Amino acid sequence of rubredoxin from *Desulfovibrio desulfuricans* strain 27774

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The amino acid sequence of a rubredoxin from *Desulfovibrio desulfuricans* (strain 27774) has been determined. Comparison with rubredoxins from other species reveals pervasive homology, including the regions known to provide the cysteine ligands to the iron atom in several rubredoxins. Neither an extra cysteinyl residue nor a unique histidyl residue in the new sequence is located in the sequence in such a way that, by homology, a functional role in the structure is suggested.

Amino acid sequence *Rubredoxin*

1. INTRODUCTION

The small iron-protein rubredoxin was first isolated in 1965 from *Clostridium pasteurianum* by Lovenberg and Sobel [1]. Since then many more have been discovered and examined by techniques ranging from electron proton resonance and Mössbauer studies of the iron atom, extended X-ray absorption fine structure analysis of the iron complex, amino acid sequence determination of the polypeptide chain, to X-ray diffraction of the three-dimensional structure of the molecule.

These studies have clearly revealed that the iron atom is ligated by the sulfurs of four cysteine residues of the polypeptide chain in a distorted tetrahedral coordination. It is not clear what role the protein plays in giving the iron complex its particular redox properties or which parts facilitate the action of the molecule as an electron mediator.

The rubredoxins from strict anaerobe microorganisms, often found along with the iron-sulfur protein ferredoxin, are composed of 50–60 amino acid residues, and have a redox potential near zero. A rubredoxin from the aerobic bacterium *Pseudomonas oleovorans* contains two iron atoms in a molecule that is more than three-times larger than the anaerobic forms [2]. Its amino acid sequence indicates that two domains in this molecule are each similar to the smaller rubredoxins [3]. The aerobic rubredoxin is known to participate in an ω-hydroxylation reaction [2] and there is some evidence that the anaerobic rubredoxins mediate electron transfer with the flavoprotein NADH rubredoxin oxido-reductase [4]. X-ray diffraction studies of three small rubredoxins show that the molecules fold in very similar configurations in spite of substantial differnces in amino acid sequence [5–7].

The rubredoxin from the sulfate and nitrate reducing strain (27774) of *Desulfovibrio desulfuricans* is being studied at the three-dimensional level [8]; its amino acid sequence is determined herein. Preliminary characterization revealed that this molecule is smaller than usual and is the first isolated from an anaerobe to contain a histidine residue [9], a feature hitherto observed only in the aerobic organism mentioned above.

Abbreviation: S-CM-, *S*-carboxymethyl-

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2. MATERIALS AND METHODS

The entire sequence was determined with less than 3 mg of protein purified essentially as for other *Desulfovibrio* rubredoxins [10]. The protein was reduced with dithiothreitol, carboxymethylated with iodoacetic acid, and cleaved at the methionyl bond as described [11]. Lysyl bonds were cleaved with *Achromobacter* protease I [12] (a gift of Dr T. Masaki, Ibaraki, Japan) in 50 mM Tris buffer, pH 9.0 containing 2 M urea at 37°C for 12 h. Phenylalanine bonds were cleaved with α-chymotrypsin (Worthington) in 0.1 M NH_4HCO_3, pH 8.0, at 37°C.

Peptides generated by cleavage at methionine, lysine or phenylalanine were purified by reversed-phase HPLC using a SynChropak RP-8 column and an acetonitrile gradient into dilute aqueous trifluoroacetic acid [13]. Amino acid compositions were determined with a Waters Picotag system [14], sequence analysis with a Beckman 890C sequencer, phenylthiohydantoins by HPLC, and homologies by the SEARCH or ALIGN programs, all described in [11].

3. RESULTS

A major portion of the overall sequence was obtained by analysis of a cyanogen bromide and a chymotryptic fragment. The remainder of the sequence and the overlaps of the two major fragments were obtained by analysis of two peptides generated by cleavage at lysyl bonds.

