PIBB 生物化学与生物物理进展
Progress in Biochemistry and Biophysics
2009, 36(5): 592~600
www.pibb.ac.cn

A Study on Drug Resistance Mechanism of HIV-1 Integrase Mutants by Molecular Modeling*

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Abstract The drug resistant mutations in human immunodeficiency virus type 1 (HIV-1) are a major impediment to successful highly active antiretroviral therapy (HAART) and new drug design. In order to understand the drug resistance mechanism of HIV-1 integrase (IN) mutually existed for multiple drug-resistant strains to the most potent IN inhibitors diketo acids (DKAs), three S-1360-resistant HIV-1 strains were selected and molecular docking and molecular dynamics (MD) simulations were performed to obtain the inhibitor binding modes. Based on the binding modes, compelling differences between the wild-type and the 3 mutants for IN have been observed. The results showed that: 1) In the mutants, the inhibitor is close to the functional loop 3 region but far away from the DNA binding site. Different binding sites lead to the decrease in susceptibility to S-1360 in mutants compared to the wild-type IN. 2) The fluctuations in the region of residues 138~166 are important to the biological function of IN. 2 hydrogen-bonds between S-1360 with residues N155 and K159 restrict the flexibility of the region. Drug resistant mutations result in a lack of the interaction, consequently, the less susceptible to S-1360. 3) In the 3 mutant IN complexes, the benzyl ring of S-1360 is far from the viral DNA binding site, thus, S-1360 can not prevent the end of the viral DNA from exposure to human DNA. 4) After T66I mutation, the long side chain of I occupied the active pocket in the 3 mutants，consequently， the inhibitor could not move into the same binding site or have the same orientation. All the above contribute to drug resistance. These results will be useful for the rational inhibitor modify and design.

Key words drug resistance, HIV-1 integrase, MD simulation
DOI: 10.3724/SP.J.1206.2008.00656

The human immunodeficiency virus type 1 (HIV-1) integrase (IN) catalyzes the insertion of retroviral DNA into host cell chromosome. It is an attractive target for the development of anti-AIDS drugs [1]. To date, there is only 1 FDA approved clinically useful IN inhibitor. Since IN has no human counterpart, the development of effective HIV IN inhibitors is very useful for the AIDS multi-drug therapy[2]. So far, 9 classes of IN inhibitors have been reported, in which the diketo acids (DKA) and its derivatives are the first compounds reported to interfere with HIV replication through a specific inhibition of the integration step and be regarded as one of the most promising classes of IN inhibitors[3, 4]. Therefore, the molecular mechanisms of HIV-1 IN drug resistance on DKAs are warranted.

IN consists of 3 distinct functional domains: the N-terminal domain, the catalytic core domain and the C-terminal domain. The central core domain (residues 50～212) contains the highly conserved DDE motif (Asp^{64}, Asp^{116}, and Glu^{152}) and is regarded to be the primary key domain of IN. The integration reaction can be described as 2 steps: 3’ processing and strand transfer.

The drug resistant mutations in HIV-1 are major impediment to successful highly active antiretroviral therapy (HARRT) and new drug design. The understanding and control of antiviral resistance and the continuous development of new drugs targeting IN are required. However, the mechanism of antiviral resistance of IN against DKA is far from being complete revealing. Recently, many experimental studies on IN mutants have been reported. These studies showed that a certain degree of viral resistance to DKAs appears when single or multiple residues are

*This work was supported by grants from The National Natural Science Foundation of China (30670497, 30500429) and Beijing Natural Science Foundattion (5072002, 7082006).
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Received: September 22, 2008 Accepted: November 25, 2008

