SOLUTION STRUCTURE OF HIV-1 PROTEASE-ALLOPHENYLNORSTATINE DERIVATIVE INHIBITOR COMPLEX OBTAINED FROM MOLECULAR DYNAMICS SIMULATION

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Structures of two enzyme-inhibitor complexes of human immunodeficiency virus-1 protease with allophenylnorstatine derivatives were obtained from molecular dynamics simulation in aqueous solution. The stronger inhibitor gave considerably smaller fluctuation at P3 site, which formed hydrogen bonding with the enzyme flap region.

KEYWORDS AIDS; HIV protease inhibitor; allophenylnorstatine; molecular dynamics; solution structure

Human immunodeficiency virus (HIV) protease inhibitors have received wide attention as attractive candidates for therapeutic drugs against AIDS,[1] and several crystallographic studies on the enzyme-inhibitor complexes have also been reported.[2] We have already reported a novel class of HIV protease inhibitors bearing allophenylnorstatine [**Apns**: (2*S*,3*S*)-3-amino-2-hydroxy-4-phenylbutyric acid],[3] including a lead tripeptide KNI-102 (**1**: IC_{50} = 89 nM)[3b] and a more potent inhibitor KNI-272 (**2**: IC_{50} = 6.5 nM).[3c, d] In this communication, we describe the structures of complexes of **1** and **2** with HIV-1 protease *in aqueous solution* calculated by molecular dynamics (MD), which may reflect physiological conditions better than a crystallographic approach.

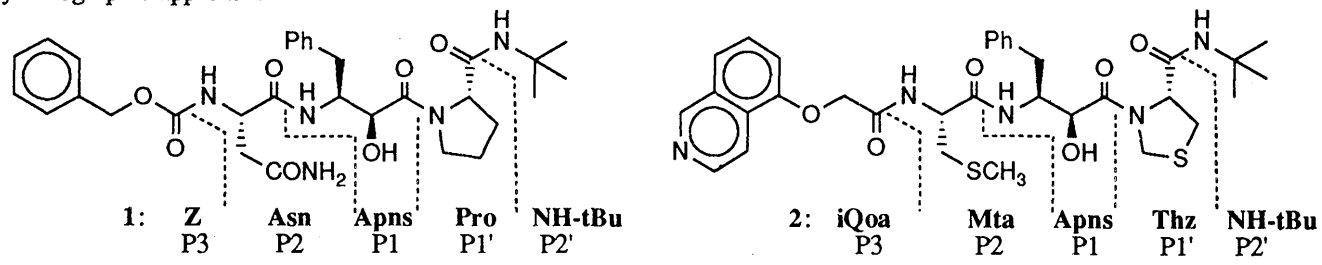

Fig. 1. Chemical Structure of KNI-102 and KNI-272

MD calculations were performed using the AMBER 4.01 program[4] with all-atom force field parameters. Atomic charges for unnatural residues (**Z**, **Apns**, **tBu**, **iQoa**, **Mta**, **Thz**)[5] were determined by electrostatic potential fitting to wave functions with a STO-3G basis set, using the geometry optimized by PM3 semi-empirical molecular orbital method.[6] One of the aspartic acids in the catalytic dyad (Asp25') was protonated,[7] and its atomic charge was taken from the literature.[8] Undecided force-field parameters were estimated from similar chemical species in the AMBER database. About 5000 solvent water molecules were treated explicitly using TIP3P model[9] in each MD-calculation. The MD simulation was performed for 150 ps by 2 fs step under periodic boundary condition without any constraint, except the SHAKE procedure.[10]

The enzyme-KNI-102 complex was modeled based on two crystal structures, a stereoview for Ro31-8959[2b] and Cartesian coordinates for JG-365[2a] (Fig. 2), followed by the MD calculation.[11] The modeling for KNI-272 was carried out

using the final structure of the KNI-102 complex.[11] Since two possible orientations of isoquinoline ring of the inhibitor were considered in the complex, two series of the MD calculations were carried out using two initial structures, one of which has the isoquinoline ring located near the flap region (**2a** in Fig.2) and the other of which bears the ring in the opposite direction (**2b** in Fig. 2).

