Supporting information

Expression, Purification, and Crystallization of pd-Toho-1 E166A/R274N/R276N

The perdeuterated enzyme was purified and crystallized as described previously [1,2]. Briefly, using an Escherichia coli-based expression system, the pd-Toho-1 E166A/R274N/R276N was expressed to a high yield in a fully deuterated minimal medium using a fed-batch fermentation protocol (35, 36). Large crystals for neutron diffraction, along with smaller crystals suitable for X-ray diffraction, were grown at 20 °C via the batch crystallization method using 300 µl of a 10 mg/ml protein concentration in a solution containing 2.0 M ammonium sulfate and 0.1 M sodium citrate (pH 6.1) prepared in D_2O. For ligand soaking crystals were placed for 2-3 h in a reservoir solution containing 2.7 M ammonium sulfate, 0.1 M sodium citrate (pH 6.1), and 5.0 mM cefotaxime. The crystals were then either mounted into a capillary for 293K data collection or placed momentarily in a reservoir solution containing a cryoprotectant (30% w/v perdeuterated trehalose) and then flash-frozen in liquid nitrogen.

Data Collection and Refinement

Time of flight (TOF) neutron diffraction data of the pd-Toho-1 E166A/R274N/R276N cefotaxime complex at 293K were recorded to 2.2 Å from a 0.4mm^3 crystal using the MaNDi instrument[3] at the Spallation Neutron Source (SNS) onsite at Oak Ridge National Laboratory (ORNL). As soon as the neutron data collection on MaNDi had been completed an X-ray diffraction data set was immediately collected at 293K on the same crystal to 1.60Å resolution. The X-ray data were processed using the XDS package[4] whereas the neutron data were processed using the Mantid package[5] and the Lauenorm program from the Lauegen package[6]. Lauenorm was used for wavelength normalization of the Laue data and scaling between Laue diffraction images. Both the neutron and X-ray data were refined to convergence using joint refinement in the *phenix.refine* program[7,8] in the PHENIX suite. Monochromatic (2.67 Å) neutron diffraction data for the pd-Toho-1 G166A/R274N/R276N ligand free structure were recorded to 1.7 Å resolution at 100K using the BIODIFF diffractometer at the FRM II research reactor[9] and reduction of that neutron data was performed using the Denzo and Scalepack packages[10]. As neutrons, unlike X-rays do not cause radiation damage, with protein crystals, most neutron datasets are collected at room temperature. However collecting neutron data at 100K

improves atomic ordering and can help extend the resolution of the final structure. These benefits are evident in the final resolution of this neutron structure which diffracts 1.7 Å yielding one of the highest resolution neutron structures to date.

High resolution monochromatic (0.69 Å) X-ray diffraction data were collected on the ligand free enzyme and the acyl-enzyme complex with cefotaxime using a helium cryostream at 10-15 K over a range of 120° (0.5° steps) using an (Area Detector Systems Corp. Quantum 315r detector) at the Advanced Photon Source (APS) on the ID19 beamline SBC-CAT. The high-resolution X-ray data were again reduced using XDS[4]. Refinement of all the X-ray and neutron models was completed using the *phenix.refine* program in the PHENIX suite[7]. The final structural models of each complex were obtained after several rounds of maximum likelihood-based refinement of individual coordinates, individual atomic displacement parameters, and occupancies. Water molecules were added to the model using *phenix.refine* and were then adjusted manually during subsequent model building rounds based on analysis of σ_A-weighted $F_o - F_c$ and $2F_o - F_c$ positive nuclear density maps. The refinement statistics for all of the neutron and X-ray structures are given in Table 1 with the Ramachandran plot quality assessment being carried out in Molprobity[11]. The data statistics for the refined structures are given in Table 1.

Table 1.

