Editor's Choice

Sulfite oxidation in the purple sulfur bacterium *Allochromatium vinosum*: identification of SoeABC as a major player and relevance of SoxYZ in the process

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In phototrophic sulfur bacteria, sulfite is a well-established intermediate during reduced sulfur compound oxidation. Sulfite is generated in the cytoplasm by the reverse-acting dissimilatory sulfite reductase DsrAB. Many purple sulfur bacteria can even use externally available sulfite as a photosynthetic electron donor. Nevertheless, the exact mode of sulfite oxidation in these organisms is a long-standing enigma. Indirect oxidation in the cytoplasm via adenosine-5’-phosphosulfate (APS) catalysed by APS reductase and ATP sulfurylase is neither generally present nor essential. The inhibition of sulfite oxidation by tungstate in the model organism *Allochromatium vinosum* indicated the involvement of a molybdoenzyme, but homologues of the periplasmic molybdopterin-containing SorAB or SorT sulfite dehydrogenases are not encoded in genome-sequenced purple or green sulfur bacteria. However, genes for a membrane-bound polysulfide reductase-like iron–sulfur molybdoprotein (SoeABC) are universally present. The catalytic subunit of the protein is predicted to be oriented towards the cytoplasm. We compared the sulfide- and sulfite-oxidizing capabilities of *A. vinosum* WT with single mutants deficient in SoeABC or APS reductase and the respective double mutant, and were thus able to prove that SoeABC is the major sulfite-oxidizing enzyme in *A. vinosum* and probably also in other phototrophic sulfur bacteria. The genes also occur in a large number of chemotrophs, indicating a general importance of SoeABC for sulfite oxidation in the cytoplasm. Furthermore, we showed that the periplasmic sulfur substrate-binding protein SoxYZ is needed in parallel to the cytoplasmic enzymes for effective sulfite oxidation in *A. vinosum* and provided a model for the interplay between these systems despite their localization in different cellular compartments.

INTRODUCTION

Anoxygenic purple sulfur bacteria like the gammaproteobacterium *Allochromatium vinosum*, a member of the family Chromatiaceae, are able to derive energy from light by using reduced sulfur compounds as photosynthetic electron donors. The substrates of purple sulfur bacteria primarily include sulfide and elemental sulfur (Imhoff, 2005a). *A. vinosum* is a metabolically especially versatile purple sulfur bacterium that can also utilize thiosulfate and sulfite (Imhoff et al., 1998; Weissgerber et al., 2011). The first major process in the oxidation of thiosulfate and sulfide is the formation of intracellularly stored sulfur globules. The oxidation of thiosulfate to sulfate with sulfur globules as an intermediate involves the periplasmic Sox proteins SoxYZ, SoxB, SoxXAK and SoxL (Pott & Dahl, 1998; Hensen et al., 2006; Welte et al., 2009). For the oxidation of sulfide, *A. vinosum* possesses the genetic equipment for at least three different enzymes catalysing this reaction, namely the soluble periplasmic flavocytochrome c and the membrane-bound sulfide:quinone oxidoreductases SqrD and SqrF. The last two are both predicted to be oriented towards the periplasm (Reinartz et al., 1998; Gregersen et al., 2011; Weissgerber et al., 2011). In *A. vinosum*, the sulfur globules are deposited in the same cellular compartment as the periplasmic thiosulfate- and sulfide-oxidizing enzymes. This is evidenced by the
presence of signal peptide-encoding sequences in the genes for the three structural proteins constituting the sulfur globule envelope (Pattaragulwanit et al., 1998; Prange et al., 2004; Frigaard & Dahl, 2008). For further oxidation of stored sulfur, the so-called Dsr system is of essential importance (Pott & Dahl, 1998; Dahl et al., 2005; Lübbe et al., 2006; Sander et al., 2006). The product of the Dsr pathway is sulfite, generated by a reverse-acting dissipatory sulfite reductase (DsrAB), an enzyme that is well known to be located in the cytoplasm (Hipp et al., 1997; Pott & Dahl, 1998; Frigaard & Dahl, 2008).

The last step is the oxidation of sulfite, yielding sulfate as the final product. Sulfate formation from sulfite is energetically favourable and carried out by a wide range of organisms (Simon & Kroneck, 2013). Many sulfite-oxidizing enzymes are located outside the cytoplasmic membrane (in the periplasm in Gram-negative bacteria). The best-characterized enzyme belonging to this group, SorAB, stems from Starkeya novella and consists of a molybdopyranopterin cofactor-carrying subunit (SorA) and a monohaem cytochrome c (SorB) (Kappler et al., 2000; Kappler & Bailey, 2005). SorA-type molybdoproteins without a SorB subunit have been termed SorF (D’Errico et al., 2006; Wilson & Kappler, 2009), but recently this discrimination has been questioned (Simon & Kroneck, 2013). A second option for oxidation of sulfite in the periplasm is the Sox system. It has been shown that sulfite is accepted in vitro as a substrate of the reconstituted Sox system from the chemotroph Paracoccus denitrificans (Friedrich et al., 2001). Notably, Friedrich and coworkers proved this reaction to be independent of the presence of SoxCD, a molybdoaemoprotein catalysing the six-electron oxidation of SoxY-cysteine-bound persulfide to sulfone sulfur. Purple bacteria that form sulfur globules during thiosulfate oxidation contain the Sox system, albeit without the SoxCD proteins (Hensen et al., 2006; Meyer et al., 2007; Frigaard & Dahl, 2008).

Oxidation of sulfite generated by the cytoplasmic Dsr proteins via the periplasmic pathways described would first require transport across the cytoplasmic membrane. However, such transport is probably not necessary in organisms that contain a further well-characterized sulfite oxidation pathway: it is firmly established that a number of purple as well as green anoxygenic phototrophic sulfur bacteria oxidize sulfite in the cytoplasm using an indirect pathway via adenosine-5’-phosphosulfate (APS) catalysed by APS reductase and ATP sulfurylase (Dahl, 1996; Sánchez et al., 2001; Frigaard & Dahl, 2008; Rodriguez et al., 2011). The electrons generated by the oxidative formation of APS from sulfite and AMP are fed into the photosynthetic electron transport chain at the level of menaquinone either by AprM or by the much better characterized QmoABC/QmoABHdrCB complex (Meyer & Kuever, 2007; Rodriguez et al., 2011; Ramos et al., 2012; Grein et al., 2013). Notably, however, neither is the APS reductase pathway generally present in purple sulfur bacteria nor is it essential in A. vinosum (Dahl, 1996; Sánchez et al., 2001).

