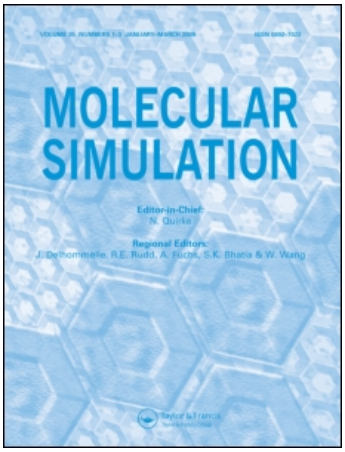

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Temperature-Dependent Changes in the Folding Patterns of the ω-Loop (Tyr181 to Tyr188) of HIV-1 Reverse Transcriptase

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TEMPERATURE-DEPENDENT CHANGES IN THE FOLDING PATTERNS OF THE Ω-LOOP (TYR181 TO TYR188) OF HIV-1 REVERSE TRANSCRIPTASE

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The segment Tyr181 to Tyr188 was dissected from the HIV-1 reverse transcriptase (RT). The segment contains two amino acids (Asp185, Asp186) of the catalytic aspartyl triad (Asp110, Asp185, Asp186) and two amino acids (Tyr181, Tyr188) of the nonnucleoside RT inhibitor (NNRTI) binding sites. In the quasicrystalline state, hydrogen-bonding forces between the folded peptide chain play the greatest role in holding two chains together and in specifying the folding pattern, an Ω-loop. To surmount the energy-barrier height during the formation of an activated complex between certain amino acids of the Ω-loop and ligands, the receptor must be in a fairly high-energy domain. This input of kinetic energy is simulated by molecular dynamics. Simulation of high and body temperatures "destroy" the hydrogen-bonding forces and therefore the ordered Ω-loop conformation found by X-ray analysis (113 Kelvin temperature) and molecular modelling (near 0 Kelvin temperature). The conformational switching of Tyr181 is larger than that of Tyr188, allowing thus improved interactions with the aromatic residues of nonnucleoside inhibitors of the HIV-1 reverse transcriptase.

Keywords: HIV-1 reverse transcriptase; Ω-loop; molecular modelling; molecular dynamics heating calculations

INTRODUCTION

To treat chemically the acquired immunodeficiency syndrome (AIDS) induced by the immunodeficiency virus type 1 (HIV-1), the viral reverse transcriptase (RT) plays a key role. Active RT is a strikingly asymmetric heterodimer composed of two differently folded 66 kDa (p66) and 51 kDa (p51) subunits. The p66 subunit is a bifunctional enzyme containing a DNA polymerase and ribonuclease H (RNase H) activity. The polymerase and RNase activities

cooperate to convert the single-stranded genomic RNA of HIV-1 into a linear double-stranded DNA that is subsequently integrated into host cell chromosomes [1–6]. During a single cycle of viral replication, HIV-1 RT catalyzes the incorporation of approximately 20,000 nucleotides. As RT does not appear to be essential in the normal functioning of mammalians, it is an attractive target for structure-based drug design.

Molecular modelling of the RT polymerase subunit has shown two subregions (amino acid positions 98–106 and 179–190) of the allosteric area. From the latter subregion, the Tyr181 to Tyr188 segment was excised. It contains two amino acids (Asp185, Asp186) of the catalytic aspartyl triad (Asp110, Asp185, Asp186) and two amino acids (Tyr181, Tyr188) of the allosteric resp. nonnucleoside RT inhibitor (NNRTI) binding sites. The Tyr183Met184Asp185Asp186 peptide is conserved in all known immunodeficiency associated retroviral RTs. Rotations of the side chains of Tyr181 and Tyr188 help to create a cavity to accommodate NNRTIs.

Quite recently, it was shown [7] that the Tyr181 to Tyr188 segment has a configuration which is like a Ω-loop dissected firstly from interleukin-1α (segment Leu41 to Val48) peptide [8]. The treatment of solvents as dielectric continuums surrounding a force field model led to changes in conformation of the Ω-loop of RT but these changes were not remarkable because the shape was maintained [9].

Since conformation analyses by X-rays and molecular modelling are mainly based on temperatures of −160 and −270 Celsius temperature, respectively, it appears important to simulate temperature-dependent folding patters of the Ω-loop at body temperature (37° Celsius) and at an energetically activated state. This kinetic activation is a condition that the Tyr181 to Tyr188 segment interacts with ligands by building a short-living transition-state peptide-ligand complex. The present paper investigates some aspects of the three-dimensional behavior of the peptide segment after the input of kinetic energy.

MATERIAL AND METHODS

All-atom molecular mechanics (MM) methods [10–12] were applied to compare the conformations of the molecules. The MM+ force field which was used in subsequent calculations is an improved MM2/MM3 force field [10, 11]. The whole procedure was repeated with Coulombs law functions using the connectivity-based iterative partial equalization of orbital electronegativity (Gasteiger charges) which does not depend on a particular geometry optimization [13].

