Solution structure of the [2Fe-2S]-ferredoxin from the halophilic archaeon *Halobacterium salinarum*

Kristian Schweimer[a], Bianca-Lucia Marg[b], Dieter Oesterhelt[b], Paul Rösch[a] and Heinrich Sticht[a]

[a]Lehrstuhl für Biopolymere, University of Bayreuth, D-95440 Bayreuth, Germany

[b]Max-Planck-Institute for Biochemistry, D-82152 Martinsried, Germany

Universität Bayreuth

Biological context

Extremely halophilic Archaea are a group of microorganisms that require high salt concentrations of up to 4.5 M for growth, that are lethal to other organisms. *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. 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 pattern 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 by coordinating water molecules and potassium ions.

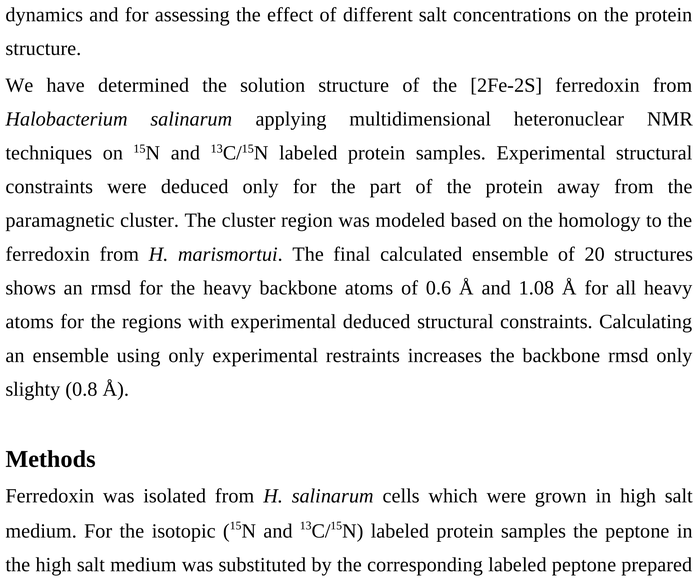
Structure-based sequence alignment of [2Fe-2S]-ferredoxins from two halobacteria (*Haloarcula marismortui*, *Halobacterium salinarum*), blue-green algae (*Anabaena PCC7120*, *Spirulina platensis*, *Synechococcus elongatus*), a green alga (*Dunaliella salina*) and plants (*Equisetum arvense*, *Oryza sativa*, *Phytolacca americana*, *Spinacia oleracea*). The numbering scheme of *H. marismortui* Ferredoxin is given at the top. Residues of the N-terminal domain of the halobacterial ferredoxins are shown in bold.

Up to present, structural information about halophilic ferredoxins is limited to the crystal structure of *Haloarcula marismortui* ferredoxin while no detailed NMR spectroscopic information is yet available for this class of proteins. NMR spectroscopic data, however, is of extreme value for the characterization of protein dynamics and for assessing the effect of different salt concentrations on the protein structure.

We have determined the solution structure of the [2Fe-2S] ferredoxin from *Halobacterium salinarum* applying multidimensional heteronuclear NMR techniques on ^{15}N and $^{13}C/^{15}N$ labeled protein samples. Experimental structural constraints were deduced only for the part of the protein away from the paramagnetic cluster. The cluster region was modeled based on the homology to the ferredoxin from *H. marismortui*. The final calculated ensemble of 20 structures shows an rmsd for the heavy backbone atoms of 0.6 Å and 1.08 Å for all heavy atoms for the regions with experimental deduced structural constraints. Calculating an ensemble using only experimental restraints increases the backbone rmsd only slighty (0.8 Å).

