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INHIBITION OF REVERSE TRANSCRIPTASE FROM FELINE IMMUNODEFICIENCY VIRUS BY ANALOGS OF 2′-DEOXYADENOSINE-5′-TRIPHOSPHATE

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Abstract—The replication of feline immunodeficiency virus (FIV) in cultured cells was inhibited by 2′,3′-dideoxyadenosine (ddA) and by 9-(2-phosphonylmethoxyethyl)adenine (PMEA) with IC_{50} values of 0.98 and 0.95 μM, respectively. The effects of the presumed active forms of these inhibitors, ddATP and PMEA-diphosphate (PMEApp), upon the FIV reverse transcriptase (RT) were examined with two different template-primer systems. Both of these compounds were potent inhibitors of the FIV RT in reactions with primed φX-174 DNA, yielding K_i values of 8.8 nM for ddATP and 5.0 nM for PMEApp. However, they were both poor inhibitors of the reaction with poly(rU)-oligo(dA); concentrations of ddATP or PMEApp greater than 10 μM were required to inhibit this reaction by 50%. Further analysis of the reaction with poly(rU)-oligo(dA) revealed that even in the absence of inhibitors the primers were extended by less than 20 nucleotides. In contrast, high molecular weight products were obtained in reactions with φX-174 DNA. These results suggest that the reaction of FIV RT with poly(rU)-oligo(dA) is not highly processive. The high degree of termination encountered during this reaction with poly(rU)-oligo(dA) may be responsible for the low inhibitory potential of ddATP and PMEApp.

Feline immunodeficiency virus (FIV)§ is a lentivirus that causes a disease in cats that is very similar to acquired immune deficiency syndrome (AIDS) in humans [1, 2]. We have shown previously that the reverse transcriptase (RT) (EC 2.7.7.49) from FIV is similar to RT from the human immunodeficiency virus type 1 (HIV) in physical properties, catalytic activities, and sensitivities to active forms of several RT-targeted antiretroviral compounds [3–5]. The FIV RT has an associated ribonuclease H activity that is also similar to the HIV enzyme in substrate specificity and sensitivity to several polyanionic inhibitors [6]. In cell culture systems the replication of FIV is inhibited by several RT-targeted antiviral compounds that have been shown to inhibit replication of HIV [3, 7]. These features make FIV an attractive *in vitro* model for studies of AIDS chemotherapy. In addition, the availability of specific pathogen-free cats and of protocols to monitor viremia and immunosuppression [8] makes this an attractive model for *in vivo* therapeutic studies.

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§ Abbreviations: AIDS, acquired immune deficiency syndrome; AZdU, 3′-azido-2′-3′-dideoxyuridine; AZG, 3′-azido-2′,3′-dideoxyguanosine; AZT, 3′-azido-3′-deoxythymidine; CrFK cells, Crandell feline kidney cells; ddA, 2′,3′-dideoxyadenosine; ddATP, ddCTP, ddGTP and ddTTP, the corresponding 2′,3′-dideoxynucleoside 5′-triphosphates; ddI, 2′,3′-dideoxyinosine; D4T, 2′,3′-dideoxy-2′,3′-didehydrothymidine; FIV, feline immunodeficiency virus; HIV, human immunodeficiency virus type 1; N_3dTTP, 3′-azido-3′-deoxythymidine 5′-triphosphate; PFA, phosphonoformic acid; PMEA, 9-(2-phosphonylmethoxyethyl)adenine; PMEApp, 9-(2-diphosphophosphonylmethoxyethyl)adenine; and RT, reverse transcriptase.

In our comparisons of the FIV and HIV RTs we have shown that these two enzymes can utilize RNA homopolymer templates (primed with complementary oligodeoxynucleotides) capable of incorporating dTTP, dCTP or dGTP, namely, poly(rA)-oligo(dT), poly(rI)-oligo(dC), and poly(rC)-oligo(dG), respectively. However, under identical conditions neither enzyme is able to effectively utilize poly(rU)-oligo(dA) for incorporation of dATP [4, 5]. Thus, we have been able to analyze inhibition of RT by only three of the four 2′,3′-dideoxynucleotides, ddTTP, ddCTP and ddGTP. Similarly, Cheng *et al.* [9] reported that the HIV RT failed to utilize poly(rG)-oligo(dC) and utilized poly(rU)-oligo(dA) poorly under their assay conditions. However, Chen and Oshana [10] reported constants for inhibition of HIV RT by ddATP using both poly(rU)-oligo(dA) and poly(dU)-oligo(dA) as template primers, although the relative velocities obtained with these templates were not compared to those with poly(rA)-oligo(dT) or other templates. A major goal of the work presented here was to develop template-primer systems that will enable study of the inhibition of FIV RT by ddATP and other dATP analogs.

