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Three-Dimensional Structural Model Analysis of the Binding Site of an Inhibitor, Nervonic Acid, of Both DNA Polymerase β and HIV-1 Reverse Transcriptase[1]

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Previously, we reported the three-dimensional molecular interactions of nervonic acid (NA) with mammalian DNA polymerase β (pol β) [Mizushina *et al.* (1998) *J. Biol. Chem.* 274, 25599–25607]. By three-dimensional structural model analysis and comparison with the spatial positioning of specific amino acids binding to NA on pol β (Leu11, Lys35, His51, and Thr79), we obtained supplementary information that allowed us to build a structural model of human immunodeficiency virus type-1 reverse transcriptase (HIV-1 RT). In HIV-1 RT, Leu100, Lys65, His235, and Thr386 corresponded to these four amino acid residues. These results suggested that the NA binding domains of pol β and HIV-1 RT are three-dimensionally very similar. The effects of NA on HIV-1 RT are thought to be same as those on pol β in binding to the rhombus of the four amino acid residues. NA dose-dependently inhibited the HIV-1 RT activity. For binding to pol β, the kinetics were competitive when the rhombus was present on the DNA binding site. However, as the rhombus in HIV-1 RT was not present in the DNA binding site, the three-dimensional structure of the DNA binding site must be distorted, and subsequently the enzyme is inhibited non-competitively.

Key words: computer simulation, inhibitor-binding site.

As reported previously, long chain fatty acids with 18 or more carbons in the backbone can inhibit mammalian DNA polymerases (*1, 2*). In particular, *cis*-configurating unsaturated fatty acids such as nervonic acid (NA) were effective in inhibiting the activity of DNA polymerase β (pol β) (*2*). This fatty acid bound to the N-terminal 8 kDa domain of pol β as a 1:1 complex (*2, 3*). The crystal structure of pol β was analyzed (*4–9*), and the NMR structure of the N-terminal 8 kDa domain of pol β has been determined (*10–12*). To obtain new information regarding the structure–function relationship between NA and other proteins, we searched for the NA-binding region on other proteins, whose three-dimensional structures have been determined, by computer analysis in comparison with the NMR data for the interaction between the 8 kDa domain of pol β and NA (*3*).

Computer analysis demonstrated NA-binding similarity in the three-dimensional structure between pol β and human immunodeficiency virus type-1 (HIV-1) reverse transcriptase (RT), although their amino acid sequences are markedly different from each other. In fact, NA was also shown to be a potent inhibitor of HIV-1 RT. NMR analysis of the three-dimensional structure of HIV-1 RT is also required. However, the HIV-1 RT molecule is too large for NMR analysis. The present study indicated that instead of NMR analysis, computer analysis can provide information regarding the relationship between the functions and three-dimensional structures of HIV-1 RT and pol β-family polymerases.

Pol β catalyzes DNA template–dependent DNA polymerization and RNA template–dependent DNA polymerization (*13*). RT has three distinct catalytic activities that enable it to convert the viral RNA genome into double-stranded DNA: (i) RNA template-dependent DNA polymerization, (ii) RNase H degradation of the RNA, and (iii) DNA template-dependent DNA polymerization (*13, 14*). Therefore, there are functional similarities between these two enzymes, and they are critical for many cellular processes including DNA replication, repair and recombination, and thus may act in harmony with each other.

This study provided novel information regarding the relationship between the functions and three-dimensional structures of pol β and HIV-1 RT. This information may help to establish new effective medicines against AIDS and/or to revive chemically those drugs that have lost their anti-viral activity.

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Abbreviations: pol β, DNA polymerase β (EC 2.7.7.7); HIV-1 RT, human immunodeficiency virus type-1 reverse transcriptase; NA, nervonic acid.

EXPERIMENTAL PROCEDURES

Search for the Nervonic Acid Interaction Interface on Other Proteins—To identify the NA binding sites of other proteins, NA binding amino acid residues of the N-terminal 8 kDa domain of DNA polymerase β (pol β) were identified by ^{1}H-^{15}N HMQC NMR mapping of the pol β 8 kDa domain with or without NA (*3*). The major shifted amino acids were Leu11, Lys35, His51, and Thr79 (*3*). These amino acids were traced on the three-dimensional structures of other proteins by two computer analysis methods; the evolutionary trace method (*15, 16*) and the geometrical method involving template-based analysis (*17–19*). The full-length amino acid sequences of reverse transcriptases of other species were retrieved from the Swiss-Prot data base (http://www.expasy.ch/sprot) for the evolutionary trace. Multiple-sequence alignment of the obtained sequences was performed. By calculation of the identity of the sequences of the reverse transcriptases, the evolutionarily important residues were determined. For geometrical analysis, the three-dimensional distances between the Cα-atoms of the four residues of Polβ (Leu11, Lys35, His51, Thr79) were determined with InsightII (Accerlys, San Diego, CA, USA), six lengths being obtained. The coordinates of the proteins in the Protein Data Bank (PDB) (http://www.rcsb.org/pdb) were downloaded to a workstation O2 (Silicon Graphics). We searched for proteins with a similar template of the above six lengths (less than 20% error) using the software "Binding Site Analysis," which is one of the modules of InsightII.

Nervonic Acid Docking Modeling—Molecular docking of NA and the 8 kDa domain of pol β (PDB ID: 1BNO) or human immunodeficiency virus type-1 (HIV-1) reverse transcriptase (RT) (PDB ID: 2HMI) was performed using the Affinity program within the Insight II modeling software (Accelrys). The calculations involved a CVFF force-field in the Discovery program and the Monte Carlo strategy using the Affinity program (*20*).