Correlation-gradient geometry optimization [14] was achieved by the following steps: The structures were refined using a conjugate gradient minimizer (Fletcher-Reeves modification of the Polak-Ribière method). Convergence was obtained when the gradient root mean square RMS was RMS < 0.05 kcal/Å · mol. The conformations were initially energy minimized using the MM+ force field without an electrostatic term. After including the partial charges, the resulting optimized conformation contains the molecular electrostatic potential and electrostatic energies.

Prior the geometry optimization, the so-called cooling-heating-cooling schedule [15] was applied: After a short mechanics run (at 0 Kelvin temperature, 0.1 ps), a molecular dynamics run (100 Kelvin temperature, 0.5 ps) was used thus invoking thermal vibrational and entropic effects, to surmount energy barriers on the potential surface and to find regions of lower potential energy. The heating is followed by a slow cooling rate (0 Kelvin temperature, 10 ps) whereby the system "freezes" to a single structure.

Molecular dynamics calculations were carried out with the geometry-optimized structure. The following options were used: starting time and temperature: 0.1 ps, 100 Kelvin temperature; running time and simulation temperature: 0.5 ps, 600 or 310 Kelvin temperature (see text); cooling time and final temperature: 0.5 ps, 0 Kelvin temperature; step size = 0.5 fs.

RESULTS

The crystal structures of complexed and unliganded HIV-1 (wild-type BH10 isolate) reverse transcriptase (E.C.2.7.7.49) were uploaded from the Brookhaven Protein Data Bank [16] by using Cartesian coordinates (Z-matrix). The resolution range of the nevirapine-liganded RT (3HVT in PDB nomenclature) was 8.0–2.9 Å, of the unliganded RT (1RTI) it was 25.0–3.0 Å; the resolutions were sufficient to permit assignment of amino acid positions through-out the structure and allows subsequent molecular modelling. Measurement occurred at −160 Celsius temperature. During nevirapine binding, significant conformational changes of the Tyr181 to Tyr188 segment occurred in comparison to the uncomplexed enzyme. Therefore, 3HVT was applied to subsequent analyses, and the hydrophilic segment Tyr181 to Tyr188 was excited [7]. Acetyl and N-methylamino were introduced as N-terminal and C-terminal blocking groups, respectively (Fig. 1).

The logarithms of the distribution coefficient P (octanol/water) were as follows [7]: $\log P = -3.87$ (neutral microspecies), $\log P = -7.70$ (at pH = 6), $\log P = -8.25$ (at pH = 7.4). The hydration energy was −35.86 kcal/mol and

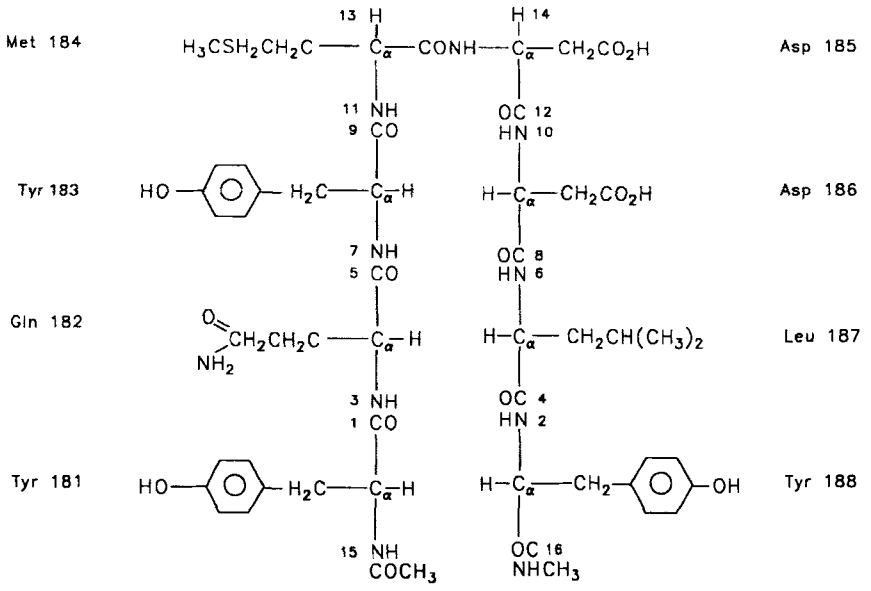

FIGURE 1 Amino acids of the Ω-loop. The numbers were used to code the intramolecular hydrogen-bonding forces.

