DOI: 10.1002/cmdc.200700223

Hyrtiosal, from the Marine Sponge *Hyrtios erectus*, Inhibits HIV-1 Integrase Binding to Viral DNA by a New Inhibitor Binding Site

Li Du,[a] Liangliang Shen,[b] Zhiguo Yu,[a] Jing Chen,[a] Yuewei Guo,*[a] Yun Tang,*[b] Xu Shen,*[a, b] and Hualiang Jiang[a, b]

HIV-1 integrase (IN) is composed of three domains, the N-terminal domain (NTD, residues 1–50), the catalytic core domain (CCD, residues 51–212), and the C-terminal domain (CTD, residues 213–288). All the three domains are required for the two known integration reactions. CCD contains the catalytic triad and is believed to bind viral DNA specifically, and CTD binds viral DNA in a nonspecific manner. As no clear evidence has confirmed the involvement of NTD in DNA binding directly, NTD has not been seriously considered and less is known about its function in viral replication. In the current work, using a SPR technology-based assay, the HIV-1 viral DNA was determined to bind directly to NTD with a K_D value of 8.8 μM, suggesting that the process of preintegrated complex formation for HIV-1 IN might involve the direct interaction of NTD with viral DNA in addition to binding of viral DNA to the catalytic core domain and C-terminal domain. Moreover, such viral DNA/IN binding could be inhibited by the marine product hyrtiosal from the marine sponge Hyrtios erectus *with an IC_{50} of 9.60 $\pm$ 0.86 μM. Molecular dynamic analysis correlated with a site-directed mutagenesis approach further revealed that such hyrtiosal-induced viral DNA/IN binding inhibition was caused by the fact that hyrtiosal could bind HIV-1 NTD at Ser17, Trp19, and Lys34. As hyrtiosal was recently discovered by us as a protein tyrosine phosphatase 1B (PTP1B) inhibitor,[1] this work might also supply multiple-target information for this marine product, and the verified HIV-NTD/HIV-1 IN interaction model could have further implications for new HIV-1 IN inhibitor design and evaluation.*

Introduction

Three HIV-1 pol gene-encoded enzymes, protease, reverse transcriptase (RT), and integrase (IN), are known to be essential for the virus replication process.[2] FDA-approved drugs targeting RT, proteases, and entry processes are currently available for the treatment of HIV/AIDS, and they are frequently used in combination as drug cocktail therapy regimens.[2] However, due to drug-resistant viral stains and severe side effects associated with the existing drugs, there is an urgent need for the development of novel anti-HIV drugs targeting other steps in viral replication process.

HIV-1 IN mediates an essential step in the viral replication cycle by catalyzing the integration of the reverse-transcribed viral cDNA into the host genome. It was reported that an IN-defective HIV-1 failed in replication.[3] Moreover, there are no cellular homologues to IN and the reactions catalyzed by IN are unique. Thus, IN is attractive target for anti-HIV drug development, and IN inhibitors are possibly a valuable complement to RT and protease inhibitors for AIDS therapy.[4,5]

As has been reported,[6] IN catalyzes two reactions in integration: 3′-end processing and strand transfer. The first reaction occurs in the cytoplasm and removes two nucleotides from the 3′-end of each viral DNA strand, leaving a 3′-hydroxyl group free. Through this reaction a preintegration complex is formed by IN and viral DNA together with some other proteins. The complex then moves into the nucleus where the second reaction takes place slowly, to join the recessed 3′-hydroxyl group at each viral DNA end to a human DNA phosphate. Joining of both viral DNA ends occurs at phosphates across the major groove of human DNA staggered by five base pairs.[7]

HIV-1 IN consists of 288 residues and is composed of three domains: the N-terminal domain (NTD, residues 1–50), the catalytic core domain (CCD, residues 51–212), and the C-terminal domain (CTD, residues 213–288).[8] All three domains are required for the two integration reactions. A functional IN appears to act as a multimer and the IN molecule in human cells

[a] *Dr. L. Du,[+] Dr. Z. Yu, Dr. J. Chen, Prof. Y. Guo, Prof. X. Shen, Prof. H. Jiang*
Drug Discovery and Design Center, State Key Laboratory of Drug Research
Shanghai Institute of Materia Medica
Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences
Shanghai 201203 (China)
Fax: (+86) 21-50806918
E-mail: ywguo@mail.shcnc.ac.cn
xshen@mail.shcnc.ac.cn

[b] *L. Shen,[+] Prof. Y. Tang, Prof. X. Shen, Prof. H. Jiang*
School of Pharmacy, East China University of Science and technology
Shanghai 200237 (China)
E-mail: ytang234@yahoo.com.cn

[+] *These authors contributed equally to this work.*

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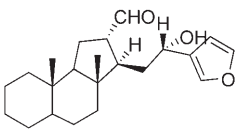
seems to be a homotetramer.[9] A CCD containing catalytic triad is believed to bind viral DNA specifically, whereas CTD binds viral DNA in a nonspecific manner. As no clear evidence has confirmed the involvement of NTD in DNA binding directly,[6] NTD has not been seriously appreciated and less is known about its function in viral replication.

NTD contains a highly conserved HHCC motif made up of residues His12, His16, Cys40, and Cys43 coordinating a Zn^{2+} ion, which was shown to promote the tetramerization and enhance the catalytic activity of the enzyme in vitro.[10] The structure of NTD has been determined in isolated form and in conjugation with CCD by means of NMR spectroscopy and X-ray crystallography techniques, separately.[11,12] However, the arrangement of NTD in the active complex with DNA substrate is unknown because of the lack of the full-length IN structure and its complex with DNA.

