Implications of HIV-1 M Group Polymorphisms on Integrase Inhibitor Efficacy and Resistance: Genetic and Structural *in Silico* Analyses[†]

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Received October 16, 2008; Revised Manuscript Received November 24, 2008

ABSTRACT: The extensive polymorphisms among HIV-1 subtypes have been implicated in drug resistance development. Integrase inhibitors represent the latest addition to the treatment of HIV-1, and their efficacy and resistance patterns among M group strains are currently under investigation. This study analyzed the intersubtype variation within 108 integrase sequences from seven subtypes. The residues associated with catalytic activity and primary resistance to raltegravir were highly conserved among all strains. Variations were observed in residues associated with secondary resistance. Molecular modeling studies indicated a two-way binding mode of raltegravir that explains the resistance pathways and the implication of nonconservative mutations in integrase−raltegravir interactions.

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Publication Date (Web): December 17, 2008 | doi: 10.1021/bi8019349

The genetic variability of HIV-1 constitutes the most striking challenge in treating HIV infection. The highly active antiretroviral therapy (HAART) includes inhibitors that target viral entry, and the viral enzymes reverse transcriptase (RT) and protease (PR). The integration of viral DNA in the human chromosome, catalyzed by integrase (IN), is an essential step for the survival of HIV and a viable target for antiviral agents (*1*).

IN is a single polypeptidic chain that consists of three structurally distinct domains, the N-terminal domain (NTD), the catalytic core domain (CCD), and the C-terminal domain (CTD). The function of IN has been linked to key motifs, primarily the DDE triad in the catalytic core domain (CCD), and to secondary motifs such as the zinc in the NTD. Point mutations in any of these residues lead to loss of activity. The DDE triad catalyzes the 3′-processing and strand transfer steps mediated by the formation of a chelating complex with Mg^{2+} (*1, 2*). The zinc motif, comprised of residues H12, H16, C40, and C43, is not directly related to the catalytic processes but has an indirect role through the multimerization of IN (*3, 4*). Host factors have been implicated in the integration process in the context of the preintegration complex (PIC). LEDGF/p75 is the most recently identified cellular partner of HIV-1 IN, for which structural information exists within the same context as IN (*5*).

To date, the only classes of validated IN inhibitors (INI) are the diketo acids (DKAs) and the pyrroloquinoline and naphthyridine carboxamide derivatives. These inhibitors bind at the interface of the viral DNA−IN and metal complex, preventing the strand transfer reaction (*6*). Recently, raltegravir, a DKA analogue developed by Merck, has been approved by the U.S. Food and Drug Administration (FDA) as a combination therapy for patients with multidrug resistance to HIV-1 (*7*). Elvitegravir, a second INI, is currently in phase III clinical trials (*8*). Current antiretrovirals are primarily designed to target HIV-1 subtype B strains, and little is known about the efficacy and drug resistance of these regimens among HIV-1 intersubtypes. Naturally existing polymorphisms when present with primary resistance mutations may affect the resistance pathway and trigger early resistance development. This effect was demonstrated with HIV-1 subtype C and AE PR (*9, 10*). Myers et al. (*11*) have recently characterized the natural polymorphisms of HIV-1 IN. Variation was greatest at the CCD around the active site. Phenotypic testing showed a minimal contribution of baseline intersubtype polymorphisms on INI efficacy (*12*); however, these polymorphisms in combination with primary resistant mutations could potentially affect INI efficacy and resistance.

This study explored the effect of the polymorphisms present within the IN gene from M group strains on IN activity and INI binding by combining genetic and computational structural analyses. The identified polymorphisms were compared to the residues associated with catalytic activity, the DDE triad (*2, 13*), the zinc motif (*3, 4*), LEDGF/p75 binding (*5*), and raltegravir (*14*−*16*) and INI (*6, 17, 18*) resistance. Computational docking (*19, 20*) of raltegravir was performed at the active site of a three-dimensional two-metal IN−DNA model that was designed to mimic the strand transfer step by including two Mg^{2+} ions at the active site and 5CITEP to mark the position of the viral DNA end and by interacting with the DNA residues Q148, Y143, and K159 (*21*). The two-metal mechanism of action is believed to be true for IN, even though only one Mg^{2+} ion per CCD is observed in the crystal structures (*22*). The study group included 108 INI-naïve Cypriot patients infected with HIV-1. The patient sequences fell into seven subtypes: subtype A, 19 sequences; subtype B, 72 sequences; subtype C, 11 sequences; subtype F1, one sequence; subtype G, three sequences; CRF02_AG, one sequence; and CRF04_cpx, one sequence (Figure S1 of the Supporting Information). The sequences of the IN gene from subtypes A and C were

[†] Funded by Marie Curie Grant FP7-PEOPLE-2007-4-3-IRG-204263, the Patti Birch 1991 Trust, and the University of Cyprus.

