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Research paper

Consensus HIV-1 subtype A integrase and its raltegravir-resistant variants: Design and characterization of the enzymatic properties

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ABSTRACT

Model studies of the subtype B and non-subtype B integrases are still required to compare their susceptibility to antiretroviral drugs, evaluate the significance of resistance mutations and identify the impact of natural polymorphisms on the level of enzymatic reactivity. We have therefore designed the consensus integrase of the HIV-1 subtype A strain circulating in the former Soviet Union territory (FSU-A) and two of its variants with mutations of resistance to the strand transfer inhibitor raltegravir. Their genes were synthesized, and expressed in *E coli*; corresponding His-tagged proteins were purified using the affinity chromatography. The enzymatic properties of the consensus integrases and their sensitivity to raltegravir were examined in a series of standard *in vitro* reactions and compared to the properties of the integrase of HIV-1 subtype B strain HXB2. The consensus enzyme demonstrated similar DNA-binding properties, but was significantly more active than HXB-2 integrase in the reactions of DNA cleavage and integration. All integrases were equally susceptible to inhibition by raltegravir and elvitegravir, indicating that the sporadic polymorphisms inherent to the HXB-2 enzyme have little effect on its susceptibility to drugs. Insensitivity of the mutated enzymes to the inhibitors of strand transfer occurred at a cost of a 30–90% loss of the efficacies of both 3′-processing and strand transfer. This is the first study to describe the enzymatic properties of the consensus integrase of HIV-1 clade A and the effects of the resistance mutations when the complex actions of sporadic sequence polymorphisms are excluded.

1. Introduction

A key step in the life cycle of the human immunodeficiency virus type 1 (HIV-1) – the insertion of the reverse transcribed viral DNA into the host genome – is performed by the viral enzyme integrase (IN) [1]. Following reverse transcription of the viral genome, IN binds both extremities of the viral DNA, forming a nucleoprotein complex that constitutes the core of the preintegration complex (PIC). Within the PIC, IN catalyzes a 3′-processing reaction, cleaving the terminal dinucleotides from both 3′-ends of the viral DNA. Once the PIC has migrated into the nucleus of the infected cell, IN mediates a strand transfer reaction, in which the viral DNA is inserted into the host cell DNA to form a provirus. Further regulated expression of the proviral genome and assembling give new virions, which leave the cell and infect other cells [1]. Because of its importance for the HIV-1 replication IN is considered as an

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attractive target for antiretroviral drug development. Several types of integration inhibitors, representing different mechanisms of action, have been reported; three of them, raltegravir (RAL), elvitegravir (EVG) and dolutegravir, are currently used as components of the highly active antiretroviral therapy (HAART) [2–4]. All these compounds interact with the IN complex with viral DNA and inhibit the process of strand transfer. Compared to the inhibitors of HIV reverse transcriptase and protease, the IN inhibitors (INI) were shown to be better tolerated by treatment-naïve and treatment-experienced patients [5,6].

HIV-1 exists as multiple subtypes and recombinant strains, distributed unevenly throughout the world. Most of the reports on the efficacy of antiretroviral therapy and the emergence of drug-resistance are based on HIV-1 subtype B, which is the prevalent subtype in North America and Western Europe [7]. However, it is other HIV-1 subtypes that feature in the majority of HIV-1 infections worldwide [8,9]. Information on susceptibility of these other subtypes to HAART and the development of drug resistance, specifically to newly introduced INI is still very limited [8,10–13]. Specifically, there is no consensus on the sensitivity of different HIV clades to INI. Some studies indicate a comparable clinical efficacy of RAL against B and non-B HIV-1 subtypes [14,15]; others point to more frequent failure of therapy in patients infected with a non-subtype B virus [16] or, on the contrary, to a higher susceptibility to INI of the non-B as compared to subtype B strains [17–19]. For example, compared to subtype B, subtype C and the circulating recombinant forms (CRF) O2_AG and A/CRF01_AE demonstrated a higher genetic barrier to acquiring resistance mutations G140S and G140C in the IN gene [17,18]. Furthermore, subtype C IN bearing resistance mutations E92Q/N155H was shown to be 10-fold more susceptible to inhibition by both RAL and EVG than subtype B IN with the same mutations [19]. Also, the prevalence of resistance-associated mutations V54I and V72I in subtype E was reportedly lower than in subtype B [20]. The discrepant data on the susceptibility to INI and on different HIV subtypes' rate of acquisition of resistance to INI point to the necessity of careful epidemiological as well as biochemical studies.

