New Crystal Form of HIV -1 Protease/Saquinavir Structure Reveals Carbamylation of N-Terminal Proline

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Supporting information

Experimental Section

Expression and purification of HIV-1 protease: HIV-1 protease (PR) (Genbank HIVHXB2CG), stabilized by five mutations (Q 7 K, L 33 I and L 63 I to minimize auto-proteolysis, and C67A and C95 to pre-vent aggregation by the formation of disulfide bonds) was expressed in BL21-Gold strain (DE3)pLysS competent cells and purified from inclusion bodies (*1*). PR was purified on FPLC with eluting buffer, 50mM MES pH 6.5, 8 M Urea. The purified PR obtained in unfolded form was stored at 4°C as eluted fractions in 50mM MES pH 6.5, 8 M Urea for a week prior to refolding with saquinavir.

Purified sample of saquinavir (SQV) was generously supplied by Professor Benedetti's group of the University of Trieste, Italy.

Refolding of PR with saquinavir: The eluted PR fractions were serially diluted in ratio 1:1000 of 50 mM formic acid pH 2.5 and PR was concentrated to 2–5 mg mL^{-1}. The pulsed dilution technique (*2*) was used to refold the PR with a 5-fold molar excess of SQV in 50 mM sodium acetate buffer, pH 4.5 (refolding buffer).

Crystallization: High throughput robotic crystallization screen using the Tecan Robot (Freedom EVO 100) was performed on HIV PR/SQV complexes. After screening optimal conditions for crystal growth, the manual hanging drop method was employed to grow crystals of suitable size for high resolution X-ray diffraction. PR/SQV crystals were grown by co-crystallization with the hanging drop, vapor-diffusion method at 20ºC. The reservoir solution contained 0.25M citrate buffer (pH 6), 10% DMSO, and 20–40% saturated $(NH_4)_2SO_4$ as precipitant. The crystallization drops were formed using 1 µL of reservoir solution and 1 µL of protein solution. Crystals of typical dimension, 0.5 x 0.3 x 0.1 mm^3 , suitable for diffraction measurements grew within 2–7 days.

Structure Determination and Crystallographic analysis: Diffraction images were collected at XRD1 Beamline at the Elettra Synchrotron (Trieste, Italy) using a monochromatic wavelength of 1.2 Å. X-ray data were collected on 165 mm MAR-CCD detector from 100 K frozen crystal with 20 % glycerol as

cryoprotecting agent. Data were processed using the Mosflm suite (*3*). Protease coordinates (PDB code: 2AVV) were used as the starting model for molecular replacement with AmoRe. The structure was refined with SHELX (*4*) and refitted with the program COOT (*5*). Alternative conformations for residues were modeled where appropriate. Structural figures were made using PyMOL (*6*). The statistics for the processed diffraction data are given in Table 1. The atomic coordinates for the PR/SQV structure have been deposited in the Protein Data Bank with accession code 3K4V.

REFERENCES

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Table 1S. X-ray data collection and refinement statistics.

Protease		PR
Inhibitor		SQV
Maximum resolution (Å)		1.39
Space group		P21
Unit cell dimensions (Å, °)		a = 51.2
		b = 62.7
		c = 59.1
		β = 98.4
Total reflections		68870
Used reflections		53954
Completeness (%)		97.8 (80.5)[a]
I/σ (I)		9.7 (2.0)[a]
Multiplicity		2.9 (2.7)[a]
Rmerge (I) (%)		8.4 (35.4)[a]
Refinement:		
R_{work} (%)		15.9
R_{free} (%)		22.7
Protein atoms		3136
Inhibitor atoms		98
Other atoms[b]		23
Water molecules		189
RMS deviation from ideality	Bond lengths (Å)	0.009
	Bond angles (Å)	0.028
Average B-factors (Å^2)		
Protein main chain, side chains		18.0, 24.7
Carbamyl group		29.7
Inhibitor atoms		15.3
Water molecules		33.2
Other atoms [c]		22.1

[a] Value in parentheses: highest resolution shell. [b] 2 YCN, 4 DMSO, 1 Cl^-

[c] DMSO, Cl^-

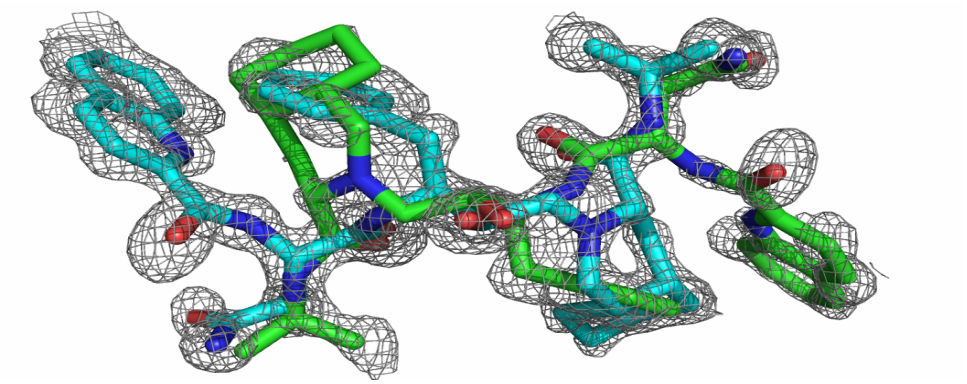

FIGURE 1S. Electron density 2Fo–Fc map (counter level at 1.0σ) of the catalytic channel of HIV-1 protease showing double orientation of saquinavir ligand.

