Application Note SC-XRD 509

SAD-phasing of Zn-substituted Pseudoazurin and the Influence of Data Multiplicity

Introduction

Pseudoazurin is a blue-copper protein found in the periplasm of denitrifying bacteria. It participates in the electron transport process leading to the reduction of nitrite to mainly nitric oxide. For the requirements of various spectroscopy experiments, substitution of the original Cu(II) by alternative transition metal ions has been carried out successfully. In order to examine the phasing potential of the very weak anomalous signal of sulphur (Δf" ~0.56 e^-) and zinc (Δf" ~0.68 e^-) atoms using an in-house Cu-Kα radiation source, we substituted the native Cu^{2+} of pseudoazurin by Zn^{2+} ion and determined its crystal structure[1] by Zn/S SAD-phasing at 2.2 Å. To achieve this, Single wavelength Anomalous Dispersion (SAD) datasets have been initially collected with high multiplicity. The lowest multiplicity required to obtain the minimal Zn/S substructure for structure determination was also investigated.

Data collection

Data were collected using a Bruker D8 VENTURE diffraction system at 100 K from a flash-cooled, cryoprotected single crystal of Zn(II)-pseudoazurin (Figure 1). The D8 VENTURE features the microfocus IμS sealed-tube source equipped with HELIOS MX optics, a KAPPA goniostat, a PHOTON 100 detector and a low temperature device. A data set consisting of 75 runs and an overall multiplicity of 87-fold was collected in 4 days without any measurable radiation damage.

Data processing

Data collection and reduction were carried out using the PROTEUM2 software suite[2]. The data were integrated and scaled using SAINT[2] and SADABS[2], respectively. Relevant data collection and processing statistics are shown in Table 1.

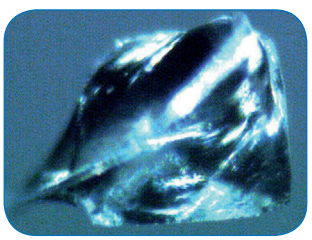

Figure 1. Zn-pseudoazurin crystal

Protein and Crystal

- 123 residues, 5 Met, 1 Cys, 1 zinc-ion
- Space group: $P6_5$
- Unit cell $a=b=49.0$, $c=98.1$ Å
- Crystal size: 0.2 x 0.2 x 0.3 mm

Effect of the data set multiplicity on SAD phasing

The multiplicity was sequentially reduced by omitting an increasing number of runs used for data integration, scaling and phasing. In all these trials the anomalous atom searches were set to find 10 peaks with XM (SHELXD)[3]. The results are shown in Table 2. Based on the latter analysis, only a small fraction (equivalent to six data-collection runs with 8.4-fold multiplicity) is sufficient for substructure determination and subsequent phasing of the protein structure. All datasets that include more than 6 runs showed the highest 6 peaks as the correct atom sites.

Structure determination and refinement

Following the determination of the substructure, density modification and phase extension were carried out with XE (SHELXE)[3] against a high resolution data set of 1.6 Å resolution also collected from the same crystal. During the initial phasing cycles, the anomalous scatterers and a few other residues of the structure were located, whereas in advanced SAD-phased electron-density maps SHELX traced 96 out of the 123 residues. No prior knowledge from known pseudoazurin structures was used for tracing of the chain. Reiterated restrained refinement with REFMAC5[4] and manual intervention employing XFIT[5] and COOT[6] revealed the electron density map shown in Figure 2.

Electron density map

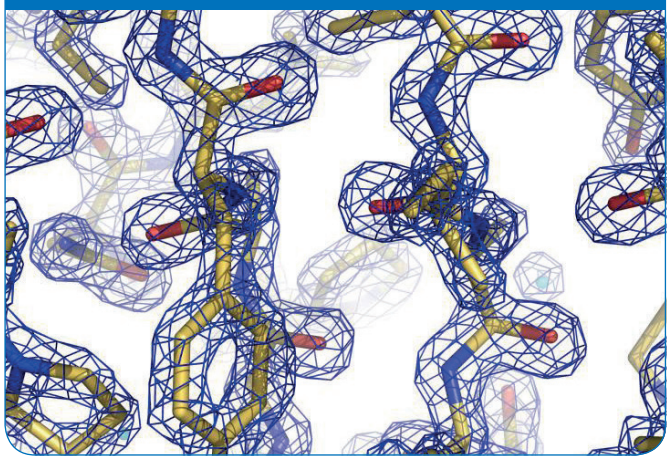

Figure 2: Electron density map after model refinement contoured at 2σ (pdb accession code 4rh4).

Data collection and processing statistics	
Resolution (Å)	2.2
Exposure time (sec)	20
Rotation angle (°)	0.8
Degrees collected (°)	8297
Multiplicity	87.2
Completeness (%)	95.6
R_{pim} (%)	2.48
$I/\sigma(I)$	15.9
R_{anom}* (%)	3.05

*The average anomalous intensity difference.

Table 1: Data collection and processing statistics

Summary

The D8 VENTURE with the PROTEUM2 data processing software provided very accurate and precise intensities. These were sufficient to obtain phases from the weak anomalous scattering of S and Zn atoms from data with multiplicity generally considered too low for successful SAD phasing.

Runs	Multiplicity	Reflections measured/ unique*	Substructure peaks at atom sites out of ten total
75	87.2	1184616/13583	Zn , 5 Met
44	65	869876/13379	Zn , 5 Met
35	52.9	707379/13378	Zn , 5 Met
29	41.5	555356/13378	Zn , 5 Met
25	36.8	492779/13376	Zn , 5 Met
15	20.3	271355/13343	Zn , 5 Met
7	10.5	139476/13326	Zn , 5 Met
6	8.4	111922/13325	Zn , 4 Met
5	6.6	87693/13289	Zn, 3 Met
4	4.6	61076/13257	Zn, 3 Met

*unmerged anomalous data

Table 2: Success of substructure detecting as a function of multiplicity

References

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