Monatshefte für Chemie 135, 1033–1046 (2004)
DOI 10.1007/s00706-004-0172-z

Monatshefte für Chemie
Chemical Monthly
Printed in Austria

Quantitative Structural Rearrangement of HIV-1 Reverse Transcriptase on Binding to Non-Nucleoside Inhibitors

Luckhana Lawtrakul[1,*], **Anton Beyer**[2,*], **Supa Hannongbua**[3], and **Peter Wolschann**[4]

[1] Department of Common and Graduate Studies, Sirindhorn International Institute of Technology (SIIT), Thammasat University, P.O. Box 22 Thammasat Rangsit Post Office, Pathumthani 12121, Thailand
[2] Research Institute of Molecular Pathology, 1030 Vienna, Austria
[3] Department of Chemistry, Faculty of Science, Kasetsart University, Bangkok 10900, Thailand
[4] Institute of Theoretical Chemistry and Structural Biology, 1090 Vienna, Austria

Received December 29, 2003; accepted January 15, 2004
Published online April 26, 2004

Summary. The crystal structures of HIV-1 reverse transcriptase (RT) and some of its inhibitor complexes have been compared quantitatively with respect to backbone and side chain dihedral angles. Furthermore distances between inhibitor molecules and surrounding amino acids have been analyzed in detail. The calculation of the conformation for all structures allows us to determine quantitative structural changes of HIV-1 RT on binding to NNIs or to *RNA*/*DNA*.

Keywords. HIV-1 reverse transcriptase; Non-nucleoside inhibitors; Protein-ligand interactions.

Introduction

Reverse transcriptase (RT) of human immunodeficiency virus type-1 (HIV-1) converts the single-stranded viral *RNA* into double-stranded *DNA*, which subsequently is integrated into the host cell genome. RT is a multifunctional enzyme and has the following activities: *RNA*-dependent *DNA* polymerase, RNase H, and *DNA*-dependent *DNA* polymerase. At the polymerase active site desoxyribonucleotide triphosphates (*dNTP*) are added to the 3′OH terminus of the primer sequence leading to chain elongation. Because this enzyme is essential for the replication of HIV-1, which is the etiological agent of Acquired Immune Deficiency Syndrome (AIDS), it is an important target for drug design efforts [1]. Two main classes of compounds

* Corresponding authors. E-mails: luckhana@siit.tu.ac.th; beyer@nt.imp.univie.ac.at

have been found to be strong inhibitors of this enzyme. Nucleoside inhibitors (NIs) are competitive inhibitors of the nucleotide substrate. After incorporation in the *DNA* strand instead of *dNTP* they cause premature termination of the newly synthesized chain [1]. NIs are also acting on other host *DNA* polymerases, which partly explains their toxicity for the host organism. Non-nucleoside inhibitors (NNIs) on the other hand act allosterically and are highly specific for HIV-1 RT and, furthermore, show lower cellular toxicity compared to NIs. The main focus of this work is a comparison of different NNIs and their mode of function. Although NNIs are surprisingly diverse chemical compounds [2], they all bind to a common site of RT near to, but distinct from the polymerase active site, and inhibit the chemical step of polymerization mainly by causing a displacement of catalytically important residues. A common major drawback of all NNIs known, is the fast emergence of drug-resistant mutants [3, 4]. Since 1992 when the first structure of RT with an inhibitor bound was published [5] a great number of structures of HIV-1 RT either in free form or in complexes have been solved by x-ray crystallography and their coordinates have been deposited subsequently in the Protein Data Bank (RCSB PDB, http://www.rcsb.org) [6]. Two variants with slightly different sequences have been used in these studies. The two sequences S1 and S2 are compared in Table 1. Bold letters indicates differences. A complete list of currently available structures is given in Table 2.

These structures include: 1) The free enzyme with no ligand bound, 2) HIV-1 RT bound to double-stranded oligonucleotide template both in the presence and in

Table 1. Sequences of HIV-1 RT (S1 and S2) contain 560 amino acid residues

S1	1	PISPIETVPV	KLKPGMDGPK	VKQWPLTEEK	IKALVEICTE	MEKEGKISKI	GPENPYNTPV	60
S2	1	PISPIETVPV	KLKPGMDGPK	VKQWPLTEEK	IKALVEICTE	MEKEGKISKI	GPENPYNTPV	60
S1	61	FAIKKKDSTK	WRKLVDFREL	NKRTQDFWEV	QLGIPHPAGL	KKKKSVTVLD	VGDAYFSVPL	120
S2	61	FAIKKKDSTK	WRKLVDFREL	NKRTQDFWEV	QLGIPHPAGL	KKKKSVTVLD	VGDAYFSVPL	120
S1	121	DEDFRKYTAF	TIPSINNETP	GIRYQYNVLP	QGWKGSPAIF	QSSMTKILEP	F**R**KQNPDIVI	180
S2	121	DEDFRKYTAF	TIPSINNETP	GIRYQYNVLP	QGWKGSPAIF	QSSMTKILEP	F**K**KQNPDIVI	180
S1	181	YQYMDDLYVG	SDLEIGQHRT	KIEELRQHLL	RWGLTTPDKK	HQKEPPFLWM	GYELHPDKWT	240
S2	181	YQYMDDLYVG	SDLEIGQHRT	KIEELRQHLL	RWGLTTPDKK	HQKEPPFLWM	GYELHPDKWT	240
S1	241	VQPIVLPEKD	SWTVNDIQKL	VGKLNWASQI	YPGIKVRQLC	KLLRGTKALT	EVIPLTEEAE	300
S2	241	VQPIVLPEKD	SWTVNDIQKL	VGKLNWASQI	YPGIKVRQLC	KLLRGTKALT	EVIPLTEEAE	300
S1	301	LELAENREIL	KEPVHGVYYD	PSKDLIAEIQ	KQGQGQWTYQ	IYQEPFKNLK	TGKYARMRGA	360
S2	301	LELAENREIL	KEPVHGVYYD	PSKDLIAEIQ	KQGQGQWTYQ	IYQEPFKNLK	TGKYARMRGA	360
S1	361	HTNDVKQLTE	AVQKITTESI	VIWGKTPKFK	LPIQKETWET	WWTEYWQATW	IPEWEFVNTP	420
S2	361	HTNDVKQLTE	AVQKITTESI	VIWGKTPKFK	LPIQKETWET	WWTEYWQATW	IPEWEFVNTP	420
S1	421	PLVKLWYQLE	KEPIVGAETF	YVDGAANRET	KLGKAGYVTN	**R**GRQKVV**T**LT	**D**TTNQKTELQ	480
S2	421	PLVKLWYQLE	KEPIVGAETF	YVDGAANRET	KLGKAGYVTN	**K**GRQKVV**P**LT	**N**TTNQKTELQ	480
S1	481	AIYLALQDSG	LEVNIVTDSQ	YALGIIQAQP	D**Q**SESELVNQ	IIEQLIKKEK	VYLAWVPAHK	540
S2	481	AIYLALQDSG	LEVNIVTDSQ	YALGIIQAQP	D**K**SESELVNQ	IIEQLIKKEK	VYLAWVPAHK	540
S1	541	GIGGNEQVDK	LVSAGIRK**V**L					560
S2	541	GIGGNEQVDK	LVSAGIRK**I**L					560