Nucleic Acids, Enzymes, and Other Reagents—Nucleotides and chemically synthesized template primers such as poly(dA), poly (rA) and oligo$(dT)_{12-18}$ were purchased from Pharmacia (Uppsala, Sweden). [^{3}H]dTTP (43 Ci/mmol) was purchased from Perkin Elmer Life Sciences. (Boston, MA, USA). Recombinant rat pol β was purified from *E. coli* JMpβ5 as described by Date *et al.* (*21*). HIV-1 RT and the Klenow fragment of DNA polymerase I were purchased from Worthington Biochemical (Freehold, NJ, USA). T4 DNA polymerase and Taq DNA polymerase were purchased from Takara (Kyoto). Bovine pancreas deoxyribonuclease I was purchased from Stratagene Cloning Systems (La Jolla, CA, USA). Nervonic acid (NA) and all other reagents of analytical grade were purchased from Wako (Osaka).

Enzyme Assays—The activities of DNA pol β and HIV-1 RT were measured by the methods described previously (*1, 2*). The activity of bovine deoxyribonuclease I was measured by the standard assays according to Lu and Sakaguchi (*22*).

RESULTS AND DISCUSSION

Docking Simulation of Nervonic Acid and DNA Polymerase β—The rat DNA polymerase β (pol β) used in this study has been studied extensively, and its amino acid sequence, and secondary and tertiary structures have been reported (*4–9, 23*). The enzyme can be divided into two domain fragments by controlled proteolysis: an 8 kDa N-terminal fragment and a 31 kDa C-terminal fragment (*24, 25*). The 31 kDa domain is the catalytic part involved in DNA polymerization, and the 8 kDa domain is the template DNA-binding domain. We prepared the whole pol β enzyme with a molecular weight (M.W.) of 39 kDa, and the two domain fragments of 8 and 31 kDa. Both fragments were obtained by controlled proteolysis, and purified by FPLC Superose 12 chromatography to near homogeneity (see Fig. 4 in Ref. *2*).

Nervonic acid (NA) contains a free carboxyl group and a double bond in the *cis*-configuration, as shown in Fig. 1. The length of the NA molecule was shown to be 23.00 Å on computer simulation (Fig. 1B).

As described previously, NA directly inhibited binding of the 8 kDa domain of pol β to the DNA template, and indirectly influenced DNA polymerization on the 31 kDa catalytic site, as determined by analyzing the products of poly(dA)-oligo$(dT)_{16}$ used as the template-primer (*2*). One molecule of NA competes with one molecule of the template-primer DNA and subsequently interferes with binding of the template-primer to the 8 kDa domain (*2*).

NMR analysis indicated that the 8 kDa domain of pol β (residues 1–87) was formed by four α-helices, packed as two antiparallel pairs (*10, 11*). The pairs of α-helices crossed one another at 50° giving them a V-like shape. The 8 kDa domain contained a "helix-hairpin-helix" motif (*10, 11*). The complex is in fast exchange on the NMR time-scale, permitting us to follow the chemical shift changes of the backbone NH and ^{15}N signals of the 8 kDa domain on complex formation by recording a series of ^{1}H-^{15}N HMQC spectra of the uniformly ^{15}N-labeled 8 kDa domain in the presence of NA. Of the 79 amides in residues 5–86 of the 8 kDa domain, 75 were assigned to the NA complex (*11*). The NA binding interface of the 8 kDa domain consisted of two regions: one consisting of Leu11 in the 1–13 unstructured linker segment, His51 in the 45–55 turn, and Thr79 in the 79–87 unstructured linker segment ("region I" in Fig. 2A), and the other consisting of Lys35 in the Ω-type loop, including helix-1 and helix-2 ("region II" in Fig. 2A) (*3*). These chemi-

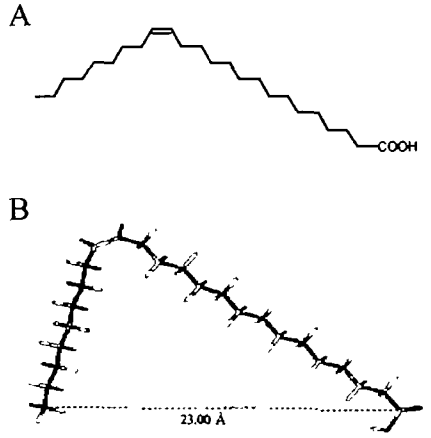

Fig. 1. **Structure of nervonic acid (NA).** (A) Chemical structure of NA. (B) Three-dimensional structure of NA.

cal shift changes can be explained in terms of NA contact and perturbation of the electrostatic charge distribution at the surface. Surface residues exhibiting chemical shift changes were predominantly, although not entirely, clustered on one side of the domain. In pol β, Lys35 is a hydrophilic amino acid and Leu11 a hydrophobic one. The carboxyl end of NA may, therefore, show a preference for binding to the hydrophilic residue of Lys35, and the other side the methyl chains may be absorbed to the hydrophobic site including Leu11. NA on the 8 kDa domain was bridged from Leu11, Lys35, His51, and Thr79, and intercalated smoothly into the pocket between helix-1 and helix-2 containing the Ω-type loop (Fig. 2). When NA bound to the domain at Lys35 and Leu11 (*i.e.* regions I and II in Fig. 2A, respectively), the 45–55 turn including His51 and the unstructured linker segment including Thr79 appeared to be adjacent to Leu11 (region I in Fig. 2A).

Docking simulation was performed by utilizing the above information. In the docking simulation, the amino moiety of the side-chain of Lys35 and the carboxyl moiety of NA were positively and negatively charged, respectively. The binding energy between NH_3^+ in Lys35 and COO^- in NA was 107.792 kcal/mol *via* a salt bridge, and the binding force consisted of coulomb (–109.038 kcal/mol) and van der Waals (+1.246 kcal/mol) forces (Table I). The binding ener-

TABLE I. **The geometrically selected groups of four amino acids in HIV-1 reverse transcriptase.**

Group	Lys	His	Leu	Thr
1	A172	A96	A349	A377
2	A172	A96	A349	A386
3	A259	A96	A349	A338
4	A259	A96	A349	A376
5	A259	A96	A349	A377
6	B22	A96	A349	A338
7	B22	A96	A349	A386
8	B32	B221	B228	B107
9	B65	B235	B100	B386
10	B66	B235	B100	B386
11	B103	B208	B214	B69
12	B223	B235	B100	B376
13	B374	B198	B205	B216

A: 66 kDa domain subunit, B: 51 kDa domain subunit.