Recently, involvement of a putative heterotrimeric membrane-bound complex named SoeABC (sulfite-oxidizing enzyme) in sulfite oxidation in the cytoplasm of the organosulfonate-degrading chemotrophic alphaproteobacterium Ruegeria pomeroyi has been reported, although not rigorously documented, by Lehmann et al. (2012). The SoeABC protein appears to be a member of the complex iron–sulfur molybdoproteins and consists of an NrfD/PsrC-like membrane anchor (SoeC) and two cytoplasmic subunits: an iron–sulfur protein (SoeB) and a molybdoprotein with an N-terminal iron–sulfur cluster binding site (SoeA). It should be noted that SoeABC and the periplasmic Sor-type sulfite dehydrogenases belong to completely different families of molybdoenzymes. SorA and relatives belong to the sulfite oxidase proteins, which bind a single molybdopterin without a second nucleotide. SoeA falls into the molybdopterin-binding MopB superfamily. In many characterized members of this family molybdopterin is present in the form of a dinucleotide, with two molybdopterin dinucleotide units per molybdenum. Similarities are obvious within each molybdoenzyme family sequence, whereas no significant homologies can be detected between members of different families (Kisker et al., 1998). Genes encoding proteins related to SoeABC are present in purple as well as green sulfur bacteria, and in the past years have repeatedly been speculated to be involved in the oxidation of sulfite generated by the Dsr system in the cytoplasm (Frigaard & Bryant, 2008; Frigaard & Dahl, 2008). Notably, soeABC-like genes co-localize with dsr genes not only in the metagenome-derived sequence of another bacterium that belongs to the Roseobacter clade like R. pomeroyi (Lenk et al., 2012), but also in several green sulfur bacteria and in Halorhodospira halophila, a purple sulfur bacterium of the family Ectothiorhodospiraceae (Dahl, 2008; Frigaard & Dahl, 2008). Independent experimental evidence for the involvement of a molybdoprotein was obtained by inhibition of sulfite oxidation in A. vinosum with the molybdate-antagonist tungstate (Dahl, 1996).

In this work, we aimed to obtain a clearer picture of the proteins involved in the oxidation of sulfite in purple sulfur bacteria and chose A. vinosum as the model organism. An array of strains carrying single and multiple deletions of relevant genes was studied with regard to the oxidation of externally supplied and internally generated sulfite. Thereby, we identified AvSoeABC (Alvin_2491-2489) as a major player in the oxidation of sulfite. In addition, we present evidence that SoxyZ is also essential in sulfite oxidation, whilst the other Sox proteins are dispensable for the process.

**METHODS**

**Bacterial strains, plasmids, PCR primers and growth conditions.** The bacterial strains, plasmids and primers used in this study are listed in Table 1. A. vinosum strains were cultivated photo-organoheterotrophically in RCV medium (Weaver et al., 1975) or photolithoheterotrophically in Pfenig medium (Pfenig & Trüper,
Table 1. Bacterial strains and plasmids used in this study

<table>
<thead>
<tr>
<th>Strain or plasmid</th>
<th>Genotype or phenotype</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em> strain</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S17-1</td>
<td>294 (<em>recA thi pro hsdR M−</em>') <em>Tp</em> <em>Sm</em> [RP4-2-Tc::Mu-Km::Tn7]</td>
<td>Simon et al. (1983)</td>
</tr>
<tr>
<td><em>Allochromatium vinosum</em> strains</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rif50</td>
<td>Rif*, spontaneous rifampicin-resistant mutant of DSM 180T</td>
<td>Lübke et al. (2006)</td>
</tr>
<tr>
<td>SM50</td>
<td>Sm*, spontaneous streptomycin-resistant mutant of DSM 180T</td>
<td>Dahl (1996)</td>
</tr>
<tr>
<td>soxX::Km</td>
<td>Km*, soxX::Km in DSM 180T</td>
<td>Hensen et al. (2006)</td>
</tr>
<tr>
<td>soxXO::Km</td>
<td>Km*, soxX::Km in DSM 180T</td>
<td>Hensen et al. (2006)</td>
</tr>
<tr>
<td>ΔsoxY</td>
<td>Rif*, in-frame deletion of soxY in Rif50</td>
<td>Hensen et al. (2006)</td>
</tr>
<tr>
<td>ΔsoxY+ YZ</td>
<td>Rif*, Km*, complementation of ΔsoxY</td>
<td>Hensen et al. (2006)</td>
</tr>
<tr>
<td>ΔsoxY ΔsoeAEEm</td>
<td>Rif′ Em′, ΔsoeA Em 51 nt upstream of soeA ATG in ΔsoxY</td>
<td>This work</td>
</tr>
<tr>
<td>aprB::ΔKm</td>
<td>Km*, aprB::ΔKm in SM50</td>
<td>Dahl (1996)</td>
</tr>
<tr>
<td>ΔsoxY aprB::ΔKm</td>
<td>Km*, Rif′, aprB::ΔKm in ΔsoxY</td>
<td>This work</td>
</tr>
<tr>
<td>ΔsoeAEEm</td>
<td>Em′ Rif′, ΔsoeA Em 51 nt upstream of soeA ATG in Rif50</td>
<td>This work</td>
</tr>
<tr>
<td>aprB:: ΔKm ΔsoeAEEm</td>
<td>Km′ Em′, Sm′, ΔsoeA Em 51 nt upstream of soeA ATG in aprB::ΔKm</td>
<td>This work</td>
</tr>
<tr>
<td>ΔsoxY aprB::ΔKm ΔsoeAEEm</td>
<td>Km′ Em′ Rif′, Em′ 51 nt upstream of soeA ATG in ΔsoxY aprB::ΔKm</td>
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<tr>
<td>Plasmids</td>
<td></td>
<td></td>
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<tr>
<td>pBBR1-MCS2</td>
<td>Km′ Mob−, rep lacZz</td>
<td>Kovach et al. (1995)</td>
</tr>
<tr>
<td>pAsox4 + YZ</td>
<td>Km′, 1.5 kb PCR fragment of soxYZ (XbaI) in XbaI of pBBR1-MCS2</td>
<td>Hensen et al. (2006)</td>
</tr>
<tr>
<td>pNTS35</td>
<td>Km′ cartrige (EcoRI) from pHP452Km in PvuII within aprB</td>
<td>Dahl (1996)</td>
</tr>
<tr>
<td>pK18mobsacB</td>
<td>Km′ Mob−, sacB oriV oriT lacZz</td>
<td>Schäfer et al. (1994)</td>
</tr>
<tr>
<td>pK18mobsacB ΔsoeA</td>
<td>Km′ HindIII fragment of PCR-amplified genome region around soeA (Alvin_2491) with deletion of 2864 bp of the soeA sequence</td>
<td>This work</td>
</tr>
<tr>
<td>pK18mobsacB ΔsoeAEEm</td>
<td>Km′ Em′, 1.1 kb blunt-ended HindIII–EcoRI fragment from pUC19Ery in SphI of pK18mobsacBΔsoeA</td>
<td>This work</td>
</tr>
<tr>
<td>pUC19Ery</td>
<td>Ap′ Em′, from pE194 in Smal of pUC19</td>
<td>Leenhouts et al. (1990)</td>
</tr>
<tr>
<td>pSUP301</td>
<td>Ap′ Km′ Mob+</td>
<td>Simon et al. (1983)</td>
</tr>
<tr>
<td>pSUP301 ΔsoeAEEm</td>
<td>Ap′ Em′, 2.9 kb HindIII fragment from pK18mobsacB ΔsoeAEEm in HindIII of pSUP301</td>
<td>This work</td>
</tr>
<tr>
<td>Primers</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Avin2491fw1</td>
<td>GGCggggGAGCttTtCCGAGATgATGCGGCT</td>
<td>This work</td>
</tr>
<tr>
<td>Avin2491rev1</td>
<td>GGCggggGAGCttTtCCGAGATgATGCGGCT</td>
<td>This work</td>
</tr>
<tr>
<td>Avin2491fw2</td>
<td>CAGGATCCAGCCATGATCCGGGAGCAA</td>
<td>This work</td>
</tr>
<tr>
<td>Avin2491rev2</td>
<td>GCGAACAGCCTCGACCGCATAGAAC</td>
<td>This work</td>
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</tbody>
</table>

Plasmid DNA from *E. coli* was purified using the QIAprep Spin Miniprep Kit (Qiagen).

