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## HIV-1 Integrase Strand Transfer Inhibitors: Novel Insights into their Mechanism of Action

**Krishan K. Pandey and Duane P. Grandgenett**

Institute for Molecular Virology, Saint Louis University Health Sciences Center, Saint Louis, MO 63104. U.S.A.

### Abstract

Human immunodeficiency virus type-1 integrase (IN) is a new and novel target for inhibitors. Strand transfer inhibitors effectively prevent concerted integration of viral DNA by IN into the host chromosomes. Raltegravir is the first approved strand transfer inhibitor for the treatment of HIV-1/AIDS. We propose a mechanistic hypothesis as to “when and where” these inhibitors are active in virus-infected cells. Using native agarose gel electrophoresis, we identified a transient synaptic complex (SC) wherein IN non-covalently juxtaposes two viral DNA ends. SC possesses many properties associated with the cytoplasmic preintegration complex (PIC) in infected cells, including concerted integration. Our results show that the strand transfer inhibitors effectively “trap” or inactivate the SC preventing concerted integration. It follows that the IN-viral DNA complex is “trapped” by the inhibitors via a transient intermediate within the cytosolic PIC before entry into the nucleus.

### Introduction

Acquired immunodeficiency syndrome (AIDS), caused by human immunodeficiency virus type-1 (HIV-1), has been a serious public health problem for approximately 25 years. There are about 34 million people infected with HIV-1 worldwide. Despite the significant advances in understanding of the biology and pathogenesis of HIV-1, efforts have not been universally successful to prevent its spread. Educational programs on how to prevent new infections have had limited success on a global basis, although there are numerous local successful programs. However, the spread can also be attributed to unique characteristics of the virus and its high mutation rate which has hindered the development of an effective vaccine. HAART (highly active antiretroviral therapy) has been very successful in the treatment of infected individuals and preventing infection of new born children. Our review will focus on the development and mode of action of inhibitors to a novel target, the HIV-1 integrase (IN).

The HIV-1 polymerase gene encodes a polyprotein which upon proteolytic processing yields three viral enzymes; reverse transcriptase, protease and IN, all of which are essential for viral replication. Integration of HIV-1 DNA by IN into the cellular genome is an obligatory step in the retrovirus life cycle resulting in permanent infection and establishment of a latent proviral reservoir for virus production. Integration mediated by IN requires two independent steps. In

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Correspondence: Krishan K. Pandey, 1100 S. Grand Blvd., Institute for Molecular Virology, E.A. Doisy Research Center, Saint Louis University of Health Sciences Center, Saint Louis, MO 63104, U.S.A. Tel: +1 314 977-8787; Fax: +1 314 977 8798; pandeykk@slu.edu.

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the cytoplasm, IN binds to the ends of reverse transcribed blunt-ended DNA and processes the 3' ends by removing two nucleotides (GT) adjacent to conserved CA motif, exposing the active 3'-hydroxyl group. In the nucleus, the second reaction occurs when IN covalently joins the processed viral DNA ends by a concerted integration mechanism into the cellular genome [1]. Due to the unique nature of IN and the absence of a cellular counterpart, IN is an obvious target for drug development. Intense efforts by Merck and Co resulted in development of first FDA approved drug Raltegravir (marketed as "Isentress") targeting HIV-1 IN (<http://www.fda.gov/bbs/topics/news/2007/new01726.html>). Raltegravir is a strand transfer inhibitor which blocks the joining of the processed viral DNA ends into the host chromosome. Raltegravir has been successful in naïve as well as patients who have undergone HAART and harbor strains resistant to combinations of drugs targeting reverse transcriptase and protease [2,3]. This article discusses our recent results to understand the mechanisms of strand transfer inhibitors that prevent the formation of concerted integration products using a reconstituted system employing purified HIV-1 IN and viral DNA substrates. Based on our results, we propose a hypothesis as to "when and where" these inhibitors are active in infected cells.

