Structure and gene expression of the *E. coli* Mn-superoxide dismutase gene

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ABSTRACT

Superoxide dismutase is an enzyme which converts superoxide O_2^- to hydrogen peroxide. Using a single synthetic oligonucleotide 33mer, we screened the *E. coli* DNA library and isolated a clone containing the *E. coli* manganese-superoxide dismutase gene. We determined the DNA sequence. The analysis of the DNA sequence and *in vivo* as well as *in vitro* transcription has shown the following. (1) The DNA sequence suggests two possible promoters. However, only one of them seems active during normal aerobic growth. Purified RNA polymerase initiates *in vitro* transcription from the same promoter. It is not clear whether the second promoter is functional. It is possible that this promoter could be activated under different growth conditions. (2) There is an inverted repeat sequence which could form a stem-loop structure downstream of the translation stop codon TAA of the Mn-SOD gene. The results of the analysis of *in vivo* and *in vitro* RNA have shown that this is the transcription termination signal. Thus, the Mn-SOD gene constitutes a single gene operon. (3) There is an almost perfect 19 base palindrome at the -35 region. The position and the size of the palindrome suggest that this could be a regulatory site.

INTRODUCTION

The univalent reduction of molecular oxygen generates the highly reactive, thus potentially toxic, free radical superoxide, O_2^- (1-4). The enzyme superoxide dismutase (SOD) catalytically converts this free radical to H_2O_2 (5). Then, catalase and/or peroxidases change H_2O_2 to H_2O (6,7). If H_2O_2 encounters ferrous ion before catalase or peroxidases, however, it is converted to a more reactive free radical, $OH^\bullet$ (8), which potentially initiates more disastrous free radical reactions including lipid peroxidation, protein and DNA damage (1-4). Thus, superoxide dismutase, catalase and peroxidases constitute important defense mechanisms against toxic oxygen free radicals (1-7).

E. coli cells can synthesize two distinct types of superoxide dismutase, one containing manganese (9), and the other iron (10). Iron-superoxide dismutase (Fe-SOD) is synthesized constitutively under most growth conditions including anaerobiosis, whereas the level of manganese-superoxide dismutase

(Mn-SOD) inside the cell varies widely according to the growth conditions (3,11-15). Mn-SOD is not synthesized during anaerobiosis, but is induced by oxygen (11,12). The intracellular level of superoxide dismutase is further increased by hyperbaric oxygen (11), or by the addition of an oxygen free radical generator such as streptonigrin (13) or methyl viologen (14). Glucose in the medium, on the other hand, lowers the intracellular level of superoxide dismutase (15).

In order to study the mechanisms controlling the synthesis of superoxide dismutase and the mechanisms governing the intracellular level of superoxide dismutase, we have cloned both the E. coli Mn- and Fe-superoxide dismutase genes by molecular cloning. Here we report the structure of the Mn-SOD gene and the preliminary characterization of its transcript. Preliminary cloning experiments of the Mn-SOD gene (16) and the Fe-SOD gene (17-19) have been reported previously.

EXPERIMENTAL

Strains & Materials. E. coli K12 K802 (from F. Blattner), N4591 tif⁻, sfi⁻ and SG5000 endI⁻, RNaseI⁻ (both from S. Gottesman) were used in most of the experiments. An E. coli DNA library in the phage vector D69 (E. coli DNA Sau3A partial; see ref. 20) was obtained from S. Mizusawa. Nitrocellulose membrane filters (BA-85) were purchased from Schleicher and Schuell. [γ -³²P] ATP was prepared using [³²P] orthophosphate purchased from New England Nuclear (21). T4 polynucleotide kinase, T4 DNA ligase, restriction enzymes were obtained from either Bethesda Research Laboratories or New England BioLabs. Calf intestine alkaline phosphatase was obtained from Boehringer-Mannheim Biochemicals. Protected nucleotide monomers and dimers, and the coupling reagent, 2-mesitylbenzensulfonyl-3-nitro-1,2,4-triazole, were purchased from Bachem, Inc.. Pyridine, tetrahydrofuran, dimethylformamide, acetic anhydride, triethylamine, tetramethylguanidine, syn-pyridine-2-carboxaldoxime and zinc bromide were obtained from Aldrich Chemicals.