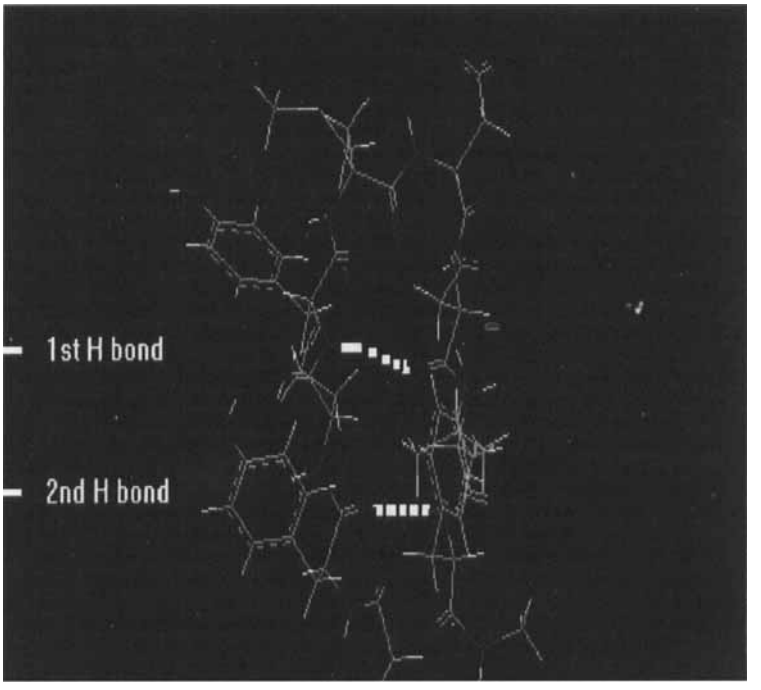

FIGURE 2 Gasteiger-MM + optimized geometry of the Ω-loop (See Color Plate I).

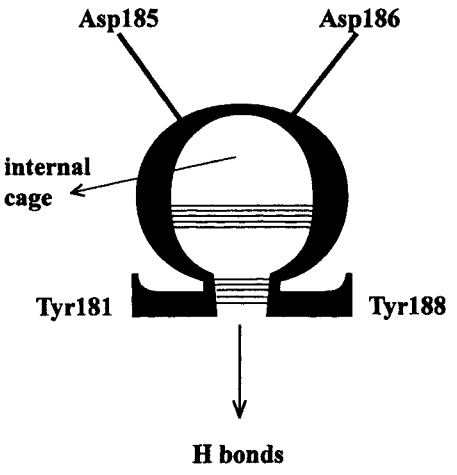

FIGURE 3 Schematic view of the Ω-loop with two "horizontal axes" that are formed by the nonnucleoside binding sites Tyr188 and Tyr188, and the two aspartyl residues Asp185 and Asp186 of the catalytic triad (Asp110, Asp185, Asp186). The strong hydrogen bonds of the peptide backbone that maintain the constrained loop are between Tyr181 and Tyr188 (independent on solvation effects), and within the internal cage (location in dependence on solvation effects).

the electrostatic part of the solvation energy was -29.66 kcal/mol. Therefore, the unliganded Tyr181–Tyr188 segment is highly hydrophilic.

The net atomic charges were determined by connectivity-based iterative partial equalization of orbital electronegativity, and the complete geometry-optimization procedure was applied. In subsequent analysis, a dielectric constant of $\varepsilon = 1$ was used.

The resulting molecule forms a highly hydrophilic Ω-like structure [7, 9] (Fig. 2). There are at least two strong O...H—N forces within the internal cage that maintain the Ω-loop. The first two amino acids that are involved are Tyr181 and Tyr188 (H bonds see code 1 and 2 in Fig. 1). At a dielectric constant of $\varepsilon = 1$, the second two amino acids are Tyr183 and Asp186 (H bond see code code 7 and 8 in Fig. 1). The other hydrogen-bonding forces are less strong and their strength depends also on solvent conditions and the dielectric constant. Figure 3 gives a schematic view of the Ω-loop.

The quasicrystalline state in kinetically inactive, a ligand cannot interact with the Ω-loop of HIV-1 RT without energetic activation. To surmount the energy-barrier height during the formation of the short-living transition state, the ligand and enzyme must be in a fairly high-energy domain. Therefore, atomic vibrations and collisions must be introduced by an input of kinetic

energy. Clearly, in warm-blooded animals the energy comes from biochemical pathways. In molecular modelling studies, the energy input is simulated by raising the temperature to achieve an activated state of the molecule by an input of kinetic energy [15] (Fig. 4).