mutated. S-1360 (a triazole analogue of DKAs) is the first IN strand transfer inhibitor to enter clinical trials, but its development was discontinued in Phase Ⅱ clinical trials[5]. Fikkert and coworkers[5] have performed experiments to investigate the development of HIV-1 resistance to S-1360 through the selection of HIV-1 strains in the presence of increasing concentrations of S-1360. At least 9 mutations were detected to be associated with resistance towards S-1360. All mutations are located in the catalytic core domain. The accumulation of the 9 mutations in this catalytic functional region may suggest the resistant mechanism. The single mutation T66I is detected in the IIIB/S-1360(#30)^{res} strain (HIV-1(IIIB) strains selected after 30 passages in the presence of S-1360), the strain showed a 3.8-fold less susceptible to S-1360 than the wild-type strain. The IIIB/S-1360 (#50)^{res} strain contains T66I/E138K/Q146K/V201I mutations showed a 7.8-fold decrease in susceptibility to S-1360 compared to the wild-type strain. The IIIB/S-1360 (#70)^{res} strain displayed a more than 62-fold reduced susceptibility to S-1360, pointing to the impact of the accumulation of the substitutions T66I/L74M/A128T/E138K/Q146K/S153A/K160D/V165I/V201I[5]. These experiment results will be helpful for further investigation of the drug-resistant mechanism of IN. Some other computational studies have been performed to reveal the partial mechanism of drug resistance towards DKAs. In this sense, Barreca and coworkers[6] have performed studies on double mutant (T66I/M154I)IN complexed with 5-CITEP. The results suggested that the loop region (residue 138~149) plays an important role on catalytic activity of IN[6~9].

Previous studies provided partial mechanism on drug resistance of HIV IN mutants, but these results are all from comparing 1 drug-resistant IN strain with wild-type IN, whether the mechanism mutually existed for multiple drug-resistant strains remains unclear. The mechanism mutually existed for multiple drug-resistant strains will be useful for gaining a 3D-pharmacophore in consideration of the conformational properties of the mutant complexes, furthermore, for improving the inhibition potency against drug-resistant IN and development of new drugs targeting IN. In this work, three S-1360-resistant HIV-1 strains were selected to illustrate several questions on the mechanism of drug resistance: the binding mode in inhibitor/mutant complexes, key amino acids involved in binding, the conformational changes of the structures close to the active site in inhibitor/mutant complexes compared with wild-type IN complex, the orientation of phenyl ring of S-1360 which has been proved to be important to prevent the exposure of the 3'-processed viral DNA ends from human DNA [10], the function of the T66I mutation which occurred frequently towards many IN inhibitors.

1 Materials and methods

1.1 Construction of IN structures

The X-ray crystallographic structure of the HIV-1 IN catalytic core domain (subunit A, residues 56～140, 145～209) complexed with the inhibitor 5-CITEP (PDB code 1QS4)[10] was used as a start structure. The missing region (residues 141～144) was added based on the homologous loop region in the crystal structure of the Avian Sarcoma Virus (ASV) integrase (PDB code 1VSH)[11]. To obtain the wild-type IN, K185 and E131 produced for crystallization in the crystal structure were changed back to F185 and W131, respectively, by using the SYBYL 7.0 /Biopolymer module[12]. 3 IN mutants in MT-4 cells in the presence of S-1360 were selected for this study: a. T66I; b. T66I/E138K/Q146K/V165I; c. T66I/L74M/A128T/E138K/Q146K/S153A/K160D/V165I/V201I [5]. These mutants were constructed by replacing the corresponding residues and designated with M#30, M#50 and M#70, respectively. All structures mentioned above were energy minimized using 5 000 steps of steepest-descent minimization, and then 1 000 steps of conjugated gradient minimization. The Kollman force field and charges were applied in these optimization procedures[12].

In order to obtain a rational IN structure for docking, the wild-type and 3 mutants of IN were used to perform 1.6 ns MD simulations with an equilibration process of 80 ps at 300 K and 1.01×10^5 Pa in the NTP ensembles with the GROMACS 3.05 program [13]. Each structure was immersed in a cubic box, and the least distance from the system to the boundary of the box was 0.7 nm. Afterward, the SPC water molecules were added in the box. The GROMOS96 43A2 force field was used, and the coordinates of the systems were saved every 2 fs for the following analysis. After these systems equilibrated, 4 average structures over the stable period of IN were obtained for molecular docking.

S-1360 was constructed by the SYBYL7.0/Biopolymer module and then was energy minimized

with 1 000 steps of the steepest-descent method and 200 steps of the conjugated gradient method. The Kollman force field and Gasteiger-Hückel charges [12] were also applied in the optimization procedures of the S-1360 system.

1.2 Molecular docking and MD simulations

S-1360 was docked into the average structure of the wild-type IN and the 3 drug-resistant mutants obtained above with the program AutoDock3.0[14]. The grid size was 2.25 nm × 2.25 nm × 2.25 nm and the grid center was set close to the atom OD2 of residue D64, the distance between the 2 adjacent grid points was 0.037 5 nm, the torsion angle step was 5.0 degrees, and 128 structures retained from docking simulations were taken into account for each system. According to the best rank and docking energy, one docked structure in each system was chosen as the complex structure for the subsequent MD simulations.

