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The Benzylthio-Pyrimidine U-31,355, a Potent Inhibitor of HIV-1 Reverse Transcriptase

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ABSTRACT. U-31,355, or 4-amino-2-(benzylthio)-6-chloropyrimidine is an inhibitor of human immunodeficiency virus type 1 (HIV-1) reverse transcriptase (RT) and possesses anti-HIV activity in HIV-1-infected lymphocytes grown in tissue culture. The compound acts as a specific inhibitor of the RNA-directed DNA polymerase function of HIV-1 RT and does not impair the functions of the DNA-catalyzed DNA polymerase or the RNase H of the enzyme. Kinetic studies were carried out to elucidate the mechanism of RT inhibition by U-31,355. The data were analyzed using Briggs–Haldane kinetics, assuming that the reaction is ordered in that the template:primer binds to the enzyme first, followed by the addition of dNTP, and that the polymerase is a processive enzyme. Based on these assumptions, a velocity equation was derived that allows the calculation of all the essential forward and backward rate constants for the reactions occurring between the enzyme, its substrates, and the inhibitor. The results obtained indicate that U-31,355 acts as a mixed inhibitor with respect to the template:primer and dNTP binding sites associated with the RNA-directed DNA polymerase domain of the enzyme. The inhibitor possessed a significantly higher binding affinity for the enzyme-substrate complexes than for the free enzyme and consequently did not directly affect the functions of the substrate binding sites. Therefore, U-31,355 appears to impair an event occurring after the formation of the enzyme–substrate complexes, which involves either inhibition of the phosphoester bond formation or translocation of the enzyme relative to its template:primer following the formation of the ester bond. Moreover, the potency of U-31,355 depends on the base composition of the template:primer in that the inhibitor showed a much higher binding affinity for the enzyme–poly (rC):$(dG)_{10}$ complexes than for the poly (rA):$(dT)_{10}$ complexes. BIOCHEM PHARMACOL 51;6:743–750, 1996.

KEY WORDS. HIV-1 reverse transcriptase; non-nucleoside inhibitors; inhibition kinetics

Several classes of non-nucleoside HIV-1 RT§ inhibitors have been described recently. These include the bis(heteroaryl)piperazines or BHAPs [1–8]; the dipyridodiazepinones [9, 10]; the benzodiazepines or TIBO compounds [11, 12]; the pyridinones [13]; the quinoline U-78,036 [14]; polysulfates and polysulfonates [15–25]; the α-anilinophenylacetamide derivatives [26]; and the HEPT (Fig. 1) or its derivatives [27, 28]. U-31,355 (Fig. 1) bears some structural similarity with this last class of compounds. It differs from the above-mentioned HEPT derivatives within the heterocylic moiety in that it contains a substituted pyrimidine, whereas the HEPT compounds contain thymine or uracil. In regard to the sulfur substituents, the HEPT compounds contain a phenylthio group, whereas U-31,355 contains a benzylthio group. U-31,355 is an inhibitor of HIV-1 RT and has antiviral activity at nontoxic doses in experimentally HIV-1 infected lymphocytes grown in tissue culture.

The current report presents data on the antiviral activity of U-31,355 in HIV-infected lymphocytes grown in tissue culture and enzymatic inhibition kinetic studies, using recombinant HIV-RT to examine the specific inhibition pattern of U-31,355 on the RNA-directed DNA polymerase function of HIV-1 RT.

MATERIALS AND METHODS

The expression of HIV-1 RT and its purification have been described [29, 30]. The enzyme was devoid of *Escherichia coli* RNase H activity and consisted of p51/p66 heterodimers as evidenced by gel electrophoresis.

The synthetic templates, primers and template:primers

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§ *Abbreviations:* HIV-1 RT, human immunodeficiency virus type 1 reverse transcriptase (EC 2.7.7.49); HIV-2 RT, HIV-2 human immunodeficiency virus type 2 reverse transcriptase; RNase H, HIV-1 ribonuclease H (EC 3.1.26.4); HEPT, 1-[(2-hydroxyethoxyl)-methyl]-6-(phenylthio)thymine; U-31,355, 4-amino-2-(benzylthio)-6-chloro-pyrimidine; and E-EPU, 1-ethoxymethyl-5-ethyl-6-(phenylthio)uracil.