TABLE II. **The binding energies of nervonic acid and DNA polymerase β or HIV-1 reverse transcriptase.**

Enzyme	NA interacting amino acid	Force	Energy (kcal/mol)
8 kDa domain of DNA polymerase β	Leu11	Coulomb	+0.008
		van der Waals	–1.136
		Total	–1.128
	Lys35	Coulomb	–109.038
		van der Waals	+1.246
		Total	–107.792
	His51	Coulomb	–0.246
		van der Waals	–0.078
		Total	–0.324
	Thr79	Coulomb	–0.242
		van der Waals	–1.794
		Total	–1.552
	All	Coulomb	–109.034
		van der Waals	–1.762
		Total	–110.796
HIV-1 reverse transcriptase	Leu100	Coulomb	+0.032
		van der Waals	–1.076
		Total	–1.046
	Lys65	Coulomb	–119.556
		van der Waals	+4.376
		Total	–115.178
	His235	Coulomb	–0.216
		van der Waals	–0.064
		Total	–0.280
	Thr386	Coulomb	–0.330
		van der Waals	–1.552
		Total	–1.882
	All	Coulomb	–120.248
		van der Waals	+1.594
		Total	–118.386

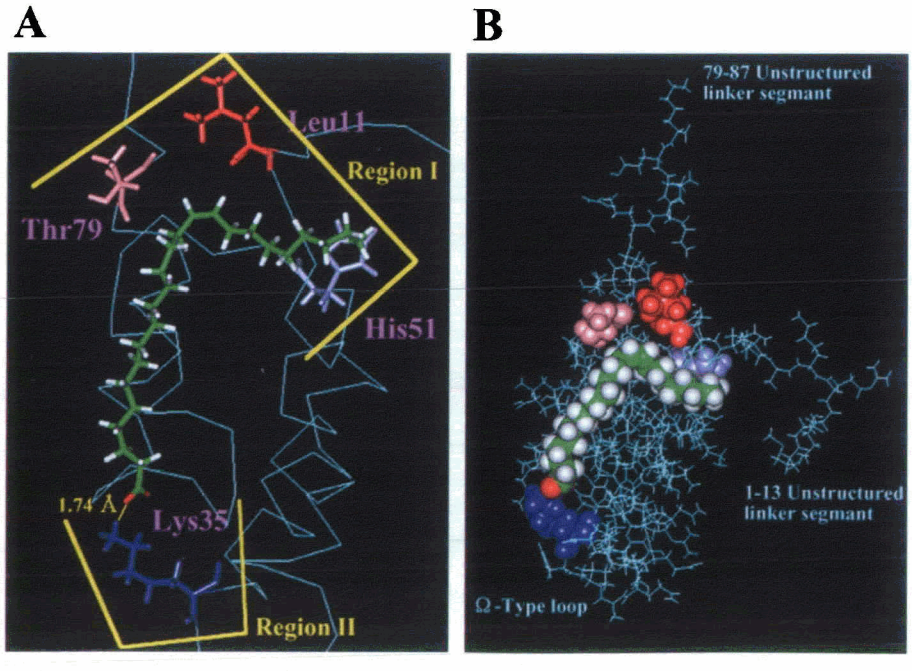

Fig. 2. **Docking simulation of the nervonic acid interaction interface on the 8 kDa domain of DNA polymerase β.** Interaction between NA and 8 kDa domain of pol β (A and B). Amino acid residues Leu11, Lys35, His51, and Thr79, which were significantly shifted as cross-peaks in ^1H-^{15}N HMQC NMR experiments, are depicted in bold (*3*). The Cα-backbone of the X-ray crystal structure of pol β is shown in blue-white. The carbons, oxygens and hydrogens of the NA structure are indicated in green, red and white, respectively. The Protein Data Bank accession code for pol β is 1BNO. This figure was prepared using Insight II (Accelrys).

gies between NA and the backbone of Leu11, between NA and the backbone of His51, and between NA and the backbone of Thr79 were –1.128, –0.324, and –1.552 kcal/mol, respectively. The distance between NA and Lys35 was 1.74 Å (Fig. 2A). We expected that residues between the Lys35 site and the Leu11/His51/Thr79 site would be important for

