Volume 244, number 2, 447-450 FEB 06805 February 1989

The crystal structure of the three-iron ferredoxin II from *Desulfovibrio gigas*

C.R. Kissinger, E.T. Adman, L.C. Sieker, L.H. Jensen and J. LeGall*

Department of Biological Structure, University of Washington, Seattle, WA 98195 and **Department of Biochemistry, University of Georgia, Athens, GA 30602, USA*

Received 19 December 1988

The crystal structure of oxidized ferredoxin II from the sulfate-reducing bacterium *Desulfovibrio gigas* has been determined and refined at 1.7 Å resolution. The folding of the polypeptide chain is similar to that of the 2[4Fe-4S] ferredoxin in *Peptococcus aerogenes*, except for an extended helical segment near the C-terminus. The single [3Fe-4S] cluster in *D. gigas* is similar to a [4Fe-4S] cluster, but lacks one Fe atom and is coordinated to Cys-8, -14 and -50. The side chain of Cys-11 is not bound to the cluster, but is rotated toward the solvent and modified by some, as yet undetermined, chemical group. Cys-18 and Cys-42 form a disulfide bridge. A previously undetected extra amino acid is found after residue 55.

Ferredoxin; Iron-sulfur cluster; Crystal structure; Anomalous scattering; Disulfide bridge; (*Desulfovibrio gigas*)

1. INTRODUCTION

Ferredoxin I (DgFdI) and ferredoxin II (DgFdII) are two distinct iron-sulfur proteins isolated from the sulfate-reducing bacterium *Desulfovibrio gigas* [1]. Both ferredoxins are composed of the same polypeptide chain, but they are reported to have significantly different molecular masses due to different states of oligomerization. The monomeric unit of DgFdI contains a single [4Fe-4S] cluster, whereas the same monomeric unit of DgFdII contains a single [3Fe-4S] cluster [2]. The [4Fe-4S] center in DgFdI exhibits a midpoint redox potential of approx. −450 mV, but the [3Fe-4S] center of DgFdII has a midpoint potential of about −130 mV [3]. Experiments by Moura et al. have shown that the two forms can be interconverted [4] and that this interconversion appears to have physiological significance, since DgFdII spontaneously undergoes conversion to DgFdI when it is used as an electron mediator in the phosphoroclastic reaction [3]. This capability for interconversion has been utilized to create new mixed-metal clusters, such as [Co,3Fe-4S] [5] and [Zn,3Fe-4S] clusters [6].

Spectroscopic studies by Mössbauer, EPR [3,7] and extended X-ray absorption fine structure (EXAFS) [8] indicate that the [3Fe-4S] cluster in DgFdII has similar geometry to the [4Fe-4S] clusters in *Peptococcus aerogenes* ferredoxin (PaFd) [9] and in high-potential iron protein (HIPIP) from *Chromatium vinosum* [10]. The clusters in these proteins have Fe–Fe and Fe–S distances of approx. 2.8 and 2.2 Å, respectively. Studies on aconitase, which has been shown to have a 3Fe cluster with similar spectral characteristics, confirms a [3Fe-4S] stoichiometry [11]. A cuboidal [3Fe-4S] geometry for the 3Fe center of ferredoxin I from *Azotobacter vinelandii* (AvFdI) was also proposed on the basis of spectroscopic evidence [12,13], but none of these studues indicated which Fe atom, with its ligating cysteine, was removed or the location of the side chain of the cysteine subsequent to becoming released from the cluster.

DgFd shows considerable sequence homology to

Correspondence address: L.C. Sieker, Department of Biological Structure, SM-20, University of Washington, School of Medicine, Seattle, WA 98195, USA

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/89/$3.50

other Fds that contain one or two [4Fe-4S] clusters [14]. The recent redetermination of the structure of AvFdI [15,16] has shown that the N-terminal half of the chain in that molecule has a fold almost identical to that in PaFd. The crystal structure of the [4Fe-4S] Fd from *Bacillus thermoproteolyticus* (BtFd) shows that its chain fold is also similar to that in PaFd, and led Fukuyama et al. [17] to predict that the chains in other Fds, including DgFdII, would fold similarly.

