The primary structure of superoxide dismutase purified from anaerobically maintained *Bacteroides gingivalis*

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The superoxide dismutase (SOD) of *Bacteroides gingivalis* can use either iron or manganese as a cofactor in its catalytic activity. In this study, the complete amino acid sequence of this SOD purified from anaerobically maintained *B. gingivalis* cells was determined. The proteins consisted of 191 amino acid residues and had a molecular mass of 21 500. The sequence of *B. gingivalis* SOD showed 44–51% homology with those for iron-specific SODs (Fe-SODs) and 40–45% homology with manganese-specific SODs (Mn-SODs) from several bacteria. However, this sequence homology was considerably less than that seen among the Fe-SOD (65–74%) or Mn-SOD family (42–60%). This indicates that *B. gingivalis* SOD, which accepts either iron or manganese as metal cofactor, is a structural intermediate between the Fe-SOD and Mn-SOD families.

Amino acid sequence; Superoxide dismutase; *Bacteroides gingivalis*

1. INTRODUCTION

Superoxide dismutases (SODs; EC 1.15.1.1) are a family of metalloproteins containing either iron (Fe-SODs), manganese (Mn-SODs) or copper plus zinc (CuZn-SODs) as cofactor(s). With some exceptions, procaryotes possess Fe-SOD, Mn-SOD, or both [1]. The Fe-SOD and Mn-SOD subfamilies have similar amino acid sequences, suggesting that these two subfamilies have diverged from a common ancestor [2–4]. The CuZn-SOD subfamily, on the other hand, differs markedly from the Fe-SOD and Mn-SOD subfamilies in both amino acid composition and sequence [2–4]. Despite such a structural similarity, metal replacement experiments showed that each of the Fe-SODs and Mn-SODs tested possessed a strict metal cofactor specificity [5–8]. Recent studies have shown that *Propionibacterium sherimanii* [9] and *Streptococcus mutans* [10] utilize the same apoprotein to form Fe-SOD or Mn-SOD depending on the metal supplied to the growth medium. It has further been reported that the apoproteins of both Fe-SOD and Mn-SOD isolated from *Bacteroides fragilis* [11,12] and *Bacteroides thetaiotaomicron* [13] accept either iron or manganese to form holoenzymes, which migrate identically on polyacrylamide gel electrophoresis. Moreover, we have found that anaerobically maintained *Bacteroides gingivalis* contains a Fe-SOD and that the denatured apoprotein of this SOD accepts iron or manganese resulting in restoration of catalytic activity [14]. These findings suggest that in certain bacteria, the apoproteins of both Fe-SOD and Mn-SOD are encoded by the same gene. However, no primary structures have yet been reported for these SODs that can bind iron or manganese to exhibit SOD activity.

In the present study, we determined the complete amino acid sequence of *B. gingivalis* SOD, which uses both iron and manganese to form the holoenzyme, and compared the determined sequence with those of Fe- and Mn-SODs possessing a strict metal cofactor specificity.

2. MATERIALS AND METHODS

The SOD from *B. gingivalis* 381 cells maintained anaerobically was purified as previously described [14]. The purified protein was denatured by dialysis for 18 h against 5 M guanidinium chloride containing 20 mM 8-hydroxyquinoline (pH 3.2) and finally dialyzed for 8 h in 5 M guanidinium chloride to remove the organic chelator. For amino acid analysis, protein and peptides were hydrolyzed in 5.7 M HCl at 110°C in evacuated, sealed tubes for 24 h. The hydrolysates were analyzed with a Hitachi 835 S amino acid analyzer (Hitachi Ltd.). The apoprotein (1–2 mg) was subjected to separate proteolysis with *Achromobacter* protease I (AP-I; Wako Pure Chemicals), endoproteinase Asp-N (Asp-N; Boehringer Mannheim GmbH) and trypsin treated with L-1(-*p*-tosylamino)-2-phenyl-ethyl chloromethyl ketone (Worthington Biochemical Co.). In the case of tryptic digestion, the apoprotein was acetylated with acetic anhydride prior to proteolysis [15]. The resulting peptide fragments were separated by HPLC using a C4 reverse phase column (0.39×15 cm, 300 Å; Millipore Ltd.). The elution of peptides was carried out with a linear gradient of organic solvent (2-propanol/acetonitrile, 7:3, v/v) from 0% to 60% (v/v) in 0.1% trifluoroacetic acid for 1 h at a flow rate of

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1 ml/min. Sequence analysis was carried out with a 477A/120 gas-phase automatic sequencer (Applied Biosystems) as previously described [16].