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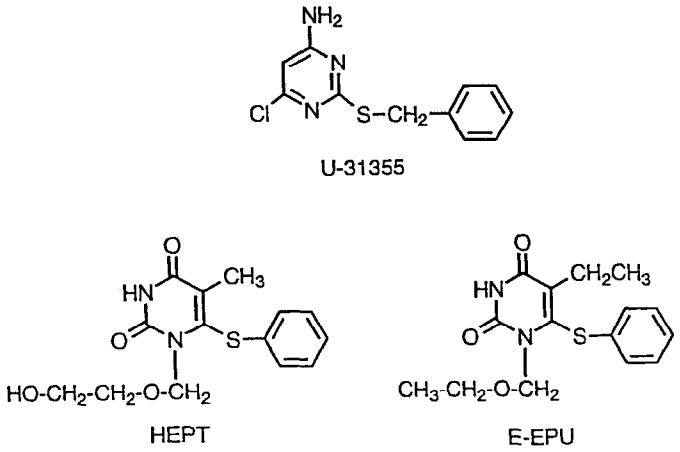

FIG. 1. Chemical structures of U-31,355, HEPT, and E-EPU.

poly(rA), $(dT)_{10}$, poly(rC), $(dG)_{10}$, and poly(dC):$(dG)_{12-18}$ were purchased from Pharmacia (Piscataway, NJ). α-^{35}S-Labeled dTTP and dGTP were purchased from Dupont NEN (Wilmington, DE). Nonidet P-40 was purchased from Sigma (St. Louis, MO).

The standard reaction mixtures for the HIV-1 RT RNA-directed DNA polymerase assay contained 20 mM dithiothreitol, 60 mM NaCl, 0.05% Nonidet P-40, 10 mM $MgCl_2$, 50 mM Tris · HCl, pH 8.3, a 10 μM concentration 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 0.0274 μM HIV RT. The total volume of the reaction mixtures was 50 μL. The samples were incubated at 37° 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 DNA-directed DNA polymerase activity of the HIV-1 RT enzyme was assessed as described above for the RNA-directed DNA polymerase assay. The synthetic template: primer used was poly(dC):$(dG)_{12-18}$ (1:1 by weight), present at a concentration of 10 μg/mL.

The RNase H assay was conducted as described [31]. 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 by weight), 50 mM Tris · HCl, pH 8.5, 5 mM $MgCl_2$, 0.02% Nonidet P-40, and 3% glycerol. Incubation was for 10 min at 25°, 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 [32] or by measuring the total amount of core p24 protein released into the culture medium and the total amount of viral RNA synthesized; the exclusion of trypan blue was used to assess cell toxicity [28]. DNA polymerases α and δ were purified from fetal calf thymus and assayed in the absence of proliferating cell nuclear antigen as described [33].

The avian myoblastoma and murine leukemia virus RT preparations were purchased from GIBCO BRL (Gaithersburg, MD). 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 approximately 0.04 nmol of dNTP in 15 min at 37°.

Michaelis–Menten kinetics, which are based on the establishment of a rapid equilibrium between the enzyme, its substrates, and the inhibitor, and the various enzyme–substrate complexes proved inadequate for the analysis of the kinetic data. The data were thus analyzed applying steady-state Briggs–Haldane kinetics as described previously [6] using Chou's graph theory of enzyme kinetics [34, 35]. The HIV RT catalyzed system considered here consists of two substrates, S_1, representing the template:primer, and S_2, representing the dNTP, and I, an inhibitor. Since the system is ordered in that the binding of S_1 to E precedes the binding of S_2, the system can be simplified significantly. Furthermore, the reactions between the inhibitor and the enzyme and various enzyme–substrate complexes are assumed to be diffusion-controlled [36–38], and the interconversion rates between E and EI, ES_1 and EIS_1, and ES_1S_2, and EIS_1S_2, respectively, can be expected to occur much faster than those between the enzyme and its substrates. Thus, although the whole system is a steady-state one, there is an equilibrium between the low molecular weight inhibitor and the enzyme and the enzyme–substrate complexes [39]. The whole system can be expressed as shown in Fig. 2, and the specific rate constants used in the following equations are defined in this figure. The equilibrium constants K_0, K_1, and K_2, respectively, are given by:

$$K_0 = \frac{[E][I]}{[EI]}, K_1 = \frac{[ES_1][I]}{[EIS_1]}, K_2 = \frac{[ES_1S_2][I]}{[EIS_1S_2]} \quad (1)$$

For such a simplified system the rate of product formation is given by the velocity (equation 2) [see Refs. 6, 7, 14, and 40 for a review)

$$\frac{d[P]}{dt} = \frac{k_{12}k_{23}(k_{2p}K_2 + k'_{+2p}[I])/(K_2 + [I])}{k_{32}k_{21} + k_{12}k_{32} + k_{12}k_{23}} e_0 \quad (2)$$

where

$$\begin{cases} k_{12} = \dfrac{(k_1K_0 + k'_{+1}[I])[S_1]}{K_0 + [I]} \\ k_{21} = \dfrac{k_{-1}K_1 + k'_{-1}[I]}{K_1 + [I]} \\ k_{23} = \dfrac{(k_2K_1 + k'_{+2}[I])[S_2]}{K_1 + [I]} \\ k_{32} = \dfrac{(k_{-2s} + k_{2p})K_2 + (k'_{-2s} + k'_{+2p})[I]}{K_2 + [I]} \end{cases} \quad (3)$$

