Acta Crystallographica Section D
Biological Crystallography
ISSN 0907-4449

Rapid screening for HIV-1 protease inhibitor leads through X-ray diffraction

Bindu Pillai,[a] K. K. Kannan,[a] S. V. Bhat[b] and M. V. Hosur[a]*

[a]Solid State Physics Division, Bhabha Atomic Research Centre, Trombay, Mumbai, India, and [b]Department of Chemistry, Indian Institute of Technology, Powai, Mumbai, India

Correspondence e-mail: hosur@magnum.barc.ernet.in

Received 19 September 2003
Accepted 22 December 2003

Knowledge of the three-dimensional structures of HIV-1 protease and of its complexes with various inhibitors has played a key role in development of drugs against AIDS. Hexagonal crystals of unliganded tethered HIV-1 protease in which the enzyme conformation is identical to its ligand-bound state can be used in combination with the soaking method in order to identify potential inhibitor leads *via* X-ray diffraction. The advantages of the soaking method are the generality of application and the rapidity of structure determination for iterative structure-based drug design. Structures of two ligand complexes with HIV-1 protease determined using this method are shown to be very similar to the structures obtained earlier *via* co-crystallization.

1. Introduction

Crystallographic structure-based drug design has led to the design and successful clinical application of six HIV-1 protease (PR) inhibitors in AIDS therapy within a short period of time (Wlodawer & Vondrasek, 1998; Tomasselli & Heinrikson, 2000). However, the emergence of drug-resistant strains of HIV has made it desirable to screen as many chemically different types of ligands as possible in the search for potential lead compounds. A rapid turnover of structural information can assist the process of drug design.

Crystals of a receptor–ligand complex are obtained by either soaking crystals of the receptor in a solution containing the ligand or by co-crystallization. The soaking method is convenient, can be applied to any small ligand molecule, uses very limited amounts of the sample and has the potential for very rapid structure determination using the direct refinement approach. However, for the soaking method to succeed, the active site must be accessible to the ligand through the solvent channel. In contrast, the co-crystallization method is sometimes more tedious since it involves an exhaustive exploration of conditions for the growth of suitable crystals and may also require *de novo* structure determination. In crystals of HIV-1 PR complexed with inhibitors, the flaps of the enzyme assume a closed conformation (Wlodawer & Erickson, 1993). In the tetragonal crystals of unliganded HIV-1 PR, the flaps were in an open conformation (Navia *et al.*, 1989; Lapatto *et al.*, 1989; Spinelli *et al.*, 1991; Wlodawer *et al.*, 1989) and because of this large difference in flap conformation, these crystals were unsuitable for soaking with active-site-binding ligands. Recently, we have reported hexagonal crystals of unliganded tethered HIV-1 PR (Pillai *et al.*, 2001) in which the flaps are in a closed conformation, thus opening up the possibility of using the soaking method for screening potential lead compounds. We report here the successful application of this method to two known HIV-1 PR inhibitors, acetyl-pepstatin and a 4-hydroxycoumarin derivative. We also report that the interactions between these inhibitors and the protein residues observed here are identical to those seen in the corresponding co-crystallized structures (Fitzgerald *et al.*, 1990; Lunney *et al.*, 1994).

2. Experimental

A tethered dimer of HIV-1 PR with Cys95 mutated to Met95 (Cheng *et al.*, 1990) was purified and crystallized according to previously-described procedures (Pillai *et al.*, 2001). Acetyl-pepstatin was purchased from Sigma Chemical Company and the 4-hydroxycoumarin derivative was synthesized at the Indian Institute of Technology, Mumbai. The two inhibitors dissolved in 100% DMSO were mixed with the crystallization buffer (Pillai *et al.*, 2001) to obtain a final inhibitor concentration of 1 m*M*. Native crystals were soaked into these solutions for varying periods of time determined by trial and error. A soaking time of 24 h was found to be optimum for obtaining good electron density for the inhibitors. Diffraction data were collected at room temperature on an R-AXIS IIC imaging-plate detector system mounted on a Rigaku RU-200HB rotating-anode X-ray generator operating at 50 kV and 100 mA. All frames in each data set were indexed in one run using *DENZO* (Otwinowski, 1993), with the starting orientation matrix obtained from the first

Table 1
Crystallographic data-collection and final refinement statistics for complexes obtained by soaking crystals of HIV-1 PR in a solution containing acetyl-pepstatin or a coumarin derivative.

