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Dissimilatory sulfite reductase from *Archaeoglobus profundus* and *Desulfotomaculum thermocisternum*: phylogenetic and structural implications from gene sequences

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Abstract The genes encoding the α- and β-subunits of dissimilatory sulfite reductase, *dsr*AB, from the hyperthermophilic archaeon *Archaeoglobus profundus* and the thermophilic gram-positive bacterium *Desulfotomaculum thermocisternum* were cloned and sequenced. The *dsr*AB genes are contiguous, and most probably comprise an operon also including a *dsr*D homolog, a conserved gene of unknown function located downstream of *dsr*AB in all four sulfate reducers so far sequenced. Sequence comparison confirms that dissimilatory sulfite reductase, Dsr, is a highly conserved enzyme. A phylogenetic analysis using the available Dsr sequences, including Dsr-like proteins from nonsulfate reducers, suggests a paralogous origin of the α- and β-subunits. Furthermore, the Dsr from sulfate reducers forms a separate cluster, with Dsr from the bacterial sulfate reducers *Desulfotomaculum thermocisternum* and *Desulfovibrio vulgaris* branching together, next to Dsr from *Archaeoglobus profundus* and *Archaeoglobus fulgidus*. Based on an alignment with the assimilatory sulfite reductase from *Escherichia coli*, the amino acid residues involved in binding of sulfite, siroheme, and [Fe_4S_4]-clusters have been tentatively identified, which is consistent with the binding of two sirohemes and four [Fe_4S_4]-clusters per $\alpha_2\beta_2$ structure. The evolution of Dsr and the structural basis for the binding of substrate and cofactors are discussed.

Key words Dissimilatory sulfite reductase · *Archaeoglobus profundus* · *Desulfotomaculum thermocisternum* · Sequence analysis · Phylogenetic analysis

Introduction

Sulfate-reducing prokaryotes constitute a large physiological group of anaerobic microorganisms with the common ability to use sulfate as the terminal electron acceptor. The 16S rRNA gene sequence data group sulfate-reducing prokaryotes in the δ-subgroup of *Proteobacteria*, the gram-positive genera *Desulfotomaculum* and *Desulfosporosinus*, the deep-branching thermophilic bacterial genera *Thermodesulfobacterium* and *Thermodesulfovibrio*, and the hyperthermophilic archaeal genus *Archaeoglobus* (Devereux et al. 1989; Henry et al. 1994; Stackebrandt et al. 1997; Woese et al. 1991). Thus, although the property of dissimilatory sulfate reduction is present in widely separated phylogenetic groups, its distribution appears to be confined to a limited number of genera.

Dissimilatory sulfite reductase, which catalyzes the terminal step in the reduction of sulfate to sulfide through a six-electron transfer, has been detected in all sulfate-reducing species investigated so far. The enzymes have been found to consist of two different polypeptides in an $\alpha_2\beta_2$ structure and to contain siroheme and [Fe_4S_4]-clusters. Based on spectral and redox properties, Dsr have been differentiated into four types: desulfoviridin, desulforubidin, P582, and desulfofuscidin (Fauque et al. 1990; Peck and Lissolo 1987). The siroheme prosthetic group is also present in assimilatory sulfite reductases, nitrite reductases, anaerobically induced sulfite reductase from *Salmonella typhimurium*, and in reverse sulfite reductase (Crane and Getzoff 1996 [and references therein]; Huang and Barrett 1991). The siroheme is directly coupled to a [Fe_4S_4]-cluster in the catalytic center of these enzymes (Crane and Getzoff 1996). An additional, ferredoxin-like cluster is present in the C-terminal region of both subunits of dissimilatory sulfite reductases (Dahl et al. 1993; Huang and Barrett 1991; Karkhoff-Schweizer et al. 1995). Sequencing of *dsr* from *Archaeoglobus fulgidus* and *Desulfovibrio vulgaris* revealed that the genes are highly conserved (Karkhoff-Schweizer et al. 1995). A third conserved open reading frame (*dsr*D) was identified downstream of *dsr*B forming a contiguous operon

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with *dsr*AB. A possible sulfite-binding function for this small lysine-rich polypeptide has been suggested (Karkhoff-Schweizer et al. 1995).

Recently, dissimilatory sulfite reductase-type genes have been cloned and sequenced from the sulfur-oxidizing phototroph *Chromatium vinosum* and the hyperthermophilic thiosulfate-reducing crenarcheote *Pyrobaculum islandicum*. Also these two enzymes probably have an $\alpha_2\beta_2$ composition with 2 sirohemes and 4 [Fe_4S_4]-clusters (Hipp et al. 1997; Molitor et al. 1998). To further characterize dissimilatory sulfite reductase from sulfate-reducing prokaryots, we have sequenced the *dsr* genes from *Archaeoglobus profundus* and the gram-positive thermophilic species *Desulfotomaculum thermocisternum*. We thereby have the opportunity to compare dissimilatory sulfite reductase sequences from mesophilic, thermophilic, and hyperthermophilic sulfate-reducing species comprising major phylogenetic groups.