Synthesis of oligonucleotide. Oligonucleotide 33mer (TTCGTCCAGTTAACAACCTCCAGAATTCTTT) was synthesized by a solid-phase triester approach (22). We coupled dimers with the overall efficiency of 25%. After synthesis, the oligonucleotide was cleaved off the resin by oximate, then the blocking groups were removed by base and acid treatment. The oligonucleotide was then passed through a Sephadex G-50 column using 50 mM triethylamine-acetate buffer, pH 7.0, as a running buffer, and lyophilized to dryness.

For end-labeling, DNA (10 μ g) was incubated with [γ -³²P] ATP (specific

activity >8000 Ci/mmol) and 20 units of T4 polynucleotide kinase in 50 μ l of 50 mM Tris-HCl, pH 7.4, 10 mM MgCl₂, 0.5 mM dithiothreitol, 0.1 mM spermidine, 0.1 mM EDTA for 1 hour at 37°C (23). Unincorporated [γ -³²P] ATP was removed by centrifugation through a Sephadex G-50 spun column (24), then the [³²P] labeled DNA was electrophoresed on a 20% polyacrylamide gel (23,24). The DNA band corresponding to 33mer was identified by autoradiography, cut out of the gel, and eluted with 0.5 M ammonium acetate, pH 7.0, 10 mM magnesium acetate, 1 mM EDTA, 0.1% SDS at 37°C overnight.

Hybridization. E. coli DNA (5 μ g) was digested for 2 hours at 37°C with restriction enzyme, HindIII (28 units) or BamHI (32 units) in 50 μ l of 10 mM Tris-HCl, pH 7.4, 10 mM MgSO₄, 1 mM dithiothreitol, 50 mM NaCl, or with EcoRI (33 units) in 50 μ l of 50 mM Tris-HCl, pH 7.4, 10 mM MgSO₄, 100 mM NaCl. DNAs were electrophoresed on a 0.8% agarose gel with 0.089 mM Tris-borate buffer, pH 8.3, 1 mM EDTA as a running buffer, then transferred onto a nitrocellulose membrane filter (S & S, BA-85)(25). The DNA filter was baked under vacuum for 2 hours at 80°C. The DNA filter was preincubated for 3 hours at 55°C in the solution containing 0.2% Ficoll, 0.2% polyvinylpyrrolidone and 0.2% bovine serum albumin (10xDenhardt), 0.9 M NaCl, 60 mM NaH₂PO₄, 6 mM EDTA, pH 7.4 (6xSSPE), 0.1% SDS (24). The medium was changed to the solution containing the same ingredient plus [³²P] labeled oligonucleotide 33mer (6x10⁵cpm/ 2 ng/ml), and the hybridization was allowed to proceed for 24 hours at 42°C. The filter was taken out and washed twice with 6xSSPE, 0.1% SDS for 5 min each at room temp., then once for 1 min at 55°C. The dried filter was autoradiographed using an intensifying screen (DuPont lightening plus).

For the plaque hybridization, phage D69 was plated with E. coli K12 N4591 on LB agar using 0.6% agarose as a top agar. Plaques were allowed to develop for 9 hours at 37°C. Plates were cooled for 1/2-1 hour in a cold room, then the duplicate plaque filters were prepared (26). Hybridization was carried out essentially as above.

Construction of plasmid pSOD-1. E2 DNA (2 μ g) and pBR322 DNA (0.18 μ g) were digested with a mixture of HindIII and NruI (5 units each), then ligated using T4 DNA ligase (2 units) for 18 hours at 4°C. This was used to transform E. coli K12 K802 (27), and Amp^RTc^S colonies were isolated. 21 such colonies were spotted onto LB agar, incubated with chloramphenicol, and the duplicate colony filters were prepared (28). To identify the plasmid carriers which contain the Mn-SOD gene, colony hybridization was carried out essentially as above.

S1 mapping. E. coli K12 SG5000 was grown in L broth to OD₆₀₀ = 0.5, then

the growth was stopped by the addition of 10 mM Na-azide and crushed frozen M9 salts. Cells were collected by centrifugation, and resuspended in 1/100 volume of 0.1 M Na-acetate buffer, pH 5.5. RNA was extracted four times at 68-70°C with an equal volume of water-saturated phenol (29), and precipitated with ethanol.