**Construction of A. vinosum single-, double- and triple-mutant strains.** For the substitution of soeA (Alvin_2491) in the genome of *A. vinosum*, as an erythromycin cassette, SOE PCR (Horton, 1995) fragments were constructed using primer pairs Avin2491fw1/Avin2491rev1 and Avin2491fw2/Avin2491rev2 (Table 1). The resulting fragment was inserted into plasmid pK18mobsacB (Schäfer et al., 1994) by HindIII restriction sites resulting in plasmid pK18mobsacB ΔsoeA. After digestion with HindIII and EcoRI the erythromycin cassette from pUC19Ery was blunt-ended using Klenow polymerase and ligated into the SphI site of pK18mobsacB ΔsoeA, resulting in pK18mobsacB ΔsoeAEEm. The *Sph* site is situated 51 nt upstream of the *soeA* ATG start codon and 110 nt downstream of the TGA stop codon of the preceding gene Alvin_2492. Thus, it was guaranteed that expression of Alvin_2492 was not affected by insertion of the antibiotic resistance cassette. The 2.9 kb HindIII fragment of pK18mobsacB ΔsoeAEEm was then inserted at the HindIII site of pSUP301. The final mobilizable construct pSUP301 ΔsoeAEEm was transferred from *E. coli* S17-1 to *A. vinosum* strains Rif50, aprB::ΔKm, ΔsoxY and ΔsoxY aprB::ΔKm by conjugation (Pattaraguwanit & Dahl, 1995). For the insertional inactivation of

1992) without reduced sulfur compound, referred to as ‘0 medium’. Sulfide or sulfite was added as electron source at the desired concentration. The cultivation was performed under anaerobic conditions and continuous illumination at 30°C either in completely filled screw-capped culture bottles or in thermostatted glass fermenters. *Escherichia coli* was cultivated in LB medium (Sambrook et al., 1989). Antibiotics used for mutant selection were applied at the following concentrations: for *E. coli*: ampicillin 100 μg ml−1, kanamycin 50 μg ml−1, erythromycin 100 μg ml−1, chloramphenicol 50 μg ml−1; for *A. vinosum*: rifampicin 50 μg ml−1, streptomycin 50 μg ml−1, ampicillin 10 μg ml−1, kanamycin 10–25 μg ml−1, erythromycin 10 μg ml−1.

**Recombinant DNA techniques.** Standard methods were used for molecular biological techniques. Chromosomal DNA of *A. vinosum* strains was obtained by a modified sarcosyl lysis (Bazael & Helinski, 1968). The genotypes of the *A. vinosum* recombinants used in this study were confirmed by Southern hybridization and PCR. The latter is the only method of choice to confirm complementation strains. Southern hybridization was performed overnight at 68°C. PCR amplifications with *Taq* DNA polymerase and *Pfu* DNA polymerase were done essentially as described previously (Dahl, 1996). DNA probes for Southern hybridization were digoxigenin labelled by PCR.
apR, plasmid pNT535 (Dahl, 1996) was conjugationally transferred from *E. coli* S17-1 to *A. vinosum* strain *AsoY*. Transconjugants were selected on RCV plates containing the appropriate antibiotics under anoxic conditions in the light. Double cross-over recombinants lost the vector-encoded ampicillin resistance. The genotype of double cross-over recombinants was verified by Southern hybridization experiments.

**Characterization of phenotypes, detection of sulfur species and protein determination.** *A. vinosum* WT and mutant strains were characterized in batch culture experiments essentially as described previously (Prange et al., 2004; Hensen et al., 2006). Cells of *A. vinosum*, grown photoheterotrophically on malate [RCV medium (Weaver et al., 1975)] for 3 days were used as an inoculum for experiments concerned with transformation of sulfide and sulfite. The culture volume of the precultures was 500 ml. Inoculum cells for experiments concerned with transformation of sulfide and sulfite were harvested by centrifugation (10 min, 2680 *g*). The culture volume for experiments concerned with transformation of sulfide and sulfite was 250 ml. Experiments in '0 medium' (Weaver et al., 1975) for 3 days were used as an inoculum and mutant strains on 5 mM sulfite was 250 ml. Experiments in '0 medium'. The culture volume for phenotypic analyses of WT and mutant strains on 5 mM sulfite was 250 ml. Experiments concerned with transformation of sulfide and sulfite were performed in 1.5 l fermenters. To maintain pH 7.0, sterile HCI (0.5 M) and Na₂CO₃ (0.5 M) solutions were added automatically.

Sulfide, thiosulfate and sulfate were determined either by HPLC (Rethmeier et al., 1997), or by classical colorimetric or turbidimetric methods as described previously (Dahl, 1996). Elemental sulfur and tetrathionate were determined colorimetrically by cyanolysis (Bartlett & Skoog, 1954; Kelly et al., 1969; Dahl, 1996). Sulfite was determined via the fuchsin method as described by Dahl (1996). Protein concentrations were determined using the Bradford reagent (Sigma) as specified by the manufacturer.

**RESULTS**

**Occurrence of sulfite oxidation-related genes in genome-sequenced purple sulfur bacteria**

A survey of all genome sequences available for a physiologically coherent group of bacteria can provide revealing insights into pathways of general, major or possibly only minor importance. We therefore searched the currently completely sequenced phototrophic members of the families *Chromatiaceae* and *Ectothiorhodospiraceae* for the presence of sulfite oxidation-related genes. Table 2 provides a compilation of our results. From these findings, it is evident that periplasmic sulfite-oxidizing systems are not present universally in purple sulfur bacteria. Whilst the sulfur substrate-binding protein SoxYZ is present irrespective of the organisms’ substrate range, with only one exception (*Thioflaviovoccus mobilis*), the presence of SoxXA(K) and SoxB appears to be strictly linked to the ability of the cell systems to utilize thiosulfate (Table 2). Nevertheless, participation of the Sox system in sulfite oxidation in those strains that contain Sox proteins is not completely excluded. Furthermore, our survey showed that only a single purple sulfur bacterium, *T. mobilis*, contains a sora-like gene (Table 2), implying that the encoded enzyme is of very restricted importance in this organism group. The cytoplasmic APS reductase pathway occurs in several, although not all, purple sulfur bacteria. The electron-accepting unit for AprBA appears to be AprM in most cases, but is replaced by QmoABHDrBC in *Thiocystis violascens* (Table 2). The only probable sulfite-oxidizing unit that is encoded in all sequenced purple sulfur bacteria is SoeABC (Table 2), pointing to a general and major importance of this membrane-bound complex iron–sulfur molybdoprotein.