The X-ray IN/5-CITEP complex, the wild-type/ S-1360 complex and the 3 IN/S-1360 complexes were used as initial structures for the second MD simulation. The same MD simulation protocol was used for all the 5 complexes as mentioned above. After these systems equilibrated, 5 average structures over the stable period of IN were obtained for subsequent analysis.

2 Results and discussion

2.1 Gaining rational starting structures of IN monomers for molecular docking

The root-mean-square deviations (RMSD) of the Cα atoms with respect to the starting and average coordinates over four IN monomers are plotted in Figure 1. It can bee seen clearly that after the first 150 ps, the 4 systems are stable during the entire remaining period of MD simulation with the average RMSD values of 0.27, 0.29, 0.24 and 0.31 nm, respectively. In consideration of protein flexibility may be influential for a correct docking result, a rational and stable IN start structure is pivotal. The average structures from 150 ps to 1 600 ps of MD trajectories of the monomer IN were obtained for the wild-type and three mutant IN structures as starting structures and used for the subsequent molecular docking.

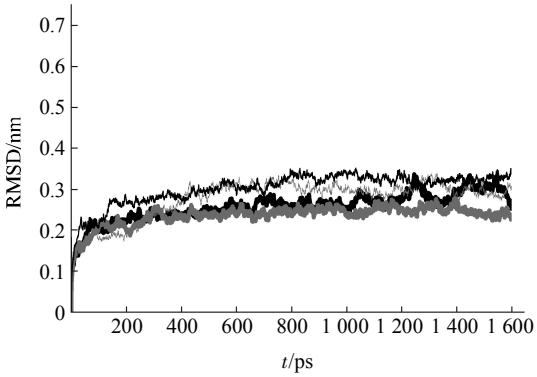

Fig. 1 RMSD of the Cα atoms for the wild-type, M#30, M#50 and M#70 IN monomers as a function of simulation time

—: Wild-type IN; —: M#30 IN; —: M#50 IN; —: M#70 IN.

The superimposed structures between the monomer IN mutants and the wild-type IN are shown in Figure 2. It can be seen that the 4 regions

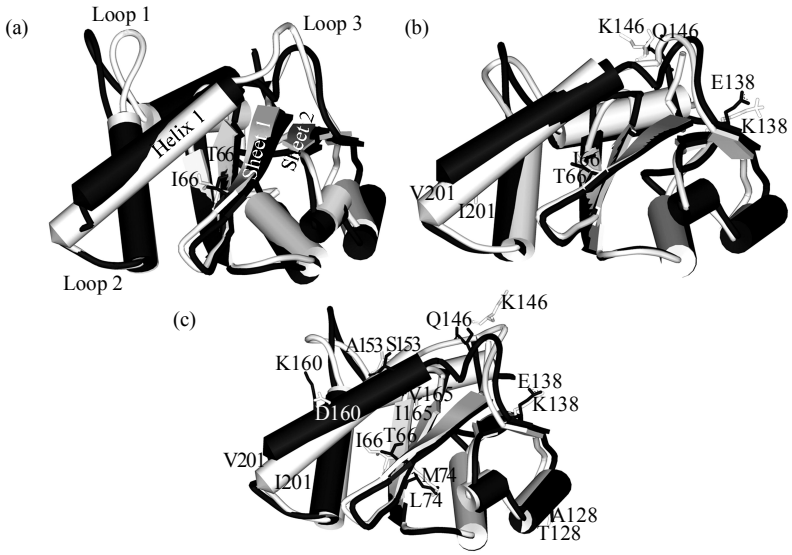

Fig. 2 The average structures over the stable period of MD trajectories of the monomer mutant IN superimposed with the wild-type IN

(a) The wild-type IN with the M#30. (b)The wild-type IN with the M#50. (c) The wild-type IN with the M#70 (the black structure is the wild-type IN and gray one is mutant IN).

containing loop 1, loop 2, helix 1 and loop 3 cannot be well superimposed. The loop 1 region is far away from the active site. Previous experiment data indicate that the functional loop 3 region located near the DDE in the active site is important for efficient biological activity[7~9, 15~17]. The highly conserved residue E152 lies on helix 1. Therefore, the structural changes and interaction with inhibitor in the loop 3 and helix 1 regions were investigated in subsequent studies.