Another compelling reason for extension of our previous work into additional template-primer systems is the promising antiviral activity of an adenosine nucleotide analog, 9-(2-phosphonylmethoxyethyl)adenine (PMEA). PMEA has shown activity against HIV *in vitro* [11, 12] and against FIV *in vitro* and *in vivo* [13]. The active form of PMEA appears to be a triphosphate analog (the diphosphorylated derivative, PMEApp), which has been shown to inhibit the HIV RT [14]. In the work presented here we have further characterized

the anti-FIV activity of PMEA and compared it to 2′,3′-dideoxyadenosine (ddA) in a cell culture system, and we have examined inhibition of the purified FIV RT by the active forms of these compounds.

MATERIALS AND METHODS

Chemicals. PMEA and its diphosphate (PMEApp) were obtained from Dr. H-T. Ho of the Bristol-Myers Squibb Co., Wallingford, CT. The 5′-triphosphate of 3′-azido-3′-deoxythymidine (AZT), N_3dTTP, was provided by Dr. Wayne Miller of the Burroughs Wellcome Co., Research Triangle Park, NC. The ddA was provided by the Developmental Therapeutics Branch, Division of AIDS, National Institute of Allergy and Infectious Diseases. Poly(rA)-oligo(dT)$_{10}$ (average template length ranged from 286 to 982 nucleotides), poly(rU) (average length 310 nucleotides), oligo(dA)$_{12-18}$, oligo(dA)$_{14}$, oligonucleotide size markers, ddATP and ddTTP were purchased from Pharmacia-LKB, Piscataway, NJ. The ϕX-174 DNA and 15-nucleotide primer were described previously [15]. All radioactive compounds were purchased from DuPont-New England Nuclear, Boston, MA.

Cells and virus. Crandell feline kidney (CrFK) cells and the Petaluma strain of FIV were grown and maintained as previously described [3]. The AZT-resistant mutant of FIV was also described previously [7]. The IC_{50} value for inhibition of this mutant by AZT is greater than 100 μM, whereas the IC_{50} value for inhibition of wild-type FIV by AZT is 1.4 μM [7].

Focal infectivity assay. Infectivity of FIV in the presence or absence of inhibitors was determined by a focal infectivity assay, which was reported previously [7]. Uninfected CrFK cells were seeded into 24 well microtiter plates at a density of 1.5×10^4 cells per well. These cells were incubated for 1 hr at 37° in growth medium or growth medium containing drug, to enable conversion of drugs to their active forms. Cells were then infected with 20–60 focus forming units of wild-type or AZT-resistant FIV. Culture medium and inhibitors were removed at 48 hr and replaced with medium containing the same concentration of inhibitor. At 96 hr, medium was removed and cells were fixed with methanol for 5 min, washed twice with 0.01 M Tris–HCl, pH 7.5, containing 0.15 M NaCl and 0.002 M EDTA, and then once with this buffer containing 1% bovine serum. Immunostaining with a double antibody technique using polyclonal anti-FIV antibody from FIV-infected specific pathogen-free cats, peroxidase-conjugated goat anti-cat IgG (Organon-Teknika, Durham, NC), and visualization of foci with 3-amino-9-ethylcarbazole were all performed as previously reported [7]. A focus is defined as a group of four or more stained cells [7]. Data were plotted as percentage of control foci (no drugs) versus inhibitor concentration, and the concentrations necessary to inhibit focus formation by 50% (IC_{50} values) were obtained directly from these plots.

Template preparation. Poly(rU)-oligo(dA) template-primers were made by annealing 20 A_{260} units of poly(rU) with oligo(dA)$_{12-18}$ at template to primer ratios (in A_{260} units) of 10:1,5:1, 2:1, and 1:1. These mixtures were heated to 65° for 1 min and then cooled to 4°, as previously described [4, 9]. Poly(rA)-oligo(dT) prepared in this way was equivalent to the poly(rA)-oligo(dT) obtained from Pharmacia-LKB in activity as a template for FIV RT. The ϕX-174 template primer was made by annealing primer to ϕX-174 DNA at a molar ratio of 2:1, as described by Preston *et al.* [15]. This mixture was heated to 95° for 1 min, and then cooled by 15-min incubations at 60°, 37°, 25° and 4°. All template-primers were stored at −20° until used.

