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

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 (Guddat 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 pipientis* (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% ^2H_2O 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-^{15}N HSQC and triple resonance HNCACB, CBCA(CO)NH, HNCO and HNCACO experiments (Bax and Grzesiek 1993) yielded data for backbone sequential assignment. Side-chain assignments were made using ^1H-^{13}C HSQC, HBHA(CO)NH, C(CO)NH-TOCSY, HCCH-TOCSY and ^1H-^{15}N-NOESY experiments. Side-chain aromatic delta and epsilon proton assignments were assisted by reference to (HB)CB(CGCD)HD and (HB)CB(CGCDCE)HE experiments (Yamazaki et al. 1993). Additional ^1H-^{15}N HSQC, CBCA(CO)NH and HNCACB spectra were obtained for the oxidised form of *Sa*DsbA 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-^{15}N HSQC spectra of oxidised and reduced *Sa*DsbA, illustrating representative backbone resonance assignments. The first eight residues from the N-terminus could not be assigned due to weak ^1H-^{15}N 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 *Vc*DsbA, the homologous DsbA enzyme in *V. cholerae* (Horne and Scanlon 2007). Of the fourteen expected ^1H-^{15}N 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-^{15}N 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 $^1H^N$ chemical shift of Q94 (3.51 ppm) and the upfield $^1H^\alpha$ 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 *Sa*DsbA have been deposited in the BioMagResBank under the accession numbers 16329 and 16330.

References

Bax A, Grzesiek S (1993) Methodological advances in protein NMR. Acc Chem Res 26:131-138.

Delaglio F, Grzesiek S, Vuister GW, Zhu G, Pfeifer J, Bax A (1995) NMRPipe: a multidimensional spectral processing system based on UNIX pipes. J Biomol NMR 6:277-293.

Dumoulin A, Grauschopf U, Bischoff M, Thöny-Meyer L, Berger-Bächi B (2005) *Staphylococcus aureus* DsbA is a membrane-bound lipoprotein with thiol-disulfide oxidoreductase activity. Arch Microbiol 184:117-118.

Guddat LW, Bardwell JC, Martin JL (1998) Crystal structures of reduced and oxidized DsbA: investigation of domain motion and thiolate stabilization. Structure 6:757-767.

Hu S-H, Peek JA, Rattigan E, Taylor RK, Martin JL (1997) Structure of TcpG, the DsbA protein folding catalyst from *Vibrio cholerae.* J Mol Biol 268:137-146.

Heras B, Kurz M, Jarrott R, Byriel KA, Jones A, Thöny-Meyer L, Martin JL (2007) Expression and crystallization of DsbA from *Staphylococcus aureus*. Acta Crystallogr F 63:953-956.

Heras B, Kurz M, Jarrott R, Shouldice SR, Frei P, Robin G, Čemažar M, Thöny-Meyer L, Glockshuber R, Martin JL (2008) *Staphylococcus aureus* DsbA does not have a destabilizing disulfide. J Biol Chem 283:4261-4271.

Horne J, d'Auvergne EJ, Coles M, Velkov T, Chin Y, Charman WN, Prankerd R, Gooley PR, Scanlon MJ (2007) Probing the Flexibility of the DsbA Oxidoreductase from *Vibrio cholerae* – a ^{15}N-^{1}H Heteronuclear NMR Relaxation Analysis of Oxidised and Reduced Forms of DsbA. J Mol Biol 371:703-716.

Horne J, Scanlon MJ (2007) Backbone and side-chain ^{1}H, ^{15}N and ^{13}C assignments for the reduced form of the oxidoreductase protein DsbA from *Vibrio cholerae*. Biomol NMR Assign 1:75-76.

Kadokura H, Katzen F, Beckwith J (2003) Protein disulfide bond formation in prokaryotes. Annu Rev Biochem 72:111-135.