Methods

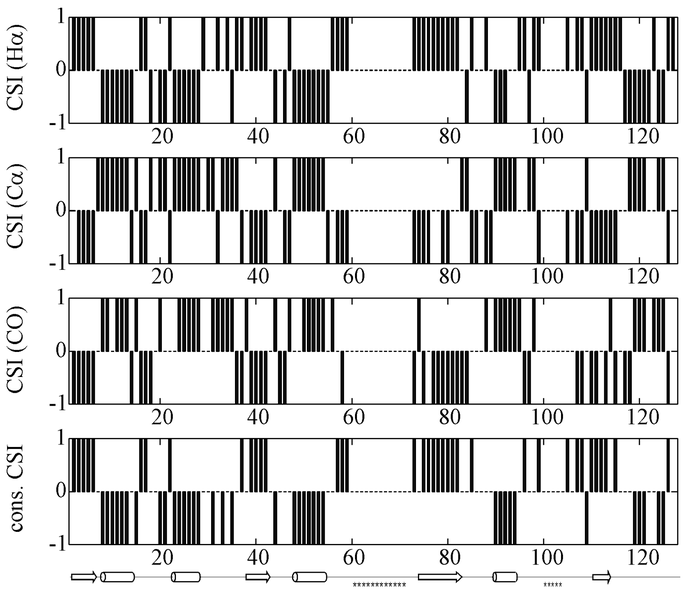
Ferredoxin was isolated from *H. salinarum* cells which were grown in high salt medium. For the isotopic (^{15}N and $^{13}C/^{15}N$) labeled protein samples the peptone in the high salt medium was substituted by the corresponding labeled peptone prepared from *Scenedesmus obliquus* algae. The purity of the ferredoxin was evaluated by its absorbance coefficient A_{420}/A_{280}. Samples with an 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%)



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. Sidechain NH_2 resonances are connected with a line. sc denotes sidechain 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 sidechains (Rsc) are marked with two asterisks.

Heteronuclear NMR spectroscopy

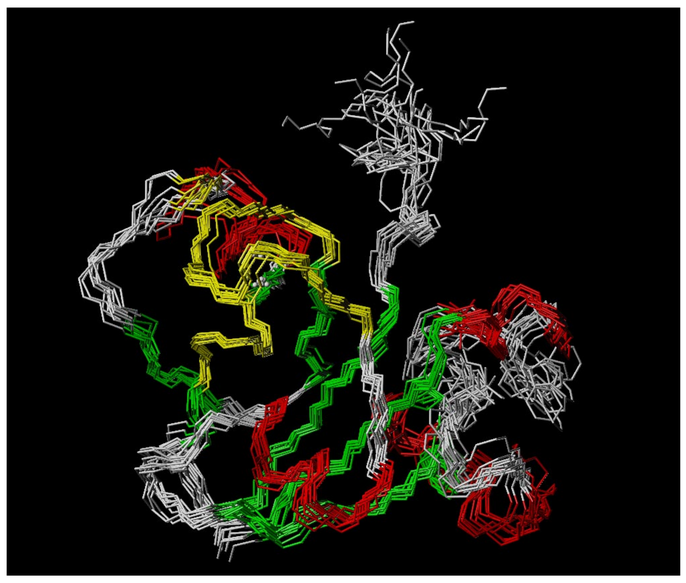
The following 3D-NMR spectra were recorded for backbone and aliphatic resonance assignment: HNCO, HNCA, HNCACB, CBCA(CO)NH, H(C)CH-COSY, HBHA(CO)NH, cp-HC(C)H-TOCSY, ^{15}N-edited TOCSY and HNHA. For the assignment of aromatic proton resonances 2D [^{1}H, ^{1}H] DQF-COSY, TOCSY, and NOESY spectra of 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. Aliphatic sidechain carbon and proton resonances were assigned by analyzing the HBHA(CO)NH, H(C)CH-COSY and cp-HCCH-TOCSY data. Aromatic proton resonance assignments were made by analysis of the homonuclear 2D NMR experiments.



Secondary chemical shift indices for HA, CA and CO nuclei of *H. salinarum* ferredoxin. The artwork at the bottom indicates the deduced secondary structure. The asterisks mark the not observed residues near the paramagnetic cluster.