In *A. vinosum*, SoeABC is encoded by genes Alvin_2491 (soeA), Alvin_2490 (soeB) and Alvin_2489 (soeC). The protein consists of the 108.95 kDa molybdoprotein SoeA carrying one [Fe₄S₄] cluster at the N-terminus, the 26.995 kDa iron–sulfur protein SoeB, which upon comparison with related structurally characterized proteins (Jormakka et al., 2008) is predicted to bind four [Fe₄S₄] clusters, and a 35.715 kDa NrfD/PsrC-like membrane protein (Simon & Kern, 2008) with eight transmembrane helices. Neither AvSoeA and AvSoeB nor any of the other purple sulfur bacterial SoeA or SoeB proteins listed in Table 2 are synthesized with cleavable TAT signal peptides that are usually present on the active-site subunits of the biochemically well-characterized periplasmic sulfur-metabolizing complex iron–sulfur molybdoproteins, i.e. polysulfide and sulfur reductases (PsrABC, SreABC), thiosulfate reductase (PhsABC) or tetrathionate reductase (TtrABC) (Krafft et al., 1992; Heinzinger et al., 1995; Hensel et al., 1999; Laska et al., 2003). We can thus state conservatively that SoeA and SoeB are located in the cytoplasm and attached to the cytoplasmic membrane by interaction with SoeC. The holoprotein would therefore be well suited for oxidation of sulfite generated in the cytoplasm.

Further indication for an involvement of SoeABC in dissimilatory sulfur oxidation in *A. vinosum* was gathered during recent genome-wide transcriptional profiling (Weissgerber et al., 2013). Relative transcription of all three *A. vinosum* soe genes was found to be increased ~3-fold during photolithoautotrophic growth on sulfide or thiosulfate compared to photo organoheterotrophic growth on malate (2.99-, 2.77- and 2.93-fold increase on sulfide and 1.96, 1.98 and 3.00-fold increase on thiosulfate, for soeA, -B and -C, respectively). Changes in the same range were observed for the genes encoding the enzymes of the APS reductase pathway when thiosulfate replaced malate, whilst relative transcript levels for the sat-aprMBA genes were 7.6- to 9.7-fold higher in the presence of sulfide compared with the presence of malate.

**Oxidation of externally supplied sulfite by *A. vinosum*: role of SoeABC and APS reductase**

To assess the importance of the SoeABC protein in *A. vinosum*, a mutant strain (*A. vinosum ΔsoeAEm*) was constructed that carries a deletion of the soeA gene and an insertion of an erythromycin resistance cassette. The latter was positioned immediately upstream of the original soeA gene, but well downstream of the stop codon of the preceding gene Alvin_2492, thereby ensuring that expression of Alvin_2492 was not affected. The same ΔsoeAEm mutation was also introduced into an *A. vinosum* strain already lacking APS reductase (Dahl, 1996). Previously, we
Table 2. Proteins related to sulfite oxidation encoded in genome-sequenced purple sulfur bacteria

With the exception of *Thiorhodospira sibirica* all organisms listed in this table contain a complete set of *dsr* genes (*dsrABEFHCMKLJOPN*). The *soxCD* genes are not listed here because they do not occur in any of the sequenced anoxygenic phototrophic bacteria. The absence of signal peptides was confirmed for all SoeA and SoeB homologues listed here. The proteins are thus predicted to be cytoplasmic.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Sulfur substrate</th>
<th>Sat</th>
<th>AprBA</th>
<th>AprM</th>
<th>Qmo</th>
<th>SorA</th>
<th>SoeABC</th>
<th>SoxXAK</th>
<th>SoxB</th>
<th>SoxYZ</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Allochromatium</em> vinosum DSM 180&lt;sup&gt;T&lt;/sup&gt;</td>
<td>Sulfide, S&lt;sub&gt;0&lt;/sub&gt;, thiosulfate, sulfite</td>
<td>Alvin_1118</td>
<td>Alvin_1120-1121</td>
<td>Alvin_1119</td>
<td>–</td>
<td>–</td>
<td>Alvin_2491-2499</td>
<td>Alvin_2168-2170</td>
<td>Alvin_2167</td>
<td>Alvin_2111-2112</td>
</tr>
<tr>
<td><em>Thiorhodovibrio</em> sp. 970</td>
<td>Sulfide, S&lt;sub&gt;0&lt;/sub&gt; (thiosulfate and sulfite not reported)</td>
<td>Thio70DRAFT_00961</td>
<td>Thio70DRAFT_00963-00964</td>
<td>Thio70DRAFT_00962</td>
<td>–</td>
<td>–</td>
<td>Thio70DRAFT_00955-00957</td>
<td>–</td>
<td>–</td>
<td>Thio70DRAFT_01660-01661</td>
</tr>
<tr>
<td><em>Thiosphaera marinea</em> 5811 (DSM 5653&lt;sup&gt;T&lt;/sup&gt;)</td>
<td>Sulfide, S&lt;sub&gt;0&lt;/sub&gt;, thiosulfate, sulfite</td>
<td>ThimaDRAFT_1689</td>
<td>ThimaDRAFT_4550</td>
<td>ThimaDRAFT_4551-4552</td>
<td>–</td>
<td>–</td>
<td>ThimaDRAFT_0331-0329</td>
<td>ThimaDRAFT_4576-4576</td>
<td>ThimaDRAFT_4579</td>
<td>ThimaDRAFT_0728-0729</td>
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<tr>
<td><em>Thiocystis violascens</em> DSM 198&lt;sup&gt;T&lt;/sup&gt;</td>
<td>Sulfide, S&lt;sub&gt;0&lt;/sub&gt;, thiosulfate, sulfite</td>
<td>Thivi_0893</td>
<td>Thivi_3300-3299</td>
<td>–</td>
<td>Thivi_3114-3111</td>
<td>–</td>
<td>Thivi_4351-4353</td>
<td>Thivi_3804-3802</td>
<td>Thivi_2200</td>
<td>Thivi_3138-3139</td>
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<tr>
<td><em>Thiorhodovibrio densus</em> AZ1 (DSM 15006&lt;sup&gt;T&lt;/sup&gt;)</td>
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<td>ThidrDRAFT_1494</td>
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<td>–</td>
<td>–</td>
<td>ThidrDRAFT_2883-2881</td>
<td>ThidrDRAFT_2416-2418</td>
<td>ThidrDRAFT_2534-2535</td>
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</tr>
<tr>
<td><em>Thioflavicoccus mobilis</em> DSM 8321&lt;sup&gt;T&lt;/sup&gt;</td>
<td>Sulfide, S&lt;sub&gt;0&lt;/sub&gt; (sulfite not tested)</td>
<td>Thimo_1948</td>
<td>Thimo_1220-1219</td>
<td>Thimo_1221</td>
<td>–</td>
<td>–</td>
<td>Thimo_2977</td>
<td>Thimo_1580-1582</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><em>Marichromatium purpuratum</em> 984 (DSM 1591&lt;sup&gt;T&lt;/sup&gt;)</td>
<td>Sulfide, S&lt;sub&gt;0&lt;/sub&gt;, thiosulfate (sulfite not tested)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><em>Thiorhodospira sibirica</em> ATCC 700588&lt;sup&gt;T&lt;/sup&gt;</td>
<td>Sulfide, S&lt;sub&gt;0&lt;/sub&gt; (sulfite not tested)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>ThissiDRAFT_1377, ThissiDRAFT_0834, ThissiDRAFT_2148</td>
<td>–</td>
<td>–</td>
<td>ThissiDRAFT_0337-0336</td>
</tr>
<tr>
<td><em>Halorhodospira halophila</em> SL1 (DSM 244&lt;sup&gt;T&lt;/sup&gt;)</td>
<td>Sulfide, thiosulfate</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>Hhal_1936-1934</td>
<td>Hhal_1948 (fused SoxX), no SoxK</td>
<td>Hhal_1939</td>
</tr>
</tbody>
</table>

The genes for the following biochemically well characterized proteins served as baits: *aprMBA* and *sat* genes from *A. vinosum* and *qmoABC* from *Desulfovibrio desulfuricans* for the APS reductase pathway, *soxYZ*, *soxXAK* and *soxL* from *A. vinosum* and *soxCD* from *Paracoccus pantotrophus* for the Sox pathway, *sorAB* from *S. novella* and *soeABC* from *R. pomeroyi*. In the case of the *soeABC* genes, a function for the encoded proteins has been reported but not rigorously documented (Lehmann et al., 2012). References for substrate ranges: Bryantseva et al. (1999), Zaar et al. (2003), Caumette et al. (2004) and Imhoff (2005a, b).
reported that crude extracts of WT *A. vinosum* catalysed sulfite-dependent ferricyanide reduction in the absence of AMP (Dahl, 1996). This activity was no longer detectable in the *A. vinosum ΔsoeAE* mutant strain. We concluded therefore that the protein encoded by the *soeABC* genes is identical with the AMP-independent sulfite-oxidizing entity in *A. vinosum*.