2.2 Binding mode analysis

One docked structure in each system with the best rank of docking and minimum docking energy was chosen as the complex structure for MD simulation. As shown in Figure 3, after the first 400 ps, the 4 systems are stable during the entire remaining period of MD simulation. The average structures from 400 ps to 1 600 ps of MD trajectories of the wild-type IN complex and the 3 IN mutant complexes were obtained to analyze the binding mode.

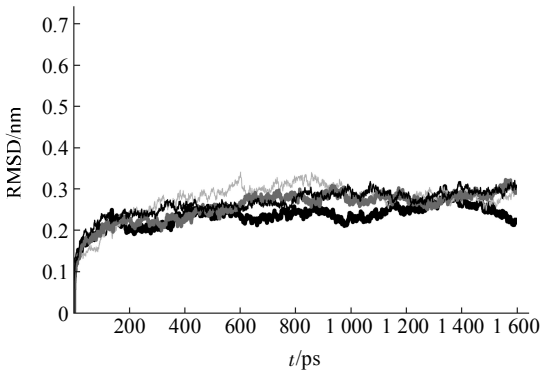

Fig. 3 RMSD of the Cα atoms for the wild-type, M#30, M#50 and M#70 IN complexes as a function of simulation time

—: Wild-type IN; —: M#30 IN; —: M#50 IN; —: M#70 IN.

It is found in Figure 4a that the keto-enol functional group is located in the position capable of chelating Mg^{2+} ions in the wild-type IN complex. This

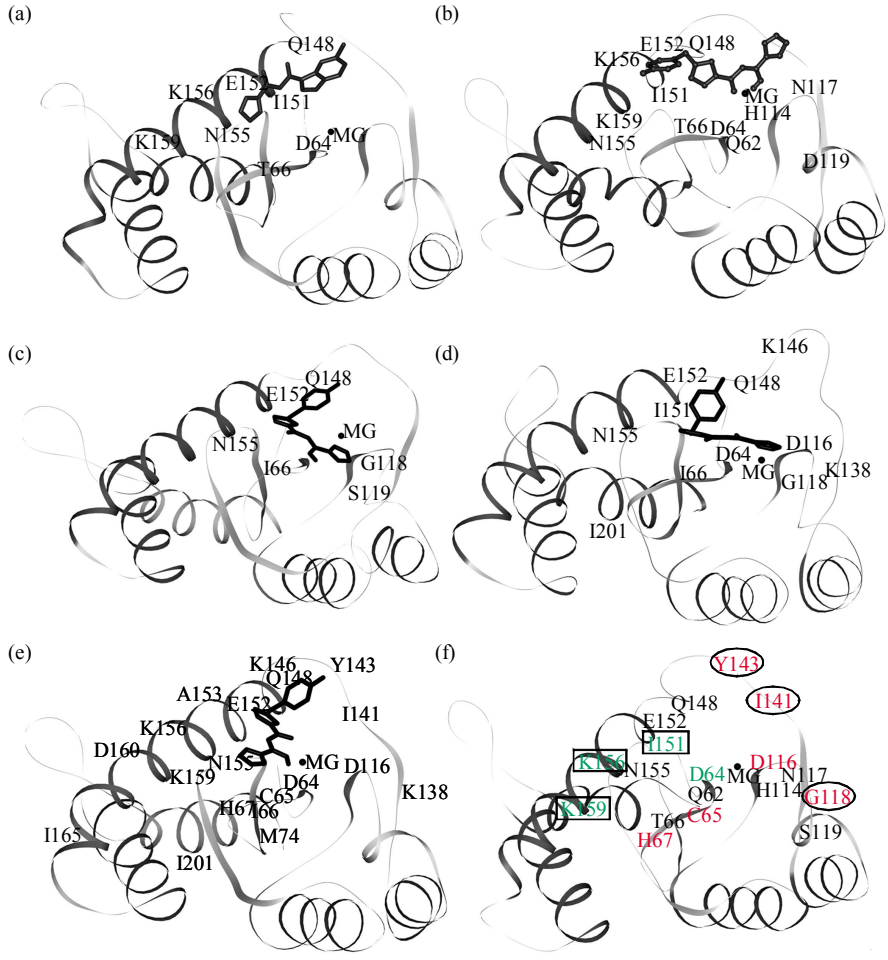

Fig. 4 The average structure of the IN-S-1360 complexes and key residues proximal to the inhibitor