Retraints used for the structure calculation

Interresidual NOEs	
sequential	(|i-j| = 1)427
medium range	(|i-j| < 5)179
long range	(|i-j| ≥ 5)325
Intraresidual NOEs	437
Dihedral constraints	
$^{3}J(H^{N},H^{a})$	56
Hydrogen bonds	46



Backbone overlay of a family of 15 *H. salinarum* ferredoxin structures. Beta-strands are colored green and helices red. The cluster region that was modeled based on the homology to the ferredoxin from *H. marismortui* is shown in yellow.

Precision of atomic coordinates (backbone / *all heavy atoms*)

	with cluster constraints	without cluster constraints
total (1-128)	1,04 / *1,56*	not calculated
region outside the cluster	0,82 / *1,34*	1,03 / *1,53*
regular secondary structure	0,66 / *1,12*	0,88 / *1,41*

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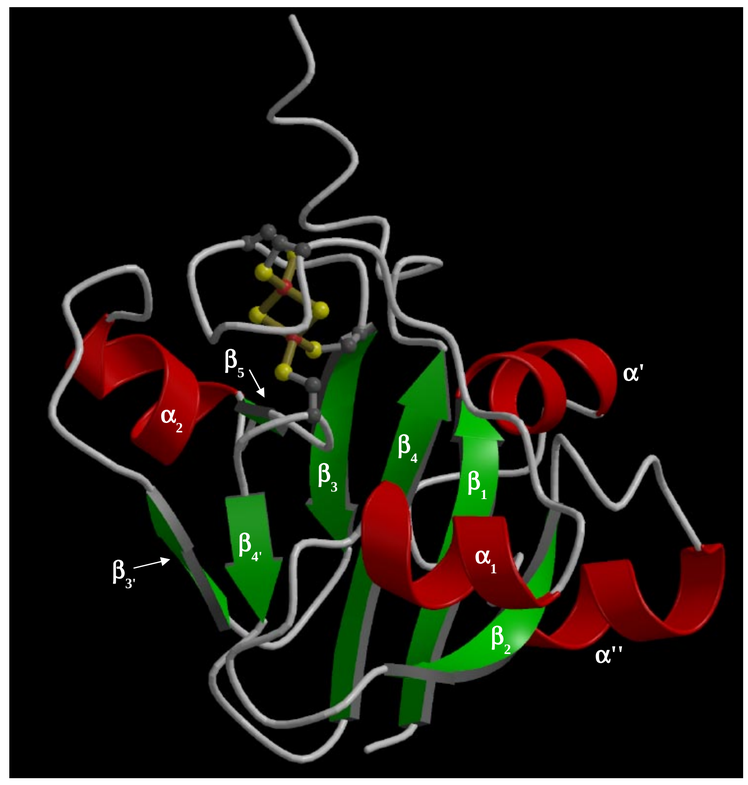
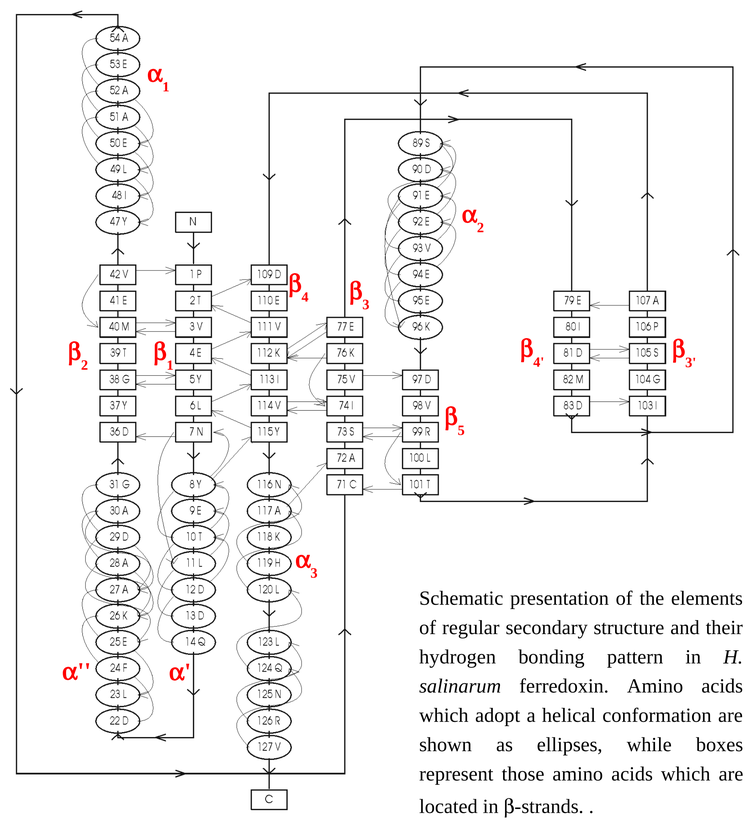
total	1-128
outside the cluster	1-59, 72-86, 88-99, 105-120
secondary structure	2-14, 24-31, 37-42, 49-56, 71-76, 79-82
	89-96, 98-100, 104-106, 110-116

