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Report:

Bacterioferritin from *Desulfovibrio desulfuricans* ATCC 27774 (BFR-Dd):

Crystallisation

Crystals of Bfr Dd were obtained in the cubic space group $P2_13$, with cell edge a=225.3 Å. Cell volume considerations led to the conclusion that there would be 8 dimers in the asymmetric unit. Since each dimer (M.W. *ca.* 52 kDa) was expected to contain 5 Fe atoms this would lead to a total of 40 Fe atoms and an estimated solvent content of about 50%. Analysis of the self rotation Patterson maps revealed the presence of non-crystallographic symmetry consistent with a 24-mer cluster (i.e., 24 monomers and 12 heme groups, or 12 dimers of Bfr). Also, it was inferred that there would be eight such clusters (two different groups of four) within the unit cell, and thus the asymmetric unit would contain 1/3 of each of the two different kinds of cluster.

Data collection

Crystals were frozen and checked at ITQB and stored in a Taylor Wharton Cryopak dewar vessel for transport to the ESRF. Although the crystals could be well indexed and characterized using the in-house facilities the quality of the data that could be measured locally was rather poor - diffraction extended to about 4 Å, with merging R statistics well above 10%. A further complication was introduced by the large cell parameter. This lead to very closely spaced diffraction spots and thus to spatial overlaps in the diffraction pattern at higher resolution, which then becomes useless for processing.

To minimize this problem, the cryo conditions were optimized so as to obtain frozen crystals with a mosaicity (0.5-0.6°) as close as possible to that obtained at room temperature (0.3°). A 3-wavelength MAD data collection was carried out at ESRF BM-14 using a MAR CCD detector. The main statistical data from this data collection are shown below.

Wavelength, Å	ResIn range (Å)	$\%R_{merg}$	$\%R_{anom}$	%Complete	Redundancy
λ_1, 1.7400, i-p	30-2.90	8.3	4.0	99. 9 (90.0)	8.8
λ_2, 1.7387, peak	30-2.90	8.8	4.0	99.9 (99.8)	10.9
λ_3, 0.992, remote	30-2.90	7.2	3.0	99.9 (90.0)	7.0

The inflexion point (i-p) and peak wavelengths were chosen from an X-ray fluorescence scan of a test crystal near the Fe K-edge. The MAD data were processed and scaled according to our standard protocol using programs DENZO / SCALEPACK / ROTAPREP / SCALA / TRUNCATE.

Structure determination (in progress)

An inspection of the Patterson map obtained using the anomalous differences from the peak data set showed several features that could be attributed to the Fe atoms in the structure, however this map was found too complex for interpretation by hand. Therefore, program Shake & Bake 2.0 was used and managed to identify 23 out of the expected 40 sites. Since there was the possibility that not all Fe atoms might be specifically bound to the protein, we thought initially that only 24 sites (3 per dimer) were present in the asymmetric unit. These sites were then input to program MLPHARE for phase determination and refinement. While attempting to locate the presumed missing 24^{th} site we realized that some of the sites found by SnB were in fact double sites. This is explained by the fact that the resolution of the data was only 2.9 Å (but the anomalous signal became very weak below about 3.5 Å) and the separation between these two sites was about 3.4 Å - under these circumstances the Fe sites could not be clearly resolved by the SnB protocol using the anomalous differences (the SnB protocol relies on the 'atomicity' of the electron density, i.e., well resolved atomic sites) and thus an 'average' site was obtained instead. Once this was realized, we could resolve the Fe sites by leaving out a suspect site from the MLPHARE refinement, carrying out a few cycles and looking at an anomalous Fourier map to identify the Fe atoms. At the same time, an improved scaling protocol was used which produced better figures of merit for the phasing (0.45 to 2.9 Å). An initial solvent flattening calculation greatly improved the overall aspect of the electron density map - many α-helices can be visible in the electron density map. A greater improvement is expected when 16-fold averaging is included in this procedure, since there are 8 independent copies of the dimer in the unit cell (or 16 independent copies of the monomer). This step is currently in progress, and once it is completed, the recently determined protein sequence will be used to build a structural model for subsequent refinement.

Concluding Remarks

It should be noted that this structure could be solved in spite of poor data statistics (R_{anom} consistently smaller than R_{merg}), very likely because the high redundancy of the peak data set allowed a very precise measurement of the weak anomalous signal. Also, it is possible that the closely spaced Fe atoms in the di-iron sites may have significantly enhanced the anomalous signal at lower resolution.