Materials and methods

Strains, plasmids, and media

Archaeoglobus profundus strain AV 18, DSM 5631 (Burggraf et al. 1990) was grown in a medium described by Möller-Zinkhan et al. (1989), modified by using 80 mM acetate and 20 mM Na_2SO_4. The pH of the medium was adjusted with 6 M HCl to 6.5 before inoculation at room temperature. After heating to 80°C the culture was pressurized with H_2/CO_2 (80:20, 200 kPa). *Desulfotomaculum thermocisternum* strain ST90, DSM 10259 (Nilsen et al. 1996) was grown under a gas phase of N_2/CO_2 (80:20) at 60°C in a bicarbonate-buffered medium (pH 7.0–7.2) with lactate as substrate (Beeder et al. 1995). Both strains were grown in 2-l rubber-stoppered glass flasks containing 1 l culture. At the end of the exponential growth phase the cells were harvested by centrifugation. *E. coli* XL1-Blue and XLOLR (Stratagene, La Jolla, CA, USA) were used as hosts for λZAP Express and pBK-CMV phagemid derivatives, respectively, and were grown as described by the supplier.

DNA preparation, cloning, and sequencing

Chromosomal DNA was prepared according to Ausubel et al. (1992), omitting the CTAB/NaCl step. After precipitation the DNA was treated with proteinase K (70 μg/ml final concentration) for 30 min at 37°C in the presence of 0.03% SDS before a second precipitation with isopropanol.

Genomic DNA was partially digested with *Sau*III A using standard protocols (Sambrook et al. 1989). DNA fragments (>3 kb) were extracted from agarose gels using QIAEX II DNA Extraction Kit (Qiagen, Valencia, CA, USA) and ligated into *Bam*HI/CIAP predigested λZAP Express vectors (Stratagene) and packed in vitro using GigaPack II packaging extract (Stratagene). *E. coli* XL1-Blue MRF was used as a host for the recombinant λ phages. Plaque hybridization was performed on Hybond N+ membranes according to the supplier (Amersham Pharmacia, Uppsala, Sweden). Hybridization was carried out overnight at 55°C in 0.5 M sodium phosphate buffer pH 7.2 with 7% SDS. A PCR product from *D. vulgaris* Hildenborough (DSM 644) obtained with conserved primers against *dsr* (Karkhoff-Schweizer et al. 1995) was used as a probe. The probe was labelled with [α-^{32}P]-dCTP (Amersham) using Ready-To-Go Labelling kit (Pharmacia, Uppsala, Sweden). Positive phages were then subjected to plasmid excision and transformed into *E. coli* XLOLR as recommended by the supplier (Stratagene). Plasmids were purified using a JetStar 2.0 Plasmid Midi Purification Kit (Genomed, Raleigh, NC, USA). DNA sequencing was carried out using the BigDye Terminator Cycle Sequencing Ready Reaction Kit with an ABI PRISM 377 DNA Sequencer (PE Applied Biosystems, Foster City, CA, USA).

Amplification of DNA by PCR

The PCR mixture consisted of 5–200 ng genomic DNA, 50 pmol primers, 4 mM of each of dNTP, 7.5 mM $MgCl_2$, enzyme buffer, and 1.25 U SuperTaq (HT Biotechnology, Cambridge, UK) in 20 μl. Thirty cycles of amplification were carried out in a Perkin Elmer 9600 Thermal Cycler. The following cycles were used: 1 min at 94°C for denaturation, 1 min at 65°C for annealing, and 4 min at 72°C for extension.

Computer analysis

DNA sequences were analyzed using the Wisconsin EGCG/GCG package version 8.1. Similarity searches were performed at NCBI using the BLAST program (Altschul et al. 1990), and multiple alignments of the predicted protein sequences were analyzed using ClustalW (Thompson et al. 1994).

DNA sequence accession numbers

The nucleotide sequences of the *dsr* loci were submitted to GenBank and were given the accession numbers AF071499, *A. profundus*, and AF074396, *D. thermocisternum*, respectively.