Table 2. The HIV-1 RT x-ray structures in the Protein Data Bank (PDB)

	PDB	Ligand	Res (Å)	Year	Ref.	Model		Mutation	
1	1RTI	*HEPT*	3.00	1995	[7]	S1	560		
2	1RT1	MKC-422 (Emivirine)	2.55	1996	[8]	S1	560		
3	1RT2	TNK-651	2.55	1996	[8]	S1	560		
4	1JLA	TNK-651	2.50	2001	[9]	S1	560	Y181C	
5	1C1B	GCA-186	2.50	1999	[10]	S1	560		
6	1C1C	TNK-6123	2.50	1999	[10]	S1	560		
7	1JLQ	739W34	3.00	2001	[11]	S1	560		
8	1HNV	8-Cl *TIBO* (R86183)	3.00	1995	[12]	S2	558	C280S	
9	1UWB	8-Cl *TIBO* (R86183)	3.20	1996	[13]	S2	558	C280S	Y181C
10	1TVR	9-Cl *TIBO* (R82913)	3.00	1996	[13]	S2	558	C280S	
11	1REV	9-Cl *TIBO* (R82913)	2.60	1995	[14]	S1	560		
12	3HVT	Nevirapine (Viramune)	2.90	1994	[15]	S2	556		
13	1VRT	Nevirapine (Viramune)	2.20	1995	[7]	S1	560		
14	1FKP	Nevirapine (Viramune)	2.90	2000	[16]	S1	543	K103N	
15	1JLB	Nevirapine (Viramune)	3.00	2001	[9]	S1	560	Y181C	
16	1JLF	Nevirapine (Viramune)	2.60	2001	[9]	S1	560	Y188C	
17	1RTH	1051U91	2.20	1995	[7]	S1	560		
18	1RT3	1051U91	3.00	1998	[17]	S1	555	D67N	K70R
								T115F	K219Q
19	1VRU	2,6-Cl_2 α-*APA* (R90385)	2.40	1995	[7]	S1	560		
20	1HPZ	2,6-Cl_2 α-*APA* (R90385)	3.00	2000	[18]	S2	560	K103N	C280S
21	1HNI	2,6-Br_2 α-*APA* (R95845)	2.80	1995	[19]	S2	558	C280S	
22	1BQM	HBY 097	3.10	1998	[20]	S2	556	C280S	
23	1BQN	HBY 097	3.30	1998	[20]	S2	558	Y188L	C280S
								E248Q	E546Q
24	1HQU	HBY 097	2.70	2000	[18]	S2	560	K103N	C280S
25	1KLM	BHAP U-90152	2.65	1997	[21]	S1	560		
26	1RT5	UC-10	2.90	1998	[22]	S1	560		
27	1RT6	UC-38	2.80	1998	[22]	S1	560		
28	1RT7	UC-84	3.00	1998	[22]	S1	560		
29	1RT4	UC-781	2.90	1998	[22]	S1	560		
30	1JLG	UC-781	2.60	2001	[9]	S1	560	Y188C	
31	1COT	BM + 21.1326	2.70	1999	[23]	S1	560		
32	1COU	BM + 50.0934	2.52	1999	[23]	S1	560		
33	1DTT	PETT-2 (PETT130A94)	3.00	2000	[24]	S1	560		
34	1JLC	PETT-2	3.00	2001	[9]	S1	560	Y181C	
35	1DTQ	PETT-1 (PETT131A94)	2.80	2000	[24]	S1	560		
36	1EET	MSC204	2.73	2000	[25]	S2	557	E478Q	
37	1IKY	MSC194	3.00	2001	[26]	S2	560	K103N	E478Q
38	1IKX	PNU142721	2.80	2001	[26]	S2	560	K103N	E478Q
39	1FK9	DMP-266 (Efavirenz)	2.50	2000	[16]	S1	543		
40	1IKW	DMP-266 (Efavirenz)	3.00	2001	[26]	S2	560	E478Q	
41	1FKO	DMP-266 (Efavirenz)	2.90	2000	[16]	S1	543	K103N	
42	1IKV	DMP-266 (Efavirenz)	3.00	2001	[26]	S2	560	K103N	E478Q
43	1JKH	DMP-266 (Efavirenz)	2.50	2001	[9]	S1	560	Y181C	
44	1EP4	S-1153	2.50	2000	[27]	S1	560		