Kouwen TRHM, van der Goot A, Dorenbos R, Winter T, Antelmann H, Plaisier M-C, Quax W, van Dijl JM, Dubois J-YF (2007) Thiol-disulphide oxidoreductase modules in the low-GC Gram-positive bacteria. Molecular Microbiology 64:984-999.

Kurz M, Iturbe-Ormaetxe I, Jarrott R, Cowieson N, Robin G, Jones A, King GJ, Frei P, Glockshuber R, O'Neill SL, Heras B, Martin JL (2008) Cloning, expression, purification and characterization of a DsbA-like protein from *Wolbachia pipientis*. Prot Exp Purif 59:266-273.

Marley J, Lu M, Bracken C (2001) A method for efficient isotopic labelling of recombinant proteins. J Biomol NMR 20:71-75.

Martin JL, Bardwell JC, Kuriyan J (1993) Crystal structure of the DsbA protein required for disulphide bond formation *in vivo*. Nature 365:464-468.

Meima R, Eschevins C, Fillinger S, Bolhuis A, Hamoen LW, Dorenbos R, Quax WJ, van Dijl JM, Provvedi R, Chen I, Dubnau D, Bron S (2002) The *bdbDC* operon of *Bacillus subtilis* encodes thiol-disulfide oxidoreductases required for competence development. J Biol Chem 277:6994-7001.

Nakamoto H, Bardwell JC (2004) Catalysis of disulfide bond formation and isomerisation in the *Escherichia coli* periplasm. Biochimica et Biophysica Acta 1694:111-119.

Rinaldi FC, Meza AN, Guimarães BG (2009) Structural and biochemical characterization of *Xylella fastidiosa* DsbA family members; new insights into the enzyme-substrate interaction. Biochemistry 48:3508-3518.

Schrödinger (2007) Jaguar version 4.5, Schrödinger LLC, New York.

Studier FW (2005) Protein production by auto-induction in high-density shaking cultures. Prot Exp Purif 41:207-234.

Vivian JP, Scoullar J, Robertson AL, Bottomley SP, Horne J, Chin Y, Wielens J, Thompson PE, Velkov T, Piek S, Byres E, Beddoe T, Wilce MCJ, Kahler CM, Rossjohn J, Scanlon MJ (2008)

Structural and Biochemical Characterization of the Oxidoreductase NmDsbA3 from *Neisseria meningitidis*. J Biol Chem 283:32452-32461.

Yamazaki T, Forman-Kay JD, Kay LE (1993) Two-dimensional NMR experiments for correlating [13]Cβ and [1]Hδ/ε chemical shifts of aromatic residues in ^{13}C-labeled proteins via scalar couplings. J Am Chem Soc 115:11054-11055.

