The Crystal Structure of Zinc-Containing Ferredoxin from a Thermoacidophilic Archaeon

Tomomi Fujii and Yasuo Hata

The crystal structure of ferredoxin from thermoacidophilic archaeon *Sulfolobus* sp. strain 7 was determined by X-ray diffraction analysis at 2.0 Å resolution. The structure of the archaeal ferredoxin consists of two parts: core fold part and the N-terminal extension part. The distinct structural feature of this archaeal ferredoxin lies in the zinc-binding center where the zinc ion is tetrahedrally ligated by four amino acid residues. The zinc ion in the zinc-binding center is located at the interface between the core fold and the N-terminal extension, and connects the β-sheet in the N-terminal extension and the central β-sheet in the core fold through the zinc ligation. Thus the zinc ion plays an important role in stabilizing the structure of the present archaeal ferredoxin by connecting the N-terminal extension and the core fold, which may be common to thermoacidophilic archaeal ferredoxins.

Key words ; X-ray analysis/ Zinc/ Iron-sulfur cluster/ Thermostability

Ferredoxins (Fds) are electron transfer proteins which have iron-sulfur clusters as their active sites, and are distributed over a wide range of living organisms. Various kinds of Fds isolated from different organisms are classified by the geometry of the Fe-S cluster into several types. The dicluster-type Fds frequently isolated from bacteria are well known to share a common protein fold known as the $(\beta\alpha\beta)_2$ fold ligating two cubane-like Fe-S clusters (2[4Fe-4S] or [3Fe-4S][4Fe-4S]). This type of Fds are also isolated from Archaea.

Archaea (archaebacteria), which grow under extreme conditions such as high temperature, strong acidity, high salinity and anaerobicity, constitute a group of organisms which is distinct from Bacteria and Eucarya [1]. *Sulfolobus* sp. strain 7 is a thermoacidophilic archaeon which grows optimally at pH 2.5-3.0 and 75-80°C [2]. Ferredoxin from *Sulfolobus* sp. strain 7 is known to serve as an electron acceptor of a 2-oxoacid:Fd oxidoreductase. The *Sulfolobus* Fd molecule consists of a polypeptide of 103 amino acid residues, and two Fe-S clusters [3]. The primary structure of the *Sulfolobus* Fd is distinct from those of bacterial Fds in two regions; the *Sulfolobus* Fd has an N-terminal extension of about 40 residues and an insertion of about 10 residues in the middle of the polypeptide chain. Such a characteristic extension and insertion in sequence has also been found in ferredoxins from other thermoacidophilic archaea. Therefore, the additional regions are expected to adopt informative conformations characteristic to thermoacidophilic archaeal Fds. In order to elucidate the stabilization mechanism and the evolutionary status of archaeal proteins by investigating structural features of thermoacidophilic archaeal Fds, we have determined the crystal structure of ferredoxin from *Sulfolobus* sp. strain 7 by X-ray analysis.

The *Sulfolobus* ferredoxin was crystallized by a batch method using ammonium sulfate as a precipitant. The crystals belong to the tetragonal space group $P4_32_12$ with

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Scope of research

Our research aims are to elucidate structure-function relationships of biological macromolecules, mainly proteins, by using physicochemical methods such as spectroscopic and X-ray diffraction methods. The following attempts have been mainly made in our laboratory for that purpose. (1) Peptide secondary or supersecondary structures in aqueous or hydrophobic environments are studied to get a principle of protein architecture, employing various spectroscopic methods. (2) X-ray diffraction studies on protein structures in crystal and in solution are carried out by crystallographic and/or small-angle X-ray scattering techniques to elucidate structure-function relationships of proteins. (3) Molecular mechanism for myosin assembly is studied by proteolytic method, electron microscopy, and computer analysis of the amino acid sequence.


Prof TAKAHASHI, Sho (D Sc)


Assoc Prof HATA, Yasuo (D Sc)


Instr HIRAGI, Yuzuru (D Sc)


Instr FUJII, Tomomi (D Sc)


Instr AKUTAGAWA, Tohru

Students:
KURIHARA, Eiji (MC)
SAKAI, Hisanobu (MC)

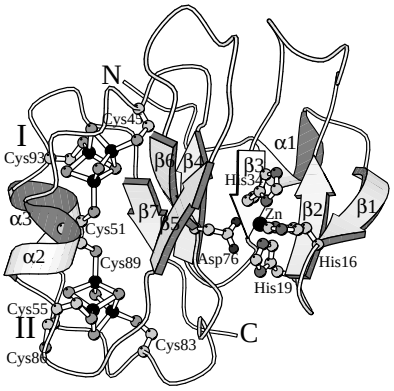

Figure 1. Schematic drawing of the *Sulfolobus* Fd molecule. β-strands are shown as gray arrows and α-helices are shown as gray spirals. The [3Fe-4S] clusters, the seven cysteines, the zinc ion and the four zinc-ligand residues are represented as ball-and-stick models. Fe-S cluster I and II are indicated as I and II, respectively.