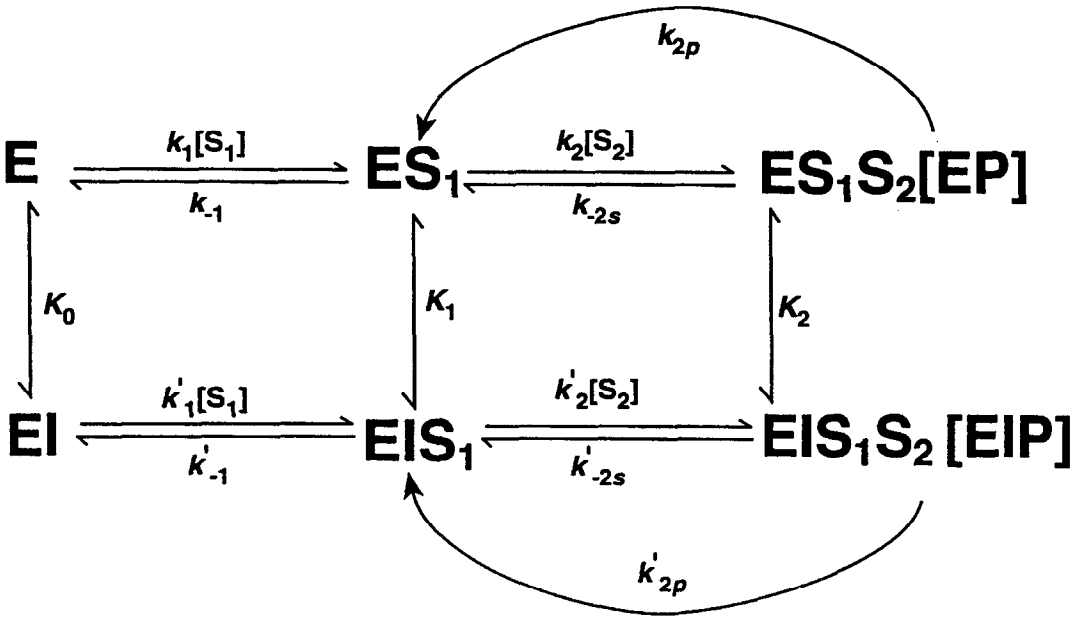

FIG. 2. Steady-state reaction scheme for HIV RT. E = enzyme; S_1 = template:primer; S_2 = dNTP; K_0, K_1, K_2 = equilibrium constants between the inhibitor (I), the enzyme, and its substrates; EP = enzyme–product complex; and EIP = enzyme–inhibitor–product complex.

RESULTS

Antiviral Activity

U-31,355 is an inhibitor of HIV-1 RT and has yielded IC_{50} values from 56 to 83 μM in the initial screening tests in the poly (rA):$(dT)_{10}$-directed enzymatic assay system described under Materials and Methods. The agent possesses 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} value was between 0.4 and 4 μM U-31,355, and the IC_{50} in terms of toxicity to the host cells was >16 μM inhibitor (Table 1). In the p24 and total viral RNA synthesis assays carried out in HIV-1 (D34 isolate)-infected peripheral blood mononuclear cells, the IC_{50} values in terms of p24 core protein released and total viral RNA synthesized were between 0.1 and 1 μM U-31,355 at both day 3 and day 4 post-infection of the cells (Table 2). Total viral RNA synthesis was suppressed completely at an inhibitor concentration of 10 μM. No severe toxic effects on the host cells were observed at 10 μM U-31,355. No inhibition of the cellular polymerases α and δ was observed at concentrations up to 398 μM. These concentrations were significantly above the ones required to inhibit HIV-1 RT to the same extent (50–80 μM).

TABLE 1. Antiviral activity of U-31,355 in the syncytia reduction assay (in HIV-1-infected MT-2 cells)

Sample	No. of syncytia/plate
Control	59
U-31,355	
39.7 μM	0
3.97 μM	0
0.397 μM	56

RNA-Dependent DNA Polymerase

This function was studied in the presence of various concentrations of poly (rA):$(dT)_{10}$ and fixed concentrations of dTTP and vice versa in the presence of different concentrations of dTTP and a fixed concentration of template:primer. Three inhibitor concentrations were used in addition to controls containing no U-31,355. Each sample was run in duplicate, and each experiment thus yielded a total of sixty-four data points for analysis. The analysis of the kinetic data was carried

TABLE 2. Antiviral activity of U-31,355 in HIV-1-infected peripheral blood mononuclear cells

Sample	% Inhibition of p24 synthesis	% Inhibition of HIV RNA synthesis	Cell viability (% of control)
Day 3			
U-31,355			
10 μM	100	100	88
1 μM	87	ND*	100
0.1 μM	7.1	ND	100
0.01 μM	0	ND	100
Day 4			
U-31,355			
10 μM	100	100	88
1 μM	73	ND	100
0.1 μM	0	ND	100
0.01 μM	0	ND	100

* ND = no data.

out with a computer using the steady-state kinetic scheme described under Materials and Methods. The experimental data were fitted to equation 2, and the thirteen kinetic parameters defined in Fig. 2 were calculated from this equation. The experimental results are shown in Fig. 3 and the calculated essential forward and backward reaction rates and equilibrium constants of the system in Fig. 4.