HIV-1 IN contains 288 amino acids (aa) and is composed of three structural domains. The N-terminal domain (1–50 aa) contains a histidine-histidine-cysteine-cysteine (HHCC) motif involved in zinc binding [4]. This domain is associated with multimerization of IN and enhances its catalytic activity. The catalytic core domain (CCD) (50–212 aa) contains the catalytic D, D-35-E motif located at positions 64, 116 and 152, respectively. The D, D-35-E motif is conserved in all retrovirus and retrotransposons INs [5]. Mutations in this motif abolish the activity of IN and viral replication [6]. The C-terminal domain (213–288 aa) makes specific contacts with viral DNA in a IN-DNA complex but also binds non-specific DNA [7]. There are no crystal structures available for full-length IN or IN complexed with DNA substrates presumably due to a flexible loop in CCD and low solubility of the protein. Individual as well as two domain structures consisting of N-terminal domain with CCD and CCD with C-terminal domain have been solved [8–10].

The first major advancement in the development of IN inhibitors came from Merck and Co in the form of diketo acid inhibitors L-731,988 and L-708,906 [11]. These compounds inhibited the strand transfer reaction catalyzed by recombinant IN using blunt-ended oligonucleotides at  $IC_{50}$ s of 80 and 150 nM for L-731,988 and L-708,906, respectively, while the  $IC_{50}$ s were 1 to 2  $\mu$ M for inhibition of HIV-1 replication in single-cycle assays. These inhibitors specifically inhibited the strand transfer reaction catalyzed by IN. The  $IC_{50}$  for inhibition of 3'OH processing by recombinant IN was  $\sim$ 70 times ( $\sim$ 6000 nM) higher for L-731,988. The next generation of successful inhibitors were the naphthyridine carboxamide inhibitors that employed a similar active diketo acid moiety (Fig. 1). The diketo acid group chelates  $Mg^{++}$  in the active site of IN required for the catalysis [12]. L-870,810 and L-870,812 were the two prominent successful members of the naphthyridine carboxamide family for inhibiting viral integration in infected cells [13] and in infected rhesus macaques [14], respectively. L-870,810 and L-870,812 also had low  $IC_{50}$ s of 8 nM and 40 nM, respectively, for inhibiting strand transfer using oligonucleotides substrates with recombinant IN [13,14].

## Discussion

Our studies with the diketo acid and naphthyridine carboxamide strand transfer inhibitors have possibly provided a new understanding of their mode of action. We investigated how these inhibitors selectively inhibit concerted integration by the use of the synaptic complex (SC) on native agarose gels [15]. IN juxtaposes two viral blunt-ended DNA molecules in a non-covalent manner in the SC (Fig. 2) which mimics properties associated with the cytoplasmic preintegration complex (PIC) found in HIV-1 infected cells. SC is the transient intermediate in the concerted integration pathway in vitro similar to the cytosolic PIC, which eventually

migrates to the nucleus and preferentially integrates into active transcription units [16]. Within SC and cytosolic PIC, the viral DNA ends are slowly processed by IN while strand transfer activity is constrained within both complexes. SC is converted to the strand transfer complex (STC) upon binding to supercoiled DNA target [15,17]. The STC contains the concerted integration product (Fig. 2), the result of concerted insertion of two donor molecules in the target DNA [17,18] which mimics the same integration reaction *in vivo*. Integration of viral DNA into the target DNA results in the characteristic HIV-1 5 bp host-site duplication at the sites of insertion [17,19–21]. IN binds to the terminal 32 nucleotides at the viral ends in SC and STC as revealed by DNaseI footprinting of the complexes suggesting a specific interaction of IN with the long terminal repeat (LTR) ends (manuscript in preparation).

With blunt-ended DNA substrates, concerted integration is inhibited by L-870,810 and L-870,812 with  $IC_{50}$ s of 55 and 102 nM, respectively [15]. The  $IC_{50}$  value for concerted integration is different from the  $IC_{95}$  values obtained *in vivo* for L-870,810. L-870,810 has an  $IC_{95}$  of 15 nM in presence of 10% FBS. The reason for this discrepancy in the potency of inhibition *in vivo* and *in vitro* could be due to several possibilities. The simplest explanation would be that concerted integration *in vitro* may not recapitulate the *in vivo* integration process in some aspects, particularly the number of active complexes in both cases. We have observed that the  $IC_{50}$  of L-870,810 is dependent on the IN concentration which is presumably related to the number of active complexes at a given concentration [15]. Secondly, concerted integration in the reconstituted system involves concerted insertion of two separate DNA molecules (1.6 kbp in length) into target DNA while *in vivo*, it is a unimolecular reaction where the PIC inserts two ends of a single viral DNA molecule prepackaged within this nucleoprotein complex into chromosomes. However, the  $IC_{50}$  value obtained with L-870,812 (102 nM) matches reasonably well with the  $IC_{95}$  value of 250 nM *in vivo* [14].