Zapun A, Bardwell JC, Creighton TE (1993) The reactive and destabilizing disulfide bond of DsbA, a protein required for protein disulfide bond formation in vivo. Biochemistry 33:5083-5092.
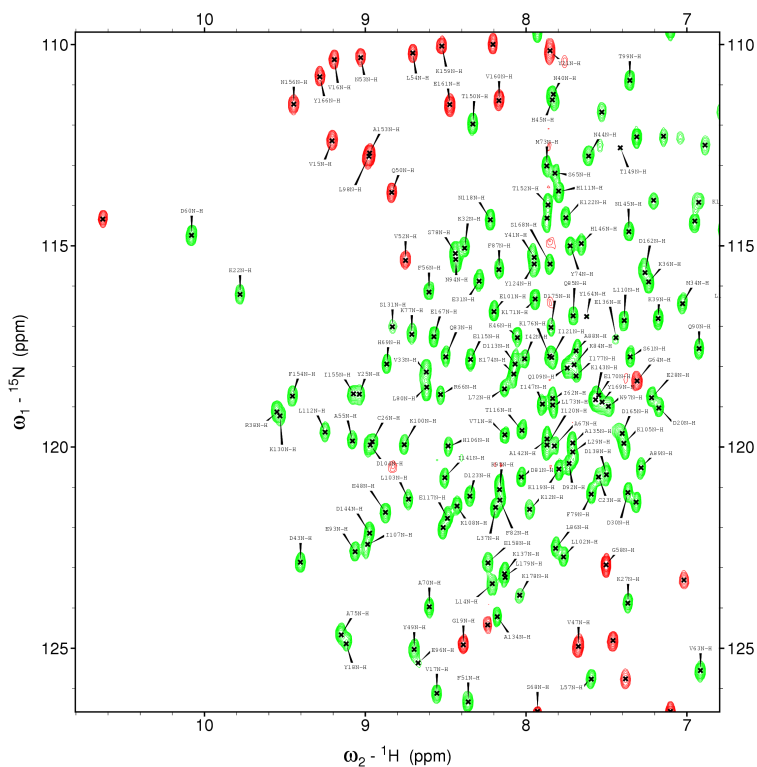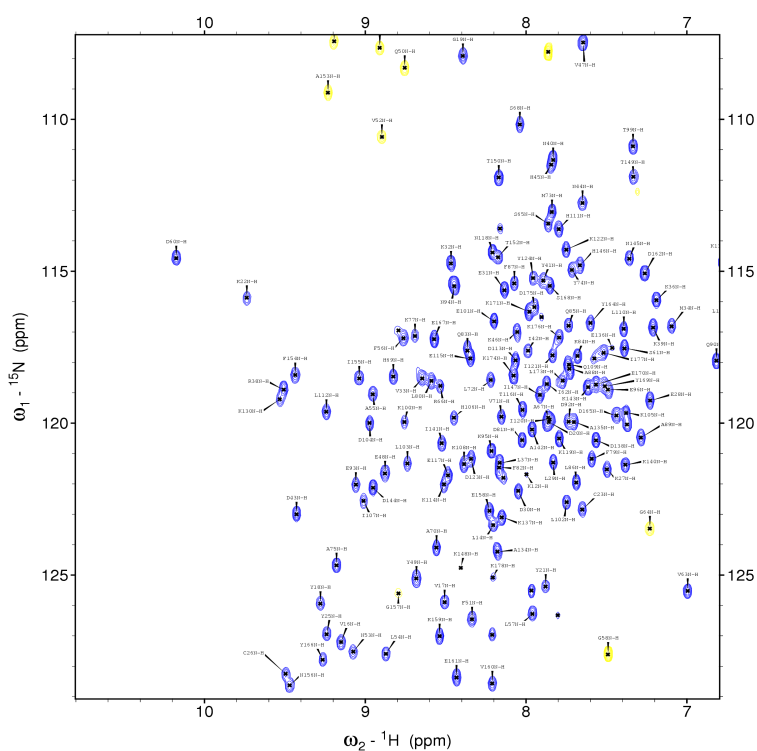
Fig 1. [^{15}N,^{1}H]-HSQC Spectra of (a) oxidised and (b) reduced forms of *Sa*DsbA. Spectra were acquired at 600MHz and 318K. Sweep widths in ω_1 were 17ppm for oxidised and 22ppm for reduced proteins, hence spectra differ in folding parameters.
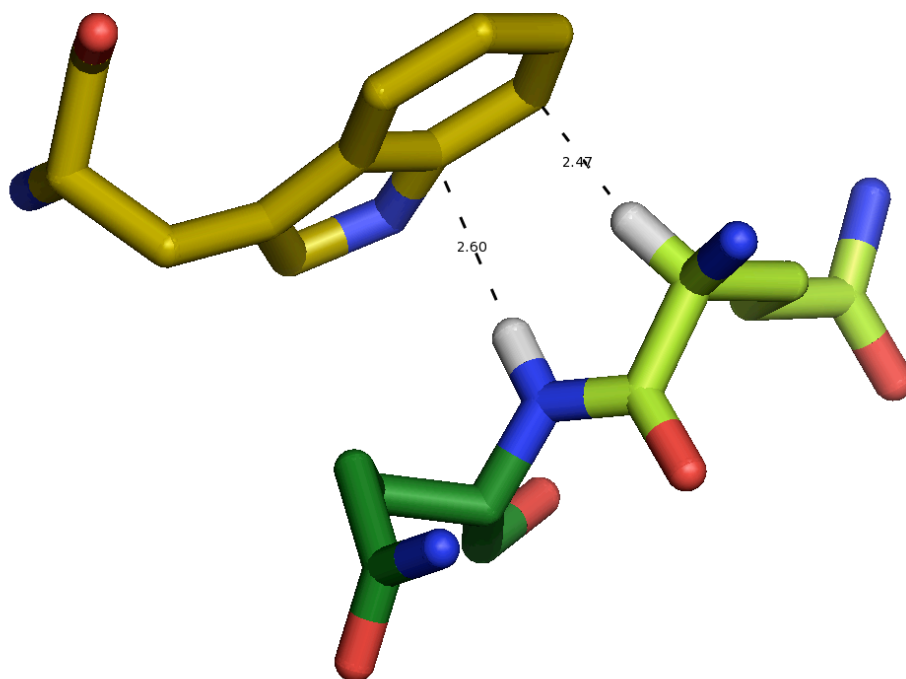