For the association constant, k_1, and the corresponding dissociation rate constant, k_{-1}, of the enzyme–poly(rA):$(dT)_{10}$ complex, values of 4.6×10^4 M^{-1} sec^{-1} and 0.3 sec^{-1} were calculated for the control reactions in the absence of the inhibitor. In the presence of U-31,355, the corresponding values were 2.8×10^4 M^{-1} sec^{-1} for k'_1 and 0.3 sec^{-1} for k'_{-1}. The forward rate constant, k_2, for the attachment of dTTP to the enzyme–poly(rA):$(dT)_{10}$ complex was 3.7×10^4 M^{-1} sec^{-1}. The backward rate constant, k_{-2s}, representing the dissociation rate of the ternary enzyme–poly(rA):$(dT)_{10}$–dTTP complex was 0.8 sec^{-1}. The corresponding values in the presence of U-31,355 were: $k'_2 = 2.8 \times 10^4$ M^{-1} sec^{-1}, and $k'_{-2s} = 0.2$ sec^{-1}. The turnover number k_{2p}, representing k_{cat}, was 1 sec^{-1} in the control reaction and the corresponding value, k'_{2p}, in the presence of the inhibitor was essentially 0. The respective inhibition constants or K_i values calculated for the enzyme (K_0), the enzyme–poly(rA):$(dT)_{10}$ (K_1), and the enzyme–poly(rA):$(dT)_{10}$-dTTP (K_2) complexes with the inhibitor U-31,355 were 88, 42 and 47 μM U-31,355. The overall fitting error for the analysis was 0.98×10^{-4}. The equilibrium constants $K_{0,\ K_1}$, and K_2, reflect the binding affinity between the inhibitor and the enzyme or the enzyme–substrate complexes. The value for K_0 was nearly twice as large as the values for K_1 and K_2, and this indicates that U-31,355 binds much tighter to both the enzyme–poly(rA):$(dT)_{10}$ and enzyme–poly(rA):$(dT)_{10}$–DTTP complexes than to the free enzyme.

Similar experiments were carried out with the homopolymeric template:primer poly(rC):$(dG)_{10}$ and the cognate nucleotide dGTP. The experimental results are given in Fig. 5, and the calculated values for the rate and equilibrium constants are shown in Fig. 6. The calculated forward rate constant, k_1, for the formation of the enzyme–poly(rC):$(dG)_{10}$ complex was 5.6×10^4 M^{-1} sec^{-1} and the corresponding backward rate, k_{-1}, was 0.25 sec^{-1}. In the presence of the inhibitor, k'_1, which is used to assess the formation of the ternary enzyme-U-31,355-poly(rC):$(dG)_{10}$ complex, was 3×10^4 M^{-1} sec^{-1}, and the value for k'_{-1}, the reverse reaction, was 0.2 sec^{-1}. Moreover, the forward reaction rate for the formation of the enzyme–poly(rC):$(dG)_{10}$–dGTP complex, k_2, was 3.7×10^4 M^{-1} sec^{-1} and the backward rate, k_{-2s}, was 0.8 sec^{-1}. In the presence of the inhibitor U-31,355, k'_2, the rate constant for the formation of the quaternary enzyme-U-31,355-poly(rC):$(dG)_{10}$-dGTP complex, was 0.9×10^4 M^{-1} sec^{-1} and the corresponding reverse rate constant, k'_{-2s}, for this complex was

