J Biomol NMR (2007) 38:161–164
DOI 10.1007/s10858-007-9155-9

NMR STRUCTURE NOTE

The NMR structure of the domain II of a chloroplastic NifU-like protein OsNifU1A

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Received: 29 January 2007 / Accepted: 8 March 2007 / Published online: 13 April 2007

Abstract NifU-like proteins are a highly conserved protein that serves as the scaffold for assembly of Fe-S clusters. Chloroplastic NifU-like proteins have tandem NifU like domains, named domain I and domain II. Although the amino acid sequences of these domains are very similar to each other, the predicted functional region for the Fe-S cluster assembly, the CXXC motif, exists only in domain I. The structure of the domain II of chloroplastic NifU-like protein OsNifU1A has an α-β sandwich structure containing two α helices located on one side of the β-sheet. The electrostatic surface potential of OsNifU1A domain II is predominantly positively charged. Chloroplastic NifU-like proteins are targeted to ferredoxin for transferring the Fe-S cluster. The ferredoxin presents an overall negatively charged surface, which may evoke an electrostatic association with OsNifU1A domain II.

Keywords *Oryza sativa* · NifU · NifU-like protein · Fe–S cluster · NMR structure

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Biological context

Fe–S cluster-containing proteins are widely distributed in nature and play important physiological roles in electron transfer, metabolic reactions, and gene regulation. The Fe–S cluster assembly requires a set of proteins for its formation and transfer to target proteins. In nitrogen-fixing bacteria, *Azotobacter vinelandii*, two proteins are essential for maturation of nitrogenase. NifS, a cysteine desulfurase, provides elemental sulfur from L-cysteine, and NifU is the acceptor for the iron and sulfur necessary to build the Fe–S cluster. The Fe–S cluster is subsequently transferred to the receptor protein, nitrogenase (Yuvaniyama et al. 2000). NifU is comprised of three distinct domains. The N-terminal domain contains three conserved cysteines and provides a scaffold for the assembly of the Fe–S cluster. The central and C-terminal domains also have four and two conserved cysteines, respectively, that can bind the Fe–S cluster. The Fe–S clusters assembled on the N-terminal and C-terminal domains of NifU are subsequently transferred to apo-nitrogenase (DosSantos et al. 2004). However, the Fe–S cluster bound to the central domain does not appear to be a precursor for nitrogenase maturation. Thus, the N- and C-terminal domains of NifU are known as complementary ‘‘transient‘‘ Fe–S cluster domains, while the central domain of NifU is referred to as a ‘‘permanent’’ Fe–S cluster domain.

In plants and cyanobacteria, the Fe–S cluster-containing proteins are also required for essential processes such as photosynthesis and respiration. Some of them have sequences similar to the C-terminal ‘‘transient’’ Fe–S cluster domain of NifU and have a strict conservation of the CXXC motif that is the predicted site for Fe–S cluster assembly. The *Arabidopsis thaliana* genome project identified five NifU C-terminal domain-like proteins (AtNFU1-5).

The AtNFUs were classified into two types, mitochondrial or chloroplastic, with a target signal sequence to each organellar (Leon et al. 2003). A mutant Arabidopsis lacking chloroplastic AtNFU exhibits a dwarf phenotype with faint pale-green leaves and has impaired ferredoxin and photosystem I accumulation in the chloroplast (Yabe et al. 2004). In vitro experiments demonstrated that chloroplastic AtNFUs assemble and transfer the Fe–S cluster to apo-ferredoxin.

All chloroplastic AtNFUs have tandem NifU C-terminal like domains, named domain I and domain II, at the downstream of the chloroplast target signal sequence (Leon et al. 2003). Although the amino acid sequences of these domains are very similar to each other, the predicted functional region for the Fe–S cluster assembly, the CXXC motif, exists only in domain I. Domain II of chloroplastic NifU-like protein, is highly conserved in higher plants but its functional role for Fe–S cluster formation remains elusive.

Here we present the NMR structure of domain II of chloroplastic NifU-like protein OsNifU1A isolated from *Oryza sativa* (OsNifU1A L154-S226). The structure of this protein may provide clues to reveal the functional roles of domain II in the biogenesis of ferredoxin and photosystem I.

