EVOLUTIONARY AND PHYLOGENETIC RELATIONSHIPS OF RUBREDOXIN-CONTAINING MICROBES

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SUMMARY- The primary structure of the Pseudomonas oleovorans rubredoxin, except for the placement of several residues, is presented. There is evidence for gene duplication in the Pseudomonas oleovorans rubredoxin. When the primary structures of this rubredoxin and the rubredoxins from anaerobic bacteria were compared, homologous sequences were observed. The best homology was obtained by creating an ancestral sequence and then comparing each of the rubredoxins with the ancestral sequence. This had led to the theory that proposed that all of the three rubredoxins have arisen from a common ancestor. A phylogenetic tree based on the primary structure data is presented.

Rubredoxin, a non-heme iron protein, was first isolated from C. pasteurianum by Lovenberg and Sobel (1). The protein has since been isolated in purified form from other anaerobes such as P. elsdenii by Mayhew and Peel (2), M. lactilyticus by Lovenberg (3), M. aerogenes by Bachmayer, et al. (4), D. desulfuricans by Newman and Postgate (5) and D. gigas by Laishley, et al (6). The primary structures of the rubredoxins from M. aerogenes and P. elsdenii have been determined by Bachmayer, et al. (4,7). McCarthy and Lovenberg (8) have shown that the rubredoxins from anaerobes are N-formylated at the NH_2-terminal end of the rubredoxin molecules. The molecular weights of the rubredoxin from

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anaerobes was shown to be 6,000 by Lovenberg and Sobel (1). The rubredoxin from the aerobic P. oleovorans which was initially characterized by Peterson, et al. (9) was shown to have a molecular weight of about 20,000 by Lode and Coon (10,11).

In the present report, we wish to report the partial sequence of the P. oleovorans rubredoxin which is complete except for the placement of two amino acid residues, and the evolutionary and phylogenetic relationships of all the rubredoxin containing organisms which are derivable from the primary structure data.

MATERIALS AND METHODS

The P. oleovorans rubredoxin was prepared as previously described Lode and Coon (10,11). The purity of the preparations was determined by disc electrophoresis at pH 7.5 and 9.5 and ultracentrifugation which indicate the presence of one component. The 280/497 nm ratio of the preparations, which is a good criterion of purity, was 6.3.

The details of the primary structure determination will be published elsewhere. However, conventional methods were used in which a suitable derivative of the protein was hydrolyzed by trypsin or chymotrypsin which was followed by purification of the peptides by ion exchange and partition chromatography. The sequence was determined manually by the Edman degradation procedure of Edman and Soquist (12) as well as by the use of the Beckman protein sequencer which is a commercial model of the instrument developed by Edman and Begg (13).

RESULTS

The primary structure of the P. oleovorans rubredoxin which has been determined thus far is shown in Fig. 1. Except for the placement of two amino acid residues in position 159 and 160 which involve a Asx and Pro residue, the sequence is complete.

When the NH_2-terminal section (residues 1-54) was compared with the COOH-terminal section residues 119-173, the MBDC (minimal base difference per codon) was 0.91. It was necessary to introduce several gaps for the