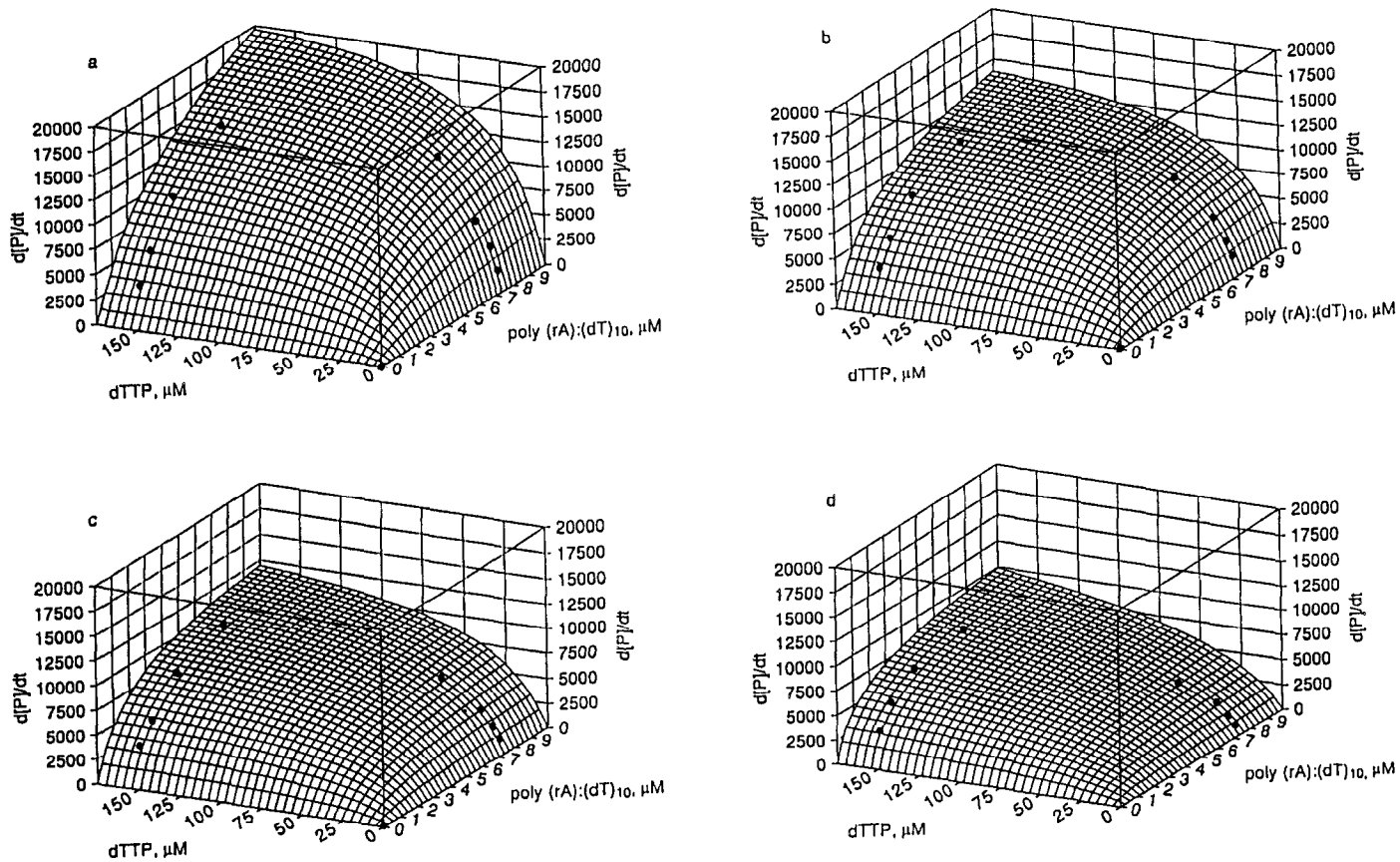

FIG. 3. Inhibition of HIV-1 RT poly(rA):$(dT)_{10}$-directed poly(dT) synthesis by U-31,355. Enzyme = 0.0274 μM, $d[P]/dt$ = μM × 10^{-6} of dTMP incorporated per sec. Key: (a) no inhibitor; (b) 25 μM, (c) 50 μM, and (d) 100 μM U-31,355.

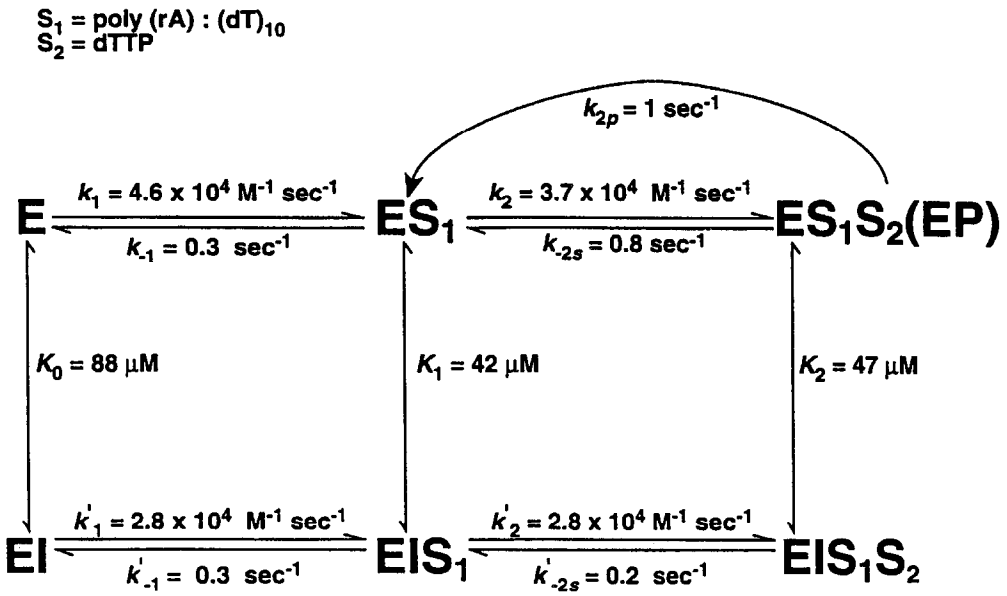

FIG. 4. Inhibition of poly(rA):$(dT)_{10}$-directed poly(dT) synthesis by U-31,355; steady-state kinetic parameters.