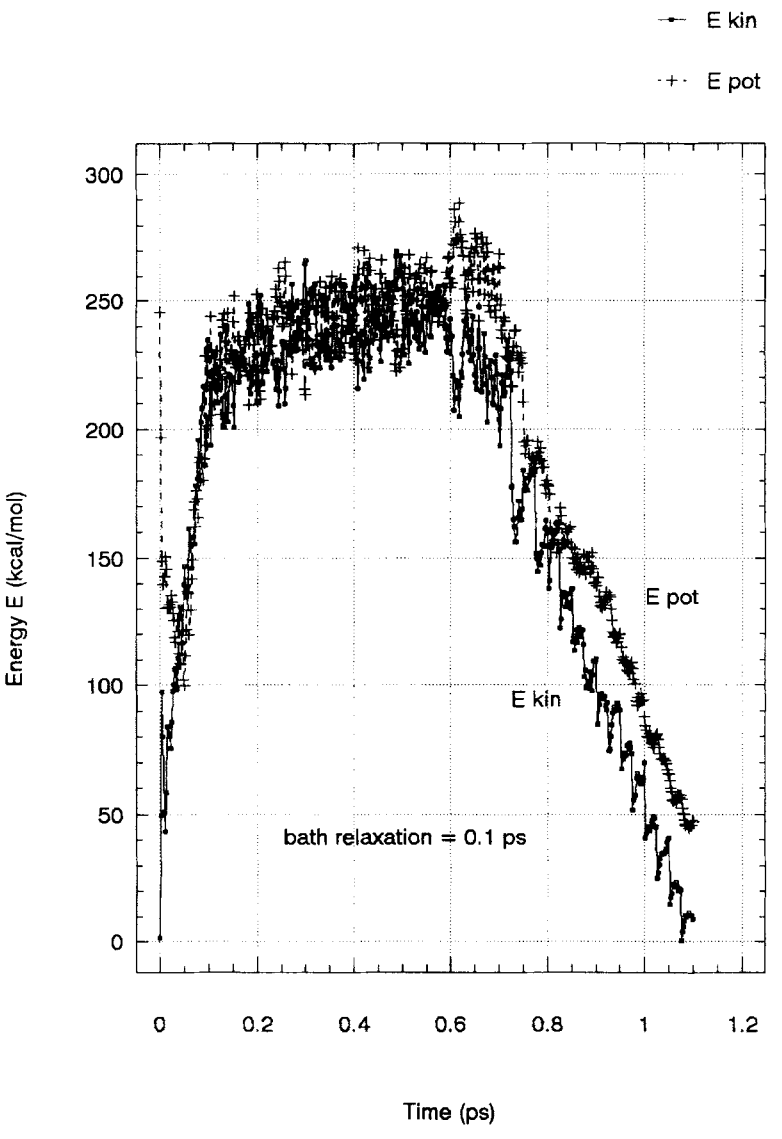

FIGURE 4 Potential and kinetic energy (kcal/mol) using the heating-simulation-cooling schedule. 600 Kelvin temperature is used to simulate an short-living, energetically activated state of the Ω-loop. See also Figure 9.

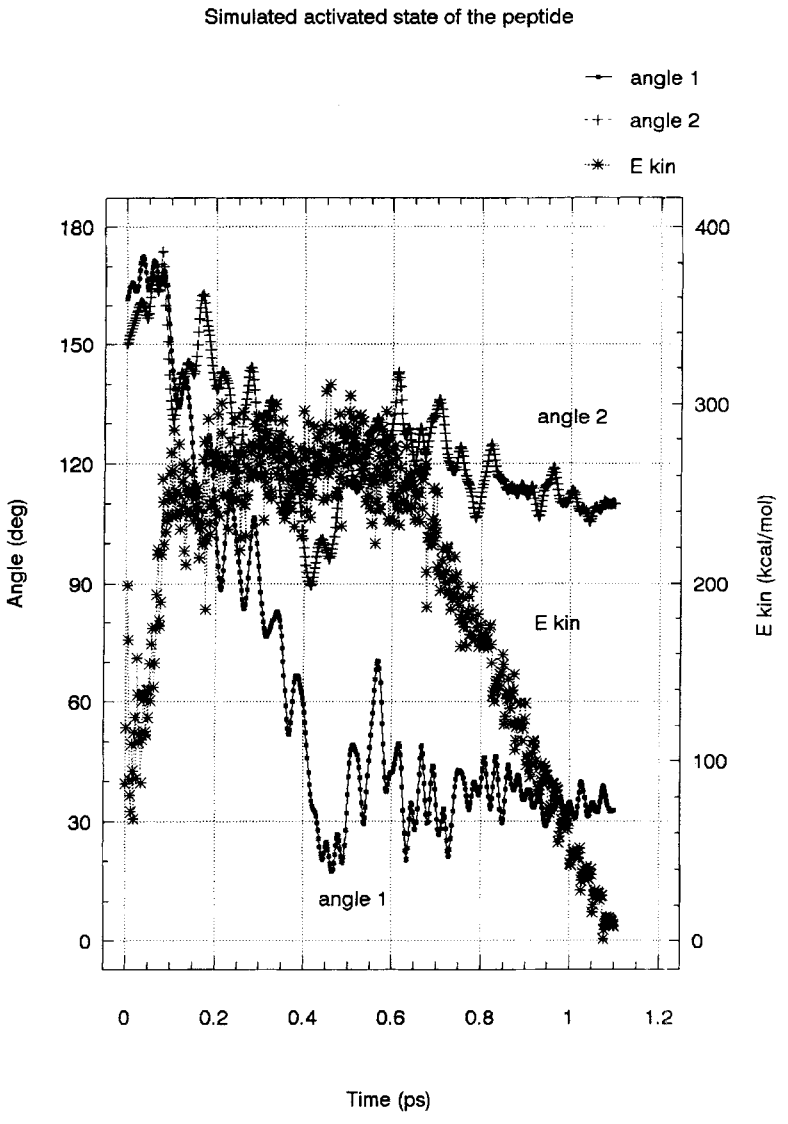

FIGURE 5 Angles (in deg unit) of the two studied hydrogen bonds, and associated kinetic energy (kcal/mol) using the heating-simulation-cooling schedule. 600 Kelvin temperature is used to simulate an short-living, energetically activated state of the Ω-loop. See also Figure 9.