Results and discussion

Amplification of dissimilatory sulfite reductase genes by PCR

Degenerate PCR primers, specific for *dsr*AB from sulfate reducers and yielding a 1.4-kb PCR product, have been designed by Karkhoff-Schweizer et al. (1995). However, using these primers we were not able to amplify *dsr* from several species of representative sulfate reducers, including *A. profundus*, *D. thermocisternum*, and *Desulfobulbus rhabdoformis* (Fig. 1). Furthermore *Thermodesulforhabdus*

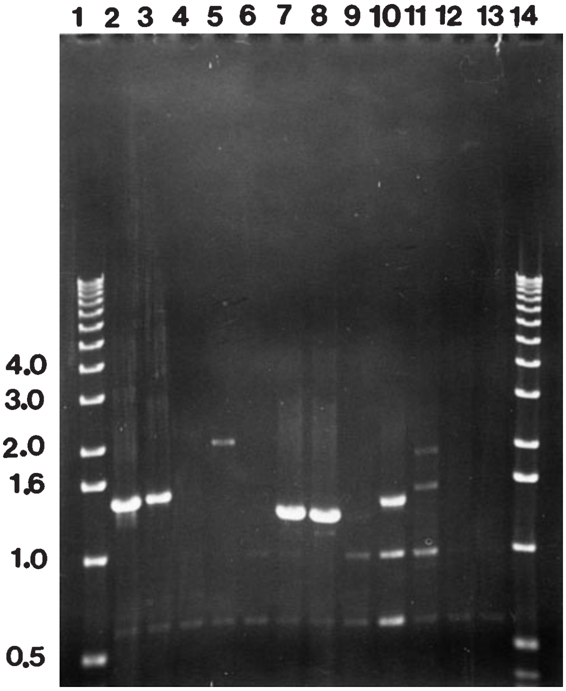


Fig. 1. PCR analysis of the genes for dissimilatory sulfite reductase using chromosomal DNA from different sulfate reducers and the sulfite-reducing bacterium *Thermotoga elfii* as template. The PCR products were electrophoresed on a 0.7% agarose gel. After electrophoresis the gel was stained with ethidium bromide and photographed. *Lanes 1* and *14*, 1-kb ladder (Gibco BRL); *lane 2*, *Desulfovibrio vulgaris* Hildenborough; *lane 3*, *Desulfobacter vibrioformis* B54; *lane 4*, *Desulfobulbus rhabdoformis* M16; *lane 5*, *Thermodesulfobacterium norvegicus* A8444; *lane 6*, *Archaeoglobus profundus* AV 18; *lane 7*, *A. fulgidus* VC-16; *lane 8*, *A. fulgidus* 7324; *lane 9*, *Desulfotomaculum thermocisternum* ST90; *lane 10*, *Desulfobacterium autotrophicum* HRM 2; *lane 11*, *Thermodesulfobacterium mobile* GFA1; *lane 12*, *Thermotoga elfii* SEBR 6459; *lane 13*, *E. coli* K12

norvegicus, *Thermodesulfobacterium mobile*, and *Desulfobacterium autotrophicum* yielded other PCR products than the expected 1.4-kb product. This result indicates that the *dsr* genes are more diverse than suggested earlier (Karkhoff-Schweizer et al. 1995), especially with regard to thermophiles. On the other hand, hybridization of the 1.4-kb PCR product from the *dsv* operon of *Desulfovibrio vulgaris* gave positive signals when hybridized to DNA from *A. profundus* and *D. thermocisternum* (data not shown), indicating variations at the PCR primer targets sequence rather than different types of enzymes.

Cloning and characterization of the *dsr* loci

To isolate the *dsr* genes from the thermophilic species *Archaeoglobus profundus* and *Desulfotomaculum thermocisternum*, genomic phage libraries were constructed, and positive clones, carrying inserts hybridizing with the *D. vulgaris* PCR product, were isolated. Phagemids excised from these phages were subjected to partial DNA sequencing. Two phagemids, designated pØLP4 and pØLS18, from *A. profundus* and *D. thermocisternum*, respectively, turned out to contain the entire *dsr* operons on 4- to 5-kb inserts. Sequencing of both clones revealed open reading frames (ORFs) with high similarity to the *dsr* genes from *A. fulgidus* and *D. vulgaris*. The ORFs were organized as for *dsr*AB of *A. fulgidus* and *dsv*AB of *D. vulgaris*. We named the ORFs *dsr*AB, and for simplicity we also refer to dissimilatory sulfite reductase from *D. vulgaris* as *dsr*AB. The *dsr*A gene from *A. profundus* was found to encode a 417-aa protein with a calculated molecular mass of 46.9 kDa showing 77% identity and 87% similarity to DsrA from *A. fulgidus*. The *dsr*A gene from *D. thermocisternum* encodes a slightly larger polypeptide with an extended 40-aa N-terminal sequence and in total 471 aa with a molecular mass of 54.1 kDa. The polypeptide shows 56% and 67% identity and 76% and 83% similarity to DsrA from *A. fulgidus* and *D. vulgaris*, respectively. In *A. profundus* the *dsr*B gene was located 11 bases downstream of *dsr*A and encodes a 373-aa polypetide with a molecular mass of 42.6 kDa showing 79% identity and 89% similarity to DsrB from *A. fulgidus*. In *D. thermocisternum,* the *dsr*B gene was located 20 bases downstream of *dsr*A, encodes a 395-aa polypeptide with a molecular mass of 44.2 kDa, and shows 54% and 66% identity and 74% and 81% similarity to DsrB from *A. fulgidus* and *D. vulgaris*, respectively. The high degree of conservation, both in sequence and gene organization, strongly suggests that the reported sequences represent the genes for the α- and β-subunit of Dsr from *A. fulgidus* and *D. thermocisternum*.

