was used and τ/T_1 ratio (0.33) was within the range of 0.07 to 0.6[5], the spectra were relatively noisy, owing to the geometry of the solenoidal radiofrequency coil and a relatively weak B_0 magnetic field. It excluded the possibility of evaluating P_i. However, it showed a marked decrease during the periods with a high perfusion rate in all experiments, which suggested an increased phosphorylation potential during enhanced oxygen consumption. Parallel enhancements of PCr and ATP with increased perfusion rate, evaluated in arbitrary units, were highly significant (t test: $p < 0.001$ for PCr; $p < 0.02$ for ATP). A confirmation of the absolute values needs simultaneous biochemical analysis.

At the low perfusion rate, the mean specific O_2 delivery, due to the high pO_2 in the perfusate, was 4.4 pmols $\cdot$ $mg^{-1} \cdot s^{-1}$ during an O_2 consumption of 2.5 pmols $\cdot$ $mg^{-1} \cdot s^{-1}$. Intramitochondrial pO_2 is considered to be well above the critical level (when the rate of electron transport begins to be limited by O_2 availability), on the basis of a following speculation. The O_2 delivery is sufficient if the vascular bed of the microcirculation remains open. Under $pO_2 > 13$ kPa, the capillary functional density declines to zero[6]. At a $pO_2 = 20$ kPa the terminal arterioles are completely closed[7]. With 5 kPa in our superfusion medium we were well below this constrictive level. Under these conditions the blood vessels/myoglobin gradient is 15 Torr/μm[8]. 97 kPa in the perfusion medium means 727 Torr, which supplies this gradient for 48 μm on both sides, which is from 1/5 to 1/10 of the distance toward the muscle surface. Approximately the same additional distance brings the gradient down to the critical 0.5 Torr, which is a sufficient pO_2 value for a maximum cytochrome turnover during an O_2 consumption rate 47 times higher than in our case[8]. Thus for a microcirculation three open capillaries, distributed toward the surface of the muscle, could be enough to achieve this critical level.

Acknowledgments. We thank Dr. Kevin Conley for his kind advice during experiments, and Mr. Rudy Stuppard for his careful technical assistance.

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0014-4754/92/11-12/1125-03$1.50 + 0.20/0

Enzymatic kinetic studies with the non-nucleoside HIV reverse transcriptase inhibitor U-9843

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Received 5 May 1992; accepted 15 July 1992

Abstract. The polymer of ethylenesulfonic acid (U-9843) is a potent inhibitor of HIV-1 RT (reverse transcriptase) and the drug possesses excellent antiviral activity at nontoxic doses in HIV-infected lymphocytes grown in tissue culture. The drug also inhibits RTs isolated from other species such as AMV and MLV retroviruses. Enzymatic kinetic studies of the HIV-1 RT catalyzed RNA-directed DNA polymerase function, using synthetic template : primers, indicate that the drug acts generally noncompetitively with respect to the template : primer binding site but the specific inhibition patterns change somewhat depending on the drug concentration. The inhibitor acts noncompetitively with respect to the dNTP binding sites. Hence, the drug inhibits this RT polymerase function by interacting with a site distinct from the template : primer and dNTP binding sites. In addition, the inhibitor also impairs the DNA-dependent DNA polymerase activity of HIV-1 RT and the RNase H function. This indicates that the drug interacts with a target site essential for all three HIV RT functions addressed (RNA- and DNA-directed DNA polymerases, RNase H).
Key words. HIV RT; inhibitor; polysulfonate; inhibition kinetics.

Polysulfates and polysulfonates such as pentosan polysulfates, dextransulfates and suramin have been identified as potent inhibitors of the HIV RT (reverse transcriptase) enzyme and have shown antiviral activity in HIV-infected lymphocytes grown in tissue culture[1–8]. We have found that U-9843, the polymer of ethylenesulfonic acid, tested as its ammonium salt form, is a potent inhibitor of HIV RT and the drug showed excellent

antiviral activity at nontoxic doses in HIV-infected lymphocytes grown in culture. The average molecular weight is 562 as determined by vapor pressure osmometry which corresponds to an average polymerization degree of about 4. Enzymatic inhibition studies were carried out with this drug to investigate the basis for its inhibitory activity against HIV RT.