Among the various hydrogen-bonding forces of the two folded peptide chains of the Ω-loop, the two strongest ones were chosen (the distance between the oxygen and nitrogen atoms of NH...O is <3.5 Å, the angle is >140 deg, the hydrogen-bonding energy is >2.2 kcal/mol). The bonds connect the

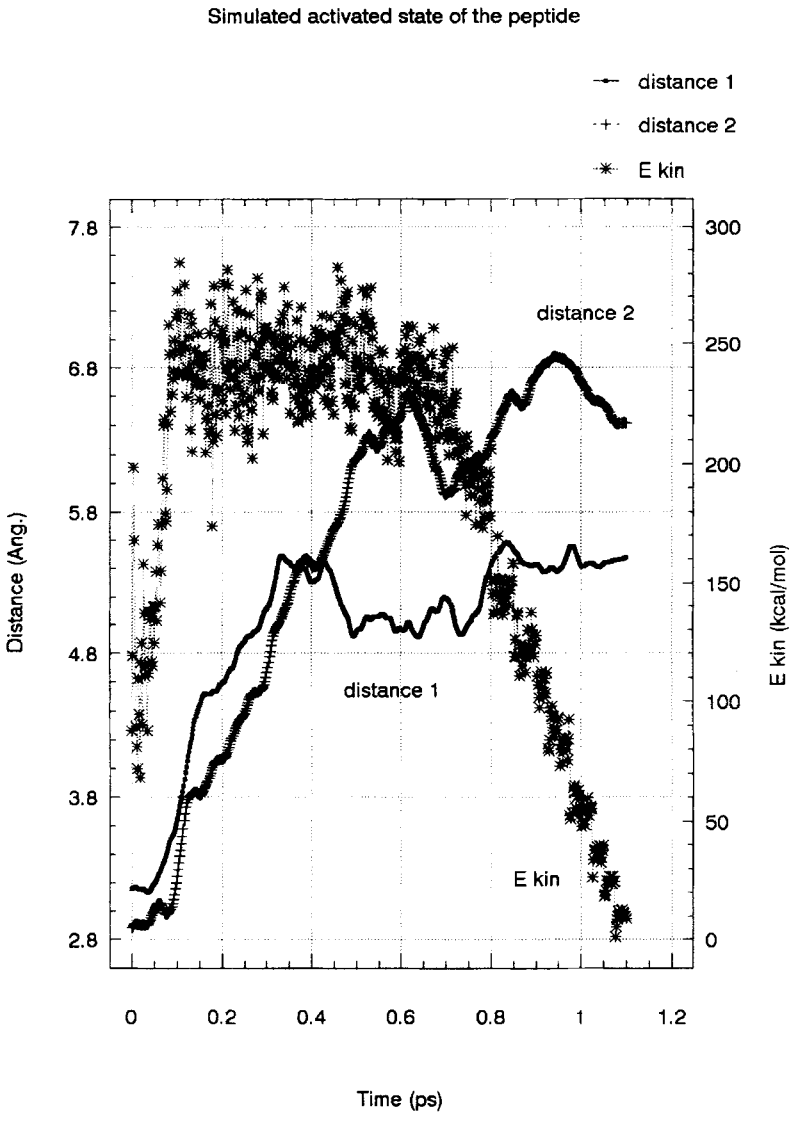

FIGURE 6 Distances (in Å unit) between the nitrogen and oxygen atoms of the two studied hydrogen bonds, and associated kinetic energy (kcal/mol) using the heating-simulation-cooling schedule. 600 Kelvin temperature is used to simulate an short-living, energetically activated state of the Ω-loop. See also Figure 9.

backbone NH atoms of Tyr183 with the backbone oxygen of Asp186 (distance 1, angle 1), and the backbone oxygen of Tyr181 with the backbone HN atoms of Tyr188 (distance 2, angle 2). The default times and temperatures of heating, simulation (600 Kelvin temperature), and cooling were given in "Material and