At 42 bp downstream of *dsr*B from *A. profundus*, an ORF encoding a polypetide of 79 aa with a molecular mass of 9.3 kDa was found that showed 57% sequence identity to the putative DsrD from *A. fulgidus*. Also in *D. thermocisternum* a DsrD homolog was located downstream of *dsr*B, whose deduced gene product showed 38% and 41% sequence identity to DsrD from *A. fulgidus* and *D. vulgaris*, respectively. The latter *dsr*D was found to encode a slightly larger polypeptide of 98 aa with a molecular mass of 11.1 kDa. Thus, the *dsr* operon from *A. profundus* and *D. thermocisternum* is organized in the same way as in *A. fulgidus* and *D. vulgaris*.

Sequencing of the flanking region of *dsr*ABD from *A. profundus* showed that the gene organization at this locus was similar to that found in *A. fulgidus* (Klenk et al. 1997), but the hypothetical gene AF0426 was not present (Fig. 2). In *D. thermocisternum* a ferredoxin gene was identified immediately upstream of *dsr*A. Multiple alignment of 23 $[Fe_4S_4]$-ferredoxins grouped the ferredoxin from *A. profundus* and *D. thermocisternum* along with ferredoxin IV from *A. fulgidus* and ferredoxin I from *D. vulgaris*, respectively (data not shown). It has been reported that a ferredoxin might be the in vivo electron donor to dissimilatory sulfite reductase (Bruschi et al. 1976; Chen et al. 1991), and this might be the reason for the apparent close genetic coupling between *dsr* and ferredoxin genes.

Further sequencing of the 5′-end of *dsr*AB from *D. thermocisternum* revealed two ORFs with high similarity scores to UDP-*N*-acetylglucosamine 1-carboxyvinyltransferase and a conserved hypothetical protein found in several bacterial genera, but with highest similarity to *yyd*A from *Bacillus subtilis* (Kunst et al. 1997). Downstream of *dsr*D from *D. thermocisternum* an ORF with significant similarity toward *O*-linked *N*-acetyl glucosaminyltransferases (gNAc) from methanogens and eukaryotes was found.

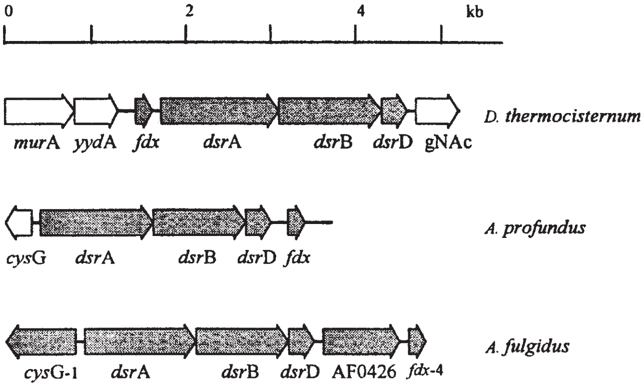


Fig. 2. Chromosomal organization at the *dsr* locus in *Desulfotomaculum thermocisternum* and *Archaeoglobus profundus* compared to the *A. fulgidus dsr* locus. ORFs were named based on *blast* search results with significant homology scores. *mur*A, UDP-*N*-acetylglucosamine 1-carboxyvinyltransferase; *yyd*A, a conserved hypothetical protein; *fdx*, ferredoxin; *dsr*ABD, dissimilatory sulfite reductase α, β, and a hypothetical δ-polypeptide; gNAc, *O*-linked *N*-acetylglucosaminyl tranferase; *cys*G, uroporphyrin-III C-methyltransferase; AF0426, conserved hypothetical protein. *Open arrows* indicate partial sequence; *arrowheads* indicate the direction of transcription. Accession number for *A. fulgidus*: AE0011075

A putative promoter-like boxA sequence, nearly identical to the one upstream from *dsr*AB in *A. fulgidus* (Dahl et al. 1993), was found 53 bp upstream of the anticipated starting codon of *dsr*A in *A. profundus* (Fig. 3a). As in *A. fulgidus* a possible transcription start sequence (boxB) was located 28 bp downstream of boxA in *A. profundus*. Transcription terminator-like sequences could not be identified between the *dsr*A and *dsr*B genes, but a hepta-thymine sequence was located between *dsr*B and *dsr*D as in *A. fulgidus* (Dahl et al. 1993). It is not known whether this sequence, or a thymine-rich stretch downstream of the stop codon of *dsr*D, is functioning as a transcriptional termination sequence in *Archaeoglobus*.