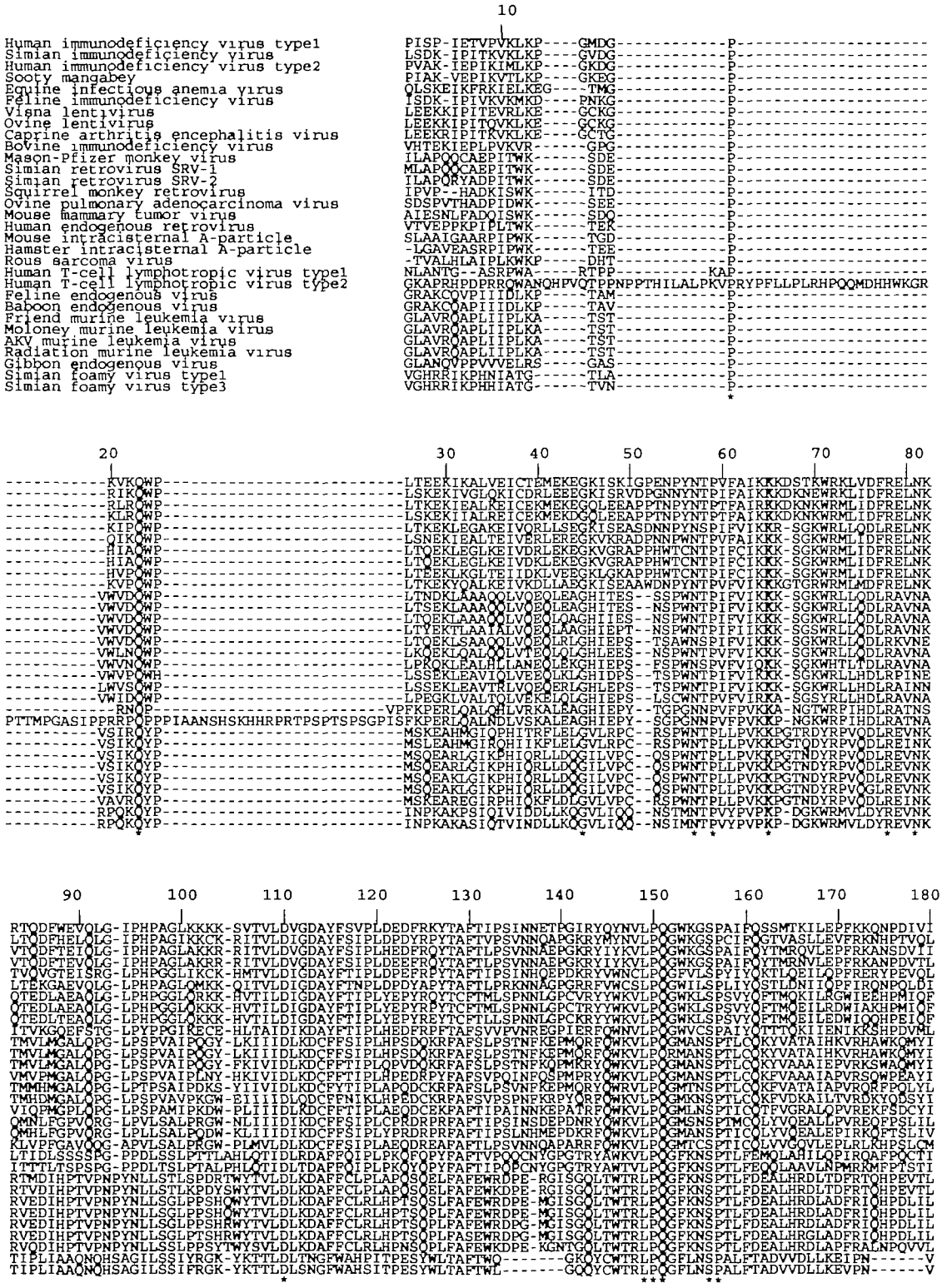

Fig. 3. **Evolutionary tracing of NA binding amino acids in the common sequence in 51 kDa domain subunits of HIV-1 reverse transcriptase (amino acid residues 1 to 430).** Asterisks (*) show conserved and specific residues within different members of the same family of amino acids. The reverse transcriptase sequences from the SWISS-PROT data base were used. Lys65 is indicated in gray.

NA binding.

Identification of Nervonic Acid Binding Residues in HIV-1 Reverse Transcriptase by Comparison with the Nervonic Acid Interaction Interface of DNA Polymerase β—We searched for the NA binding sites of the other proteins three-dimensionally, with reference to the spatial positions of NA binding amino acids on pol β, by computer analysis. For this analysis we used two methods; the geometrical