Sequenator analysis of the intact S-CM-protein (4 nmol) identified only 17 residues (fig.1). However, only 8% of the protein was susceptible to Edman degradation, suggesting that the amino-terminal methionine is largely blocked. As a result, Cys-6 and Cys-9 were not identified in this analysis.

Fragmentation at the amino-terminal methionine with cyanogen bromide provided one major fragment, M1, which yielded the sequence of residues 2–23. A low yield was also observed in this degradation due to partial blockage by cyclization of the amino-terminal glutamine during cyanogen bromide cleavage. Cleavage at lysyl residues provided peptide K1, which overlapped M1 and extended the sequence to residue 30. In addition, a hexapeptide K2 (residues 40–45), was

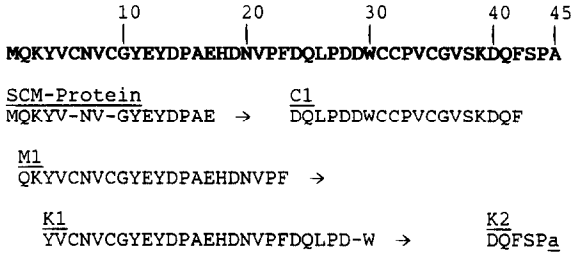

Fig.1. Summary of the proof of sequence. The sequences of specific peptides (names are underlined) are given in one-letter code below the summary sequence (bold type). Prefixes M, K and C denote peptides generated by cleavage at the carboxyl-side of Met, Lys and Phe, respectively. (–) Unidentified amino acid; (→) longer unidentified sequence. The carboxyl-terminal alanine (a) is placed on the basis of the composition of K2 (table 1). Approx. 92% of the protein was blocked.

recovered. Since no other peptide in this digest lacked lysine, it appeared that K2 was derived from the carboxyl-terminus. The carboxyl-terminal alanine of K2 was placed on the basis of amino acid composition and confirmed by the composition of a chymotryptic peptide C2 (table 1). Cleavage at phenylalanine bonds with α-

Table 1

Amino acid compositions[a]

Residue no.	Rubredoxin 1–45	Peptide K2 40–45	Peptide C2 43–45
Asp/Asn (D/N)	8.5 (8)	0.8	
Glu/Gln (E/Q)	5.2 (5)	1.1	
Ser (S)	2.2 (2)	1.0	1.0
Gly (G)	2.7 (2)		
His (H)	0.8 (1)		
Ala (A)	2.0 (2)	1.0	1.0
Pro (P)	5.7 (5)	1.1	0.9
Tyr (Y)	2.4 (3)		
Val (V)	5.0 (5)		
Met (M)	0.8 (1)		
CM-Cys (C)	5.0 (5)		
Leu (L)	1.2 (1)		
Phe (F)	1.8 (2)	0.9	
Lys (K)	1.2 (2)		
Trp (W)	N.D. (1)		

[a] Residues/molecule by amino acid analysis or (in parentheses) from the sequence

chymotrypsin also provided peptide C1, which overlapped K1 and K2, providing residues 29 and 31–39.

4. DISCUSSION

The amino acid sequence of the rubredoxin from *D. desulfuricans* is clearly established by the strategy outlined in fig.1, although the group that blocks ~92% of the amino-terminus has not been identified. In this respect it may resemble the rubredoxin of *C. pasteurianum*, which has *N*-formyl-methionine at its amino-terminus [16] or of *D. gigas* that is also blocked [17]. Since other rubredoxins are not blocked the difference is unlikely to be crucial to function.