To map the 5' end of RNA, pSOD-1 DNA was cleaved with restriction enzyme NcoI, dephosphorylated with calf intestine alkaline phosphatase (24), then kinased with [³²P] ATP using T4 polynucleotide kinase. To map the 3' end of RNA, pSOD-1 DNA was cleaved with restriction enzyme PstI, then the 3' end was labeled with [³²P] cordycepin 5' triphosphate (New England Nuclear) using terminal transferase (30). [³²P] DNA (0.1 µg) thus obtained was mixed with 50 µg E. coli RNA in 30 µl hybridization buffer (40 mM Pipes buffer, pH 6.4, 1 mM EDTA, 0.4 M NaCl, 80% formamide)(24), heated for 10 min at 73°C, then incubated for 3 hours at 51°C. S1 nuclease treatment (700-1000 units) was carried out for 30 min at 37°C, and the remaining DNA was analysed on a 6% polyacrylamide gel containing 7 M urea.

Transcription in vitro. RNA polymerase and termination factor ρ were prepared using polymin P from the same E. coli cells (K12 SG5000)(J.P. Richardson, personal commun. and ref. 31). For in vitro transcription, pSOD-1 DNA (2.6 µg) and RNA polymerase (7 µg; RNA polymerase/DNA = 5) were preincubated in 50 mM Tris-HCl, pH 7.5, 10 mM MgCl₂, 80 mM KCl, 10% glycerol for 10 min at 37°C. Then, RNA synthesis was initiated by the addition of nucleotide mixture (4 ribonucleoside triphosphate, each at 100 µM, 0.1 mCi/ml [³²P] ATP and 50 µg/ml heparin) and continued for 15 min at 37°C. RNA synthesis was stopped by the addition of 20 mM EDTA. RNA products were analysed on a 6% polyacrylamide gel containing 7 M urea.

RESULTS

Cloning of the Mn-SOD gene. Steinman (32) reported the complete amino acid sequence of Mn-SOD of E. coli B. Considering the preference in usage of the genetic code in bacteria (33-35), we chemically synthesized the 33 bases long oligonucleotide "TTCGTCCAGTTAACAACGTTCAGAAATTCITT" corresponding to the amino acid sequence Lys186-Glu-Phe-Trp-Asn-Val-Val-Asn-Trp-Asp-Glu196 of Mn-SOD (32). This was end-labeled with [³²P] ATP, and used to hybridize with E. coli DNA Southern blot. As shown in Fig. 1, this DNA probe hybridizes a unique DNA fragment. The size of the hybridizing EcoRI or BamHI DNA fragment is estimated to be 7.0 kb, and that of the EcoRI and BamHI double digest to be 4.9 kb.

We then used the same [³²P] end-labeled oligonucleotide to screen the E.

Figure 1. Southern blot hybridization. *E. coli* DNA was digested with restriction enzyme, HindIII (H), EcoRI (R), BamHI (B) or in their combination, then the digests were electrophoresed on a 0.8% agarose gel. DNA was transferred onto the nitrocellulose filter (25), and the hybridization was carried out as in Methods.

coli DNA library in phage D69 (20). Five positive plaques were obtained when approximately 10,000 plaques were screened. Preliminary studies indicated that all of them contained the Mn-SOD gene. We have characterized one isolate called "E2", which contains the Mn-SOD gene in the middle of the 5.8 kb *E. coli* insert (see Fig. 2a). Using the HindIII and NruI sites in phage DNA just outside of this insert, the *E. coli* DNA fragment was cloned into plasmid pBR322. The resulting plasmid is called pSOD-1 (Fig. 2b).

Structure of the Mn-SOD gene. The restriction map was constructed (Fig. 3), and the 1053 bases long AvaI fragment was sequenced according to Maxam & Gilbert (23) (Fig. 4). The DNA sequence confirmed the amino acid sequence (32), except at two places. The reported amino acid sequence His80-Asn81 is in fact Asn80-His81, while Leu164 is Met164. The latter discrepancy could be due to strain difference, because the amino acid sequence was determined for the superoxide dismutase from *E. coli* B, and the nucleotide sequence for the DNA from *E. coli* K12.

The DNA sequence suggests two possible promoters designated as p_1 and p_2 (Fig. 4). The -10 region of the promoter p_1 has exactly the same sequence as the consensus sequence TATAAT (36), while the -35 region has two mismatches

Figure 2. The DNA structure of the Mn-SOD gene in (A) phage clone E2, and (B) plasmid clone pSOD-1. Restriction sites are abbreviated as R1, EcoR1; P, PstI; H, HindIII; Nde, NdeI; Nco, NcoI; A or Ava, AvaI; B, BamHI; Nru, NruI, respectively. DNA length is represented by either bp (base-pair) or Kb (killo base-pair).

from the consensus sequence TTGACA (36). On the other hand, both the -10 and -35 regions of the promoter p_2 have one mismatch from the consensus sequences (36). Experiments described below show that only the p_1 promoter is active in vivo as well as in vitro. There is an almost perfect 19 base palindrome at

Figure 3. Restriction map of the 1053 bp AvaI DNA fragment and the DNA sequencing strategy.

nucleotide 45 to 63 (from the AvaI site), overlapping the -35 region of this promoter. The Shine-Dalgarno sequence (37) is identified 6 bases upstream of the ATG codon. The DNA sequence indicates an inverted repeat at nucleotide 817 to 839 preceding "T" stretch. This is downstream of the translation termination codon TAA of the Mn-SOD gene, and could be a transcription terminator for this gene (38).

