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Letter to the Editor: Sequence-specific ^{1}H, ^{13}C and ^{15}N resonance assignments and secondary structure of [2Fe-2S] ferredoxin from *Halobacterium salinarum*

Kristian Schweimer[a], Bianca-Lucia Marg[b], Dieter Oesterhelt[b], Paul Rösch[a] & Heinrich Sticht[a,*]
[a]*Lehrstuhl für Biopolymere, Universität Bayreuth, Universitätsstrasse 30, D-95440 Bayreuth, Germany*
[b]*Max-Planck-Institute for Biochemistry, D-82152 Martinsried, Germany*

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Biological context

Extremely halophilic Arachaea are a group of microorganisms that require high salt concentrations of up to 4.5 M for growth. *Halobacterium salinarum*, one of the halophilic organisms most thoroughly studied, encodes a [2Fe-2S] ferredoxin with a size of 128 amino acids that was reported to serve as an electron carrier in the decarboxylation of α-ketoacids (Kerscher and Oesterhelt, 1977). The core fold of this halophilic ferredoxin, which coordinates the iron–sulfur cluster, shares a high sequence similarity with plant-type [2Fe-2S] ferredoxins and the patterns of the cluster-ligating cysteines is identical. One major difference to plant-type [2Fe-2S]-ferredoxins is the presence of an additional domain (residues 6–38) in *H. salinarum* ferredoxin that contains a large excess of negative charges, and was suggested to play a major role in halophilic adaption (Hase et al., 1977).

Up to present, structural information about halophilic ferredoxins is limited to the crystal structure of *Haloarcula marismortui* ferredoxin (Frolow et al., 1996) while no detailed NMR spectroscopic information is yet available for this class of proteins.

We assigned ^{1}H, ^{13}C, and ^{15}N resonances and deduced the secondary structure of *H. salinarum* ferredoxin from multidimensional heteronuclear NMR data. For [2Fe-2S]-ferredoxins, the availability of high resolution NMR data in the cluster vicinity is hampered by paramagnetic relaxation, with the extent of unobserved resonances depending on the sample conditions and NMR experiments applied (Im et al., 1998).

*To whom correspondence should be addressed. E-mail: Heinrich.Sticht@uni-bayreuth.de

Methods and results

Ferredoxin was isolated from *H. salinarum* cells which were grown in high salt medium. For the isotopic (^{15}N and ^{13}C/^{15}N) labeled protein samples the peptone in the high salt medium was substituted by the corresponding labeled peptone prepared from *Scenedesmus obliquus* algae as described previously (Patzelt et al., 1997). The cell lysate was prepared following protocol 6 for the isolation of purple membranes until step 3 (Robb et al., 1995). Ferredoxin was purified from the supernatant by ammonium sulfate precipitation followed by a Sepharose 4B column and a DEAE-Sephadex column according to Werber and Mevarech (1978). Samples with an A_{420}/A_{280} absorbance ratio greater than 0.3 proved to be sufficiently pure for the NMR spectroscopic studies. NMR sample conditions were : 0.8–1.0 mM oxidized ferredoxin, 50 mM potassium phosphate, pH 6.5, 450 mM sodium chloride in H_2O/D_2O (9:1). Samples for the measurements in D_2O were prepared by dissolution of the lyophilized protein in D_2O (99.996%).

All NMR experiments were acquired on a Bruker DRX 600 spectrometer at a temperature of 15 °C. The following 3D-NMR spectra were recorded for backbone and aliphatic resonance assignment: HNCO, HNCA, HNCACB, CBCA(CO)NH, H(C)CH-COSY, HBHA(CO)NH, cp-HC(C)H-TOCSY, ^{15}N-edited TOCSY and HNHA (Bax and Grzesiek, 1993; Sattler et al., 1999). For the assignment of aromatic proton resonances 2D [^{1}H,^{1}H] DQF-COSY, TOCSY,

