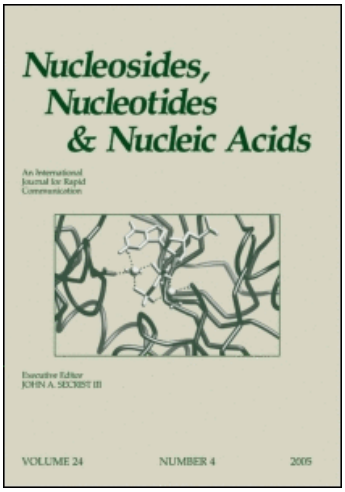

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UNDERSTANDING THE MODE OF ACTION OF L-NUCLEOSIDES AS ANTIVIRAL AGENTS: A MOLECULAR MODELING APPROACH

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ABSTRACT

Computer modeling studies have been performed on the several pairs of D- and L-nucleoside inhibitors with the HIV-1 RT model. Additionally, clinically important M184V mutation, which confers the viral resistance against 3TC and FTC, were studied by the same modeling system.

Correlation of the antiviral activity of nucleoside reverse transcriptase inhibitors (NRTIs) (1) with their structural and stereochemical features is of considerable significance, not only for understanding the molecular mechanism, but also for developing more effective and less toxic agents. Since the discovery of 3TC 1(b), a number of nucleosides with the unnatural L-configuration have emerged as potent antiviral agents. However, even though both 3TC and FTC showed potent antiviral activity against HIV and hepatitis B virus (HBV) with favorable pharmacokinetic and toxicity profiles, there has not been enough understanding about the conformational preferences and interaction patterns of the unnatural L-enantiomers at the active site of the target enzymes.

On the other hand, a major obstacle in the successful treatment of AIDS patients with NRTIs is the emergence of resistant variant strains. Among those the M184V mutant, which appears rapidly in monotherapy with 3TC or FTC and exhibits cross-resistance to ddI and ddC, has been extensively studied because of its

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clinical importance (2), but the detailed mechanism of drug resistance conferred by mutations is not clear yet. In an attempt to understand the interactions of D- and L-nucleoside triphosphates with HIV-1 RT and the mutated RT conferring resistance to 3TC (M184V), molecular modeling studies were performed (3). The enzyme site of the enzyme-ligand complex was constructed based on the truncated X-ray structure (4) of covalently trapped catalytic complex of HIV-1 RT with dTTP and the primer-template duplex (5). In case of 3TC-resistant mutation study, the enzyme site of M184V mutant was constructed with the same size of the wild type site by point mutation of the amino acid residue Met184 to Val184. The two major conformations, north (3′-endo, 2′-exo) and south (2′-endo, 3′-exo), of each inhibitor were constructed based on the X-ray coordinates (AZT, FTC, ddC) (6) and/or conformational analysis. The enzyme site and the inhibitor were merged to form a catalytic complex and then the heterocyclic base moiety was situated in such an orientation that it could be paired with its complementary base in the template strand. The enzyme-inhibitor complexes were minimized by using Kollman-All Atom Force Field until the energy change from one iteration to the next was less than 0.05 kcal/mol. The binding affinity of the examined structures toward HIV-1 RT was estimated by means of the relative binding energy differences (Er) between the inhibitor-enzyme complex and the dTTP-RT or dCTP-RT complex in the energy-minimized states. After energy minimization of AZTTP complexed with the enzyme site model, the 3′-azido moiety of AZTTP fitted nicely in the 3′-OH pocket (5) of the enzyme, along with the hydrogen bonding to the NH group of Tyr 115, which may contribute to the enhanced binding affinity (Er = 70.4 kcal/mol) of this inhibitor (Fig. 1). Also, we have found that only the 3′-endo (north) conformation of AZTTP fits into the active site of HIV-1 RT, while it has been known that 2′-endo (south) conformation of AZT is favored in the kinase phosphorylation level (7). The result suggests that AZT, by virtue of its flexible nature, may adopt the south conformation required for the initial phosphorylation, and subsequently switch to a north conformation at the triphosphate state for a better interaction with the active site of HIV-1 RT (8). In case of L-AZTTP, the binding affinity (Er = −174.7 kcal/mol) seems much lower than that of the natural substrate, dTTP or AZTTP. It appears that its L-configuration and 3′-azide group do not allow L-AZTTP to bind at the active site, especially at the 3′-OH pocket.