(a) The X-ray IN complex. (b) Wild-type IN complex. (c) The M#30 IN complex. (d) The M#50 IN complex. (e) The M#70 IN complex. (f) Key residues proximal to the inhibitor (the residues selected by rectangle are in wild-type IN complex, the residues selected by ellipse are in mutant complexes).

is consistent with the pharmacophore model proposed by Deng *et al*.[18] The key residues nearby the inhibitor include the Q62, D64, T66, Q148, I151, E152, N155, K156, K159, H114, D116 and N117. Figure 4b shows the X-ray structure of IN complex, in which the inhibitor 5-CITEP occupies the active site including D64, T66, Q148, I151, E152, N155, K156, K159. Previous studies reported that the orientation of 5-CITEP obtained by the docking approach is slightly different from the X-ray complex[9, 17, 19, 20], whereas the binding location and the proximal key residues are the similar. The binding mode obtained in the present work is consistent with previous docking studies.

The mutations attenuate IN activity and confer resistance to IN inhibitors, suggesting that DKAs interact with IN[5]. The lowest docking energy for the wild-type IN combined with S-1360 is –23.40 kJ/mol. After drug resistance mutations, the lowest binding energy were –22.75 kJ/mol for the M#30 IN, –22.913.8 kJ/mol for the M#50 IN and –22.87 kJ/mol for the M#70 IN, respectively. The experimental study showed that the IC_{50} of S-1360 in the wild-type IN is 20 nmol/L, whereas a 3.8-fold, 7.8-fold and 62-fold decrease in susceptibility to S-1360 were obtained in M#30, M#50 and M#70 IN as compared with the wild-type IN. The results reveal that these mutations attenuate the binding between IN and S-1360, this is consistent with experimental results.

The average structures of M#30, M#50 and M#70 mutant complexes obtained from the stable MD simulations were shown in Figure 4c～e. It is found that the binding modes of the 3 mutant complexes are distinctly different from that of the wild-type complex, and the obvious conformational change is that S-1360 is more close to the functional loop 3 and sheet 2 regions. Hu *et al*[17]. have reported the similar observation in a G140S IN mutant- LCA complex. In detail, T66I mutation in M#30 mutant as shown in Figure 4c made S-1360 far away from residues I151, K156 and K159 while close to residues G118 and S119. In the case of M#50 mutant as shown in Figure 4d, the E138K and Q146K mutations locate at the two end of the loop 3 region. It is noted that the E138K mutation is from an acidic amino acid to a basic amino acid, and the Q146K mutation is from a neutral amino acid to a basic amino acid. It is also found that S-1360 in the M#50 mutant is also far away from residues K156 and K159 while close to residue G118. As shown in Figure 4e, in the M#70 complex, the E138K/Q146K mutations lie on the loop 3 region and the Q146K/S153A/K160D/V165I mutations lie on the helix1 region. S-1360 is far from residues I151, K156 and I66 while close to residues I141, Y143, D116, C65 and H67. It shows clearly in Figure 4f that the binding groove in mutant complexes (residues selected by rectangle) is close to the regions of loop 3, sheet 1 and sheet 2, while in the wild-type IN complex (residues selected by ellipse), the binding groove is more close to the helix 1 region.

Karki *et al*. [9, 21～23] have identified that several residues near the active site are critical for binding viral DNA, they are Q148, K156, K159, K160, R166, K186, R187, K188. Except for Q148, all the other residues locate on the loop 1, loop 2 and helix 1 regions. Residues H67, E69, K71, N117, K159 and R166 lie proximal to the human DNA. Residues Q62, H67, N120, N144, Q148 and N155 are critical for HIV-1 integration and replication [21]. Residues Q62, H67 lie on the sheet 1 region, residue N155 lies on the helix 1 region, other 3 residues lie on the loop 3 region. A binding mode obtained from 31 DKA inhibitors indicates that DKA inhibitors probably chelate the metal ion in the catalytic site, and also possess another set of substituents may occupy the position contacted with the viral DNA to prevent the exposure of the 3′-processed end of the viral DNA from human DNA[10]. While in the mutant complexes, S-1360 is tend to be far from the helix 1 region, and its binding groove excludes the critical residues K156 for binding viral DNA and K159 for both viral DNA and human DNA. Therefore, these drug-resistant mutations cause the change of the binding mode and decrease the effect of S-1360.