Although many classes of IN inhibitors have been reported and some have even entered into clinical trials, no IN inhibitor is approved by the FDA.[8,13–15] The most promising drug candidates are β-diketo containing compounds, which belong to the only class of IN inhibitors with a clear inhibition mechanism. Among them, compounds S-1360 and L-870, 810 entered phase II clinical trials in 2003 and 2004, but were discontinued for some reasons.[8,14] So far, the first IN inhibitor in phase III clinical trial, MK-0518, was announced by Merck in early 2006 and related safety and efficacy experimental results were reported.[16,17] Recently, several potent and active IN inhibitors were reported for their excellent inhibitory activity and research on the binding sites and IN–inhibitor interactions has provided enough data to elucidate the mechanism of action.[18–20]

As all the three domains of IN are required for integration, each domain might be the target for inhibitor binding. However, almost all the reported HIV-1 IN inhibitors target CCD.[15] Guanosine quartets or G-quartets, such as T30177, were reported to require NTD for binding,[21] but further investigations demonstrated that they actually bound to CCD.[22] Small molecular compounds targeting NTD or CTD might inhibit IN activity as



those targeting CCD enhance the inhibition efficacy when used together.

In the current work, the marine natural product hyrtiosal, a recently reported protein tyrosine phosphatase 1B (PTP1B) inhibitor[1] was discovered to competitively inhibit HIV-1 integrase (IN) binding to the viral DNA. SPR binding assay results indicated that this IN–viral DNA binding inhibition was caused by hyrtiosal's specific binding to the NTD of HIV-1 IN. Molecular dynamic analysis correlating with site-directed mutagenesis further revealed that hyrtiosal–HIV-1 IN NTD binding involved the binding sites of Ser17, Trp19, and Lys34. This work is expected to facilitate the understanding of the function of HIV-1 IN NTD and supply a new potential system for the discovery of HIV-1 IN inhibitors. As hyrtiosal was already discovered as a PTP1B inhibitor, this work might also supply multiple-target information for this marine product.

Results and Discussion

Hyrtiosal competitively inhibited HIV-1 integrase binding to viral DNA by acting as N-terminal domain binder

It is known that the binding of HIV-1 (IN) to viral DNA is one of the key steps for IN to take within virus infection, and exploration of the interrupter against such HIV-1 IN–viral DNA interactions is of great importance for HIV-1 IN inhibitor discovery. Interestingly, by screening against the in-house marine product library (≈1500 compounds), the marine product hyrtiosal from the marine sponge *Hyrtios erectus* was discovered to competitively inhibit HIV-1 IN binding to viral-DNA. As shown in Figure 1, the SPR technology-based competitive inhibition assay revealed that the resonance unit (RU) value of HIV-1 IN binding to viral DNA significantly decreased with increasing hyrtiosal concentrations, thus suggesting that hyrtiosal could compete with the binding of HIV-1 IN to the immobilized viral DNA. The IC_{50} value for hyrtiosal was thus evaluated as 9.60 ± 0.86 μM by fitting the inhibition data to a dose-dependent curve using a logistic derivative equation (Origin 6.1). This

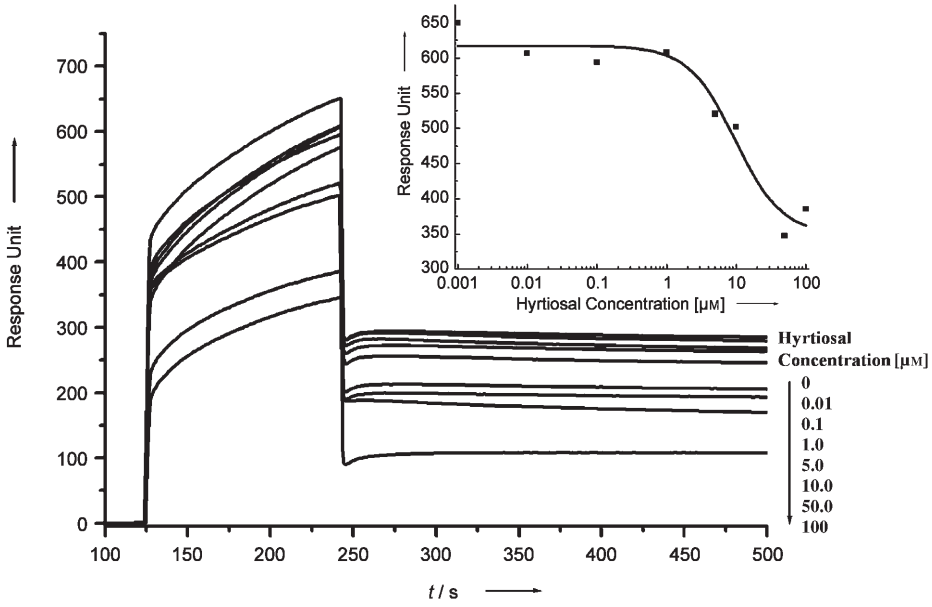


Figure 1. Competitive inhibition assay of hyrtiosal. Representative sensorgrams obtained with IN preincubated with 0, 0.01, 0.1, 1, 5, 10, 50, and 100 μM of hyrtiosal (curves from top to bottom). Insets represent the dose-dependences of RU values for hyrtiosal.

result implies that this marine natural product might function as a potential inhibitor of HIV-1 IN/viral DNA interaction.