In contrast to AZTTP, D- and L-pairs of 2′,3′-dideoxynucleoside triphosphate such as 3TCTP and ddCTP, have only two chiral centers, which seem to be well tolerated at the RT level (9). The results from our modeling study suggest that the binding mode of 3TCTP (Er = 70.4 kcal/mol) and L-ddCTP (Er = 9.9 kcal/mol) to the dNTP pocket appears to be similar to that of the natural D-nucleotide. The conformational preference of the triphosphates was not found in the case of 3TC and ddC, since any conformers could fit into the active site, including the 3′-OH pocket. Therefore, the differences in antiviral activity between D- and L-isomers seem mainly derived from the different substrate specificity of other cellular enzyme, such as deoxycytidine kinase, thymidine kinase or deaminase.

Based on these findings, we may conclude that only 3′-substituted-2′,3′-dideoxy nucleoside triphosphates in the D-configuration may effectively bind to

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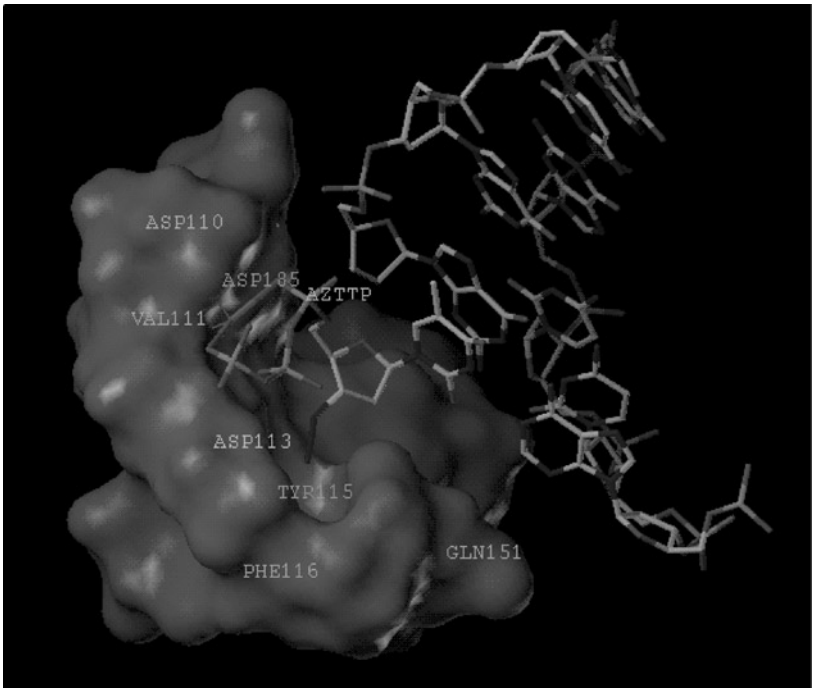

Figure 1. AZTTP complexed with the enzyme site.

the active site of RT, while in the case of 3′-unsubstituted 2′,3′-dideoxy nucleosides, both the D- and L-nucleosides can be effectively bound to the enzyme.

The M184V mutation is likely to affect the position, stability as well as reactivity of the bound analogue because the amino acid 184 is located in the neighborhood of the incoming nucleotide. Small changes in the contacting residues could markedly influence the rate of nucleotide incorporation 6(a). The methionine side chain contacts with the sugar and the base of the 3′-nucleotide in the primer, and the introduction of a β–branched side chain, such as valine, might create a contact with the dNTP sugar ring. Particularly, 3TCTP incorporation into the active site of M184V mutant may be severely interfered by this substitution due to its 1,3-oxathiolane ring as well as the β–L-configuration. Results from the energy minimization of 3TCTP in the M184V mutant site (Er $= -2318.8$ kcal/mol) showed that the structure of the enzyme-inhibitor complex was distorted in order to avoid the steric conflict of 3TCTP with the side chain of Val184, which resulted in instability of overall conformations of the complex, including the primer:template duplex. This aspect is also well represented as the significant decrease in the relative binding energy change of 3TCTP in the M184V mutant site model, which correlates with the loss of anti-HIV-1 activity (10).

As expected, the binding affinity of AZTTP was not significantly affected by mutation (Er $= 49.7$ kcal/mol) probably due to the fact that the sugar ring of AZTTP is positioned distant from the amino acid residue Val184 in comparison to that of

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3TCTP). These results may explain the ability of M184V mutant to recognize and incorporate other dNTPs as substrates, yet reject 3TC or FTC triphosphates.