In a first set of experiments, we studied the phenotypes of *A. vinosum* WT, the APS reductase and *SoeABC*-deficient single-mutant strains as well as that of the corresponding double mutant regarding the oxidation of externally supplied sulfite under anoxic conditions in the light. The experiments were performed in batch culture in 250 ml bottles that were completely filled with medium containing 5 mM sulfite as the only source of sulfur and electrons. The *A. vinosum* strain lacking only APS reductase exhibited a phenotype clearly discernible from that of the WT, i.e. sulfite was oxidized with a significantly lower rate (Table 3). This finding is in agreement with results reported by Sánchez et al. (2001) who compared sulfite oxidation by *A. vinosum* WT and the APS reductase-deficient strain in continuous culture. While Sánchez et al. (2001) observed only small differences between WT and APS reductase-deficient strains at light-limiting irradiances, the presence of the APS reductase pathway was clearly advantageous for *A. vinosum* at high irradiances. This finding explains why initial batch culture experiments performed in 1.5 l culture vessels allowing only limited light penetration to the centre of the culture failed to demonstrate an effect of APS reductase deficiency (Dahl, 1996).

As evident from Table 3, the *SoeABC*-deficient *A. vinosum* mutant exhibited an even more apparent phenotype with regard to the oxidation of externally added sulfite than the mutant solely lacking APS reductase. The specific sulfite oxidation rate of the former amounted to only 17 ± 10 % of the WT (Table 3). This finding is again in full agreement with Sánchez et al. (2001), who reported that under all the conditions tested an APS reductase-independent pathway was responsible for most of the sulfur flow to sulfate (69–90 %) in *A. vinosum*. A knockout of both *aprB* and *soeA* led to an additive effect with a residual specific rate of sulfite oxidation for whole cells of only ~7 % of the WT (Table 3).

### Table 3. Sulfite oxidation rates in *A. vinosum* WT and mutant strains lacking functional APS reductase, *SoeABC*, *SoxYZ* or combinations thereof

<table>
<thead>
<tr>
<th>Strain</th>
<th>Sulfite oxidation rate [nmol min⁻¹ (mg protein)⁻¹]</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>64.6 ± 4.6</td>
</tr>
<tr>
<td><em>aprB::ΩKm</em></td>
<td>26.0 ± 8.2</td>
</tr>
<tr>
<td>ΔsoeAEEm</td>
<td>10.9 ± 8.0</td>
</tr>
<tr>
<td><em>aprB::ΩKm ΔsoeAEEm</em></td>
<td>4.5 ± 0.5</td>
</tr>
<tr>
<td>ΔsoxY</td>
<td>5.2 ± 0.4</td>
</tr>
<tr>
<td>ΔsoxY <em>aprB::ΩKm</em></td>
<td>6.4 ± 1.2</td>
</tr>
<tr>
<td>ΔsoxY ΔsoeAEEm</td>
<td>6.5 ± 0.6</td>
</tr>
<tr>
<td>ΔsoxY <em>aprB::ΩKm ΔsoeAEEm</em></td>
<td>4.0 ± 1.2</td>
</tr>
</tbody>
</table>

Initial sulfite concentration: 5 mM. Experiments were performed in completely filled 250 ml culture bottles. A background rate of chemical sulfite oxidation was determined for medium incubated under the same experimental conditions (0.33 nmol min⁻¹ ml⁻¹) and has already been deducted from the rates given. Initial protein concentration: 56–134 μg ml⁻¹.

### Oxidation of externally supplied sulfite by *A. vinosum*: role of Sox proteins

As already outlined in the Introduction, it has been demonstrated convincingly that sulfite is accepted in vitro as a substrate of the reconstituted Sox system from the chemotroph *P. denitrificans* (Rother et al., 2001). In this case, the reaction cycle has been proposed to be simpler than that needed for oxidation of thiosulfate. The cycle would start with SoxXA-catalysed oxidative coupling of sulfite to the cysteine in the C-terminal SoxX ‘GGCGG’ motif followed by a SoxB-mediated hydrolysis reaction releasing sulfate (Sauvé et al., 2007; Kappler & Maher, 2013). An involvement of the periplasmic Sox proteins in the oxidation of sulfite in purple sulfur bacteria is thus not a priori excluded, especially where the oxidation of external sulfite is concerned. We therefore conducted a series of experiments in which we studied the effect of a deficiency in various Sox proteins on sulfite oxidation in *A. vinosum*. The assumption that coordinated action of the *A. vinosum* Sox proteins is necessary for their in vivo function originally led us to the conclusion that, if any phenotype of Sox protein deficiency was detectable, it should be the same or at least very similar, irrespective of which specific Sox protein or which combination thereof is removed. However, surprisingly this was not the case. While both an *A. vinosum* mutant lacking functional SoxXAK and a mutant additionally lacking SoxB behaved virtually indiscernibly from the WT (Table S1, available in Microbiology Online), the ΔsoxY mutant showed a significant decrease in sulfite oxidation rate (Tables 3 and S1). It should be emphasized that the experiments compiled in Tables 3 and S1 were not performed under exactly the same experimental conditions. The experiments summarized in Table S1 were run in 1.5 l culture vessels and thus light intensities in the centre of the cultures were lower than in the 250 ml cultures used for the experiments compiled in Table 3. It is evident that the specific sulfite oxidation rates for WT *A. vinosum* are higher at higher irradiances [64.6 ± 4.6 versus 36.2 ± 1.6 nmol min⁻¹ (mg protein)⁻¹ in 250 ml and 1.5 l cultures, respectively]. The lack of a protein important for sulfite oxidation (SoxYZ in this case) has a more drastic effect under high irradiances, just as has already been found for APS reductase (Sánchez et al., 2001). Reintroduction of *soxYZ* into the ΔsoxY-deficient mutant (strain *A. vinosum ΔsoxY +YZ*) resulted in complete restoration of the WT.
phenotype with regard to oxidation of externally supplied sulfite (Table S1). These findings suggested the periplasmic sulfur substrate-binding protein SoxYZ as another important player in sulfite metabolism in *A. vinosum*, whilst SoxXAK and SoxB do not appear to play a significant role in this process.

