymerase was 7.6 ± 0.2 μM and that for partially purified DNA polymerase was 1.2 ± 0.2 μM. The apparent K_i for poly(U) was estimated to be 8.8 ± 0.3 μM and 1.0 ± 0.2 μM for virus-associated and partially purified DNA polymerase, respectively. The kinetic parameters for other template:primer and inhibitors are listed in Table 2. It is noteworthy that there is a six to eight-fold reduction in apparent K_s for poly(C):oligo(dG) and apparent K_i for poly(U) when virus-associated and partially purified SiSV DNA polymerase activities are compared. Similarly, apparent K_s for poly(A):oligo(dT) decreases from 15.9 ± 0.7 μM to 5.2 ± 0.6 μM and apparent K_i for poly(A) from 30.5 ± 1.5 μM to 6.7 ± 0.9 μM when the two polymerase activities are compared (Table 2). Changes in kinetic parameters of this magnitude were not observed in the

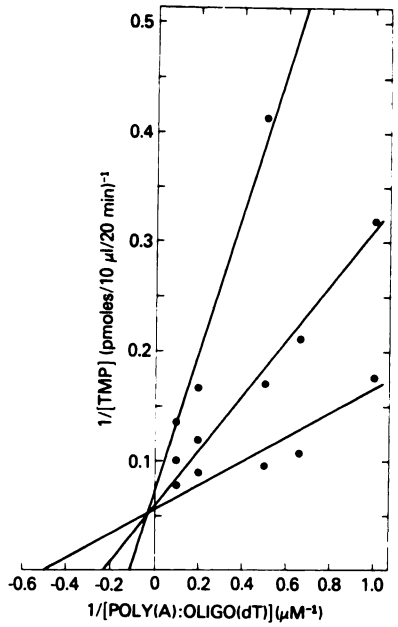

FIG. 5. *Kinetics of poly(I) inhibition of poly(A): oligo(dT)-directed partially purified MMTV DNA polymerase.*

The reaction mixture contained 50 mM Tris-HCl (pH 7.9), 80 mM KCl, 20 mM magnesium chloride, 10 mM dithiothreitol, 25 μM [^{3}H]TTP (1,400 cpm/pmole), various concentrations of poly(A):oligo(dT), 1 or 5 μM poly(I), and 10 μl aliquot of poly(C)-agarose purified enzyme preparation. The reaction mixture was incubated at 37° for 20 min and precipitated with trichloroacetic acid. Poly(T) synthesis in the absence (-●-●-) and presence of poly(I) (-⊖-⊖-, 2 μM; -◐-◐-, 5 μM).

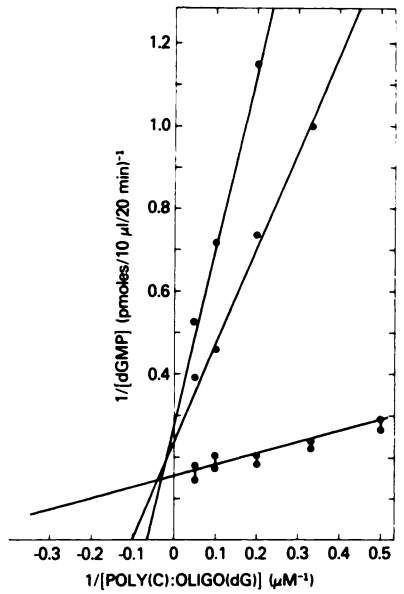

FIG. 6. *Kinetics of poly(U) inhibition of poly(C): oligio(dG)-directed partially purified MMTV DNA polymerase.*

The reaction conditions were the same as described for Fig. 5, except poly(C):oligo(dG) replaced poly(A): oligo(dT), [^{3}H]dGTP replaced [^{3}H]TTP, and poly(U) replaced poly(I). Poly(dG) synthesis in the absence (-●-●-) and presence of poly(U) (-⊖-⊖-, 2 μM; -◐-◐-, 5 μM).

case of MMTV DNA polymerase activities (Table 1).

