Conditions for PaDsbA2 crystallization

Wild-type PaDsbA2 in 10 mM Hepes pH 7.0 and 100 mM NaCl buffer at three different protein concentrations was used for screening. The protein concentrations used were 2.9 mg/ml, 5.8 mg/ml and 11.6 mg/ml. The 2.9 mg/ml and 5.8 mg/ml protein concentrations were made from 11.6 mg/ml stock. JBScreen Cryo HTS, JBScreen JCSG++ HTS and JBScreen Classic 5-8 from Jena Bioscience were used to screen using 3 well Intelli plates. The drops were 100 nl protein to 100 nl crystallization solution and were set up using a robot. The crystallization was by vapor diffusion using sitting drops. After three weeks of incubation at 20°C, one hit was found in 20 % PEG 400, 30 % PEG 4000, 100 mM sodium citrate pH 5.6 50 mM ammonium citrate and another hit in 0.1 mM Bis-Tris pH 5.5, 2.0 M ammonium sulfate. Further optimization by changing ammonium sulfate concentration gave fewer and bigger crystals in 1.5 M ammonium sulfate.

Data collection

Before data collection, the crystals were cryo-protected in mother liquor containing 20 % glycerol before flash freezing in liquid nitrogen. Highly redundant data for S-Sad phasing were collected in house on a rotating anode, Rigaku Micromax-007 HF from Rigaku comprising a Saturn944+ detector at 100 K by changing the kappa angle from 0° to 25° in increments of 5° for low and high resolution data. The redundant high resolution datasets were collected at a detector distance of 50 mm with 4 s exposure time and 1° oscillation range while redundant low resolution data sets were collected at a detector distance of 80 mm, 2.5 s exposure time and 1° oscillation range. Native high-resolution dataset was collected at a detector distance of 50 mm and 2θ -30°.

Data processing

All data sets were integrated and scaled with Denzo and Scalepack (1) respectively. The redundant data set for phasing had an average multiplicity of 61.3 to 2.1 Å resolution with an overall R-merge of 0.02 and 99% completeness. The data set for refinement was integrated and scaled to 1.3 Å resolution with an overall R merge of 0.016 and 99.8% completeness. Statistics are shown in the table below.

	S-SAD DsbA2	**Native DsbA2**
Beamline	Home source	Home source
Wavelength (Å)	1.54178	1.54178
Space group	P212121	P212121
Unit cell a (Å)	47.815	47.815
b (Å)	59.280	59.280
c (Å)	59.879	59.879
Resolution of phasing (A)	30-2.1	42.049-1.30
Number of measured reflections	635872	215929
Number of unique reflections	10377	41116
Completeness	99.8 (99.2)	96.5 (83.8)
R_{merge}	0.060 (0.136)	0.120 (0.773)
$\langle I/\sigma(I) \rangle$	16.73 (2.8)	15.58 (3.53)
Redundancy	61.3(44.2)	5.3 (3.2)
FOM		
R_{free}-factor		0.2196
Ramachandran profile		
Core		95.68 %
Other allowed		4.32 %
Disallowed		0.00%
R.m.s. deviations		
Bond lengths (Å)		0.023
Bond angles (°)	-	2.283
		-
R-factor		0.1932
PDB entry		

Structure solution and refinement

Shelxc (2) was used to find the sulfur sites with data from 30 Å to 2.1 Å. In total five sulfur sites could be identified, though seven were the input. The heavy atom sites were input into MlPhare (3) for phasing. The initial phases and their figures of merit (FOM) were input into DM for density modification and subsequently into Buccaneer for automatic model building. Buccaneer automatically traced 80% of the structure with correct side chains and the rest the structure was built manually by iterative model building and refinement using Coot and Refmac (4) respectively using the 1.3-Å dataset.

Analysis of the electrostatic surface

The PDB2PQR server (5) was used to convert the protein files into PQR format and charges were assigned using the PARSE force field (6). The APBS (Adaptive Poisson−Boltzmann Solver) plugin for PyMOL (DeLano Scientific; http://www.pymol.org) was used to map the electrostatic potential (±3 *kT/e*) on to the molecular surface of the protein.

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