THE N-TERMINAL SEQUENCE OF SUPEROXIDE DISMUTASE FROM THE STRICT ANAEROBE *DESULFOVIBRIO DESULFURICANS*

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1. Introduction

Superoxide dismutase (EC 1.15.1.1.) which is widely distributed among the oxygen metabolizing organisms [1], catalyzes the dismutation reaction of superoxide free radical ($O_2^- + O_2^- + 2\ H^+ \rightarrow H_2O_2 + O_2$) generated in a variety of biological oxidations [2–5] and has been assumed to play an important role in the protection of cells against the detrimental effects of this radical [6]. Thus far three different groups of superoxide dismutase, with respect to the metal in the enzyme, have been described in the aerobic organisms. A cuprozinc protein with a molecular weight of 32 000 and composed of two identical subunits has been isolated from a wide range of eukaryotes [7–9] whereas a Mn-containing enzyme has been obtained from prokaryotes [10,11] and from eukaryotic mitochondria [12]. In addition, an iron-containing superoxide dismutase, similar to the mangano-enzyme has been purified only from prokaryotes [13–16]. The molecular weight of the bacterial Mn- and Fe-containing dismutases is about 40 000 whereas that of the mitochondrial Mn-enzyme is 80 000; each contains subunits of molecular weight 20 000. As compared to the Cu–Zn-protein these Mn- and Fe-containing enzymes represent a distinct class of superoxide dismutases which exhibit similar amino-acid composition and N-terminal sequence [17]. These results led Steinman and Hill [17] to suggest that the two classes of dismutase present in the aerobic organisms are probably composed of different proteins of independent evolutionary origin. The data of Bridgen et al. [18] on the partial sequence of the *B. stearothermophilus* Mn-enzyme provided additional evidence for this view.

In contrast to the report of McCord et al. [1], Hewitt and Morris [19] detected superoxide dismutase activity in a wide range of obligate anaerobes. The enzyme was previously reported to be present in some sulfate-reducing bacteria of the genus *Desulfovibrio* [20]. Recently, an iron-containing superoxide dismutase has been isolated and characterized from the strict anaerobe *Desulfovibrio desulfuricans* [21,22]. Its physico-chemical properties including molecular weight, subunits structures, EPR and absorption spectra and amino acid composition [21] have been shown to be in most respect similar to those of the Fe-enzymes from aerobic organisms [13–16]. It was then of interest from an evolutionary point of view to determine the N-terminal sequence of the superoxide dismutase of an organism that has retained many primitive characters.

The present paper is dealing with the establishment of the sequence of the 30 N-terminal residues of the iron-containing dismutase from *D. desulfuricans*. The results indicate that the superoxide dismutase of this strict anaerobe belong to the same family of homologous proteins constituted by the Fe- and Mn-dismutases of the aerobic organisms.

2. Materials and methods

The superoxide dismutase used in this work was purified from *D. desulfuricans* strain Norway 4 by the procedure previously described [21]. The pure dismutase gave a single band by gel-electrophoresis with and without 0.1% SDS.

Sequence analysis by automated Edman degrada-

tion were performed in a Socosi Protein Sequencer (P.S. 100) using the standard quadrol double-cleavage program [23]. Dimethylbenzylamine buffer was also used [24] and appeared to be suitable for low molecular weight proteins. The quantitative determination of the PTH derivatives was done relative to known amounts of the appropriate standards on gas-chromatography GC45 using SP400 as stationary phase according to Pisano et al. [25]. An analysis of the silylated PTH amino acids was always performed. PTH Amino acids were also characterized by thin-layer chromatography on silica-gel containing an ultraviolet fluorescent indicator (Silica Gel GF254, Merck) as described by Edman [26–28]. In some instances, PTH derivatives have been characterized by amino acid analysis after conversion to the parent amino acid by hydrolysis with chlorhydric acid [29] or hydriodic acid [30] for serine residues.

After denaturation of the protein in 8 M urea under nitrogen at + 4°C, the two cysteine residues of the protein were converted to *S*-amino-ethylated cysteines after reduction with dithiothreitol and reaction with ethyleneimine according to Keresztes-Nagy and Margoliash [31]. The content of *S*-aminoethylated cysteine was determined by automatic amino acid analysis.

3. Results and discussion

Two sequencer analysis of the *S*-β-aminoethyl cysteinyl superoxide dismutase were performed. One using the double-cleavage quadrol program and another using the DMBA buffer. With the DMBA buffer 35 steps could be identified and only 26 in the quadrol buffer. The sequence of the 30 first residues of the molecule is reported on fig.1.

