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Letter to the Editor: ^{1}H, ^{13}C and ^{15}N backbone resonance assignment of the arsenate reductase from *Staphylococcus aureus* in its reduced state

Doris M. Jacobs[a], Joris Messens[b], Rainer W. Wechselberger[c], Elke Brosens[b], Rudolph Willem[a], Lode Wyns[b] & José C. Martins[a,*]

[a]*High-resolution NMR Centre (HNMR), Vrije Universiteit Brussel, Pleinlaan 2, B-1050 Brussels, Belgium;* [b]*Department of Ultrastructure (ULTR), Flemish Interuniversitary Institute for Biotechnology (VIB), Vrije Universiteit Brussel, Paardenstraat 65, B-1640 Sint-Genesius-Rode, Belgium;* [c]*SON NMR Large Scale Facility, Bijvoet Center for Biomolecular Research, Padualaan 8, 3584 CH Utrecht, The Netherlands*

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Biological context

In *S. aureus*, resistance to the metal(III)oxyanions arsenite $As(III)O_2^-$ and antimonite $Sb(III)O_2^-$ is mediated by two proteins, ArsB and ArsR, encoded in the *ars* operon of plasmid pI258 (Silver, 1999). ArsR acts as the transcription repressor, which is de-repressed in the presence of intracellular oxy(III)anions (Ji and Silver, 1992). ArsB is an integral membrane protein that functions as an ATP-independent transporter selective for arsenite and antimonite (Bröer et al., 1993). Resistance against arsenate $As(V)O_4^{3-}$ involves the intervention of the third and final protein coded in the *ars* operon, ArsC (Chen et al., 1985). This protein reduces intracellular arsenate $As(V)O_4^{3-}$ to arsenite $As(III)O_2^-$, which can subsequently be extruded by ArsB. This more elaborate handling of arsenate is thought to avoid phosphate starvation of the bacterium (Silver, 1998). The arsenate reductase (ArsC) of *S. aureus* is a 14.8 kDa (131-residue) monomeric protein, containing four cysteinyl residues, two of which (Cys82, Cys89) were identified (Messens et al., 1999) as the redox pair reducing arsenate to arsenite. ArsC from *S. aureus* requires coupling via thioredoxin and thioredoxin reductase to NADPH to continuously regenerate the enzymatically active, reduced state of ArsC (Ji et al., 1994; Messens et al., 1999). Interestingly, *S. aureus* ArsC displays no sequence homology with ArsC proteins from Gram-negative bacteria, which also present a different mechanism for arsenate reduction. The N-terminal Ile9-Gln18 segment of ArsC does, however, present considerable sequence identity with the conserved active site sequence motif in the family of low molecular weight protein tyrosine phosphatases. Herein, we report the backbone and C^{β} resonance assignment of ArsC in its active, reduced state, as the first step towards a better understanding of this redox protein at the structural level.

*To whom correspondence should be addressed. E-mail: jose@hnmr.vub.ac.be

Methods and experiments

Expression and purification of labelled ArsC. The *arsC* wild type gene from *S. aureus* was cloned into the pET-11a vector by introducing restriction sites NdeI and BamHI with PCR. The *E.coli* strain BL21 (DE3) was transformed with the pET-11a *arsC* wild type plasmid and was grown for 5 h at 37 °C in 2TY medium with 100 μg/ml ampicillin. This preculture was diluted 1/100 in M9 minimal medium with 100 μg/ml ampicillin containing 1 g/l $^{15}NH_4Cl$ for [^{15}N]-ArsC. For [^{13}C,^{15}N]-ArsC, 4 g/l $^{13}C_6$-glucose was added as well. Induction at a cell density of $OD_{600} = 0.9$ was carried out overnight with 1 mM IPTG at 28 °C. Cells were harvested ($OD_{600} = 4$), disrupted and purified as described before (Messens et al., 1999, 2000) except for the following changes. The gel filtration buffer for the Superdex75 PG (16/90) run was 20 mM Tris/HCl, pH 8.0, 150 mM KCl, 50 mM K_2SO_4, 0.1 mM EDTA and 1 mM DTT. The sample was concentrated to at least 2 mM, dialysed to a 50 mM K-phosphate buffer at pH 6.7, containing 50 mM K_2SO_4, 0.1 mM EDTA and 1 mM DTT. The

