HIV-1 Integrase Inhibitor Interactions at the Active Site: Prediction of Binding Modes Unaffected by Crystal Packing

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Received April 3, 2000

Crystal structures of an inhibitor or substrate bound to an enzyme generally constitute the preferred starting point for structure-based drug design.[1−5] Although this approach is normally very effective for the rational optimization of lead compounds,[6] limitations can arise when the crystal environment significantly affects the observed binding mode and leads to differences with respect to the solution phase. Such situations have the potential to be misleading for working hypotheses about the enzyme−inhibitor interaction. Here, we outline a computational docking study of these issues for HIV-1 integrase (IN), the enzyme responsible for the integration of reversely transcribed viral DNA into host cell DNA.[7−9]

IN represents an important, but yet unexploited target for drugs that could complement the combination therapy focused on reverse transcriptase and protease.[10−12] A problem for the design of IN inhibitors is the current lack of detailed structural information about IN−inhibitor interactions. The recently published X-ray structure of the IN catalytic core domain complexed with inhibitor 5CITEP (1-(5-chloroindol-3-yl)-3-(tetrazolyl)-1,3-propanedione enol) is at the moment the only available IN structure with a ligand bound to the active site[13] (PDB ID 1QS4). Although it has been presented as a "platform for antiviral drug design" and is definitely of value in this context, the experimental data[13] and a visual analysis of the structure suggest that crystal packing effects influence the observed position of the inhibitor. Here, we present computational docking studies which support this hypothesis and reveal preferred binding modes in the absence of the crystal environment as refined starting points for further design efforts.

The crystals of the IN catalytic core domain soaked with the inhibitor contain three molecules of IN within the asymmetric unit (A, B, C). Only for the inhibitor associated with subunit A, however, is the density sufficiently well defined to allow unambiguous determination of the inhibitor coordinates. This is most probably related to the fact that in the crystal the active sites of two A subunits (A and A′) are positioned close to each other, forming a cavity that keeps the ligand more tightly in place. The ligand bound to A makes additional contacts with the symmetry-related protein A′ and, more importantly, with the second inhibitor molecule bound to it. To verify whether this crystallographic environment actually influences the binding mode of the inhibitor, docking studies were carried out for both the crystallographic dimer with one prebound inhibitor molecule as well as the isolated monomeric protein as "receptor" for the ligand. The latest version of AutoDock (3.0)[14] was used to thoroughly search the available configuration space. It allows torsional flexibility in the ligand and incorporates an efficient Lamarckian genetic search algorithm together with an empirical free energy function (further details can be found in the Supporting Information).

Docking of the inhibitor to the crystallographic dimer reproduced the X-ray finding with a root-mean-square deviation (rmsd) of 1.5 Å (cf. Figure 1). The corresponding result was ranked with the best binding energy (estimated $\Delta G_{bind} = -9.0$ kcal/mol) and found 12 times in 50 independent docking runs. In the remaining runs, 7 alternative positions were found, but all with larger deviations from the experimental position (ranging from 3.3 to 11.0 Å rmsd) and all ranked with less favorable energy. The deviation of 1.5 Å in the best result is mainly due to a slight shift of the chloroindole ring leading to somewhat improved interactions of the chlorine atom and the indole *NH* with the catalytic center. Despite this small difference, the applied docking procedure is successful not only in finding the correct binding mode but also in attributing the most favorable ΔG_{bind} to it, which underlines the quality of the energy function. (In this context it is also worth noting that the AutoDock free energy function[14] provides a good balance between hydrogen bonds and hydrophobic interactions, in line with the recent reevaluation of their relative importance in drug−receptor interactions.[1])

In contrast to the results for the dimer, docking of the inhibitor to the monomer alone resulted in the two main binding positions shown in Figure 2. In the most frequently found and most favorable position (36 times in 50 independent runs, −6.9 kcal/mol), the chloroindole ring is located in proximity to Gln 148, the keto−enol oxygens point toward Glu 152, and the tetrazole ring is placed close to Lys 159. The main molecular plane is aligned "horizontally" within the active site (i.e. perpendicular to the orientation of the ligand in the X-ray structure). This provides a large surface area for van der Waals interactions with buried residues, especially Thr 66, Asn 155, and Gln 148. More specific interactions are formed between the negatively charged tetrazole and the ammonium group of Lys 159 (2.3 Å minimum distance), between the keto−enol oxygens and the amide of Asn 155 (2.0−2.3 Å), as well as between the indole NH and the Asp 116 side chain (2.1 Å H···O distance). The catalytic residue Asp 64 is a further interaction partner.

The second result of the monomer docking occurred less frequently (10/50), but is associated with the same free energy as the top result, thus representing an energetically degenerate position. Although largely overlapping with the first result, the molecular plane is no longer oriented "horizontally", but tilted with respect to the first docking result in such a way that the chlorine points toward the protein and is completely buried, while the keto−enol oxygens are oriented toward the Asn 155 amide, showing a tighter interaction with this residue. In contrast, the hydrogen bond between the indole and Asp 116 is no longer formed, since the corresponding part of the ligand remains solvent

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10.1021/ja001152x CCC: $19.00
Published on Web 06/07/2000

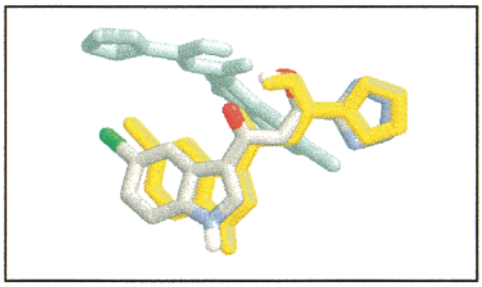

Figure 1. Docking to the dimer structure with one prebound inhibitor molecule (shown in light blue): comparison of the top-ranked docking result (yellow) with the experimental position of the second inhibitor molecule.

exposed. The tetrazole ring is still interacting with Lys 159, but now placed between the two lysine residues 156 and 159.