Enzyme and assays. RT was purified from wild-type FIV (Petaluma strain) and, unless stated otherwise, was assayed with poly(rA)-oligo(dT)$_{10}$, as previously described [4]. In all reactions with RT the following precautions were taken to minimize degradation of RNA templates by RNase: all glassware, plasticware, salt solutions and water used for preparation of reaction buffers were autoclaved (121° and 18 psi for 25 min) prior to use; Tris and bovine serum albumin used in these assays were nuclease-free, molecular biology grade reagents. One unit of RT is the amount of enzyme that catalyzes incorporation of 1 nmol of dTTP into acid-insoluble product per hr at 37°.

For reactions with poly(rU)-oligo(dA)$_{12-18}$, 0.0625 A_{260} units of the poly(U) annealed to the indicated amount of oligo(dA) were incubated with 1 unit of FIV RT and various concentrations of [^{3}H]-dATP under assay conditions otherwise as described above. For reactions with ϕX-174 DNA the mixtures contained 0.0065 A_{260} units of primed DNA and 1 unit of RT in 20 mM Tris–HCl, pH 8.5, 25 mM KCl, 10 mM $MgCl_2$, 2 mM dithiothreitol, 0.0125% Triton X-100, 0.1 mg of nuclease-free bovine serum albumin per mL, 10 μM each of dCTP, dGTP, and dTTP, and various concentrations of [^{3}H]dATP.

Primer extension assays. Reaction mixtures for primer extension assays contained 20 mM Tris–HCl, pH 8.5, 25 mM KCl, 2 mM dithiothreitol, 10 mM $MgCl_2$, 0.1 mg nuclease-free bovine serum albumin/mL, 0.032 units of primed template [ϕX-174 DNA or poly(rU)-oligo(dA)$_{14}$], 10 μM [^{32}P]dATP (1.25 μCi) and, for reactions with ϕX-174 DNA, 10 μM each of dCTP, dGTP and dTTP in a total volume of 12.5 μL, essentially as described by Townsend and Cheng [16]. Mixtures were incubated for 30 min at 37° and reactions were terminated by the addition of 12.5 μL of 7 M urea containing 0.3% xylene cyanol and 0.3% bromophenol blue, heated for 5 min in a boiling water bath, and then placed on ice. Aliquots (10 μL) were loaded onto 17.5% acrylamide/7 M urea gels and electrophoresis was carried out for 1.5 hr at 40 mA constant current. After electrophoresis, gels were wrapped in polyethylvinyl film (plastic wrap) and radiolabeled products were visualized by autoradiography with Kodak X-OMAT-AR film.

RESULTS

Initial attempts to develop a template primer capable of utilizing dATP were directed toward modification of the reaction conditions with poly(rU)-oligo(dA). As we previously reported [4], the FIV

Table 1. Activity of FIV RT using various templates relative to velocity obtained with poly(rA)·oligo(dT)$_{10}$

Template	Template concentration (A_{260} U/mL)	Ratio of primer to template*	Relative activity†
Poly(rA)·oligo(dT)$_{10}$	0.5	1:1	1.000
Poly(rU)·oligo(dA)$_{12-18}$	1.25	1:1	0.054
Poly(rU)·oligo(dA)$_{12-18}$	1.25	1:2	0.022
Poly(rU)·oligo(dA)$_{12-18}$	1.25	1:5	0.011
Poly(rU)·oligo(dA)$_{12-18}$	1.25	1:10	<0.001
ϕX174 DNA	0.13	2:1	0.125

* Ratios are expressed as A_{260} units primer to A_{260} units template for RNA homopolymers, and moles primer to moles template for the ϕX174 DNA template.

