Improved Sulfur-SAD phasing of Thaumatin with Cu-Kα radiation obtained in-house with a Bruker Nonius MICROSTAR rotating anode generator

Significant improvement of speed and precision of Sulfur-SAD phasing of Thaumatin with Cu-Kα radiation obtained in-house with a Bruker Nonius MICROSTAR rotating anode generator.

The Bruker Nonius MICROSTAR is a micro-focus generator delivering up to 2.7kW on a 100 micron focus. Due to the new design of the electron optics, the brilliance and the divergence of the X-rays have been significantly improved. With data collected using this instrument we have solved the structure of Thaumatin on native data only. To do this, we applied Sulfur SAD phasing on data collected in-house with Cu-Kα on a 150 micron crystal in one day.

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Introduction

Single wavelength Anomalous Dispersion (SAD) phasing is a technique which uses the anomalous signal of heavy atoms to solve the structure of proteins.

Phasing on the anomalous signal of sulfur atoms only, using Cu-Kα radiation from an in-house rotating anode can be done, but as this signal is very small at this wavelength, the data to be collected have to be very accurate. This can be achieved by collecting data with a large number of multiple observations and by using a goniometer with multiple axes.

A number of test cases (trypsin, insulin & thaumatin) of SAD phasing have been investigated and published by Debreczeni *et al*. Thaumatin proved to be one of the harder examples. The "sulfurs were hard to find" and only part of the chain could be traced in the experimental map.

We have repeated the latter experiment on a smaller crystal (150 µ) and with a MICROSTAR generator equipped with Montel200 optics as opposed to the traditional rotating anode with 300 µ focus used in the publication. Both experiments were carried out using a SMART 6000 detector mounted on a PROTEUM R 3-circle goniometer.

Experimental

Some data on the crystal and on the experiment are shown in Table 1 and 2.

Using the MICROSTAR generator, a much shorter crystal-to-detector distance (60 mm instead of 120 mm) could be used. This is due to the lower divergence of the X-ray beam of the MICROSTAR. At this distance it was still possible to resolve the 150 Å axis of thaumatin as can be seen in Figure 1. A total of 800° was measured in 0.3° images as omega and phi scans at theta settings ranging from 10° to 35°.

Table 1:

Thaumatin	
Space group	$P4_12_12$
Unit cell parameters (Å)	a=b=58.07, c=150.48
Number of anomalous scatterers	17 (8 Cys-Cys, 1 Met)

Table 2:

Experiment	With traditional RAG	With MICROSTAR
Focal spot size	300 µ	100 µ
X-ray optics	Osmic blue	Montel200 optics
Power	4.5 kW	2.4 kW
Detector	SMART 6000	SMART 6000
Goniometer	3-circle goniostat	3-circle goniostat
Crystal size	300 µ	150 µ
Crystal-Detector distance	120mm	60mm
Exposure time	600 sec/°	67 sec/°
Total data collection time	2.94 days	1.08 days

Table 3:

Resolution	#Data	#Theory	%Complete	Multiplicity	<I>	<I/σ>	R(int)	R(σ)
Inf - 1.55	38434	38450	100.0	25.47	141.4	43.66	0.0536	0.0164
1.65 - 1.55	6430	6430	100.0	17.95	23.8	11.42	0.2656	0.838

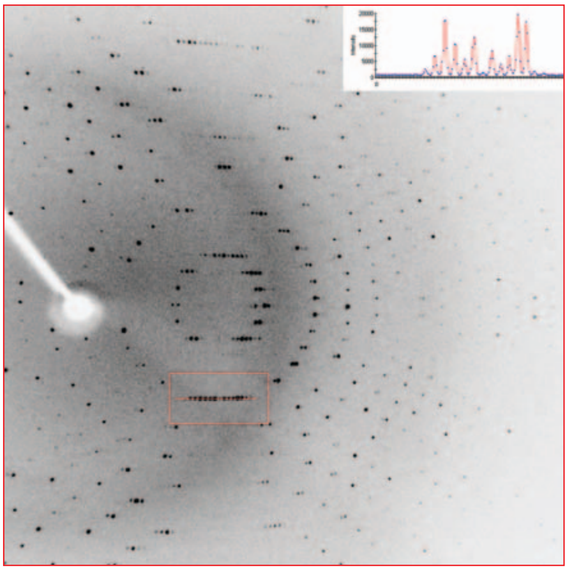

Figure 1: A 0.3° rotation image of thaumatin. The insert shows the intensity profile of the reflections of the 150 Å axis (highlighted in the red box).

