Title: Backbone and side chain 1H, 15N and 13C assignments for the oxidised and reduced forms of the oxidoreductase protein DsbA from Staphylococcus aureus

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Corresponding Author: Dr Martin J Scanlon

Corresponding Author's email: Martin.Scanlon@pharm.monash.edu.au

Corresponding Author's Institution: Monash University

First Author: Martin L Williams

Order of Authors: Martin L Williams; David K Chalmers, PhD; Jennifer L Martin, PhD; Martin J Scanlon, PhD

Addresses of Authors: Martin Williams . David Chalmers . Martin Scanlon (✉)
Medicinal Chemistry and Drug Action, Monash Institute of Pharmaceutical Sciences
Monash University (Parkville Campus), 381 Royal Parade, Parkville
VIC 3052, Australia

Jennifer Martin
Institute for Molecular Bioscience,
University of Queensland, Brisbane QLD 4072, Australia

Abstract: The function and dynamics of the thiol-disulfide oxidoreductase DsbA in the low-GC gram positive bacterium, Staphylococcus aureus, are yet to be elucidated. Here we report 13C, 15N and 1H assignments for the oxidised and reduced forms of SaDsbA as a prelude to further studies on the enzyme.
Backbone and side chain $^1$H, $^{15}$N and $^{13}$C assignments for the oxidised and reduced forms of the oxidoreductase protein DsbA from *Staphylococcus aureus*

Martin L. Williams, David K. Chalmers, Jennifer L. Martin and Martin J. Scanlon.

**Biological context**

Thiol-disulfide oxidoreductase (TDOR) enzymes are the principal catalysts of disulfide bond formation during the post-translational phase of extracytoplasmic protein synthesis in prokaryotes. The structure of the prototype enzyme in this class, DsbA from *E. coli*, has been solved and its catalytic mechanisms studied extensively (Gudat et al. 1998; Martin et al. 1993). The structures of DsbA homologues from other Gram-negative bacteria including *Vibrio cholerae* (Hu et al. 1997, Horne et al. 2007), *Neisseria meningitidis* (Vivian et al. 2008), *Wolbachia pipiensis* (Kurz et al. 2008) and *Xylella fastidiosa* (Rinaldi et al. 2009) have also been determined, and DsbA enzymes of Gram-negative bacteria have been implicated in functional aspects including motility and expression of virulence factors (Kadokura et al. 2003).

Homologues of DsbA have also been identified in a smaller range of Gram-positive bacteria, including *B. subtilis* (Meima et al. 2002) and *S. aureus* (Dumoulin et al. 2005), however the role of Dsb enzymes in the process of disulfide bond formation in Gram-positive bacteria remains unresolved. Indeed, natural substrates of Gram-positive TDORs are yet to be conclusively identified, although complementation studies have demonstrated that *S. aureus* DsbA (*Sa*DsbA) can restore functionality to *bdb* *B subtilis* mutants (Kouwen et al. 2007). Of the Dsb enzymes isolated from Gram-positive bacteria, only the oxidised form of *Sa*DsbA has been crystallised and its structure solved (Heras et al. 2007, Heras et al. 2008). The monomeric 21 kDa enzyme is a membrane-bound lipoprotein (Dumoulin et al. 2005); however, the structure of the soluble *Sa*DsbA enzyme lacking the N-terminal membrane anchor is very similar to the Gram-negative DsbAs, consisting of a thioredoxin domain with an inserted α-helical domain. In common with all DsbA enzymes so far identified, *Sa*DsbA possesses two key features. The first is the strictly conserved catalytic motif of two cysteines separated by two other amino acids (CxxC) located on the first α-helix and the second is a cis-Proline residue that in the Gram-negative DsbA enzymes has been implicated in substrate recognition and binding. *Sa*DsbA has a strong oxidative redox potential of -131 mV, similar to Gram-negative DsbAs, but differs in subtle but significant respects from the prototypical *E. coli* DsbA (*Ec*DsbA) with which it shares 15% sequence homology. Firstly, a hydrophobic groove located in the thioredoxin domain, below the active site of *Ec*DsbA, is shallower and significantly truncated in *Sa*DsbA, and secondly it lacks an adjacent hydrophobic patch that has been implicated in substrate binding to *Ec*DsbA (Nakamoto and Bardwell 2004). Additionally, the oxidised and reduced forms of *Sa*DsbA have been found to be energetically equivalent (Heras et al. 2008) in contrast to the Gram-negative DsbA enzymes where the reduced form of the protein has been demonstrated to be more thermodynamically stable in *Ec*DsbA (Zapun et al. 1993) and *Vc*DsbA (Horne et al. 2007).

Previous studies on *Vc*DsbA have examined the dynamics of the oxidised and reduced forms of the enzyme through measurement of NMR relaxation data, in efforts to elucidate the significance of inter-domain movement for catalytic activity (Horne et al. 2007). Our current study aims to extend that approach to the low-GC Gram-positive bacteria, of which *S. aureus* is a prominent example, and so we report here the near-complete backbone and side chain assignments for the oxidised and reduced forms of *Sa*DsbA.