Materials and methods

The expression of HIV-1 RT and its purification have been described[9]. For the polymerase assays, a partially purified RT preparation was used which was judged as 90–95% pure based on SDS polyacrylamide gel electrophoresis. This preparation was devoid of *Escherichia coli* RNase H activity and consisted of p51/p66 heterodimers of RT, with no evidence of monomeric RT in the form of p66 or p51 alone. Three forms of highly purified recombinant RT were used in the RNase H assay including the p66/p66 homodimer, the p51/p66 heterodimer and the C-terminal p15 domain. Their expression and purification have been described[10–12].

The synthetic template : primers poly (rA), oligo $(dT)_{10}$, poly (rC), oligo $(dG)_{10}$, poly (dA) : $(dT)_{12-18}$ and poly (dC) : $(dG)_{12-18}$ were purchased from Pharmacia. α-[^{35}S]-labeled dTTP and dGTP as well as [^{3}H]GTP were purchased from Dupont NEN.

The standard reaction mixtures for the RNA-directed DNA polymerase assay contained 20 mM dithiothreitol, 60 mM NaCl, 0.05% NP-40, 10 mM $MgCl_2$, 50 mM Tris-HCl, pH 8.3, 8 μM of the cognate α-[^{35}S]-labeled deoxyribonucleotide-5′-triphosphate (final specific activity 1 Ci/mmol), 10 μg/ml of RNA template [poly (rA) or poly (rC)], 5 μg/ml of the appropriate primer $(dT)_{10}$ or $(dG)_{10}$, and 0.45 μg of purified HIV-1 RT. The total volume of the reaction mixtures was 50 μl. The samples were incubated at 37 °C for 15 min. The reactions were terminated by the addition of equal volumes of 10% trichloroacetic acid. Incorporation of radiolabeled precursor was determined by collecting the precipitates on glass fiber filters, drying and counting the samples.

The AMV and MLV RT preparations were purchased from BRL. These enzymes were assayed in the same standard reaction mixture as described above for the HIV RT. Sufficient amounts of the latter RT species were added per reaction mixture to incorporate 0.04 nmol of dNTP in 15 min at 37 °C.

The DNA-directed DNA polymerase activity of the RT enzyme was assessed as described above for the RNA-directed DNA polymerase assay. The synthetic template : primers used were poly (dA) : $(dT)_{12-18}$ or poly (dC) : $(dG)_{12-18}$ present at concentrations of 10 μg/ml.

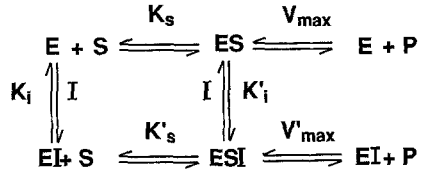
The RNase H assay was conducted as described[13]. In general, the assay follows the loss of trichloroacetic acid precipitable radiolabeled RNA : DNA hybrid as a function of time. The specific assay mixtures contained 2.5 μg and 2 μCi/ml of [^{3}H]poly (rG) : poly (dC) (1 : 1), 50 mM Tris-HCl, pH 8.5, 5 mM $MgCl_2$, 0.02% NP-40, and 3% glycerol. Incubation was for 10 min at 25 °C and the reactions were terminated by the addition of equal volumes of 10% trichloroacetic acid. The loss of substrate was determined by collecting the precipitates on glass filters, drying and counting the samples.

The infectivity assays in HIV infected lymphocytes grown in culture were carried out by the syncytia reduction method[14] or by measuring the total amount of core p24 protein released into the culture medium and the total amount of viral RNA synthesized[15].

The kinetic assay results were statistically analyzed with two computer programs using Michaelis-Menten kinetics. One was the Enzfitter program (Sigma) which calculates the V_{max} and K_m values for each single curve. This program was used to analyze the data in the experiments with varying template : primers where the specific inhibition patterns were found to change with the drug concentrations. The second program is based on a nonlinear least square fit method[16]. This latter program averages all the experimental data points to fit one specific equation. The general equation used for this program was:

$$v_0 = \frac{(V_{max} + V'_{max}\, I/K'_i)\, S}{S(1+I/K'_i) + K_m(1+I/K_i)}$$

where v_0 = initial reaction velocity in cpm/15 min, V_{max} = maximum initial velocity of product formation by the enzyme-substrate or [ES] complex in cpm/15 min, V'_{max} = maximum initial velocity of product formation by the enzyme-inhibitor-substrate or [EIS] complex in cpm/15 min, S = concentration of limiting substrate (μM dNTP or primer), I = inhibitor concentration (μM), K_m = Michaelis-Menten constant for the limiting substrate (μM), K_i = dissociation constant of the enzyme-inhibitor or [EI] complex (μM), K'_i = dissociation constant of the enzyme-inhibitor-substrate or [EIS] complex (μM). Data generated by the programs were plotted using the experimental data points and the computer-generated curves defined by the indicated equation for best fit. The general reaction scheme outlining the inhibition kinetics is shown in figure 1. Assays for each kinetic experiment were carried out in duplicate and the results were confirmed in at least 3–4 repeat experiments.