† Velocity was converted to total nanomoles dNTP incorporated per hour and normalized as fractions of FIV RT activity with poly(rA)·oligo(dT)$_{10}$ template. Velocity values represent the average of two determinations.

and HIV RTs utilize this template-primer poorly. Interestingly, an increase in the ratio of oligo(dA) to poly(rU) resulted in increased velocity of the FIV RT (Table 1) with a maximum achieved when template was saturated with primer. The velocity achieved when template and primer were present in a 1:1 ratio (moles of nucleotide) was 5% of that obtained with poly(rA)-oligo(dT). The K_m for dATP in this reaction was 5.1 μM. This is only slightly higher than the K_m of 3.4 μM dTTP obtained in the reaction of FIV RT with poly(rA)-oligo(dT) [5]. When the inhibitory properties of ddATP and PMEApp were examined, these compounds were found to be 100- to 1000-fold less effective as inhibitors of the FIV RT than were ddTTP, ddCTP or ddGTP, which we previously examined with appropriate homopolymer templates [5]. Concentrations of ddATP and PMEApp required to inhibit FIV RT by 50% (IC_{50}) were determined to be 12 and 17 μM, respectively. These IC_{50} values were determined from reactions carried out with 20 μM dATP under our standard assay conditions. Accurate K_i values could not be determined for these two compounds because of the low levels of [^{3}H]dATP incorporation even in the absence of inhibitors.

The poor activities of ddATP and PMEApp as inhibitors of the FIV RT are surprising in view of the known antiviral activities of ddA, 2′,3′-dideoxyinosine (ddI) and PMEA. We have shown previously that ddI (the active form of which is presumed to be ddATP [17, 18]) inhibits replication of FIV in CrFK cells with an IC_{50} of 2.1 μM [7]. The data presented in Fig. 1 confirm that FIV is sensitive to ddA and PMEA with IC_{50} values of 0.98 and 0.95 μM, respectively. The ability of these compounds to inhibit an AZT-resistant isolate of FIV was also examined. The IC_{50} values for inhibition of this mutant by ddA (1.1 μM) and PMEA (1.3 μM) were not significantly different from values obtained with wild-type FIV. Thus, the poor activity of ddATP and PMEApp against the FIV RT in this system is not due to an inherent resistance of FIV to ddA or PMEA.

The low velocity and requirement for high primer

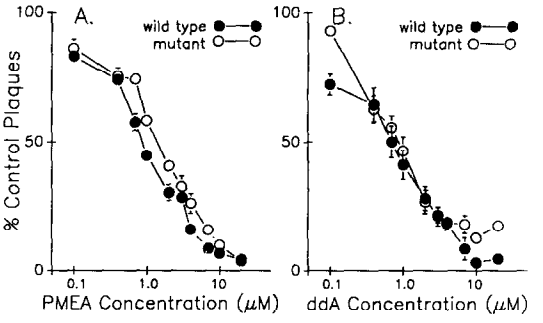

Fig. 1. Inhibition of wild-type and AZT-resistant FIV by PMEA (A) and ddA (B). Determinations were performed by the focal infectivity assay, as described in Materials and Methods. Each point is the mean ± SEM of a minimum of four determinations. Control values in individual experiments ranged from 20 to 60 focus forming units per well.

concentration, as well as the relative insensitivity to dATP analogs of the reaction with poly(rU)-oligo(dA), prompted us to examine DNA products of the reaction. For these experiments a reaction with poly(rU)-oligo(dA)$_{14}$ and [^{32}P]dATP was carried out and the product size analyzed on polyacrylamide gels. The results shown in Fig. 2 (lane 1) reveal that the products of this reaction were of low molecular weight. The largest product detectable was less than 20 nucleotides longer than the primer. The failure to produce high molecular weight products was not due to degradation of the poly(rU) template during the reaction. We were able to confirm this by 5′-[^{32}P] end-labeling the poly(rU), running an RT reaction with non-radioactive dATP, and then analyzing the products on a polyacrylamide/urea gel. The length of poly(rU) after the reaction (average length about 300 nucleotides) was not different from the starting material (data not shown). This inability to produce high molecular weight products in the reaction with poly(rU)-oligo(dA) explains the dependence of the reaction on the