2.3 Conformational changes of the loop 2, helix 1 and loop 3 regions

As mentioned in Figure 2, the loop 1, loop 2, helix 1 and loop 3 regions have larger fluctuations. To explore which parts of those regions were more flexible and affected by the drug-resistant mutations, we monitored the root-mean-square fluctuations (RMSF) of the Cα atoms for each residue during MD simulations. It can be seen from Figure 5 that residues from 138 to 166 in the loop 2, helix 1 and loop 3 regions and from 187 to 195 in the loop 1 region show larger fluctuations in the 3 mutant complexes as compared with the wild-type complex. Since the loop 1 region is far away from the active site, we focused on the loop 3, helix 1 and loop 2 regions

which are closed to the active site. The result is consistent with previous studies in which the mobility of the loop 3 is lower in the wild-type complex as compared with the mutant IN complexes[6~8, 24, 25].

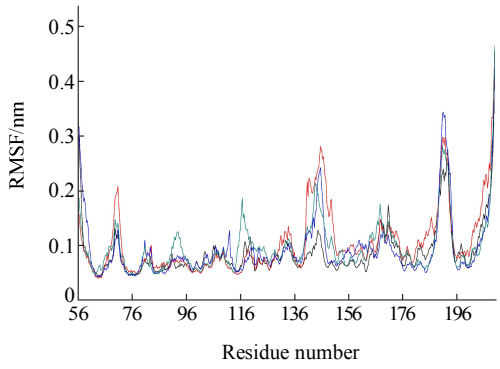

Fig. 5 RMSF of the Cα atoms as a function of residue number

—: Wild-type IN; —: M#30 IN; —: M#50 IN; —: M#70 IN.

The reason for the larger fluctuations of the loop 2, helix 1 and loop 3 regions in the 3 mutant IN complexes is the lack of 2 important hydrogen-bonds interaction between S-1360 and residues in these regions. There are 2 hydrogen-bonds between S-1360 and residues N155 and K159 located at the helix 1 region in the wild-type IN complex, those hydrogen bonding interactions restrict the flexibility of the helix 1 and loop 2 regions. The fluctuations in the region of residues 138 ~ 166 are important to the biological function of IN. As mentioned above, residue K159 is critical for the binding of both viral DNA and human DNA, and residue N155S is critical for HIV-1 integration and replication and it is also associated with resistance to S-1360[26, 27]. According to the X-ray analysis, the 5-CITEP inhibitor is hydrogen-bonded to several residues in the active site, such as T66, Q148, N155, E152, K156 and K159 [6]. These results may partially explain that the inhibitory mechanism of DKA inhibitors is by decreasing the mobility of the region, this lower mobility is caused by competitively interacting with critical residues for binding viral or human DNA, and simultaneously interacting with critical residue for HIV-1 integration and replication. In contrast, there is no hydrogen-bond between S-1360 and the residues located in the helix1 and loop2 region in the M#30 IN complexes. In the M#50 IN complex, S-1360 just forms one hydrogen-bond with the residue Q148 which lies on loop3. In the M#70 IN complex, S-1360 forms hydrogen-bonds with residues D64 and C65 located in the sheet1 region. These results indicated that the lack of the important interaction between S-1360 and the critical residues for binding viral or human DNA and for HIV-1 integration and replication in the drug-resistant mutants is the pivotal reason for the larger mobility of the loop 2, helix 1 and loop 3 regions, and for the decreasing of the inhibition effect of S-1360.

2.4 The orientation of phenyl ring of S-1360

DKA inhibitors probably prevent the exposure of the 3′-processed end of the viral DNA from human DNA[9]. Biochemical experiment data indicate that the benzyl ring group of S-1360 interacts with both 3′ and 5′ terminal nucleobases of the viral DNA by hydrophobic/stacking contact to perform the prevention [28]. In order to compare the orientation of the benzyl ring group in the wild-type IN complex with the 3 drug-resistant mutant complexes, we monitor the distance between the DDE center and the phenyl ring center of S-1360. As shown in Figure 4a, b, the benzyl ring group is closed to the residues I151, E152, N155, K156 and K159 in the wild-type and X-ray IN complex. They are the critical residues for viral DNA binding and integration, and all lie on the helix1 region. As shown in Figure 6, the average distance between the active center of DDE and the center of the