Homology among the rubredoxins has been described previously and is extended by the present analysis (figs 2 and 3). Conservation of the four cysteine iron ligands is apparent in fig.3. The extra

	RUDVDS	RUCLEP	RUDV	RUDVEG	RUPE	RUME	RUPSEO-a
RUDVDS	--						
RUCLEP	12.7						
RUDV	16.7	17.4					
RUDVEG	14.8	17.2	17.6				
RUPE	11.7	15.4	12.7	11.8			
RUME	12.6	18.3	13.9	13.7	15.3		
RUPSEO-a	5.9	11.5	9.8	9.2	7.2	10.1	
RUPSEO-b	3.9	8.7	7.4	9.3	9.6	8.6	8.9

RUDVDS - Current work (Desulfovibrio desulfuricans)
RUCLEP - Rubredoxin (Clostridium pasteurianum)
RUDV - Rubredoxin (Desulfovibrio vulgaris)
RUDVEG - Rubredoxin (Desulfovibrio gigas)
RUPE - Rubredoxin (Peptococcus aerogenes)
RUME - Rubredoxin (Megasphaera elsdenii)
RUPSEO-a - Rubredoxin (Pseudomonas oleovorans - residues 1-55)
RUPSEO-b - Rubredoxin (Pseudomonas oleovorans - residues 119-180)

Fig.2. Sequence similarities among selected rubredoxins, as quantified by the ALIGN program [15]. Alignment scores greater than 4 and 10 correspond to probabilities of less than 10^{-4} and 10^{-22} that a randomly generated sequence of the same composition would produce a better score by chance.

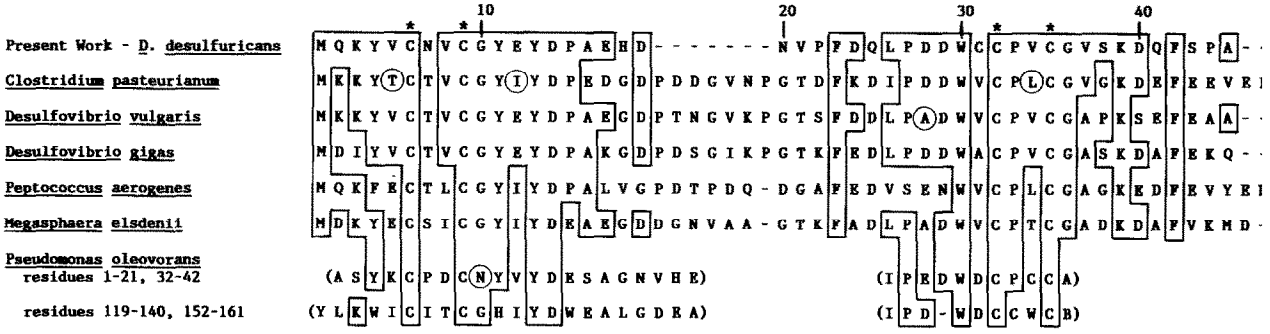

Fig.3. Sequence alignment indicating the homology between the rubredoxin of *D. desulfuricans* and those of other organisms [3,17–19]. Residues identical with the *D. desulfuricans* sequence (numbered at the top) are boxed. (*) Iron ligands identified by crystallography in *D. desulfuricans, C. pasteurianum, D. vulgaris* and *D. gigas*. The protein from *P. oleovorans* is larger and contains two iron atoms (see text). Only segments surrounding its Cys residues (in parentheses) are aligned. (–) Gaps introduced to optimize alignments.

cysteine residue at residue 31 does not align with any homolog, including the two segments of *P. oleovorans* rubredoxin that each also possess an extra cysteine residue. The unique histidyl residue 18 is not adjacent to the Cys ligands in the linear sequence; nor is it in a locus corresponding to His residues in the rubredoxin of the aerobe *P. oleovorans*.

As indicated by earlier composition and X-ray studies [8,9], this rubredoxin is 7 residues shorter than the others derived from anaerobes. The missing heptapeptide appears to correspond to residues 20–26 in the other *Clostridium* and *Desulfovibrio* species (fig.3), and this is in concordance with the X-ray diffraction analysis underway at 1.5 Å resolution.

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