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Letter to the Editor: ^{1}H, ^{13}C and ^{15}N resonance assignments of a *Bacillus subtilis* arsenate reductase

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Biological context

Arsenic compounds are toxic to nearly all kinds of life forms, while some microorganisms such as bacteria, fungi and algae utilize unique arsenical systems to confer arsenic resistance. In most such microorganisms, arsenic compounds are firstly reduced from pentavalent arsenicals to trivalent derivatives by arsenate reductase (ArsC), and then pumped out of the cells via membrane transport systems, though arsenite (III) is at least about 1000 times toxic than arsenate (Cervants et al., 1994). The X-ray structure of the ArsC protein from *Bacillus subtilis*, was reported previously (Bennett, et al., 2001). The X-ray structure shows that the ArsC protein packs into four ArsC molecules per asymmetric unit, among which, the crucial functional segment (Cys82-Val96) is missing in two of them, while it is visible in the other two ArsC molecules. This may imply an uncommon flexibility. In order to determine the solution structures and obtain further insights into the structure-function correlations of the arsenate reductase, here we report the nearly complete sequence-specific backbone and side-chain ^{1}H, ^{13}C and ^{15}N resonance assignment of the ArsC protein from *Bacillus subtilis* in the reduced form.

Methods and experiments

The *B. subtilis arsc* gene was cloned into pET28a expression vector and expressed in *E. coli* strain BL21 (DE3)/plysS cells. The *E. coli* cells were cultured overnight in 50 ml Luria–Bertani broth (LB) medium with 100 μg/ml kanamycin and 35 μg/ml chloramphenicol at 35 °C. The overnight culture was then transferred into 1L of LB medium with antibiotics. When the OD_{600} of the cell culture reached 0.8–1.0, the cells were harvested by centrifugation at 4000 *g* for 5 min and then re-suspended in 250 ml M9 minimal medium with antibiotics and 1 g/l $^{15}NH_4Cl$, and without or with $^{13}C_6$-glucose at 4 g/l concentration for ^{15}N-labeled, and ^{13}C/^{15}N-labeled sample, respectively (Marley et al., 2001), the cells were allowed to grow for half an hour before isopropyl-1-thio-β-D-galactopyranoside (IPTG) was added to a final concentration of 0.4 mM to induce the ArsC expression. Six h after induction the cells were harvested by centrifugation at 14 000 *g* for 20 min at 4 °C. The ArsC protein was purified by the anion-exchange chromatography (Mono Q column) and Gel-filtration (Superdex-75 column) with an AKTA FPLC system (Pharmacia, USA). The purity of the protein was identified of more than 95% by SDS-PAGE.

NMR samples were prepared in 20 mM Tris-HCl buffer at pH 6.85, with 40 mM KCl, 20 mM Na_2SO_4, 20 mM DTT and 20 mM Urea in 95% H_2O/5% D_2O and are nitrogen saturated for stabilization. All NMR experiments were performed at 298 K on Bruker Avance 500 MHz equipped with cryoprobe and Bruker Avance 800 MHz spectrometers, both equipped with four RF channels and a triple-resonance probe with pulsed field gradients. The chemical shifts were referenced to internal DSS. ^{13}C and ^{15}N chemical shifts are referenced indirectly to DSS, using the absolute frequency ratios. The NMR spectra were processed with the software package NMRPipe (Delaglio et al., 1995) and analyzed by NMRView (Johnson and Blevins, 1994).