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REFERENCES

1. (a) Daluge, S. M.; Purifoy, D. J.; Savina, P. M.; St Clair, M. H.; Parry, N. R.; Dev, I. K.; Novak, P.; Ayers, K. M.; Reardon, J. E.; Roberts, G. B.; Fyfe, J. A.; Blum, M. R.; Averett, D. R.; Dornsife, R. E.; Domin, B. A.; Ferone, R.; Lewis, D. A.; Krenitsky, T. A. *Antimicrob. Agents Chemother*. **1994**, *38*, 1590. (b) Schinizi, R. F.; Lloyd, R. M.; Nguyen, M. -N.; Cannon, D. L.; McMillan, A.; Ilksoy, N.; Chu, C. K.; Liotta, D. C.; Bazmi, H. Z.; Mellors, J. W. *Antimicrob. Agents Chemother*. **1993**, *37*, 875. (c) Balzarini, J.; Cooney, D. A.; Dalad, M.; Kang, G. J.; Cupp, J. E.; De Clercq, E.; Broder, S.; Johns, D. G. *Pharmacol*. **1987**, *32*, 798. (d) Mitsuya, H.; Broder, S. *Proc. Natl. Acad. Sci. USA* **1986**, *82*, 1911. (e) Lin, T. -S.; Liu, M. -C.; Pai, S. B.; Dutschman, G. E.; Cheng, Y. -C. *J. Med. Chem*., **1994**, *37*, 798. (f) Daluge, S. M.; Good, S. S.; Faletto, M. B.; Miller, W. H.; St Clair, M. H.; Boone, L. R.; Tisdale, M.; Parry, N. R.; Reardon, J. E.; Dornsife, R. E.; Averet, D. R.; Krenitsky, T. A. *Antimicro. Agents Chemother*. **1997**, *41*, 1082.
2. Tisdale, M.; Kemp, S. D.; Parry, N. R.; Larder, B. A. *Proc. Natl. Acad. Sci. USA* **1993**, *90*, 5653.
3. Molecular modeling studies were performed with Sybyl (Tripos Associates, Version 6.5), with all minimizations performed using the Kollman-all Atom force Field within Biopolymer module on a Silicom Graphics Indigo 2 or Octane workstation.
4. The enzyme site is consisted of amino acid residues 51–242 from the p66 domain, 7:4 double stranded DNA template:primer, two magnesium ions and dNTP.
5. Huang, H.; Chopra, R.; Verdine, G.; Harrison, S. *Science*, **1998**, *282*, 1669.
6. (a) Birnbaum, G. I.; Giziewicz, J.; Gabe, E. J.; Lin, T. S.; Prusoff, W. H. *Can. J. Chem*., **1987**, *65*, 2135. (b) Van Roey, P.; Pangborn, W. A.; Schinazi, R. F.; Painter, G.; Liotta, D. C. *Antiviral Chem. Chemother*., **1993**, *4*, 369. (c) Birnbaum, G. I.; Lin, T. S.; Prusoff, W. H. *Biochem. Biophys. Res. Commum*., **1988**, *151*, 608.
7. (a) Birnbaum, G. I.; Giziewicz, J.; Gabe, E. J.; Lin, T. S.; Prusoff, W. H. Can. *J. Chem*., **1987**, *65*, 2135. (b) Van Roey, P.; Salerno, J. J.; Duax, W.; Chu, C. K.; Ahn, M. K.; Schinazi, R. F. *J. Am. Chem. Soc*., **1988**, *110*, 2277. (c) Van Roey, P.; Salerno, J. J.; Chu, C. K.; Schinazi, R. F. *Proc. Natl. Acad. Sci. USA*, **1989**, *86*, 3929.
8. Marquez, V. E.; Ezzitouni, A.; Russ, P.; Siddiqui, M. M.; Ford, H.; Feldman, R. J.; Mitsuya, H.; George, C.; Barchi, Jr., J. J. *J. Am. Chem. Soc*., **1998**, *120*, 2780.
9. Schwach, D. S.; Liotta, D. C.; Schinazi, R. F. *Biochem. Pharmacol*. **1993**, *45*, 1540.
10. Sarafinanos, S. G.; Das, K.; Clark Jr. A. D.; Ding, J.; Boyer, P. L.; Hughes, S. H.; Arnold, E. *Proc. Natl. Acad. Sci. USA*, **1999**, *96*, 10027.

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