**Oxidation of externally supplied sulfite by *A. vinosum*: connection of cytoplasmic systems and SoxYZ**

To further dissect the importance of SoxYZ in relation to the cytoplasmic sulfite-oxidizing systems we constructed *A. vinosum* mutants lacking SoxYZ as well as SoeABC and/or APS reductase. It now appeared that the specific sulfite oxidation rates of neither the double nor the triple mutants were significantly different from that of the ΔsoxY single mutant (Table 3). These observations suggest that SoxYZ is needed for oxidation of externally added sulfite in *A. vinosum* even in the presence of both cytoplasmic sulfite-oxidizing systems. The requirement of SoxYZ is not absolute, though, because we found conditions under which the SoxYZ-deficient mutant still exhibited a sulfite oxidation rate well above background (Table S1).

**Oxidation of sulfide in *A. vinosum* strains deficient in SoeABC, APS reductase and/or SoxYZ**

We now set out to define the roles of the newly detected cytoplasmically oriented membrane-bound molybdenum cofactor-containing SoeABC complex as well as the periplasmic SoxYZ protein in the very complex transformation of sulfur compounds occurring during oxidation of sulfide to sulfate. To this end, a suitable set of *A. vinosum* mutant strains was studied in 1.5 l fermenter cultures under pH control. This experimental design guaranteed full comparability of results obtained for this multi-step process and enabled sampling for various different sulfur compounds at ample time points. We could thus lay a special focus on intermediates of the process as well as the final sulfur product(s).

The WT *A. vinosum* control cultures behaved as expected: sulfide was rapidly converted (Fig. 1a) to sulfur, which transiently accumulated in sulfur globules (Fig. 1b). Polysulfides were formed as intermediates in the process (Prange et al., 2004), but are not shown here for better clarity. In the next step, sulfite was generated in the cytoplasm by the Dsr proteins. In WT cultures, sulfite does not accumulate in the medium to more than 30 μM (Dahl, 1996) (Fig. 1c). Finally, the end product sulfate appeared in the medium (Fig. 1d). Thiosulfate and tetrathionate concentrations were below detection limits once sulfide was used up. In accordance with previous results (Dahl, 1996), the phenotype of the APS reductase-negative mutant was indiscernible from the WT under the conditions chosen and is therefore not shown in Fig. 1.

Like the apr-negative *A. vinosum* mutant strain, neither the SoeABC-deficient mutant nor the tested double mutant was affected with regard to sulfide removal from the

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**Fig. 1. Sulfide oxidation in *A. vinosum* WT and mutant strains.** Representative experiments of biological triplicates are shown. (a) Sulfide, (b) intracellular sulfur, (c) sulfite, and (d) sulfate, thiosulfate and tetrathionate. *A. vinosum* strains: WT (●), ΔsoeAEm (□), ΔsoxY (Δ), aprB::Vkm ΔsoeAEm (■), ΔsoxY aprB::Vkm ΔsoeAEm (○). Thiosulfate and tetrathionate concentrations are depicted in (d) using dotted and dashed lines, respectively. Initial protein concentration: WT 0.19 mg ml$^{-1}$, ΔsoeAEm 0.17 mg ml$^{-1}$, ΔsoxY 0.11 mg ml$^{-1}$, aprB::Vkm ΔsoeAEm 0.13 mg ml$^{-1}$ and ΔsoxY aprB::Vkm ΔsoeAEm 0.14 mg ml$^{-1}$. Note the break in timescale. The period between 84 and 134 h was omitted to enable a good overview of the ongoing sulfur transformations during the first 3 days of the experiments and to depict the final situation for the triple-mutant strain in the same figure.
cultures, whilst the ΔsoxY aprB::ΩKm ΔsoeAEm triple mutant exhibited a significantly reduced sulfide oxidation rate (Table 4). We conclude that neither SoxYZ nor APS reductase and SoeABC is involved directly in oxidation of sulfide to intracellular sulfur in *A. vinosum*. Oxidation rates for intracellularly stored sulfur were found to be reduced by ~50% in the SoeABC-deficient and the SoxYZ-deficient mutant, whilst the rate was even further reduced in the strain lacking both cytoplasmic sulfite-oxidizing systems, and found to be only 20% of the original rate in the triple mutant (Table 4).

Quantitative analysis of inorganic sulfur compounds in the medium revealed that all mutants lacking SoeABC and/or SoxYZ accumulated massive amounts of sulfite up to a maximum of 1.2 mM in the *aprB*::ΩKm ΔsoeAEm strain that is no longer capable of cytoplasmic sulfite oxidation. Up to 0.43 mM sulfite accumulated in the ΔsoeAEm strain, again underlining the major importance of direct SoeABC-catalysed sulfite oxidation over the indirect APS reductase pathway. In both tested mutants still containing SoxYZ, i.e. ΔsoeAEm and *aprB*::ΩKm ΔsoeAEm mutants, sulfate was formed as the only detectable final product (Fig. 1d). Thiosulfate was not detected at any time in these cultures. In contrast, the ΔsoxY mutant produced not just sulfate, but also thiosulfate (Fig. 1d). Part of this thiosulfate was further oxidized to tetrathionate, which can be explained by the action of the periplasmic thiosulfate dehydrogenase (Denkmann *et al.*, 2012). The amount of thiosulfate consumed (0.473 mM in the experiment shown in Fig. 1d) and that of tetrathionate formed (0.210 mM) adhered exactly to the predicted 2:1 stoichiometry. Once formed, thiosulfate could obviously no longer be transformed into sulfate in the ΔsoxY strain. This result is in complete agreement with earlier findings that tetrathionate is the only product of thiosulfate-exposed *A. vinosum* ΔsoxY cells (Hensen *et al.*, 2006). The inability of the ΔsoxY strain to form sulfate from thiosulfate is clearly due to the incompleteness of the Sox system. Nevertheless, the mutant formed sulfate as the major end product of the oxidation of sulfide. This is explained by the presence and action of the APS reductase/SoeABC systems in the cytoplasm. In contrast, the triple mutant lacking SoxYZ as well as the cytoplasmic sulfite-oxidizing enzymes appeared to be completely incapable of sulfate formation. The small amount of sulfate found in the medium after prolonged incubation (144 h) is completely accounted for by chemical formation from sulfite. The main product in the sulfide-exposed triple-mutant strain was thiosulfate, which to some extent was again transformed to tetrathionate by the action of the periplasmic thiosulfate dehydrogenase.

**DISCUSSION**

In this work, we identified the membrane-bound iron–sulfur molybdoprotein SoeABC as a major enzyme catalysing direct oxidation of sulfite to sulfate in the cytoplasm of *A. vinosum*. The function of SoeABC was proven by strongly reduced specific oxidation rates for externally supplied sulfite and by massive excretion of sulfate into the medium during oxidation of sulfide in *A. vinosum* SoeABC-deficient strains. Our conclusion is corroborated by the lack of AMP-independent sulfite-oxidizing activity in the crude extract of SoeABC-deficient *A. vinosum*. SoeABC appears to be of general importance in purple sulfur bacteria because it is encoded in all genomes of organisms belonging to this group (Table 2).