E = enzyme
S = substrate
P = product
I = inhibitor

V_{max} = max. velocity of uninhibited reaction
V'_{max} = max. velocity of inhibited reaction
K_s = substrate constant, uninhibited reaction
K'_s = substrate constant, inhibited reaction
K_i = dissociation constant of EI complex
K'_i = dissociation constant of ESI complex

Figure 1. General reaction scheme for enzyme inhibition.

Results

Antiviral activity. The polyethylenesulfonate U-9843 is a potent inhibitor of HIV RT and has shown excellent antiviral activity at nontoxic doses in HIV-1 infected lymphocytes grown in tissue culture. In the syncytia reduction assay, using MT-2 cells infected with HIV-1 (IIIb isolate), the IC_{50} was < 10 μM of U-9843 and the IC_{50} in terms of cytotoxicity to the host cells was > 178 μM of drug (table 1). This corresponds to a toxicity/activity ratio in excess of 17.8. In the p24 release and total viral RNA synthesis assays, carried out in HIV-1 (H34 isolate) infected peripheral blood mononuclear cells, the IC_{50} values in terms of p24 core protein released and total viral RNA synthesized were < 10 μM of U-9843 both at days 3 and 4 post-infection of the cells (table 2). No apparent significant toxic effects on the host cells were observed.

RNA-directed DNA polymerase. Enzymatic kinetic studies were performed with U-9843 and synthetic template : primers to determine the type of inhibition pattern on the RNA-directed DNA polymerase function of HIV-1 RT with respect to the dNTP and template : primer binding sites.

In one set of experiments the template : primer combination poly (rA) : $(dT)_{10}$ was varied and three concentrations of inhibitor (0.5, 0.875, and 1 μM) were studied in addition to the control containing no drug. The analysis of the data by the nonlinear least square fit program, which averages all the experimental data points and fits them to a common equation, showed that the general inhibition pattern was characteristic for a mixed inhibitor. The V'_{max} was 0, the K_i was 1.4 μM (SD = 0.7) of U-9843 and the K'_i was 0.5 μM (SD = 0.1) of drug. The K_m was 3.3 μM (SD = 0.7) of $(dT)_{10}$ for the control containing no inhibitor (table 3). However, the kinetic inhibition patterns were not uniform and changed depending on the drug concentration. For this reason the curve for each drug concentration within the experimental set was generated separately from the others with the Enzfitter program and transformed into a Lineweaver-Burk plot (fig. 2a). At the lower drug concentrations tested (0.5, 0.875 μM), the inhibition patterns were nearly uncompetitive as evidenced by a drop in both the V_{max} and the apparent K_m values as compared to the control reaction. At the highest drug concentration of 1.25 μM, the K_m was the same as in the control reaction and the V_{max} showed a decrease which is characteristic for a noncompetitive inhibitor. This reveals a pleiotropic effect of U-9843 in the sense that the specific inhibition pattern is concentration-dependent in the system as the drug acts

Table 3. Kinetic constants for the inhibition of HIV-1 RT (RNA-dependent DNA polymerase) by U-9843

Variable substrate	Fixed substrate	K_m μM	K_i μM	K'_i μM	Inhibition type
dTTP	$(dT)_{10}$	39.0	0.63	0.62	Noncompetitive
$(dT)_{10}$	dTTP	3.3	1.4	0.5	Mixed
dGTP	$(dG)_{10}$	11.0	0.36	0.36	Noncompetitive
$(dG)_{10}$	dGTP	3.0	0.38	0.38	Noncompetitive

Table 1. Antiviral activity of U-9843 in the syncytia reduction assay in HIV-1 infected MT-2 cells

Sample		Number of syncytia per plate
Control		32
U-9843,	178 μM	0
	17.8 μM	0
	1.78 μM	7
Control		51
U-9843,	17.8 μM	16
	1.78 μM	40
	0.178 μM	39

The cytotoxicity in MT-2 cells was > 178 μM of U-9843 in 6 separate assays.