Very little is known about the promoter sequences from *Desulfotomaculum*. Comparison of promoter regions from pØLS18 with promoters from *Clostridium* (Graves and Rabinowitz 1986) did not reveal any promoter-like sequences. However, comparison of the upstream regions of *fdx*, *dsr*A, and gNAc identified a putative −10 promoter with the consensus sequence $^{A}/_{T}{}^{T}/_{A}$ATTT. This sequence was located 30 bp upstream of the anticipated start codon of *dsr*A in *D. thermocisternum*. No obvious termination-like sequences could be identified neither between *dsr*B and *dsr*D nor between *dsr*D and gNAc. Comparison of the *dsr*A and *dsr*B intergenic sequence from *A. fulgidus* has shown that *dsr*A and *dsr*B are translationally coupled with the stop codon of *dsr*A overlapping with the start codon of *dsr*B (Dahl et al. 1993). In *A. profundus*, *dsr*A and *dsr*B are separated by 11 bp, and thus in this respect the organization is more similar to the bacterial *dsr* operons (Fig. 3b).

(a)

BoxA BoxB rbs
A.f AAATTTCGAAATT**CTTATATAT**TGCCTAAAAAGAGGGGGAC**TTGC**AA.TTAC**AGGAGG**TGAACGA**ATG**
A.p TAATTTCGAAAT.**CTTATATAT**GGTTAATGGAGAGATAAAT**ACGA**ACCGTTTG**GGAGG**TGGTTAA**ATG**

(b)

LeuLysLysArgGlyMetTrp MetValValGlu
A.f: TTGAAGAAG**AGGGGG**ATGTGGTA**ATG**GTAGTTGAG

LeuGlnGlu MetAlaGluGlu
A.p: TTGCAGGAGTGA**GGTGG**TTTGTT**ATG**GCTGAGGAG

HisLysArg MetAlaLeuSer
D.t: CACAAGAGGTAAAATA**AGGG**GGTTTGTTGG**ATG**GCTCTTTCC

HisLeuArg MetAlaPheIle
D.v: CACCTGAGATAAGAAG**AGGG**GTGCAGAAAG**ATG**GCATTCATA

HisMetArg MetAlaPheIle
D.g: CACATGAGATAAGGGG**AGGA**GAGAAAAG**ATG**GCTTTCATT

HisLeuArg MetAlaPheIle
D.a: CACCTGAGATAAGGG**AGGG**AGAATAACCA**ATG**GCTTTTATA

Fig. 3. a Comparison of the promoter region of *dsr*AB in *Archaeoglobus*. Putative boxA, boxB, and ribosome-binding sequence (rbs), and start codons (ATG) are given in *bold*. Identical sequences outside BoxA and BoxB are *underlined*. **b** Comparison of the intergenic sequence between *dsr*A and *dsr*B in sulfate-reducing microorganisms. Stop codons are *underlined*. Putative rbs and start codons are given in *bold*. *A.f*, *A. fulgidus* (accession no. M95624); *A.p*, *A. profundus*; *D.t*, *D. thermocisternum*; *D.v*, *D. vulgaris* (accession no. U16723); *D.g*, *Desulfovibrio gigas* (accession. no. U80961); *D.a*, *Desulfobacterium autotrophicum* (accession no. Y15478)