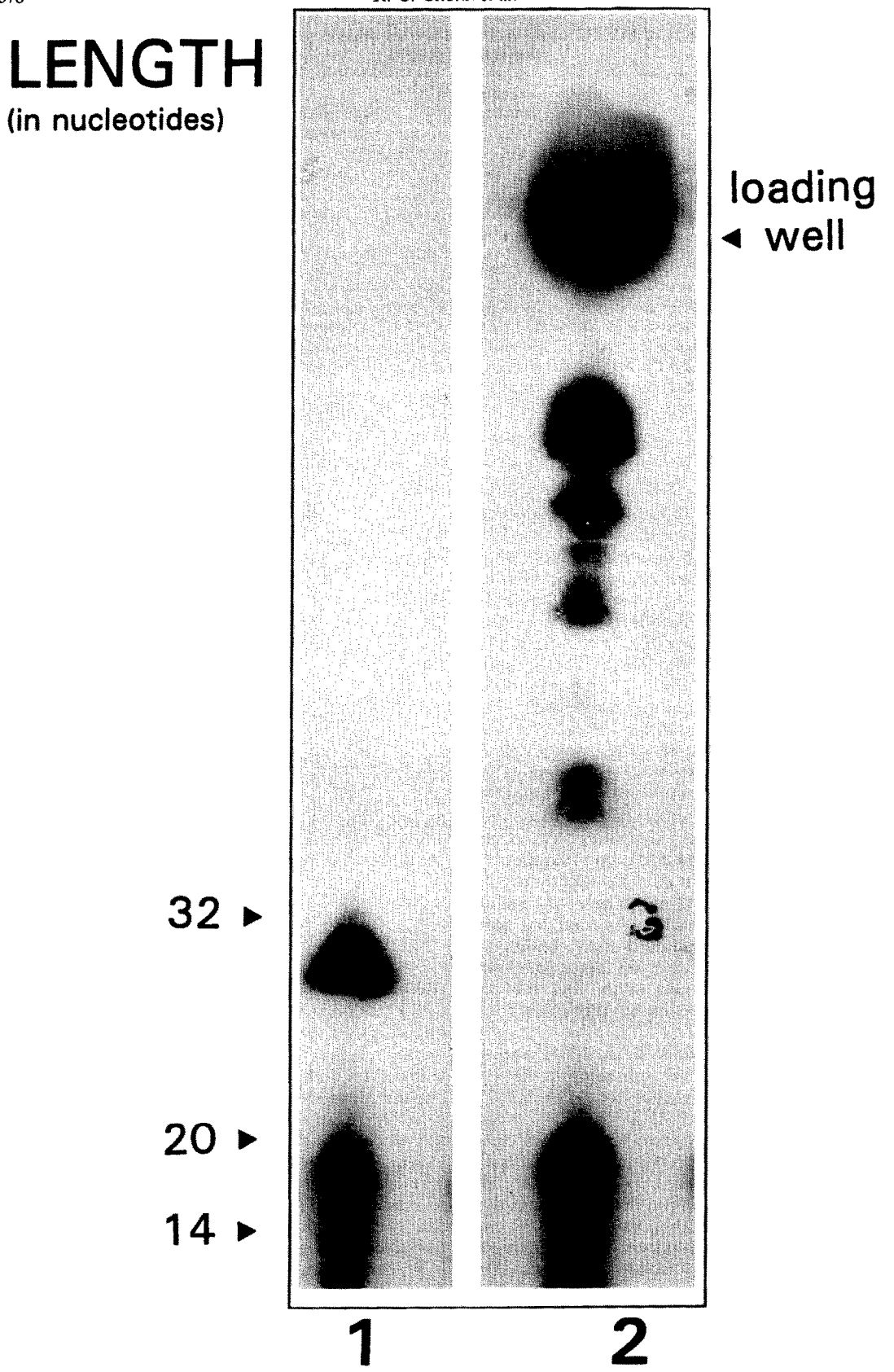

Fig. 2. Product analysis of FIV RT activity with poly(rU) RNA (lane 1) and ϕX-174 DNA (lane 2) templates. Reactions were carried out as described in Materials and Methods. Arrows on the left side indicate the length of oligonucleotide markers. The arrow on the right indicates the position where samples were loaded onto the gel (origin).