Database searches revealed the presence of three linked genes related closely to *soeABC* and encoding the three subunits of a cytoplasmically oriented membrane-bound iron–sulfur molybdoprotein not only in phototrophic sulfur oxidizers, but also in a large number of chemotrophic bacteria. Examples are compiled in Table S2. Whilst the so-far sequenced epsilon- and deltaproteobacteria do not appear to contain *soeABC*, these genes occur in several chemotrophic sulfur oxidizers belonging to the gammaproteobacteria including the bacterial endosymbionts of *Riftia pachyptila* and *Tevnia jerichonana*, *Thioalkalivibrio* species and *Acidithiobacillus* species. Furthermore, a number of known sulfur oxidizers belonging to the betaproteobacteria contain the genes, including *Sulfuricella denitrificans*, *Thiomonas* species as well as *Thiobacillus denitrificans*. Among the alphaproteobacteria containing *soeABC*, *Magnetospirillum* species and *Magnetococcus* species are established sulfur

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**Table 4. Rates for oxidation of externally added sulfide and intracellular sulfur in *A. vinosum* WT, single- and double-mutant strains defective in *soe*, *apr* and/or *sox* genes**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Sulfide oxidation rate [nmol min⁻¹ (mg protein)⁻¹]</th>
<th>Sulfur oxidation rate [nmol min⁻¹ (mg protein)⁻¹]</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>69.3 ± 4.5</td>
<td>26.5 ± 4.0</td>
</tr>
<tr>
<td>ΔsoeAEm</td>
<td>69.0 ± 6.3</td>
<td>15.3 ± 1.3</td>
</tr>
<tr>
<td>ΔsoxY</td>
<td>94.8 ± 5.8</td>
<td>13.4 ± 1.2</td>
</tr>
<tr>
<td><em>aprB</em>::ΩKm ΔsoeAEm</td>
<td>73.3 ± 20.9</td>
<td>10.2 ± 2.3</td>
</tr>
<tr>
<td>ΔsoxY <em>aprB</em>::ΩKm ΔsoeAEm</td>
<td>10.5 ± 5.4</td>
<td>4.4 ± 0.8</td>
</tr>
</tbody>
</table>

Initial sulfide concentration: 4 mM. Protein concentration at the onset of fermentations: 110–193 μg ml⁻¹.
oxidizers. The presence and genomic linkage of soeABC with dsr and sox genes in bacteria belonging to the Roseobacter clade has recently been noted by Mussmann and coworkers (Lenk et al., 2012). The genes are also present in several genome-sequenced Roseovarius species and Roseobacter species. Among the soeABC-containing alpha- and betaproteobacteria, we find a number of species for which sulfur-oxidizing capabilities have not been described and/or tested (e.g. Herminimonas arseneoxydans and several Burkholderia species) or in which sulfur-oxidizing capabilities are restricted to oxidizing sulfite released as an intermediate during organosulfur compound degradation, as in the Roseobacter clade bacterium R. pomeroyi (Lehmann et al., 2012). We can thus state that the A. vinosum and the currently not well described R. pomeroyi soeABC genes encode the prototypes of a new protein family within the complex iron–sulfur molybdoenzymes that is widespread among several branches of proteobacteria. We propose that the so-far largely unrecognized SoeABC sulfite dehydrogenase is of major importance for the oxidation of sulfite in the cytoplasm generated either by the Dsr system or by cytoplasmic desulfonating enzymes like sulfocetaldehyde acetyltransferase (Xsc) in a wide range of physiologically and phylogenetically diverse bacteria.

Furthermore, the compilation of the occurrence of cytoplasmic sulfite-generating and sulfite-oxidizing systems in selected proteobacteria in Table S2 shows that there is no tight correlation between specific modes of sulfite generation (here, Dsr or Xsc) and the sulfite-oxidizing module present (here, SoeABC or AprBA combined with either AprM or QmoABHdRB). In most cases, the presence of Xsc is coupled with the presence of SoeABC; however, ‘Candidatus Pelagibacter ubique’ is a well-recognized exception (Meyer & Kuever 2007, Table S2). The Dsr system may occur in parallel with SoeABC alone, with AprBA alone (e.g. in ‘Candidatus Ruthia magnifica’, see Table S2) or with a combination of both systems. The possession of the APS reductase pathway in addition to or instead of SoeABC may be advantageous because additional energy is gained by substrate phosphorylation in the ATP sulfurylase-catalysed step by transferring the AMP moiety of APS onto pyrophosphate (Parey et al., 2013). Furthermore, it may be especially advantageous to be equipped with the Qmo-related electron-accepting unit for APS reductase. Irrespective of whether SoeABC or AprMBA is used, the electrons stemming from sulfite are proposed to enter the electron transport chain at the energetic level of quinones (via SoeC or AprM). However, the presence of the HdrA-like QmoA in the Qmo complex opens the possibility that – in a reverse manner to the mechanism suggested for sulfate reducers (Ramos et al., 2012; Grein et al., 2013) – an electron bifurcation occurs that could result in simultaneous reduction of low-potential electron acceptors like ferredoxin or NAD\(^+\). Such a process would be of significant energetic advantage especially for chemolithoautotrophic growth because it would result in a lower energy demand for reverse electron flow.

In the current work, we furthermore collected evidence for an involvement of the periplasmic sulfur substrate-binding protein SoxYZ in the sulfite metabolism in A. vinosum, whilst other Sox proteins (SoxXAK and SoxB) do not appear to play a significant role in this process. We suggest that SoxYZ may serve the same function – and thus be of considerably higher importance than previously assumed – in other purple sulfur bacteria as well. This is suggested by our observation that genes encoding the protein are almost universally present in this group of organisms irrespective of whether the organism contains further sox genes and is able to metabolize thiosulfate (Table 1). The exact nature of SoxYZ participation in sulfite metabolism cannot yet be deduced from in vitro experiments, but nevertheless can be pinpointed by our experiments with A. vinosum mutant strains. One important conclusion from these studies is that SoxYZ cannot be a component of a sulfite-oxidizing pathway running independently of the cytoplasmic pathways. Mutants lacking SoxYZ and mutants lacking both cytoplasmic sulfite oxidation pathways are each very strongly affected with regard to degradation of external sulfite as well as with regard to turnover of sulfite formed as an intermediate by the cells. The effects are not additive, rather both SoxYZ and the cytoplasmic pathways need to co-exist. Obviously, both the periplasmic SoxXZ and the cytoplasmic sulfite-oxidizing enzymes are needed in parallel for effective sulfite oxidation in A. vinosum, suggesting some kind of interplay between these systems despite localization in two different cellular compartments.