To inspect the possible hyrtiosal binding segment of HIV-1 IN, the interactions of hyrtiosal with full-length HIV-1 IN and its relevant segments IN^{1-55}, IN^{1-210}, IN^{52-210}, and IN^{52-288} (see Experimental Section) were separately investigated by the SPR technology-based assay, and the related binding details are given in Table 1. For kinetic analysis, various concentrations of hyrtiosal were injected for 60 s at a flow rate of 30 $\mu L\,min^{-1}$ to allow for interaction with the proteins immobilized on the surface of the CM5 chip. For example, RU values evaluating hyrtiosal interaction with IN^{1-210} revealed an obvious concentration-dependent manner interaction. The concentration series were thus fitted using a 1:1 (Langmuir) binding model supplied in the Biacore software, resulting in the K_D value of 0.937 μM (Table 1). This result indicates that hyrtiosal could strongly bind to the IN^{1-210} segment. Moreover, the fact that hyrtiosal could bind to the IN^{1-55} segment but fail to bind to either IN^{52-210} or IN^{52-288} (Table 1, Figure 2) indicated that hyrtiosal bind specifically to the N-terminal domain of HIV-1 IN.

Table 1. Binding affinities[a] of hyrtiosal to the full-length HIV-1 IN, IN fragments, and IN^{1-210} mutants, determined by SPR.

IN	IN^{1-210}				IN^{1-55}	IN^{52-210}	IN^{52-288}
	Wild-type	S17A	W19A	K34A			
0.197 ± 0.018	0.937 ± 0.102	17.0 ± 0.72	12.9 ± 1.03	27.0 ± 1.47	11.1 ± 0.41	–[b]	–[b]

[a] Equilibrium dissociation constant; K_D values in μM. [b] No binding was detected.

As hyrtiosal could bind to NTD, the SPR technology-based assay was performed to evaluate possible NTD binding to viral DNA. For this assay, the investigation of full-length IN binding to viral DNA was carried out as a positive control, the viral DNA was immobilized on the SA chip and NTD or full length HIV-1 IN protein was treated as analyte. As indicated in Figure 3, NTD could directly bind to viral DNA with a K_D value of 8.8 μM, which is ≈20 times weaker than the binding affinity between the full length IN and the viral DNA ($K_D = 0.4\,\mu M$). It is suggested that such a binding affinity difference might be due to the ability of CCD to bind viral DNA and the nonspecific DNA binding characteristic of CTD for the full length HIV-1 IN.[23,24] Therefore, our SPR-based result that HIV-1 NTD could bind to viral DNA supports the previously published suggestion that NTD can bind viral DNA[25] and implies that the process of preintegrated complex formation of IN possibly involves the direct interaction between NTD and viral DNA, in addition to viral DNA–CCD binding. This result thereby confirms that hyrtiosal might inhibit HIV-1 IN interaction with viral DNA through its binding to NTD.

To further investigate whether hyrtiosal could directly inhibit the binding of IN NTD to DNA, the binding assay of IN^{1-55} preincubated with hyrtiosal and DNA was performed on Biacore 3000. The results shown in Figure 4a indicated that hyrtiosal could inhibit the binding of IN^{1-55} to DNA in a dose-dependent manner. To prove that the inhibition of hyrtiosal specifically involved NTD, the competitive binding assay of hyrtiosal and the two fragments IN^{52-210} and IN^{52-288} to DNA was preformed. During the assay, the interaction of the two IN fragments (IN^{52-210} and IN^{52-288}) with DNA was also evaluated and the binding affinities showed that these two segments could still bind to DNA (Supporting Information Figure S1), thereby indicating that they are folded in a functional way. From Figure 4b and 4c, the sensorgrams of the proteins showed almost no difference from those preincubated with hyrtiosal, which implied that hyrtiosal had no effects on the interaction of IN^{52-210} (IN^{52-288}) with DNA, further confirming that hyrtiosal inhibited the IN–DNA binding through its specific binding to NTD.

In comparison with CCD, the NTD of HIV-1 IN has been paid less attention with respect to HIV-1 IN-targeted inhibitor discovery. In 1996, Mazumder et al. reported that G quartets required the zinc finger region of NTD for inhibition of IN activity, whereby the zinc finger was supposed to stabilize ligand binding.[21] However, subsequent experiments demonstrated that the G quartets actually bound to CCD.[22] Our SPR results have suggested that hyrtiosal exhibited a highly specific binding affinity to HIV-1 NTD and revealed a new potential binding site for a HIV-1 IN inhibitor.

Molecular docking technology based hyrtiosal binding site investigation

Although the literature indicates that IN catalyzed the integration reaction as a tetramer,[26,27] the crystal structures of IN CCD and IN^{1-210} and the NMR structure of IN^{1-55} all existed as dimers.[11,28] Therefore, to explore the possible hyrtiosal binding site at NTD, hyrtiosal was docked with a NTD dimer structure derived from the protein data bank (PDB entry code 1WJA).[29]

As shown in Figure 5a, the docking poses of hyrtiosal were located in a small pocket near the zinc finger motif composed by the residues from both monomers, and hyrtiosal aligned in the possible binding pocket of NTD. All the possible docking geometries superposed well with each other. The binding details of hyrtiosal to the NTD dimer are shown in Figure 5b, which demonstrated that hyrtiosal interacts with NTD through three groups. The tricarbocyclic skeleton of hyrtiosal interacts with the side chains of Trp 19, Lys 34, Val 37, Ala 38 in monomer A, and Ala 38, Ser 39 in monomer B via hydrophobic interactions, and the carboxyl group of hyrtiosal forms a salt bridge with the NH_3^+ group of Lys 34, whereas the hydroxyl group of hyrtiosal as a donor hydrogen binds to the hydroxyl group of Ser 17.