(*continued*)

Table 2 (*continued*)

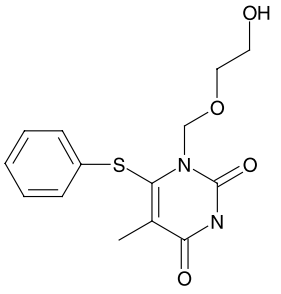
	PDB	Ligand	Res (Å)	Year	Ref.	Model		Mutation	
45	1HMV	–	3.20	1994	[28]	S2	560		
46	1RTJ	–	2.35	1995	[29]	S1	560		
47	1DLO	–	2.70	1996	[30]	S2	556	C280S	
48	1HQE	–	2.70	2000	[18]	S2	560	K103N	C280S
49	1JLE	–	2.80	2001	[9]	S1	560	Y188C	
50	1QE1	–	2.85	1999	[31]	S2	558	C280S	M184I
51	2HMI	*DNA*/FAB	2.80	1998	[32]	S2	558	C280S	
52	1C9R	*DNA*/FAB	3.50	1999	[31]	S2	556	C280S	M184I
53	1RTD	*DNA*/*dNTP*	3.20	1998	[33]	S1	553	Q258C	R461K
								T468P	E478Q
								Q512E	
54	1HYS	*RNA*/*DNA*	3.00	2001	[34]	S2	553	C280S	

the absence of *dNTP* substrate, 3) HIV-1 RT complexed with different NNIs, and 4) Structures of various HIV-1 RT mutants important for drug resistance, either in free form or with inhibitor.

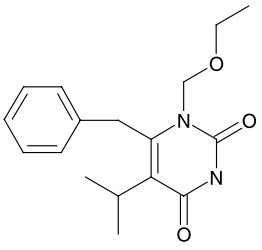
This work compares all available structures of free HIV-1 RT to those with some selected NNIs bound, and the complex of RT with double-stranded DNA together with *dNTP*. Some selected non-nucleoside inhibitors, which are studied in more detail, are shown in Fig. 1.

To study local conformational changes which occur on binding to different NNIs we calculated distances between RT and inhibitor, and also backbone and side chain dihedral angles of amino acid residues which are in close contact with NNIs, and also for important residues belonging to the active site. This was done for 54 crystal structures available in PDB (44 with NNIs). Beyond these local changes, which are described by dihedral angles, domain movements of the enzyme on binding either to *DNA* or to NNIs were investigated by superposition of some selected structures.

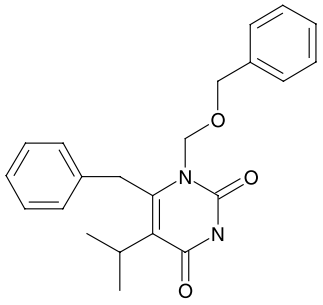
Fig. 1. Chemical structures of some non-nucleoside inhibitors: 1-[(2-hydroxyethoxy)methyl]-6-(phenylthio)thymine (*HEPT*) (**1**); 6-benzyl-1-(ethoxymethyl)-5-isopropyluracil (MKC-442) (**2**); 6-benzyl-1-[(benzyloxy)methyl]-5-isopropyluracil (TNK-651) (**3**); 6-(3′,5′-dimethylbenzyl)-1-ethoxymethyl-5-isopropyluracil (GCA-186) (**5**); 6-cyclohexylthio-1-ethoxymethyl-5-isopropyluracil (TNK-6123) (**6**); (+)-(*S*)-4,5,6,7-tetrahydro-8-chloro-5-methyl-6-(3-methyl-2-butenyl)imidazo[4,5,1jk][1,4]benzodiazepin-2(1*H*)-thione (8-CI *TIBO*) (**8**); (+)-(*S*)-4,5,6,7-tetrahydro-9-chloro-5-methyl-6-(3-methyl-2-butenyl)imidazo[4,5,1jk][1,4]benzodiazepin-2(1*H*)-thione (9-CI *TIBO*) (**10**); 11-cyclopropyl-5,11-dihydro-4-methyl-6*H*-dipyrido(3,2-b:2′,3′-e)(1,4)diazepin-6-one (Nevirapine) (**12**); 6,11-dihydro-11-ethyl-6-methyl-9-nitro-5*H*-pyrido[2,3-b][1,5]benzodiazepin-5-one (1051U91) (**17**); α-(2,6-dichlorophenyl)-α-(2-acetyl-5-methylanilino) acetamide (2,6-Cl_2 α-*APA*) (**19**); α-(2,6-dibromophenyl)-α-(2-acetyl-5-methylanilino) acetamide (2,6-Br_2 α-*APA*) (**21**); (*S*)-4-isopropoxycarbonyl-6-methoxy-3-(methylthiomethyl)-3,4-dihydroquinoxaline-2(1*H*)-thione (HBY 097) (**22**); (−)-6-chloro-4-cyclopropylethynyl-4-trifluoromethyl-1,4-dihydro-2*H*-3,1-benzoxazin-2-one (DMP-266) (**39**)



