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Functional participation of a *nifH–arsA2* chimeric fusion gene in arsenic reduction by *Escherichia coli*

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Abstract

The NifH (dimer) and ArsA proteins are structural homologs and share common motifs like nucleotide-binding domains, signal transduction domains and also possible similar metal center ligands. Given the similarity between two proteins, we investigated if the NifH protein from *Azotobacter vinelandii* could functionally substitute for the ArsA1 half of the ArsA protein of *Escherichia coli*. The chimeric NifH–ArsA2 protein was expressed and detected in the *E. coli* strain by Western blotting. Growth comparisons of *E. coli* strains containing plasmids encoding for complete ArsA, partial ArsA (ArsA2) or chimeric ArsA (NifH–ArsA2) in media with increasing sodium arsenite concentrations (0–5 mM) showed that the chimeric NifH–ArsA2 could substitute for the ArsA. This functional complementation demonstrated the strong conservation of essential domains that have been maintained in NifH and ArsA even after their divergence to perform varied functions.
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Keywords: *nifH*; *arsA*; Arsenite reduction; Chimeric gene; Complementation

Nitrogen fixation and arsenic resistance are both processes that had developed very early in the evolution of life [1,2]. Nitrogen fixation is driven by the nitrogenase enzyme and its major structural components are the NifH, D, and K proteins [2–4]. The NifH is the obligate electron donor to the NifDK protein for the nitrogen fixation reaction. The biosynthesis and assembly of the [4Fe–4S] cluster found in NifH and the FeMoco and P-Cluster found in NifDK is carried out by a host of *nif* accessory factors that mainly include the NifE, N, M, B, Q, V, and X proteins ([5–8], for reviews, see [9,10]). In case of arsenic resistance, the most well studied mechanism of arsenic resistance is the ArsAB pump that is encoded by the *ars* operon of conjugative R-factor R773 in *Escherichia coli* and is part of the *arsR-DABC* operon [11]. The *arsA* gene encodes for a 583-amino acid catalytic subunit (63 kDa) with two ABC domains, whereas the *arsB* gene encodes a 429-amino acid (45.5 kDa) inner membrane protein that serves as both the anion channel and an anchor for the ArsA protein [12,13].

It has been speculated that nitrogen fixation and arsenite detoxification evolved from an inter-related process in the ancient past since the NifH protein of the nitrogenase complex and the ArsA protein of the arsenite detoxification pump share many structurally homologous features as revealed by the crystallized structure of the ArsA protein [14,15]. ArsA has homologous N-terminal (A1) and C-terminal (A2) halves, "indicating an evolutionary gene duplication and fusion" [16]. This ATPase contains two consensus nucleotide-binding sites (NBSs), one each in the A1 and A2 halves. The A1 and A2 halves are held together by a 25-residue linker peptide [17]. ArsA is twice the size of NifH, yet since it consists of two similar domains connected by a short linker, each ArsA monomer could actually be considered to be a pseudodimer [15].

The percentage protein sequence similarity between NifH and ArsA1 is 53.7% and between NifH and ArsA2 is 48.7% (alignment shown in Fig. 1A). Further information obtained on ArsA (PDB ID: 1F48) and NifH (PDB