Table 2. Inhibition of FIV RT by analogs of dATP and dTTP using ϕX174 DNA and ribohomopolymer templates

Template	Substrate	K_m* (μM)	Inhibitor	K_i (nM)	IC_{50} (nM)
ϕX174 DNA	dATP	0.14 ± 0.06	ddATP	8.8 ± 3.4	80
			PMEApp	5.0 ± 0.9	75
Poly(rU)·oligo(dA)$_{12-18}$	dATP	5.1 ± 2.8	ddATP	ND†	12,000
			PMEApp	ND	17,000
ϕX174 DNA	dTTP	2.3 ± 1.1	ddTTP	14.0 ± 2.1	
			N_3dTTP	15.3 ± 0.9	
Poly(rA)·oligo(dT)$_{10}$‡	dTTP	3.4 ± 0.1	ddTTP	6.7 ± 3.3	
			N_3dTTP	3.3 ± 1.6	

* K_m and K_i values are reported as the mean ± SD of at least three determinations.

† K_i values could not be determined due to the poor linear response of the assays. The IC_{50} values are provided only to illustrate the insensitivity of FIV RT to these compounds when using this template-primer.

‡ From North *et al.* [5].

amount of primer, and is likely responsible for the low velocity and relative insensitivity to the dATP analogs.

We have also examined the activity of FIV RT with a ssDNA template that requires all four dNTP substrates, namely, ϕX-174 ssDNA primed with a 15 nucleotide primer. The velocity obtained by FIV RT in a reaction with this template-primer was 12% of that obtained with poly(rA)-oligo(dT), as shown in Table 1. DNA products made in reactions with ϕX-174 DNA were of much higher molecular weight that those obtained in reactions with poly(rU)-oligo(dA), as confirmed by primer extension assays (Fig. 2). Most of the product was greater than 100 nucleotides in length, although the presence of bands coresponding to lengths of approximately 30, 50 and 80 nucleotides longer than the primer suggests the presence of pause sequences.

Kinetic analyses were performed for inhibition of FIV RT by ddATP and PMEApp with the ϕX-174 DNA template. The K_m for dATP was determined to be 0.14 μM (Table 2), which is considerably lower than the value obtained in reactions with poly(rU)-oligo(dA). Both ddATP and PMEApp were competitive with respect to dATP in their inhibition of reactions, and yielded K_i values of 8.8 and 5.0 nM, respectively. This K_i value for ddATP is more consistent with the inhibition constants we reported for the other ddNTPs [5].

To compare kinetic data obtained with the ϕX-174 DNA to values previously obtained with an RNA homopolymer template, the effects of dTTP analogs upon the reaction with ϕX-174 DNA were compared to values we reported previously for inhibition of the reaction with poly(rA)-oligo(dT). The K_m for dTTP in the reaction with ϕX-174 DNA was 2.3 μM, which is comparable to the value of 3.4 μM that we obtained with poly(rA)-oligo(dT) [5]. Both ddTTP and N_3dTTP were competitive with respect to dTTP in their inhibition of reactions with either template. The K_i values for inhibition of the reaction with ϕX-174 DNA were determined to be 14.0 nM for ddTTP and 15.3 nM for N_3dTTP, which are 2- to 3-fold higher than values obtained in the reaction with poly(rA)-oligo(dT) [5]. These data are summarized in Table 2.

DISCUSSION

The two adenosine nucleoside analogs we have investigated in this study are potent inhibitors of FIV replication. In our focus reduction assay, the IC_{50} values for ddA and PMEA were both approximately 1.0 μM. These are slightly lower than our reported values for AZT (1.4 μM) and for ddI (2.1 μM) [7]; the IC_{50} values for ddA and PMEA were also lower than values we obtained for 2',3'-dideoxycytidine (>3 μM, Tandberg and North, unpublished data), or reported for 2',3'-dideoxy-2',3'-didehydrothymidine (D4T), phosphonoformate (PFA), 3'-azido-2',3'-dideoxyuridine (AZdU) or 3'-azido-2',3'-dideoxyguanosine (AZG), which are all greater than 6 μM [7]. Our finding that ddA was more potent than ddI as an inhibitor of FIV replication is in contrast to HIV, which was reported to be equally sensitive to these two dideoxynucleosides [19]. This could be due to metabolic differences between the feline cell line we use and human cells. The IC_{50} we determined for inhibition of FIV replication by PMEA in CrFK cells was only slightly higher than the value (0.6 μM) reported by Egberink *et al.* for inhibition of FIV replication in cultured feline thymocytes [13].