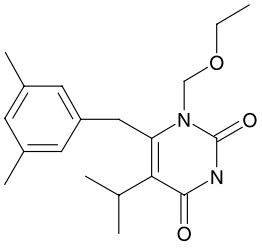
HEPT (**1**) 1RTI



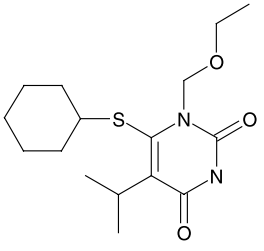
MKC-442 (**2**) 1RT1



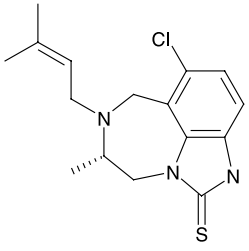
TNK-651 (**3**) 1RT2



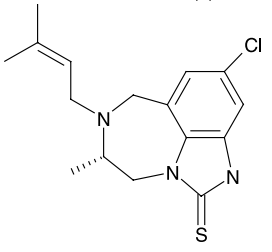
GCA-186 (**5**) 1C1B



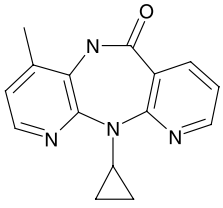
TNK-6123 (**6**) 1C1C



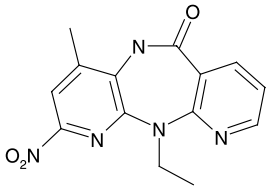
8-Cl *TIBO* (**8**) 1HNV



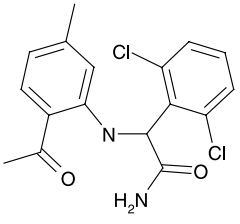
9-Cl *TIBO* (**10**) 1TVR



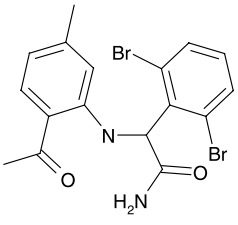
Nevirapine (**12**) 3HVT



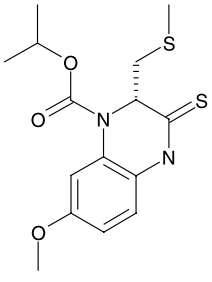
1051U91 (**17**) 1RTH



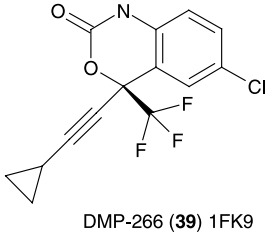
2,6-Cl_2 α-*APA* (**19**) 1VRU



2,6-Br_2 α-*APA* (**21**) 1HNI



HBY 097 (**22**) 1BQM



DMP-266 (**39**) 1FK9

Results and Discussion

Structural Features of HIV-1 RT

HIV-1 RT is a heterodimer containing two separate chains with identical amino acid sequences but of different length with a molecular weight of 66 kDa (p66) and 51 kDa (p51) [5] (see also Table 1). p66 contains the N-terminal polymerase part (pol, 440 residues) and the RNase H domain at the C-terminus (120 residues). The pol subunit is folded into four domains called fingers, thumb, palm, and connection domain. Overall, p66 resembles a human right hand that holds on to the nucleotide chain. The connection domain is located between the hand of the pol subunit and the RNase H domain. Structural details of p66 including domain boundaries and secondary structure description are outlined in Fig. 2.

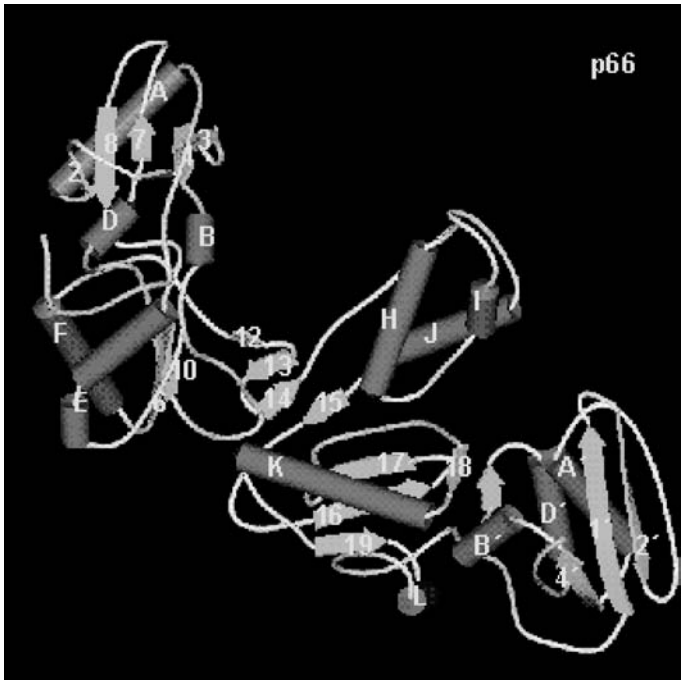
Palm and connection domain can be roughly described as five-stranded beta sheets with two alpha helices on one side. The thumb consists of mostly four helices. The second chain, p51 lacks the RNase H domain but still contains the connection domain. It is processed by proteolytic cleavage of p66.

Although the corresponding domains of p66 and p51 have identical sequences and rather similar internal folds their relative arrangement in space is strikingly different. p66 has a large cleft between fingers, thumb, and palm, where the nucleic acid binding site is located. Three important catalytic residues of the polymerase part (*Asp*110, *Asp*185, and *Asp*186) are exposed in the cleft, but are buried in p51, which lacks this cleft. The reason is a large movement of the connection domain. In p51 this domain is fixed in a central position and contacts all other domains, whereas in p66 it contacts only the RNase H and the thumb domain. The oligonucleotide chain is fixed in the cleft of p66, and the 3′-OH of the primer strand comes close to the three aspartic acid residues where chain elongation takes place. This idea is further supported by a hydrogen bond between 3′-OH and *Asp*185 which has been observed [32b]. Other important residues of the active site are *Tyr*183, *Met*184, *Tyr*115, and *Gln*151. These residues are involved in holding the nucleotide triphosphate in the right place and they are essential for maintaining the topology of this site. In addition, residues 224 to 235 (hairpin β12–β13) are responsible for proper positioning of the *RNA*/*DNA* chain.