We report here the structure of oxidized DgFdII as determined by X-ray diffraction at 1.7 Å. Complete details of the crystallographic solution, interpretation of the electron density map and refinement of the DgFdII model will appear separately. The detailed geometry of the [3Fe-4S] cluster from this study has already been reported [18].

2. EXPERIMENTAL

The C2 crystal form of DgFdII [19], which has a single monomer in the asymmetric unit, was used for this analysis. Because no heavy-atom derivatives were found, the technique of resolved anomalous phasing was used to solve the structure [20].

Initial attempts to solve the Bijvoct Patterson at 2.5 Å [21] for the structure of the iron atoms resulted in ambiguity regarding the nature of the Fe core. Although the spectroscopic evidence strongly indicated a 3-iron core, our model distances did not refine to values consistent with either the 4Fe PaFd core or the then reported 3Fe AvFdI core [22]. Extension of the diffraction data to 1.7 Å led to a Bijvoet Patterson map that was interpretable in terms of a 3Fe center with Fe–Fe distances of approx. 2.8 Å. Confidence in this geometry, coupled with test calculations using the resolved anomalous phasing method [20] on a known structure (rubredoxin from *D. vulgaris* [23]), encouraged us to proceed and to phase the X-ray reflections by this technique. The model derived from the resulting electron density map has been refined to an R value of 0.176 with a nearly complete solvent model.

3. RESULTS AND DISCUSSION

Fig.1 is a stereo drawing of the C^{α} chain, showing that its fold is similar to the fold in PaFd with a helical segment as predicted by Fukuyama et al. [17]. The [3Fe-4S] cluster corresponds to a [4Fe-4S] cluster minus an Fe atom, one of the models proposed by Beinert and Thomson [24], and it is ligated by Cys-8, -14 and -50 but not Cys-11. The average Fe–Fe and Fe–S distances are 2.75 and 2.25 Å, respectively.

The side chain of Cys-11 is rotated away from the cluster, extending toward solvent, and is not involved in any crystal packing interactions. As refinement of the structural model has progressed, it has become apparent that Cys-11 is modified. At this point, S^{γ} of Cys-11 appears to be covalently linked to a group of atoms resembling $S(CH_3)_n$ where n is 1 or 2. The shape and density of this group indicate clearly that it is not an adventitiously bound heavy atom. It is interesting to note that methanethiol has been found as a product of *D. gigas* metabolism [25] and that cysteinyl residues modified by addition of this compound have been detected in a streptococcal proteinase [26]. These facts could indicate that the [3Fe-4S] DgFdII is not a purification artifact and that it has physiological significance.

The molecule has an additional amino acid inserted after Ile-55, bringing the total number of residues to 58. The electron density is consistent with a valine at this location. The addition of a residue places the C-terminal segment of DgFdII in better agreement with the amino acid sequence of Fd from *D. desulfuricans* (strain Norway 4 [14]).

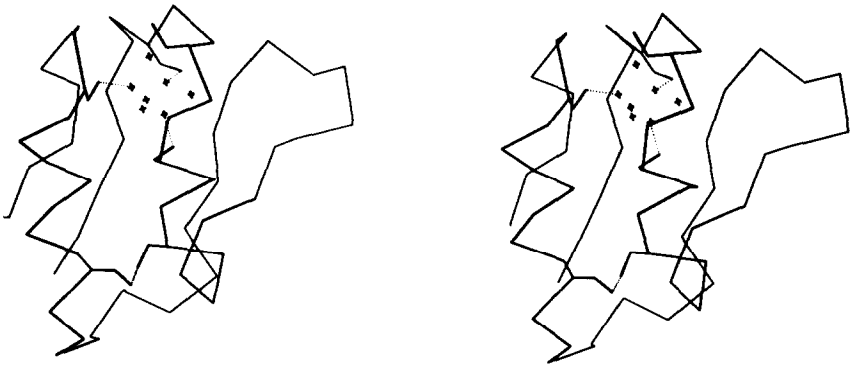

Fig.1. Stereo line drawing of the α-carbon chain folding. The six cysteine residues are also shown.