Table 2. Antiviral activity of U-9843 in HIV-1 infected peripheral blood mononuclear cells

Sample		Day	ng HIV p24/ml	pg HIV RNA/ml	Cell viability % of control
Control		3	50.4	1225	–
U-9843,	10 μM		6.6	0	–
	1 μM		49.4	1375	–
	0.1 μM		50.4	1736	–
Control		4	49.3	1490	–
U-9843,	10 μM		12.5	110	79
	1 μM		47.4	1462	82
	0.1 μM		48.6	1590	77

The assay methods were as described under Materials and methods.

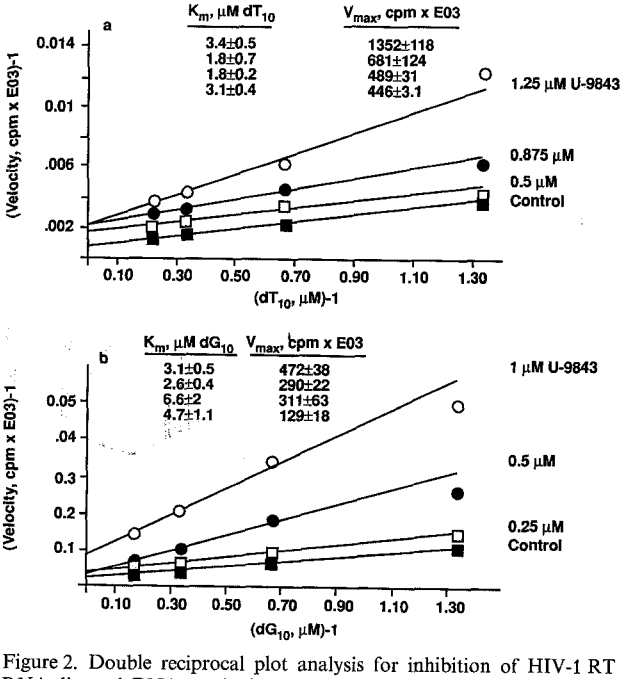


Figure 2. Double reciprocal plot analysis for inhibition of HIV-1 RT RNA-directed DNA synthesis by U-9843. *a* Various concentrations of poly (rA) : $(dT)_{10}$ (1 : 0.5) were tested in the presence of excess dTTP (160 μM). *b* Various concentrations of poly (rC) : $(dG)_{10}$ (1 : 0.5) were tested in the presence of excess of dGTP (84 μM). The remainder of the reaction mixtures were as described under Materials and methods.

uncompetitively at very low concentrations and noncompetitively at higher concentrations.

A similar experiment was carried out in the presence of various amounts of the template : primer poly (rC) : $(dG)_{10}$ (table 3). The drug was tested at concentrations of 0.25, 0.5, and 1 μM. The data analysis with the nonlinear least square fit program yielded a K_m of 3 μM (SD = 0.5) of $(dG)_{10}$ in the control reaction containing no drug. The V'_{max} was 0 and the dissociation constants K_i and K'_i were equal and amounted to 0.38 μM (SD = 0.03) of U-9843 which indicates that the inhibitor acts on the average noncompetitively with respect to the $(dG)_{10}$ binding site. However, when each curve was analyzed separately with the Enzfitter program (fig. 2b), the V_{max} values showed a decrease and the apparent K_ms an increase as compared to the controls at the higher drug concentrations (0.5, 1 μM), which is indicative for a mixed inhibitor. Hence, the inhibition pattern with respect to the poly (rC) : $(dG)_{10}$ binding site was noncompetitive at low drug concentrations and mixed at higher concentrations. This reveals, as discussed above for the poly (rA) : $(dT)_{10}$ system, a pleiotropic concentration-dependent effect of U-9843 in the poly (rC) : $(dG)_{10}$ system.

The effect of varying amounts of the mononucleotide dTTP was investigated in the presence of 0.25, 0.5, and 1 μM of U-9843 (fig. 3a, table 3). The kinetic analysis of the data by the nonlinear least square fit program indicated that the V'_{max} was zero and the K_i and K'_i values were equal and yielded 0.62 μM (SD = 0.03) of dTTP. This shows that the inhibitor binds equally well to the free enzyme as to the enzyme-substrate complex. The K_m for dTTP was 39 μM (SD = 3) in the control reaction containing no drug. This kinetic inhibition pattern is indicative for a noncompetitive inhibitor and shows that the drug does not compete for the dTTP binding site. The analysis of the data with the Enzfitter program yielded essentially the same conclusion.