DISCUSSION

The polymerization of nucleotide precursors into DNA catalyzed by DNA polymerase is a mutlicomponent reaction. The enzyme system is similarly complex. At least three components require binding sites on the enzyme: template, primer and nucleoside triphosphate precursor. Binding at all three sites may be subject to modification by co-factors such as divalent cations. Because a readily analyzable model for enzyme: template:primer:precursor complex is not available, we have resorted to a simplified model. The template:primer was employed as a substrate (in the presence of excess precursor), and rate of incorporation of precursor into DNA was taken as a measure of template:primer utilization or velocity of the reaction. Analysis of double-reciprocal (Lineweaver-Burk) plots obtained from the experimental data indicated that, in nearly all cases, both MMTV and SiSV DNA polymerases displayed complex inhibition kinetics. Both K_s and V_m were affected, consistent with neither simple competitive nor noncompetitive inhibition (19). The inhibition kinetics thus appeared to be of the mixed type (19). We have previously made similar observations for polynucleotide inhibition of MuLV DNA polymerase. More recently, Yamamura and Cavalieri (20) obtained a mixed type of inhibition kinetics for non-primer tRNA inhibition of AMV DNA polymerase. A mixed type inhibition results when the inhibitor not only affects the binding of the substrate to the enzyme but also interferes with the breakdown of active complex (19). Consistent with this concept is the hypothesis that the polynucleotide binding site(s) on the enzyme consists of a collection of

TABLE 2

Polyribonucleotide inhibition of SiSV RNA-directed DNA polymerase

Template:primer	Inhibitor	K_s[a] (μM)	K_i[a] (μM)
(a) *Virus-associated DNA polymerase*			
Poly(A):oligo(dT)	—	15.2, 16.6[b]	—
	Poly(I)	—	1.3, 1.0
Poly(C):oligo(dG)	—	7.4, 7.7[b]	—
	Poly(A)	—	29.1, 32.0
	Poly(U)	—	9.1, 8.5
(b) *Partially purified DNA polymerase*			
Poly(A):oligo(dT)	—	4.6, 5.8[b]	—
	Poly(I)	—	0.7, 0.6
Poly(C):oligo(dG)	—	1.4, 1.1[b]	—
	Poly(A)	—	5.8, 7.6
	Poly(U)	—	0.96, 0.86

[a] The kinetic constants were estimated from Lineweaver-Burk plots of experimental data analyzed by the least-squares method. The apparent K_i's were computed from the relative slopes of the lines representing uninhibited and inhibited reaction.

[b] The amount refers to the concentration of the template strand. The concentration of the primer strand was one-fifth of the template strand.

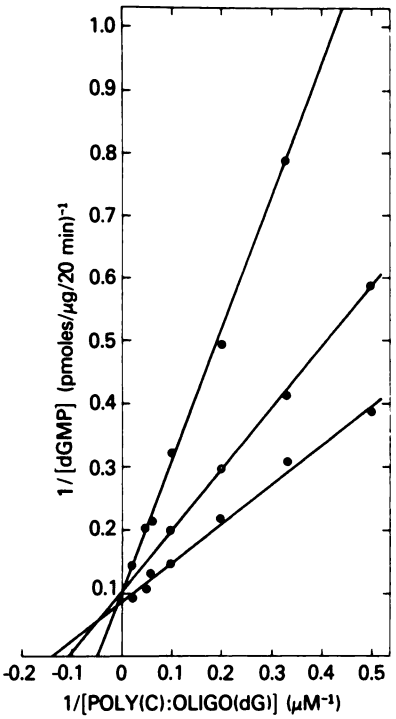

FIG. 7. *Kinetics of poly(U) inhibition of poly(C):oligo(dG)-directed virus-associated SiSV DNA polymerase.*

The reaction conditions were the same as described for Fig. 4, except 1 mM manganese chloride replaced magnesium chloride. Poly(dG) synthesis in the absence (-●-●-) and presence of poly(U) (-⊖-⊖-, 5 μM; -◐-◐-, 20 μM).

subsites (4, 21), each of which may be affected differentially by the inhibitor polynucleotides. Such interactions could result in the type of complex inhibition kinetics observed. Other explanations are also possible. For example, inhibitor polynucleotide may introduce specific conformational changes in the enzyme. The interaction of polynucleotides with molecular species contaminating DNA polymerase preparations that affect catalytic activity may be another factor.