Part of the recorded differences between HIV subtypes/CRFs' susceptibility to drugs and rate of acquisition of resistance may be due to the interference of natural viral polymorphisms, including secondary or accessory resistance mutations [21]. The prevalence of natural viral mutations conferring resistance to INI in treatment-naïve patients is very low (unlike the prevalence of similar mutations in reverse transcriptase or protease) [22–24]. Phenotypic testing showed that the influence of baseline inter-subtype polymorphisms on the efficacy of INI is minimal [24,25]. However, reports are forthcoming on mutations (mostly accessory, very few primary) that have not yet been associated with treatment failure but can potentially interfere with INI [26–29]. Several mutation pathways that lead to INI-resistance and no virological suppression on INI-containing treatment regimens have already been characterized [30,31]. Importantly, both an early development of resistance and the choice of resistance pathway were explained by a combined effect of primary resistance mutations and inter-subtype polymorphisms [32]. This has been demonstrated for HIV-1 of subtypes C and AE [33,34]. Altogether, this motivates model studies of subtype B and non-subtype B HIV INs that would compare their susceptibility to antiretroviral drugs, evaluate the significance of early resistance mutations and identify the role of natural polymorphisms in the level of enzymatic activity.

With this in mind, we have analyzed the naturally occurring variations in 34 full-length INs of HIV-1 subtype A strain predominant in the territory of the former Soviet Union (FSU-A) [35–40]. RAL-sensitive and resistant variants of the consensus FSU-A IN were created, and their enzymatic properties, including sensitivity to RAL and EVG, were examined *in vitro*. The data presented here constitute the first report on the effects of primary and secondary mutations of resistance to RAL in a non-subtype B enzyme relieved, by the consensus approach, from the heterologous unspecified influences of the sporadic sequence polymorphisms.

2. Materials and methods

2.1. Design of the consensus integrases of HIV-1 FSU-A

Full-length HIV-1 clade A IN sequences from 34 treatment-naïve patients isolated in the territory of the former Soviet Union were selected from HIV-1 databases at http://www.hiv.lanl.gov/content/index, entries DQ167216_E, DQ823360_U DQ823359_U DQ823358_U DQ823357_U DQ823356_U DQ823361_U DQ823366_U DQ823365_U DQ207944 G, AF413987 U, AY500393 R, BY_04BY062, BY_04BY069, BY_04BY073, BY_06BY086, BY_06BY086, BY_06BY086, BY_06BY087, BY_06BY089, BY_06BY096, A1_UA_00_9, AF193275_B, AY829203_U, AY829205_U, AY829206 U, AY829208_U, AY829209_U, AY829210_U, A1_UZ_02_0, AF413972 U, AF413971 U, AF413970 U, AF413969 U. IN consensus was created using BioEdit software (Ibis Biosciences, Carlsbad, CA). The enzyme was N-terminally extended by dipeptide Met-Gly. A polynucleotide sequence encoding the consensus IN of FSU-A (IN_a) was designed and synthesized by Evrogen (Moscow, Russia).

2.2. Cloning of the synthetic integrase genes

To create prokaryotic expression vectors, a DNA fragment encoding IN_a was PCR-amplified using Pfu polymerase (Promega) and primers: forward 5′-TGACCATATGGGCTTCCTGGAGGG-3′ and reverse 5′-TGACGGATCCTAGTCCTCATCCTGTCTGCTG-3′, containing *NdeI* and *BamHI* restriction sites. PCR product was digested with *NdeI* and *BamHI* (Fermentas, Vilnius, Lithuania) and ligated into the *NdeI*/*BamHI*-cleaved plasmid pET15b in frame with the codons for the N-terminal 6His-tag in replacement of the coding sequence of HIV-1 HXB2 IN. The ligation mixture was transformed into the competent OneShotTop10 *E. coli* cells (Invitrogen, Carlsbad, CA) by heat shock. Clones obtained on the selective media were screened by PCR using cloning primers. Plasmid pETIN_a containing the insert of IN_a coding sequence (as confirmed by sequencing; Eurofins MWG Operon, Germany) was selected and purified using a miniprep kit (Qiagen, Chatsworth, CA). Further, pETIN_a was subjected to the site-directed mutagenesis to introduce into the IN_a gene the mutations of resistance to RAL: L74M, E92Q, V151I, N155H, G163R (pattern 1; IN_r1), and E138K, G140S and Q148K (pattern 2; IN_r2). Site mutagenesis was done using the QuikChange II Site-Directed Mutagenesis kit (Agilent Technologies, USA) according to the manufacturers' instructions. The resulting plasmids encoding IN_r1 (pETIN_r1) and IN_r2 (pETIIN_r2) were purified using a miniprep kit (Qiagen) and sequenced (Eurofins MWG Operon).

2.3. Protein expression and purification

IN variants of HIV-1 subtype A bearing a 6His-tag were expressed in *E. coli* BL21(DE3) host strain (Novagen®, Merck Millipore, Darmstadt, Germany and Billerica, MA, USA) with pRARE plasmid from Rosetta (DE3) strain (Novagen). Protein expression was induced by adding IPTG. The recombinant IN variants were purified as described previously [41]. Fractions were analyzed by electrophoresis in 12% SDS-PAGE with subsequent Coomassie-staining. Quantitative image analysis of the Coomassie-stained gels, using Image-QuantTM 4.1 software (Amersham Biosciences Corp, Piscataway, NJ), revealed each IN preparation to be at least 80% pure. Protein concentration in the purified IN preparations was

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