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10.1021/bi8019349 CCC: $40.75 © 2009 American Chemical Society
Published on Web 12/17/2008

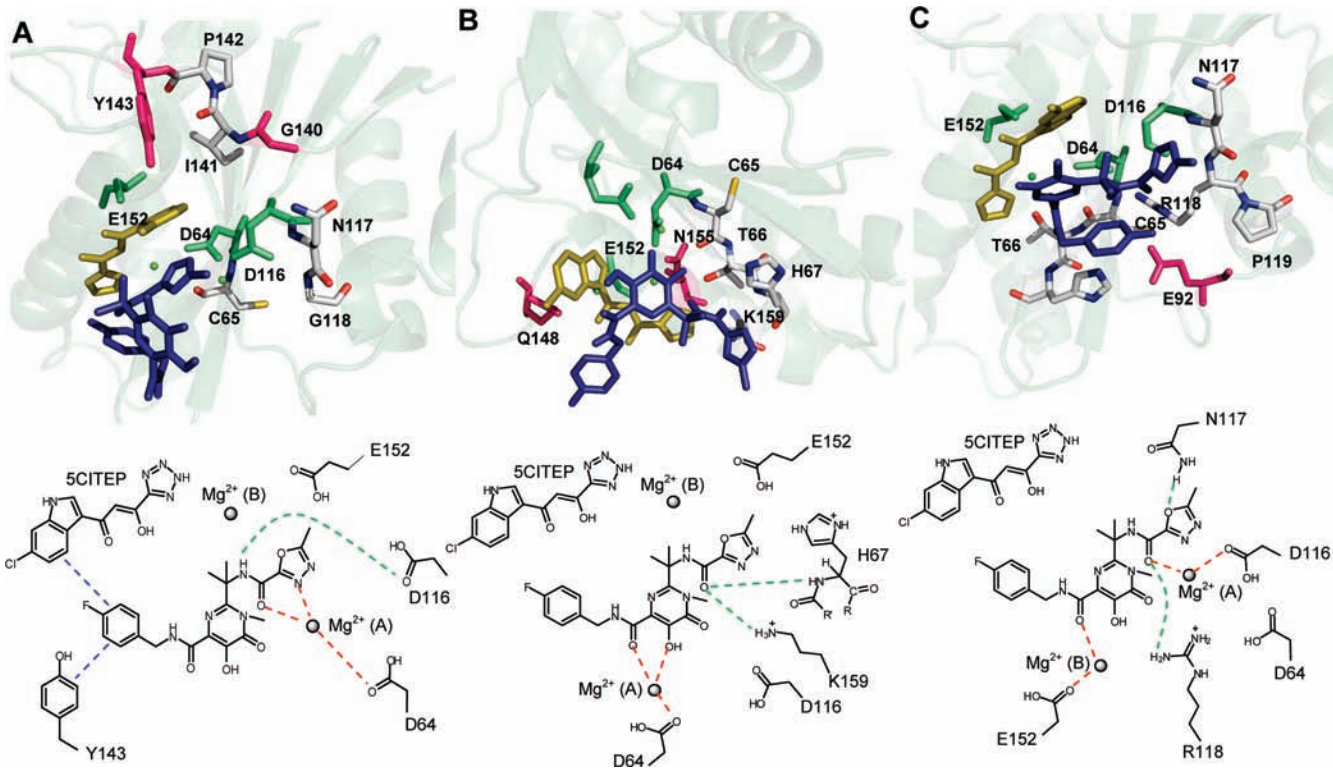

FIGURE 1: Docked conformations of raltegravir in the active site of IN. DDE triad (green stick representation), amino acids associated with raltegravir resistance (magenta), the remaining amino acids within 15 Å of the root of raltegravir (gray), 5CITEP (orange), and raltegravir (blue stick representation). Mg^{2+} ions are presented as green spheres. Red lines represent dipole−metal interaction, green lines hydrogen bonding, and blue lines π-stacking. Cutoff values for metal coordination set to 2 Å, those for hydrogen bonding to 3 Å, and those for π-stacking to 4 Å. (A) For subtype B IN, $\Delta G_{bind} = -6.07$ kcal/mol. (B) For subtype B IN, $\Delta G_{bind} = -5.36$ kcal/mol. (C) For subtype C IN, $\Delta G_{bind} = -6.13$ kcal/mol. The IN−raltegravir models were optimized using the energy minimization module of the SPDBV (Supporting Information).

compared to the sequence of consensus B. In subtype A, 47 mutations were identified in 42 positions, and in subtype C, 32 mutations were identified in 29 positions.

Variants occurring at a frequency of more than 10% can be found in Table S1 of the Supporting Information. Both the DDE and zinc motif are phylogenetically conserved, in agreement with other studies (*11*) and as expected from the fact that these residues are conserved in all retroviral INs. In terms of host factor interactions, the IN residues that interact with LEDGF/p75 have been mapped to α4/5 connector residues R166, D167, Q168, A169, E170, and H171. Of these, the H171Y substitution was identified as a natural polymorphism of subtype A IN. This substitution represents a nonconservative change from a basic to a polar amino acid, the effect of which should be determined experimentally on isolates of subtype A IN.

Resistance to raltegravir is obtained through three distinct pathways, Q148H/K/R, N155H (*15*), and less frequently Y143H (*14*). Secondary mutations associated with the resistance pathways are L74M, E138A, E138K, G140S, E92Q, T97A, G163K/R, V151I, D232N, and E157Q (*16*). Resistance mutations identified for other INI in clinical and preclinical development, including