Site-directed mutagenesis technique-based hyrtiosal binding site validation

To validate the binding sites of hyrtiosal against NTD as suggested above, site-directed mutagenesis technique with SPR

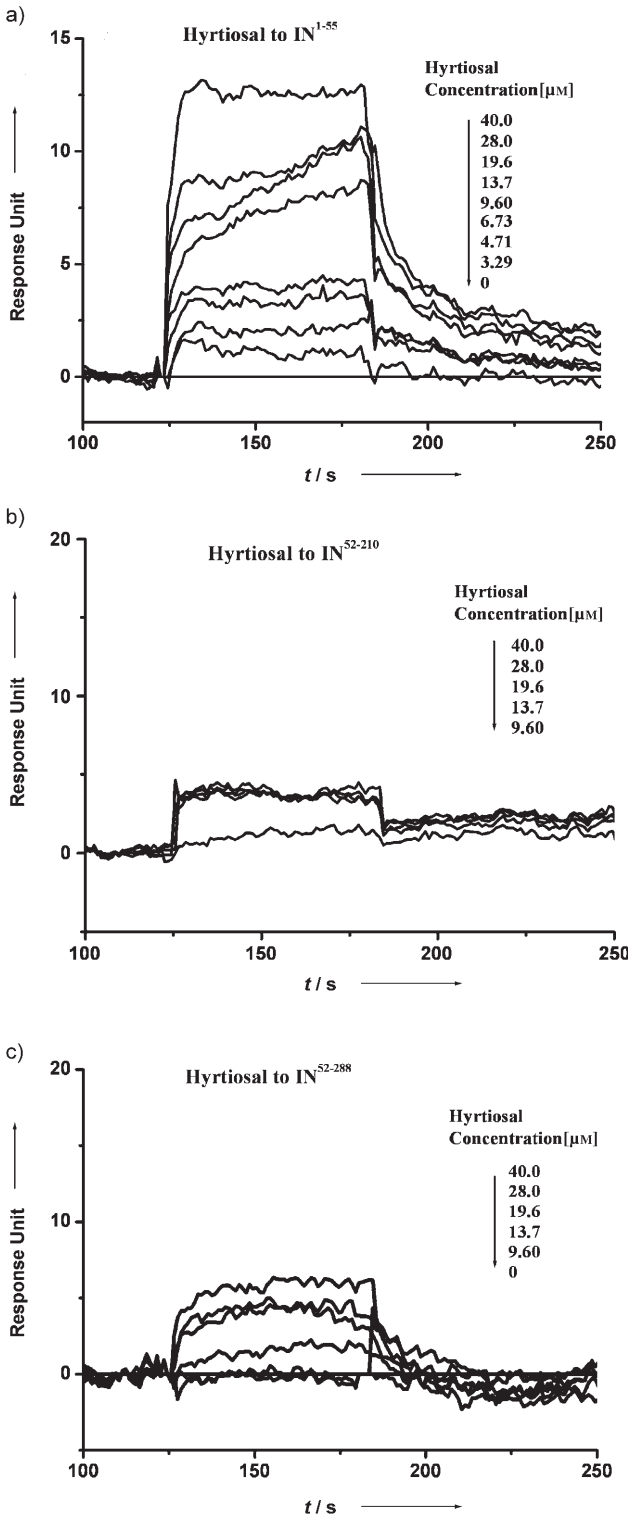


Figure 2. Binding of hyrtiosal to a) HIV-1 IN^{1-55}, b) IN^{52-288}, and c) IN^{52-210} as determined by SPR assay. Representative sensorgrams obtained with hyrtiosal at concentrations of a) 40, 28, 19.6, 13.72, 9.604, 6.7228, 4.706, 3.294, 0 μM, b) and c) 40, 28, 19.6, 13.72, 9.604 M (curves from top to bottom) are shown. The compound was injected for 60 s, and dissociation was monitored for 120 s.

