Pergamon

Biochemical Pharmacology, Vol. 47, No. 11, pp. 2017–2028, 1994.
Copyright © 1994 Elsevier Science Ltd
Printed in Great Britain. All rights reserved
0006-2952/94 $7.00 + 0.00

0006-2952(94)E0095-3

KINETIC STUDIES WITH THE NON-NUCLEOSIDE HUMAN IMMUNODEFICIENCY VIRUS TYPE-1 REVERSE TRANSCRIPTASE INHIBITOR U-90152E

IRENE W. ALTHAUS, JAMES J. CHOU,* ANDREA J. GONZALES,† MARTIN R. DEIBEL, KUO-CHEN CHOU, FERENC J. KEZDY, DONNA L. ROMERO, RICHARD C. THOMAS, PAUL A. ARISTOFF, W. GARY TARPLEY and FRITZ REUSSER‡

The Upjohn Company, Kalamazoo, MI 49001, U.S.A.

(*Received* 21 *October* 1993; *accepted* 12 *January* 1994)

Abstract—The bisheteroarylpiperazine U-90152E is a potent inhibitor of human immunodeficiency virus type 1 (HIV-1) reverse transcriptase (RT) and possesses excellent anti-HIV activity in HIV-1-infected lymphocytes grown in tissue culture. The compound inhibits both the RNA- and DNA-directed DNA polymerase functions of HIV-1 RT. Kinetic studies were carried out to elucidate the mechanism of RT inhibition by U-90152E. Michaelis–Menten kinetics, which are based on the establishment of a rapid equilibrium between the enzyme and its substrates, proved inadequate for the analysis of the experimental data. The data were thus 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, which 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-90152E acts exclusively as a mixed inhibitor with respect to the template : primer and dNTP binding sites for both the RNA- and DNA-directed DNA polymerase domains of the enzyme. The inhibitor shows a significantly higher binding affinity for the enzyme–substrate complexes than for the free enzyme and consequently does not directly impair the functions of the substrate binding sites. Therefore, U-90152E 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.

Key words: HIV-1 reverse transcriptase; non-nucleoside inhibitors; inhibition kinetics

Certain bisheteroarylpiperazine derivatives are potent inhibitors of HIV-1§ RT (EC 2.7.7.49) [1–7]. Their mechanistic inhibitory activity differs from the one exerted by nucleoside analogs such as AZT, ddI, and ddC, which serve as dNTP substrates for the polymerase. Several classes of non-nucleoside RT inhibitors have been described recently. These include the dipyridodiazepinones [8, 9], the benzodiazepines or TIBO compounds [10, 11], the HEPT or 1-[(2-hydroxyethoxyl)-methyl]-6-(phenylthio)thymine derivatives [12, 13], the pyridinones [14], the quinoline U-78036 [15], polysulfates and polysulfonates [16–25], the α-anilinophenylacetamide derivatives [26], and the bisheteroarylpiperazines or BHAPs [1–7].

The bisheteroarylpiperazine U-90152E {1-(5-methanesulfonamido-1*H*-indol-2-yl-carbonyl)-4-[3-(1-methyl-amino)pyridinyl]piperazine} (Fig. 1) is a member of this last class of compounds [2, 4, 5]. The agent is a potent inhibitor of HIV-1 RT and has excellent antiviral activity at nontoxic doses in experimentally HIV-1-infected lymphocytes grown in tissue culture. In this report, we describe enzymatic kinetic studies, using recombinant HIV-1 RT, to examine the inhibitory effects of U-90152E on both the RNA- and DNA-dependent DNA polymerase functions of HIV-1 RT.

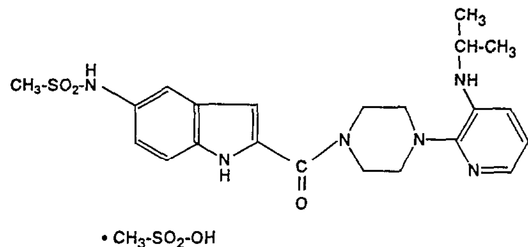

Fig. 1. Chemical structure of U-90152E.

* Current address: Department of Physics, University of Michigan, Ann Arbor, MI 48109.

† Current address: Curriculum for Toxicology, University of North Carolina, Chapel Hill, NC 27599.

‡ Corresponding author. Tel. (616) 385-7152; FAX (616) 385-7373.

§ Abbreviations: HIV-1, human immunodeficiency virus type 1; and RT, reverse transcriptase.

MATERIALS AND METHODS

The expression of HIV-1 RT and its purification have been described [27, 28]. The enzyme was devoid

of *Escherichia coli* RNase H activity and consisted of p51/p66 heterodimers as evidenced by gel electrophoresis.