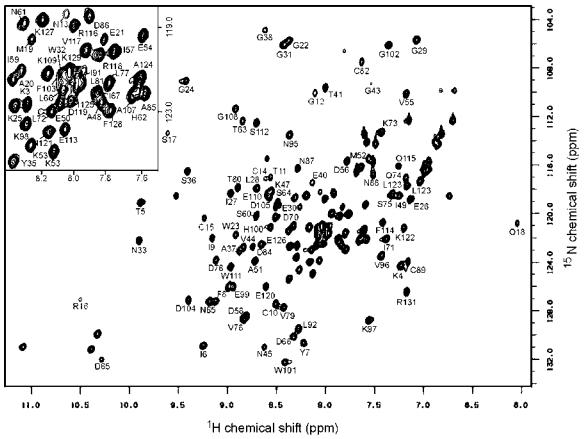

Figure 1. ^{1}H-^{15}N HSQC spectrum at 25 °C and 500 MHz of 1.8 mM ArsC, pH 6.7. Unlabelled cross peaks correspond to the side-chain amide (Gln, Asn) or indole NH (Trp) resonances. Correlation peaks that only become visible when K_2SO_4 is present in solution are highlighted in grey.

protein yield was approximately 11 mg/l culture for ^{15}N ArsC and 5 mg/l culture for ^{13}C,^{15}N ArsC. All buffers were argon flushed for several minutes prior to their use.

NMR spectroscopy. Samples for NMR contained 1.8 mM [^{15}N] or [^{13}C,^{15}N] ArsC in phosphate buffer pH 6.7, with 50 mM K_2SO_4, 1 mM DTT, 0.1 mM EDTA and 5% D_2O and were transferred to a 5 mm Shigemi tube. Under these conditions ArsC remained in its reduced state for the duration of the 3D triple resonance acquisition, performed at 298 K. All chemical shifts were referenced against internal DSS, either directly (^{1}H) or indirectly (^{13}C, ^{15}N) (Markley et al., 1998). Spectra were recorded at 500, 600 and 750 MHz on Bruker AMX, Bruker Avance and Varian Inova spectrometers, respectively. Backbone assignments relied mainly on the combination of data obtained from ^{1}H-^{15}N HSQC (Figure 1), CBCA(CO)NH, HNCACB, HNCO, HN(CA)CO, HNCA and HN(CO)CA (Sattler et al., 1999). In addition, ^{15}N-edited HSQC-TOCSY and HSQC-NOESY spectra were used to confirm the assignments.

The presence of 50 mM sulphate was necessary to reveal the NH correlations of residues Ser11 to Ser17. Interestingly, this corresponds to the segment displaying sequence homology with the phosphatase active site, mentioned above. Matching the chemical shift index calculated from the H^{α}, C^{α} and C' chemical shift values (Wishart and Sykes, 1994) with the distribution of medium range backbone NOE contacts, allowed to identify the presence of four strands and four helices: Lys4–Cys10 (β1), Arg16–Glu26 (α1), Trp32–Gly38 (β2), Pro46–Ile57 (α2), Asn69–Ser75 (α3), Leu77–Leu81 (β3), Lys98–Trp101 (β4) and Trp111–Arg131 (α4). The interstrand NOEs are consistent with a parallel β-sheet organised as β2β1β3β4, indicating that ArsC adopts a structure with mixed α/β topology.

Extent of assignments and data deposition

The resonances of the N-terminal residue Met1, as well as His42 were lacking in all spectra, as were the NH of Asp2, Ile39 and Ser83. Pro93, which is followed by another proline, could not be assigned either. In all, the resonance assignment presented here is 98% complete and has been deposited in the BMRB (http://www.bmrb.wisc.edu) database under accession number BMRB-4944.

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