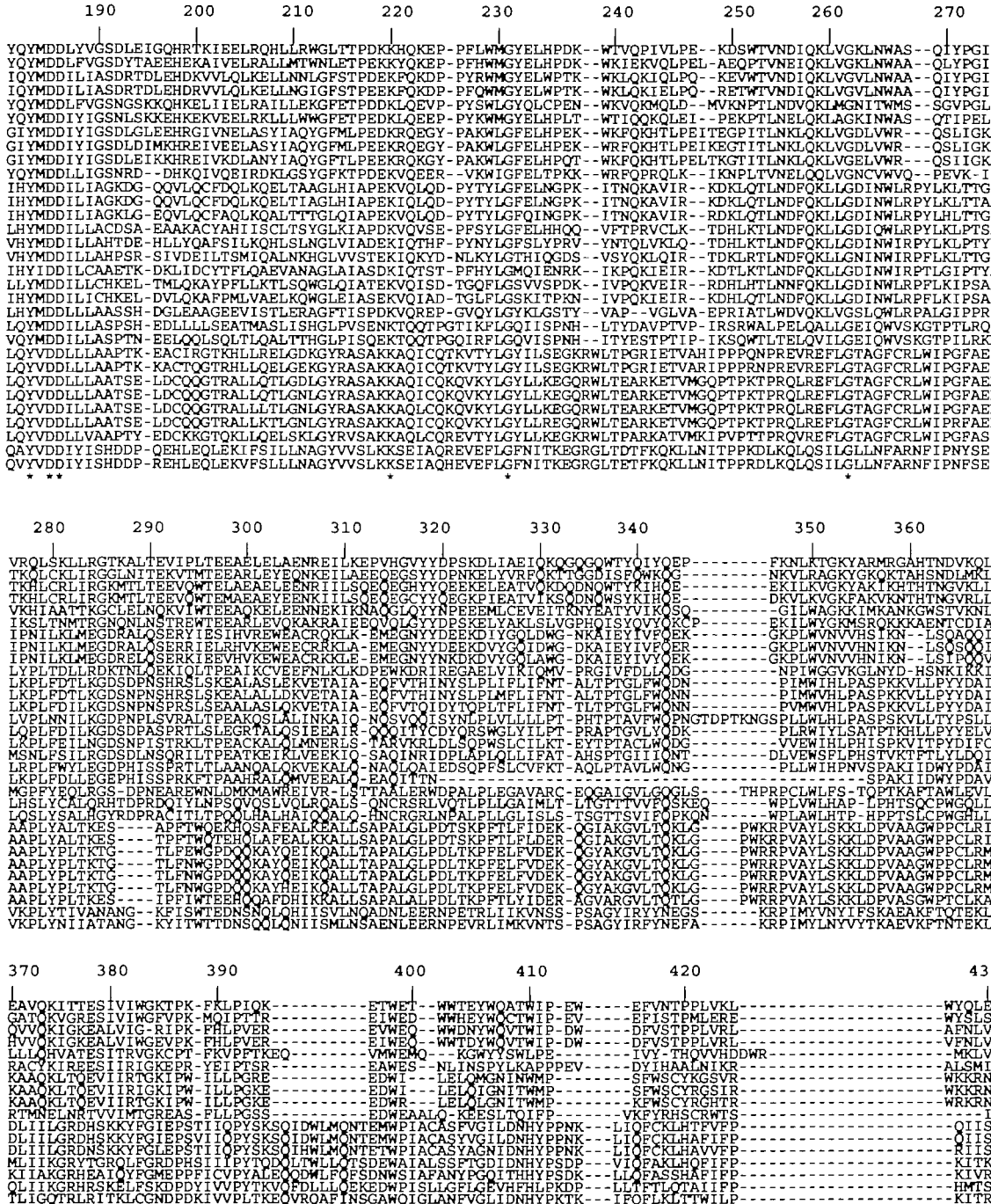

Fig. 3-2

method for template-based finding and the evolutionary trace method. Subsequently, human immunodeficiency virus type-1 reverse transcriptase (HIV-1 RT) was subjected to computer analysis. HIV-1 RT is composed of two subunits of 66 and 51 kDa (p66 and p51) (*26–28*). The N-terminal 440 amino acids of the p66 subunit constitute the polymerase domain, and the C-terminal 120 amino acids comprise the RNase H domain. The p51 subunit of HIV-1 RT consists of 430 amino acids, and corresponds to the polymerase domain of the p66 subunit. The active sites of the dimer associate covalently with DNA break-points. The amino acid sequence in the DNA binding site is completely different from that of the 8 kDa domain of pol β. There were no similar amino acid sequence stretches in the two molecules.

The geometrical method for template-based finding in the case of HIV-1 RT was highly constrained by distance constraints between the four Cα-atoms of NA binding

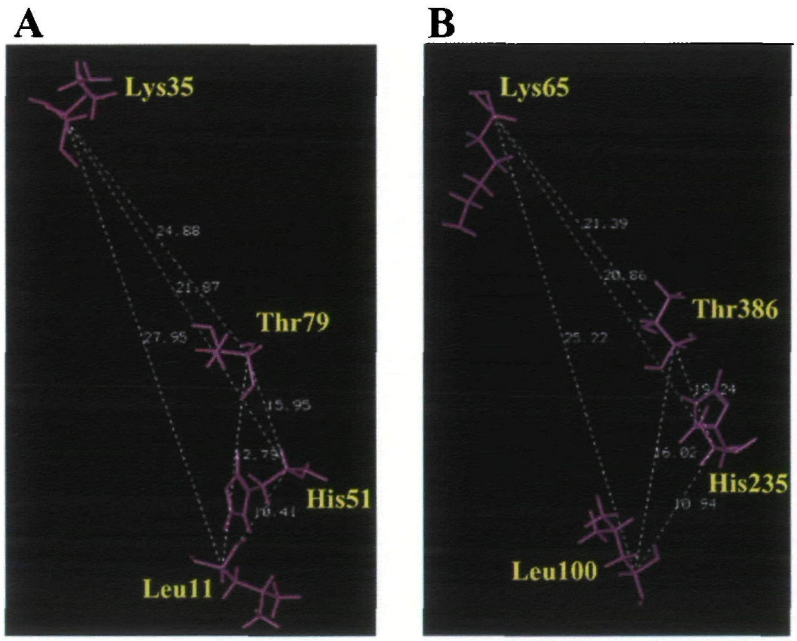

Fig. 4. **Geometrical tracing of NA binding amino acids mapped onto the structures of DNA polymerase β and HIV-1 reverse transcriptase.** The distances of the Cα-carbons of NA binding amino acids of pol β (A) and HIV-1 RT (B) are shown. The Protein Data Bank accession codes for the N-terminal 8 kDa domains of pol β and HIV-1 RT are 1BNO and 2HMI, respectively. This figure was prepared using Insight II (Accelrys).