These strand transfer inhibitors are significantly less effective with the use of 3'OH pre-processed substrates, which are effectively recruited by IN for near immediate strand transfer reactions since no 3'OH processing is necessary with this pre-processed substrate for concerted integration [15,22]. The  $IC_{50}$ s for inhibiting the concerted integration product formed with pre-processed substrates are higher; 1400 nM and 9600 nM for L-870,810 and L-870,812, respectively. Similar results were obtained with diketo acid L-841,411;  $IC_{50}$ s for inhibition of concerted integration were 110 nM and 4100 nM with blunt and pre-processed substrates, respectively [15].

The reason(s) for paradoxical nature of preferential inhibition of concerted integration using blunt-ended DNA as substrate is not clear. However, it suggests that inhibitors interact significantly differently within SC formed with blunt and pre-processed substrates *in vitro*. Here, it is important to point out again that after viral entry into the cell and reverse transcription of the viral RNA genome in the cytoplasm, the resulting DNA in the PIC is blunt-ended. The 3'OH processing of the viral DNA is a slow and time-dependent process and by the time the PIC enters the nucleus, approximately 70%–90% of the viral DNA molecules have processed ends [23,24]. Hence, in cells infected with HIV-1, the inhibitor first encounters PICs that contain blunt-ended DNA which is undergoing 3'OH processing. Similar  $IC_{50}$ s obtained in inhibiting concerted integration *in vitro* as well as inhibiting the HIV-1 replication *in vivo* suggest that IN complexed with blunt-ended DNA undergoing 3'OH processing is the preferred nucleoprotein complex for inhibitor binding to IN.

In support of our data, cytoplasmic PIC isolated from HIV-1 infected cells grown for 5 h in presence of L-731,988 was found to be catalytically inactive [11]. The results suggested that the inhibitor had modified the PIC before entry into the nucleus and rendered the cytoplasmic PIC inactive. As stated previously, these inhibitors do not affect 3'OH processing of the viral DNA ends either *in vivo* or within the SC at low nM concentrations *in vitro*.

The partially ineffective strand transfer inhibition with 3'OH pre-processed oligonucleotides substrates in comparison to blunt-ended substrates by inhibitors has been observed by other groups [25,26]. The reactions containing blunt-ended DNA oligonucleotides are inhibited with an  $IC_{50}$ s two to 10-fold lower than that observed in the reactions with pre-processed DNA ends. The inhibitors need to be in close proximity to the IN active-site within the SC for effective inhibition. Our assay does not allow us to determine exactly when the inhibitor interacts with IN to block strand transfer activity and yet not affecting 3'OH processing. However, it appears that the inhibitor may interact with a transient structural IN-DNA intermediate arising prior to or immediately after the 3'OH processing event. Inhibitor may bind to IN-DNA complex before processing but most likely would not yet be in right orientation to block the active site and hence does not inhibit 3'OH processing. Conformational change in IN-DNA complex caused by 3'OH processing gives inhibitor an access to the active site and block the strand transfer reaction [27,28]. In any case, the inhibitors appear to have the best opportunity to “trap” the IN-DNA complex in the cytosolic PIC before it encounters the chromosomal DNA for integration.

Summarizing the above observations, we speculate that inhibitors likely bind to a transient IN-DNA structure formed prior to or upon 3'OH processing within SC or the cytosolic PIC. We observed an accumulation of this trapped transient structure (“trapped” SC) (Fig. 2) in our inhibitor titration experiments which supports this possibility [15]. A similar interfacial inhibition mechanism was proposed for HIV-1 IN strand transfer inhibitors [29]. In our assays with pre-processed substrate, the transient conformation in IN observed during 3'OH processing does not exist and hence, refractive to effective inhibition in comparison to blunt-end substrate. To further support our hypothesis, we formed the SC containing one blunt-ended and one pre-processed DNA to determine if only one blunt-end is sufficient for effective inhibition at low nM concentrations of strand transfer inhibitors. L-870,810 inhibited the formation of concerted integration products with this SC containing one blunt-ended and one pre-processed DNA ends with an  $IC_{50}$  of 60 nM [22]. This  $IC_{50}$  value with L-870,810 was nearly double than what we observed with the SC formed with two blunt-ended DNA molecules ( $IC_{50}$  of 30 nM) under identical experimental conditions [22]. Similar results were obtained with L-870,812. The data suggest a single blunt-end in SC is sufficient to effectively inhibit concerted integration, a possibility which also occurs within the PIC as it transverses the cytoplasm in the presence of inhibitors.