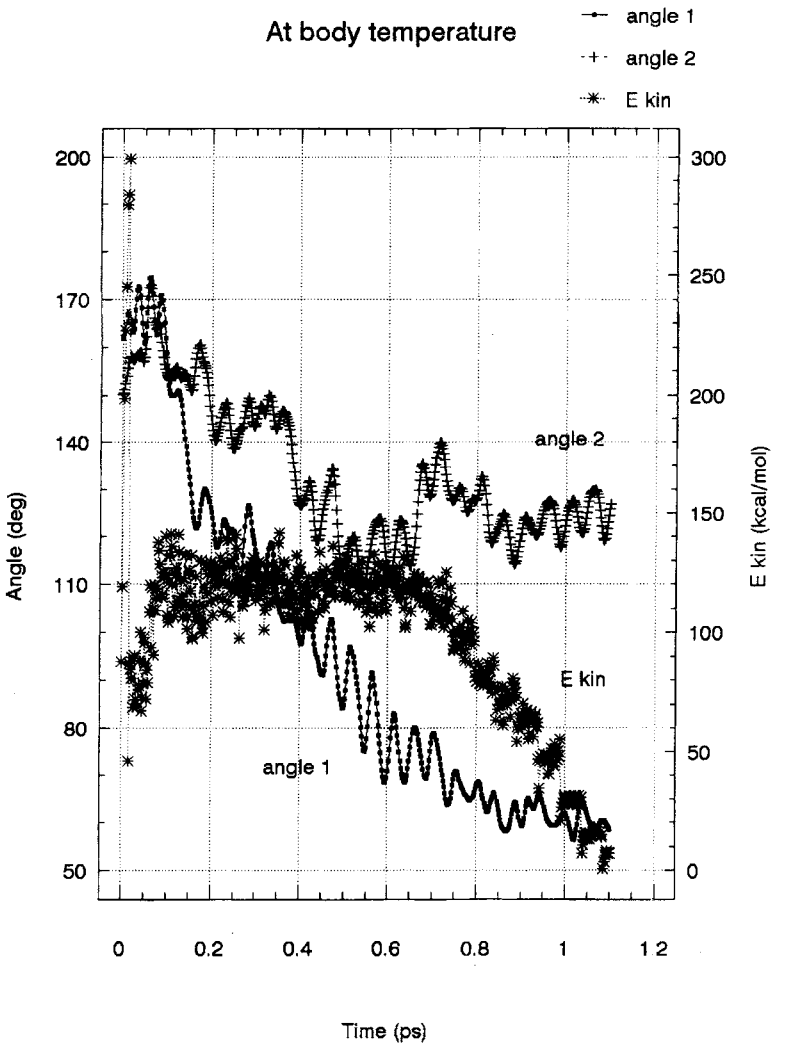

FIGURE 7 Angles (in deg unit) of the two studied hydrogen bonds, and associated kinetic energy (kcal/mol) using the heating-simulation-cooling schedule. 310 Kelvin temperature is used to simulate body temperature.

Methods". The results are illustrated in Figures 5 and 6 (the final angles and distances were angle 1 = 32.6 deg, distance 1 = 5.45 Å, angle 2 = 109.9, distance 2 = 6.41 Å). The conformation will be given in Figure 9 (see below).

Using equivalent conditions with a simulation temperature of 310 Kelvin (body temperature), the hydrogen-bond breaking effect of temperature is

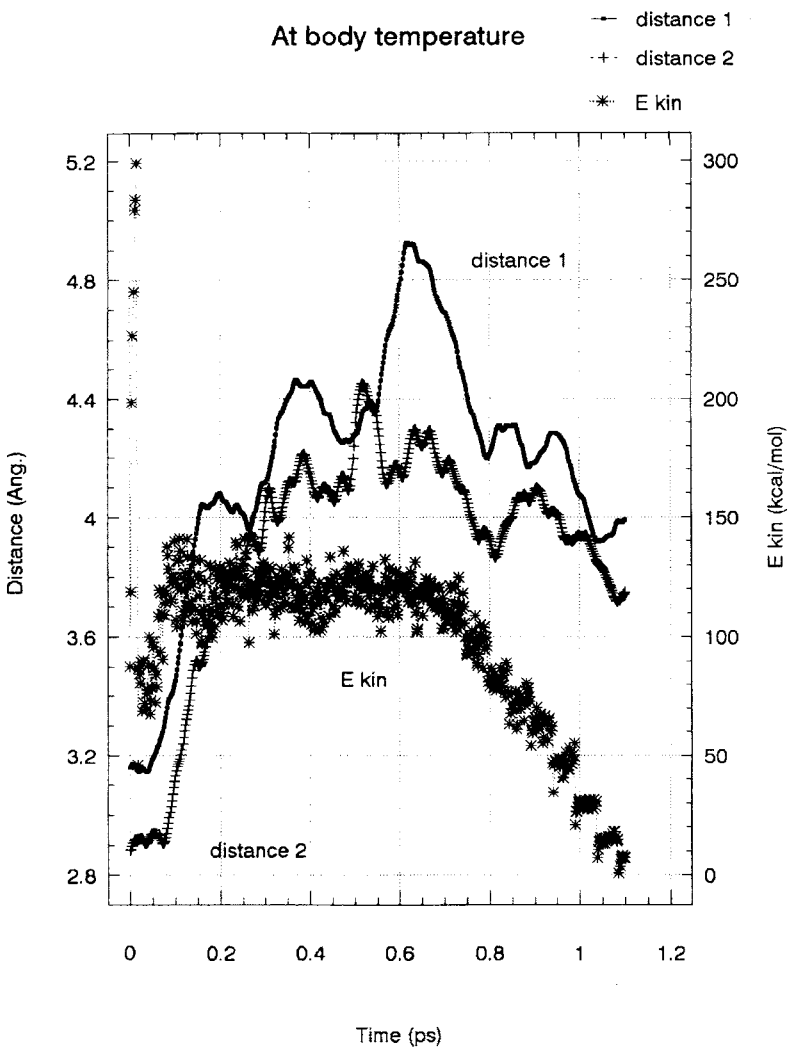

FIGURE 8 Distances (in Å unit) between the nitrogen and oxygen atoms of the two studied hydrogen bonds, and associated kinetic energy (kcal/mol) using the heating-simulation-cooling schedule. 310 Kelvin temperature is used to simulate body temperature.

weaker but also remarkable (Figs. 7 and 8; angle 1 = 58.6 deg, distance 1 = 3.99 Å, angle 2 = 126.9, distance 2 = 3.75 Å).