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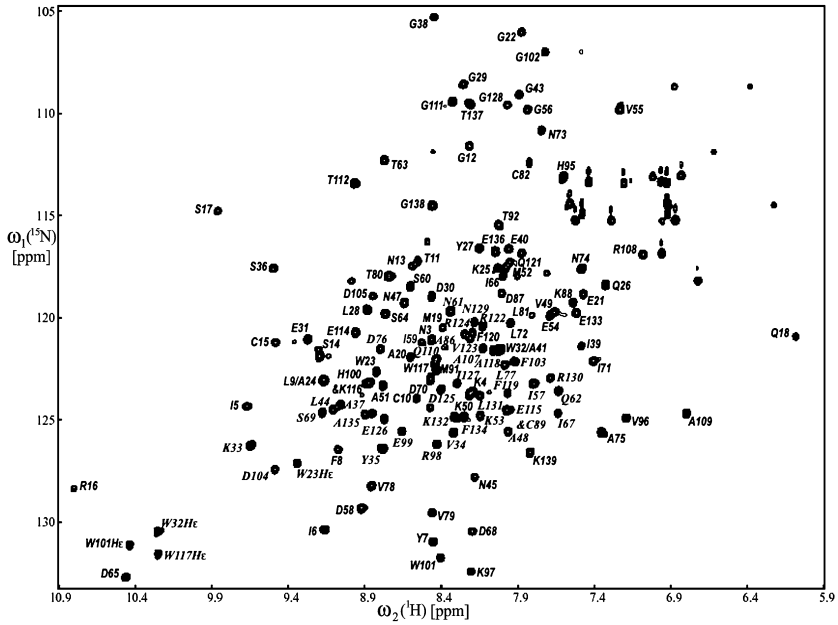

Figure 1. ^{15}N-edited HSQC spectrum of the uniformly ^{15}N labeled reduced ArsC protein in 95% H_2O/5% D_2O at pH 6.85 and 298 K. The assignments are annotated by the resonance peaks with the one-letter amino acid code and the sequence number. The Trp side-chain assignments are also shown.

3D triple resonance spectra of HNCA, HNCO, CBCA(CO)NH, HNCACB, were performed for the backbone assignments. 3D HBHA(CO)NH, C(CO)NH, HC(CO)NH, HCCH-COSY and HCCH-TOCSY (mixing time 17 ms) were performed for the side-chain assignments. The 3D NOESY-HSQC with mixing time of 100 ms was collected to confirm the backbone assignments. The side-chain assignments were confirmed with a 3D ^{15}N-edited HSQC-TOCSY (mixing time 80 ms) experiment.

Extent of assignment and data deposition

Figure 1 shows the 2D ^{1}H-^{15}N HSQC spectrum of the protein ArsC. With the high quality of spectra from the 3D triple-resonance experiments, we obtained nearly complete backbone and side-chain assignments of ^{1}H, ^{13}C and ^{15}N for the entire protein except for M1, G83, D84 and P93. The side chain ^{1}H, ^{13}C resonance assignments were obtained for over 95% of the residues. Total 39 slow exchange amide protons were identified from a series of H/D exchange experiments. Deviations of the ^{1}H, and ^{13}C chemical shifts from random coil values (Wishart et al., 1995), combined with sequential and medium range NOEs indicate that the ArsC protein consists mainly of 4 β-strands and 4 α-helices. A table of the ^{1}H, ^{13}C and ^{15}N chemical shift assignments of the ArsC protein has been deposited in the BioMagRes Bank (http://www.bmrb.wisc.edu) under the accession number 6075.

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References

Bennett, M.S., Guan, Z., Laurbeg, M. and Su, X.D. (2001) *Proc. Natl. Acad. Sci. USA*, **98**, 13577–13582.

Cervants, C., Ji, G.Y. and Silver, S. (1994) *FEMS Microbiol. Rev.*, **15**, 355–367.

Delaglio, F., Grzesiek, S., Vuister, G.W., Zhu, G., Gfeifer, J. and Bax, A. (1995) *J. Biomol. NMR*, **6**, 277–293.

Johnson, B.A. and Blevins, R.A. (1994) *J. Biomol. NMR*, **4**, 603–614.

Marley, J., Min, L. and Brachen, C. (2001) *J. Biomol. NMR*, **20**, 71–75.

Wishart, D.S., Bigam, C.G., Holm, A., Hodges, R.S. & Sykes, B.D. (1995) *J. Biomol. NMR*, **5**, 67–81.