Structure of rubredoxin from the bacterium *Desulfovibrio desulfuricans*

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The X-ray crystallographic structure of rubredoxin from *Desulfovibrio desulfuricans* strain 27774 is described. This molecule is 15% smaller than previously studied rubredoxins, lacking a seven-residue loop of chain but containing a histidine and a free-sulfhydryl cysteine. Except for solvent exposure of the single invariant tryptophan, no other major difference occurs in the molecule.

Rubredoxin *Fe-protein* *Histidine* *Sulfhydryl group* *Crystallography* *Electron transport*

1. INTRODUCTION

Rubredoxins (RBs) are small Fe-proteins believed to be involved in electron transfer reactions in many anaerobic bacteria. Typically, these small metalloproteins comprise 52–54 amino acid residues and have a molecular mass of approx. 6 kDa [1]. RBs from different sources have somewhat different primary sequences but the four cysteine ligands of the single Fe atom in these RBs are invariant and only conservative changes in the aromatic residues have been reported.

These iron-proteins show species-specific interactions with a rubredoxin oxidoreductase [2] and a small but measurable range in redox potential [3]. Structural studies of several rubredoxins are being carried out to provide a structural basis for understanding the differences in redox potentials, to determine any detectable difference in the Fe-S_4 complexes, and to compare structural features for clues to the differences in specificity of electron transfer.

X-ray diffraction studies of one clostridial and two desulfovibrio type RBs have shown the virtual identity of chain folding, the aromatic and cysteine residues having essentially the same positions and conformations [4–6]. Fig.1 shows the amino acid

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                          5                  10                  15
RBCP  f-MET-Lys-Lys-Tyr-Thr-CYS-Thr-Val-CYS-GLY-TYR-Ile-TYR-ASP-Pro-
RBDV                      Val                            Glu
RBDG        Asp Ile       Val                            Glu
RBPE        Asp           Glu      Ser Ile                           Glu
RBPA        Gln      Phe  Glu           Leu
RBDD  f-MET-Gln-Lys-Tyr-Val-CYS-Asn-Val-CYS-GLY-TYR-Glu-TYR-ASP-Pro-

                          20                  25                  30
RBCP    Glu-Asp-Gly-Asp-Pro-Asp-Asp-Gly-Val-Asn-Pro-GLY-Thr-Asp-PHE-
RBDV    Ala Glu                Thr Asn           Lys                 Ser
RBDG        Lys                      Ser       Ile-Lys             Lys
RBPE        Glu            ---       Gly Asn       Ala Ala         Lys
RBPA        Leu Val Gly              Thr Pro Asp Gln Asp       --- Ala
RBDD    Ala-Glu-His-Asp --- --- --- --- --- --- --- Asn-Val-Pro-PHE-

                          35                  40                  45
RBCP    Lys-Asp-Ile-Pro-Asp-Asp-TRP-Val-CYS-PRO-Leu-CYS-GLY-Val-Gly-
RBDV    Asp      Leu      Ala                        Val           Ala Pro
RBDG    Glu      Leu                    Ala         Val           Ala Ser
RBPE    Ala      Leu      Ala                        Thr           Ala Asp
RBPA    Glu      Val Ser Glu Asn                                   Ala
RBDD    Asp-Gln-Leu-Pro-Asp-Asp-TRP-Cys-CYS-PRO-Val-CYS-GLY-Val-Ser-

                          50                  55
RBCP    LYS-Asp-Glu-PHE-Glu-Glu-Val-Glu-Glu
RBDV        Ser              Ala Ala --- ---
RBDG             Ala         Lys Gln --- ---
RBPE             Ala     Val Lys Met Asp ---
RBPA        Glu Asp          Val Tyr      Asp
RBDD    LYS-Asp-Gln-PHE-Ser-Pro-Ala --- ---
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Fig.1. The amino acid residues of the other rubredoxins are placed in the appropriate column where they differ from the rubredoxin of *Clostridium pasteurianum*. Deletions are shown as dashes in the column. The complete sequence of RBDD is given. Invariant residues are capitalized. RBPE, rubredoxin from *Peptostreptococcus elsdenii*; RBPA, rubredoxin from *Peptococcus aerogenes*.