Transcription of the Mn-SOD gene. Transcription of the Mn-SOD gene in vivo was analysed by S1 mapping. Experimental results shown in Fig. 5 locate the 5' end of the transcript 120-122 bases upstream of the unique NcoI site, and the 3' end 410 bases downstream of the unique PstI site within the gene. This locates the 5' end of transcript at around nucleotide 90 (+/-2 nucleotides)(Fig. 4), suggesting that p_1 is the active in vivo promoter. The result also locates the 3' end of the transcript at around nucleotide 840, which coincides with the end of the stem-loop structure (Fig. 4). Thus, it appears that the Mn-SOD gene constitutes a single gene operon.

We carried out transcription in vitro. As can be seen in Fig. 6(A), pSOD-1 DNA linearized with restriction enzyme NcoI and PstI yields approximately 120 bases and 340 bases long transcripts, respectively. The results of S1 mapping experiments using the in vitro transcript locate the 5' end 120 bases upstream of the NcoI site [Fig. 6(B)]. Thus, purified RNA polymerase also recognizes the p_1 promoter to initiate transcription. Supercoiled pSOD-1 DNA is a poorer template (roughly one-fifth to one-tenth as active) for in vitro transcription (data not shown). However, it appears that transcription, if any, is still initiated from the same promoter [Fig. 6(B)]. Transcription using the 1053 base-pairs AvaI DNA fragment as a template yields approximately 750 bases long transcript (data not shown). Thus, in vitro

