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Crystallization and preliminary X-ray diffraction studies of the peptide methionine sulfoxide reductase B domain of *Neisseria meningitidis* PILB

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Peptide methionine sulfoxide reductases (Msr) are ubiquitous enzymes that catalyse the reduction of free and protein-bound methionine sulfoxide back to methionine *via* sulfenic acid chemistry. Two classes of Msrs, MsrA and MsrB, have been described. The fact that the two Msrs display opposite stereoselectivities and have no sequence identity suggests that there is no structural similarity between the two classes. No three-dimensional structure of a MsrB is known. In the present report, the MsrB subdomain of *Neisseria meningitidis* PILB was used to grow orthorhombic crystals by the hanging-drop vapour-diffusion technique. The crystals belong to space group $P2_12_12_1$, with unit-cell parameters a = 44.0, b = 118.6, c = 138.5 Å. Crystals of selenomethionine-substituted MsrB were grown under the same conditions in order to use the MAD method for structure determination. Three diffraction data sets at 1.8 Å resolution were collected. The positions of the Se atoms were determined and should result in a full structure determination.

1. Introduction

Methionine residues are easily oxidized to methionine sulfoxides (MetSO) by activated oxygen and nitrogen species produced either during aerobic respiration, after exposure to various agents or after pathogenic bacteria invasion (Vogt, 1995; Nathan & Shiloh, 2000). Therefore, it is essential for cells to possess enzymes that can restore the biological function of the modified MetSO proteins. This is the role of the methionine sulfoxide reductases (Msr), which reduce peptidic MetSO back to methionine. Two Msr activities (MsrA and MsrB) have been described so far (Grimaud *et al.*, 2001; Moskovitz *et al.*, 2002; Olry *et al.*, 2002; Kryukov *et al.*, 2002). Both enzymes are monomeric and share a similar catalytic mechanism. This includes (i) formation of a sulfenic acid intermediate with a concomitant release of one mole of methionine per mole of Msr and (ii) regeneration of the oxidized Cys *via* formation of one or two intradisulfide bonds followed by reduction by thioredoxin (Boschi-Muller *et al.*, 2000; Olry *et al.*, 2002). In contrast, they differ in stereoselectivity toward the sulfoxide function (Grimaud *et al.*, 2001; Moskovitz *et al.*, 2002; Olry *et al.*, 2002; Kryukov *et al.*, 2002).

MsrA and MsrB are produced either as distinct proteins as in *Escherichia coli* or as fused domains as in pathogenic bacteria such as *Streptococcus pneumoniae*, *Neisseria meningitidis* and *N. gonorrhoeae* (Wisemann *et al.*, 1996). In *Neisseria* species, MsrA and MsrB are included in a large polypeptide called PILB that is likely to be a virulence factor involved in *Neisseria* pathogenicity (Taha *et al.*, 1988). PILB is composed of three subdomains: an N-terminal part (residues 1–194), which may encode a disulfide oxidoreductase (Taha *et al.*, 1991), a central domain (residues 195–375) and a C-terminal domain (residues 376–521). Recently, the central subdomain and the C-terminal subdomain of *N. meningitidis* were produced as independent folded entities (Olry *et al.*, 2002). They were shown to display MrsA and MsrB activities, respectively.

The fact that MsrA and MsrB exhibit opposite stereoselectivities towards sulfoxide function and present no sequence homology in particular around the cysteines involved in the catalytic mechanism argues in favour of a three-dimensional structure of MsrBs that is different from that of MsrAs. The X-ray structures of the MsrA from *E. coli* and *Bos taurus* (Tete-Favier *et al.*, 2000; Lowther *et al.*, 2000) have been determined, whereas no three-dimensional structure of an MsrB is known so far. Structural analysis of MsrB will permit definition of the amino acids involved not only in the catalytic mechanism but also in the stereoselectivities towards sulfoxide function. It is in this context that X-ray determination of the *N. meningitidis* MsrB structure was undertaken. Since no significant sequence similarity was found with any protein of known structure, the use of selenomethionine-substituted MsrB ([Se-Met]-MsrB) and multi-wavelength anomalous diffraction phasing

Table 1
MsrB crystallization conditions and X-ray characterization.

Protein concentration (mg ml^{-1})	75
Precipitant solution	0.5 *M* KH_2PO_4
Cryoprotectant solution	20%(*v*/*v*) MPD
Unit-cell parameters (Å)	*a* = 44.0, *b* = 118.6, *c* = 138.5
Space group	$P2_12_12_1$
Molecules per asymmetric unit	4
V_M (Å^3 Da^{-1})	2.76
Solvent content (%)	51
Useful diffraction limit (Å)	1.82

Table 2
Statistics of X-ray diffraction data collection for the [SeMet]-MsrB crystals.

Values in parentheses refer to data in the highest resolution shell.

Wavelength (Å)	0.9796 (inflection)	0.9791 (peak)	0.9765 (remote)
Resolution (Å)	30–1.82 (1.86–1.82)	30–1.82 (1.86–1.82)	30–1.82 (1.86–1.82)
No. of independent reflections	65709	64482	65003
Completeness (%)	97.1 (92.2)	93.6 (84.1)	97.0 (91.9)
R_{sym} (%)	4.5 (18.5)	5.8 (12.2)	4.5 (17.6)
$\langle I\rangle/\langle\sigma(I)\rangle$	12.7 (3.1)	16.1 (2.5)	13.1 (2.9)
Mean figure of merit after *SHARP*		0.5986	
Mean figure of merit after *SOLOMON*		0.8543	

measurements (MAD) was chosen as the strategy to solve the structure. Here, we describe the crystallization, data collection and initial analyses for MsrB and [SeMet]-MsrB.

