5A, NW12A, NE3A /2007G212, 2008G524

High Resolution X-ray Structure Analysis of HIV-1 Protease in Complex with Potent Inhibitors

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Introduction

Currently, the development of the HIV-1 protease inhibitors is regarded as a major success of structure-based drug design, and the inhibitors of HIV-1 protease are important compounds to establish highly active antiretroviral therapy for AIDS. Despite this remarkable success, adverse effects linked to the use of the HIV-1 protease inhibitors and the emergence of HIV-1 mutants resistant to current drug remains critical factors in clinical failure in antiviral therapy. The mutant HIV-protease (L10F/V32I/M46I/I47V/Q58E/I84V) derived from A17 strain (A17-HIVPR) resistant to Lopinavir and Ritonavir [1] was prepared and crystallized to compare the complex structure with that of wild-type. Besides Lopinavir and Ritonavir, other potent inhibitors (KNI-764[2] and KNI-1657[3]) containing allophenylnorstatine with hydroxyl-methylcarbonyl(HMC) isostere were used to analyze interaction with the drug resistant mutant.

Crystallization and Data Collection

The WT- and A17-HIVPRs were prepared as reported previously[4]. After the inhibitors were dissolved into dimethylsulfoxide at a concentration of 11.5 mM, 2-fold molar excess amount of inhibitors were added to a 2.0 mg/mL protein solution. The protein solution was mixed with the reservoir solution containing 126 mM phosphate buffer (pH 5.0), 63 mM sodium citrate and either 0-30% saturated ammonium sulfate or 20-30% PEG4000 as precipitant. Crystallization was performed using hanging drop vapor diffusion method at 293 K. For data collection, crystals were soaked into the reservoir solution containing 25-45% (w/v) glycerol, then flash-frozen under a N_2 gas cryo-stream (100 K). Collected images were processed using the HKL2000 program suite, and the data collection and the refinement statistics are summarized in Table 1.

Refinement and Structure

The structure models were built using the program XtalView and were refined using the program SHELX97 (Table 1). Electron density maps belonging to the inhibitors bound to the WT- and A17-HIVPRs were clearly observed. Any significant differences of overall structure between the WT- and A17-HIVPRs were not observed. Although most of the positions of catalytically important hydrogen atoms were not determined at the resolution range employed here, accurate information regarding to the interaction between the bound inhibitor and protein obtained from high resolution X-ray structure analysis provides us important information for designing new drug candidates.

References

[1] Mo H., et al., Antiviral Res. 59, 173-180 (2003).
[2] Mimoto T., et al., J. Med. Chem. 42, 1789-1802 (1999).
[3] Hidaka K., et al., J. Med. Chem. 52, 7604-7617 (2009).
[4] Adachi M., et al., Proc. Natl. Acad. Sci. USA, 106, 4641-4646 (2009).

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Table 1 Data collection and refinement statistics

HIV-1 protease	WT-HIVPR	WT-HIVPR	A17-HIVPR	A17-HIVPR	A17-HIVPR
Inhibitor	Lopinavir	KNI-764	KNI-1657	Ritonavir	KNI-764
Beam line	BL5A	NW12A	NE3A	NE3A	NW12A
Data collection					
Space group	$P2_12_12$	$P2_12_12$	$P2_12_12_1$	$P2_12_12$	$P2_12_12_1$
Cell constants (Å) *a, b, c*	59.6, 85.6, 46.4	57.4, 85.9, 46.3	28.7, 66.3, 92.5	60.2, 86.0, 46.5	28.8, 65.6, 92.0
Resolution (Å)	1.10	0.93	0.98	1.00	0.95
Unique reflections	93297	148138	101279	129304	109216
Completeness	95.6	95.9	99.3	98.8	98.6
R_{merge} (%)	8.4	4.5	6.8	8.0	7.6
$I/\sigma I$	51.1	54.0	61.5	63.0	49.3
Average redundancy	9.2	7.7	9.2	12.1	5.7
Refinement					
Resolution range (Å)	48.9-1.10	47.7-0.93	31.2-0.98	49.3-1.00	37.7-0.95
R_{work} (%)	13.5	11.1	14.8	14.4	15.6
R_{free} (%)	16.9	13.1	17.0	16.8	19.6