The synthetic template:primers 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 the Sigma Chemical Co., 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), present at a concentration of 10 μg/mL.

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 (see, for instance, Refs. 25 and 29). The reason for this inadequacy resides in the fact that the enzyme needs to form an initiation complex with its respective substrates before elongation of the primer can commence. Therefore, no immediate equilibrium is established between the reactants involved. The experimental data were thus analyzed using steady-state Briggs–Haldane kinetics. These kinetics assume that the enzyme–substrate complex is not necessarily in equilibrium with the enzyme and its substrate. However, shortly after initiation of the reaction, enzyme–substrate complex is formed at the same rate as it dissociates. A steady-state scheme that includes all the forward and backward reaction rate constants to be considered here yields complex velocity equations that are impractical to solve. However, the general steady-state kinetic system used in this study was simplified significantly, as detailed in Fig. 2. The specific rate constants used in the following text are defined in this figure. The steady-state kinetics were limited to the reactions occurring between the enzyme and its substrates, and rapid equilibrium kinetics were applied to the reactions between the inhibitor on the one hand and the free enzyme and the various enzyme–substrate complexes on the other. This treatment is admissible since the low molecular weight inhibitor will react instantly with the respective components of the reaction. Moreover, an ordered mechanism was assumed, whereby the template:primer complex binds first to the enzyme, followed by the addition of dNTP [30, 31]. The polymerase is a processive enzyme and, after the addition of the first nucleotide, translocation occurs along the template, resulting in the incorporation of further nucleotides into the growing chain [30]. Under these conditions the formation of the phosphoester bond can be considered as irreversible as the reverse reaction occurs at an extremely low rate and the dissociation of the enzyme–product complex into its components is also negligible during the initial phase of the reaction. Thus, the enzyme–product does not differ from the initial enzyme–template:primer complex in that the former shuttles back to the enzyme–template:primer state, and this reaction rate constant (k_{2p}) is equal to k_{cat}, representing the turnover number. The quaternary enzyme–inhibitor–template:primer–dNTP complex should be non-productive as no translocation to the enzyme–inhibitor–template:primer state should occur and k'_{2p} should be 0. These simplifications reduce the number of parameters to be considered in the system to twelve or thirteen if we include k'_{2p}.

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. The reactions between the enzyme (E) and the low molecular weight inhibitor can be deemed as diffusion-controlled reactions [32–34]. Consequently, the conversions between E and EI, ES_1 and EIS_1, and ES_1S_2 and EIS_1S_2 occur at a much faster rate than the interconversions between the enzyme and its substrates. Thus, although the whole system is a steady-state one, there is an equilibrium between E and EI, ES_1 and EIS_1, and ES_1S_2 and EIS_1S_2. Accordingly, these three pairs of enzyme species can be treated as three combined species, i.e. E + EI, ES_1 + EIS_1, and ES_1S_2 + EIS_1S_2, where the fractions of E, EI, ES_1, EIS_1, ES_1S_2 and EIS_1S_2 are:

$$\begin{cases} f_E = \dfrac{[E]}{[E]+[EI]} = \dfrac{K_0}{K_0+[I]} \\ f_{EI} = \dfrac{[EI]}{[E]+[EI]} = \dfrac{[I]}{K_0+[I]} \\ f_{ES_1} = \dfrac{[ES_1]}{[ES_1]+[EIS_1]} = \dfrac{K_1}{K_1+[I]} \\ f_{EIS_1} = \dfrac{[EIS_1]}{[ES_1]+[EIS_1]} = \dfrac{[I]}{K_1+[I]} \\ f_{ES_1S_2} = \dfrac{[ES_1S_2]}{[ES_1S_2]+[EIS_1S_2]} = \dfrac{K_2}{K_2+[I]} \\ f_{EIS_1S_2} = \dfrac{[EIS_1S_2]}{[ES_1S_2]+[EIS_1S_2]} = \dfrac{[I]}{K_2+[I]} \end{cases} \quad (1)$$

and the equilibrium constants K_0, K_1 and K_2 are defined as follows:

$$K_0 = \frac{[E][I]}{[EI]}, \quad K_1 = \frac{[ES_1][I]}{[EIS_1]}, \quad K_2 = \frac{[ES_1S_2][I]}{[EIS_1S_2]}. \quad (2)$$

Therefore, instead of the mechanism depicted in Fig. 2 which contains six enzyme species, we can consider a simplified mechanism in which there are only three independent enzyme species, as shown in Fig. 3. In doing so, however, the corresponding rate

Download English Version:

https://daneshyari.com/en/article/2516385

Download Persian Version:

https://daneshyari.com/article/2516385

Daneshyari.com