3. RESULTS AND DISCUSSION

Fig. 1 shows the amino acid sequence of *B. gingivalis* SOD, together with the peptides used for the sequence determination. The sequence was determined on the basis of the complete set of overlapping AP-I peptides obtained by Asp-N digestion and of a tryptic peptide (Ac-T) obtained from acetylated protein, which provided evidence for the alignment of AP-I peptides (A12 and -13). The subunit of *B. gingivalis* SOD consisted of 191 amino acids and had a molecular mass of 21 500. Fig. 2 compares the sequence of *B. gingivalis* SOD with previously determined sequences of Fe- and Mn-SODs [2-4,17-19]. Gaps have been inserted to maximize the homologies among the sequences, and the resulting scores are listed in Table I. The *B. gingivalis* SOD showed 43.5-51.3% homology with other Fe-SODs, although the homology among the other three Fe-SODs was 65.1-74.0%. The homology between *B. gingivalis* SOD and the Mn-SODs was 39.8-45.0%, whereas those among the Mn-SOD was 42.2-59.9%. In the sequence of *B. gingivalis* SOD, 41 residues were found at identical positions of both Fe-SODs and Mn-SODs (Fig. 2). In addition to these residues, 18 and 14 residues of *B. gingivalis* SOD were at identical positions of the other Fe-SODs and Mn-SODs, respectively. These results suggest that this SOD, which binds either iron or manganese without loss of activity, is a structural intermediate between Fe-SOD and Mn-SOD. Furthermore, as shown in Fig. 2, glycine residues, which often have a specific structural role in the folding of the polypeptide chain [20-22], were present at a similar level and position in all SODs, implying that the three-dimensional structure of *B. gingivalis* SOD is similar to those of Fe- and Mn-SOD as determined by X-ray diffraction studies. Judging from the results of X-ray studies for Fe-SODs from *E. coli* [20] and *Ps. ovalis* [21] and Mn-SOD from *Thermus thermophilus* [22], His^{27}, His^{82}, Asp^{171} and His^{175} in the sequence of *B. gingivalis* SOD might be ligands to iron.

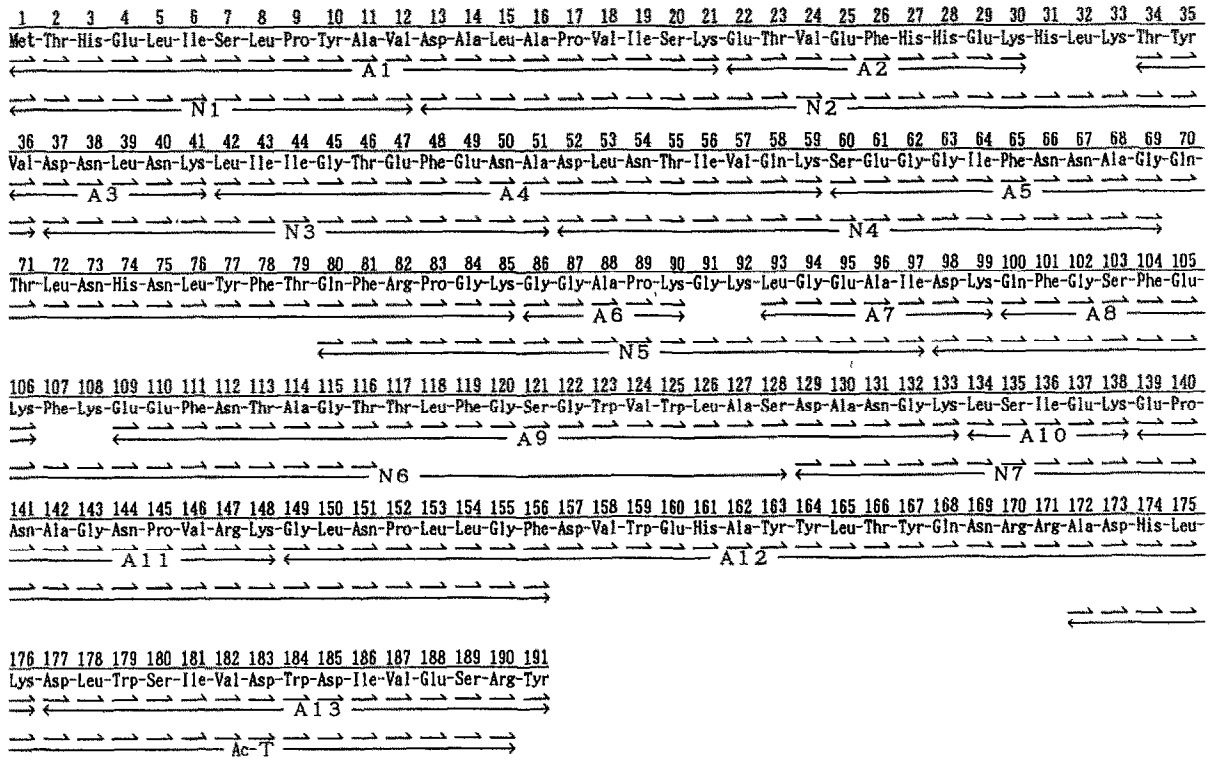

Fig. 1. Amino acid sequence of Fe-SOD from *B. gingivalis* 381. Residues arrowed (→) were identified by Edman degradation. A and N denote the peptides obtained by proteolysis with *Achromobacter* protease I and endoproteinase Asp-N, respectively. Ac-T denotes the peptide obtained by proteolysis with trypsin after acetylation of α- and ε-amino groups of the apoprotein.