0.2 sec^{-1}. The rate of translocation (k_{2p}) or turnover number in the reaction without inhibitor was 0.4 sec^{-1}, and the corresponding value, k'_{2p}, for the quaternary enzyme-U-30,355–poly(rC):$(dG)_{10}$–dGTP complex was essentially zero. The equilibrium constant, K_0, was 62 μM U-31,355 for the enzyme-inhibitor complex but decreased dramatically to 7.6 μM for K_1, the enzyme–inhibitor–template:primer complex, as well as for K_2, the enzyme–inhibitor–template:primer–dGTP complex. The fitting error was 0.55×10^{-4} for this system. The value for K_0 exceeds the values for K_1 and K_2 by about 8-fold,

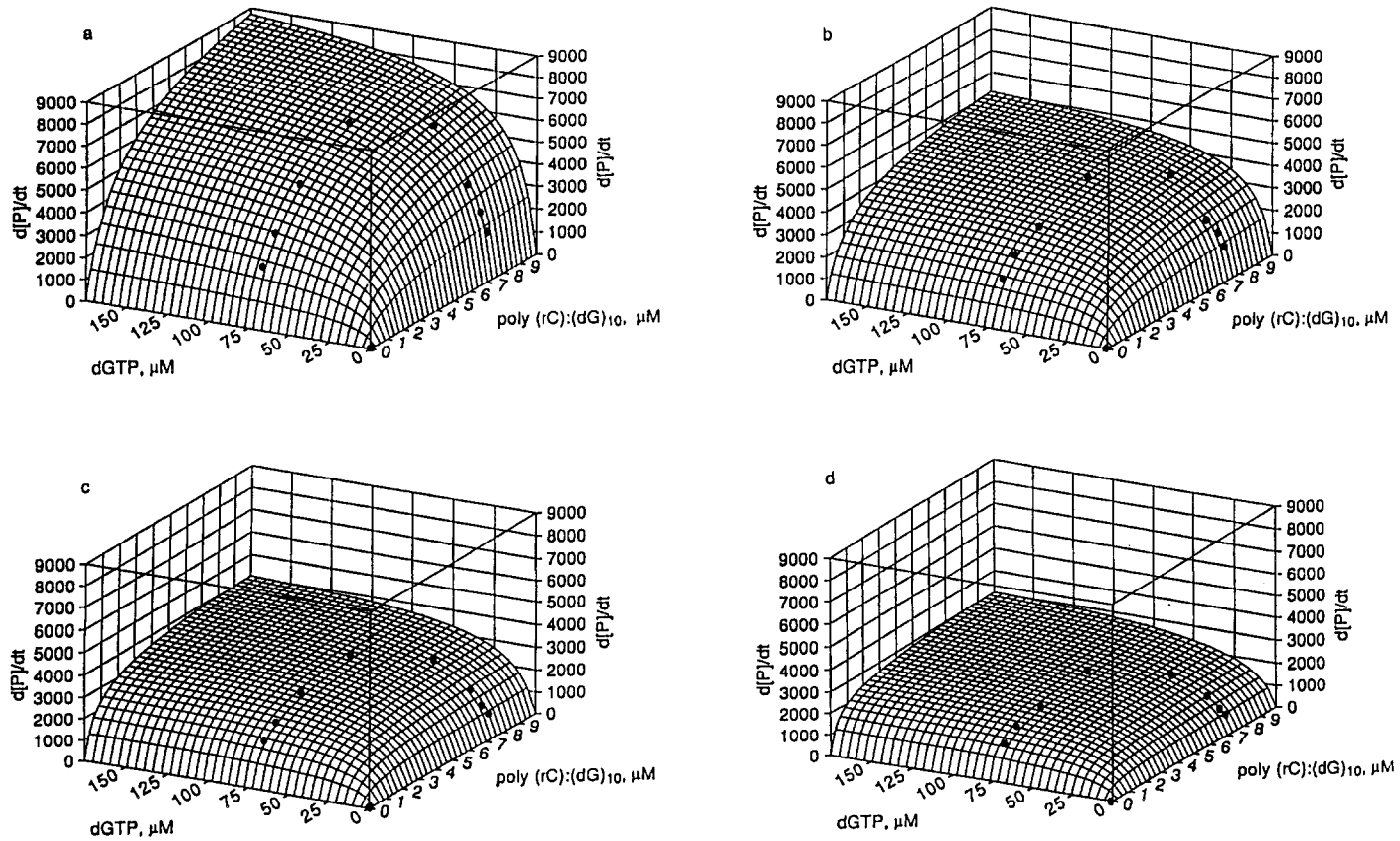

FIG. 5. Inhibition of poly(rC):$(dG)_{10}$-directed poly(dG) synthesis by U-31,355. Enzyme = 0.0274 μM, $d[P]/dt$ = μM × 10^{-6} of dGMP incorporated per sec. Key: (a) no inhibitor; (b) 2.5 μM, (c) 7.5 μM, and (d) 15 μM U-31,355.

