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Supporting Information

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SI Text

α -domain:

Fig. S1. Multiple sequence alignment of PvdQ from *Pseudomonas aeruginosa* PA01 with PvdQ-like AHL-acylases from other *Pseudomonas* species, aculeacin acylase from *Actinoplanes utahensis* and penicillin V acylase from *Streptomyces mobaraensis*. Residue numbering is according to PvdQ. The secondary structure of PvdQ is shown at the top, helices in red, β -strands in blue. The conserved cysteines are indicated in bold red, the disulfide bridges are numbered in green below the sequence. The residues that form the substrate binding site (L) are indicated in bold orange while the catalytic residues are indicated in bold green: N-terminal nucleophile (*), the nucleophile activating residue (A) and the oxyanion hole residues (O). Residues in the α -chain between brackets (<>) were visible in the electron density. The conserved Arg297 is indicated in blue.

β-domain:

Fig. S1 (continued).

Fig. S2. Catalytic mechanism of the N-acyl homoserine lactone acylase PvdQ. (a) Water 1 is involved in the activation of the Serβ1 nucleophile by relaying the proton from the O_γ to the α-amino group. (b) Upon nucleophile activation the N-terminal nucleophile attacks the carbonyl carbon of the scissile bond in the substrate. The transition state is stabilized by the oxyanion hole formed by a backbone amide and a side chain amide (c). Upon protonation of the α-amino group by Wat1 the transition state collapses into the free amine and an ester intermediate, which is subsequently attacked by the Wat1 hydroxylate (d). Upon the nucleophilic attack by the hydroxylate the tetrahedral transition state is again stabilized by the oxyanion hole (e). The collapse of the transition state results in the free acid product (f).

Table S1.

	apo	3-oxo-C ₁₂	C ₁₂	Covalent C ₁₂
Data collection				
Beam line (ESRF)	ID14-2	ID29	BM16	ID14-2
Resolution (Å)	40.0–1.8 (1.9–1.8)	40.0–1.9 (2.0–1.9)	39.1–1.9 (2.0–1.9)	44.5–2.1 (2.2–2.1)
Reflections	85309	75009	73345	56251
Space group	C222 ₁	C222 ₁	C222 ₁	C222 ₁
Cell dimensions (Å)	120.1, 163.9, 93.6	120.8, 166.5, 94.3	120.8, 166.4, 94.3	121.1, 167.1, 94.3
R _{sym} [¶] (%)	3.8 (34.0)	5.6 (35.6)	7.8 (47.1)	11.8 (57.0)
Wavelength (Å)	0.933	0.933	0.979	0.933
I/σ(I)	24.4 (4.4)	15.5 (3.7)	14.1 (2.2)	11.0 (2.6)
Completeness (%)	99.7 (99.7)	99.9 (100.0)	98.1 (88.6)	99.7 (98.5)
Redundancy	4.1 (4.0)	3.7 (3.7)	4.2 (2.9)	4.3 (4.3)
Refinement				
Resolution (Å)	40.0–1.8	40.0–1.9	39.1–1.9	43.5–2.1
Number reflections	81004	70962	69623	53387
R _{work} /R _{free} [§] (%)	16.0/18.9	16.3/19.0	16.7/19.4	17.7/21.2
Number of atoms				
Protein	5667	5625	5629	5596
Ligand/water	42/516	73/396	76/432	36/325
B-factors				
Protein	28.3	26.8	27.0	23.9
Water	26.4	21.3	23.9	17.9
rms deviations				
Bond lengths (Å)	0.010	0.009	0.010	0.009
Bond angles (°)	1.20	1.12	1.14	1.12
Ramachandran				
Favored/outliers (%)	96.9/0	97.2/0	97.2/0	97.1/0

$$^1R_{\text{sym}} = \sum_{hkl} \sum_i (hkl) - \langle I(hkl) \rangle / \sum_{hkl} \sum_i I_i(hkl)$$
$$R = \sum |F_{\text{obs}}(hkl) - F_{\text{calc}}(hkl)| / \sum |F_{\text{obs}}(hkl)|$$

R_{free} is the R value calculated for 5% of the data that were not included in the refinement.