	Acetyl-pepstatin	Coumarin derivative
Resolution (Å)	2.4	2.2
Space group	$P6_1$	$P6_1$
Unit-cell parameters (Å)		
a	63.24	63.14
b	63.24	63.14
c	83.39	83.4
No. molecules per asymmetric unit	One tethered dimer	One tethered dimer
Unique reflections	7170	9243
Multiplicity	3.2	4.5
R_{merge} (%)	5.3	7.7
Completeness (%)	96.3	96.5
R factor (%)	18.9	19.8
R_{free} (%)	27.8	25.4
No. of water molecules	62	65
R.m.s.d. from ideal values		
Bond lengths (Å)	0.006	0.006
Bond angles (°)	1.45	1.28
$B_{average}$ (Å^2)		
Main-chain atoms	33	26.2
Side-chain atoms	33	30.5
$B_{average}$ for both inhibitor orientations (Å^2)	27	26

oscillation frame in the respective data set. The R_{merge} values were very similar when the processed data were merged in either space group $P6_1$ or $P6_122$, although they were slightly lower for the former, especially as the resolution increased. Since the unit-cell parameters were very similar to those reported previously for hexagonal crystals, the crystals were assumed to belong to space group $P6_1$, consistent with previous studies on hexagonal crystals of HIV-1 PR–inhibitor complexes (Erickson *et al.*, 1990; Dreyer *et al.*, 1992). Also, consistent with the assignment of $P6_1$ as the appropriate space group, simulated-annealing $2F_o - F_c$ omit maps calculated by omitting residues 95 and 1095 appear to distinguish the methionine and cysteine residues expected at these positions. (These maps are provided as supplementary material[1].) Additional reasons for the choice of space group $P6_1$ for hexagonal crystals of HIV-1 PR are listed by Wlodawer & Erickson (1993). Since these crystals were isomorphous with those of the unliganded enzyme, the structures of the complex were determined by difference Fourier methods using as a model the protein part of the unliganded tethered HIV-1 PR dimer (PDB code 1g6l). The structures were refined with *CNS* (Brünger *et al.*, 1998) and model building was carried out using the program *O* (Jones *et al.*, 1991). The acetyl-pepstatin model was extracted from the PDB (PDB code 5hvp; Fitzgerald *et al.*, 1990). The coordinates for the coumarin derivative were generated using the software *CHEM3D* (Cambridge Soft Corporation, USA). Crystallographic statistics are given in Table 1.

3. Results and discussion

3.1. Acetyl-pepstatin complexed to HIV-1 PR

All six residues of acetyl-pepstatin (Ace-Val-Val-Sta-Ala-Sta) were clearly defined in the $2F_o - F_c$ maps contoured at 1σ (Fig. 1*a*). Two equally populated binding modes (*A* and *B*) for acetyl-pepstatin related by a pseudo-twofold axis were revealed in these maps. In both the orientations, acetyl-pepstatin was bound in an extended conformation; the hydrogen-bonding pattern for orientation *A* is illustrated in Fig. 2.

Two orientations (twofold-related) of acetyl-pepstatin were also reported in the 2 Å orthorhombic co-crystallized structure (Fitzgerald *et al.*, 1990). A least-squares alignment of the two structures resulted in an r.m.s.d. of 0.6 Å for 198 protein C^{α}-atom pairs and an r.m.s.d. of 0.2 Å for the C^{α}-atom pairs of acetyl-pepstatin. The interactions between acetyl-pepstatin and the protein residues were similar in the soaked and the co-crystallized structures (Fig. 2). The density for a conserved flap water molecule (Wlodawer & Vondrasek, 1998) is clearly seen in the present structure (Fig. 2).

As expected, the overall conformation of the enzyme in the crystal was similar before (PDB code 1g6l) and after soaking in acetyl-pepstatin, with an r.m.s.d. of 0.2 Å. However, the backbone atoms of residues comprising the flaps have moved by about 0.4–0.7 Å (Fig. 3) in a direction towards the inhibitor to form tighter interactions. The side chains of residues 23/1023, 27/1027, 29/1029, 47/1047, 48/1048, 82/1082 and 84/1084 which