1
Ala-Ser-Tyr-Lys-Cys-Pro-Asp-Cys-Asn-Tyr-Val-Tyr-Asp-Glu-Ser-Ala-Gly-Asn-Val-His-Glu-Gly-Phe-

24
Ser-Pro-Gly-Thr-Pro-Trp-His-Leu-Ile-Pro-Glu-Asp-Trp-Asp-Cys-Pro-Cys-Cys-Ala-Val-Arg-

45
Asp-Lys-Leu-Asp-Phe-Met-Leu-Ile-Glu-Ser-Gly-Val-Gly-Glu-Lys-Gly-Val-Thr-Ser-

64
Thr-His-Thr-Ser-Pro-Asn-Leu-Ser-Glu-Val-Ser-Gly-Thr-Ser-Leu-Thr-Ala-Glu-Ala-Val-Val-Ala-Pro-Thr-Ser-Leu-

90
Glu-Lys-Leu-Pro-Ser-Ala-Asp-Val-Lys-Gly-Gln-Asp-Leu-Tyr-Lys-Thr-Glu-Pro-Pro-Arg-Ser-Asp-Ala-Glu-

114
Gly-Gly-Lys-Ala-Tyr-Leu-Lys-Trp-Ile-Cys-Ile-Thr-Cys-Gly-His-Ile-Tyr-Asp-Trp-Glu-Ala-Leu-Gly-

137
Asp-Glu-Ala-Glu-Gly-Phe-Thr-Pro-Gly-Thr-Arg-Phe-Glu-Asp-Ile-Pro-Asp-Trp-Asp-

156 174
Cys-Cys-Trp-Cys(Asx,Pro)Gly-Ala-Thr-Lys-Glu-Asn-Tyr-Val-Leu-Tyr-Glu-Glu-Lys

Figure 1. The partial amino acid sequence of the P. oleovorans rubredoxin.

best alignment but this is an accepted convention among protein evolutionists. Since the comparison involves a large number of amino acid residues, the MBDC value may possibly be considered to indicate gene duplication during the evolution of the P. oleovorans rubredoxin organism.

The amino acid sequences of the various rubredoxins which have been sequenced thus far include the P. elsdenii and M. aerogenes rubredoxins by Bachmayer, et al. (7,4). When the sequences of the anaerobic rubredoxins and the residues 1-53 and the residues 119-172 were aligned as shown in Fig. 2, there was some evidence for homology in all the sequences under comparison. The MBDC when all of the sequences were compared with one another is shown in Table I. The MBDC value alone are slightly high to indicate homology but it is evident even by visual inspection of the sequences that there is a possibility of

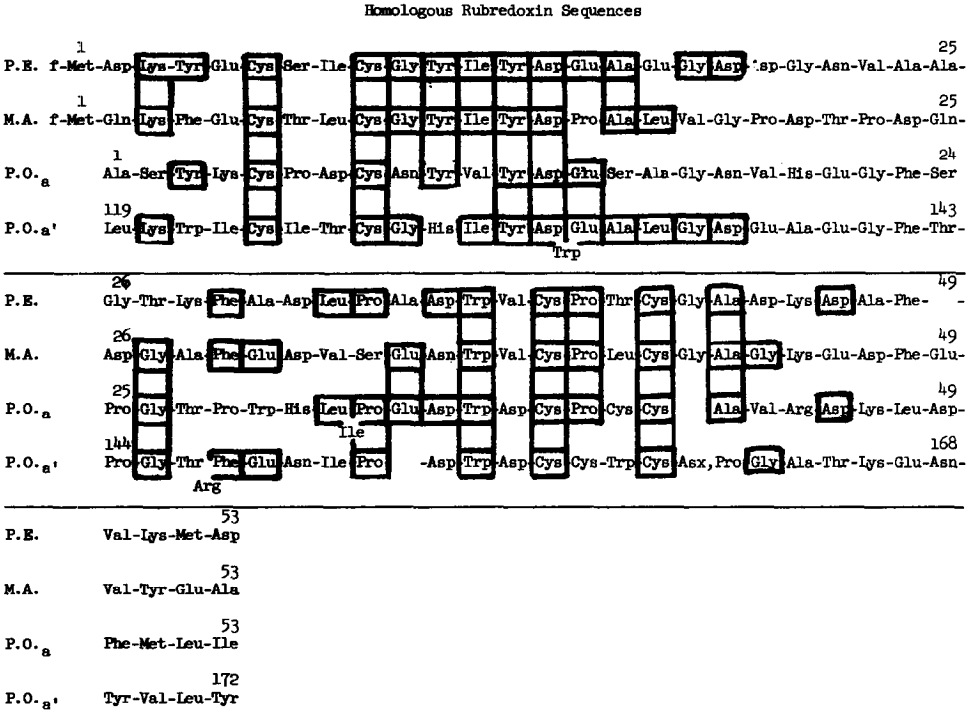

Figure 2. Homologous sequences in the P. elsdenii, M. aerogenes and P. oleovorans rubredoxin. $P.O_a$ and $P.O_{a'}$ stands or residues 1-53 and residues 119-172, respectively, of the P. oleovorans rubredoxin.