Fig 2. Relative orientations of Trp residue W100 (olive) with α-proton of Gln residue Q93 (light green) and amide proton of Gln residue Q94 (dark green). The associated ring-current effects result in the strong upfield $^1H^N$ chemical shift of Q94 (3.51 ppm) and the significant upfield $^1H^\alpha$ shift of Q93 (2.23 ppm). Diagram based on published X-ray crystal structure 3BCI (Heras *et al*. 2008).
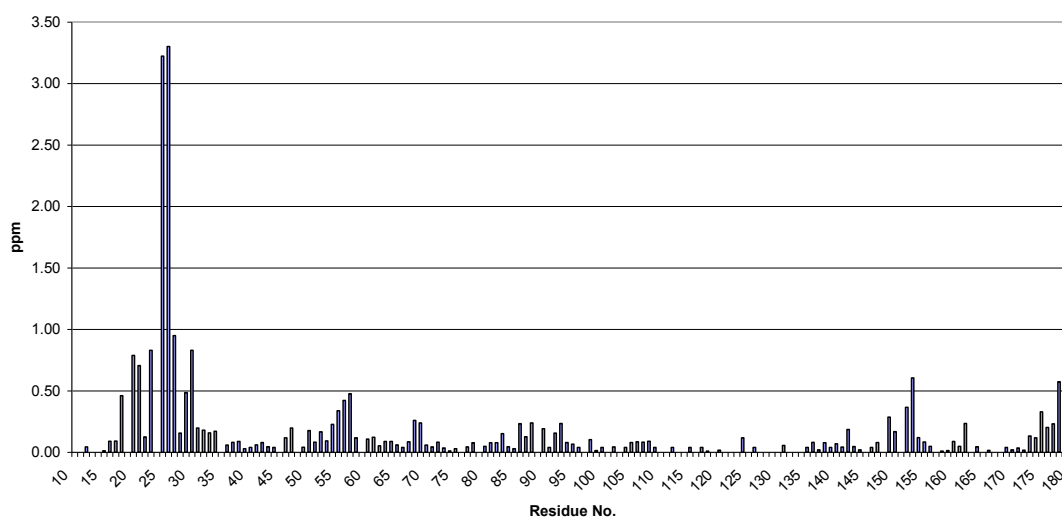

Fig 3. Amide chemical shift differences between oxidised and reduced forms of *Sa*DsbA for residues 10 - 179, calculated by the formula $\Delta\delta = \sqrt{(0.154\delta_N^2 + \delta_H^2)}$.