Table 1a. Ligand free X-ray Diffraction Data Collection (15K) and Refinement Statistics

PDB Accession Code	5A91
X-ray data collection	
Unit-cell parameters (Å)	a = 72.40, b = 72.40, c = 97.19 α = β = 90° and γ = 120°
Space group	$P3_221$
No. of unique reflections	86849
Resolution range (Å)	38.41-1.20 (1.26-1.20)
Multiplicity	5.8 (5.7)
I/σ(I)	8.4 (2.5)
R_{merge}[a] (%)	7.3 (34.0)
R_{pim}[a](%)	2.9 (13.7)
Data completeness (%)	96.9 (94.6)
Crystallographic refinement	
R_{factor} (%)	12.94
R_{free} (%)	15.05
$RMSD_{Bonds}$[b] (Å)	0.011
$RMSD_{Angles}$[b] (°)	1.492
Ramachandran plot[c]	
Outliers (%)	0.40
Favored (%)	97.4
Rotamer Outliers (%)	2.30

Highest resolution shell is shown in parentheses

Table 1b. Cefotaxime acyl-enzyme complex X-ray Diffraction Data Collection (15K) and Refinement Statistics

PDB Accession Code	5A92
X-ray data collection	
Unit-cell parameters (Å)	a = 72.54, b = 72.54, c = 98.19 α = β = 90° and γ = 120°
Space group	$P3_221$
No. of unique reflections	138,609
Resolution range (Å)	38.68-1.05 (1.11-1.05)
Multiplicity	5.4 (5.4)
I/σ(I)	8.0 (1.8)
R_{merge}[a] (%)	7.9 (48.1)
R_{pim}[a](%)	3.4 (20.5)
Data completeness (%)	99.5 (100.0)
Crystallographic refinement	
R_{factor} (%)	13.34
R_{free} (%)	15.43
$RMSD_{Bonds}$[b] (Å)	0.015
$RMSD_{Angles}$[b] (°)	1.453
Ramachandran plot[c]	
Outliers (%)	0.70
Favored (%)	96.6
Rotamer Outliers (%)	0.90

Table 1c. Cefotaxime acyl-enzyme complex X-ray and Neutron Diffraction Data Collection (293K) and Joint Refinement Statistics

X-ray data collection	
PDB Accession Code	5A93
Unit-cell parameters (Å)	a = 73.31, b = 73.31, c = 98.87 α = β = 90° and γ = 120°
Space group	$P3_221$
No. of unique reflections	41094
Resolution range (Å)	19.50-1.60 (1.68-1.60)
Multiplicity	6.8 (5.8)
I/σ(I)	6.1 (2.8)
R_{merge}[a] (%)	7.7 (29.7)
R_{pim}[a] (%)	2.9 (11.8)
Data completeness (%)	99.5 (97.6)
Neutron data collection	
Unit-cell parameters (Å)	a = 73.31, b = 73.31, c = 98.87 α = β = 90° and γ = 120°
Space group	$P3_221$
No. of unique reflections	11,803
Resolution range (Å)	15.21-2.20 (2.28-2.20)
Multiplicity	3.90 (2.2)
I/σ(I)	9.4 (3.9)
R_{merge}[a] (%)	17.1 (25.2)

R_{pim} (%)	7.50 (15.2)
Data completeness (%)	71.40 (49.70)
X-ray Crystallographic refinement	
R_{factor} (%)	13.35
R_{free} (%)	15.58
Neutron Crystallographic refinement	
R_{factor} (%)	21.84
R_{free} (%)	23.72
$RMSD_{Bonds}$[b] (Å)	0.012
$RMSD_{Angles}$[b] (°)	1.240
Ramachandran plot[c]	
Outliers (%)	0.40
Favored (%)	98.1
Rotamer Outliers (%)	1.30

Highest resolution shell is shown in parentheses

Table 1d. Ligand free Neutron Diffraction Data Collection (100K) and Refinement Statistics

PDB Accession Code	5A90
Neutron data collection	
Unit-cell parameters (Å)	a = 73.24, b = 73.24, c = 98.53 α = β = 90° and γ = 120°
Space group	$P3_221$
No. of unique reflections	30,302
Resolution range (Å)	40.00-1.70 (1.74-1.70)
Multiplicity	3.9 (3.2)
I/σ(I)	6.5 (1.8)
R_{merge}[a] (%)	19.1 (62.6)
R_{pim} (%)	10.7 (39.7)
Data completeness (%)	88.4 (76.8)
Crystallographic refinement	
R_{factor} (%)	19.19
R_{free} (%)	22.65
$RMSD_{Bonds}$[b] (Å)	0.006
$RMSD_{Angles}$[b] (°)	0.938
Ramachandran plot[c]	
Outliers (%)	0.40
Favored (%)	97.6
Rotamer Outliers (%)	1.40

Highest resolution shell is shown in parentheses

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