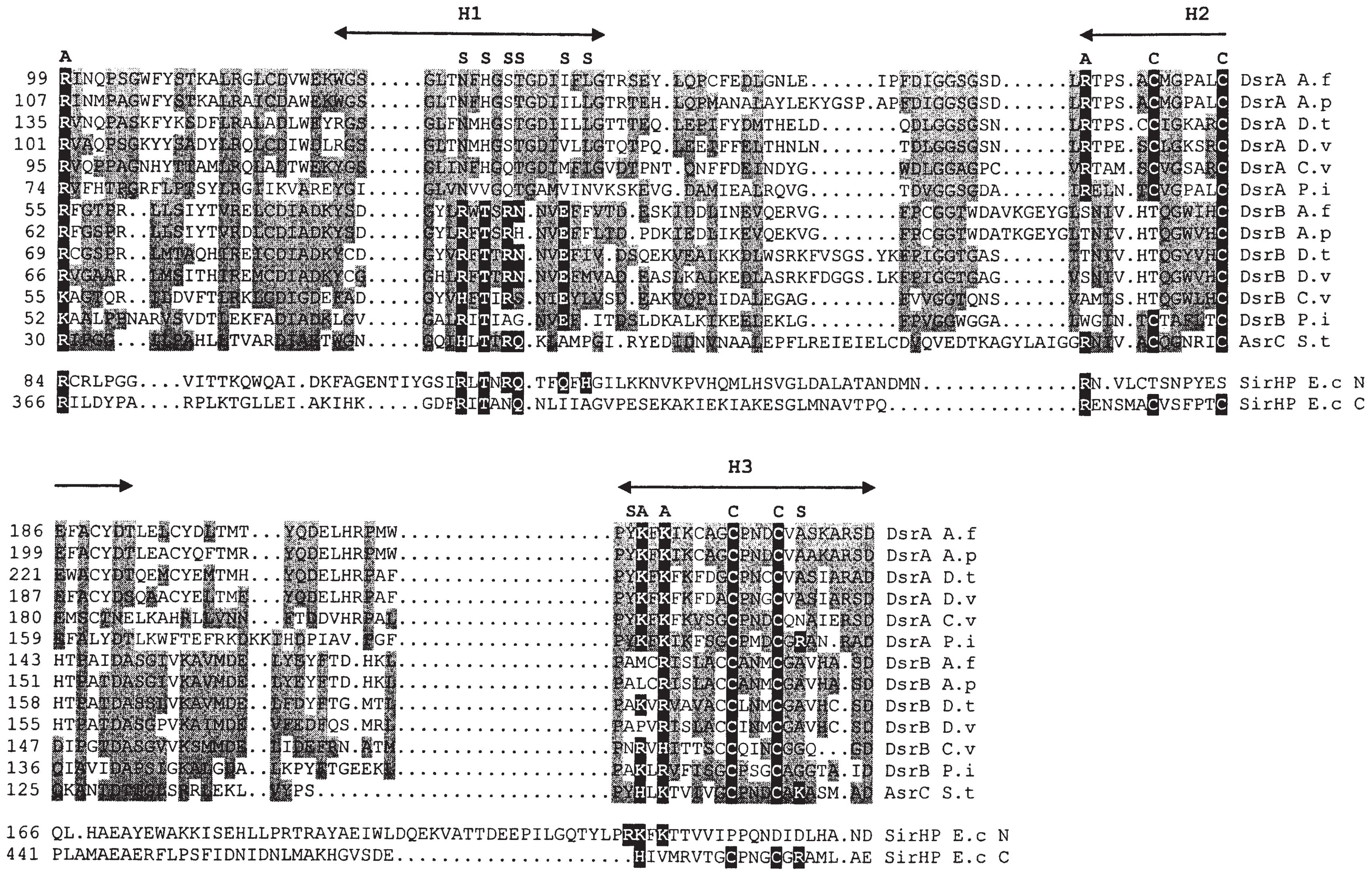
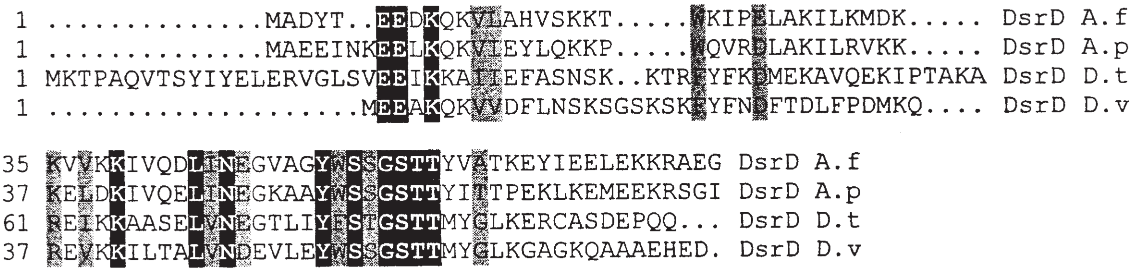


Fig. 4. Sequence comparison of the homology regions H1, H2, and H3, identified by Crane et al. (1995) and Crane and Getzoff (1996), for DsrA, DsrB, and AsrC. For comparison the assimilatory sulfite reductase hemoprotein subunit (SirHP) from *E. coli* is included. Residues similar to the residues in SirHP that interact with siroheme (*S*), the FeS-cluster (*C*), or bind the sulfite anion (*S*) are *boxed in black*. Other similar residues are grouped according to Dayhoff et al. (1978) and shaded in gray. *S.t*, *S. typhimurium* (Accession no. M57706); *E.c N*, N-terminal part of *E. coli* SirHP; *E.c C*, C-terminal part of *E. coli* SirHP (accession no. M23008)