An analogous experiment was performed with varying amounts of the mononucleotide dGTP (fig. 3b, table 3). The drug concentrations used were 0.25, 0.5 and 1 μM. Again, the results were analyzed with the nonlinear least square fit program. The K_m was 10.9 μM (SD = 1.2) of dGTP in the control reaction. Moreover, the V'_{max} was zero and the K_i and K'_i values were equal and calculated to be 0.36 μM (SD = 0.02) of dGTP. Thus, as in the case with dTTP, this indicates that the drug binds equally well to the free enzyme as to the enzyme-substrate complex and the resulting inhibition pattern is characteristic for a noncompetitive inhibitor. The analysis of the data with the Enzfitter program yielded the same conclusion.

DNA-directed DNA polymerase. U-9843 was also tested for its effect on the DNA-directed DNA polymerase function of RT. The poly (dA) : (dT) homopolymer is a very inefficient template : primer for RT operating in the DNA-directed DNA synthesis mode[17]. Using poly (dC) : oligo (dG) as a template : primer the reaction proceeded at rates comparable to the RT driven RNA-directed DNA polymerase function. Thus, with poly (dC) : oligo (dG) as the template : primer, the IC_{50} value for U-9843 as determined in the standard assay described under Methods was 2.7 μM (SD = 0.3) which is higher than the corresponding value in a poly (rC) : oligo (dG) primed assay (table 4).

RNase H assay. The drug U-9843 was also tested for inhibitory activity against HIV-1 RNase H. The substrate for the reaction consisted of a full length poly (rG) : poly (dC) hybrid with the RNA component being [^{3}H]-labeled. Initially, the dose-response relationship for U-9843 was established with the heterodimeric p51/p66 RNase form of RT and the IC_{50} for the drug was 1.6 μM (SD = 0.2) in this system (table 5). Recently, a p15 protein, consisting of the C-terminal RNase H domain

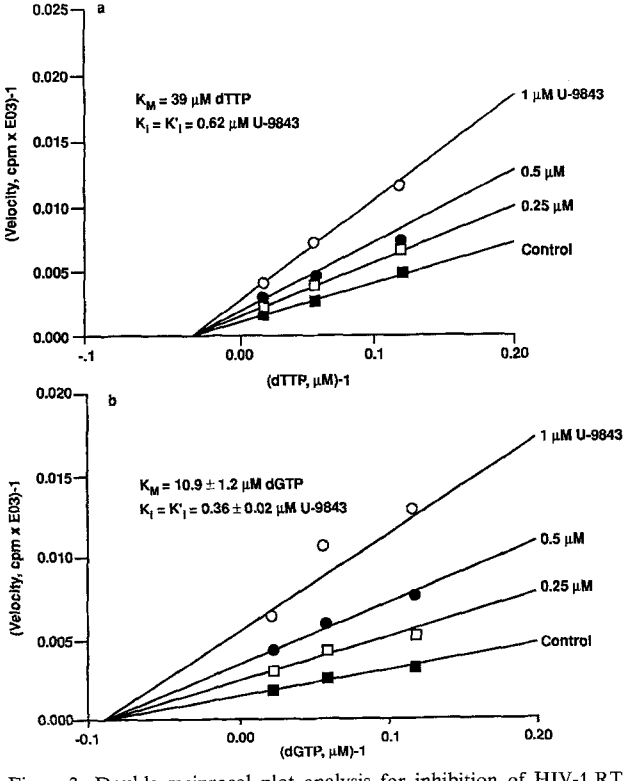


Figure 3. Double reciprocal plot analysis for inhibition of HIV-1 RT RNA-directed DNA synthesis by U-9843. *a* Various concentrations of dTTP were tested in the presence of excess amounts of poly (rA) : $(dT)_{10}$ (7.5 μM). *b* Various concentrations of dGTP were tested in the presence of excess poly (rC) : $(dG)_{10}$ (7.5 μM). The remainder of the reaction mixtures were as described under Materials and methods.