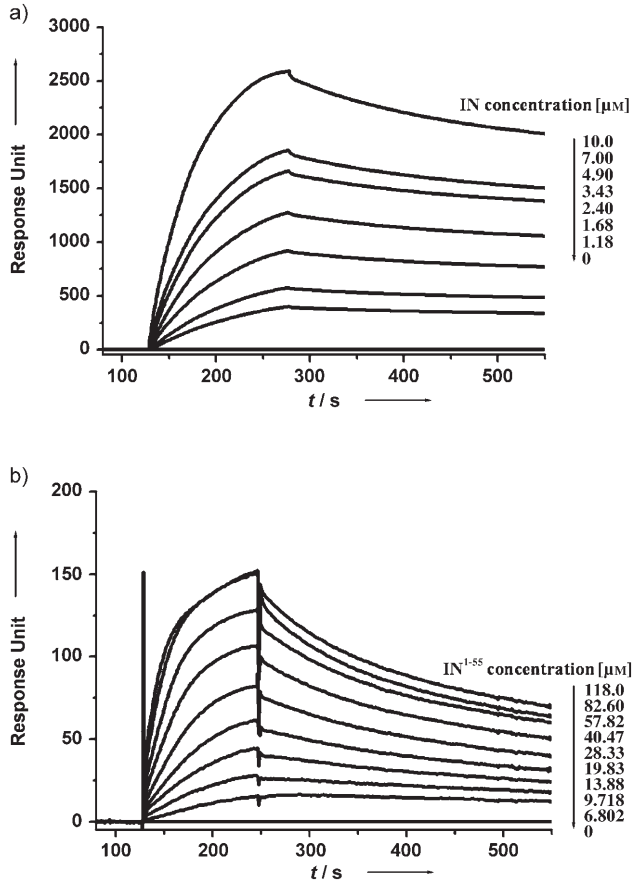


Figure 3. a) The binding of HIV-1 integrase binding to the immobilized viral DNA. Representative sensorgrams obtained with IN at concentrations of 10.0, 7.00, 4.90, 3.43, 2.40, 1.68, 1.18, and 0 μM (curves from top to bottom) are shown. b) The binding of HIV-1 integrase N-terminal domain (NTD) to the immobilized viral DNA. Representative sensorgrams obtained with IN^{1-55} at concentrations of 118.0, 82.60, 57.82, 40.47, 28.33, 19.83, 13.88, 9.718, 6.802, and 0 μM (curves from top to bottom) are shown.

technology-based assays were preformed. During the assays, the three important residues Ser 17, Trp 19, and Lys 34 suggested by the above molecular docking studies were substituted by alanine. As indicated in Table 1 and Figure 6, the substitutions of alanine for Ser 17 and Trp 19 could significantly reduce hyrtiosal binding to IN^{1-210}, and the mutation of Lys 34 to Ala could almost abolish hyrtiosal binding to IN^{1-210}. Such mutagenesis experiments clearly imply that residues Ser 17, Trp 19, and Lys 34 of IN^{1-210} might be involved in the interaction of HIV-1 NTD with hyrtiosal. Moreover, Lys 34 probably played a key role in this interaction. Thus, we inferred that hyrtiosal probably inhibits the interaction of HIV-1 IN with viral DNA by competitively binding to the residues of NTD near Lys 34.

In our previous report,[1] hyrtiosal was discovered as a protein tyrosine phosphatase 1B (PTP1B) inhibitor and shows extensive cellular effects on PI3K/AKT activation, glucose transport, and TGFβ/Smad2 signaling. This current work might supply multiple-target information for the marine product hyrtiosal, and it has also been determined as a valuable probe to address a new inhibitor binding site for HIV-1 IN.

As indicated in Figure 5, the hyrtiosal binding site is located on the side surface of the isolated NTD dimer, small but well organized. Most of the pocket surface is hydrophobic. The resi-

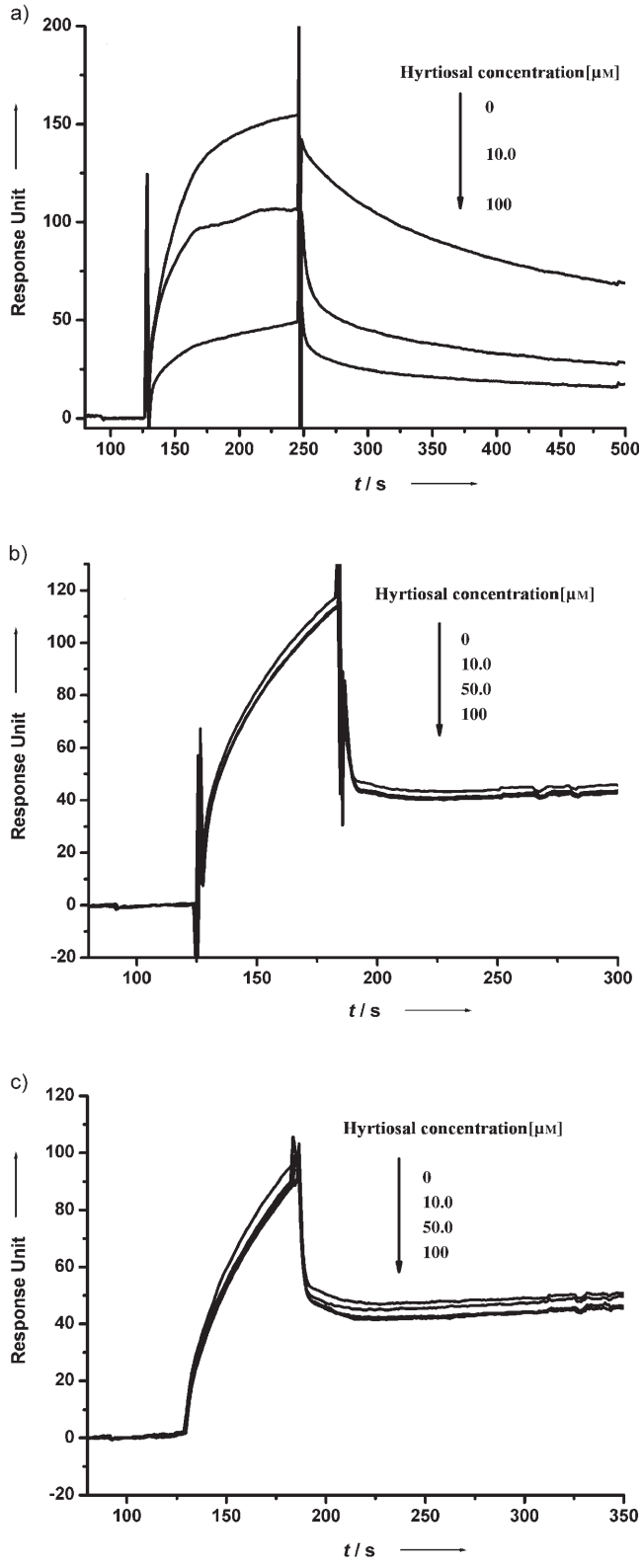


Figure 4. Competitive inhibition assay of a) hyrtiosal to IN^{1-55}, b) IN^{52-210}, and c) IN^{52-288} binding to viral DNA. Representative sensorgrams obtained with IN preincubated with different concentrations of hyrtiosal (curves from top to bottom).