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1  ..............MADYT..EEDKQKVLAHVSKKT.....WKIPELAKILKMDK..... DsrD A.f
1  ..............MAEEINKEELKQKVIEYLQKKP.....WQVRDLAKILRVKK..... DsrD A.p
1  MKTPAQVTSYIYELERVGLSVEEIKKAIIEFASNSK..KTRFYFKDMEKAVQEKIPTAKA DsrD D.t
1  ....................MEEAKQKVVDFLNSKSGSKSKFYFNDFTDLFPDMKQ.... DsrD D.v

35 KVVKKIVQDLINEGVAGYWSSGSTTYVATKEYIEELEKKRAEG DsrD A.f
37 KELDKIVQELINEGKAAYWSSGSTTYITTPEKLKEMEEKRSGI DsrD A.p
61 REIKKAASELVNEGTLIYFSTGSTTMYGLKERCASDEPQQ... DsrD D.t
37 REVKKILTALVNDEVLEYWSSGSTTMYGLKGAGKQAAAEHED. DsrD D.v
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Fig. 5. Sequence alignment of the *dsr*D-encoded polypeptide (DsrD). Identical residues are shaded in *black* and similar residues in *gray* according to Dayhoff et al. (1978)

Sequence comparison

Comparison of the DsrAB sequences from *A. profundus* and *D. thermocisternum* to other dissimilatory sulfite reductases revealed strong homology throughout the entire polypeptide. The high sequence similarity suggests that Dsr from *A. profundus* and *D. thermocisternum* will be a $\alpha_2\beta_2$-tetramer with two sirohemes and four [Fe_4S_4]-clusters as for Dsr from *A. fulgidus* and *D. vulgaris*.

So far no dissimilatory sulfite reductases have been crystallized, but the structure of the assimilatory sulfite reductase (SirHP) from *E. coli* has recently been determined (Crane et al. 1995). Although dissimilatory sulfite reductases show low overall sequence similarity with assimilatory sulfite reductases, alignments of Dsr and SirHP sequences indicate that residues involved in binding of siroheme, [Fe_4S_4]-cluster, and sulfite are conserved, suggesting a similar active site structure (Crane and Getzoff 1996). These residues are located in three homology regions termed H1, H2, and H3 (Fig. 4).

From the alignment in Fig. 4, the residues involved in cofactor and substrate binding in Dsr from *A. profundus* and *D. thermocisternum* could be putatively identified. The residues involved in sulfite binding (A) are totally conserved only in DsrA, indicating that sulfite is bound by the DsrA subunit. The same holds true for the siroheme-linked [Fe_4S_4]-cluster, where the residues involved in and surrounding the [Fe_4S_4]-cluster ($C\text{-}X_5\text{-}C\text{-}X_n\text{-}C\text{-}X_3\text{-}C$) is more conserved in DsrA than in DsrB. Thus, DsrA probably also binds the siroheme-[Fe_4S_4]-cluster. The amino acid residues involved in siroheme binding appear to be less conserved. It has been suggested by Crane et al. (1995) that one single Dsr subunit does not alone provide the necessary structural elements for binding of sulfite, [Fe_4S_4]-cluster, and siroheme, but that Dsr functions as an α/β-heterodimer. Our comparison of sulfite reductases shows that the siroheme-binding residues in the SirHP H1 region are conserved in the DsrB sequence, while corresponding residues in region H3 are not conserved. However, comparison of AsrC from *Salmonella typhimurium* and Dsr sequences identified a conserved tyrosine in the H3 region of DsrA, which might be important in siroheme binding. The analysis of the *A. profundus* and *D. thermocisternum* Dsr sequences supports previous suggestions that both subunits are needed for the formation of the active site. Thus, because DsrA binds sulfite and the [Fe_4S_4]-cluster, the binding of siroheme in the active center must involve interactions with amino acid residues from both the α- and β-subunits.

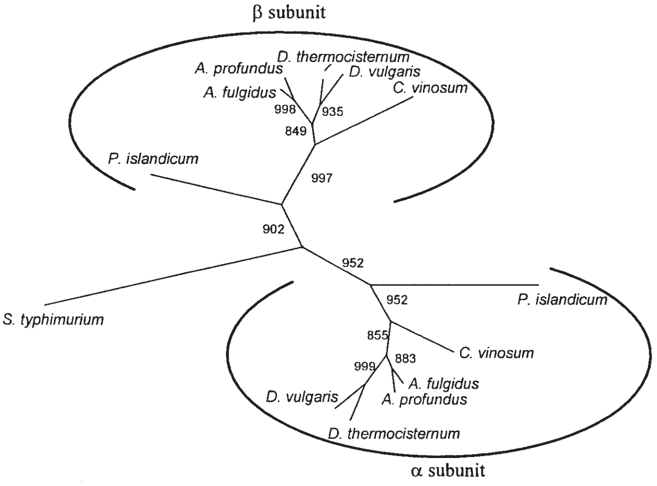

Fig. 6. An unrooted neighbor-joining tree of DsrA, DsrB, and AsrC. The alignment was constructed by CLUSTALW (Thompson et al. 1994) and visualized by TREEVIEW (Page 1996). Position with gaps were excluded and correction for multiple substitutions was made. The *numbers* between nodes are bootstrap values and refer to how often the topology shown occurred in 1000 trees tested