Table 4. DNA-directed DNA polymerase of HIV-1 RT; dose-response relationship of U-9843

Sample		dTMP incorporated, cpm	% Inhibition	IC_{50}, μM
Control		827352	0	2.7 μM (SD = 0.3)
U-9843,	1 μM	587395	29	
	2 μM	523110	37	
	4 μM	314733	62	

The reaction mixtures were as described under Materials and methods. The template was poly (dC) : $(dG)_{12-18}$.

Table 5. RNase H function of p51/p66 HIV-1 RT; dose-response relationship of U-9843

Sample	Unhydrolyzed substrate remaining, cpm	% Inhibition	IC_{50}, μM
Control, no RT	41033		
Control + RT	21602	0	1.2 μM (SE = 0.2)
U-9843, 0.5 μM	26017	22	
2.5 μM	34140	65	
5 μM	45477	100	

The reaction mixtures were described in Materials and methods. The concentration of p51/p66 enzyme was 12 μg/ml.

Table 6. Inhibition of diverse HIV-1 RNase H forms by U-9843

Enzyme form	Unhydrolyzed substrate remaining, cpm			
	No enzyme	Enzyme	Enzyme + U-9843, 10 μM	% Inhibition
p66/p66	64400	24295	60887	90
p51/p66	73113	43059	71901	80
p15	59396	24236	25685	4

The enzyme concentrations used were 9.3 μg/ml for p66/p66 RNase H, 9 μg/ml for p51/p66 RNase H and 124.8 μg/ml for p15 RNase H, respectively. The remainder of the reaction mixtures were as described under Materials and methods.

of the p66 RT enzyme was cloned and expressed in *E. coli*[12]. The construct retains RNase H activity and is devoid of any polymerase activity. In addition, we also prepared unprocessed p66/p66 homodimer RT[10]. The drug, present at concentrations of 10 μM, was then tested for inhibitory activity against all three RNase H forms at hand, meaning the naturally occurring p51/p66 heterodimer, the unprocessed p66/p66 homodimer and the p15 C-terminal fragment (table 6). Confirming the results discussed above, the compound under study did inhibit the p51/p66 form of the enzyme (80% inhibition) and also potently inhibited the unprocessed p66/p66 form (90% inhibition). However, U-9843 did not inhibit the RNase H activity of p15. This observation demonstrates that the inhibitor does not interact directly with the catalytic site of the esterase but instead impairs a function residing within the p51 domain of the RT.

Other retroviral RT species. The drug was also tested for its inhibitory activity against the RNA-directed DNA polymerase functions of HIV-2 RT, AMV RT, and MLV RT. The results showed that the IC_{50} values were 1.45 μM (SD = 0.2) of U-9843 for HIV-2 RT, 1.0 μM (SD = 0.2) for the AMV enzyme, and 0.4 μM (SD = 0.01) for the MLV RT, respectively (table 7).

Table 7. Inhibition of diverse RT enzymes (RNA-directed DNA polymerase) by U-9843

Enzyme species	U-9832 μM	dTMP incorporated, cpm	% Inhibition	IC_{50}, μM
HIV-2 RT	0	383498	0.0	1.4 (SD = 0.2)
	0.25	354277	7.7	
	0.5	290684	24.2	
	1	214452	44.1	
	1.5	199502	48.0	
AMV RT	0	119149	0.0	1.0 (SD = 0.2)
	0.25	85156	28.6	
	0.5	80970	32.1	
	1	57017	52.0	
MLV RT	0	219248	0.0	0.4 (SD = 0.01)
	0.25	188786	13.9	
	0.5	99123	54.8	
	0.75	28007	87.2	
	1	8152	96.3	

The enzyme concentrations were 3.7 μg/ml for HIV-2 RT, 17.5 units/ml for AMV RT and 800 units/ml for MLV RT. The template : primer used was poly (rA) : $(dT)_{10}$. The remainder of the reaction mixtures were as described under Materials and methods.