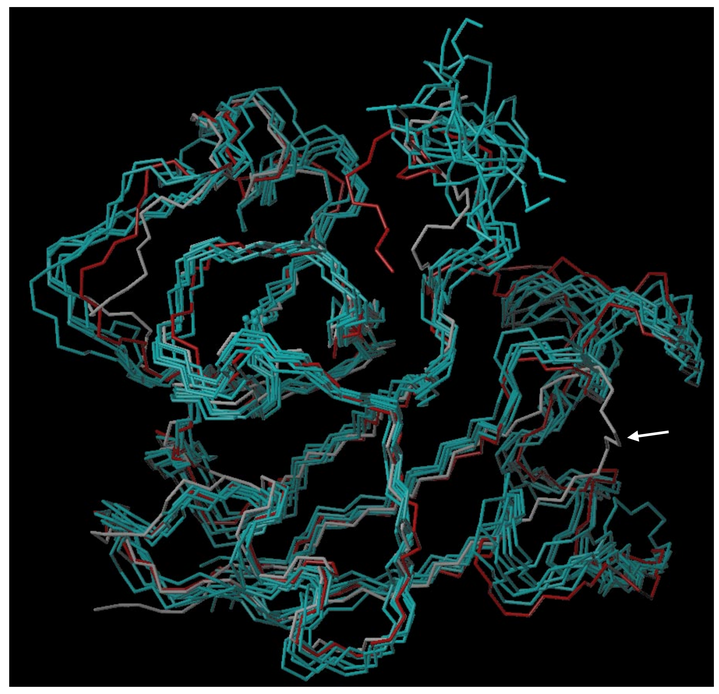


MOLSCRIPT drawing of *H. salinarum* ferredoxin. The elements of regular secondary structure are emphasized as ribbon representation. The iron-sulfur cluster and the ligating cysteines are shown in ball-and-stick representation.



Schematic presentation of the elements of regular secondary structure and their hydrogen bonding pattern in *H. salinarum* ferredoxin. Amino acids which adopt a helical conformation are shown as ellipses, while boxes represent those amino acids which are located in β-strands. .

The ferredoxin contains a five-stranded mixed β-sheet with the arrangement β2, β1, β4, β3, β5. Strands β1 and β4 are oriented in a parallel fashion, while all other strands show an antiparallel orientation. Strand β3' and β4' form an additional short β-sheet. There are five helical regions present in *H. salinarum* ferredoxin; two of them (α' and α'') are located in the additional N-terminal domain that is exlusively found in halophilic ferredoxins.



Overlay of a family of six *H. salinarum* ferredoxin structures (cyan) with the crystal structures of *H. marismortui* (red) and *Anabaena* 7120 (white) ferredoxin, revealing the similarities in the overall fold. The lack of the additional N-terminal domain in the plant-type ferredoxin from *Anabaena* is marked by an arrow.