MATERIALS AND METHODS

Protein isolation and crystallization

Bacillus pasteurii urease (BPU) was isolated and purified to a specific activity of ca. 2500 units/mg as previously reported.[1] Crystallization of boric acid inhibited BPU was achieved at 20 °C using the hanging drop method. 3 μL of a solution containing 11 mg/mL of BPU in 50 mM sodium phosphate at pH = 7.5, containing 50 mM Na_2SO_3, was diluted with 3 μL of the well solution (1.6-1.8 M ammonium sulfate (AMS) in 100 mM boric acid and 100 mM citric acid brought to pH 6.3 with sodium hydroxide) and equilibrated against 1000 μL of precipitant. Rice-shaped single crystals, of about 0.4 x 0.4 x 0.7 mm^3 on average, formed in few days.

Data collection and evaluation

Diffraction data were collected on a single crystal of the inhibited BPU at 100 K using synchrotron radiation from the X-13 line of the DORIS storage ring at the EMBL outstation at DESY, Hamburg (Germany). The detector was a MAR-CCD 162 mm. For cryo-protection, a single crystal, of dimensions 0.3 x 0.3 x 0.5 mm^3, was transferred from the crystallization drop into 10 μL of the well solution, with the AMS concentration increased to 2.2 M to prevent the crystal from dissolving and containing 20% ethylene glycol. The crystal was scooped up in a cryo-loop and rapidly exposed to a cold nitrogen stream.

The data were processed using the program DENZO and merged with SCALEPACK.[2] The data collection statistics are reported in Table 1. The cell is isomorphous with that of the native enzyme (Table 1) and contains four urease molecules lying on the special positions of point symmetry three. Therefore the asymmetric unit consists of one third (one $\alpha\beta\gamma$ moiety[4]) of the $\alpha_3\beta_3\gamma_3$ urease molecule.

Structure determination and refinement

Initial phases were obtained from the refined model of the native enzyme (PDB code 2UBP[4]) from which the sulfate anion, the flexible loop region, and all water molecules were omitted. The protein regions displaying different conformations were manually rebuilt with the program O.[5] The structure was refined using REFMAC[6] with established geometric targets.[7] Randomly selected reflections (2 % of the total) were used as an R_{free} set for cross validation. Automatic solvent building was performed using the program ARP, keeping only those water molecules having density greater than 1.5 σ in the 2Fo-Fc electron density map,[8] and that after visual inspection resulted to be in good density regions. No restraints were imposed on the Ni-ligand distances. Omit maps were calculated after several refinement cycles of a model from which the BO_3 atoms were excluded. Using isotropic temperature factors the refinement converged to R and R_{free} of 17.7% and 20.4 %, respectively. The stereo-chemistry of the final model was checked using the programs WHATIF[9] and PROCHECK.[10] The refinement statistics are reported in Table 2. The refined crystallographic coordinates and structure factor amplitudes for BO_3-inhibited BPU have been deposited in the Protein Data Bank under the accession code 1S3T.

References:

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Table 1. X-ray data collection statistics and data reduction for $B(OH)_3$-inhibited BPU

Wavelength (Å)	0.802
Resolution range (Å)	24.8 -2.10
R_{merge} [1]	0.13
Raw measurements	458791
Unique reflections	56215
Redundancy	8.16
High resolution bin (Å)	2.14 -2.10
% Completeness	99.7
% Completeness in high resolution bin	99.9
% Greater than 3σ	71.6
% Greater than 2σ in high resolution bin	49.9
Redundancy in high resolution bin	6.8
I/σ	13.9
I/σ in high resolution bin	3.5
Space group	$P6_322$
$a = b$ (Å)	130.91
c (Å)	189.37

[1] $R_{merge}=\Sigma|I_i - \langle I\rangle| / \Sigma\langle I\rangle$, where I_i is an individual intensity measurement, and $\langle I\rangle$ is the average intensity for this reflection with summation over all the data.

Table 2. Summary of the crystallographic analysis and refinement for $B(OH)_3$-inhibited BPU

Protein atoms	5959
Solvent atoms	406
Bound metal ions	2 Ni
Bound inhibitor atoms	4
Temperature factors for protein atoms (Å^2)	21.5
Temperature factors for solvent (Å^2)	28.6
Temperature factors for Ni(1), Ni(2) (Å^2)	21.75, 20.94
Average Temperature factors for BO_3 atoms (Å^2)	24.27
R.m.s. bond length deviation (Å)	0.012
R.m.s. bond angle deviation (degrees)	1.286
R.m.s. planes deviation (Å)	0.011
R.m.s. on final 2*Fo-Fc* electron density map (electrons/Å^3)	0.34
Ramachandran most favored region (%)	89.8
Ramachandran additional allowed region (%)	9.2
Ramachandran generously allowed region (%)	0.9
R-factor (R-free)[1] (%)	17.8 (20.4)

[1] R-factor = $\Sigma \, | \, |F_O| - |F_C| \, | / \Sigma |F_O|$; R-factor and R-free are calculated by using the working and free reflection sets, respectively; the free reflections (2 % of the total) were held aside throughout the refinement.