elvitegravir, are T66I/A/K, V72I, F121Y, T125K, G140C, S147G, Q148H, V151I, S153Y, M154I, and S230R (*6*, *17*, *18*). None of the mutations associated with primary resistance (N155H, Q148H/K/R, Y143, T66, and E92) were observed. Variation was greater at residues involved in secondary resistance. Substitutions V72I, L74I, T125A, and M154I were identified as natural polymorphisms of subtype A [16% (V72I), 45% (L74I), 100% (T125A), and 0.5% (M154I)] and subtype C [64% (V72I) and 91% (T125A)]. Substitutions V72I and T125A were also present in subtypes F1, G, CRF02, and the additional CRF04 for T125A. Variation was also present at G140 in one subtype C and E157 in two subtype B subjects.

In addition to the drug resistant residues, the computational docking analysis of this study identified residues around the active site of IN that may be involved in important interactions with raltegravir. Considering their location proximal to the active site, and their possible involvement in interactions with raltegravir, these residues could be potentially involved in drug resistance. Two distinct conformations of raltegravir were identified at the active site of IN that differ in the nature of these interacting residues. The affinity of raltegravir for the active site residues is described by the estimated free energy of binding (ΔG_{bind}). In the first conformation ($\Delta G_{bind} = -6.07$ kcal/mol), raltegravir is positioned in the proximity of the DDE triad, Y143 associated with primary and secondary drug resistance, G140 associated with secondary drug resistance, and additional residues P142, I141, G118, N117, and C65 (Figure 1A). In the second conformation ($\Delta G_{bind} = -5.36$ kcal/mol), raltegravir is placed in the interacting proximity of the DDE triad, N155, Q148 associated with primary drug resistance, and additional residues K159, H67, T66, and C65 (Figure 1A).

The fact that two different groups of interacting residues are identified from the docking experiments implies a differential binding mode of raltegravir, one leading to the Y143 and another to the N155 and Q148 resistance pathway. This hypothesis is further justified by the experimental drug resistance data (*14*). In vivo, the primary resistance mutation Y143H is not observed as often as the Q148H/K/R and N155H mutations.