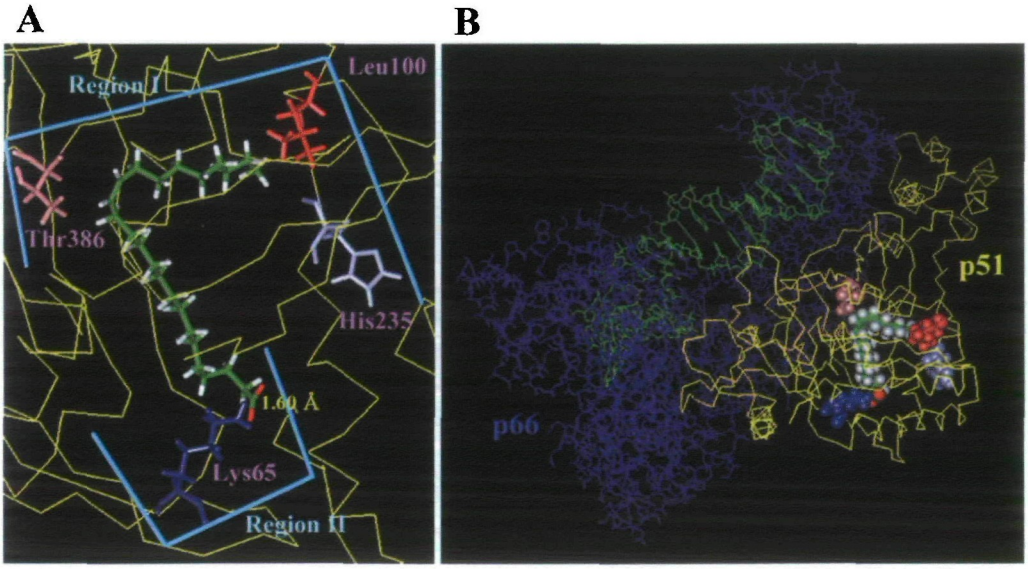

Fig. 5. **Docking simulation of the nervonic acid interaction interface on HIV-1 reverse transcriptase.** Interaction between NA and HIV-1 RT (A and B). Evolutionary tracing and the geometrical method were carried out with Insight II/Binding Site Analysis (Accelrys, San Diego, CA), and amino acid residues Lys65, Leu100, His235 and Thr386, which bind to NA, are depicted in bold. The Cα-backbones of the X-ray crystal structures of p51 and p66 of HIV-1 RT are shown in yellow and blue-white, respectively. The carbons, oxygens and hydrogens of the NA structure are indicated in green, red and white, respectively. The Protein Data Bank accession code for HIV-1 RT is 2HMI. This figure was prepared using Insight II (Accelrys).

amino acids of pol β (*i.e.* Leu11, Lys35, His51, and Thr79) (Fig. 2). There were 13 geometrically selected groups of four amino acids in HIV-1 RT (Table II). The amino acid residues of each of these groups were analyzed by the evolutionary trace method adjusted to maximize either specificity or sensitivity, and thereby the focus of the trace was shifted from residues that are functionally essential to the Lys65 residue of group 9, which regulates specific functional features; the search was performed using the SWISS-PROT data base (Fig. 3). The evolutionary trace method is a systematic, transparent and novel predictive technique that allows the identification of active sites and functional interfaces in proteins with known structures (*15, 16*). This method can be used to determine the conserved and specific residues within different members of the same family, and therefore can be used to determine the relationships between the functions and sequences of different members. In Fig. 3, the asterisks (*) show the conserved amino acids, and indicate the amino acids in the catalytic site and functional interface in the common regions between the p51 and the p66 subunits of HIV-1 RT. Amino acid residues 65 to 262 in HIV-1 RT were well conserved (Fig. 3). In pol β and HIV-1 RT, the four amino acid residues that were located at the same three-dimensional positions were Leu11, Lys35, His51, and Thr79 for pol β, and Lys65, Leu100, His235, and Thr386 for the p51 subunit of HIV-1 RT. The geometrical method indicated that the distances of the Cα-atoms of the amino acids in pol β were almost the same as those in HIV-1 RT (Fig. 4). The longest distance, *i.e.* that between Leu11 and Lys35 of pol β, was calculated to be 27.95 Å, which was almost the same as that between Leu100 and Lys65 of HIV-1 RT (25.22 Å). The error of the distances between the two enzymes was within 2.73 Å. The length of the NA molecule was 23.00 Å (Fig. 1B). Therefore, NA must be small enough to enter the pocket formed by these three amino acids in both pol β (Fig. 4A) and HIV-1 RT (Fig. 4B).

Docking Simulation of the Nervonic Acid Interaction Interface on HIV-1 Reverse Transcriptase—As described above, the combination of geometrical and evolutionary tracing is a new method for uncovering functionally important residues in proteins. Modeling of the NA interaction interface on HIV-1 RT obtained by computer analysis was compared with pol β modeling using the results of NMR analysis of the 8 kDa domain of pol β with NA. NA on HIV-1 RT was bridged from Lys65, Leu100, His235, and Thr386, and intercalated smoothly into the pocket (Fig. 5). In HIV-1 RT, the hydrophilic amino acid Lys65 or the hydrophobic amino acid Leu100 could bind to the carboxyl group or methyl chains of NA, respectively. When NA bound to the domain at Lys65 and Leu100 (*i.e.* regions I and II in Fig. 5A, respectively), the turn including His235 and the turn including Thr386 appeared to be adjacent to Leu100 (region I in Fig. 5A). NA could lie in the pocket across the space between helix-10 and helix-14 in the p51 subunit of HIV-1 RT. In this docking simulation, the amino moiety of the side-chain of Lys65 and the carboxyl moiety of NA were positively and negatively charged, respectively. The distances between NA and the surface residues of HIV-1 RT were within 4.50 Å. The binding force of NA and HIV-1 RT consisted of the salt bridge between NH_3^+ of Lys65 and COO^- of NA, and the hydrogen bond between the hydroxyl group of NA and the backbone functional group of Leu100, His235, or Thr386. The binding energies of Lys65-NA, Leu100-NA, His-235-NA, and Thr386-NA were −115.178, −1.046, −0.280, and −1.882 kcal/mol, respectively (Table I). The NA-Lys65 binding energy was much greater than that of NA-Leu100, NA-His-235, or NA-Thr386. The total binding energy of NA and pol β (−110.796 kcal/mol) was almost the same as that of NA and HIV-1 RT (−118.386 kcal/mol). Since these very low binding energy values were due to the above initial settings, estimation of these values cannot be simply performed. However, the energy values for pol β have a similar tendency to those for HIV-1 RT. The distance between NA and Lys65 was 1.60 Å (Fig. 5B).

The hydrophilic end (carboxyl group) and hydrophobic sites (methyl chain) in NA played crucial roles in the inhibition, because after modification of the carboxyl group to a carboxyl ester the compound did not inhibit the activity (*1, 2*). Therefore, the carboxyl group, *i.e.* the hydrophilic site, in NA might be important for the inhibition of pol β or HIV-1 RT.