Methods and results

The sample preparation of ^{15}N- and ^{13}C/^{15}N-labeled OsNifU1A domain II has been previously described (Katoh et al. 2005). Two- and three-dimensional NMR experiments were carried out on Varian UNITY inova spectrometers operating at 800 and 600 MHz. Spectra were processed by NMRPipe (Delaglio et al. 1995) and data analysis was assisted by Sparky program (Kneller and Goddard 1997). ^{1}H, ^{13}C and ^{15}N resonance assignments were carried out using a set of standard spectra; ^{1}H-^{15}N HSQC, ^{1}H–^{13}C HSQC, HN(CO)CA, HNCA, CBCA(CO)NH, HNCACB, HNCO, (HCA)CO(CA)NH, HBHA(CO)NH, HN(CA)HA, C(CO)NH, H(CCO)NH, and HC(C)H–TOCSY. Figure 1 shows a ^{1}H–^{15}N HSQC spectrum of OsNifU1A domain II in which all of the backbone amide resonances were assigned except for Ala199 (Fig. 1). Nearly complete side-chain resonances were also assigned. The ^{1}H, ^{13}C and ^{15}N chemical shifts were referenced to DSS according to the IUPAC recommendation. Interproton distance restraints for structural calculation were obtained from ^{13}C-edited NOESY-HSQC and ^{15}N-edited NOESY-HSQC spectra using a 150 ms mixing time. The structures were calculated using the CYANA software package (Herrmann et al. 2002). As input for the final calculation of the three-dimensional structure of OsNifU1A domain II, a total of 2,830 distance restraints were used (Table 1). At each stage, 100 structures were calculated using 10,000 steps of simulated annealing, and a final ensemble of 20 structures was selected based on CYANA target function values.

The overlay of the 20 structures and the ribbon model of the mean structure of OsNifU1A domain II are shown in Fig. 2A and B, respectively. The structure of OsNifU1A domain II has two α-helices (α1: Asn157–Ala173 and α2:

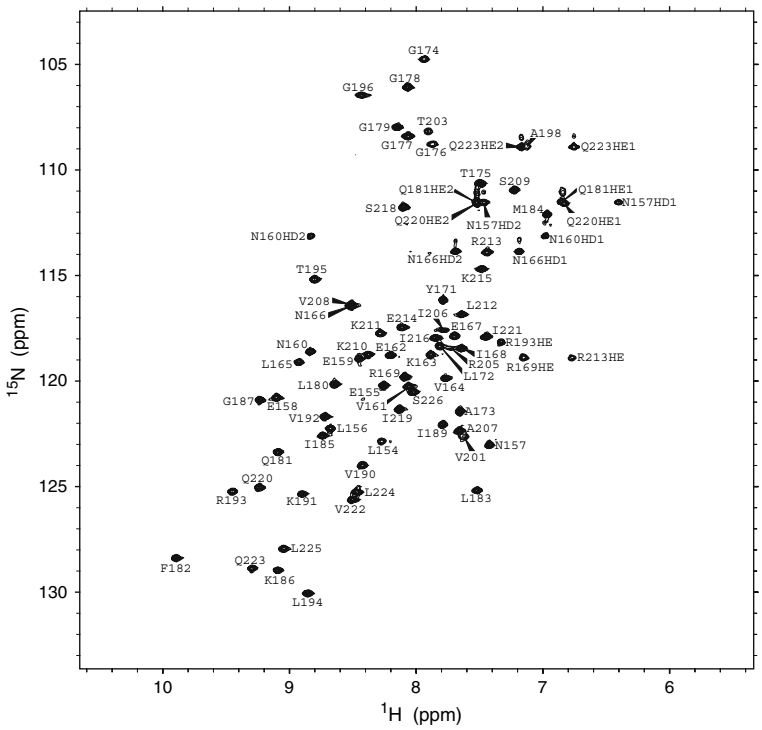

Fig. 1 2D ^{1}H–^{15}N HSQC spectrum of OsNifU1A domain II at 600 MHz and 25°C. The assignments of the backbone amide groups are labeled

Table 1 Structural statistics of OsNifU1A domain II

NOE distance constraints	2,830
Short range (intraresidue and sequential)	2,145
Medium range ($2 \leq \|i-j\| \leq 4$)	314
Long range ($\|i-j\|>4$)	371
Number of distance violation > 0.15 Å	0
Structural coordinates rmsd (Å)	
Backbone atoms	0.50
All heavy atoms	0.88
Ramachandran plot	
Most favored regions	738 (60.5%)
Additionally allowed regions	425 (34.8%)
Generously allowed regions	45 (3.7%)
Disallowed regions	12 (1.0%)