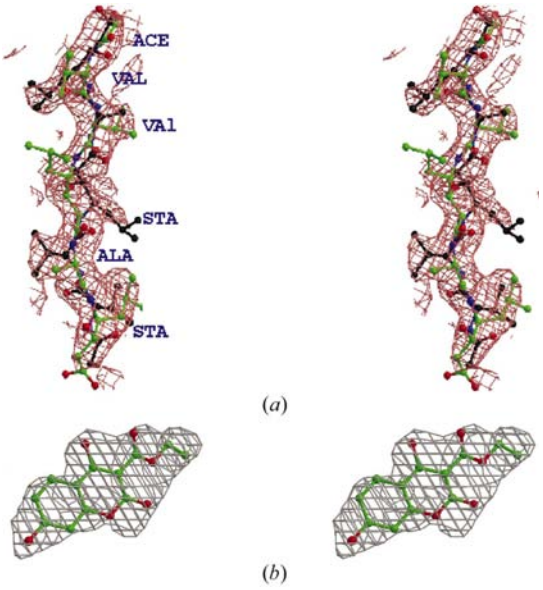

Figure 1
(*a*) Stereoview of the $2F_o - F_c$ map contoured at 1σ corresponding to acetyl-pepstatin in orientation *A* (green). Orientation *B* (black) was not used in the calculation of the maps. (*b*) $2F_o - F_c$ omit map contoured at 1σ showing the density corresponding to the coumarin compound.

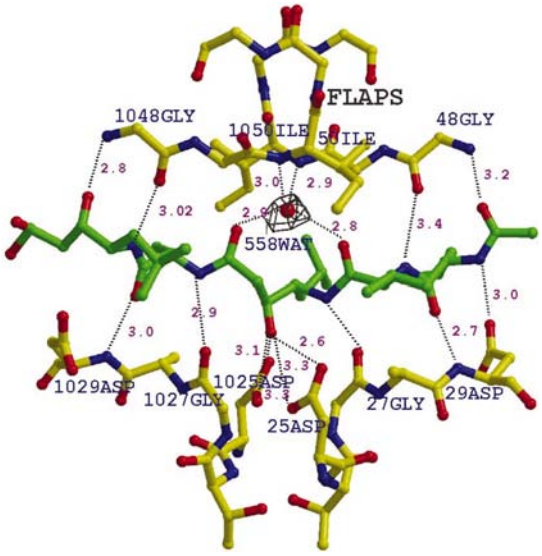

Figure 2
Hydrogen-bonding interactions of acetyl-pepstatin (green) in orientation *A* bound in the active-site cavity of HIV-1 PR (yellow).

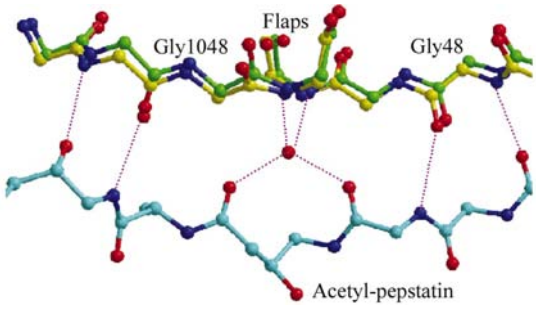

Figure 3
Movement of the flap backbone in the active site of soaked HIV-1 PR crystals (yellow) from its position in 1g6l (green) on binding the inhibitor (cyan).

[1] Supplemetary material has been deposited in the IUCr electronic archive (Reference: SX5009). Details of how to access this material are given at the back of the journal.

form the inhibitor-binding pockets lining the active-site cavity were found to adopt slightly different conformations in response to inhibitor binding.

3.2. Structure of coumarin derivative–HIV-1 PR complex

The general applicability of this method was evaluated by soaking the crystals in a solution containing a non-peptidic 4-hydroxycoumarin-based inhibitor of HIV-1 PR (K_i = 20 μM). After soaking, the crystals diffracted to 2.2 Å resolution and the structure was solved using the direct refinement approach. Electron-density maps (Fig. 2*b*) clearly revealed the inhibitor bound to the active site in orientations similar to that reported for the co-crystallized structure (Lunney *et al.*, 1994). The 4-hydroxyl group of the coumarin ring was located between the catalytic aspartates, while both O atoms of the lactone were engaged in hydrogen bonds with residues 50 and 1050 of the flap region. These hydrogen-bonding interactions were well conserved in the two structures, suggesting that they might determine the mode of binding for this class of compounds.

4. Conclusions

Munshi and coworkers have described a ligand-replacement method for studying HIV-1 PR–inhibitor complexes of a series of potent inhibitors (Munshi *et al.*, 1998). Orthorhombic crystals of HIV-1 PR co-crystallized with a weak inhibitor were used for inhibitor exchange in these studies. However, this method suffers from the following drawbacks: (i) it requires the preparation of a co-crystal of HIV-1 PR with a suitable inhibitor, (ii) it requires prior knowledge of the relative affinities of the lead compounds and (iii) replacing one bound ligand by another in the crystal can cause a drastic decrease in diffraction resolution. In contrast, the method suggested here of soaking into closed-flap hexagonal tethered HIV-1 PR crystals does not require prior knowledge of relative inhibitor-binding affinities and has general applicability; even weakly binding leads can be identified using this method. Since the active-site cavity of the native enzyme contains only loosely bound water molecules, the inhibitor is able to compete and replace them without significant loss in diffraction quality.