Regardless of the complex kinetics, kinetic parameters can be computed from double-reciprocal plots. The apparent K_s can be obtained from the intercept on $1/_{[S]}$-axis of the line representing uninhibited reaction and the apparent K_i can be computed from relative slopes of the lines representing uninhibited (K_s/V_m) and inhibited (K_s/V_m [1 + $^{I}/K_i$]) reaction (19). Accordingly, we have computed apparent K_i's from relative slopes. This is satisfactory for our primary purpose of comparing various polynucleotides and enzyme preparations.

As indicated in Tables 1 and 2, different enzyme preparations respond differentially to various polynucleotides. Apparent K_s and K_i for partially purified enzyme are less than those observed with virus-associated polymerase activity. In the case of MMTV, this difference is modest (two fold), but with SiSV it is four to eight fold [the apparent K_i for poly(I) is an apparent exception in this regard]. Similar results have been reported for AMV DNA polymerase (5) and for MuLV DNA polymerase (7, 8, 10). The reason(s) for these observations is not clear. It is possible that extraneous molecular species present in detergent-disrupted virus preparations interfere with the interaction of polynucleotide with DNA polymerase. They could, for example, interact with the added polynucleotide, thus reducing its effective concentration for the polymerase reaction. If such is the case, these

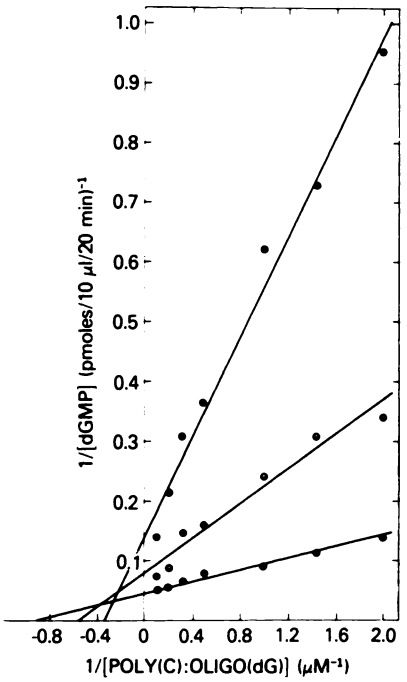

FIG. 8. *Kinetics of poly(U) inhibition of poly(C):oligo(dG)-directed partially purified SiSV DNA polymerase.*

The reaction conditions were the same as described for Fig. 6, except 1 mM manganese chloride replaced magnesium chloride. Poly(dG) synthesis in the absence (-●-●-) and presence of poly(U) (-⊖-⊖-, 2 μM; -○-○-, 10 μM).

interacting species must be more effective in the case of SiSV than MMTV. Further, they display a degree of specificity, since kinetic parameters for various template: primer and inhibitor polynucleotides are affected differentially. This could be due to different concentrations of such species in the two virus preparations or such species may be intrinsically different with respect to their interaction with polynucleotides. Among such candidate species could be the specific RNA binding phosphoproteins recently described for some type C viruses (22). Additionally, the interacting species may be the viral genomic RNA that is present in detergent-disrupted virus preparations but is absent from purified polymerase preparations. The concentration of genomic RNA in our reactions, estimated to be 1.2 μM based on the known mass ratio of protein to RNA in virus particles (23), conceivably is sufficient to be a factor.