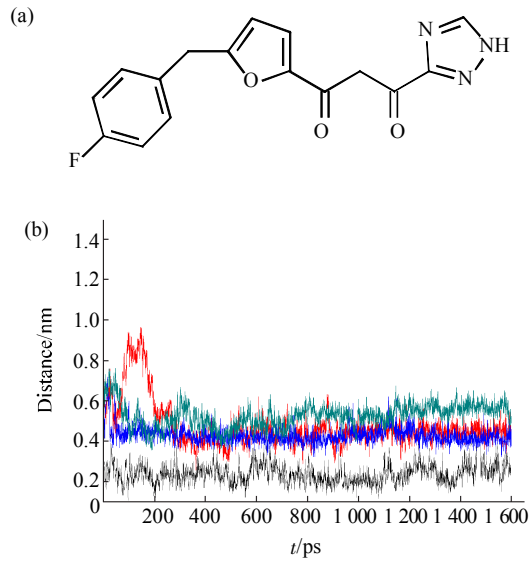

Fig. 6 Distance from the active center of DDE to the center of the phenyl ring of S-1360

(a) S-1360. (b) Distance from DDE center to phenyl ring of S-1360. —: Wild-type IN; —: M#30 IN; —: M#50 IN; —: M#70 IN.

phenyl ring of S-1360 is 0.25 nm. Whereas in the 3 mutant IN complexes, the benzyl ring group extends towards the loop3 region. It is depicted in Figure 4c～e that the benzyl ring group is closed to the residues I141, Y143, Q148, I151, E152. In Figure 6, the average distance between the DDE center and the phenyl ring group center is 0.45 nm, 0.44 nm and 0.54 nm for M#30, M#50 and M#70 IN complexes，respectively. These results may explain a portion of the drug resistance mechanism of IN mutants. In the 3 mutants, the benzyl ring group of S-1360 is apart away from the viral DNA binding site, therefore the inhibitor can not prevent the exposure of the 3′-processed end of the viral DNA from human DNA as that of in the wild-type IN complex, so these drug-resistant mutant strains show less susceptible to S-1360 than the wild-type strain.

2.5 Role of the T66I mutation

T66I drug-resistant mutation is found to occur frequently in IN strains when one of the inhibitors S-1360, 5-CITEP, L-708906, L-731988 or GS-9137 was used respectively [29], whereas its contribution to drug resistance of the 3 IN mutants has not been declared. The hydroxyl of T66 points to the active pocket in the wild-type complex. After T66 mutates to I66, the long side chain of I66 will occupy the space of the pocket in the 3 mutants (Figure 7). This means that the inhibitor in the mutant IN could not move into the same binding site in the wild-type IN complex or have the same orientation in the pocket. This result is consistent with the observation of a former study[6].

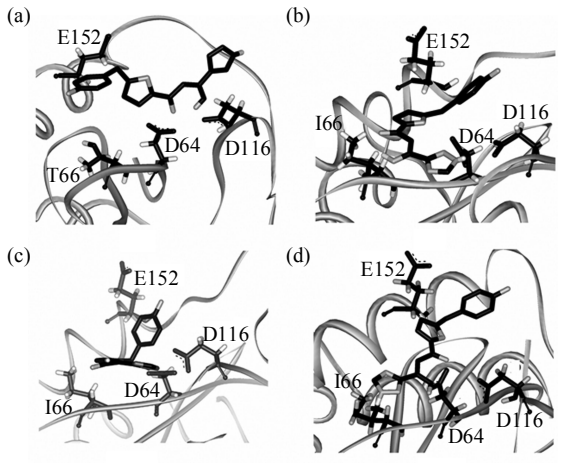

Fig. 7　The orientations of the T66, I66 and the three conserved residues DDE

(a) The wild-type IN complex. (b) The M#30 IN complex. (c) The M#50 IN complex. (d) The M#70 IN complex.

3 Conclusion

In the present study, 3 strains of S-1360-resistant IN were chosen for molecular docking and molecular dynamics simulations study. Compelling differences of binding modes have been observed between the wild-type IN complex and the 3 mutant IN complexes. Based on the binding modes, the flexibility of the regions close to the active site was explored to determine which part is more affected by the drug-resistant mutations. Furthermore, the orientation of the phenyl ring of S-1360 which can prevent the exposure of viral DNA end from human DNA have been analyzed to clarify the mechanism of drug resistance towards DKAs. At last, the contribution of the T66I mutation to the drug resistance was described because the T66I mutation is resistant to multiple DKA inhibitors.

