Supplementary material

Aqueous Subtilisin, with/without Cs soaking, wavelength 2.070 Å

1. Crystallization, cryo-protection and Cs soaking

Subtilisin Carlsberg was purchased from Sigma (product code: P5380) and used for crystallization without further purification. The protein powder was dissolved in 330 mM Na cacodylate buffer at pH 5.6 to a concentration of 10 mg/mL. Subtilisin was crystallized by the batch method from buffer solution saturated with Na_2SO_4 ~13% (w/v) as precipitant[1]. Long needle-like crystals grew over a period of two weeks to typical dimensions 50 x 50 x 400 μm3. Native crystals were cryo-protected with a solution of 25% Glycerol and 75% buffer reservoir. Cs derivative was prepared by soaking a single crystal with cryoprotectant (25% Glycerol; 75% Buffer reservoir) containing 2 M CsCl for 1 minute.

2. Data collection and refinement

Data collection was performed on SRS BL10[2] Daresbury Laboratory. Diffraction data were collected using softer X-rays[3] at 2.070 Å and processed using the HKL2000 package[4]. Initial phases were calculated from PDB model code 2wuw[1] for the aqueous subtilisin model and 2wuv[1] for the Cs derivative subtilisin model. Models were refined with Refmac 5.0[5] and the protein regions displaying different conformations were rebuilt with COOT[6]. Anomalous difference Fourier maps were calculated and electron density peaks contoured at 4sigma level or higher were taken into consideration. Ions were assigned on the basis of coordination geometry, map peak height, B-factor, and occupancy of each site. Cl^- and Cs^+ Ion occupancies were refined so to model a B-factor equal to the average of the surrounding protein atoms within a radius of 7 Å of each ion. This adjustment was performed using *ION_GRINDER*, a Python script which uses the Python interfaces of the Computational Crystallography Toolbox (CCTBX)[7] to work on PDB models. Final R_{factor} (R_{free}) were 15.9 (22.6) and 15.6 (23.4) for the aqueous subtilisin model and for the Cs derivative subtilisin model respectively.

Table 2 supplementary. Data collection, data processing and refinement statistics for aqueous subtilisin, with and without Cs soak.

	Aqueous	**Cs-Aqueous**
PDB deposition code	4c3v	4c3u
Crystallographic data		
Wavelength (Å)	2.070	
Cell parameter (a, b, c)	51.89, 55.07, 75.31	52.17, 55.23, 75.85
Space group	$P2_12_12_1$	
[ab]R_{merge}	0.07 (0.236)	0.124 (0.210)
[a]Completeness	100.0 (100.0)	99.9 (99.2)
Unique reflections	10628 (1025)	10346 (994)
[a]Redundancy	10.1 (8.5)	5.8 (4.6)
[a]Resolution	50.0-2.26 (2.34-2.26)	50.0-2.28 (2.36-2.28)
[a]I/σ(I)	55.61 (14.3)	10.6 (6.9)
Average B_{factor} from Wilson plot (Å**2)	20.07	23.63
Matthew Coefficient (Vm)	1.98	2.01
Solvent Content (Vs)	37.7	38.7
Refinement results		
R_{factor} (R_{free})	15.9 (22.6)	15.6 (23.4)
bond lengths refined atoms (rms; weight)	0.016; 0.019	0.014; 0.019
bond angles refined atoms (rms; weight)	1.840; 1.948	1.718; 1.941

Cruickshank's DPI for coordinate error (Å)	0.39	0.41
DPI based on free R factor (Å)	0.23	0.25
Number of ions		
Waters	120	118
Cesium	--	12
Chlorine	--	6
Calcium	1	1
Sodium	3	1
Sulfate	3	--
[c]**Ramachandran Plot**		
most favoured regions (%)	89.0	87.3
additional allowed regions (%)	11.0	12.7
generously allowed regions (%)	0.0	0.0
disallowed regions (%)	0.0	0.0

[a]The values in parentheses are for the highest-resolution shell
[b]Rmerge= ΣhklΣi|Ii(hkl)-<I(hkl)>|/ ΣhklΣi |Ii(hkl)|
[c]Data taken from PROCHECK[8]

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