TABLE 1

MBDC values obtained from comparison of various sequences

	P.E.	M.A.	$P.O._a$	$P.O._b$
P.E.	0	0.73	0.88	0.93
M.A.		0	1.13	1.18
$P.O._a$			0	0.91
$P.O._b$				0

homology. Therefore, we obtained a common ancestral sequence from the P. elsdenii, the M. aerogenes and the residues 1-53 and 119-172 of the P. oleovorans rubredoxin by comparing the various sequences to the hypothetical precursor. Very respectable MBDC values in the range of 0.59 were obtained. We have tentatively taken this evidence to indicate that all of the rubredoxins under consideration have arisen from the same common ancestor.

It is assumed by evolutionists (14) that anaerobic bacteria were one of the first organisms to evolve from the proto-organism, the first distinct living cell. If this theory is correct, it is possible that the M. aerogenes and P. elsdenii evolved when the atmosphere was still reducing but as far as the P. oleovorans is concerned, since it is an aerobic organism, it must have evolved after the atmosphere changed to the present day type. The possible phyletic relationship of the various rubredoxin containing microbes are summarized in Fig. 3.

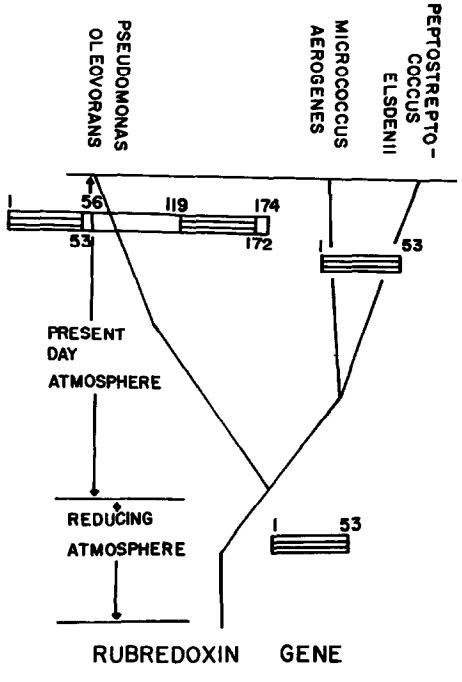

Figure 3. A possible evolutionary pathway of the rubredoxin gene in the rubredoxin containing organisms.

The rubredoxin from anaerobic bacteria have been shown to replace ferredoxins in some reactions but the exact function is not clearly known (15). On the other hand, the rubredoxin from P. oleovorans functions as an electron carrier in an enzyme system which hydroxylates alkanes and fatty acids (9). Lode and Coon (11) have shown that the anaerobic rubredoxins have only barely detectable activity in the alkane hydroxylation system of the P. oleovorans. Thus, if our hypothesis is correct that the various rubredoxins have all arisen from the same common ancestor, the changes in the structure of rubredoxin which are seen in P. oleovorans rubredoxin drastically altered the biological activity of the molecule. Moreover, Lode and Coon (10,11) have recently demonstrated that the active site of the enzyme is in the COOH-terminal half of the molecule. Although iron can also be incorporated into the NH_2-terminal half which contains five cysteine residues, it does not increase the biological activity of the rubredoxin molecule. A further point of interest is concerned with the biological function of the center portion (residues 54-118) which does not appear to be homologous to the other halves of the molecule. Therefore, we are undertaking a study of the primary structure of other rubredoxins in order to clarify the dilemma concerning the structure-function relationship as well as to obtain further confirmation for the proposed homologies of the various rubredoxins. Evans and coworkers (16) have isolated rubredoxin from a phylogenetic bacteria and the primary structure of this rubredoxin may provide valuable confirmatory data.

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