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      10          20          30          40          50          60
TCGGGCAITTT TCCTGCAAAA CCATACCCTT ACGAAAAGTA CGGCATTGAT ATCATTTTC
      70          80          90          100
AATATCAATTT AATTAACTAT AATGAACCA CTGCTTACGC GGCATTAACA ATCGGCCGC
      110          120          130          140          150
CGACAATACT GGAGATGAAT ATG AGC TAT ACC CTG CCA TCC CTG CCG TAT GCT
      160          170          180          190          200
TAC GAT GCC CTG GAA CCG CAC TTC GAT AAG CAG ACC ATG GAA ATC CAC CAC
      210          220          230          240          250
TYR ASP ALA LEU GLU PRO HIS PHE ASP LYS GLN THR MET GLU ILE HIS HIS
      260          270          280          290          300
ACC AAA CAC CAT CAG ACC TAC GTA AAC AAC GCC AAC GCG GCG CTG GAA AGC
      310          320          330          340          350
THR LYS HIS HIS GLN THR TYR VAL ASN ASN ALA ASN ALA ALA LEU GLU SER
      360          370          380          390          400
CTG CCA GAA TTT GCC AAC CTG CCG GTT GAA GAG CTG ATT ACC AAA CTG GAC
      410          420          430          440          450
LEU PRO GLU PHE ALA ASN LEU PRO VAL GLU GLU LEU ILE THR LYS LEU ASP
      460          470          480          490          500
CAG CTG CCA GCA GAC AAG AAA ACC GTA CTG CGC AAC AAC GCT GGC GGT CAC
      510          520          530          540          550
GLN LEU PRO ALA ASP LYS LYS THR VAL LEU ARG ASN ASN ALA GLY GLY HIS
      560          570          580          590          600
GCT AAC CAC AGC CTG TTC TGG AAA GGT CTG AAA AAA GGC ACC ACC CTG CAG
      610          620          630          640          650
ALA ASN HIS SER LEU PHE TRP LYS GLY LEU LYS LYS GLY THR THR LEU GLN
      660          670          680          690          700
GGT GAC CTG AAA GCG GCT ATC GAA CGT GAC TTC GGC TCC GTT GAT AAC TTC
      710          720          730          740          750
GLY ASP LEU LYS ALA ALA ILE GLU ARG ASP PHE GLY SER VAL ASP ASN PHE
      760          770          780          790          800
AAA GCA GAA TTT GAA AAA GCG GCA GCT TCC CGC TTT GGT TCC GGC TGG GCA
      810          820          830          840          850
LYS ALA GLU PHE GLU LYS ALA ALA ALA SER ARG PHE GLY SER GLY TRP ALA
      860          870          880          890          900
TGG CTG GTG CTG AAA GGC GAT AAA CTG GCG GTG GTT TCT ACT GCT AAC CAG
      910          920          930          940          950
TRP LEU VAL LEU LYS GLY ASP LYS LEU ALA VAL VAL SER THR ALA ASN GLN
      960          970          980          990          1000
GAT TCT CCG CTG ATG GGT GAA GCT ATT TCT GGC GCT TCC GGC TTC CCG ATT
      1010          1020          1030          1040          1050
ASP SER PRO LEU MET GLY GLU ALA ILE SER GLY ALA SER GLY PHE PRO ILE
      1060          1070          1080          1090          1100
ATG GGC CTG GAT GTG TGG GAA CAT GCT TAC TAC CTG AAA TTC CAG AAC CGC
      1110          1120          1130          1140          1150
MET GLY LEU ASP VAL TRP GLU HIS ALA TYR TYR LEU LYS PHE GLN ASN ARG
      1160          1170          1180          1190          1200
CGT CCG GAC TAC ATT AAA GAG TTC TGG AAC GTG GTG AAC TGG GAC GAA GCA
      1210          1220          1230          1240          1250
ARG PRO ASP TYR ILE LYS GLU PHE TRP ASN VAL VAL ASN TRP ASP GLU ALA
      1260          1270          1280          1290          1300
GCG GCA CGT TTT GCG GCG AAA AAA TAA TCATTGGC GCCTGCTGCA ATGAGGCGTA
      1310          1320          1330          1340          1350
ALA ALA ARG PHE ALA ALA LYS LYS
      1360          1370          1380          1390          1400
TAGGCCGCAT ATCAGCTTAA AAAATGAACC ATCGCCAAC GCGGTGGTTT TTTGTGATC
      1410          1420          1430          1440          1450
AATTTCAAA TAAAAACAAT GATCCGAAT AAAATAAAAC AGCGTTTCAA TTGATGTGGT
      1460          1470          1480          1490          1500
TTTGACACTT TTATGATTAA ATGAATGTCT ATCTTCGTTT CCATCAACAC TGATGCTCCA
      1510          1520          1530          1540          1550
TTGAGGAATT ACGCATCAGC CCTTAAAAAT ATGCCGACAG GTGATGGAAG TGCAGATAAA
      1560          1570          1580          1590          1600
ACGCTCGATT GAGAAAAATC CGG

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Figure 4. Nucleotide sequence of the 1053 bp *Ava*I fragment containing the *E. coli* Mn-SOD gene. The -35 and -10 regions of putative promoters p_1 and p_2 are indicated by boxes. Probable transcription start points are indicated at nucleotide 89 and 90. SD, Shine-Dalgarno sequence. Arrows indicate inverted repeats. Waved line (nucleotide 36-46) indicates one of the best (among several) IHF binding sites within and around the palindrome.

transcription seems to terminate at the same termination site as *in vivo*. Transcription termination occurs without termination factor rho, but if rho factor is present, termination is more effective (about twofold). Thus, *in vitro* transcription mimics *in vivo* transcription.

Figure 5. S1 mapping of *in vivo* transcription. To map the 5' end of RNA, pSQD-1 DNA was cleaved with restriction enzyme *Nco*I, and end-labeled with [32 P]ATP using T4 polynucleotide kinase. To map the 3' end, pSQD-1 DNA was cleaved with restriction enzyme *Pst*I and end-labeled with [32 P] cordycepin 5'-triphosphate using terminal transferase. This was hybridized with *in vivo* RNA and S1 resistant DNA was analysed on a 6% polyacrylamide gel containing 7 M urea. First lane from the left; [32 P] ϕ X174 *Hae*III markers. Lane 2; 5' end mapping. Lane 3; 3' end mapping.