The AZT-resistant mutant of FIV that we have isolated remained sensitive to both PMEA and ddA. The IC_{50} values determined for inhibition of the mutant were approximately 1 μM and were not significantly different from values for inhibition of wild-type FIV. This mutant was shown previously to be cross-resistant to two azidonucleosides, AZdU and AZG, but similar to wild-type FIV in sensitivity to ddI, D4T and PFA [7]. This FIV mutant appears similar to AZT-resistant mutants of HIV that arise in humans after prolonged therapy with AZT, most of which are resistant to AZT, AZdU and AZG, but remain sensitive to dideoxynucleosides, D4T, PFA and PMEA [20, 21].

Studies of the inhibition of FIV RT by active

forms of ddA and PMEA required templates that utilize dATP as a substrate. We previously showed that poly(rU)-oligo(dA), at a primer to template ratio of 1:5 (moles of nucleotide), was a poor template for the FIV RT [4]. However, our present studies show that the velocity is dependent upon template:primer ratio, and can increase 5-fold when this ratio is increased to 1:1 (see Table 1). The highest velocity obtained with this template was low relative to that obtained for incorporation of dTTP into poly(rA)-oligo(dT). Moreover, the products of this reaction were less than 20 nucleotides longer than the primer. This failure to synthesize high molecular weight products suggests a distributive rather than processive mode of action with this template. It has been shown that the rate-limiting step for RT in a non-processive mode of polymerization is association and dissociation of the template-primer from the enzyme rather than the catalytic elongation step [22]. The high concentration of ddATP or PMEApp necessary to inhibit this reaction (>10 μM) is most likely a reflection of a non-processive reaction and the inability of these inhibitors to affect the rate-limiting step (association and dissociation of the template-primer).

The ϕX-174 ssDNA appears to be a good template for the FIV RT because of the high activity obtained with this template [12% of that obtained with poly(rA)-oligo(dT)], and the high molecular weight DNA produced. The K_m for dATP in this reaction (0.14 μM) was 35-fold lower than with poly(rU)-oligo(dA), and the concentrations of ddATP and PMEApp necessary to inhibit this reaction by 50% were more than 100-fold lower when the DNA template was used. The K_i value we determined for inhibition of FIV RT by ddATP in this reaction (8.8 nM) was much lower than the value for inhibition of HIV RT reported by Chen and Oshana [10], who used poly(rU)-oligo(dA) and poly(dU)-oligo(dA) in their studies. The K_i value we determined for ddATP using the ϕX-174 template was similar to K_i values reported for other ddNTPs against RTs from HIV [9, 10] and FIV [5]. The K_i we determined for inhibition of FIV RT by PMEApp (5.0 nM) was also substantially lower than the value for inhibition of the HIV RT in a reaction with poly(rU)-oligo(dA) (90 nM) reported by Balzarini *et al.* [14].

To determine whether reaction kinetics with this ϕX-174 DNA template were different from those obtained in our previous studies with other RNA homopolymers, we analyzed the inhibition of this reaction with ddTTP and N_3dTTP. We previously showed that both of these are competitive inhibitors of FIV RT in a reaction with poly(rA)-oligo(dT) with K_i values of 6.7 and 3.3 nM for ddTTP and N_3dTTP, respectively [5]. These compounds were also competitive inhibitors of the reaction with ϕX-174 DNA, although K_i values of 14 nM for ddTTP and 15.3 nM for N_3dTTP were somewhat higher than those obtained with the RNA homopolymer. This might reflect the lower frequency of template A residues and thus fewer opportunities for chain termination by ddTTP and N_3dTTP in ϕX-174 DNA compared to poly(rA).

Other templates we have attempted to use include 23S rRNA primed with oligodeoxyribonucleotides, and poly(A)$^+$-mRNA primed with oligo (dT). The reaction was barely detectable with the former and very low with the latter template [less than 0.1%, and approximately 1%, respectively, of the velocity with poly(rA)-oligo(dT), data not shown]. We have also used several preparations of DNase I gapped DNA and obtained variable results with velocities ranging from 3 to 10% of that with poly(rA)-oligo(dT) (data not shown). The velocity obtained with ϕX-174 DNA was consistently higher than with any preparation of gapped DNA we tested.

The ϕX-174 DNA template is the best of those we have screened for studies of the inhibition of FIV RT by dATP analogs. The problems we have encountered with poly(rU)-oligo(dA) underscore the need to ascertain that the DNA product of an RT reaction is highly polymerized DNA. This primed ϕX-174 DNA is also an efficient template for the HIV RT [15] and it should prove suitable for studies of its inhibition by dATP analogs.

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