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Short sequence-paper

Nucleotide sequence of the genes, encoding the pentaheme cytochrome (*dmsC*) and the transmembrane protein (*dmsB*), involved in dimethyl sulfoxide respiration from *Rhodobacter sphaeroides* f. sp. *denitrificans* [1]

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Abstract

The nucleotide sequence of the genes encoding a pentaheme cytochrome (*dmsC*) and a transmembrane protein (*dmsB*) were determined upstream of the *dmsA* gene encoding dimethyl sulfoxide reductase from *Rhodobacter sphaeroides* f. sp. *denitrificans*. *dmsC* and *dmsB* encode proteins of 404 and 226 amino acid residues, which show 40% and 26% identity to the pentaheme cytochrome TorC and the transmembrane protein TorD, respectively, of the trimethylamine N-oxide reduction system in *Escherichia coli*.

Keywords: Cytochrome *c*; *dmsCBA* operon; Dimethylsulfoxide respiration; (*R. sphaeroides* f. sp. *denitrificans*)

Under dark conditions the phototrophic bacterium *Rhodobacter sphaeroides* f. sp. *denitrificans* can use dimethyl sulfoxide (DMSO) as an electron acceptor for growth in the absence of oxygen [1]. DMSO reductase, the terminal enzyme of the DMSO respiration, is inducibly synthesized by DMSO and trimethylamine N-oxide (TMAO) [2] and is secreted into the periplasmic space of the cell [3]. This enzyme contains a molybdenum cofactor in the active center and catalyzes reduction of some sulfoxides and N-oxides with some artificial electron donors such as methyl or benzyl viologens. However, no information has been obtained about physiological electron donors of DMSO reductase in the phototroph.

The gene encoding DMSO reductase has been cloned from the genomic DNA of *R. sphaeroides* f. sp. *denitrificans* [4] and *R. sphaeroides* strain WS8 [5]. The *dmsA* gene of *R. sphaeroides* f. sp. *denitrificans* [4] are analyzed to encode a signal peptide and the periplasmic enzyme DMSO reductase. The deduced amino acid sequence of DMSO reductase contains six homologous regions conserved in twelve bacterial enzymes containing molybdenum cofactor. Among them three enzymes of *Escherichia coli* are highly homologous with DMSO reductase of the phototroph: TMAO reductase TorA (48% of identical residues), biotin sulfoxide reductase BisC (44%), and DMSO reductase DmsA (28%). TMAO reductase is a

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[1] The nucleotide sequence data reported in this paper have been submitted to the GSDB, DDBJ, EMBL and NCBI databases under the accession number D82820.

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terminal enzyme in anaerobic respiration using TMAO as an electron acceptor [6]. The enzyme is encoded in the *torCAD* operon with the membrane-bound pentaheme cytochrome *c* TorC and the transmembrane protein TorD [7]. The transcription of the *torCAD* operon is activated by TMAO under anaerobic conditions [8,9]. A deduced amino acid sequence with homology to a C-terminal region of the *torD* gene product is found upstream of the *dmsA* gene of the phototroph, whereas a termination signal-like