Thr203–Ile216) and an antiparallel/parallel β-sheet comprised of three strands (β1: Met184–Lys186, β2: Ile189–Val192 and β3: Ile221–Leu224), where α1 is kinked at Pro170 (Fig. 2). This protein has an α–β sandwich structure containing two α helices located on one side of the β-sheet. The loop region between β2 and α2, where the CXXC motif is located in domain I, has low convergence of backbone structure (Fig. 2A) because residues in this region had very weak or no cross-peaks on the ^{1}H–^{15}N HSQC spectrum. This suggests exchange broadening due to motion on an intermediate time scale. The atomic coordinates have been deposited in the Protein Data Bank (PDB code: 1TH5).

Discussion and conclusion

We determined the solution structure of the domain II of OsNifU1A. This domain has sequence similarity to the C-terminal domain of NifU protein, but is lacking the CXXC motif; i.e., the predicted Fe–S cluster assemble site. In order to elucidate the functional roles of this domain in the assembly and transfer of the Fe–S cluster to apo-receptor proteins, its structure was compared with those deposited in the Protein Data Bank using the Dali search engine (Holm and Sander 1995). The most similar structures were NifU-like proteins with PDB code 1VEH (Z score 6.6); that is, the C-terminal domain of mouse HIRA-interacting protein 5 (HIRIP5). The mouse HIRIP5 is located in the mitochondrial matrix where it appears to be involved in iron metabolism (Schilke et al. 1999) and has a high sequence homology with Arabidopsis mitochondrial NifU-like proteins (more than 60%). Figure 2C shows the overlay of the NifU-like protein from HIRIP5 with the domain II of OsNifU1A. The RMSD between these proteins is 2.0 Å for the main chain atoms. These proteins have a common α–β sandwich architecture, but a major difference is found in the loop between β2 and α2, where the CXXC motif is inserted in HIRIP5 (shown in the stick model in Fig. 2C).