Global Structural Changes in HIV-1 RT and its Complexes

One can distinguish between three different conformational states of RT. The free enzyme, RT bound to *RNA*/*DNA*, and RT bound to a non-nucleoside inhibitor. This is demonstrated in Table 3 and Fig. 3 showing a superposition of three structures, using the palm domain as template: Free RT (1DLO), RT with *dNTP* bound (1RTD), and RT complexed with inhibitor (1RT1). Both *dNTP* and the inhibitor were artificially kept in their original position and hypothetical distances were calculated between amino acid residues and *dNTP* or the inhibitor for all three structures.

At the *dNTP* binding site the greatest changes occur at *Lys*65, *Arg*72 from the tip of the fingers domain and *Val*111. These residues are held in place when *dNTP* is bound. The distance separations of three essential aspartic acid residues (*Asp*110,



Finger (1–84)	
β1	7–12
αA	28–44
β2	49–51
β3	56–63
β4	73–77
αB	78–83

Palm (85–119)	
β5a	86–90
β5b	94–96
β6	105–112
αC	114–117

Finger (120–150)	
αD	122–127
β7	128–134
β8	141–147

Palm (151–243)	
αE	155–174
β9	178–183
β10	186–191
αF	195–212
β11a	214–217
β11b	219–222
β12	227–229
β13	232–235
β14	238–242

Thumb (244–322)	
αH	255–268
αI	278–286
αJ	298–311
β15	316–321

Connection (323–437)	
β16	326–333
β17	336–341
β18	350–358
αK	364–382
β19	388–391
αL	395–404
β20	408–412
β21	421–424
β22	427–430

RNase H (438–556)	
$\beta1'$	438–447
$\beta2'$	452–459
$\beta3'$	462–470
$\alpha A'$	474–488
$\beta4'$	492–497
$\alpha B'$	500–508
$\alpha D'$	516–527
$\beta5'$	530–536
$\alpha E'$	544–555

Fig. 2. Folding diagram showing the arrangement of secondary structure elements within the p66 polymerase subunit of HIV-1 RT; the tubes represent α-helices and the arrows represent β-strands; domain boundaries are from Ref. [28], and secondary structural elements are according to *Rodgers et al.* [32a]; these boundaries are only marginally different from those given in Ref. [7]

Table 3. The distances (Å) between the C_α-atoms of the corresponding amino acid residues of the polymerase catalytic site and the NNIs binding pocket, when *dNTP* and MKC-442 are put to the active sites

	1DLO	1RTD	1RT1
dNTP binding site			
*Ile*63	>6.0	>6.0	>6.0
*Lys*65	>6.0	2.8	>6.0
*Arg*72	>6.0	2.8	>6.0
*Leu*74	>6.0	>6.0	>6.0
*Asp*110	3.5	3.5	3.0
*Val*111	2.8	3.5	6.0
*Gly*112	3.0	3.5	2.8
*Asp*113	3.0	3.5	3.5
*Ala*114	3.5	3.5	5.0
*Tyr*115	2.8	3.0	2.8
*Phe*116	5.0	5.0	>6.0
*Gln*151	3.0	3.0	2.8
*Gly*152	>6.0	>6.0	>6.0
*Met*184	5.0	5.0	5.0
*Asp*185	1.4	2.8	2.8
*Asp*186	>6.0	>6.0	>6.0
NNIs binding site			
*Pro*95	5.0	5.0	5.0
*Leu*100	2.8	2.8	3.5
*Lys*101	3.0	3.5	2.8
*Lys*103	4.0	4.0	3.5
*Val*106	3.5	3.5	4.0
*Val*179	4.0	3.5	4.0
*Tyr*181	2.8	2.8	3.5
*Tyr*188	1.4	1.4	3.0
*Gly*190	4.0	3.5	3.5
*Phe*227	3.0	3.0	3.0
*Trp*229	1.4	1.4	4.0
*Leu*234	2.8	2.8	4.0
*His*235	5.0	5.0	5.0
*Pro*236	>6.0	>6.0	4.0
*Tyr*318	3.5	3.5	3.5
*Glu*138(p51)	>6.0	>6.0	>6.0

*Asp*185, and *Asp*186) of the polymerase catalytic site are about 2.53 Å in average with only little variation. Nevertheless the conformations of *Asp*186 in the RT/*DNA* complex differ remarkably from those in RT/NNI complexes. In the NNI binding site the most pronounced differences are found for residues *Tyr*181, *Tyr*188, *Trp*229, *Leu*234, and *Pro*236. As can be seen in Fig. 3 binding of either

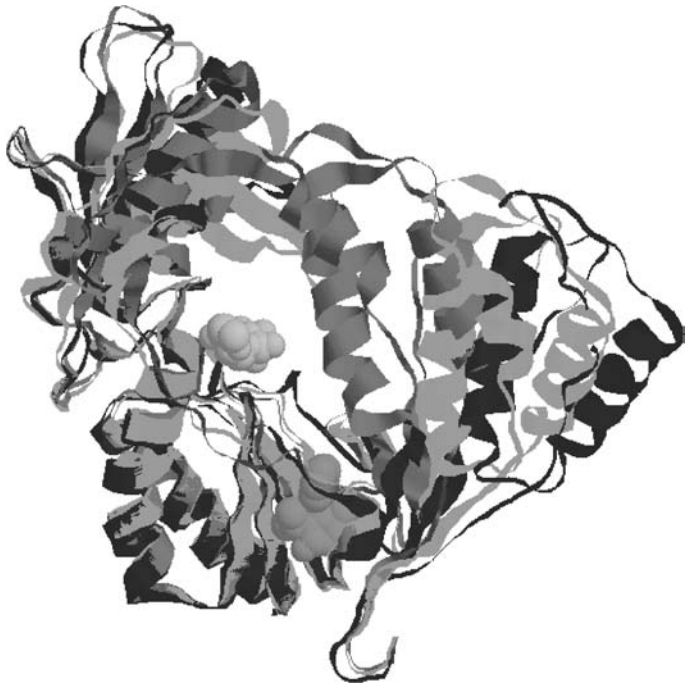


Fig. 3. Superpositions of the fingers, palm, and thumb subdomains in p66 of unliganded RT (dark grey) together with RT/*DNA*/*dNTP* complex (grey) and RT/MKC-442 complex (black); dNTP and MKC-442 are presented in their binding sites