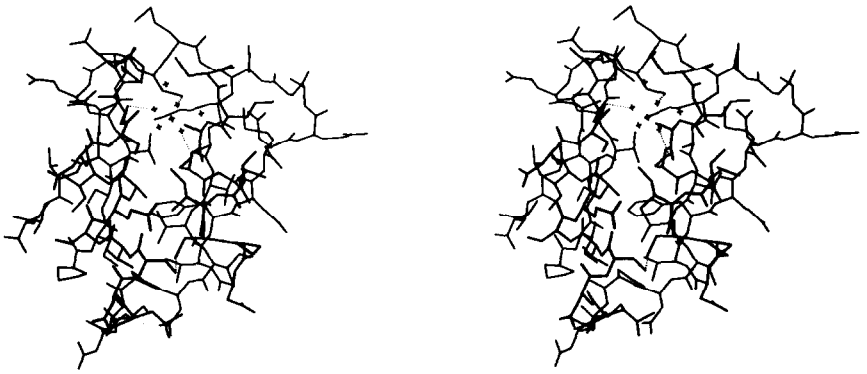

Fig.2. Stereo drawing of the current model of the DgFdII molecule. Several external side chain positions have not been unambiguously determined and therefore some of their atoms are not shown.

Another previously undetected feature in this structure is a disulfide bridge between Cys-18 and Cys-42, linking the remaining two cysteines of the four that bind the second cluster of PaFd. The presence of a disulfide bridge in a ferredoxin has not been reported heretofore, and its significance deserves further scrutiny. Since the BtFd does not contain a disulfide bridge and yet has a fold similar to DgFdII [17], the disulfide is not necessary to maintain the overall structure of single cluster ferredoxins. However, the bridge may stabilize the chain folding in such a way that the cluster interconversion in DgFd is facilitated.

Fig.2 is a stereo view of the current model of the molecule. The [3Fe-4S] cluster is protected from solvent by the chain segment 8–14. The figure shows that a simple rotation about the C^{α} -C^{β} bond of Cys-11 will bring the S^{γ} atom into the fourth iron position, so that adding a fourth iron would require a readjustment of the polypeptide chain in this region.

The association of monomers in this crystal form is primarily through hydrogen bonding of Asp and Glu residues about the crystallographic 2-fold axes. Since crystals can only be obtained at pH 5.0 from a solution adjusted to be 2.5 M ammonium sulfate [19], and the two oligomeric forms were isolated and studied at pH 7 [1–4], it is not clear that this association is relevant to the functional forms.

Acknowledgements: We gratefully acknowledge the help of Drs I. Moura, J.J.G. Moura and A.V. Xavier in the purification and supply of the material and for their continuing interest in this study. Partial funding for this research is from NIH grants GM-13366 (L.H.J.), GM-31770 (E.T.A.), GM 41482 (J.L.), NSF grant DMB-8602789 (J.L.), and the Norwegian Marshall Fund (E.T.A.).