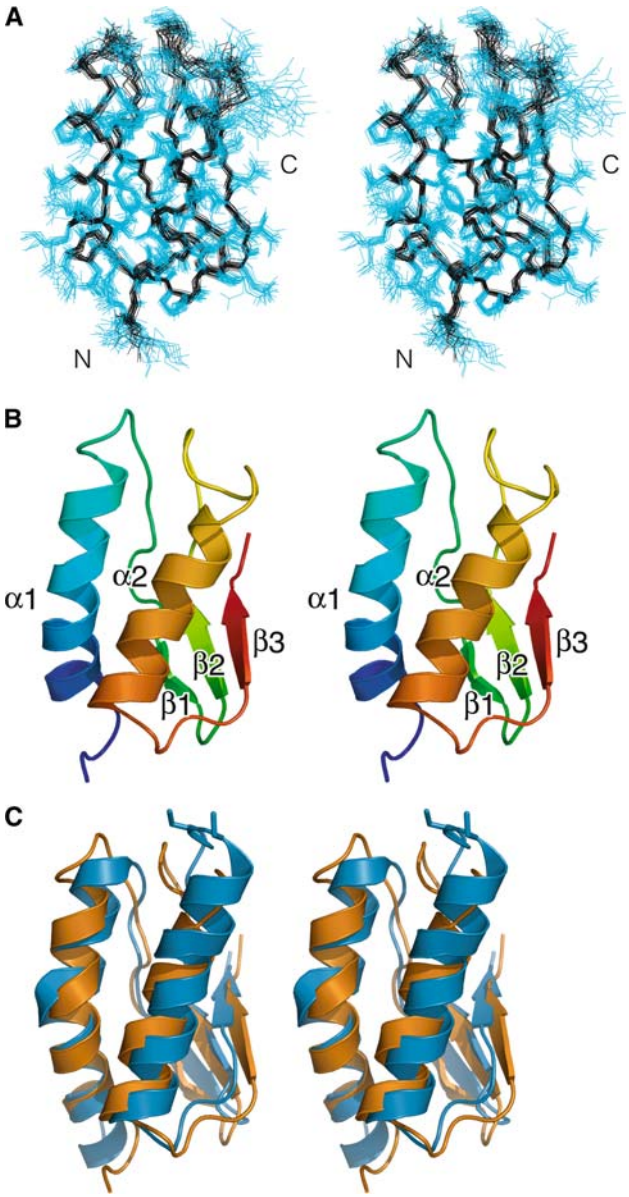

Fig. 2 (**A**) The overlay of the ensemble of the final energy-minimized CYANA 20 structures of OsNifU1A domain II in stereo where the backbone atoms (N, Ca, C′, and O) and side chain atoms from Asn157 to Leu225 are superimposed. The structure is drawn using the program MOLMOL (Koradi et al. 1996). (**B**) The Ribbon diagrams of the mean structure of OsNifU1A domain II (PDB code: 1TH5) in stereo. (**C**) Overlay of the backbone structures of mouse HIRIP5 C-terminal domain (blue) (PDB code: 1VEH) with OsNifU1A domain II (orange) in stereo. The side chains of the CXXC motif in the HIRIP5 C-terminal domain are shown as a stick model

The electrostatic surface potential of OsNifU1A domain II, calculated using APBS tools in the PyMOL program

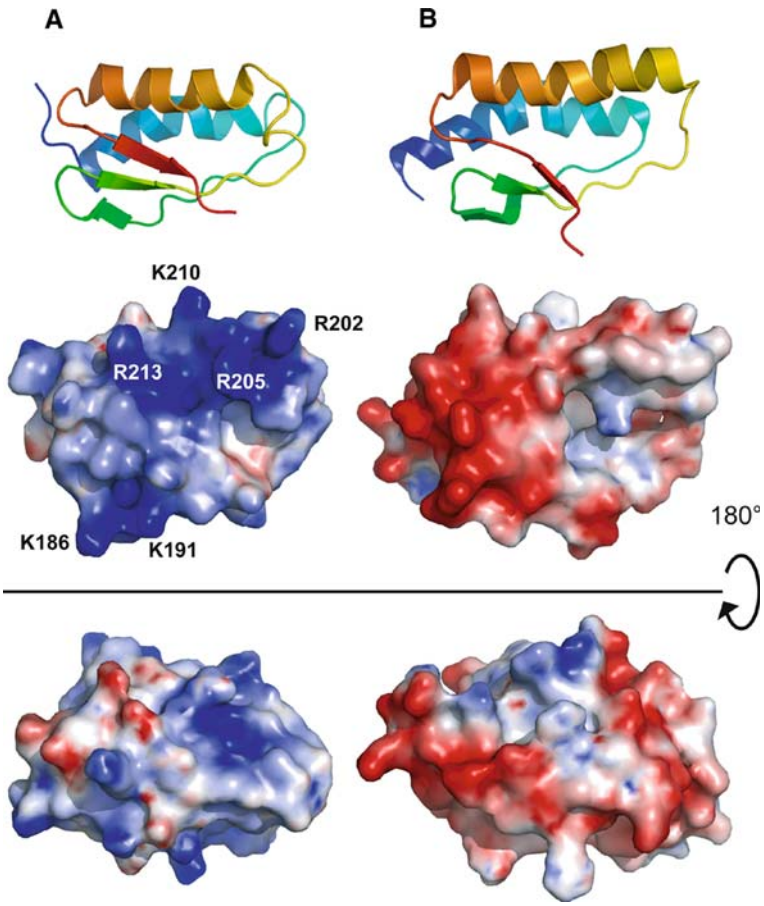

Fig. 3 Electrostatic surface potential of (**A**) OsNifU1A domain II and (**B**) mouse NifU-like protein. Top panel: the ribbon model of two proteins. Middle panel: the electrostatic surface potential presented in the same orientation as in the top panel. Lower panel: Rotation by 180° along the axis. Positive and negative charge densities are colored red and blue, respectively. The structure is drawn using the program PyMOL with APBS tools (http://www.pymol.org/)

(http://www.pymol.org/), is predominantly positively charged, as anticipated from the high isoelectric point (pI 10.6) (Fig. 3A). The positively charged surface is formed on the α2 helix and β-sheet, comprised of K186, K191, R202, R205, K210, and R213, while the opposite surface is less positively charged. In contrast, the electrostatic surface potential of the NifU-like domain of mouse HIRIP5 presents negatively charged surfaces (Fig. 3B). Chloroplastic NifU-like proteins are targeted to ferredoxin for transferring the Fe–S cluster (Yabe et al. 2004). The ferredoxin presents an overall negatively charged surface, which may evoke an electrostatic association with OsNifU1A domain II. Therefore, we can expect that OsNifU1A domain II associates with ferredoxin to facilitate the efficient transfer of the Fe–S cluster from domain I to ferredoxin.

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