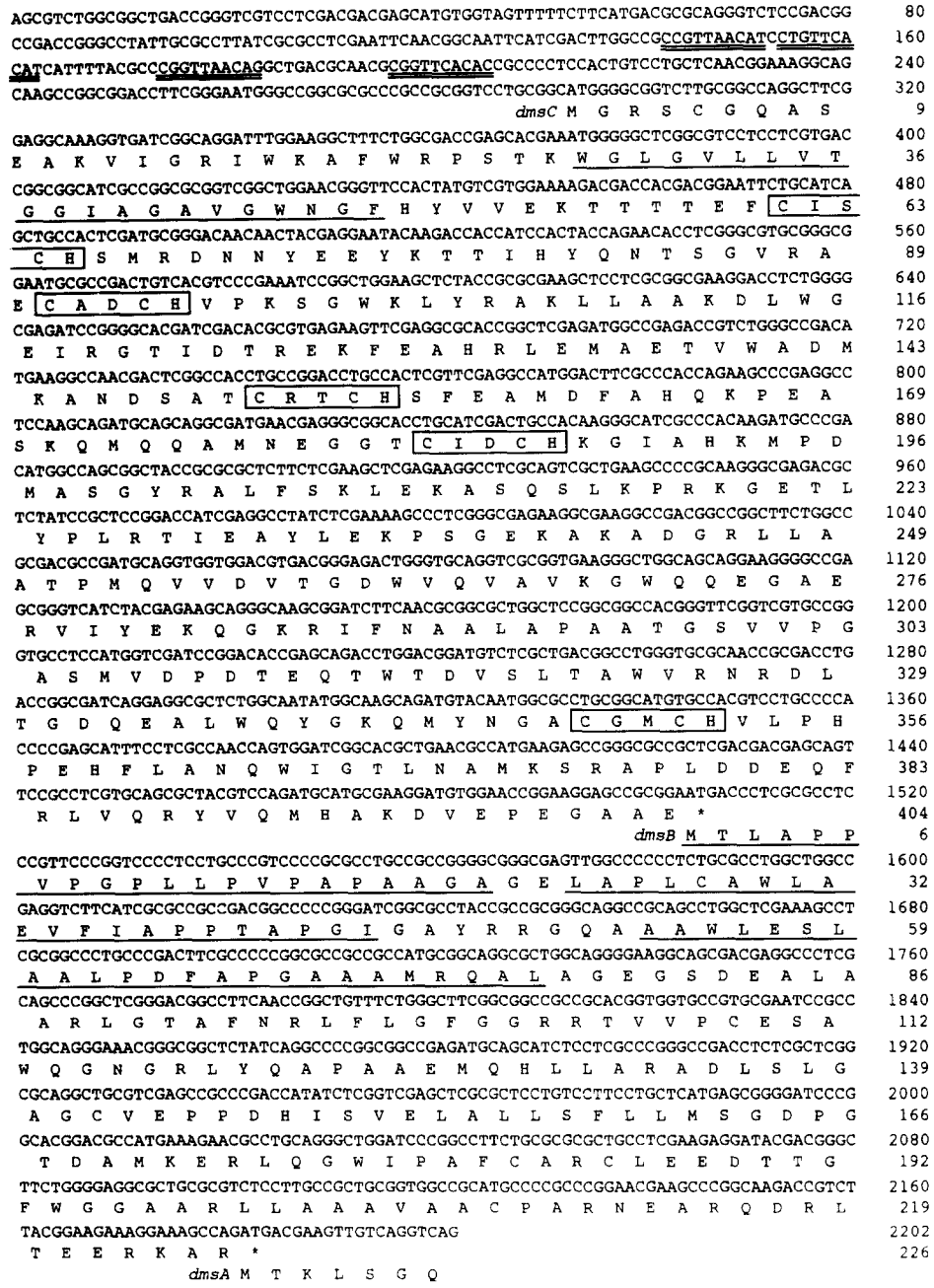

Fig. 1. Nucleotide and deduced amino acid sequences of the *dmsC* and *dmsB* genes of *R. sphaeroides* f. sp. *denitrificans* and 5′-end of the *dmsA* gene. Direct repeats of the decameric nucleotides are double-underlined. Putative membrane-spanning regions in the DmsC and DmsB proteins are underlined. Boxed amino acid residues indicate the motif for binding of *c*-type heme.

hairpin-stem loop is downstream of the gene [4], suggesting that genes relating to DMSO reduction lie just upstream of *dmsA*. In this paper we report the nucleotide sequence of two genes associated with DMSO reduction as well as the *dmsA* gene.

To analyze nucleotide sequence upstream of *dmsA*, the 1.7-kilobase pairs (kbp) *Cfr*9I- and 3.4-kbp *Eco*RI-digested DNA fragments prepared from NW6, which contains the *dmsA* gene [4], were subcloned into pUC118 (Takara Shuzo Co.), producing plasmids pDMS31 and pDMS32, respectively. DNase I-digests of the 1.7-kbp *Cfr*9I fragment and the 0.6-kbp *Sph*I fragment obtained from pDMS32 were ligated with the phage M13mp18/19 to obtain overlaps of DNA fragments. Single-stranded DNA templates were prepared and sequenced in the both directions by the dideoxy-chain termination method with a dye-primer (Applied Biosystems Inc.) and *Bca*BEST DNA polymerase Sequencing Kit (Takara Shuzo Co.). The sequence was electrophoresed and analyzed with a DNA autosequencer (Applied Biosystems Inc.). The sequencing data were analyzed using the program GENETYX (Software Development Co.) in the Center of Gene Science, Hiroshima University.

The nucleotide sequence of 2202 bases and the deduced amino acid sequences are shown in Fig. 1. Two open reading frames were found to be arranged in the same transcriptional orientation as *dmsA*: one (named *dmsC*) consists of 1215 bp (from position 295 to 1509) and the other (*dmsB*) of 681 bp (from position 1506 to 2186). The stop codons of the upstream genes overlap the start codons of the downstream genes at the nucleotides ATGA between *dmsC* and *dmsB*, and between *dmsB* and *dmsA*. The overlap between the start and stop codons of the two open reading frames has been proposed to facilitate translational coupling [10].