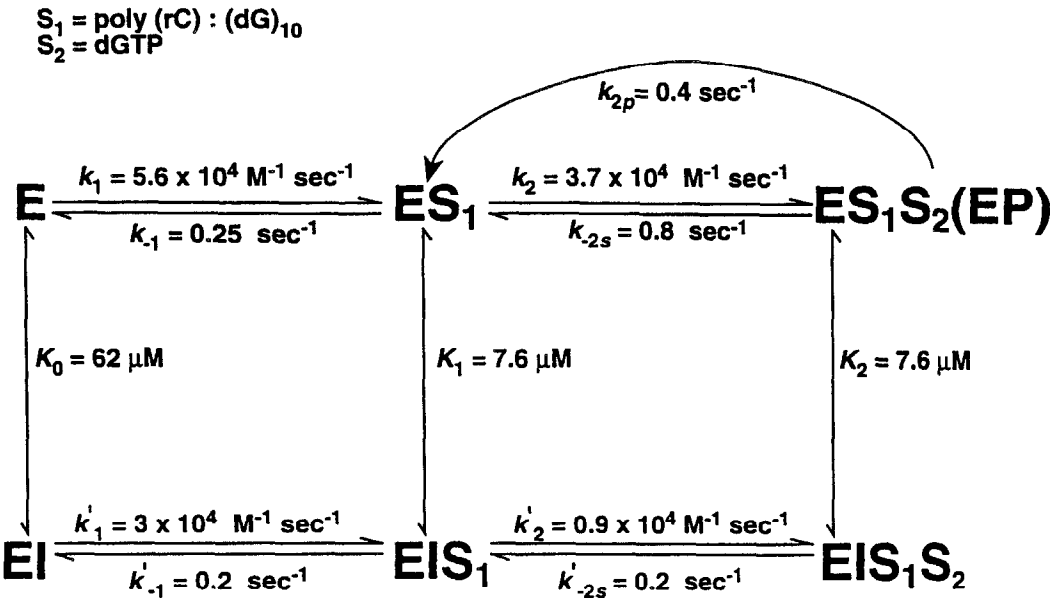

FIG. 6. Inhibition of poly(rC):$(dG)_{10}$-directed poly(dG) synthesis by U-31,355; steady-state kinetic parameters.

and K_1 is equal to K_2. This demonstrates that U-31,355 binds much more tightly to the enzyme–substrate complexes than to the free enzyme if the template:primer is poly(rC):$(dG)_{10}$ and the cognate nucleotide is dGTP. The values for K_1 and K_2 were smaller than the corresponding ones obtained with the poly(rA):$(dT)_{10}$-catalyzed systems where K_1 was 32 µM and K_2 was 46 µM, and this demonstrates that the potency of U-31,355 depends on the base composition of the template:primer.

DNA-Directed DNA Polymerase of HIV-1 RT

U-31,355 was also tested for its effect on the DNA-directed DNA polymerase function of RT using poly(dC):oligo(dG) as the template:primer. No inhibition of this enzyme activity was observed with U-31,355 at concentrations up to 100 µM.

RNase H Assay

U-31,355 did not inhibit this RT activity when tested at concentrations up to 100 µM.

Other Retroviral RT Species

The compound was also tested for its inhibitory activity against the RNA-directed DNA polymerase functions of HIV-2 RT, avian myoblastoma virus RT, and murine leukemia virus RT. None of these enzyme species was inhibited by U-31,355 when tested at concentrations up to 100 µM.

DISCUSSION

The inhibition kinetics of U-31,355 on the RNA-directed DNA polymerase of HIV-1 RT were studied with respect to the nucleic acid and dNTP substrate binding sites using homopolymeric template:primers. The analysis of the experimental results was carried out using a modified Briggs–Haldane kinetic scheme [6]. The rate equation 2 derived for such a reaction system contains thirteen rate and equilibrium constants, as defined in Fig. 2. Equation 2 allows the calculation of these constants as it relates the rate of product formed by the RT enzyme to the two substrates, the template:primer and the dNTP. The turnover numbers k_{2p} or k_{cat} for the RNA-directed polymerase functions ranged from 0.3 to 1 sec^{-1}. Moreover, the equilibrium constants, K_0, for the enzyme–inhibitor (EI) complexes significantly exceeded the ones for the enzyme–inhibitor–substrate complexes (EIS_1 and EIS_1S_2), although the values for K_1 and K_2 were nearly equal within each system studied. This indicates that U-31,355 possesses a much higher binding affinity for the enzyme–substrate complexes as compared to the free enzyme. In addition, the inhibitor did not compete directly with the template:primer nor the dNTP substrate binding sites of the RT enzyme and acts thus as a mixed inhibitor with respect to the two substrate binding sites. As the values for K_1 and K_2 were nearly equal, it follows that the formation of the binary enzyme–template:primer complex solely accounts for the amplification of the binding affinity of U-31,355, and no further enhancement results following the binding of the second substrate, the dNTP. Moreover, as the functions of the substrate binding sites remain unaffected by U-31,355, the inhibitor must impair an event occurring after the formation of the enzyme–substrate complexes, which includes either the formation of the phosphoester bond or the translocation of the enzyme relative to the template:primer following the generation of the ester bond. The potency of U-31,355 was also dependent on the base composition of the template:primers as the K_i values were much lower for the poly (rC):$(dG)_{10}$-directed system than for the poly (rA):$(dT)_{10}$-directed one. Moreover, U-31,355 was a specific inhibitor of the RNA-directed DNA polymerase function of HIV-1 RT