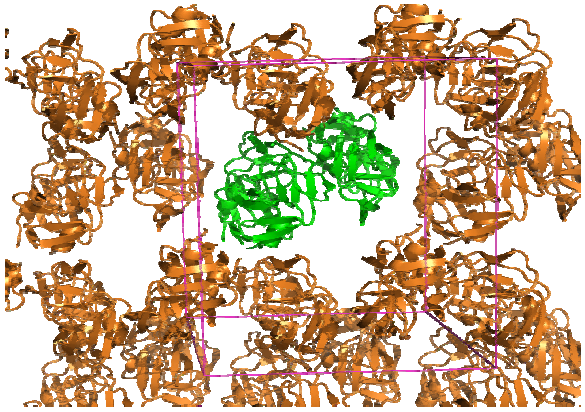

FIGURE 2S. Crystal packing. The monoclinic crystal form in space group $P2_1$ has two dimers in the asymmetric unit shown in green.

Liquid Chromatography / Mass Spectrometry analysis. PR crystals were picked and washed in the mother liquor (reservoir solution) before putting into 5µL of MQ H_2O. This was gently crushed with a needle and final dissolution of the crystals was achieved with the addition of 1µL of 2.5% trifluoroacetic acid (TFA). This solution was diluted to 25 µL with MQ H_2O. The sample containing about 100 ng of protein and 0.1% TFA was analysed by a 1200 series Agilent capillary HPLC system using an Agilent column (0.5 mm ϕ, C18 SB300, 15 cm long). Proteins were separated by means of a linear water /acetonitrile gradient (water 0.05% TFA: solvent A, acetonitrile 0.05% TFA: solvent B. gradient: from 20% B to 70 %B in 50 min). Eluting proteins were directly analysed by ESI-MS using an HCT-ultra (Bruker) ion trap mass spectrometer scanning from 600 to 1500 (m/z). Protein molecular masses (Da) were obtained by deconvoluting m/z spectra.

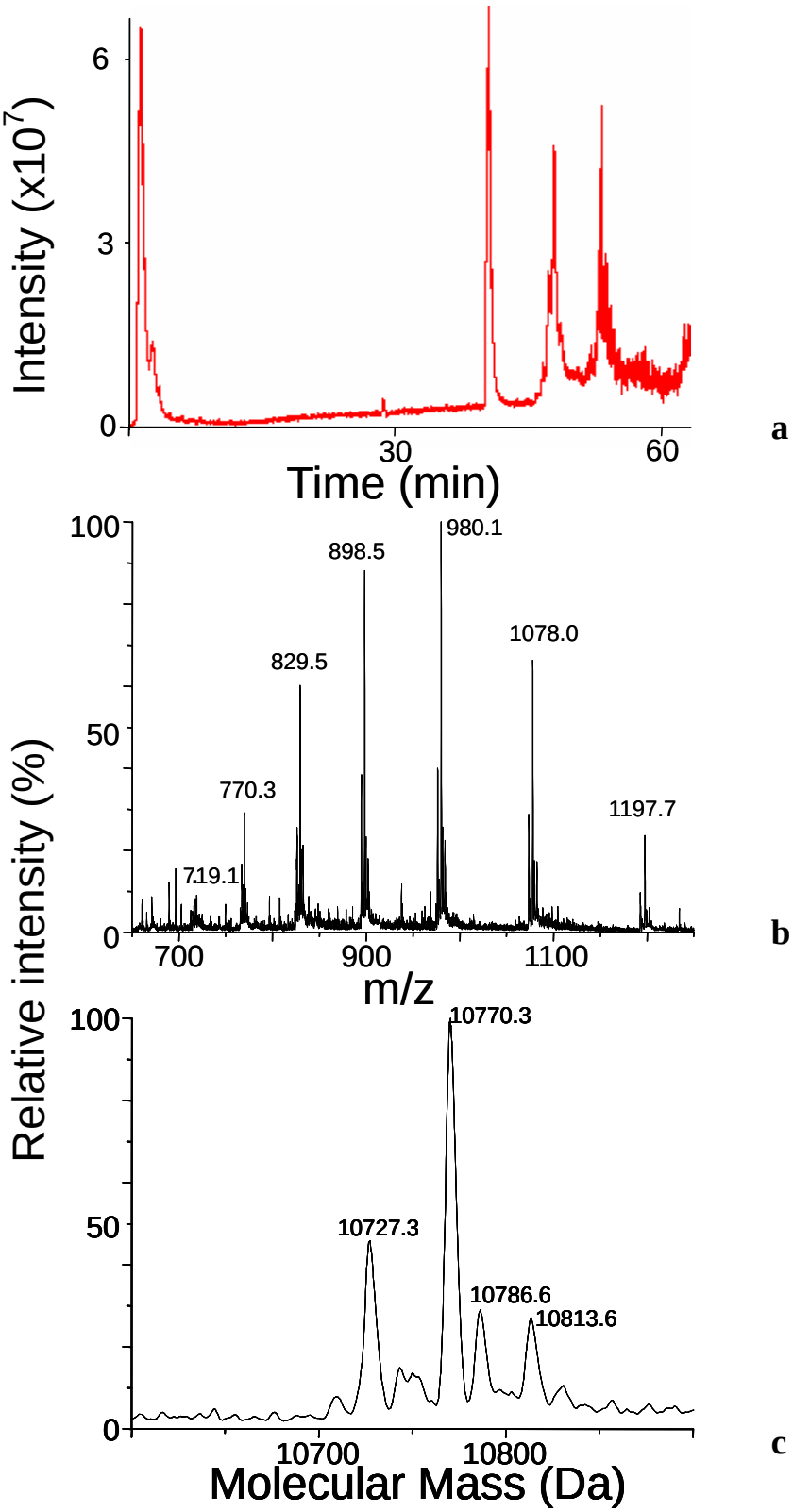

FIGURE 3S. Liquid Chromatography / Mass Spectra: (a) LC Chromatogram; (b) m/z spectra; (c) deconvoluted m/z spectra.