The deduced *dmsC* gene product of 404 amino acid residues (molecular mass of 45086 Da) contained five boxes of the heme-binding motif CXXCH (Fig. 1). A transmembrane hydrophobic sequence was found in the N-terminal region (from residue 28 to 48) of the protein DmsC, indicating that DmsC anchors to the cytoplasmic membrane of the cell. Heme-staining of cell-free extracts on a gel of SDS-PAGE [11] showed that the phototroph grown anaerobically with DMSO contained a considerable amount of a hemoprotein with a molecular mass of 44 kDa in

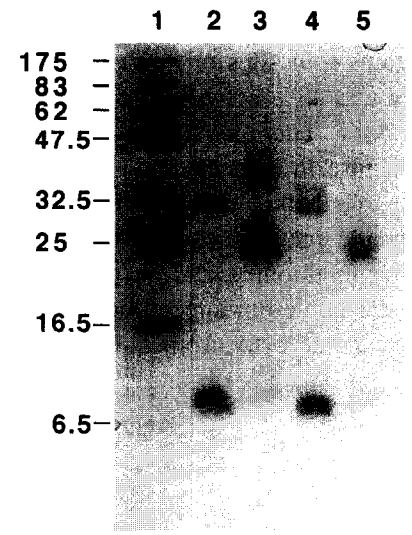

Fig. 2. Identification of proteins containing *c*-type heme. The cell extracts loaded on SDS-PAGE (12.5%) were stained for heme with 3, 3′, 5, 5′-tetramethylbenzidine [11]. Samples are: prestained protein markers (lane 1), the soluble fractions (lanes 2 and 4) and the membrane fractions (lanes 3 and 5) prepared by centrifugation at 100000× *g* for 1 h of sonic extracts obtained from the phototroph. The cells were grown anaerobically with 0.2% DMSO (lanes 2 and 3) and without DMSO (lanes 4 and 5). Each lane contains 25 μg of protein.

the membrane fraction, whereas a faint band of a 44-kDa protein was observed in the cell grown without DMSO. The 44-kDa hemoprotein induced by DMSO under anaerobic conditions seems to be the product of the *dmsC* gene.

A search of SwissProt data base revealed that the deduced protein DmsC was homologous to the pentaheme protein TorC of *E. coli* [7] and the tetraheme protein NirT of *Pseudomonas stutzeri* [12]. Cytochromes orf1 of *Chromatium vinosum* [13] and CycB of *Nitrosomonas europaea* [14] also showed homologies to DmsC (Fig. 2). TorC, which is the membrane-bound cytochrome *c* involved in TMAO reduction, have five sites of the heme-binding motif (CXXCH), as DmsC does. Homology between DmsC and TorC is 40% with identical residues in alignment of their amino acid sequences. The NirT has a feature of a membrane-bound tetraheme cytochrome *c* and works as an electron donor to nitrite reductase on the periplasmic side of the cytoplasmic membrane. The amino acid sequence of NirT which consists of 201 residues corresponds to an N-terminal half (208

residues) of DmsC; that is, the N-terminal half of DmsC contains four sites of the heme-binding motif, and identity was 48% between the N-terminal half of DmsC and NirT. This fact suggests that DmsC has a domain structure in respect to positions of heme-binding sites: four hemes are clustered in the N-terminal domain and one heme is alone in a C-terminal domain. One methionine and two histidine residues outside of the heme-binding motif are conserved in the three tetraheme cytochromes and in the N-terminal halves of the two pentaheme cytochromes (Fig. 2).

The deduced *dmsB* gene product was composed of 226 amino acid residues (Fig. 1) and had a molecular mass of 23480 Da. Hydropacy plot showed that this protein DmsB contained three transmembrane regions (from residue 1 to 21, 24 to 44, and 53 to 76). The protein DmsB is homologous to TorD, which is the transmembrane protein associated with TMAO reductase in *E. coli* [7]. Identical residues are 26% in the