and did not inhibit the DNA-catalyzed DNA polymerase. The HEPT analog E-EPU (Fig. 1) was characterized as a noncompetitive inhibitor with respect to the poly (rA):$(dT)_{10}$ and dGTP binding sites and competitive with respect to the dTTP binding site of the RT enzyme [28]. The specific inhibition patterns for U-31,355 were found to be mixed in that the inhibitor bound much more tightly to the enzyme–substrate complexes than the free enzyme as described in the present study, and these results thus differ significantly from the ones obtained with the HEPT compound E-EPU. In terms of their chemical structures, the two inhibitors differ from each other in that the heterocyclic moiety in E-EPU is 5-ethyl-1-ethoxymethyl-uracil; U-31,355, on the other hand, contains a 4-amino-6-chloro-pyrimidine. Focussing on the sulfur substituent, E-EPU contains a phenylthio group whereas U-31,355 contains a benzylthio group.

Kinetic studies with other non-nucleoside HIV-1 RT inhibitors have been described. The benzodiazepine or TIBO compound R82150 appears to be a specific inhibitor of the HIV-1 RT catalyzed RNA-directed DNA polymerase function [12]. The inhibitor acts uncompetitively with respect to the nucleic acid binding site and noncompetitively with respect to the dNTP site. The IC_{50} for the DNA-directed DNA polymerase was forty times higher than the one required to inhibit the RNA-directed DNA polymerase, and the RNase H activity was not impaired by the inhibitor. The dipyridodiazepinone nevirapine acts as a mixed inhibitor with respect to the poly-(rA):$(dT)_{10}$ and poly(rC):$(dG)_{10}$ binding sites and noncompetitively with respect to the dNTP binding sites during RNA-directed DNA synthesis by HIV-1 RT [41]. The pyridinone derivative L-697,639 indicated noncompetitive inhibition with respect to dGTP and poly(rC):$(dG)_{12-18}$ [13]. The quinoline U-78,036 acts as a mixed to noncompetitive inhibitor with respect to both the substrate binding sites of the enzyme [14]. Moreover, this compound is a specific inhibitor of the RNA-catalyzed DNA polymerase function of RT as it has no inhibitory effect on the DNA-catalyzed one. We had previously studied the inhibition kinetics of the bis(heteroaryl)piperazines U-87,201E, U-88,204E and U-88,204E and U-90,152E [6–8]. All three compounds share the common property in that they inhibit both the RNA- and DNA-directed DNA polymerase functions of RT but not the RNase H function. Their specific kinetic inhibition patterns differ in some significant ways. U-87,201E acts predominantly as a noncompetitive inhibitor with respect to both substrate binding sites of the enzyme regardless of the template:primer used [6]. U-88,204E acts as a noncompetitive inhibitor if the template:primers are either poly(rA):$(dT)_{10}$ (RNA-directed DNA polymerase) or poly(C):$(dG)_{12-18}$ (DNA-directed DNA polymerase), but it acts as a mixed inhibitor if the template:primer is poly(rC):$(dG)_{10}$ [7]. U-90,152E, on the other hand, acts as a mixed inhibitor in all the systems tested in that it possesses a higher binding affinity for the enzyme–substrate complexes than for the free enzyme [8].

Compared with these other classes of RT inhibitors, the inhibition kinetics of U-31,355 differ from all of them except for the quinoline U-78,036 [14]. Both compounds act as specific inhibitors of the RNA-directed DNA polymerase function without any effect on the DNA-catalyzed DNA polymerase or the RNase H domains of the RT enzyme. Moreover, the potency of both inhibitors depends strictly on the base composition of the template:primer in that both compounds show a much higher binding affinity for the enzyme–poly (rC):$(dG)_{10}$ complex than for the enzyme-poly (rA):$(dT)_{10}$ complex. The two compounds differ, however, from each other in regard to their specific inhibition patterns in that the quinoline U-78,036 acts as a noncompetitive to mixed inhibitor with respect to the substrate binding sites of the RNA-directed DNA polymerase, whereas U-31,355 acts as a mixed inhibitor. Hence, although the two compounds differ structurally from each other, they appear to interact within the same general functional domain of the RT enzyme. It should be noted, however, that all of the kinetic data cited, except for the ones obtained with U-31,355, the quinoline U-78,036, and the arylpiperazines U-90,152E, U-87,201E, and U-88,204E, were derived from rapid equilibrium kinetics and not Briggs–Haldane kinetics.

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