dues Val 37, Ala 38, Cys 43, Ala 38′, and Ser 39′ (the prime indicates a residue from the other monomer in the dimer) are important for lining the pocket. However, this pocket is different from the binding patch previously identified by an inhibitory monoclonal antibody.[30] That binding surface, mapped to a region within residues Asp 25–Glu 35, is located on top of the isolated NTD dimer, which is possibly involved with binding to other proteins.[31] Previous literature reported that Lys 34 is required for preintegration complex function and viral replication,[32] and is probably involved in viral DNA binding.[30] Based on the present study, we propose a potential hyrtiosal inhibition mechanism whereby hyrtiosal might interrupt the preintegration complex formation for HIV-1 IN through direct binding to NTD.

Conclusion

In the work presented herein, the marine natural product hyrtiosal, a PTP1B inhibitor,[1] has been discovered to competitively inhibit HIV-1 IN binding to viral DNA. SPR binding-assay results demonstrated that hyrtiosal specifically binds to the N-terminal domain. Molecular docking provided a possible binding mode for hyrtiosal and HIV-1 IN interaction at atomic level. The importance of three key amino acid residues (Ser 17, Trp 19, and Lys 34) involved in the binding was identified by site-directed mutagenesis. This study is expected to facilitate the understanding of the function of the N-terminal domain of HIV-1 integrase thus supplying a new potential system for designing new types of IN inhibitors targeting NTD.

Experimental Section

Materials: The marine natural product, hyrtiosal, was isolated from marine sponge as described previously.[33] All solvents and reagents were purchased commercially and were used without further purification. Plasmid extraction was performed with the GenElute Plasmid Mini-prep Kit (Sigma–Aldrich). QuikChange site-directed mutagenesis kit was purchased from Stratagene (La Jolla, CA, USA).

Plasmid construction and protein preparation: The wild-type HIV-1 IN DNA coding for HIV-1 integrase (GenBank No. AF 040373) was synthesized with an Applied Biosystems DNA synthesizer (Shanghai Sangon Biological Engineering and Technology and Service Co. Ltd.) and cloned into glutathione S-transferase (GST) expression vector pGEX-4T-1 to construct the plasmid pGEX-4T-1-IN. The F185K substitution was introduced to construct the mutant plasmid pGEX-4T-1-IN (F185K) to increase the solubility.[34] The plasmid pGEX-4T-1-IN (F185K) was used as the template DNA to construct the deletion mutants pGEX-4T-1-IN^{1-55}, pGEX-4T-1-IN^{52-210}, pGEX-4T-1-IN^{1-210}, and pGEX-4T-1-IN^{52-288}, which encode the residues of the HIV-1 IN N-terminal domain (amino acid 1–55), or core domain (amino acids 52–210, IN^{52-210}), or N-terminal with core domain (amino acids 1–210, IN^{1-210}), or core with C-terminal domain (amino acids 52–288, IN^{52-288}), respectively. Site-directed mutagenesis was performed based on the plasmid pGEX-4T-1-IN^{1-210} using the QuikChange site-directed mutagenesis system (Stratagene, La Jolla, CA, USA). Codons for Ser 17, Trp 19, and Lys 34 were mutated to alanine by using the following duplex oligonucleotides. S17A: 5′-GAA CAT GAA AAA TAT CAC **GCT** AAT TGG AGA GCA ATG GC-3′; W19A: 5′-GAA AAA TAT CAC AGT AAT **GCG** AGA GCA ATG GCT AGT G-3′; K34A: 5′-C CTG CCA CCT GTA GTA GCA **GCA** GAA ATA GTA GCC AGC TG-3′. The substituted nucleotide is shown in boldface and