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This trend was also observed in the docking experiments. In 75% of the docked poses, raltegravir interacts with Q148 and N155 (Figure 1B), while in 25% of the docked poses, raltegravir interacts with Y143 (Figure 1A). Closer examination of the interactions between raltegravir and the active site residues further supports the differential binding mode hypothesis. Figure 1 displays the detailed interactions of the two conformations. In both conformations, certain functionalities of raltegravir are at an ideal distance for formation of dipole−charge complexes with Mg^{2+}(A) but not with Mg^{2+} (B). However, in each conformation, it is a different functionality of raltegravir that interacts with the metal cation, colored red in Figure 1. In addition, in the first conformation, the benzene ring of raltegravir is located in an ideal position for π-stacking interactions with the indole ring of 5CITEP, suggesting an in vivo interaction of raltegravir and the terminal portion of the 3′-processed viral DNA. These observations are in agreement with the suggested mechanism of inhibition of the DKA inhibitors that act on the interface between the IN−DNA and Mg^{2+} complex (*23*). This π-stacking interaction is not observed in the second conformation. Residue Y143, implicated in raltegravir resistance, is in an ideal location for π-stacking interactions with the benzene ring of raltegravir (*24*). In the second conformation, H67 and K159 are involved in direct hydrogen bonding with raltegravir while Q148 and N155, which coincide with raltegravir resistance, are probably involved in dipole−dipole interactions with either the inhibitor or DNA.

Considering their potential involvement in the interactions with raltegravir, the interacting residues were examined against the 108 IN sequences to determine their variability. Among these residues, the G118R and G140R substitutions were identified in a single subtype C strain. The possible effect of these substitutions on the binding of raltegravir was examined by generating *in silico* a mutated two-metal IN−DNA model that includes polymorphic residues I60V, F100Y, L101I, T112V, G118R, S119P, K136Q, G140R, E198D, and V201I of the particular subtype C strain and repeat docking of raltegravir. In this case, the docked conformations displayed similar interacting residues, with the most prevalent being N155, P119, R118, N117, E92, H67, T66, and C65. The DDE triad was present in all conformations. In the lowest-energy docked conformation ($\Delta G_{bind} = -6.13$ kcal/mol), raltegravir is placed in the interacting proximity of the DDE triad, E92 associated with secondary drug resistance, and additional residues P119, R118, N117, H67, T66, and C65 (Figure 1C). The energetics of binding were not affected by these point mutations; in contrast, a single trend is observed in all docked poses. Raltegravir is placed at an ideal distance to interact with both Mg^{2+} cations, hydrogen bonding with the side chain of N117 and R118, and dipole−dipole interactions with T66 and E92. Both Mg^{2+} ions are involved in interactions with raltegravir in contrast to what is observed at the active site of the "wild-type" two-metal IN−DNA model (Figure 1A,B). These observations imply that strains with particularities in key amino acids may have an effect on the binding mechanism of raltegravir. More experiments, including functional assays, with intersubtype HIV-1 patients are currently under way to further elucidate the intersubtype efficacy of raltegravir.

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SUPPORTING INFORMATION AVAILABLE

Methods, Table S1, and Figure S1. This material is available free of charge via the Internet at http://pubs.acs.org.