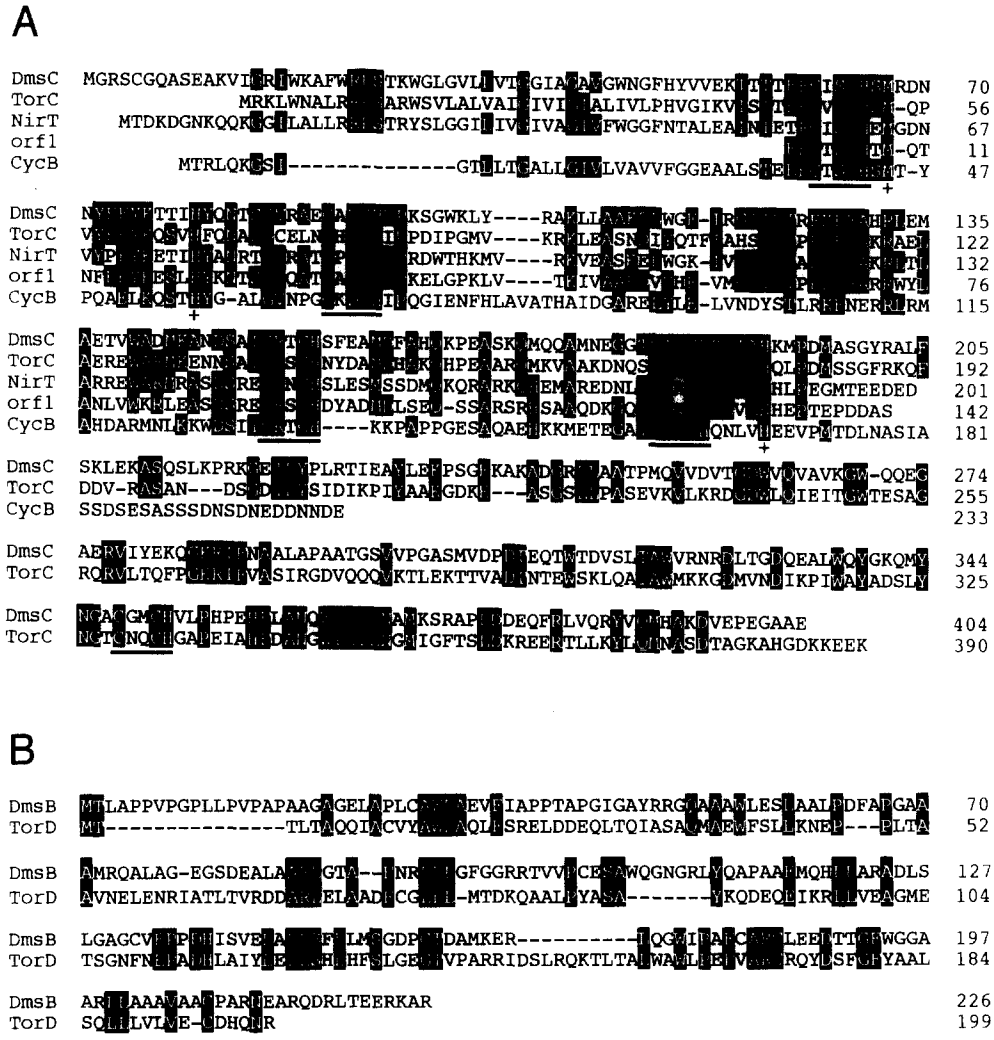

Fig. 3. Alignments of amino acid sequences. A, the deduced DmsC polypeptide and related proteins. TorC, the pentaheme cytochrome *c* of the *torCAD* operon in *E. coli*; NirT, the electron donor to nitrite reductase in *P. stutzeri*; orf1, a part of the tetraheme cytochrome *c* found in *C. vinosum*; CycB, the membrane-bound tetraheme cytochrome *c* of *N. europaea*. The underlined amino acid residues indicate the motif involved in binding of heme *c*. Plus symbols indicate M and H residues conserved in the five cytochromes, except of the heme-binding motif. B, the amino acid sequences of DmsB and the *E. coli* TorD. Identical residues at a given position are marked in reverse contrast letters.

alignment of their amino acid sequences (Fig. 3). Though the involvement of cytochrome *b* as well as cytochrome *c* in TMAO reduction is reported in *E. coli* [15,16], neither DmsB nor TorD seems to be cytochrome *b* based on the sequences characteristic for heme-binding regions of cytochrome *b* [17]. Physiological functions of these two proteins are still unknown.

It appears that the *dmsCBA* genes compose a transcriptional operon unit and the DMSO reductase system involves at least three proteins, the membrane-bound pentaheme cytochrome *c* DmsC, the transmembrane protein DmsB, and the periplasmic enzyme DMSO reductase. The composition of the gene products is very similar to the *E. coli torCAD* operon encoding TMAO reductase system [7], though the order of gene arrangement is different from each other. The expression of the *torCAD* operon is induced by TMAO under anaerobic conditions, and its transcriptional activation is mediated by the TorR protein [7] which is a response regulator in a two-component regulatory system [18]. The TorR regulatory protein is recognized to bind four direct repeats of the decameric sequence 5′-CTGTTCATAT in the *torC* regulatory region of *E. coli* [9] and of *Salmonella* species [19]. Four homologous sequences, which have 7–10 matches of the 10 nucleotides, are also present upstream of the *dmsC* gene (Fig. 1). The synthesis of DMSO reductase is also induced by DMSO. These facts suggest that transcription of the *dmsCBA* genes in the phototroph would be controlled by a regulatory protein responsive to a signal occurred by sensing DMSO present in growth environments.

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