Comparison of the N-terminal sequence of the *Desulfovibrio* superoxide dismutase with N-terminal sequences of other dismutases shows that there is a high degree of sequence homology with the prokaryotic dismutases and the mitochondrial enzyme but not with the bovine erythrocyte Cu–Zn-superoxide dismutase.

The sequence of the superoxide dismutase from *D. desulfuricans* can be aligned for maximum homology with the other known sequences of iron- and mangano-enzymes but there is an additional residue in the N-terminal sequence. In the first 30 residues, 9 residues are totally conserved through the five homologous sequences. It concerned the hydrophobic residues, leucine, proline, and alanine, the aromatic residue tyrosine and the basic residue lysine. The striking feature of the iron- and mangano-dismutases is the high amount of hydrophobic residues. In the case of the enzyme of *Desulfovibrio*, near 50% of the 30 N terminal residues are hydrophobic. This suggests that the N-terminal extremity may be oriented to the hydrophobic core of the protein.

The enzyme of *D. desulfuricans* shows greater homology with the iron-containing enzyme from *E. coli*: there is 56% of homology on 30 residues. It exhibits only 40% of homology with the mangano-enzyme from *E. coli* and 43% respectively, with the Mn-enzymes from *B. stearothermophilus* and chicken liver mitochondria.

The fact that mitochondrial and bacterial dismutases are structurally related and have evolved from a common ancestor seems to be clearly established in spite of the phylogenetic distance between chickens and bacteria. These sequence comparisons involve only the N-terminal part of the protein which may be a highly conservative part of the molecule; thus any definitive statement on the significance of SOD for phylogenetical studies must await more complete sequencing data.

From the results reported here, the problem of significance of the presence in a strict anaerobe of a

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                                        10                  15
Ser–Ile–Phe–Val–Leu–Pro–Asp–Leu–Pro–Tyr–Ala–Lys–Asp–Ala–Leu–( )–Pro–
      20                  25                  30
Lys–Ile–Ser–Ala–Lys–Thr–Phe–Asp–Phe–( )–( )–Gly–Lys
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Fig.1. N-terminal sequence of superoxide dismutase after reduction and aminoethylation. Residues identified by using the Socosi automatic sequencer. Parentheses indicate that the residues in these positions could not be identified.

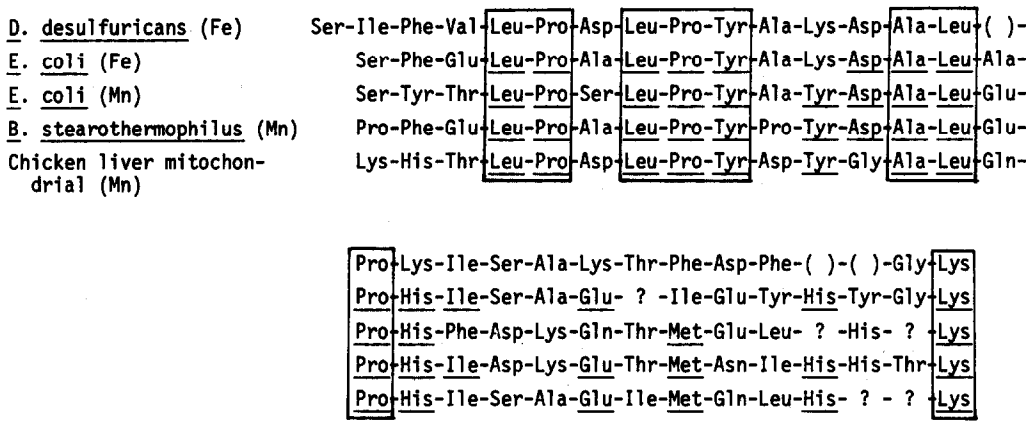

Fig.2. Comparison of the N-terminal region of superoxide dismutase from *D. desulfuricans* with the N-terminal sequences of three other bacterial and one mitochondrial dismutases. Residues identical in all these proteins are included in boxes.

superoxide dismutase very close to the ferri- and mangano-dismutases of aerobic microorganisms is posed. Two explanations are possible:

(i) One is that, as suggested by Lumsden and Hall [32], it was an early acquisition of the strict anaerobes for protection against some molecular oxygen produced in the primitive oceans by photolysis of water by ultra violet irradiation.

(ii) The second is that it would be a more recent acquisition allowing the survival of the bacteria when they are accidentally put in contact with oxygen.

Furthermore, the somewhat erratic presence of the enzyme in both sulfate-reducing bacteria [22] and Clostridia [1,19] has to be pointed out. This could be an indication that the information for the biosynthesis of the protein comes from a plasmid and not from the bacterial genome. This question should also be cleared out before utilizing SOD sequences for phylogenetical studies [22].

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