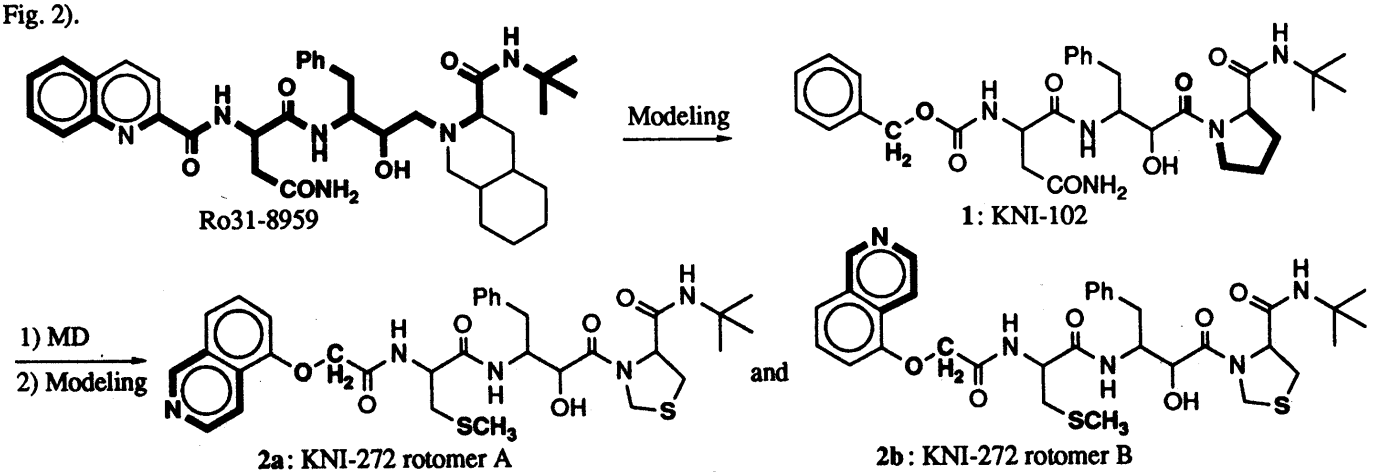

Fig. 2. Strategy for the Modeling of the Inhibitors Bound to the HIV-1 Protease
The template from Ro31-8959, attached parts, and replaced parts are shown in bold.

Interestingly, a bridge water which exists in most of the crystal structures[11] went out of the enzyme during all the simulations. This observation supports the report by Mulochak *et al.*[12] that the water which is important for crystallization is not essential for the enzyme-inhibitor binding in solution.

After the two series of MD calculations for KNI-272, each MD-averaged structure over a 30-picosecond period from 120 ps to 150 ps was minimized and the free energy of association was calculated using Eisenberg's parameters.[13] The structure obtained from **2a**, whose binding free energy was more favorable by 0.7 kcal/mol[14] than that of **2b**, was adopted as the complex between KNI-272 and HIV-1 protease. In this structure, the hydrophobic isoquinoline ring is covered with the flap region[15] and a hydrogen bond is formed between the nitrogen of iQoa and NH of Gly48 in the flap region. More than ten-fold higher inhibitory activity of iQoa derivatives compared with the corresponding 8-quinolinyloxyacetyl derivative with different nitrogen position (our unpublished data) can be clearly rationalized by this hydrogen bond formation. In addition, orientation of hydroxy and carbonyl of **Apns** suggests that they possibly interact with the catalytic Asp25 and Asp25'.

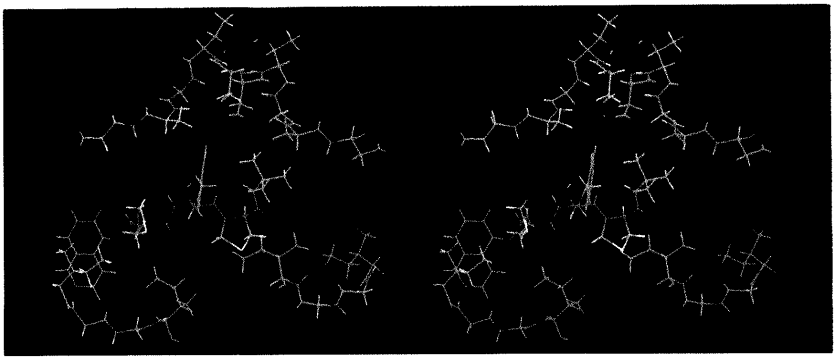

Fig. 3. Steroview of the Complex of KNI-272 with HIV-1 Protease Calculated from the Initial Structure of **2a**
The inhibitor backbone is shown in green. The residues within a 4.2 Å from each moiety of the inhibitor were (Ile47, Gly48, Gly49, Ile50, Pro81') for iQoa, (Val32, Ile47, Ile50, Leu76) for Mta, (Asp25, Gly27, Ala28, Val32, Ile84, Leu23', Asp25', Ile50', Pro81', Val82', Ile84') for Apns, (Val32, Ile54, Pro79, Thr80, Pro81, Ile84, Ile50') for Thz, and (Asp25, Ile84, Asp25', Gly27', Ala28', Asp29', Ile50') for NH-tBu, respectively.