Discussion

The inhibition kinetics of U-9843 on the RNA-directed DNA polymerase domain of HIV-1 RT were investigated with respect to the template : primer and dNTP binding sites. The data indicate that the inhibition pattern with respect to the poly (rA) : $(dT)_{10}$ binding site ranges from uncompetitive to noncompetitive in response to increasing concentrations of drug. This indicates that at low drug concentrations the inhibitor does not bind to the free enzyme but binds to the enzyme-substrate complex while at higher concentrations it interacts equally with both the free enzyme and the enzyme-substrate complex. Similarly, the inhibition pattern with respect to the poly (rC) : $(dG)_{10}$ binding site changes from noncompetitive to mixed with increasing drug concentrations which means that the inhibitor binds equally to the free enzyme and the enzyme-substrate complex at low inhibitor concentrations but interacts preferentially with the enzyme-substrate complex at higher concentrations. Moreover, U-9843 does not interfere with dNTP binding and is a noncompetitive inhibitor with respect to this latter site. These results suggest that U-9843 interacts with a site distinct from either the template : primer and the dNTP binding sites. The drug was also tested for its inhibitory activity against other RT species including HIV-2, AMV and MLV RT. U-9843 is also a potent inhibitor of these three enzyme species which indicates that the drug lacks exclusive specificity for HIV-1 RT. The potency of the drug is largely independent of the base composition of the template : primer [poly (rA) : $(dT)_{10}$ vs poly (rC) : $(dG)_{10}$] as indicated by the near identical ratios between the respective K_m values of the template : primers and their cognate K_i values. The agent also inhibits the other two functions of HIV-1 RT, the DNA-directed DNA polymerase and the RNase H of the p51/p66 and p66/p66 dimeric forms of the RT but not the RNase H activity of the p15 C-terminal peptide. Hence, U-9843 apparently interferes with an essential site involved within the active domains of all three main activities of the RT enzyme addressed. Moreover, since only the RNase H activity of the p51/p66 and p66/p66 dimeric forms of RT is inhibited by the drug but not the function of the p15 form, the inhibitor impairs a function residing within the p51 subunit of RT.

HIV-inhibiting sulfated polysaccharides (dextran sulfate, heparin, carboxymethylchitosan) exert their antiviral activity by interfering with the adsorption of the viral particles to the target cell, but also inhibit the RT and RNase H activity[18,19]. In addition to the polyethylenesulfonate U-9843 described in this paper, a few other polysulfonates were reported to inhibit HIV such as suramin, naphthalenesulfonic acids and other sulfonic acid dyes[18,20]. All of these agents have shown RT inhibitory activity.

Recently, other non-nucleoside classes of HIV-1 RT inhibitors have been described. These include the benzodiazepines or TIBO compounds[21,22]; the dipyridodiazepinones[23,24]; the HEPT derivatives[15,25]; the pyridinone derivatives[26] and the bisheteroarylpiperazines (BHAP compounds)[27]. The TIBO compound R82150 appears to be a specific inhibitor of HIV-1 RT-catalyzed RNA-directed DNA synthesis[22]. Kinetic studies suggest that the inhibitor acts uncompetitively with respect to the nucleic acid binding site and noncompetitively with respect to the dNTP site. The IC_{50} for DNA-directed DNA synthesis was 40 times higher than the one required for effective inhibition of RNA-directed DNA synthesis. Moreover, R82150 did not inhibit RNase H. The dipyridodiazepinone BI-RG-587 acts noncompetitively with respect to dGTP during RNA-directed DNA synthesis by RT[23]. This drug was also found to inhibit the function of RNase H but it did not inhibit the RNA-directed DNA synthesis of RT enzymes from other species such as simian and feline retroviruses. The HEPT compounds are pyrimidine analogs. One member of this class, E-EPU [5-ethyl-1-benzyloxymethyl-6-(phenylthio)uracil] was reported to act as a competitive inhibitor with respect to dTTP and noncompetitively with respect to dGTP or poly (rA) : oligo (dT)[15]. The pyridinone derivatives[26], like the TIBO compounds mentioned above, also seem to act as uncompetitive inhibitors with respect to the nucleic binding site and noncompetitive with respect to the dNTP binding site. Amongst these classes of non-nucleoside RT inhibitors mentioned, U-9843 is unique in that it is the only drug which inhibits all three main RT enzyme functions including the RNA- and DNA-directed DNA polymerases and RNase H. Moreover, U-9843 is the only inhibitor able to inhibit other RT enzyme species in addition to HIV-1 RT.

Acknowledgments. We thank R. S. Goody, Max-Planck-Institute, Heidelberg, for his generous gift of HIV-2 RT and D. P. Brunner for providing some of the *E. coli* (RT +) cells used to prepare purified HIV-1 RT for the enzyme kinetic studies. These studies were supported in part by a grant from the NCDDG and the Gunerick and Aussen Research Fund of Miami, Florida, USA.

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