dNTP or an inhibitor involves a major reorientation caused by rotation of the thumb domain of p66. To further investigate the global motions of HIV-1 RT domains, we superimposed the different structures of p66 as well as the domains alone. The calculated RMS values are shown in Table 4. The RMS values for the whole structure are much larger than for the different domains. The mean RMS values are 4.37 Å for p66, and 1.4, 1.8, 1.3, 0.9, and 0.8 Å for fingers, palm, thumb, connection, and RNase H domain respectively.

These structural changes in the different states of the enzyme, can also be seen in Fig. 4 where distance separations of C_α atoms of some characteristic amino acids of superimposed proteins are shown.

In this figure the diagrams on the left show distance separations between residues at the active site, whereas on the right side the distances at the NNI binding pocket can be seen. The distances between the free enzyme and RT bound with *DNA* is shown in the first row. Pronounced changes between free and active enzyme at the *dNTP* binding site can be observed in the fingers domain (*Ile*63-*Leu*74; light grey). There are no large differences for the other amino acids, particularly at the primer grip (*Met*230, *Gly*231; dark grey). The other diagrams result from comparison of the *DNA*-bound structure (active form) with the enzyme bound to different inhibitors (inactive forms). The differences are again rather large at the fingers domain, but additionally large differences appear at the primer grip (residues 230 and 231). This is a clear indication that the active site of RT is severely distorted on inhibitor binding. At the inhibition pocket drastic changes occur for

Table 4. Root mean square value (RMS) of the backbone atoms of p66 and its domains

		1DLO	1RTD	1RT1	1HNV	1VRT	1VRU
p66[a]	1DLO	0.00					
	1RTD	5.03	0.00				
	1RT1	6.67	3.47	0.00			
	1HNV	6.75	3.64	2.76	0.00		
	1VRT	7.79	4.38	2.36	2.10	0.00	
	1VRU	7.85	4.38	2.41	2.24	0.71	0.00
Fingers[b]	1DLO	0.00					
	1RTD	1.50	0.00				
	1RT1	1.44	1.58	0.00			
	1HNV	1.42	1.47	1.17	0.00		
	1VRT	1.34	1.40	1.32	1.23	0.00	
	1VRU	1.47	1.45	1.41	1.38	1.06	0.00
Palm[c]	1DLO	0.00					
	1RTD	1.07	0.00				
	1RT1	2.54	2.39	0.00			
	1HNV	2.18	2.15	1.80	0.00		
	1VRT	2.36	2.24	1.41	1.25	0.00	
	1VRU	2.38	2.30	1.38	1.27	0.45	0.00
Thumb[d]	1DLO	0.00					
	1RTD	1.65	0.00				
	1RT1	1.00	1.65	0.00			
	1HNV	1.97	1.00	1.88	0.00		
	1VRT	1.46	0.97	1.27	1.27	0.00	
	1VRU	1.45	0.98	1.23	1.28	0.31	0.00
Connection[e]	1DLO	0.00					
	1RTD	0.76	0.00				
	1RT1	1.16	1.04	0.00			
	1HNV	1.07	1.05	1.25	0.00		
	1VRT	0.76	0.84	1.19	0.89	0.00	
	1VRU	0.74	0.83	1.19	0.90	0.30	0.00
RNase H[f]	1DLO	0.00					
	1RTD	0.54	0.00				
	1RT1	1.08	1.12	0.00			
	1HNV	0.71	0.77	1.23	0.00		
	1VRT	0.59	0.50	1.08	0.81	0.00	
	1VRU	0.73	0.62	1.13	0.87	0.56	0.00

[a] p66 (*Pro*4-*His*539); [b] Fingers (*Pro*4-*Thr*84 and *Leu*120-*Pro*150); [c] Palm (*Gln*85-*Pro*119 and *Gln*151-*Pro*243); [d] Thumb (*Ile*244-*Ser*322); [e] Connection (*Lys*323-*Ala*437); [f] RNase H (*Glu*438-*His*539)

the amino acids *Phe*227, *Trp*229, *Leu*234, *His*235, and *Pro*236 going from the active to the inactive form. On the other hand the data are quite similar in complexes with different NNIs, supporting the idea that NNIs have similar binding modes. Binding to NNIs also affects the movement of both fingers and thumb