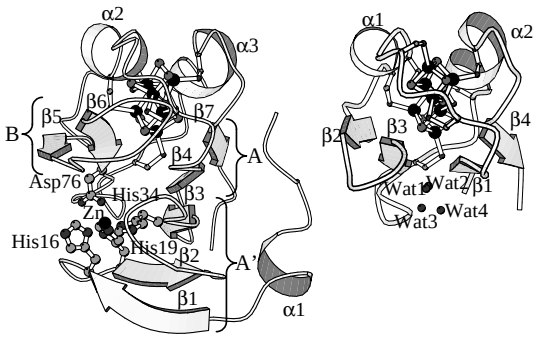

Figure 2. Schematic drawing of bacterial Fd molecules, viewed from the top of Figure 1. (left) The *Sulfolobus* Fd, (right) *Clostridium acidurici* Fd. The Fe-S clusters and the residues which participate in interaction between β-sheets A and B are represented with ball-and stick models. The atoms of the Fe-S cluster ligating cysteine residues are only represented with small balls. β-Sheets A (strands β4 and β7), A' (strands β1, β2 and β3) and B (strands β5 and β6) in the *Sulfolobus* Fd are indicated as A, A' and B, respectively.

cell dimensions of $a = b = 50.12$ Å and $c = 69.52$ Å. The structure was determined by the isomorphous replacement method for the uranium and platinum derivatives, supplemented with anomalous dispersion effects from the iron atoms of the Fe-S clusters as well as the uranium atoms in the derivative. In the course of the structural analysis, an uninterpreted high density peak coordinated by three histidines and one aspartate in a tetrahedral manner suggested that the present Fd molecule might contain a divalent metal cation such as Zn^{2+}. The X-ray crystallographic identification of the metal ion was performed using anomalous dispersion effects characteristic for zinc which were measured with a tuneable synchrotron radiation source. Finally, the whole model of the Fd molecule was refined to an R factor of 0.173 at 2.0 Å resolution.

The overall structure of the *Sulfolobus* Fd molecule is depicted in Fig. 1. The protein folding of the *Sulfolobus* Fd can be divided into two parts by a structural comparison of the present archaeal Fd with other bacterial dicluster Fds (Fig. 2). One part is the core-fold part (residues 37-103), which binds two [3Fe-4S] clusters and has a topologically conserved protein folding; a $(\beta\alpha\beta)_2$ fold, common to bacterial dicluster Fds, forms two antiparallel β-sheets, A (β4 and β7) and B (β5 and β6), and two α-helices, α2 and α3. The other part is the N-terminal extended part (residues 1-36), which is mainly formed by one-turn α-helix α1 and antiparallel β-sheet A' of strands 1-3. β-Sheet A' in the N-terminal part interacts with the terminal β-sheet A in the core-fold part through hydrogen bonds between strands β3 and β4 to form a larger five-stranded β-sheet, A'+A. As described above, the *Sulfolobus* Fd molecule contains a novel zinc-binding center, which had never been found in ferredoxins, at the interface between the core-fold part and the N-terminal extended part (Fig. 1). The zinc ion is tetrahedrally ligated by four amino acid residues, His 16, His 19 and His 34 from the N-terminal part, and Asp 76 from the core-fold part. Three ligand histidines, His 16, His 19 and His 34, to the zinc ion are located at the C-, N- and C-terminal ends of three β-strands, β1-β3, respectively, which form β-sheet A' in the N-terminal extended part. The zinc ion binds tightly through the Zn-His coordinate bonds to β-sheet A' which interacts with β-sheet A through hydrogen bonds between β3 and β4 to form the larger sheet, A'+A. The last zinc ligand, Asp 76, is located at the C-terminal end of strand β6 in β-sheet B. In this way, two β-sheets, A and B, in the core-fold part, are indirectly linked by both the zinc ligation between β-sheets B and A' and the hydrogen bonds between β-sheets A' and A (Fig. 2).

The core fold common to bacterial Fds adopts a quite simple $(\beta\alpha\beta)_2$ fold. In the core-fold part of bacterial dicluster Fds, β-sheets A and B are usually so apart from each other that they do not interact directly (Fig. 2). The unfolding of the core fold may begin with dissociation of the two β-sheets. If the interaction between the β-sheets is strong, the unfolding of the core fold would be unlikely to occur. Indeed, the *Sulfolobus* Fd seems to utilize the zinc ion, the N-terminal extension and the insertion in order to enhance the stability of the core fold through the indirect interaction between the two β-sheets. Therefore, thermoacidophilic archaeal Fds may acquire thermostability primarily by inserting the zinc ion in the interface between the extended N-terminal and the core-fold parts, as observed in the *Sulfolobus* Fd. It is expected that biochemical and biophysical studies on the roles of the zinc ion in thermoacidophilic archaeal Fds might lead to elucidation of the role of metal ions in protein thermostability.

References

1. Woese C R , Kandler O and Wheelis M L, *Proc. Natl. Acad. Sci. U.S.A.*, **87**, 4776-4579 (1990).
2. Wakagi T and Oshima T, *Biochim. Biophys. Acta*, **817**, 33-41 (1985).
3. Wakagi T, Fujii T and Oshima T, *Biochem. Biophys. Res. Commun.*, **225**, 489–493 (1996).