One of the most interesting results derived from the MD simulations on the protease-inhibitor complex is concerned with thermal motions of the inhibitor molecules. The fluctuation of P3 moiety in KNI-272 was markedly smaller than that of KNI-102, which is illustrated in Fig.4 as a superimposition of snapshots of MD trajectories. The iQoa moiety of KNI-272 is considered to afford the tight enzyme-inhibitor binding through the van der Waals and electrostatic interactions, whereas the Z moiety of KNI-102 may interact non-specifically with the hydrophobic regions on the enzyme surface ($Pro^{81'}$, $Val^{82'}$). This smaller fluctuation excellently rationalizes the higher inhibitory activity of KNI-272.

Our MD simulations were able to clarify some of the interesting features of the allophenylnorstatine derivative inhibitors, especially of KNI-272.[16] Further molecular dynamics studies are now in progress.

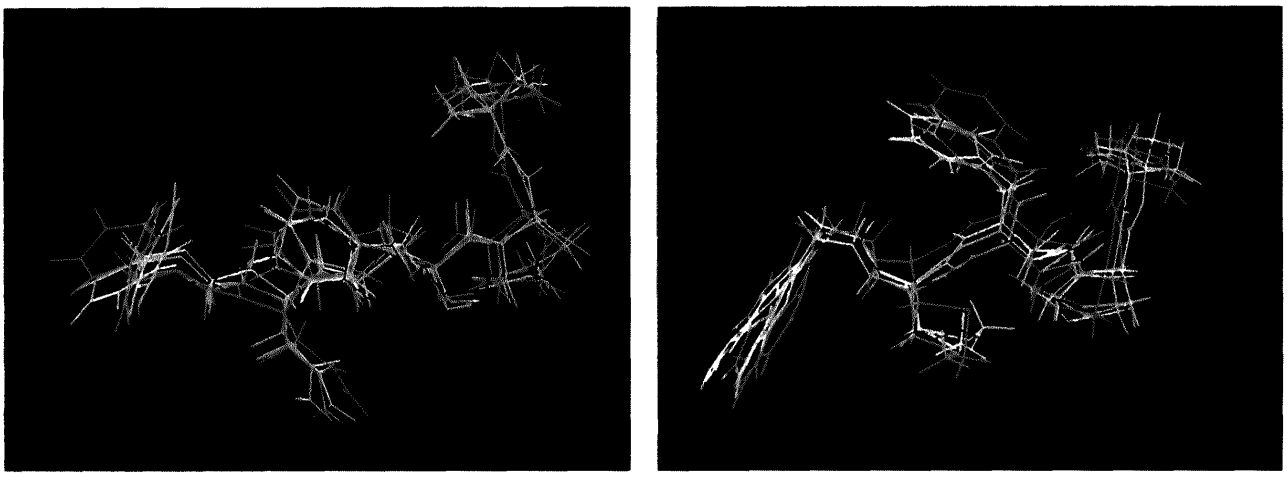

Fig. 4. Superimposition of Snapshots of the MD Simulation for KNI-102 (left) and KNI-272 (right) at 110 ps (Red), 120 ps (Green), 130 ps (Blue), 140 ps (Yellow) and 150 ps (White), Respectively

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5) Abbreviations: Z = benzyloxycarbonyl, tBu = *tert*-butyl, iQoa = 5-isoquinolinyloxyacetyl, Mta = L-methylthioalanine = (*R*)-2-amino-3-methylthiopropionic acid, Thz = L-thioproline = (*R*)-thiazolidine-4-carboxylic acid.
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11) A bridge water which exists in most of the crystal structures (ref. 2) was put into the initial coordinates to form hydrogen bonds between the enzyme flaps (Ile^{50} and $Ile^{50'}$ amide hydrogens) and the inhibitor (P2 and P1' carbonyl oxygens).
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14) The free energy difference corresponds to 3-fold strong inhibitory activity.
15) Recently, examples of the interaction between the P3 moiety and the flap were crystallographically reported in ref. 2c.
16) Crystal structure of the KNI-272 complex will soon be reported by J. W. Erickson *et al.*

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