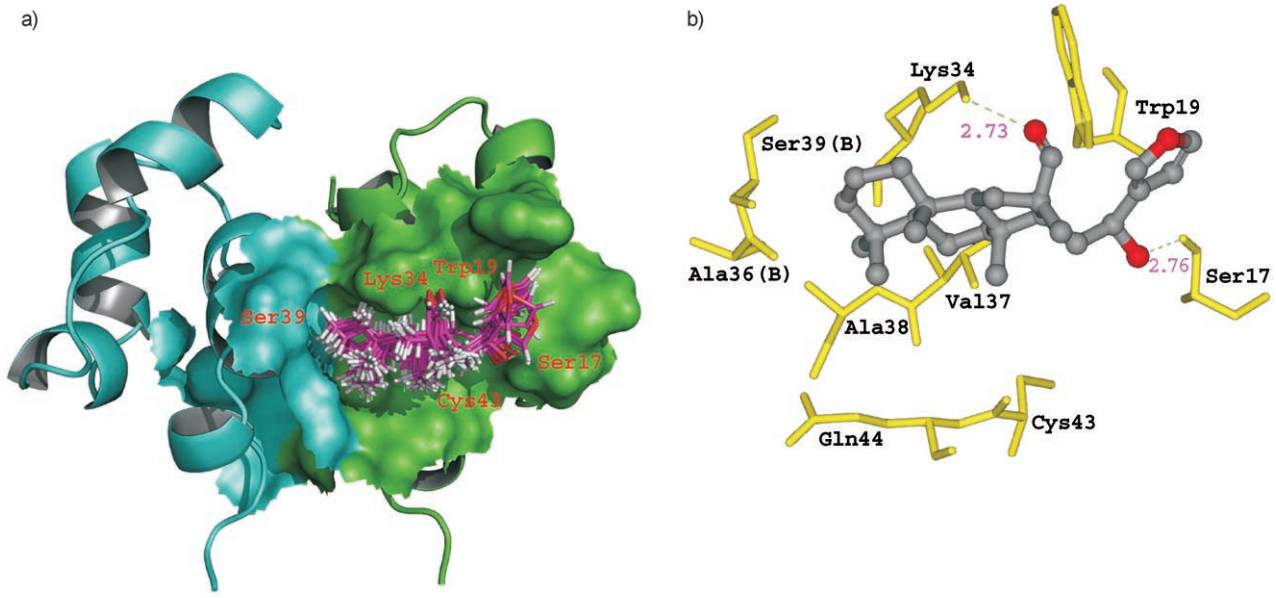


Figure 5. a) Alignment of GOLD-derived poses of hyrtiosal on the binding site of N-terminal domain (NTD). The pocket was shown in solvent-accessible surface, whereas the others are shown in ribbon. The two NTD monomers were colored green and blue, respectively. b) The detailed interactions of N-terminal domain (NTD) residues with hyrtiosal. Hyrtiosal is shown in ball-and-stick and residues in stick only. Green dashed lines represent hydrogen bonds.

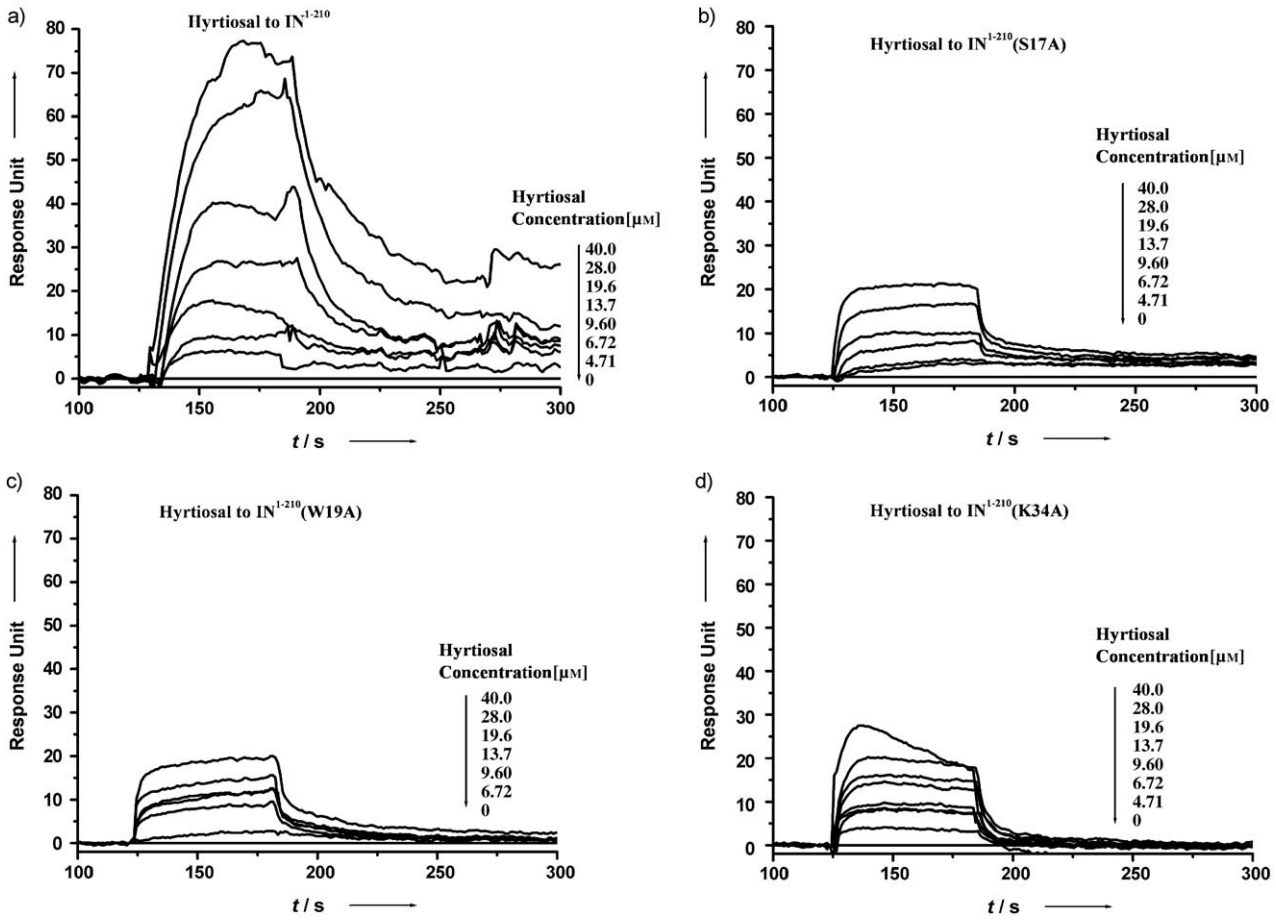


Figure 6. Binding of hyrtiosal to a) HIV-1 IN^{1-210},b) IN^{1-210} (S17A), c) IN^{1-210} (W19A), and d) IN^{1-210} (K34A) as determined by SPR assay. Representative sensorgrams obtained with hyrtiosal at concentrations of 40, 28, 19.6, 13.72, 9.604, 6.7228, 4.706, and 0 μM (curves from top to bottom) are shown. Hyrtiosal was injected for 60 s, and dissociation was monitored for 120 s.

the mutated codon is underlined. All clones were verified by sequencing.