**Methods and Experiments**

*Sa*DsbA was expressed in *E. coli* BL21(DE3)/pLysS, in uniformly $^{15}$N isotope-labelled form by Autoinduction (Studier 2005) and in $^{15}$N/$^{13}$C isotope-labelled form by induction with IPTG (1 mM) according to the method of Marley et al. (2001). In each case, recombinant protein bearing a C-terminal hexahistidine tag was purified by nickel-chelate affinity chromatography using a HisTrap HP 5mL column (GE Healthcare, Piscataway, NJ, USA) followed by ion-exchange chromatography using a Mono S 5/50GL column (GE Healthcare). After purification, *Sa*DsbA was chemically oxidised using copper phenanthroline (1.5 mM) or reduced with dithiothreitol (100-fold excess). Copper
phenanthroline and DTT were removed using a HiPrep 16/10 Desalting column (GE Healthcare). Samples for NMR analysis were concentrated using Amicon Ultra centrifugal filtration units with 10kDa cutoff (Millipore, Bellerica, MA, USA), before addition of 10% ²H₂O to give a final protein concentration of approximately 240uM. The buffer used throughout purification and acquisition of NMR spectra was 10mM HEPES, 50mM NaCl, pH 6.8.

NMR data were acquired at 318K with a Varian INOVA 600MHz spectrometer equipped with a cryogenically cooled probe. Data were processed using NMRPipe (Delaglio et al. 1995) and analysed in SPARKY. ¹H-¹⁵N HSQC and triple resonance HNCACB, CBCA(CO)NH, HNCO and HNCACO experiments (Bax and Grzesie 1993) yielded data for backbone sequential assignment. Side-chain assignments were made using ¹H-³¹C HSQC, HBHA(CO)NH, C(CO)NH-TOCSY, HCCl-TOCSY and ¹H-¹⁵N-NOESY experiments. Side-chain aromatic delta and epsilon proton assignments were assisted by reference to (HB)CB(CGCD)HD and (HB)CB(CGDC)HE experiments (Yamazaki et al. 1993). Additional ¹H-¹⁵N HSQC, CBCA(CO)NH and HNCACO spectra were obtained for the oxidised form of SaDsbA at 298K with a Bruker Avance 800MHz spectrometer equipped with TCI cryoprobe.

Assignments and Data Deposition

The expressed protein consisted of 180 residues plus a C-terminal His-tag, which was not assigned in this analysis. Backbone amide resonance assignments were determined for 92% of non-proline residues in both oxidised and reduced forms. Figure 1 presents ¹H-¹⁵N HSQC spectra of oxidised and reduced SaDsbA, illustrating representative backbone resonance assignments. The first eight residues from the N-terminus could not be assigned due to weak ¹H-¹⁵N HSQC signals. Residues K128 and 130KDS132, constituting a loop at the intersection of the helical domain and the thioredoxin domains, also could not be assigned. This loop was also unassigned in VcDsbA, the homologous DsbA enzyme in V. cholerae (Horne and Scanlon 2007). Of the fourteen expected ¹H-¹⁵N HSQC peak pairs corresponding to side-chain amides of glutamines and asparagines, ten pairs and two individual peaks could be observed in the spectrum. Of these, seven were assigned by reference to the ¹H-¹⁵N NOESY spectrum.

Assignments have been made for over 80% of all aliphatic side-chain carbons and protons, and aromatic delta and epsilon protons in tyrosine and phenylalanine residues.

The unique upfield ¹H α chemical shift of Q94 (3.51 ppm) and the upfield ¹H δ shift of Q93 (2.23 ppm) are likely due to ring current effects exerted by W100, as depicted in Figure 2. These effects were corroborated by ab initio calculation of isotropic shielding factors for residues Q93 and Q94 in the presence and absence of W100, using the Jaguar package of Maestro at the 6-31G** basis set level (Schrödinger, 2007). These calculations yielded significant differences in Isotropic Shielding factors for the chemical shifts of those protons in the presence and absence of the proximal indole ring system of W100 (data not shown).

The weighted chemical shift differences between the oxidised and reduced forms of the enzyme are depicted for backbone amide nitrogens and protons in Figure 3. Overall, the differences in chemical shifts between the oxidised and reduced forms are small, the most significant being localised close to the direct region of the catalytic site.

The chemical shifts of oxidised and reduced SaDsbA have been deposited in the BioMagResBank under the accession numbers 16329 and 16330.

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References


Fig 1. \[^{15}\text{N},^{1}\text{H}\]-HSQC Spectra of (a) oxidised and (b) reduced forms of \(\text{SaDsbA}\). Spectra were acquired at 600MHz and 318K. Sweep widths in \(\omega_1\) were 17ppm for oxidised and 22ppm for reduced proteins, hence spectra differ in folding parameters.
Fig 2. Relative orientations of Trp residue W100 (olive) with α-proton of Gln residue Q93 (light green) and amide proton of Gln residue Q94 (dark green). The associated ring-current effects result in the strong upfield $^1$H$_N$ chemical shift of Q94 (3.51 ppm) and the significant upfield $^1$H$_\alpha$ shift of Q93 (2.23 ppm). Diagram based on published X-ray crystal structure 3BC1 (Heras et al. 2008).

Fig 3. Amide chemical shift differences between oxidised and reduced forms of SaDsbA for residues 10 - 179, calculated by the formula $\Delta \delta = \sqrt{0.154\delta_N^2 + \delta_H^2}$. 

\[ \Delta \delta = \sqrt{0.154\delta_N^2 + \delta_H^2} \]