

## **Structure determination of iron superoxide dismutase (FeSOD) from *Vigna unguiculata* by MAD**

Eukaryotic iron superoxide dismutase (FeSOD) consists of one protein of 26 kDa that binds a Fe atom as a cofactor. The protein can be found in a dimeric or tetrameric form depending on the organisms where it works. It belongs to the superoxide dismutase family of metalloproteins, which act defending the organism by dismuting the superoxide radical to molecular oxygen, therefore constituting a fundamental protection against free radicals, which can damage essential cellular mechanisms. The protein was cloned and overexpressed in *E. coli* with a N-terminal His-tag. Crystallisation experiments of the protein resulted, after several refined screenings, in crystals suitable for X-ray diffraction analysis.

In order to solve the phase problem, we collected a data set at the anomalous peak of iron (1.731 Å). Previously we recorded a Xanes scan using a mounted crystal. Although the scan was noisy, a clear signal at the Fe edge was observed. After inspection of the data, no anomalous signal was detected and the anomalous Patterson did not yield useful information to locate the Fe atom. Unfortunately this data set was not good enough to help us to solve the structure by MAD.

Using the data set described in Table 1, the crystal structure of the dimeric Fe(III) superoxide dismutase from *Vigna unguiculata* was determined using the structure of *Pseudomonas ovalis* FeSOD as a search model in molecular replacement, and ultimately refined at 1.8 Å resolution to a crystallographic R-factor of 16.9 % and R-free of 23.5 %. The monomer has a molecular weight of 26 kDa and consist of 238 amino acid residues of which 222 are visible and modelled into the electron density map, together with one Fe atom and 200 water molecules

Table 1. Data collection statistics for the FeSOD

<i>Data collection<sup>a</sup></i>	
Environment	130 mm MarCCD ESRF, beamline BM14S
Wavelength	1.033 Å
Cell dimensions (Å, °), space group C2	a=82.5, b=48.4, c=64.3, β=119.6
Resolution (Å)	30.0-1.80 (1.90-1.80)
Unique reflections	20443
Average multiplicity	3.3 (3.1)
Completeness	99.0 (99.0)
$R_{\text{merge}}^b$	0.10 (0.77)
$\langle I/\sigma(I) \rangle$	6.2 (0.9)

<sup>a</sup> Values in the highest resolution shell are given in parentheses.

$$^b R_{\text{merge}} = \frac{\sum_{\eta} \sum_i |I_{\eta,i} - \langle I_{\eta} \rangle|}{\sum_{\eta} \sum_i |I_{\eta,i}|}$$

## Reference

Crystallisation and preliminary X-ray diffraction studies of the eukaryotic iron superoxide dismutase (FeSOD) from *Vigna unguiculata*. Muñoz, I. G., Morán, J. F., Becana, M. & Montoya, G. In press at *Acta Cryst. D*.