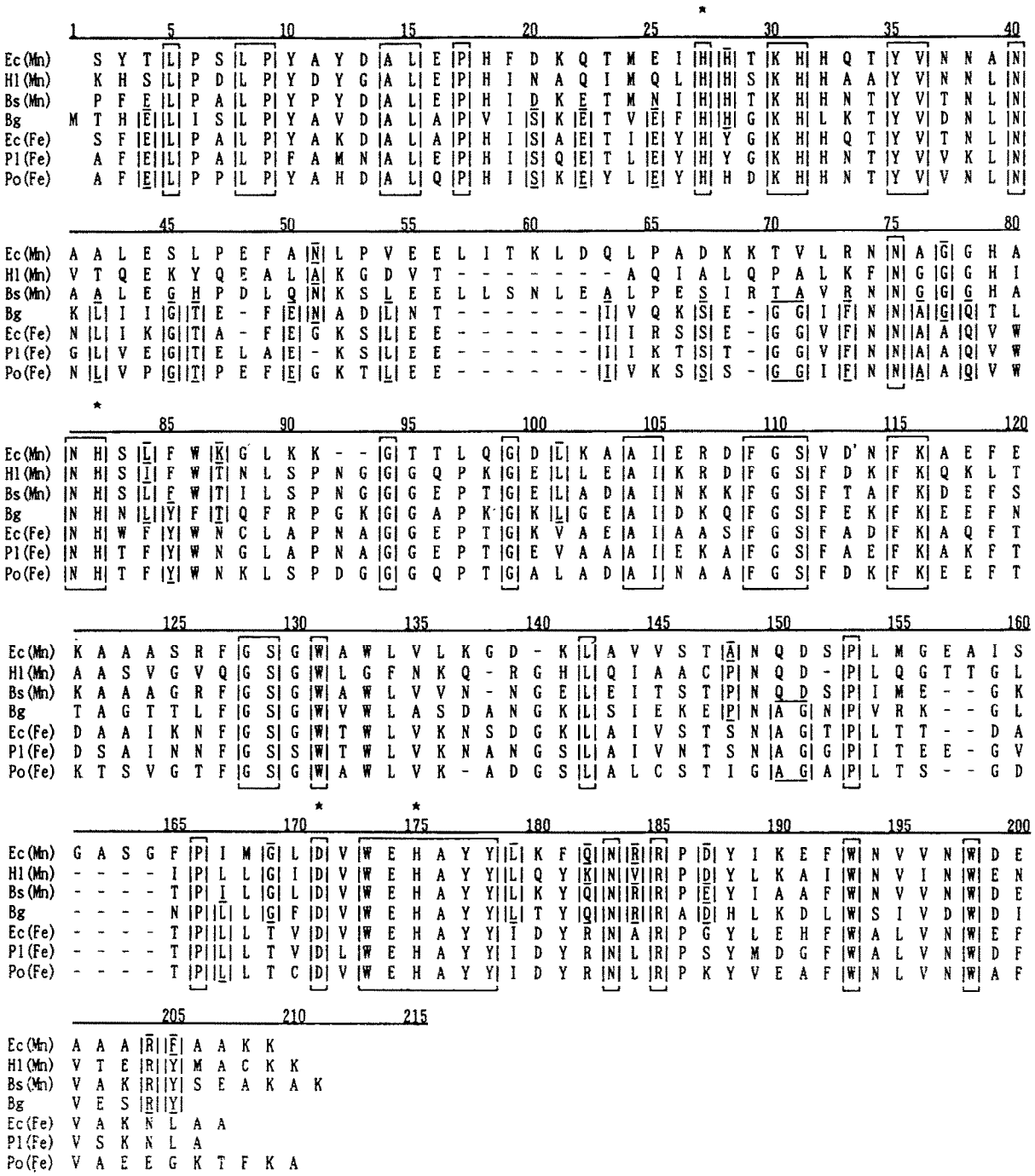

Fig. 2. Comparison of the amino acid sequences of *E. coli* (Ec), human liver (Hl), *Bacillus stearothermophilus* (Bs), *B. gingivalis* (Bg), *Ph. leiognathi* (Pl) and *Ps. ovalis* (Po) SODs. Gaps have been introduced to obtain maximal homologies amongst the sequences. Boxes indicate positions at which residues are identical. Asterisks (*) indicate positions regarded as metal ligands.

Table I
Sequence homology between Fe-SOD and Mn-SOD

	E. coli (Fe)	*Ph. leiognathi* (Fe)	*Ps. ovalis* (Fe)	*Ba. stearothermophilus* (Mn)	*E. coli* (Mn)	Human liver (Mn)
B. gingivalis	51.3	43.5	47.6	45.0	40.3	39.8
E. coli (Fe)		74.0	67.2	52.6	45.3	39.6
Ph. leiognathi (Fe)			65.1	49.8	39.3	35.0
Ps. ovalis (Fe)				52.7	42.2	39.4
Ba. stearothermophilus (Mn)					59.9	47.8
E. coli (Mn)						42.2

Values are given as percentage of identical residues among the total residues aligned in Fig. 2

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