REFERENCES

1. Loizidou, E. Z., Nicolaou, C., Nicolaides, A., and Kostrikis, L. G. (2007) *Curr. HIV Res. 5*, 365–388.
2. Engelman, A., and Craigie, R. (1992) *J. Virol. 66*, 6361–6369.
3. Lee, S. P., Xiao, J., Knutson, J. R., Lewis, M. S., and Han, M. K. (1997) *Biochemistry 36*, 173–180.
4. Zheng, R., Jenkins, T. M., and Craigie, R. (1996) *Proc. Natl. Acad. Sci. U.S.A. 93*, 13659–13664.
5. Cherepanov, P., Ambrosio, A. L. B., Rahman, S., Ellenberger, T., and Engelman, A. (2005) *Proc. Natl. Acad. Sci. U.S.A. 102*, 17308–17313.
6. Hazuda, D. J., Felock, P., Witmer, M., Wolfe, A., Stillmock, K., Grobler, J. A., Espeseth, A., Gabryelski, L., Schleif, W., Blau, C., and Miller, M. D. (2000) *Science 287*, 646–650.
7. Evering, T. H., and Markowitz, M. (2008) *Expert Opin. Invest. Drugs 17*, 413–422.
8. Shimura, K., Kodama, E., Sakagami, Y., Matsuzaki, Y., Watanabe, W., Yamataka, K., Watanabe, Y., Ohata, Y., Doi, S., Sato, M., Kano, M., Ikeda, S., and Matsuoka, M. (2008) *J. Virol. 82*, 764–774.
9. Clemente, J. C., Coman, R. M., Thiaville, M. M., Janka, L. K., Jeung, J. A., Nukoolkarn, S., Govindasamy, L., Agbandje-McKenna, M., McKenna, R., Leelamanit, W., Goodnow, M. M., and Dunn, B. M. (2006) *Biochemistry 45*, 5468–5477.
10. Coman, R. M., Robbins, A. H., Fernandez, M. A., Gilliland, C. T., Sochet, A. A., Goodenow, M. M., McKenna, R., and Dunn, B. M. (2008) *Biochemistry 47*, 731–743.
11. Richard, E., and Myers, D. P. (2008) *J. Virol. 82*, 9228–9235.
12. Van Baelen, K., Van Eygen, V., Rondelez, E., and Stuyver, L. J. (2008) *AIDS 22*, 1877–1879.
13. Kulkosky, J., Katz, R. S., Mack, J. P., and Skalka, A. M. (1992) *Mol. Cell. Biol. 12*, 2331–2338.
14. Hazuda, D. J., Miller, M. D., Nguyen, B. Y., and Zhao, J. (2007) *Antiviral Ther. 12*, S10.
15. Johnson, V. A., Brun-Vezinet, F., Clotet, B., Gunthard, H. F., Kuritzkes, D. R., Pillay, D., Schapiro, J. M., and Richman, D. D. (2007) *Top. HIV Med. 15*, 119–125.
16. Malet, I., Delelis, O., Valantin, M. A., Montes, B., Soulie, C., Wirden, M., Tchertanova, L., Peytavin, G., Reynes, J., Mouscadet, J. F., Katlama, C., Calvez, V., and Marcelin, A. G. (2008) *Antimicrob. Agents Chemother. 52*, 2069–2078.
17. Hazuda, D. J., Anthony, N. J., Gomez, R. P., Jolly, S. M., Wai, J. S., Zhuang, L., Fisher, T. E., Embrey, M., Guare, J. J. P., Egbertson, M. S., Vacca, J. P., Huff, J. R., Felock, P. J., Witmer, M. V., Stillmock, K. A., Danovich, R., Grobler, J., Miller, M. D., Espeseth, A. S., Jin, L., Chen, I.-W., Lin, J. H., Kassahun, K., Ellis, J. D., Wong, B. K., Xu, W., Pearson, P. G., Schleif, W. A., Cortese, R., Emini, E., Summa, V., Holloway, M. K., and Young, S. D. (2004) *Proc. Natl. Acad. Sci. U.S.A. 101*, 11233–11238.
18. Fikkert, V., Van Maele, B., Vercammen, J., Hantson, A., Van Remoortel, B., Michiels, M., Gurnari, C., Pannecouque, C., De Maeyer, M., Engelborghs, Y., De Clercq, E., Debyser, Z., and Witvrouw, M. (2003) *J. Virol. 77*, 11459–11470.
19. Morris, G. M., Goodsell, D. S., Halliday, R. S., Huey, R., Hart, W. E., Belew, R. K., and Olson, A. J. (1998) *J. Comput. Chem. 19*, 1639–1662.
20. Huey, R., Morris, G. M., Olson, A. J., and Goodsell, D. S. (2007) *J. Comput. Chem. 28*, 1145–1152.
21. Savarino, A. (2007) *Retrovirology 4*, 21–35.
22. Goldgur, Y., Craigie, R., Cohen, G. H., Fujiwara, T., Yoshinaga, T., Fujishita, T., Sugimoto, H., Endo, T., Murai, H., and Davies, D. R. (1999) *Proc. Natl. Acad. Sci. U.S.A. 96*, 13040–13043.
23. Deng, J., Kelley, J. A., Barchi, J. J., Sanchez, T., Dayam, R., Pommier, Y., and Neamati, N. (2006) *Bioorg. Med. Chem. 14*, 3785–3792.
24. Zeinalipour-Yazdi, C. D., and Pullman, D. P. (2006) *J. Phys. Chem. B 110*, 24260–24265.

BI8019349