When partially purified DNA polymerase activities of MMTV and SiSV are compared, no major qualitative differences are apparent that would reliably differentiate the polymerases of the two virus types. Both polymerases are inhibited by the polyribonucleotides thus far investigated. However, there are significant quantitative differences. Whereas there is no appreciable difference in the apparent K_s's for poly(A):oligo(dT) and poly(C):oligo(dG) for MMTV DNA polymerase, the corresponding apparent K_s's for SiSV DNA polymerase differ by a factor of about four. These results suggest that while template binding site(s) on MMTV DNA polymerase does not discriminate between poly(A) and poly(C), such site(s) on SiSV DNA polymerase displays higher affinity for poly(C) than poly(A). Similar results have been previously reported for AMV DNA polymerase (5) and MuLV DNA polymerase (4, 7). Thus, preferential binding to poly(C) sequences may be a common feature of DNA polymerases of type C viruses.

There is a notable difference between the apparent K_i's for poly(I) and poly(U) for MMTV DNA polymerase; poly(U) is considerably more potent as an inhibitor than poly(I), but this is not the case for SiSV DNA polymerase. Poly(A) appears to be three to four times more potent as an inhibitor of SiSV DNA polymerase compared with MMTV DNA polymerase. These results again suggest sequence-specific differences in the polynucleotide binding site(s) on DNA polymerases. It should be recognized that these inferences are based on analyses of kinetics of DNA synthesis and are not based on direct measurements of binding of polynucleotides to viral DNA polymerases.

In summary, the responses of MMTV and SiSV DNA polymerases to synthetic polynucleotides revealed no qualitative features that would reliably and predictably distinguish these two DNA polymerases. However, significant quantitative differences were noted. In addition, partially purified DNA polymerase activities were more strongly inhibited than virus-associated DNA polymerase activity. These find-

ings suggest that purified enzyme may be a more suitable target than virus-associated DNA polymerase for screening assay of potential inhibitors. On the other hand, if viral DNA synthesis *in vivo* occurs in the cores of virus particles, the virus-associated DNA polymerase may be a more appropriate target.

ACKNOWLEDGMENTS

We thank Drs. Jack Gruber and Charles Benton for providing the preparations of mouse mammary tumor virus and simian sarcoma virus. We thank Dr. Cephas Patch for his assistance with computations. S.K.A. is an IPA research scientist at the National Cancer Institute, on leave of absence from Roswell Park Memorial Institute, Buffalo, New York.