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sequences of the RBs from *Clostridium pasteurianum* (RBCP), *Desulfovibrio vulgaris* (RBDV) and *D. gigas* (RBDG), along with the sequences of the RBs from *Peptostreptococcus elsdenii* (RBPE) and *Peptococcus aerogenes* (RBPA). The major variations are in RBPA where phenylalanine is substituted for tyrosine at position 4 and tyrosine with its aromatic side chain is found at position 52.

Recently, a strain of *D. desulfuricans* capable of reducing sulfur and nitrate (strain 27774) was discovered to possess an RB (RBDD) that is significantly smaller than previously found and has histidine in its composition [12]. We report here a brief description of the 3D structure of RBDD. A more extensive account covering the solution of the structure and the refinement of the model will appear elsewhere.

2. EXPERIMENTAL

The crystals of RBDD are triclinic, space group P1. The unit cell parameters are the following: a = 24.91 Å, b = 17.78 Å, c = 19.71 Å, α = 101.0°, β = 83.4°, γ = 104.5°, and there is one molecule per unit cell. Diffraction data were collected to 1.5 Å resolution, including both reflections of each Friedel pair. These were averaged to cancel the effects of anomalous scattering from the Fe and sulfur atoms. The fact that the position of the origin is arbitrary in P1 enables one to solve the structure solely by applying the rotation function [13]. The correct solution of a rotation function calculated using the RBDV structure as the model served as the basis for calculating the initial electron density map. Most of the invariant features of the RB molecules were recognized at the outset, but it was apparent that some residues were missing from the hairpin loop (see plate I in [14]) between residues 16 and 27. However, the close packing in this region placed the C-terminal part of the chain of a neighboring molecule in the vicinity of the missing residues. This fact, coupled with the discrete solvent structure and the uncertainty in the amino acid sequence based on those of the other RBs, complicated the map interpretation, making it difficult to determine the chain deletions in the hairpin loop, the position of the histidine residue which was later shown to be located in the same region [15], and the number of residues beyond the phenylalanine near the C-terminus. It proved impossible to interpret the map until the amino acid sequence was available despite the fact that approx. 85% of the model was correct, and it had been refined to R = 0.20 for the 1.5 Å data set.

Subsequently the amino acid sequence, which appears in fig.1, revealed seven missing residues in the hairpin loop, three additional residues following the phenylalanine near the C-terminus and the location of the histidine at position 18. An additional cysteine immediately precedes the third cysteine ligand of the Fe atom [15]. The C-terminal residue is the equivalent of position 52, numbered according to RBCP.

Once the sequence was known, the remaining density in the electron density map could be readily interpreted. The α-carbon plot of RBDD is shown in fig.2a and is to be compared with that for RBDV in fig.2b. The positions of His 18 and Cys 38 are also shown in fig.2a, and Trp 37 appears in both plots.

3. RESULTS AND DISCUSSION

Several interesting features are found in the RBDD structure. The major differences in chain folding involve residues 18 and 19 which reorient to bridge across to position 27 of the other RBs, eliminating essentially the whole hairpin loop of the usual RB fold. Beyond this point, the chain follows its normal path. The side chain of Cys 38 is disordered, the γ-sulfur occupying two positions so that it appears as valine in the electron density map. Indeed, this residue had been identified as valine before the sequence was determined, being in accord with several other RB sequences appearing in fig.1. Without the amino acid sequence, this residue would almost certainly have remained identified as a valine, attesting to the value of the sequence information even for structures that appear to fit the normal pattern.