A multiple alignment of the four available DsrD sequences revealed several conserved regions (Fig. 5), especially an octa-peptide ($Y^W/_F S^S/_T GSTT$) at residues 52 to 60 (*A. fulgidus* numbering). A profile search using the programs POFILEMAKE and PROFILESEARCH, included in the GCG package, did not reveal other homologs of this polypeptide. DsrD has a high content of lysine residues, and it has been suggested that the function of the polypeptide is in binding or transport of sulfite (Karkhoff-Schweizer et al. 1995). This function is plausible because sulfite is highly toxic also to sulfate-reducing bacteria (Widdel and Bak 1992).

Phylogeny of dissimilatory sulfite reductase

The phylogenetic tree of dissimilatory sulfite reductases (Fig. 6) showed nearly a dyad symmetry centered around a trichotomy node branching into three clusters encompassing DsrA, DsrB, or the *S. typhimurium* AsrC sequences.

This implies that the α and β Dsr subunits are results of an early duplication of an ancestor *dsr* gene that must have taken place before the divergence of the bacterial and archaeal lineage and are thus paralogous. The Dsr sequences from the sulfate reducers are clearly separated into a bacterial and an archaeal cluster with a close relationship to each other. Dsr from the sulfur-respiring archaeon *Pyrobaculum islandicum* and the sulfide-oxidizing photographic bacterium *Chromatium vinosum* form separate branches in the DsrA and DsrB lineage. Dsr from *P. islandicum* appears to have branched quite early from the other sequences. The *S. typhimurium* AsrC sequence branches out about midway between the DsrA and DsrB clusters, and clearly represents an evolutionary line different from the one leading to Dsr from sulfate reducers. This branching pattern contradicts the 16S rRNA phylogeny, where the domain Archaea is monophyletic (Woese 1987), but resembles tree topologies observed from several other proteins such as V-ATPases and Hsp70 where Archaea groups together with Bacteria (Forterre 1997).

The strong sequence conservation among Dsr from sulfate reducers can be explained by a lateral gene transfer of *dsr* from bacterial to archaeal groups, which would also explain the limitation of sulfate respiration to a single archaeal genus and the placement of *Archaeoglobus* next to bacterial *dsr* rather than next to the archaeon *P. islandicum*. As previously noted, the main difference between *A. fulgidus* and *D. vulgaris dsr*AB codon usage is a strong bias in *A. fulgidus* toward AG(A/G) as arginine codon (38 of 39 codons), whereas *D. vulgaris* has a bias toward CG(T/C) (40 of 43 codons) (Dahl et al. 1993; Karkhoff-Schweizer et al. 1995). In *A. profundus*, the *dsr*AB arginine codons are exclusively AG(A/G) while *D. thermocisternum* has an intermediate frequency with 26% of the arginines in Dsr encoded by AG(G/A). This bias conforms well with the fact that the average arginine codon usage in the *A. fulgidus* genome is more than 90% AG(A/G) (http://www.dna.affrc.go.jp/nakamura/CUTG.html). Thus, codon usage frequencies indicate that dissimilatory sulfite reductase is an ancient enzyme in those genera of sulfate reducers from which it has been sequenced, as changes in codon usage are believed to be a slow process (Diaz-Lazcoz et al. 1995). It is more likely that the strong sequence conservation among sulfate reducers results from the specific function of this enzyme in the process of dissimilatory sulfate reduction, compared to the enzyme from the nonsulfate reducers *C. vinosum*, *P. islandicum*, and *S. typhimurium*. It has previously been suggested that the different sulfite reductase-type proteins might have originated before the divergence into Bacteria and Archaea (Hipp et al. 1997; Molitor et al. 1998), but additional Dsr-type proteins from other sulfate reducers and nonsulfate-reducers must be characterized before any rigorous conclusions about the evolutionary path of this protein family can be made.

Note added in proof. A phylogenetic comparison of sulfite reductases based on partial amino acid sequences has recently been published by Wagner et al. [Wagner M, Roger AJ, Flax JL, Brusseau GA, Stahl DA (1998) Phylogeny of dissimilatory sulfite reductases supports an early origin of sulfate respiration. J Bacteriol 180:2975–2982].

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