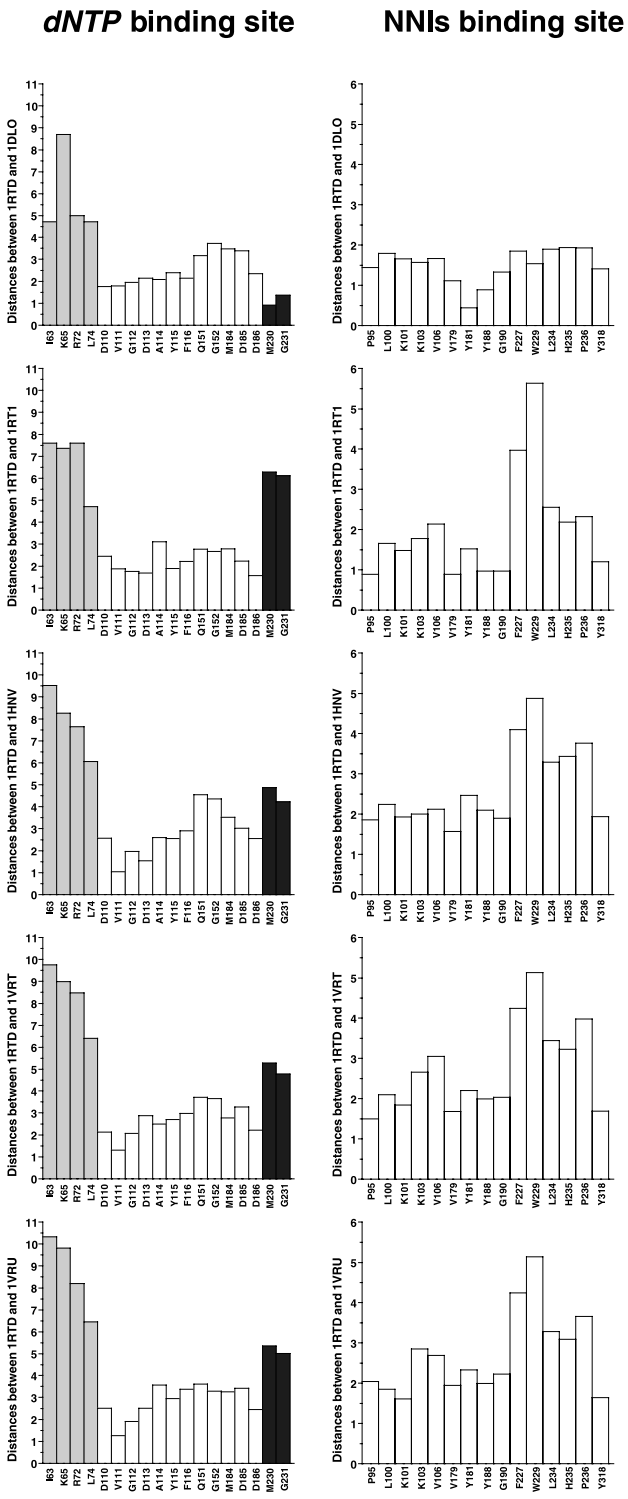


Fig. 4. Distance separations of amino acid residues in the *dNTP* and NNI binding site of unliganded RT (1DLO) compared to RT/NNIs complexes (1RT1, 1HNV, 1VRT, and 1VRU) and an RT/*DNA*/*dNTP* complex (1RTD)

Table 5. C_α atoms' distances between the top of the fingers (*Pro*133) and the thumb (*Ala*288) subdomains of p66

PDB	Ligand	Gap (Å)
1DLO	–	16.60
1RTD	*DNA*	35.95
1RTI	*HEPT*	45.40
1RT1	MKC-442	43.17
1RT2	TNK-651	43.59
1HNV	8-Cl *TIBO*	47.66
1UWB	8-Cl *TIBO*	47.20
1TVR	9-Cl *TIBO*	47.91
1VRT	Nevirapine	50.51
1RTH	1051U91	45.47
1VRU	2,6-Cl_2 α-*APA*	51.24
1HNI	2,6-Br_2 α-*APA*	45.30

domains. This is indicated by the large distance change between the top of the fingers (*Pro*133) and the top of thumb (*Ala*288), which is shown in Table 5.

Methods

The following software packages were used: SYBYL 6.5 [35] for the visualisation of the structures, TINKER 3.6 [36] using the AMBER force field [37] did the superpositions of the molecules.

Acknowledgements

This investigation was supported by The Thailand Research Fund (TRF) project MRG4680111. *S. Hannongbua* is grateful to TRF and KURDI for research fellowship (RSA4480001). The quantum chemical calculations were performed on the Cluster of Digital Alpha Servers (2100 4/275) of the computer centre of the University of Vienna. Generous supply of computer time on this installation is grateful acknowledged. The authors thank *S. Maurer-Stroh* for carefully reading the manuscript.

References

[1] Jonckheere H, Annè J, De Clercq E (2000) Med Res Rev **20**: 129
[2] De Clercq E (1998) Antiviral Res **38**: 153
[3] Shafer RW, Kantor R, Gonzales MJ (2000) AIDS Rev **2**: 211
[4] Richman D, Shih CK, Lowy I, Rose J, Prodanovich P, Goff S, Griffin J (1991) Proc Natl Acad Sci USA **88**: 11241
[5] Kohlstaedt LA, Wang J, Friedman JM, Rice PA, Steitz TA (1992) Science **256**: 1783
[6] Berman HM, Westbrook J, Feng Z, Gililand G, Bhat TN, Weissig H, Shindyalov IN, Bourne PE (2000) Nucleic Acids Res **28**: 235
[7] Ren J, Esnouf R, Garman E, Somers D, Ross C, Kirby I, Keeling J, Darby G, Jones Y, Stuart D, Stammers D (1995) Nat Struct Biol **2**: 293
[8] Hopkins AL, Ren J, Esnouf RM, Willcox BE, Jones EY, Ross C, Miyasaka T, Walker RT, Tanaka H, Stammers DK, Stuart DI (1996) J Med Chem **39**: 1589