REFERENCES

[1] Bruschi, M., Hatchikian, E.C., LeGall, J., Moura, J.J.G. and Xavier, A.V. (1976) Biochim. Biophys. Acta 449, 275–284.
[2] Kent, T.A., Moura, I., Moura, J.J.G., Lipscomb, J.D., Huynh, B.H., LeGall, J., Xavier, A.V. and Munck, E. (1982) FEBS Lett. 138, 55–58.
[3] Moura, J.J.G., Xavier, A.V., Hatchikian, E.G. and LeGall, J. (1978) FEBS Lett. 89, 177–179.
[4] Moura, J.J.G., Moura, I., Kent, T.A., Lipscomb, J.D., Huynh, B.H., LeGall, J., Xavier, A.V. and Munck, E. (1982) J. Biol. Chem. 257, 6259–6267.
[5] Moura, I., Moura, J.J.G., Munck, E., Papaefthymiou, V. and LeGall, J. (1986) J. Am. Chem. Soc. 108, 349–351.
[6] Surerus, K., Munck, E., Moura, I., Moura, J.J.G. and LeGall, J. (1987) J. Am. Chem. Soc. 109, 3805–3807.
[7] Huynh, B.H., Moura, J.J.G., Moura, I., Kent, T.A., LeGall, J., Xavier, A.V. and Munck, E. (1980) J. Biol. Chem. 255, 3242–3244.
[8] Antonio, M.R., Averill, B.A., Moura, I., Moura, J.J.G., Orme-Johnson, W.H., Teo, B.K. and Xavier, A.V. (1982) J. Biol. Chem. 257, 6646–6649.
[9] Adman, E.T., Sieker, L.C. and Jensen, L.H. (1973) J. Biol. Chem. 248, 3987–3996.
[10] Carter, C.W. jr, Kraut, J., Freer, S.T., Xuong, N.H., Alden, R.A. and Bartsch, R.G. (1974) J. Biol. Chem. 249, 4212–4225.
[11] Beinert, H., Emptage, M.H., Dreyer, J.L., Scott, R.A., Hahn, J.E., Hodgson, K.O. and Thomson, A.J. (1982) Proc. Natl. Acad. Sci. USA 80, 393–396.
[12] Johnson, M.K., Bennett, D.E., Fee, J.A. and Sweeney, W.V. (1987) Biochim. Biophys. Acta 911, 81–94.
[13] Stephens, P.J., Morgan, T.V., Devlin, F., Penner-Hahn, J.E., Hodgson, K.O., Scott, R.A., Stout, C.D. and Burgess, B.K. (1985) Proc. Natl. Acad. Sci. USA 82, 5661–5665.

[14] Bruschi, M. and Guerlesquin, F. (1988) FEMS Microbiol. Rev. 54, 155–176.
[15] Stout, G.H., Turley, S., Sieker, L.C. and Jensen, L.H. (1988) Proc. Natl. Acad. Sci. USA 85, 1020–1022.
[16] Stout, C.D. (1988) J. Biol. Chem. 263, 9256–9260.
[17] Fukuyama, K., Nagahara, Y., Tsukihara, T., Katsube, Y., Hase, T. and Matsubara, H. (1988) J. Mol. Biol. 199, 183–193.
[18] Kissinger, C.R., Adman, E.T., Sieker, L.C. and Jensen, L.H. (1988) J. Am. Chem. Soc., in press.
[19] Sieker, L.C., Adman, E.T., Jensen, L.H. and LeGall, J. (1984) J. Mol. Biol. 179, 151–155.
[20] Hendrickson, W.A. and Teeter, M.M. (1981) Nature 290, 107–113.
[21] Sieker, L.C., Adman, E.T., Jensen, L.H. and LeGall, J. (1984) Acta Crystallogr. suppl. A40, 363.
[22] Ghosh, D., O'Donnell, S., Furey, S. jr, Robbins, A.H. and Stout, C.D. (1982) J. Mol. Biol. 158, 73–109.
[23] Adman, E.T., Sieker, L.C., Jensen, L.H., Bruschi, M. and LeGall, J. (1977) J. Mol. Biol. 112, 113–120.
[24] Beinert, H. and Thomson, A.J. (1983) Arch. Biochem. Biophys. 222, 333–361.
[25] Hatchikian, E.C., Chaigneau, M. and LeGall, J. (1976) in: Microbial Production and Utilization of Gases (Schlegel, H.G. et al. eds) pp. 109–118, Goltze, Göttingen.
[26] Lo, S.-S., Fraser, B.A. and Liu, T.-Y. (1984) J. Biol. Chem. 259, 1041–1145.