REFERENCES

1. Tuominen, F. W. & Kenney, F. T. (1971) Inhibition of the DNA polymerase of Rauscher leukemia virus by single-stranded polyribonucleotides. *Proc. Natl. Acad. Sci. U. S. A.*, **68**, 2198–2202.
2. Tennant, R. W., Farrelly, J. G., Ihle, J. N., Pal, B. C., Kenney, F. T. & Brown, A. (1973) Effects of polyadenylic acids on functions of murine RNA tumor viruses. *J. Virol.*, **12**, 1216–1225.
3. Pitha, P. M., Teich, N. M., Lowry, D. R. & Pitha, J. (1973) Inhibition of murine leukemia virus replication by poly(vinyl-uracil) and poly(vinyladenine). *Proc. Natl. Acad. Sci. U. S. A.*, **70**, 1204–1208.
4. Arya, S. K., Carter, W. A., Alderfer, J. L. & Ts'o, P. O. P. (1974) Inhibition of RNA directed DNA polyerase of murine leukemia virus by 2′-0-alkylated polyadenylic acids. *Biochem. Biophys. Res. Commun.*, **59**, 608–615.
5. Erickson, R. J. & Grosch, J. C. (1974) The inhibition of avian myeloblastosis virus deoxyribonucleic acid polymerase by synthetic polynucleotides. *Biochemistry*, **72**, 1987–1993.
6. De Clercq, E., Billiau, A., Hobbs, J., Torrence, P. F. & Witkop, B. (1975) Inhibition of oncornavirus functions by 2′-azido polynucleotides. *Proc. Natl. Acad. Sci. U. S. A.*, **72**, 284–288.
7. Arya, S. K., Carter, W. A., Alderfer, J. L. & Ts'o, P. O. P. (1975) Inhibition of ribonucleic acid-directed deoxyribonucleic acid polymerase of murine leukemia virus by polyribonucleotides and their 2′-O-methyl derivatives. *Mol. Pharmacol.*, **11**, 421–426.
8. Arya, S. K., Carter, W. A., Alderfer, J. L. & Ts'o, P. O. P. (1976) Inhibition of synthesis of murine leukemia virus in cultured cells by polyribonucleotides and their 2′-O-alkyl derivatives. *Mol. Pharmacol.*, **12**, 234–241.
9. 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.
10. Arya, S. K. (1977) Mechanism of polyribonucleotide inhibition of ribonucleic acid tumor virus replication. Effect of poly(inosinic acid) on the synthesis of proviral deoxyribonucleic acid of murine leukemia virus. *Mol. Pharmacol.*, **13**, 585–597.
11. Tennant, R. W., Hanna, M. G. & Farelly, J. G. (1974) Effect of poly (2′-O-methyladenylic acid) on susceptibility and autogenous immunity to RNA tumor virus oncogenesis. *Proc. Natl. Acad. Sci. U. S. A.*, **71**, 3167–3171.
12. Arya, S. K., Carter, W. A., Alderfer, J. L. & Ts'o, P. O. P. (1975) Inhibition of murine leukemia virus replication in cell culture and spleen focus formation in mice by polyadenylic acids. *Mol. Pharmacol.*, **11**, 501–505.
13. Vengris, V. E., Pitha, P. M. Sensenbrenner, L. L. & Pitha, J. (1978) Polymeric drugs: Direct compared with indirect inhibition of leukemia virus replication in mice. *Mol. Pharmacol.*, **14**, 271–277.
14. Michalides, B. & Schlom, J. (1975) Relationship in nucleic acid sequences between mouse mammary tumor virus variants. *Proc. Natl. Acad. Sci. U. S. A.*, **72**, 4635–4639.
15. Sarin, P. S. & Gallo, R. C. (1974) RNA directed DNA polymerase, in *MTP International Review of Science*, Biochemistry Series One, Vol. 6, Burton, K. (ed.), University Park Press, Baltimore, 219–253.
16. Dion, A. S., Vaidya, A. B. & Fout, G. S. (1974) Cation preference for poly(rC)·oligo(dG)-directed DNA synthesis by RNA tumor viruses and human milk particulates. *Cancer Res.*, **34**, 3509–3515.
17. Gross, L. (1970) *Oncogenic Viruses*, ed. 2; Pergamon Press, Oxford.
18. Marcus, S. L., Sarkar, N. H. & Modak, M. J. (1976) Purification and properties of murine mammary tumor virus DNA polymerase. *Virology*, **71**, 242–254.
19. Webb, J. L. (1963) *Enzyme and Metabolic Inhibitors*, Vol. 1, Academic Press, New York, 149–153.
20. Yamaura, I. & Cavalieri, L. F. (1978) Inhibition of reverse transcription of 70S and 35S avian myeloblastosis RNAs by nonprimer tRNAs. *J. Virol.*, **27**, 300–306.
21. Marcus, S. L., Modak, M. J. & Cavalieri, L. F. (1974) Evidence for template-specific sites on DNA polymerases. *Biochem. Biophys. Res. Commun.*, **56**, 516–521.
22. Sen, A. & Todaro, G. J. (1977) The genome associated, specific RNA binding proteins of avian and mammalian type C viruses. *Cell*, **10**, 91–99.
23. Tooze, J., ed. (1973) *Molecular Biology of Tumor Viruses*, Cold Spring Harbor Laboratory, New York, p. 509.