We thank the National Facility for Macromolecular Crystallography, BARC for providing the biochemical and X-ray diffraction equipment.

References

Brünger, A. T., Adams, P. D, Clore, G. M., DeLano, W. L., Gros, P., Grosse-Kunstleve, R. W., Jiang, J.-S., Kuszewki, J., Nilges, M., Pannu, N. S., Read, R. J., Rice, L. M., Simonson, T. & Warren, G. L. (1998). *Acta Cryst.* D**54**, 905–921.

Cheng, Y. E., Yin, F. H., Foundling, S., Blomstrom, D. & Kettner, C. A. (1990). *Proc. Natl Acad. Sci. USA*, **87**, 9660–9664.

Dreyer, G. B., Lambert, D. M., Meek, T. D., Carr, T. J., Tomazek, T. A., Fernandez, A. V., Bartus, H., Cacciavillani, E., Hassell, A. M., Minnick, M., Petteway, S. R. & Metcalf, B. W. (1992). *Biochemistry*, **31**, 6646–6659.

Erickson, J. W., Neidhart, D. J., Vandrie, J., Kempf, D. J., Wang, X. C., Norbeck, D. W., Plattner, J. J., Rittenhouse, J. W., Turon, M., Wideburgh, N., Kohlbrenner, W. E., Simmer, R., Helfrich, R., Paul, D. A. & Knigge, M. (1990). *Science*, **249**, 527–533.

Fitzgerald, P. M. D., McKeever, B. M., Van Middlesworth, J. F., Springer, J. P., Heimbach, J. C., Leu, C. T., Herber, W. K., Dixon, R. A. F. & Darke, P. L. (1990). *J. Biol. Chem.* **265**, 14209–14219.

Jones, T. A., Zou, J. Y., Cowan, S. W. & Kjeldgaard, M. (1991). *Acta Cryst.* A**47**, 110–119.

Lapatto, R., Blundell, T., Hemmings, A., Overington, J., Wilderspin, A. M., Wood, S., Merson, J. R., Whittle, P. J., Danley, D. E., Geoghegan, K. F., Hawrylik, S. J., Lee, S. E., Scheld, K. & Hobart, P. M. (1989). *Nature (London)*, **342**, 299–302.

Lunney, E. A., Hagen, S. E., Domagala, J. M., Humblet, C., Kosinski, J., Tait, B. D., Warmus, J. S., Wilson, M., Ferguson, D., Hupe, D., Tummino, P. J., Baldwin, E. T., Bhat, T. N., Liu, B. & Erickson, J. W. (1994). *J. Med. Chem.* **37**, 2664–2677.

Munshi, S., Chen, Z., Li, Y., Olsen, D. B., Fraley, M. E., Hungate, R. W. & Kuo, L. C. (1998). *Acta Cryst.* D**54**, 1053–1060.

Navia, M. A., Fitzgerald, P. M. D., McKeever, B. M., Leu, C. T., Heimbach, J. C., Herber, W. K., Sigal, I. S., Darke, P. L. & Springer, J. P. (1989). *Nature (London)*, **337**, 615–620.

Otwinowski, Z. (1993). *Proceedings of the CCP4 Study Weekend. Data Collection and Processing*, edited by L. Sawyer, N. Isaacs & S. Bailey, pp. 56–62. Warrington: Daresbury Laboratory.

Pillai, B., Kannan, K. K. & Hosur, M. V. (2001). *Proteins Struct. Funct. Genet.* **43**, 57–64.

Spinnelli, S., Liq, Q. Z., Alzari, P. M., Hirel, P. H. & Poljak, R. J. (1991). *Biochimie*, **73**, 1391–1393.

Tomasselli, A. G. & Heinrikson, R. L. (2000). *Biochim. Biophys. Acta*, **1477**, 189–214.

Wlodawer, A. & Erickson, J. W. (1993). *Annu. Rev. Biochem.* **62**, 543–585.

Wlodawer, A., Miller, M., Jaskolski, M., Sathyanarayana, B. K., Baldwin, E., Weber, I. T., Selk, L. M., Clawson, L., Schneider, J. & Kent, S. B. H. (1989). *Science*, **245**, 616–621.

Wlodawer, A. & Vondrasek, J. (1998). *Annu. Rev. Biophys. Biomol. Struct.* **27**, 249–284.