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NOTES

Isolation, Expression, and Nucleotide Sequence of the *sod* Gene from *Porphyromonas gingivalis*

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The *sod* gene coding for the Mn/Fe-dependent superoxide dismutase (SOD) enzyme has been isolated on a 5.9-kb DNA fragment from *Porphyromonas gingivalis* ATCC 53977. SOD activity can be expressed from the *P. gingivalis* fragment and from a subcloned fragment in *Escherichia coli*. However, the enzyme does not appear to be expressed from its own promoter in *E. coli* cells. The nucleotide sequence of the gene has been determined, and the deduced amino acid sequence of the enzyme is nearly identical to that of the enzyme purified from *P. gingivalis* 381 and shares extensive sequence similarity with comparable enzymes from *E. coli*.

The gram-negative anaerobic organism *Porphyromonas* (formerly *Bacteroides*) *gingivalis* has been implicated in the etiology of human periodontal diseases (15). However, unlike some other anaerobic organisms implicated in these diseases, *P. gingivalis* strains are oxygen tolerant (3). This property appears to be correlated with the presence of superoxide dismutase (SOD; EC 1.15.1.1) activity. In addition, this enzyme may be important in resisting the inhibitory effects of superoxide-producing neutrophils (4).

Recently, the Mn/Fe SOD enzyme from *P. gingivalis* 381 was purified to apparent homogeneity (1) and its amino acid sequence was determined (2). However, there are no published reports concerning the characterization of the *sod* gene from these organisms. The present communication reports the isolation, expression, and nucleotide sequence of the *sod* gene from strain ATCC 53977 (previously designated A7A1-28) of *P. gingivalis*.

In the course of sequencing the *prtC* gene from strain ATCC 53977, which expresses collagenase activity (17), it was observed that an open reading frame (ORF) sharing amino acid sequence similarity with the SOD enzymes from *Escherichia coli* and *Bacillus stearothermophilus* could be detected downstream from the *prtC* gene on a 5.9-kb DNA fragment isolated on plasmid vector pPL-lambda (Pharmacia-LKB Biotech, Inc., Piscataway, N.J.) (Fig. 1, plasmid pS1). The complete nucleotide sequence of this ORF was determined from both DNA strands by using the dideoxynucleotide-sequencing strategy (11) with overlapping DNA fragments cloned into M13 mp18 and M13 mp19 sequencing vectors. The deduced amino acid sequence of the complete ORF (Fig. 2) showed extensive similarity to the SOD proteins of *E. coli* (41% identity with the Mn-dependent enzyme [16] and 50% identity with the Fe-dependent enzyme [12]) (Fig. 3) as well as 46% identity with the *B. stearothermophilus* Mn-dependent enzyme (5; data not shown). In addition, the amino acid sequence of the strain ATCC 53977 SOD enzyme differed from that of the strain 381 enzyme determined by protein sequencing (2) at only three positions in the entire sequence: position 12 (Tyr replaced by Asp), position 28 (Gly replaced by Glu), and position 111 (Asp replaced by Asn). In addition, after submission of the present communication, the complete nucleotide sequence of the *sod* gene from *P. gingivalis* ATCC 33277, representing a different serotype than ATCC 53977, was reported (10). The deduced amino acid sequence of the latter SOD protein differed from the sequence reported here at only two positions: position 12 (Tyr replaced by Asp) and position 111 (Asp replaced by Asn). Therefore, the ORF corresponds to the *sod* gene of *P. gingivalis* ATCC 53977.

Examination of the nucleotide sequence immediately upstream from the *sod* gene did not reveal any sequences with strong homology to the ribosome-binding site consensus sequences of *E. coli* (14). However, the sequence AGA (position 101) is homologous to the 3′ terminus of the 16S rRNA from *Bacteroides fragilis* (17). In addition, potential −10 and −35 regions (positions 76 and 53) homologous to the *E. coli* consensus promoter sequences (8) could be identi-