Thus, high and body temperatures "destroy" the hydrogen-bonding forces and therefore the ordered Ω-loop conformation. In both cases, the conformational switching of Tyr181 is larger than that of Tyr188, allowing thus

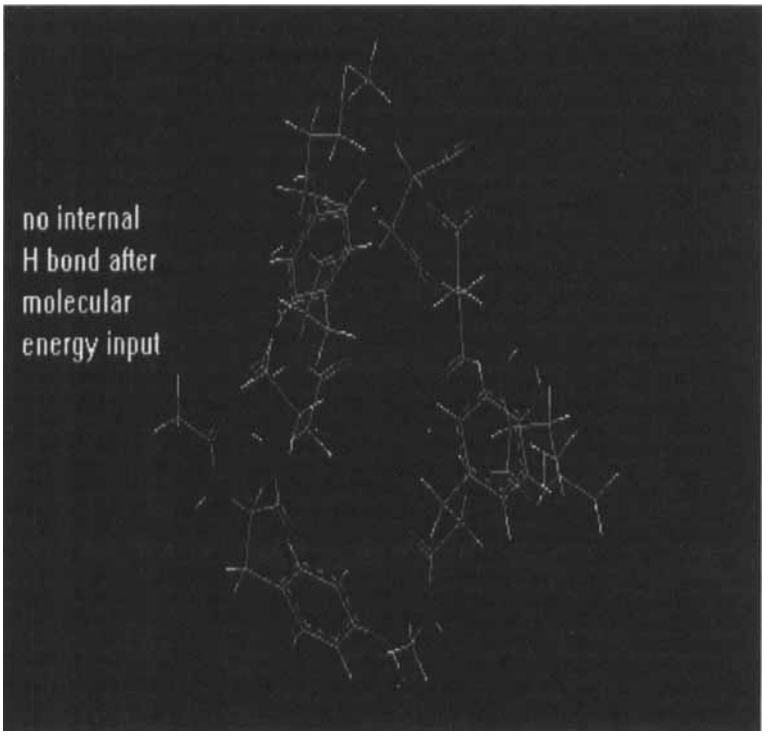

FIGURE 9 Molecular conformation of the Ω-loop after the final step of calculation using the heating-simulation-cooling schedule. 600 Kelvin temperature is used to simulate an short-living, energetically activated state of the Ω-loop (See Color Plate II).

improved interactions with the aromatic residues of nonnucleoside inhibitors of the HIV-1 reverse transcriptase.

The analysis of the amino acid positions 181 to 188 yielded the secondary structure RRLRRPRR after strong heating (where R denotes a random coil, L is a left-handed α-helix, and P is a parallel β-sheet). Therefore, there is a tendency to form a random coil. After heating, this transition to a random coil is not reversible while it is without heating (PPM, unpublished). Furthermore, the side chains of Tyr181 and Tyr188 rotate out of the Ω-loop (Fig. 9).

DISCUSSION AND CONCLUSIONS

The hydrophilic segment Tyr181 to Tyr188 was dissected from HIV-1 reverse transcriptase. The segment contains two amino acids (Asp185, Asp186) of the

catalytic aspartyl triad (Asp110, Asp185, Asp186) and two amino acids (Tyr181, Tyr188) of the nonnucleoside RT inhibitor (NNRTI) binding sites. The aspartyl residues Asp185 and Asp186 of the segment are involved in binding the triphosphate moiety of an incoming deoxyribonucleoside triphosphates *via* cheleated Mg^{2+} ions [1–6]. The conformationally stabilized configuration links two folded peptide chains consisting each of four amino acids. The structure-specifying forces are based on at least two intramolecular hydrogen-bonding forces which hold the two chains together and specify the folding patterns so that a constrained Ω-loop configuration is obtained.

There is much water around the highly polar Ω-loop and within the internal cage of the folded pattern. It might be expected that this internal microenvironment has considerably lower dielectric than the bulk aqueous solution. The treatment of solvents as dielectric continuums surrounding the force field model has shown changes in distances and bonding angles of the intramolecular hydrogen-bonding forces of the peptide backbone and the residues [7, 9]. However, these changes did not influence the formation of the Ω-loop shape, in contrast to temperature-dependent folding patterns. High and body temperatures "destroy" the hydrogen-bonding forces and therefore the ordered Ω-loop conformation found by X-ray analysis (113 Kelvin temperature) and molecular modelling (near 0 Kelvin temperature). In both cases, the conformational mobility of Tyr181 is larger than that of Tyr188. Such a kinetically activated mobile portion of the Ω-loop of HIV-1 RT seems to be one of the conditions that the enzyme will be inhibited by noncompetitive, allosteric antagonists.