IN, IN^{1-55}, IN^{52-210}, IN^{52-288}, IN^{1-210}, and IN^{1-210} mutants S17A, W19A, and K34A were expressed and purified according the GST gene fusion system handbook (Amersham Bioscience). IN, IN^{52-288}, IN^{1-210}, and IN^{1-210} mutants S17A, W19A, and K34A were purified in GST-fusion form and IN^{1-55} and IN^{52-210} with GST tag removed. The purity of all proteins was confirmed by SDS-PAGE.

Competitive inhibition assay: The compound's competitive inhibition assay against IN and the viral DNA binding was performed using the SPR biosensor technology (Biacore 3000, Biacore AB, Uppsala, Sweden).[30] During the assay, a 21 bp 5′-biotinylated oligonucleotide (5′-GTGTGGAAAATCTCTAGGTGT-3′) hybridized with a nonbiotinylated complementary oligonucleotide was immobilized on the streptavidin matrix-coated sensor chip (SA chip), 200 nM IN incubated with 0 to 0.2 mM compounds for 1 h at 4 °C flowed over the chip surface. The compound inhibition against IN binding to DNA was demonstrated by monitoring the RU decrease with the addition of the compounds at different concentrations. All the sensorgrams were processed by using automatic correction for nonspecific bulk refractive index effects.

Binding assays: The binding affinity of the two marine compounds GYWYF2 and GYWYF9 to HIV-1 IN, IN^{52-210}, IN^{52-288}, IN^{1-210}, IN^{1-210} (S17 A), IN^{1-210} (W19A), and IN^{1-210} (K34A) in vitro was determined using the surface plasmon resonance (SPR) biosensor technology. The measurement was performed using the dual flow cell Biacore 3000 instrument (Biacore AB, Uppsala, Sweden). Immobilization of the wild-type and mutant IN proteins to the hydrophilic carboxymethylated dextran matrix of the sensor chip CM5 (Biacore) was carried out by the standard primary amine coupling method. The protein to be covalently bound to the matrix was diluted in 10 mM sodium acetate buffer (pH 4.5) to a final concentration of 0.2 mg mL^{-1}, and the resonance signal reached about 8500 resonance units (RUs). Equilibration of the baseline was completed by a continuous flow of HBS-EP buffer (10 mM HEPES, 150 mM NaCl, 3 mM EDTA and 0.01 % P20, pH 7.4) through the chip for 4–5 h. For the GST fusion protein IN, IN^{1-210}, IN^{52-288} (S17A), IN^{1-210} (W19A), and IN^{1-210} (K34A) binding assays, the reference flow cell surface was immobilized at a parallel level (4500 RU) using GST as a control. All the sensorgrams were processed by using automatic correction for nonspecific bulk refractive index effects. The specific binding profiles of the compounds to the immobilized protein were obtained after subtracting the response signal from the control flow cell. All the Biacore data were collected at 25 °C with HBS-EP as running buffer at a constant flow of 30 μL min^{-1}. The equilibrium dissociation constants (K_D) evaluating the protein–ligand binding affinity were determined using 1:1 binding model (Langmuir) and the curve fitting efficiency was checked by residual plots and χ^2.

Molecular modeling: Molecular modeling was conducted with the software package SYBYL v7.0[35] on Dell Precision 670 running Redhat WS 3.0. The 3D structures of ligands were sketched and energetically minimized with Tripos force field[36] and Gasteiger–Hückel charges.[37] The experimental structures of IN NTD were obtained from the protein data bank (PDB entry code 1WJA).[29] All the ionizable residues were kept in their standard protonation states. The domain was relaxed 400 steps using Tripos force field with Kollman all-atom charges[38] in SYBYL. GOLD version 3.0.1[39] was employed to investigate the binding mode between the inhibitors and IN NTD. The whole NTD dimer was treated as the binding pocket. The default parameters of genetic algorithms (GA) were applied to search the reasonable binding conformation of ligands. To find more accurate geometries, the option "allow early termination" was turned off. The maximum number of GA runs was set to 10 for each compound. The ChemScore function[40] was used to evaluate the docking conformations.

Abbreviations

HIV: human immunodeficiency virus; IN: integrase; NTD: N-terminal domain; CCD: catalytic core domain; CTD: C-terminal domain; GST: glutathione S-transferase; PCR: polymerase chain reaction; PDB: Protein Data Bank; IPTG: isopropyl-β-D-thiogalactopyranoside; PBS: phosphate-buffered saline; SPR: surface plasmon resonance; RU: response unit; GA: genetic algorithms; *E. coli*: *Escherichia coli*.

Acknowledgements

This work was financially supported by the National Natural Science Foundation of China (grants 30525024, 20472095, and 20572023), the National "863" Project of China (2006AA609Z447 and 2006AA609Z41), Shanghai Pujiang (Program No. 05J14034), Shanghai Key Basic Research Project (grants 06JC14080 and 05JC14092), the State Key Program of Basic Research of China (grants 2004CB58905 and 2006AA09Z447), and a grant from CAS (grant KSCX2-YW-R-18).

Keywords: HIV-1 integrase · hyrtiosal · molecular docking · N-terminal domain · site-directed mutagenesis

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Received: August 26, 2007
Published online on October 17, 2007