DISCUSSION

The DNA sequence has shown that 30 out of 33 nucleotides of our DNA probe are correct sequence. Because of this, we obtained unique hybridization very easily in Southern blot as well as in library screening. By screening a cosmid DNA library, Touati (16) isolated a clone which overproduced Mn-superoxide dismutase. She named the gene *sod-A* and mapped at 87.5 min on the *E. coli*

Figure 6. Analysis of *in vitro* transcription. (A) RNA synthesis. pSOD-1 DNA was digested with restriction enzyme *Nco*I or *Pst*I, and RNA synthesis was carried out using purified RNA polymerase as in Methods. The products were analysed on a 6% polyacrylamide gel containing 7 M urea. First lane from the left; [32 P] ϕ X174 *Hae*III markers. Lane 2; *Nco*I digest as template. Lane 3; *Pst*I digest as template. (B) S1 mapping. RNA synthesis was carried out using cold ribotriphosphate on linear pSOD-1 DNA (*Ava*I digest) or on supercoiled pSOD-1 DNA (pretreated with DNA ligase). S1 mapping of 5' end was carried out as in Fig. 5. First lane from the left; [32 P] ϕ X174 *Hae*III markers. Lane 2; *in vivo* RNA. Lane 3; *in vitro* RNA made on linear DNA. Lane 4; *in vitro* RNA made on supercoiled DNA.

linkage map (16). Her restriction map (16) and ours are almost identical. The *Eco*R1 fragment, containing the Mn-SOD gene, is 7 kb long; the *Eco*R1-*Bam*H1 and *Pst*I-*Bam*H1 fragments are 4.9 kb and 1.25 kb long, respectively, whereas we estimated these fragments to be 4.8 kb and 1.35 kb (see Fig. 1 & 2). Thus, we conclude that both groups have isolated the same Mn-SOD gene.

The DNA sequence has suggested two putative promoters, p_1 and p_2 . However, the S1 mapping experiments have shown that RNAs prepared from *E. coli* grown in L broth (aerobic growth) contain only the transcript initiated at the

p_1 promoter. RNA polymerase purified from *E. coli* grown similarly starts transcription in vitro from the same promoter. Thus, p_1 seems to be the active promoter during normal aerobic growth. We do not know whether the putative promoter p_2 is active or not. If it is an active promoter, it may be activated by growth conditions not tested here. It is known that the synthesis of many oxygen regulated proteins are subjected to multiple regulatory signals (39). Mn-superoxide dismutase is induced by oxygen (11,12), but the enzyme level can be further elevated by hyperbaric oxygen (11) or a superoxide generator (13,14). Similarly, catalase and peroxidases are induced by oxygen (40), and their enzyme levels increase in the presence of hydrogen peroxide (41,42). Recently, Christman et al. (43) have isolated a positive regulator oxyR in *Salmonella* as a gene responsible for the cell's adaptation to hydrogen peroxide. A mutation in oxyR does not affect the oxygen induction or the synthesis of normal levels of enzymes, but abolishes the adaptation synthesis of catalase, peroxidases and possibly superoxide dismutase (43). Considering the dual regulation exerted on the superoxide dismutase synthesis, the putative second promoter p_2 might be responsible for the adaptation or emergency synthesis of superoxide dismutase and can be activated by the *E. coli* oxyR equivalent. We are currently testing this possibility by isolating a fusion of the p_2 promoter to the lac or gal gene.

Sequence analysis has revealed a 19 base palindrome at the -35 region. Judging from the position and the size of the palindrome, this could be a binding site for a regulatory protein. We note the following in this context.

(1) If this palindrome is actually a regulatory site, and the SOD gene is under a coordinate oxygen regulation with some other genes, we may expect to find a similar sequence at the 5' flanking region of other oxygen regulated genes. A computer search for such sequences in the bacterial GenBank has identified a similar sequence at the 5' flanking region of *E. coli* tnaA (tryptophanase) gene; some 50 bases upstream of the CAP site of this gene (44), but none at the 5' flanking region of any of the sequenced oxygen regulated genes. At present we do not know whether this sequence has any role in the regulation of sod and/or tnaA gene expression.

(2) Catabolite-gene activator protein, CAP, regulates expression of many genes by binding at the -35 region (45,46). Since the synthesis of superoxide dismutase is subject to a glucose effect (15), we compared the 5' region of the SOD gene sequence with the sequences of CAP binding sites (46,46). We found a CAP core consensus sequence within this palindrome (TGAT.....TCA)(see Fig. 4). However, since the homology is not very extensive, direct experiments will

be needed to determine the involvement of CAP, if any, in this gene system. In fact, previous workers (15) have suggested that the glucose effect may be indirect, since the addition of cAMP did not relieve depression of the superoxide dismutase synthesis.

(3) We have identified several possible integration host factor, IHF (himA-hip complex, see ref. 47), binding sites within and around this palindrome (see Fig. 4). It remains to be determined whether IHF is actually involved in the regulation of superoxide dismutase synthesis.

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