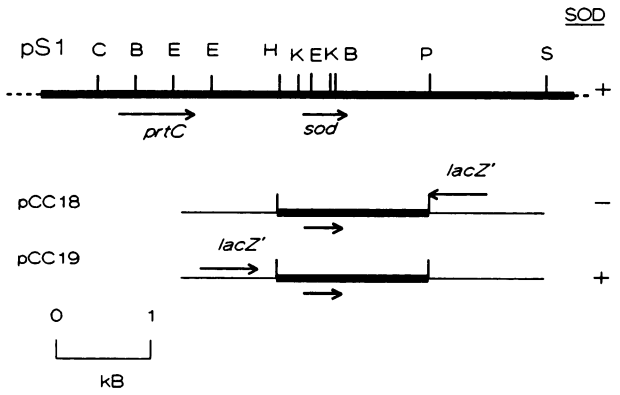

FIG. 1. Subcloning of the *P. gingivalis sod* gene. *sod* was subcloned as a *Pst*I-*Hin*dIII fragment into plasmid vectors pUC18 and pUC19. ----, Plasmid vector pPL-lambda; ▬, *P. gingivalis* DNA; ——, pUC18 or 19. Restriction enzyme sites: B, *Bgl*II; C, *Cla*I; E, *Eco*RI; H, *Hin*dIII; K, *Kpn*I; P, *Pst*I; S, *Sma*I.

* Corresponding author.

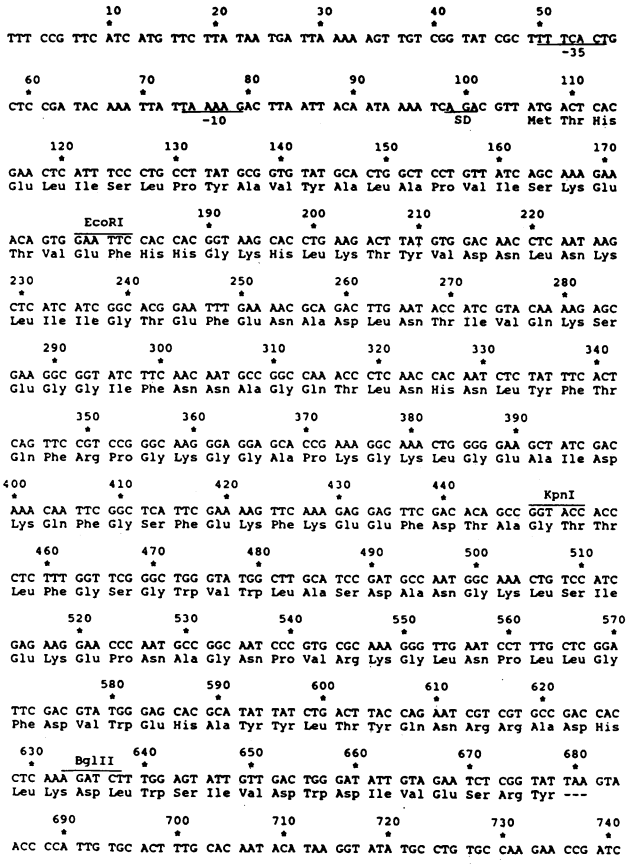

FIG. 2. Nucleotide sequence and deduced amino acid sequence of the *P. gingivalis sod* gene. Potential −10 and −35 promoter sequences as well as a potential ribosome-binding site (SD) are underlined.

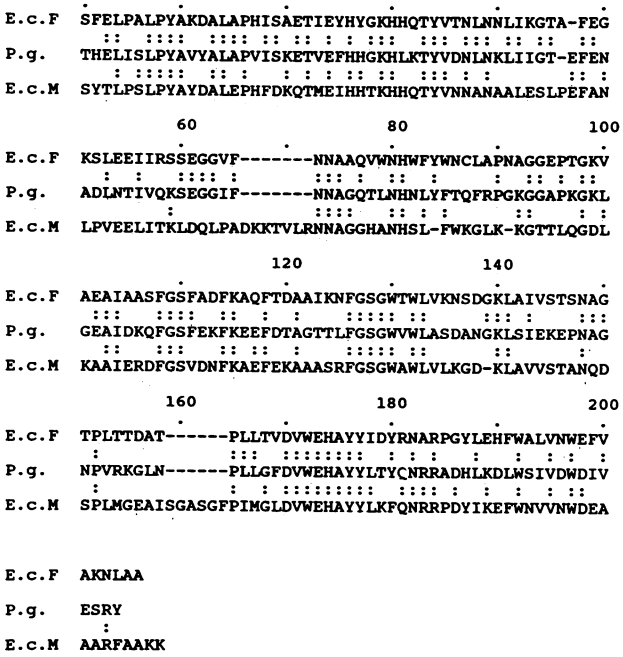

FIG. 3. Sequence similarities between the SOD proteins of *P. gingivalis* and *E. coli*. Numbering begins with the first amino acid after f-Met. Gaps have been introduced for maximum alignment, with colons indicating identical amino acid positions between the P.g. (*P. gingivalis*) and either the E.c.M (*E. coli* Mn-dependent SOD or *E.c.F* (*E. coli* Fe-dependent SOD) sequences.

fied. However, transcription start site determination will be necessary to accurately identify the promoter region for this gene. No sequences resembling potential strong transcription terminators could be identified downstream from the *sod* gene. The calculated G+C ratio for the *sod* gene (48%) corresponds well to the G+C ratio of 46 to 48% previously determined for *P. gingivalis* strains (13).