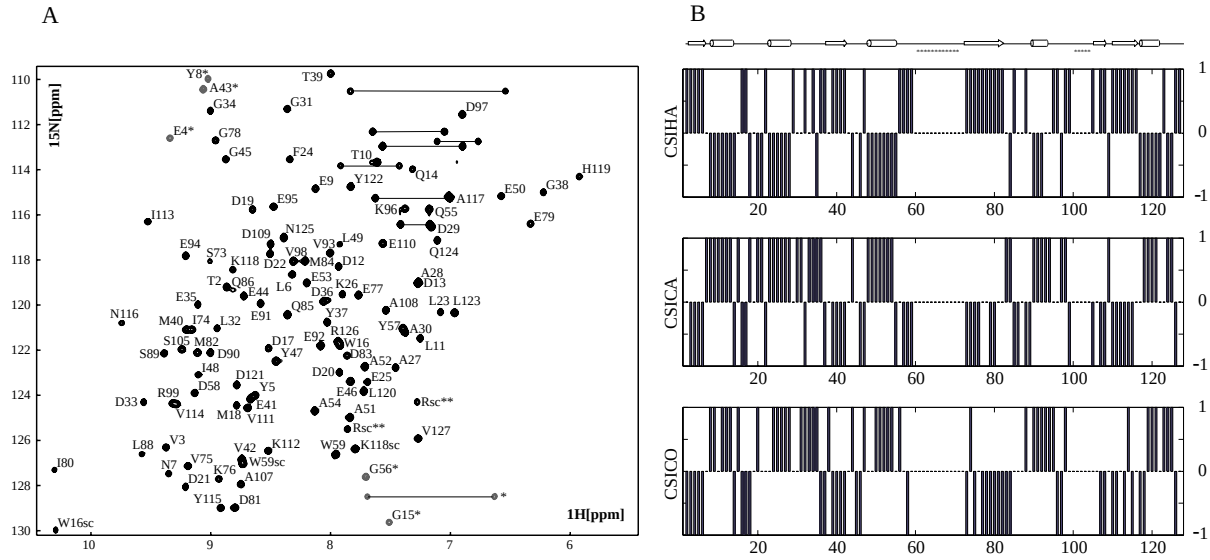

Figure 1. (A) 600 MHz [^{15}N,^{1}H]-HSQC spectrum of ^{13}C/^{15}N labeled *H. salinarum* ferredoxin at 15 °C. Resonances are labeled with the corresponding sequence positions. Side-chain NH_2 resonances are connected with a line. sc denotes side-chain resonances of arginines (HE1), of Trp 16, 59 (HE1) and acetylated Lys 118 (HZ1). Aliased resonances are marked with an asterisk, doubly aliased resonances of arginine side chains (Rsc) are marked with two asterisks. (B) Secondary chemical shift indices for HA, CA and CO nuclei of *H. salinarum* ferredoxin. The artwork at the top indicates the deduced secondary structure. The asterisks mark the not observed residues near the paramagnetic cluster.

and NOESY spectra of an unlabeled sample in D_2O were recorded. The backbone resonances were automatically assigned with an in-house written search algorithm using inter- and intraresidual C^{α} and C^{β} chemical shifts for sequential linking of amide resonances and amino acid type determination.

Extent of assignments and data deposition

The analysis of the triple resonance spectra allowed the identification and sequential assignment of 107 backbone amide resonances. No amide resonances could be found for I128 and the stretches P60–C71, L100–G104 as well as for I87 because of the proximity to the paramagnetic cluster. For all assignable residues the C^{α} and C^{β} chemical shifts could be assigned. The H^{α} assignment is complete for all observed residues with the exception of Q86. H^{β} chemical shifts could be assigned for all resonances except P1, Q86, L88, E95, R99, R126 and V127 (92% completeness). The aromatic proton resonance assignment is complete, and the other aliphatic proton and carbon side-chain resonances could be assigned to an extent of 75%. The assignment of the aliphatic resonances was hampered due to the severe overlap with degradation products and high chemical shift similarities.

The analysis of the secondary chemical shifts of HA, CA and CO nuclei (Figure 1B) allowed the identification of the secondary structure elements of *H. salinarum* ferredoxin. The secondary structure pattern is highly similar to that found for the *H. marismortui* ferredoxin. The assigned ^{1}H, ^{13}C, ^{15}N chemical shifts of *H. salinarium* ferredoxin have been deposited in the BioMagResBank, accession code 4444.

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