2. Material and methods

2.1. MsrB and [SeMet]-MsrB production and purification

MsrB was overexpressed and purified as described by Olry *et al.* (2002). The *E. coli* strain B834(DE3) containing plasmid pETMsrB (pETMsrB containing the PILB-MsrB coding sequence (amino acids 376–521) under the control of the T7 promotor) was cultured as described by Ramakrishnan *et al.* (1993) and was used as a source of [SeMet]-MsrB. [SeMet]-MsrB was purified using a procedure similar to that described for MsrB. Substitution of the methionine residues by selenomethionines was checked by electrospray mass analysis: the difference between the MsrB mass (16372.0 ± 0.5 Da) and the [SeMet]-MsrB mass (16467.2 ± 0.7 Da) corresponds precisely to the introduction of two Se atoms, indicating full substitution since MsrB contains two methionine residues at positions 463 and 508.

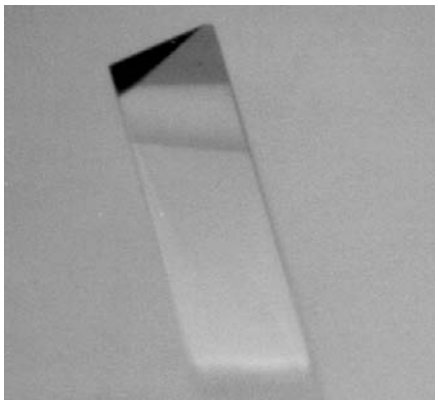

Figure 1
Orthorhombic MsrB crystals, obtained using conditions described in Table 1, with approximate dimensions 330 × 250 × 250 µm.

2.2. Crystallization

MsrB and [SeMet]-MsrB were crystallized using the same procedure. The purified enzyme was treated with 50 m*M* DTT, dialysed twice in 50 m*M* Tris–HCl pH 8.0 buffer and finally concentrated to 75 mg ml^{-1}. Crystallization was achieved by the hanging-drop vapour-diffusion method in Linbro multiwell tissue-culture plates. The crystals were grown from 8 µl droplets composed of equal volumes of the protein solution and of the precipitant solution (Table 1) and equilibrated against 700 µl reservoirs at 293 K. Crystals quickly appeared and reached their maximal size within 3 d (Fig. 1).

2.3. Crystal X-ray characterization and data collection

X-ray diffraction experiments were performed at 100 K. To provide cryoprotection of the crystals upon flash-freezing, crystals were briefly soaked in cryoprotectant solution (Table 1). Cryocooling was performed by fast immersion of crystals in a nitrogen-gas stream at 100 K. Crystals belong to space group $P2_12_12_1$, with unit-cell parameters *a* = 44.9, *b* = 118.9, *c* = 137.7 Å.

A MAD experiment was performed on beamline BM30A (FIP) at the ESRF. Three different wavelengths were chosen from inspection of the fluorescence spectrum using the program *CHOOCH* (Evans & Pettifer, 2001), corresponding to the inflection point, peak and high-energy remote of the selenium *K* edge. All data sets were processed using *DENZO* (Otwinowski & Minor, 1997) and scaled using *SCALEPACK* (Otwinowski & Minor, 1997). Further details are given in Table 2.

3. Results and discussion

The orthorhombic crystals showed diffraction to 1.8 Å resolution and three data sets were collected at three different wavelengths to this resolution (Table 2). On the basis of the solvent-content evaluation, these crystals contain four molecules per asymmetric unit. The non-crystallographic symmetry has been analysed from the self-rotation function calculated by the program *GLRF* (Tong & Rossmann, 1990). It showed several weak peaks with general rotation angle (κ value in the polar angle convention). The native Patterson function calculated using *FFT* from the *CCP*4 package does not reveal any translation vector between the four molecules. The monomers are thus randomly positioned in the asymmetric unit, as confirmed subsequently by calculation of the superpositioning operators determined from the models obtained after the automatic building procedure.

Searches for the position of a maximum of 12 Se atoms were performed from the data set collected at the peak of the selenium *K* edge with the program *Shake-and-Bake* v.2.1 (Weeks & Miller, 1999). In any case, the same eight major sites were obtained with very high confidence after only 30 cycles of searching with *SnB* (Fig. 2). Their positions and occupancies were refined using *SHARP* (de La Fortelle & Bricogne, 1997). Peak-wavelength structure factors were phased by *SHARP* to 2 Å resolution and these phases were extended to 1.82 Å with solvent flattening using *SOLOMON* (Abrahams &

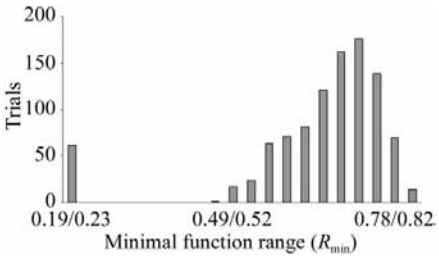

Figure 2
Histogram of the number of trials and their corresponding minimal function values R_{min} obtained in *Shake-and-Bake* (*SnB*). Using 500 reflections selected in the resolution range 25–1.8 Å and 5000 invariants, a maximum of 12 sites were searched for in 1000 trials of 20 cycles each. Eight sites were found in 61 successful trials. The best value of R_{min} is 0.196. R_{min} is one of the figures of merit calculated by *SnB*.

Leslie, 1996) (see Table 2). Automatic model building with *ARP*/*wARP* (Perrakis *et al.*, 1999) allowed the positioning of 465 of 588 residues with their side chains.

Note added in proof: During the reviewing of this paper, the structure of the MsrB from *N. gonorrhoeae* was published by Lowther *et al.* (2002).

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