## Conclusions

L-870,810, L-870,812 and L-841,411 are the earlier generation inhibitors of Raltegravir (Fig. 1). However, the mechanism of the inhibition caused by Raltegravir is similar [26,30]. Surely new insights into the mechanism associated with the strand transfer inhibitors will be coming from different avenues of research. It is essential that ongoing efforts along multiple lines of research be continued because IN drug-resistant mutants are cross-resistant to a variety of structurally distinct strand transfer inhibitors [22,26,31]. For patients, it will be of great significance that resistant virus mutants against an IN inhibitor and mutants resistant to HAART remain sensitive to other structurally distinct inhibitors targeting IN.

## Acknowledgments

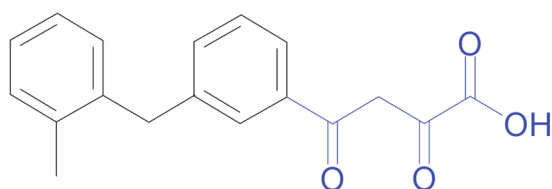
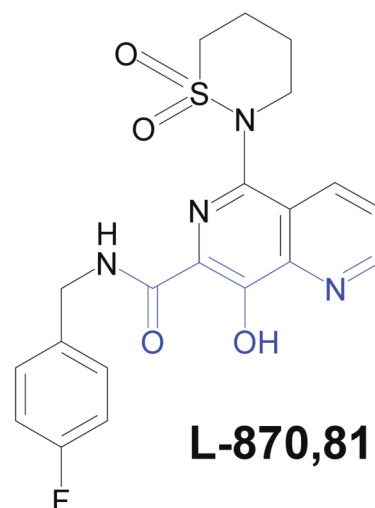
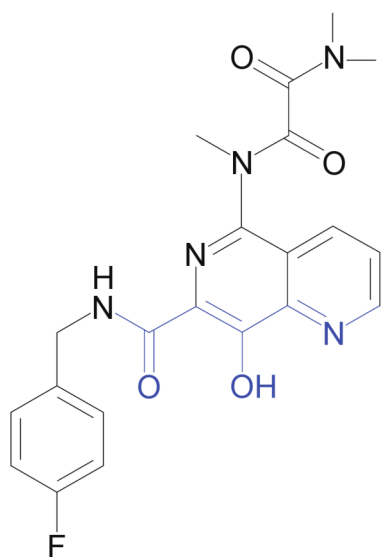
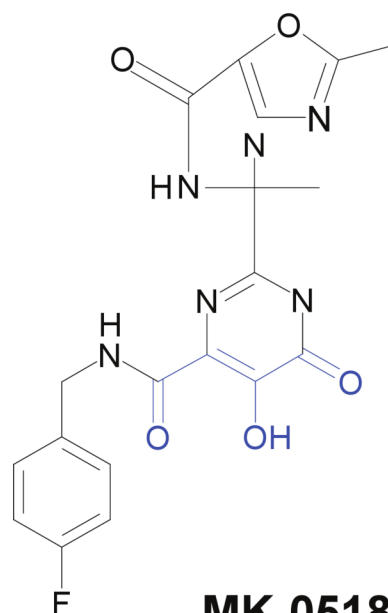
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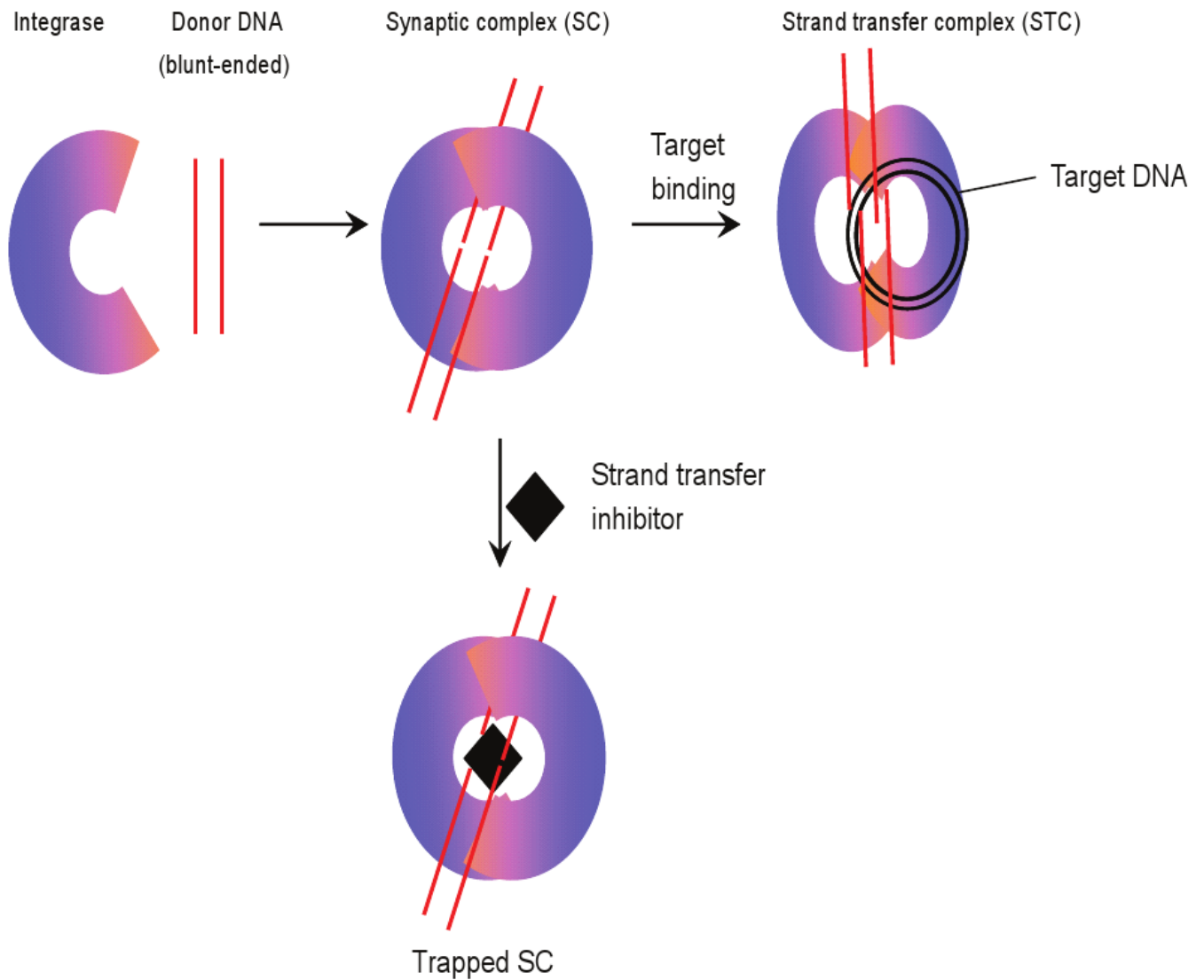
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**L-841,411****L-870,810****L-870,812****MK-0518  
(Raltegravir)****Figure 1.**

Chemical structures of IN strand transfer inhibitors used in this study. L-841,411 belongs to the diketo acid family. L-870,810 and L-870,812 are the members of naphthyridine carboxamide family. MK-0518, the first FDA approved inhibitor targeting IN is also shown and belongs to the hydroxypyrimidinone carboxamide group. The diverse class of IN strand transfer inhibitors contain the diketo acid moiety (shown in blue) which interacts with  $Mg^{++}$  in the IN active site.



**Figure 2.**

Mechanism of IN strand transfer inhibitors. IN juxtaposes two viral DNA ends forming the synaptic complex (SC). The viral DNA is processed by IN cleaving the terminal dinucleotide (GT) from their 3' OH ends. Processing of the viral DNA induces a conformational change in SC which facilitates the binding of target DNA, forming the strand transfer complex. However, when SC is formed in presence of a strand transfer inhibitor, the inhibitor binds near the catalytic site which prevents target binding. The 3' OH processing of the viral DNA ends is not affected by inhibitor at low nM concentrations. The inhibitor alters the structure of SC rendering it catalytically inactive and results in the accumulation of the "trapped" SC, analogous to the inhibitor inactivated preintegration complex in vivo.