Effects of Nervonic Acid on Various DNA Metabolic Enzymes—As reported previously, NA was a potent and selective inhibitor of mammalian pol β. Interestingly, NA also potently inhibited human immunodeficiency virus type-1 (HIV-1) reverse transcriptase (RT) activity at the same concentration (Fig. 6). The inhibition by NA was dose-dependent, with 50% inhibition of pol β and HIV-1 RT by NA at doses of 5.0 and 4.8 μM, respectively, and almost complete inhibition (more than 80%) at 8 and 8 μM, respectively. The inhibitory dose of NA was lower than not only that of dideoxyTTP, a well-known pol β inhibitor, but also those of other newly found DNA polymerase inhibitors

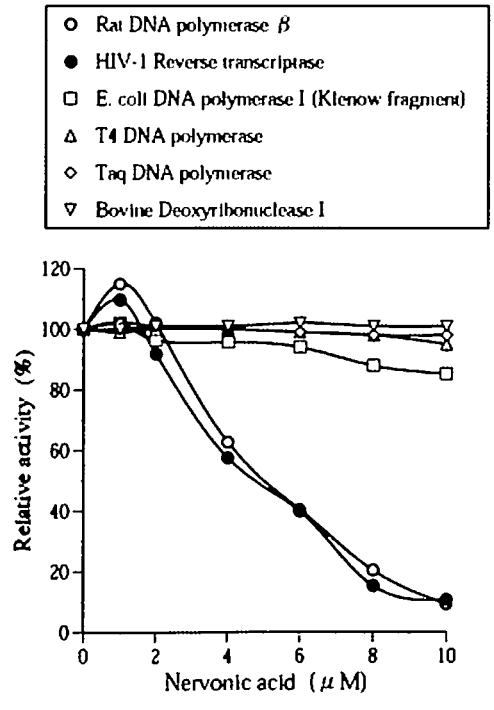

Fig. 6. **Dose-response curves of nervonic acid.** The various DNA polymerases (0.05 units each) were: rat DNA polymerase β (E), HIV-1 reverse transcriptase (J), *E. coli* DNA polymerase I (Klenow fragment) (G), T4 DNA polymerase (C), Taq DNA polymerase (A), and bovine deoxyribonuclease I (S).

such as a few flavonoids (*29, 30*), phospholipids (*31, 32*) and terpenoids (*33–37*). On the other hand, the activities of prokaryotic DNA polymerases such as the Klenow fragment of *E. coli* DNA polymerase I, T4 DNA polymerase and Taq DNA polymerase, and DNA metabolic enzymes such as bovine deoxyribonuclease I were not inhibited by NA (Fig. 6). As shown in Fig. 6, NA significantly inhibited the activities of pol β and HIV-1 RT. NA should, therefore, be referred to as a pol β-preferring and/or HIV-1 RT-preferring inhibitor.

Mode of Inhibition of DNA Polymerase β and HIV-1 Reverse Transcriptase by Nervonic Acid—Next, to elucidate the mechanism of inhibition, the extent of inhibition was studied as a function of the template-primer or dNTP substrate concentration (Table III). For kinetic analyses, poly(dA)/oligo$(dT)_{12-18}$ and dTTP, for pol β, and poly(rA)/oligo$(dT)_{12-18}$ and dTTP, for HIV-1 RT, were used as the DNA template-primer and nucleotide substrate, respectively. Double reciprocal plots of the results indicated that NA-mediated inhibition of the pol β activity was competitive with the DNA template-primer and the nucleotide substrate (Table III). In the case of the DNA template-primer, 144, 255, and 460% increases in the Michaelis constant (K_m) were observed in the presence of 1, 4 and 6 μM NA, respectively, whereas the apparent maximum velocity (V_{max}) was unchanged at 111 pmol/h (Table III). The V_{max} for the nucleotide substrate (dTTP) was 62.5 pmol/l, and the K_m for the nucleotide substrate increased from 3.05 to 18.7 μM in the presence of 10 μM NA (Table III). The K_i values for the DNA template-primer and dTTP were 4.0 and 3.5 μM, respectively (Table III).

On the other hand, the inhibition of HIV-1 RT by NA was non-competitive with the RNA template-primer, since there was no change in the apparent K_m (1.40 μM), while the V_{max} decreased from 27.7 to 6.65 pmol/h template-primer, in the presence of 0 to 4 μM NA (Table III). Similarly, the apparent K_m for the substrate dTTP was unchanged at 3.65 μM, whereas a 7.7-fold decrease in the V_{max} was observed in the presence of 6 μM NA (Table III). The NA inhibition was, therefore, non-competitive with respect to the substrate dTTP. The inhibition constant (K_i) values for HIV-1 RT, obtained from Dixon plots, were 1.2 μM and 1.6 μM for the RNA template-primer and substrate dTTP, respectively (Table III). The affinity of NA appeared to be 1.3-fold higher

TABLE III. **Kinetic analysis of the inhibition by nervonic acid of the activities of rat DNA polymerase β and HIV-1 reverse transcriptase, as a function of the DNA template-primer dose and the dTTP substrate concentration.**

Enzyme (0.05 unit each)	Substrate	Nervonic acid concentration (μM)	K_m (μM)	V_{max} (pmol/h)	K_i (μM)	Inhibitory mode
pol β	Template-primer[a]	0	6.74	111	4.0	Competitive
		1	9.69			
		4	17.2			
		6	31.0			
	Nucleotide[b]	0	3.05	62.5	3.5	Competitive
		4	3.54			
		6	4.80			
		10	18.7			
HIV-1 RT	Template-primer[c]	0		27.7	1.2	Non-competitive
		1	1.40	17.3		
		4	6.65			
	Nucleotide[b]	0		109.0	1.6	Non-competitive
		1	3.65	72.7		
		4		43.6		
		6		14.1		

[a]poly(dA)/oligo$(dT)_{12-18}$ (= 2/1). [b]dTTP. [c]poly(rA)/oligo$(dT)_{12-18}$ (= 2/1).