The binding sites are similar in the wild-type and the 3 mutants, but the proximal residues are different. In the mutants, the inhibitor is more close to the functional loop 3 region and far away from the viral DNA and human DNA binding site residues, such as K156 and K159. The conformational flexibilitiy of the functional loop 3, helix 1 and loop 2 regions (residues 138 ～166) are important for efficient biological activity of IN. The larger fluctuations appeared in these regions of the 3 mutants. The reason is the lack of 2 important hydrogen-bonds interaction between S-1360 and residues N155 and K159 in these regions. Residue K159 is critical for binding viral or host DNA, N155 is critical for HIV-1 integration. The 2 hydrogen bonding interactions can restrict the flexibilities of these regions. Better DKA inhibitors can interact with critical residues both for binding viral or host DNA and HIV-1 integration and replication. Whereas no similar hydrogen-bond in the mutant IN complexes. The lack of the important interaction between S-1360 and the critical residues for binding DNA and for HIV-1 integration in the drug-resistant mutants is the pivotal reason for the decreasing of the inhibition effect of S-1360. The experiment data suggested that the benzyl ring group of S-1360 interacts with both 3′ and 5′ terminal nucleobases of the viral DNA to prevent the end of the viral DNA from exposuring to human DNA[10, 29], while in the 3 mutant IN complexes, the benzyl ring group extends towards the loop3 region and it is far from the viral DNA binding site, therefore these drug-resistant mutant strains show less

susceptible to S-1360 than the wild-type strain. T66I drug-resistant mutation is resistant to multiple IN DKA inhibitors such as S-1360, 5-CITEP, L-708906, L-731988 or GS-9137. The T66I mutation makes the long side chain of I66 occupied the active pocket space in the three mutants so that the inhibitor S-1360 could not move into the same binding site or possesses the same orientation. All these factors contribute to the drug resistance of IN to S-1360. In accordance with previous experimental data, the simulation results presented in this study will be helpful for the rational inhibitor design of novel IN inhibitors. Based on these drug-resistant mechanisms, our aim is to gain a 3D-pharmacophore in consideration of the conformational properties of the mutant complexes, furthermore, to modify and design suitable ligands for improving the inhibition potency against drug-resistant IN.

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用分子模拟方法研究 HIV-1 整合酶突变体的耐药性机理 *

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摘要 二酮酸类化合物(DKAs)是目前最有前景的 HIV-1 整合酶(integrase, IN)抑制剂. 为了解 DKAs 引起的多种耐药株共有的耐药性机理，选择 3 种 S-1360 引起的 IN 耐药突变体，用分子对接和分子动力学模拟，研究了野生型和突变型 IN 与 S-1360 的结合模式，基于该结合模式探讨了 3 种耐药突变体所共有的耐药性机理. 结果表明：在突变体中，S-1360 结合到耐药突变 IN 核心区中的位置靠近功能 loop 3 区却远离与 DNA 结合的关键残基，结合位置不同导致 S-1360 的抑制作用部分丧失；残基 138 到 166 区域的柔性对 IN 发挥生物学功能很重要，S-1360 能与 DNA 结合的关键残基 N155 及 K159 形成氢键，这 2 个氢键作用降低了该区域的柔性，突变体中无类似氢键，因而该区域柔性增高；在突变体中，S-1360 的苯环远离病毒 DNA 结合区，不能阻止病毒 DNA 末端暴露给宿主 DNA；T66I 突变导致残基 I 的长侧链占据 IN 的活性口袋，阻止抑制剂以与野生型中相同的方式结合到活性中心，这均是产生抗药性的重要原因. 这些模拟结果与实验结果吻合，可为抗 IN 的抑制剂设计和改造提供帮助.

关键词 耐药性，HIV-1 整合酶，分子动力学

学科分类号 O641，R978.7 **DOI:** 10.3724/SP.J.1206.2008.00656

* 国家自然科学基金(30670497, 30500429)和北京市自然科学基金(5072002, 7082006)资助项目.

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收稿日期：2008-09-22，接受日期：2008-11-25