References

[1] Ren, J. S., Esnouf, R., Garman, E., Somers, D., Ross, O., Kirby, I., Keeling, J., Darby, G., Jones, Y., Stuart, D. and Stammers, D. (1995). "High Resolution Structures of HIV-1 RT from Four RT-inhibitor Complexes", *Nature Struct. Biol.*, **2**, 293–302.

[2] Ding, J., Das, K., Tantillo, C., Zhang, W., Clark, A. D., Jessen, S., Lu, X., Hsiou, Y., Jacobomolina, A., Andries, K., Pauwels, R., Moereels, H., Koymans, L., Janssen, P. A. J., Smith, R. H., Koepke, M. K., Michejda, C. J., Hughes, S. H. and Arnold, E. (1995). "Structure of HIV-1 Reverse Transcriptase in a Complex with the Non-nucleoside Inhibitor Alpha-APA R 95845 at 2.8 Angstrom Resolution", *Structure*, **3**, 365–379.

[3] Boyer, P. L., Ferris, A. L., Clark, P., Whitmer, J., Frank, P., Tantillo, C., Arnold, E. and Hughes, S. H. (1994). "Mutational Analysis of the Fingers and Palm Subdomains of Human Immunodeficiency Virus Type-1 (HIV-1) Reverse Transcriptase", *J. Mol. Biol.*, **243**, 472–483.

[4] Pauwels, R., Andries, K., Janssen, P. A. J. and Arnold, E. (1994). "Locations of Anti-AIDS Drug Binding Sites and Resistance Mutations in the Three-Dimensional Structure of HIV-1 Reverse Transcriptase – Implications for Mechanisms of Drug Inhibition and Resistance", *J. Mol. Biol.*, **243**, 369–387.

[5] Tantillo, C., Ding, J., Jacobo-Molina, A., Nanni, R. G., Boyer, P. L., Hughes, S. H., Pauwels, R., Andries, K., Janssen, P. A. J. and Arnold, E. (1994). "Locations of Anti-AIDS Drug Binding Sites and Resistance Mutations in the Three-dimensional Structure of HIV-1 Reverse Transcriptase", *J. Mol. Biol.*, **243**, 369–387.

[6] Kohlstaedt, L. A., Wang, J., Friedman, J. M., Rice, P. A. and Steitz, T. A. (1992). "Crystal Structure at 3.5 Å Resolution of HIV-1 Reverse Transcriptase Complexed with an Inhibitor", *Science*, **256**, 1783–1790.

[7] Mager, P. P. and Walther, H. (1996). "A Hydrophilic Ω-Loop (Tyr181 to Tyr188) in the Nonsubstrate Binding Area of HIV-1 Reverse Transcriptase", *Drug Design and Discovery*, [in press].

[8] Sarabu, R., Lovey, K., Madison, V. S., Frey, D. C., Greeley, D. N., Cook, C. M. and Olson, G. L. (1993). Design, "Synthesis, and Three-Dimensional Structural Characterization of a Constrained Ω-Loop Excised from Interleukin-1α", *Tetrahedron*, **29**, 3629–3640.

[9] Mager, P. P. (1996). "Molecular Simulation of the Folding Patterns of the Ω-Loop (Tyr181 to Tyr188) of HIV-1 Reverse Transcriptase", *Drug Design and Discovery*, [in press].

[10] Allinger, N. L. and Yan, L. (1993). "Molecular Mechanics (MM3). Calculation of Furan, Vinyl, Ethers, and Related Compounds", *J. Am. Chem. Soc.*, **115**, 11918–11925.

[11] Tai, J. C., Yang, L. and Allinger, N. L. (1993). "Molecular Mechanics (MM3). Calculation on Nitrogen-Containing Aromatic Heterocyclics", *J. Am. Chem. Soc.*, **115**, 11906–11917.

[12] Weiner, S. J., Kollman, P. A., Nguyen, D. T. and Case, D. A. (1986). "An All Atom Force Field for Simulations of Proteins and Nucleic Acids", *J. Comput. Chem.*, **7**, 230–252.

[13] Gasteiger, J. and Marsili, M. (1980). "Iterative Partial Equalization of Orbital Electronegativity – a Rapid Access to Atomic Charges", *Tetrahedron*, **36**, 3219–3288.

[14] Frey, R. F., Coffin, J., Newton, S. Q., Ramek, M., Cheng, V. K. W., Momany, F. A. and Schäfer, L. (1992). "Importance of Correlation-Gradient Geometry Optimization for Molecular Conformational Analyses", *J. Am. Chem. Soc.*, **114**, 5369–5377.

[15] Mager, P. P. (1994). "Interactive Multivariate Modelling of ArgGlyAsp (RGD) Derivatives", *Med. Res. Rev.*, **14**, 75–126.

[16] Brookhaven Protein Data Bank (PDB), files 3HVT.ENT and 1RTI.ENT.