In order to examine the expression of the *sod* gene in *E. coli*, sonic extracts of strain NTS1 (HB101 containing plasmid pS1) were prepared (10 min at 4°C) with a Heat Systems (Farmingdale, N.Y.) Ultrasonic Cell Disruptor and assayed directly for SOD activity (9), and the activities were compared with extracts from *E. coli* HB101. Significant SOD activity over and above the activity expressed by the *E. coli sod* genes (6) could be detected (Table 1). In order to more clearly demonstrate expression in *E. coli*, the *P. gingivalis sod* gene was subcloned as a 2.3-kb *Pst*I-*Hin*dIII fragment into plasmid vector pUC19 and transformed into *E. coli* QC779 (6), in which the two *sod* genes have been insertionally inactivated. The transformed cells containing the resultant plasmid pCC19 exhibited moderate SOD activity, while the *E. coli* mutant lacking the plasmid displayed low activity (Table 1). In order to determine whether transcription of the *sod* gene was initiated within the *P. gingivalis* insert or from the *lacZ* promoter of pUC19, the *Hin*dIII-*Pst*I fragment was inserted into vector pUC18. No SOD activity above background could be detected in these latter plasmid constructs (Table 1). Therefore, it appears that *sod* transcription in pCC19 is initiated from the *lacZ* promoter despite the presence of potential promoter sequences in the *P. gingivalis* DNA insert. In addition, the relatively modest effect of IPTG (isopropyl-β-D-thiogalactopyranoside) on *sod* induction (Table 1) might suggest that efficient translation of the *sod* gene was also limiting expression in *E. coli*. Difficulty in expressing at least one other *P. gingivalis* gene in *E. coli* has also been documented (7). However, it has not been established whether transcription of the *sod* gene in plasmid pS1 is initiated from the λ_L vector promoter or from a promoter in

TABLE 1. SOD expression in *E. coli*[a]

Strain + treatment	SOD activity (units/mg)[b]
HB101	8.07
HB101(pS1)	10.99
QC779	0.33
QC779(pCC18)	0.31
QC779(pCC19)	0.92
QC779(pCC19) + IPTG (0.14 mM)	1.90
QC779	0.50
QC779(pS1)	0.70

[a] Crude extracts of each strain were prepared and assayed for SOD activity as described in the text. Each value is derived from the activities determined with five different dilutions of each extract in the standard assay. Each grouping represents separate experiments.

[b] One unit of SOD activity is defined as the amount of enzyme required to produce 50% inhibition of superoxide synthesis from xanthine oxidase in the standard reaction mixture.

the *P. gingivalis* insert. The higher levels of SOD activity expressed from pS1 in strain HB101 compared with those in QC779 indicate that host strain factors may also influence *sod* expression. Furthermore, the increased SOD activity expressed from pCC19 in *E. coli* QC779 compared with that from pS1 suggests that genes flanking the *Hin*dIII site (Fig. 1) are not required for *sod* expression. It is more likely that a potential *sod* repressor gene may be present upstream from the *sod* gene. Further characterization of this region will be required to substantiate this possibility.

Characterization of the SOD purified from *P. gingivalis* 381 indicated that these organisms produced a single enzyme activated by either Mn^{2+} or Fe^{2+} (1). In this regard, the addition of Fe^{2+} (0.2 mM) or Mn^{2+} (0.12 mM) to the strain ATCC 53977 SOD enzyme resulted in 50 and 83% stimulation of activity, respectively. In addition, as with Fe SODs (12), H_2O_2 (1.0 mM) produced 70% inhibition of cloned SOD activity.

The present results represent an initial description of the isolation and expression of the *P. gingivalis sod* gene in *E. coli* and determination of its nucleotide sequence. It should now be possible to utilize the gene to directly examine the role of the *P. gingivalis* SOD enzyme in oxygen tolerance and sensitivity to neutrophil killing.

Nucleotide sequence assession number. The *P. gingivalis sod* gene sequence has been deposited in the GenBank directory as accession no. M60401.

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