The intensities were integrated with the Bruker Nonius data processing package SAINT+ and subsequently scaled with SADABS. This resulted in a total of 979232 reflections up to 1.55Å with an average I/sigma(I) of 9.0.

The statistics were calculated and the reflections were merged with the program XPREP. Final merging statistics are given in Table 3.

Determination of ΔF values for Single Anomalous Scattering (SAS) was performed by XPREP. The anomalous signal to noise ratio gives an indication of the strength of the sulfur signal (1.0 is random). For this thaumatin data set, the values were around 4.3 at low resolution, going down to 1.25 at 1.9Å and 1.16 at 1.6Å.

For the determination of the sulfur atom substructure we used SAS data up to 1.9Å, resulting in 17527 SAS ΔF values.

Anomalous scatterers were found using the dual-space recycling algorithm implemented in SHELXD[4, 5, 6].
The program was instructed to locate 17 sulfurs (8 disulfides and a methionine).

The SHELXD solution with the highest correlation coefficient was input directly into SHELXE[3] for phase calculation and improvement without further refinement of the atom positions or occupancies. The final phasing statistics by SHELXD & SHELXE is shown in Table 4.

Table 4:

SHELXD output	
Data truncated to (Å)	1.9
CC	26.57
CC (weak)	13.23
No. of peaks located	17
Success rate (%)	3
SHELXE output	
Expanded to (Å)	1.55
No. of cycles	50
Contrast	0.68
Contrast (inverted)	0.21
Solvent content	0.45
Final map CC	0.910

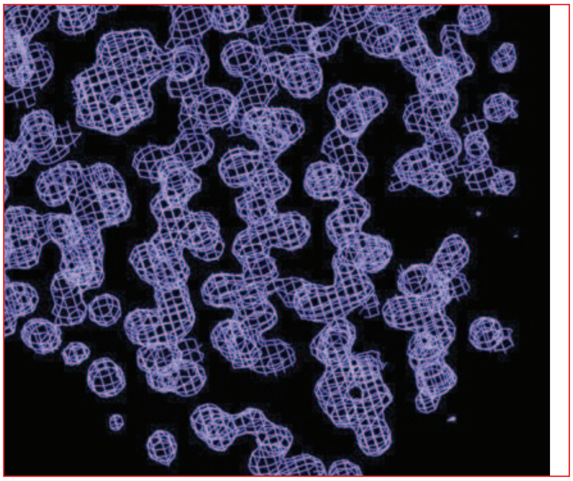

Figure 2: Experimental electron density after SAD phasing

Part of the map calculated with the phases of SHELXE is shown in Figure 2. The main chain beta strands and the side chains of a Trp and a Tyr can be clearly seen.

The final SHELXE phases were fed into ARP/wARP[2] for chain tracing and side-chain docking. wARP was able to autotrace 203 of the 207 residues. Only the N-terminal Ala, cis-Pro 84 and C-terminal Thr & Ala were not automatically docked.

A part of the autotraced thaumatin in the native density is shown in figure 3.

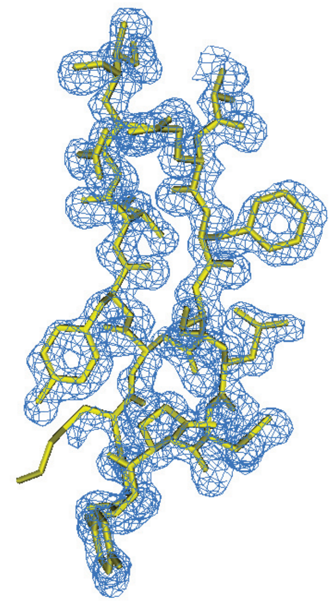

Figure 3: Part of the autotraced thaumatin model in the native density.

Conclusion

Sulfur SAD phasing from Cu-Kα data, obtained in-house with a Bruker Nonius MICROSTAR microfocus rotating anode generator and a PROTEUM R CCD detector on a 3-circle goniometer system, can now be achieved with greater ease than before.

With such a system:

- Data can be collected much faster
- Data are more accurate
- More data can be collected

References:

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