Comparing the monomer docking results with the experimental position, the overall location within the active site and the relative positioning of the three functional units (tetrazole, keto−enol, chloroindole) are similar. Nevertheless, the rmsd relative to the X-ray structure is 2.8 Å for the first result and 3.2 Å for the second (a measurement relative to the dimer docking result gives 3.4 Å in both cases). The reason for the discrepancy is the different orientation in the binding site and is most probably related to the crystal packing in the experimental structure. In the absence of the crystal environment no contacts with the symmetry-related complex can be formed and it is likely that the ligand prefers a different orientation, an assumption made plausible also by visualization of the experimental structure without the symmetrical complex.

The estimated binding energy for the experimental (dimer) position calculated with the AutoDock function in the presence of the monomer alone is −4.9 kcal/mol and thus 2 kcal/mol less favorable than for the docking results. This explains why a reasonably converged docking run will never end up in this position. Although by a 1.3 Å movement from the experimental position a binding energy of −6.2 kcal/mol can be obtained, the two docked orientations achieve better energy (−6.9 kcal/mol) by placing the tetrazole closer to the lysine residues, moving the keto−enol oxygens farther away from the Glu 152 carboxylate and providing tighter interactions for the chloroindole ring. The estimated K_i (298 K) for these positions is 8.7 μM, which compares well with the experimental IC_{50} value of 2.1 to 2.3 μM,[13] although a direct comparison of K_i and IC_{50} values is of course not feasible. Note also that the estimated K_i for the docked position in the dimer is 0.25 μM and thus an order of magnitude lower; this is perfectly in line with the expectation that the ligand can bind with higher affinity in the dimer cavity than in the exposed monomeric active site.

For the purpose of ligand design, an exclusive focus on the X-ray result may not be useful. Instead, consideration of the docking results could provide better indications of which regions should be explored to arrive at a tighter fit even in the absence of a blocking crystal environment. MD simulations have been started to further clarify the binding mode of 5CITEP, taking into consideration solvation and flexibility of the protein. The poorly defined density for the compound in subunits B and C of the crystal structure suggests, in fact, that the ligand retains considerable mobility and/or adopts different orientations within the binding site. The simulations together with docking studies of other known inhibitors should help to obtain a consensus view of inhibitor interactions at the active site and will provide important support to structure-based design efforts as long as the current difficulties in obtaining experimental structures of IN−inhibitor complexes persist.

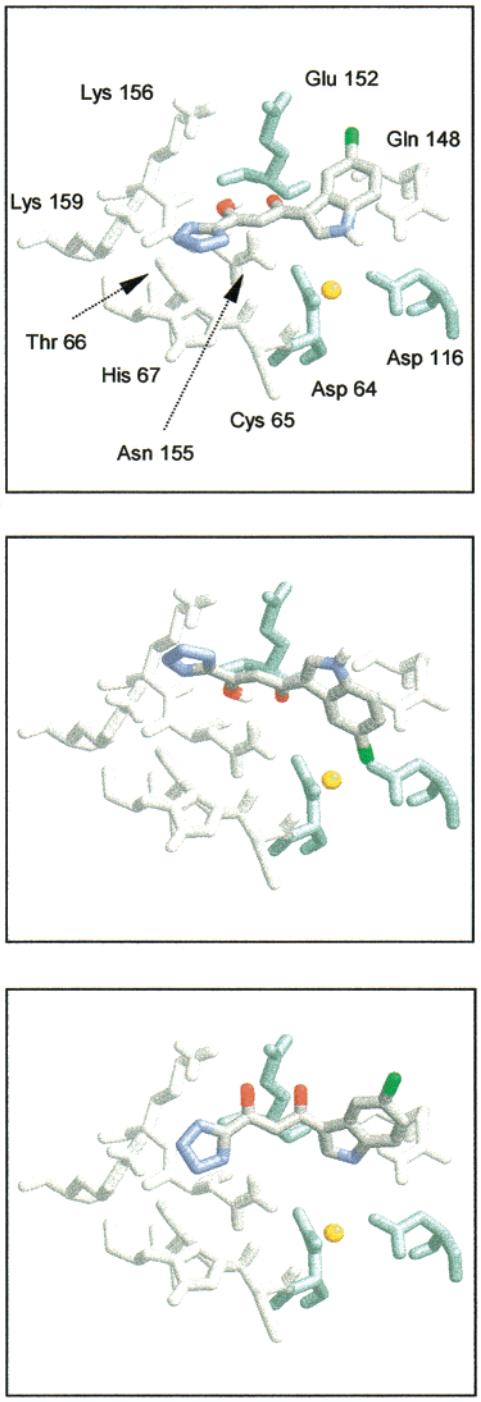

Figure 2. Comparison of the first result (shown at the top) and second result (shown in the middle) for docking to the monomer structure. The figure at the bottom shows the experimental position. The catalytic residues are highlighted in light blue, the metal ion in yellow.

Acknowledgment. C.A.S. is grateful to the Austrian Science Fund for a postdoctoral fellowship (J1758-GEN). We thank MSI for the donation of the QUANTA and CHARMm software and Dr. Arthur Olson for the AutoDock program. We also thank Dr. Heather Carlson for helpful comments. This work is supported by NIH grant GM56553 and through grants of supercomputer time from the NSF.

Supporting Information Available: Details about the setup of the structures and the docking method (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

JA001152X