The aromatic ring of His 18 is located on the surface of the molecule and is exposed to solvent. Similarly, the tryptophan residue in this molecule is exposed to solvent in contrast to the RBCP, RBDV and RBDG molecules where it is shielded by the hairpin loop. This difference in solvent exposure suggests the possibility of a measurable difference in fluorescence quenching of the tryptophan compared to the other RBs. At the pre-

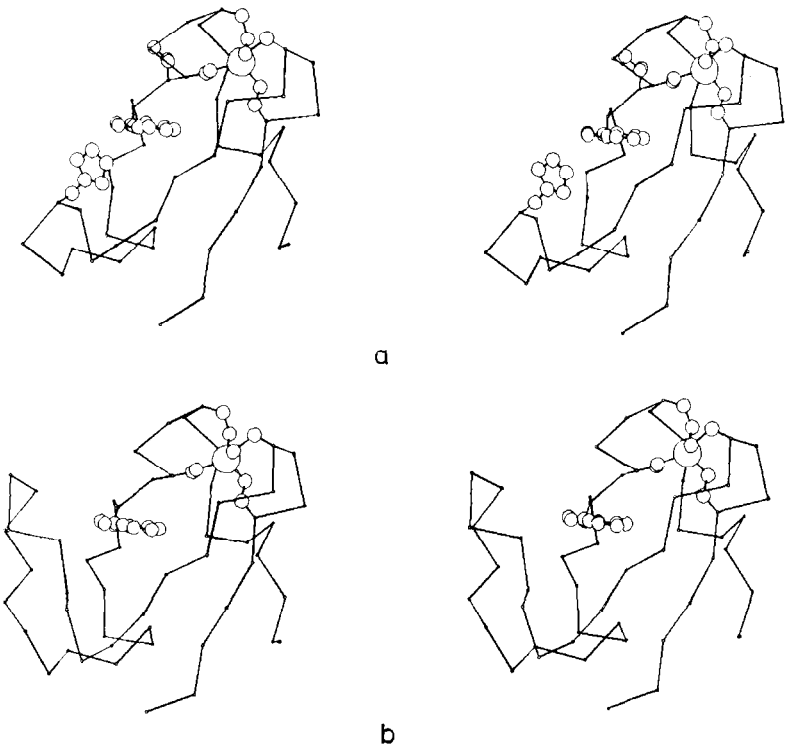

Fig.2. Stereo views of α-carbons and selected side chains for (a) rubredoxin from *Desulfovibrio desulfuricans* and (b) rubredoxin from *Desulfovibrio vulgaris*.

sent state of refinement, the geometric parameters of the Fe-S_4 complex in RBDD differ little from the corresponding ones in RBCP and RBDV.

We note that the only other RB to contain histidine and adjacent cysteine residues in the polypeptide chain is found in the aerobic bacterium *Pseudomonas oleovorans*. This RB is atypical in the sense that the molecule is much larger, and has two domains, each containing an Fe-S_4 complex [16]. This molecule has been shown to be involved in ω-hydroxylation of certain fatty acids, and although each domain has a cysteine-cysteine segment in the chain, the free SH is not necessary for the molecule to function in enzymatic oxygenation [17].

The discovery of an atypical RB from *D. desulfuricans* (27774) has sharpened interest in the relationship between structure and function in these anaerobic RBs. Indeed, this molecule emphasizes the importance of structural studies of naturally occurring variants to complement similar studies of site-specific mutations in determining the residues and structural features that enable the molecules to function as redox agents. This structure also emphasizes the difficulty of assessing a prior which amino acid residues or structural features are not essential to the functional molecule. Until this structure was determined, there was no evidence that the loss of such a major structural feature as the hairpin loop in the typical RB molecule could be accommodated without loss of function. RB interacts with other molecules, possibly as a type of cofactor, and it is possible, of course, that they possess structural elements which compensate for the missing loop. This could be a means of introducing elements of specificity into the redox activities associated with RB.

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