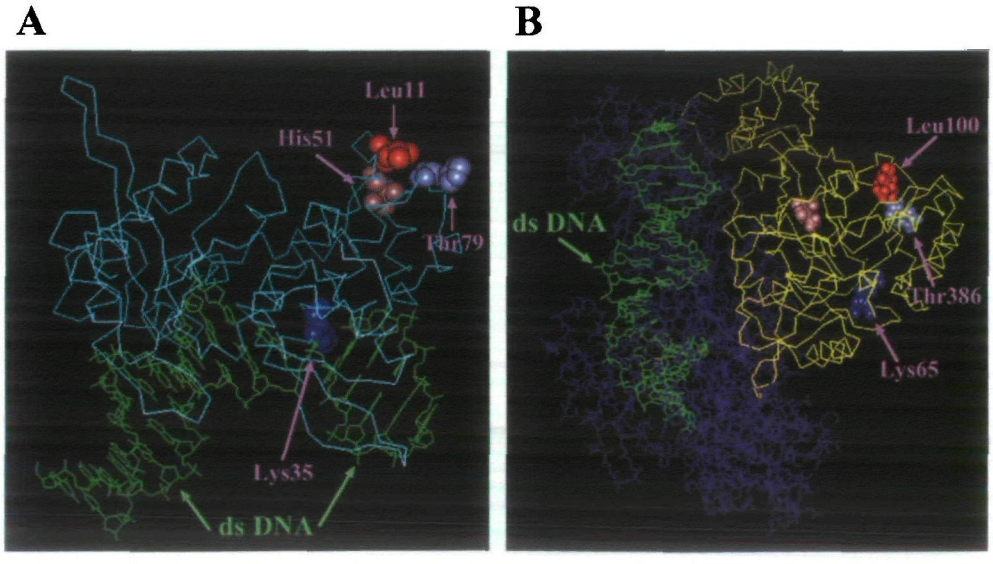

Fig. 7. **Three-dimensional structures of the nervonic acid interaction interfaces on DNA polymerase β and HIV-1 reverse transcriptase.** Interactions between NA and pol β (A) or HIV-1 RT (B). The Cα-backbone of the X-ray crystal structure of pol β is shown in blue-white. The Cα-backbones of the X-ray crystal structures of p51 and p66 of HIV-1 RT are shown in yellow and blue-white, respectively. dsDNA is indicated in green. The Protein Data Bank accession codes for pol β and HIV-1 RT are 1BNP and 2HMI, respectively. This figure was prepared using Insight II (Accelrys).

at the RNA template-primer binding site than at the nucleotide substrate binding site.

Prasad *et al.* (*12*) reported that template DNA [*i.e.* $p(dT)_8$] binding activity was impaired in site-directed mutants of Phe25, Lys35, Lys60, or Lys68. The helix-3-hairpin-helix-4 motif and residues in an adjacent Ω-type loop connecting helix-1 and helix-2 form the ssDNA interaction surface (*12*) NA bound to the ssDNA binding region of the 8 kDa domain and competed for binding with template DNA. The only amino acid residue shifted by both NA binding and ssDNA binding was Lys35 in the Ω-type loop. Leu11, His51, and Thr79 are different from the other DNA binding sites (*i.e.* Phe25, Lys35, and Lys68). NA probably competes with template DNA at residue Lys35 and binds to the site, which subsequently inhibits ssDNA binding activity on the 8 kDa domain (Table III).

The effect of NA on HIV-1 RT was suggested to be the same as that on pol β based on the rhombus composed of four amino acid residues, although NA inhibits HIV-1 RT activity in a different manner from the action on pol β, *i.e.* non-competitively (Table III). Lys35 of the 8 kDa domain of pol β is not only a fatty acid binding amino acid but has also been shown to be essential for binding to DNA (Fig. 7A). Lys65 in HIV-1 RT does not bind to DNA as this residue is not present in the DNA binding region (Fig. 7B), indicating that NA does not bind directly to the DNA binding site of HIV-1 RT. These results may reflect the relationship between the results of kinetic analyses and three-dimensional structural model analyses as follows: When the rhombus is present on the DNA binding site, the kinetics are competitive. However, when the rhombus is absent, the three-dimensional structure of the DNA binding site must be distantly distorted, and subsequently the enzyme is inhibited non-competitively. The present study showed that the three-dimensional positioning of specific amino acids binding to molecular probes provides supplementary information facilitating the building to structural models. Such methods with molecular probes would be widely applicable for three-dimensional structural modeling analysis, although they require at least NMR data. The results of the present study indicated that instead of NMR analysis, computer analysis can provide information regarding the relationship between the functions and three-dimensional structures of HIV-1 RT and pol β–family polymerases.

Moreover, three-dimensional positioning may facilitate the design of new agents for the treatment of AIDS. HIV-1 RT is an important target for the screening of anti-AIDS agents *in vitro*. For example, 3′-azido-3′-deoxythymidine (AZT), 2′,3′-dideoxyinosine (ddI), 2′,3′-dideoxycitidine (ddC), and 2′,3′-didehydro-2′,3′-dideoxythymidine (4dT) have been demonstrated to possess significant ability as inhibitors of HIV-1 RT (*38*). These drugs, however, have not shown satisfactory prevention of the progression of AIDS. Based on the present results, and the idea that as long chain fatty acids bind directly to HIV-1 RT and inhibit its activity agents containing fatty acids could act as inhibitors of HIV-1 RT, we screened for new anti–HIV-1 RT agents with fatty acids. We subsequently found many such agents, e.g. chemically synthetic sulfoquinovosyl-acylglycerols (*39–41*). The modeling analysis reported here may facilitate the computer design of new sulfoquinovosyl-acylglycerols capable of functioning as new stronger and more selective anti–HIV-1 RT agents, *i.e.* new anti-AIDS drugs.

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