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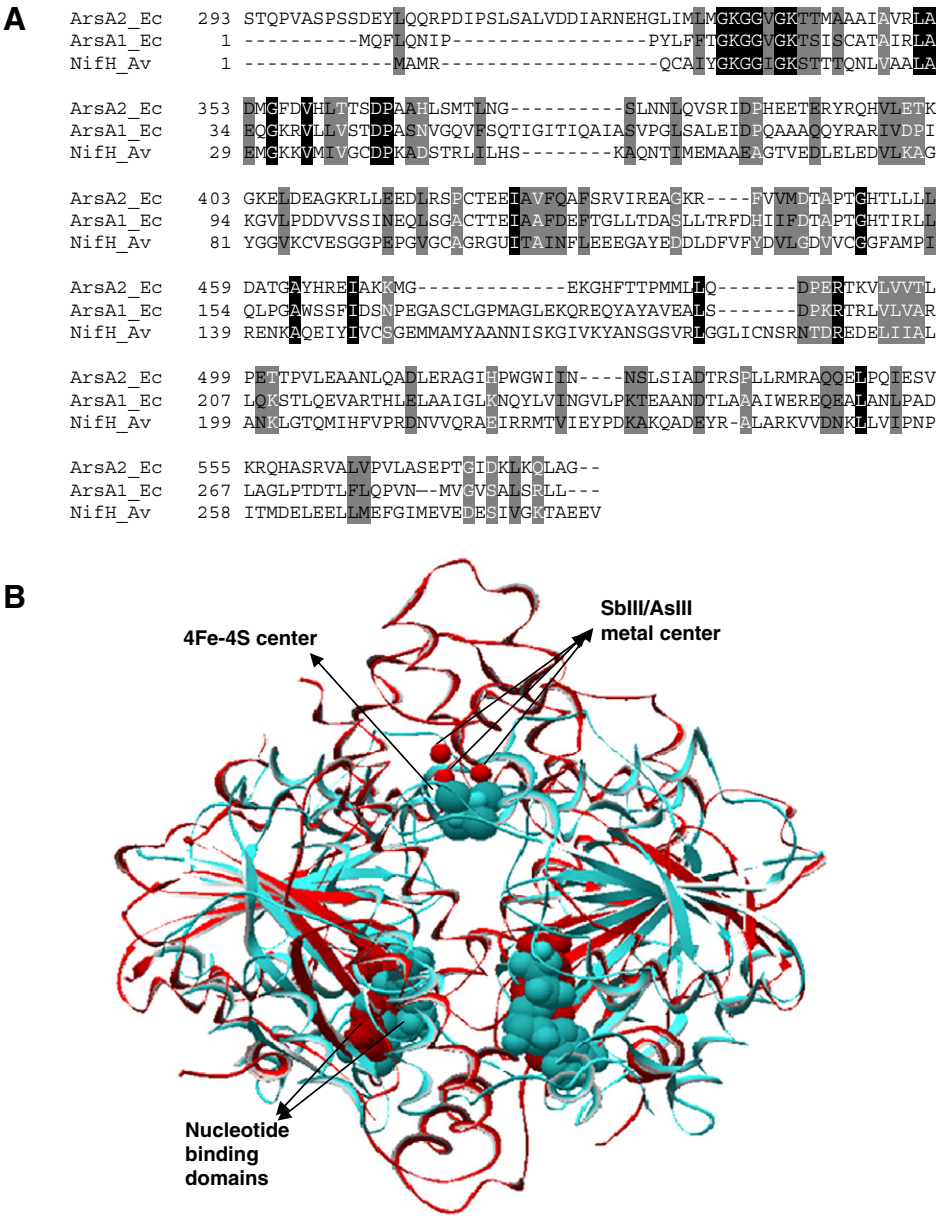

Fig. 1. (A) ClustalW alignment of the ArsA1 (*E. coli* plasmid R773), ArsA2 (*E. coli* plasmid R773), and NifH (*A. vinelandii*) amino acid sequences. The ArsA1 spans the first 292 residues of ArsA and ArsA2 spans the remaining 293–583 residues of ArsA (ArsA2 includes the linker region). Identical residues highlighted in black, conservation of strong groups, dark gray and conservation of weak groups, dark gray, with white letters; (B) superimposition of ArsA (red) on NifH dimer (cyan). Near coincidence of their metal clusters and a good fit of the β-strand structures is shown. The [4Fe–4S] metal center of NifH, the Sb(III)/As(III) metal center of ArsA, the nucleotide-binding sites of NifH and ArsA are shown as spacefilled residues and labeled. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

ID: 1FP6) protein families from the SCOP database [18] and their comparison based on structural modeling revealed that the core β-sheet pattern in both proteins, made up of at least seven parallel β-strands, could fit with a minimum root square deviation of 1.54 Å upon superimposition [14] (Fig. 1B). ArsA is a homodimer in its catalytically active form [19] and dimerization is favored by the formation of a three-coordinate complex among three specific cysteine thiolates (Cys-113, Cys-172, and Cys-442) and the effector, Sb(III) or As(III) [20,21]. Comparably, the [4Fe–4S] cluster in NifH is bound by the Cys97 and Cys132 ligands from each NifH monomer [17]. A close coincidence of the metalloid centers of ArsA and NifH can also be observed when the ArsA protein structure is superimposed on a NifH dimer. Moreover, the $D^{142/447}$TAPTGH$^{148/453}$ signature sequence of ArsA1 and ArsA2 [15] has an exact counterpart in NifH and corresponds to the Switch II region of G-proteins [22].

Similar to NifH, ArsA exists in a more open conformation in the presence of ADP and contains two NBSs. The two NBSs are each filled with MgADP and lie at the interface between ArsA1 and ArsA2. In each NBS, Mg^{2+} is

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