JOURNAL OF THE AMERICAN CHEMICAL SOCIETY
J. Am. Chem. Soc., 1998, 120(38), 9748-9752, DOI:10.1021/ja9818001

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Methods. Crystals[20] were soaked, at 4°C, in a saturated imipenem solution in 20 mM Na,K-phosphate, pH 7.8, containing 50% of saturated ammonium sulfate. After 20 min, half of the solution was replaced by a fresh saturated imipenem solution, and the soaking was pursued for another 20 min. The crystal was mounted in a cryoloop, transferred in a 50% (v/v) PEG 20K solution for a few seconds and frozen in a 100 K nitrogen flux. Diffraction data were collected on a MarResearch imaging plate at the X31 synchrotron beamline of the EMBL outstation at DESY (Hamburg, Germany), with detector distance 120 mm, wavelength 1.1271 Å and frame size 1°. Data were processed using MOSFLM 5.40[21] and CCP4[22]. A rigid body refinement, using the TEM-1 structure (PDB entry 1btl), was performed to account for the cell parameters variation between the native and derivatized crystals. All reflections between 18 and 1.8 Å were used in refinement with REFMAC[23]. An external bulk solvent contribution was computed with X-PLOR[24] and updated after each major refinement cycle. The electron density map after the first refinement cycle allowed identification of all the substrate atoms. Water molecules were automatically introduced/removed and refined using a procedure that alternates the use of REFMAC[23] and ARP[25]. The final crystallographic R and Rfree[26] values are 0.173 and 0.238, respectively. The final model comprises all proteins and substrate atoms, 8 sulfate ions and 347 water molecules. Alternate conformations were assigned to 13 side chains.

The energy minimization procedures and molecular dynamics simulations were performed using the AMBER 4.1 package[27]. In all starting conformations, the ester oxygen in the canonical acyl-enzyme species was in the oxyanion hole, making hydrogen bonds to the backbone nitrogens of Ser-70 and Ala-237. The crystallographic water molecules were retained and the complexes were immersed in a shell of water of 12 Å thickness from the surface of the enzyme complex. The energy-minimizated (10,000 cycles) complexes were used as the starting conformations for the molecular dynamics simulations. The structures were warmed from 0-300 K in steps of 20 K per 1 ps. Simulations were carried out for 165 ps for each of the two complexes at 300 K The coordinates were collected for every 0.05 ps during the first 20 ps, and for every 0.2 ps thereafter. They were measured using the CARNAL module in AMBER package and plotted as a function of time.

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Table 1. Summary of crystallographic analysis

Data collection

Resolution (Å)	Reflections Measured/unique		Completeness[1]	I/<σI>	R_{sym}[2]
18- 1.8	84, 646	21, 770	98.3 (96.8)	20.9 (8.7)	5.9 (11.6)

Refinement

Atoms				Temperature factors (Å^2)			R.m.s. deviations			
protein	imipenem	solvent and ions	R / R_{free}[3]	protein	inhibitor	solvent	Bonds	Angles	Dihedrals	Impropers
2090	20	387	0.173 /0.238	9.50	13.47	21.42	0.010 Å	1.9°	23.4°	1.6°

[1]Numbers in parenthesis refer to the highest resolution shell (1.89-1.80 Å).

[2] $R_{sym} = \sum\sum|\langle I\rangle - I_i| / \sum\sum I_i$

[3] $R = \sum||F_o| - |F_c|| / \sum|F_o|$

The R_{free} value was calculated from a random set of reflections (10%) which were omitted from structure refinement. The R value was calculated from the remaining reflections.

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