[9] Ren J, Nichols C, Bird L, Chamberlain P, Weaver K, Short S, Stuart DI, Stammer DK (2001) J Mol Biol **312**: 795
[10] Hopkins AL, Ren J, Tanaka H, Baba B, Okamato M, Stuart DI, Stammers DK (1999) J Med Chem **42**: 4500
[11] Chan JH, Hong JS, Hunter RN III, Orr GF, Cowan JR, Sherman DB, Sparks St M, Reitter BE, Andrews CW III, Hazen RJ St, Clair M, Boone LR, Ferris RG, Creech KL, Roberts GB, Short SA, Weaver K, Ott RJ, Ren J, Hopkins A, Stuart DI, Stammers DK (2001) J Med Chem **44**: 1866
[12] Ding J, Das K, Moereels H, Koymans L, Andries K, Janssen PA, Hughes SH, Arnold E (1995) Nat Struct Biol **2**: 407
[13] Das K, Ding J, Hsiou Y, Clark AD Jr, Moereels H, Koymans L, Andries K, Pauwels R, Janssen PAJ, Boyer PL, Clark P, Smith RH Jr, Kroeger Smith MB, Michejda CJ, Hughes SH, Arnold E (1996) J Mol Biol **264**: 1085
[14] Ren J, Esnouf R, Hopkins A, Ross C, Jones Y, Stammers D, Stuart D (1995) Structure **3**: 915
[15] a) Smerdon SJ, Jäger J, Wang J, Kohlstaedt LA, Chirino AJ, Friedman JM, Rice PA, Steitz TA (1994) Proc Natl Acad Sci USA **91**: 3911; b) Wang J, Smerdon SJ, Jäger J, Kohlstaedt LA, Rice PA, Friedman JM, Steitz TA (1994) Proc Natl Acad Sci USA **91**: 7242
[16] Ren J, Milton J, Weaver KL, Short SA, Stuart DI, Stammers DK (2000) Structure **8**: 1089
[17] Ren J, Esnouf RM, Hopkins AL, Jones EY, Kirby I, Keeling J, Ross CK, Larder BA, Stuart DI, Stammers DK (1998) Proc Natl Acad Sci USA **95**: 9518
[18] Hsiou Y, Ding J, Das K, Clark AD, Boyer PL, Lewi P, Janssen PAJ, Kleim J-P, Roesner M, Hughes SH, Arnold E (2001) J Mol Biol **309**: 437
[19] Ding J, Das K, Tantillo C, Zhang W, Clark AD Jr, Jessen S, Lu X, Hsiou Y, Jacobo-Molina A, Andries K, Pauwels R, Moereels H, Koymans L, Janssen PAJ, Smith RH Jr, Kroeger Koepke M, Michejda CJ, Hughes SH, Arnold E (1995) Structure **3**: 365
[20] Hsiou Y, Das K, Ding J, Clark AD Jr, Kleim JP, Rosner M, Winkler I, Riess G, Hughes SH, Arnold E (1998) J Mol Biol **284**: 313
[21] Esnouf RM, Ren J, Hopkins AL, Ross CK, Jones EY, Stammers DK, Stuart DI (1997) Proc Natl Acad Sci USA **94**: 3984
[22] Ren J, Esnouf RM, Hopkins AL, Warren J, Balzarini J, Stuart DI, Stammers DK (1998) Biochemistry **37**: 14394
[23] Ren J, Esnouf RM, Hopkins AL, Stuart DI, Stammers DK (1999) J Med Chem **42**: 3845
[24] Ren J, Diprose J, Warren J, Esnouf RM, Bird LE, Ikemizu S, Slater M, Milton J, Balzarini J, Stuart DI, Stammers DK (2000) J Biol Chem **275**: 5633
[25] Hogberg M, Sahlberg C, Engelhardt P, Noreen R, Kangasmetsa J, Johansson NG, Oberg B, Vrang L, Zhang H, Sahlberg BL, Unge T, Lovgren S, Fridborg K, Backbro K (1999) J Med Chem **42**: 4150
[26] Lindberg J, Sigurosson S, Lowgren S, Sahlberg C, Noreen R, Fridborg K, Unge T (2002) Eur J Biochem **296**: 1670
[27] Ren J, Nichols C, Bird LE, Fujiwara T, Sugimoto H, Stuart DI, Stammers DK (2000) J Biol Chem **275**: 14316
[28] Rodgers DW, Gamblin SJ, Harris BA, Ray S, Culp JS, Hellmig B, Woolf DJ, Debouck CD, Harrison SC (1995) Proc Natl Acad Sci USA **92**: 1222
[29] Esnouf R, Ren J, Ross C, Jones Y, Stammers D, Stuart D (1995) Nat Struct Biol **2**: 303
[30] Hsiou Y, Ding J, Das K, Clark AD Jr, Hughes SH, Arnold E (1996) Structure **4**: 853
[31] Sarafianos SG, Das K, Clark AD Jr, Ding J, Boyer PL, Hughes SH, Arnold E (1999) Proc Natl Acad Sci USA **96**: 10027
[32] a) Jacobo-Molina A, Ding J, Nanni RG, Clark AD, Lu X, Tantillo Ch, Williams RL, Kamer G, Ferris AL, Clark P, Hizi A, Hughes SH, Arnold E (1993) Proc Natl Acad Sci USA **90**: 6320;

b) Ding J, Das K, Hsiou Y, Sarafianos SG, Clark AD, Jacobo-Molina A, Tantillo Ch, Hughes SH, Arnold E (1998) J Mol Biol **284**: 1095
[33] Huang H, Chopra R, Verdine GL, Harrison SC (1998) Science **282**: 1669
[34] Sarafianos SG, Das K, Tantillo Ch, Clark AD, Ding J, Whitcomb JM, Boyer PL, Hughes SH, Arnold E (2001) EMBO J **20**: 1449
[35] Tripos Associates, Inc. 1996 SYBYL Molecular Modelling Software, Version 6.5; St. Louis, MO
[36] Jay Ponder Lab, Dept. of Biochemistry & Molecular Biophysics; Washington University School of Medicine 1998 TINKER-Software Tools for Molecular Design, St. Louis, Missouri
[37] Weiner SJ, Kollman PA, Case DA, Singh UC, Ghio C, Alagona G, Profeta S, Weiner P (1984) J Am Chem Soc **106**: 765