The simplest model into which these results and considerations can be integrated is the suggestion that SoxYZ may act as a sulfite-binding protein in the periplasm of A. vinosum and that in this function the protein acts independently of the other Sox proteins, i.e. SoxXAK and SoxB. Originally, we rationalized that binding of sulfite to SoxYZ may only be possible in an oxidative step yielding SoxY-cysteine-sulfonate catalysed by a protein containing a redox-active site. However, in vivo such a process is obviously not exerted by the most likely candidate SoxXA since the sulfite oxidation capability of the SoxXA-deficient strain is undisturbed. An alternative mode for attachment of sulfite to SoxYZ not involving a redox reaction is the reaction of the thiolate of the conserved SoxY-cysteine (Sox-Cys\(^-\)) with aqueous sulfite yielding SoxY-cysteine-S-sulfinate (SoxY-Cys-SO\(_2\)\(^-\)). Such a reaction is indeed feasible at the moderate pH values prevalent in the bacterial periplasm (Steudel & Steudel, 2010). However, it should be kept in mind that the reaction has so far only been shown for free cysteine and not for an active-site residue of a complex protein. We also considered an alternative mechanism for the generation of SoxY-cysteine-S-sulfinate. In aqueous solution sulfite can form disulfite by a chemical reaction (2 HSO\(_3\)\(^-\) (aq) \(\rightleftharpoons S_2O_5^{2-}\) (aq) + H\(_2\)O, equation 1). This disulfite could serve as substrate for SoxZY according to equation 2: SoxZY-Cys\(^-\) + [O\(_2\)S\(_2\)SO\(_4\)]\(^2+\) + H\(^+\) \(\rightleftharpoons\) SoxZY-Cys-SO\(_2\)\(^-\) + HSO\(_3\)\(^-\). Based on this mechanism, SoxYZ could act as a binding protein not
only shielding components of the periplasm from chemical reactions with sulfite, but possibly also delivering sulfite for transport into the cytoplasm. We suggest that the SoxY-cysteine-S-sulfinate may act as a substrate donor for an as-yet unknown transport system importing sulfite into the cytoplasm. Either sulfite could be released by the transporter by simple addition of water to the SoxY-cysteine-S-sulfinate or disulfite may be released in reverse of equation 2 shown above. In Fig. 2, the role of SoxYZ as a donor for a sulfite-importing system is depicted for the simplest case, i.e. the degradation of externally available sulfite. The A. vinosum genome contains an array of genes encoding putative (ABC) transporters that could in principle serve such a function and work together with a sulfite-binding protein. Once in the cytoplasm, sulfite is immediately oxidized to sulfate via the direct SoeABC and/or the indirect APS reductase pathway. At present, we cannot discount the possibility that sulfite is also taken up into the cytoplasm in the absence of SoxYZ – albeit with a significantly slower rate – as the AsoXY mutant shows residual sulfite-oxidizing capability under light-limited conditions (Table S1).

We also present a model for the interplay of SoxYZ and cytoplasmic sulfite oxidation when sulfide, the most readily available substrate in its natural habitat, is metabolized by A. vinosum. Here, the picture is much more complicated. It was already outlined above that zero-valent sulfur stored in sulfur globules is an intermediate during the oxidation of this most highly reduced sulfur compound to sulfate. Sulfite is also a well-established intermediate further downstream in the process. Although this intermediate is formed in the cytoplasm, it appears to be excreted, i.e. transported across the cytoplasmic membrane, to some extent even by WT cells. This is evidenced by the detection of sulfite in WT culture supernatants albeit at only very low concentrations (Dahl, 1996). Once formed, sulfite is obviously removed with high efficiency. Two principally different mechanisms appear to play a role here. The first is the oxidation to sulfate in the cytoplasm either by SoeABC alone or by a combination of the SoeABC/APS reductase pathways depending on the organism (Table 1). The second mechanism working in parallel to avoid any accumulation of sulfite in the cytoplasm would be transfer across the cytoplasmic membrane into the periplasm. Transporters mediating such a sulfite efflux have not yet been described for purple sulfur bacteria. Putative permeases resembling sulfite exporters of the TauE/SafE family (Pfam accession no. PF01925) (Weinitschke et al., 2007; Krejcík et al., 2008) are encoded in some purple sulfur bacteria including A. vinosum (Alvin_1107), but not in all genome-sequenced members of this group. Other described sulfite efflux pumps stem from fungal sources, e.g. SSU1 from Saccharomyces cerevisiae (Park & Bakalinsky, 2000; Nardi et al., 2010) or Aspergillus fumigatus (Léchenne et al., 2007). However, genes encoding closely related proteins are not present in the currently sequenced purple sulfur bacteria with the exception of Thiorhodococcus drewoii. Once sulfite anions reach the periplasm, they are possibly bound by SoxYZ just as outlined above.

We considered the possibility that either the SoxY-Cys-sulfinate could act as a substrate for oxidation to SoxY-Cys-sulfonate or sulfite could be oxidatively bound to the conserved cysteine of SoxY in a reaction catalysed by a periplasmic enzyme other than SoxXA. The membrane-bound trihaem cytochrome c DsrJ would be a candidate for catalysing such a reaction as it contains a putative active site similar to that of SoxXA. It has been proposed that DsrJ catalyses oxidation of a sulfur substrate and uses the released electrons for transmembrane electron transfer to a disulfide of DsrC via the other components of the DsrMKJOP complex (Grein et al., 2010a, b). However, in such a process, production of each sulfite molecule in the cytoplasm would require oxidation of exactly one molecule of sulfite in the periplasm. Sulfite oxidation in the cytoplasm would be obsolete and thus such a mechanism is discounted. It is more likely that SoxYZ acts as a ‘sulfite buffer’ again shielding periplasmic components from reaction with free sulfite (see above) and also keeping sulfite on hold for reimport into the cytoplasm. We attribute the accumulation of external sulfite in the AsoX single mutant to the latter of the proposed functions. When the major cytoplasmic sulfite-oxidizing system is absent (SoeABC) or sulfite oxidation in the cytoplasm is no longer possible at all, as in the A. vinosum SoeABC/APS reductase double mutant, the whole system runs out of balance even in the presence of SoxYZ and free sulfite starts to accumulate outside of the cells in the medium. When cells still contain periplasmically stored zero-valent sulfur in such a situation, this sulfur is in principle accessible to abiotic attack by the strong nucleophile sulfite. Such a reaction would yield thiosulfate (Roy & Trudinger, 1970;
Suzuki, 1999). In cells with a complete Sox system, such as the ΔsoeAΔm and the ΔsoeAΔm aprB::ΩKm mutants, thiosulfate should still be completely degraded to sulfate. The oxidized sulfone group of thiosulfate would be released immediately by SoxB, whilst the sulfane sulfur would be hooked up again to stored sulfur, which would in turn be transformed to cytoplasmic sulfite by the Dsr proteins. Sulfite would then be transported out of the cytoplasm, leading to thiosulfate formation and the same series of reactions would restart. Finally, sulfate would be the only detectable end product. In fact, this is exactly what we observed in our experiments: degradation of stored sulfur took significantly longer in the ΔsoeAΔm and the ΔsoeAΔm aprB::ΩKm mutants than in the WT, and the final product in all cases was sulfate. In these cultures, thiosulfate never accumulated, whilst this was clearly the case for cells that lacked SoxYZ and could thus not run a complete Sox cycle.

The only transformation that thiosulfate underwent in SoxYZ-deficient cultures was oxidation to tetrathionate leading to thiosulfate formation and the same series of reactions that finally lead to sulfate accumulation. This pattern is observed in our experiments: degradation of stored sulfur along with our finding that the respective genes occur even in purple sulfur bacteria that do not contain other sox genes and have accordingly been reported not to be capable of thiosulfate utilization (Table 1). The only exception to this pattern is *T. mobilis*. However, *T. mobilis* is the only genome-sequenced purple sulfur bacterium so far that contains a gene encoding a periplasmic sulfite dehydrogenase (Denkmann et al., 2012). In the *A. vinosum* triple mutant, neither sulfite nor thiosulfate could be further metabolized, leading to a very significant reduction not only of the rate of degradation, but also of formation of sulfur globules and furthermore to a complete inability of the cells to produce sulfate.

Our proposal for a more general role of SoxYZ not only as a thiosulfate- but also as a sulfite-binding protein goes well along with our finding that the respective genes occur even in purple sulfur bacteria that do not contain other sox genes and have accordingly been reported not to be capable of thiosulfate utilization (Table 1). The only exception to this pattern is *T. mobilis*. However, *T. mobilis* is the only genome-sequenced purple sulfur bacterium so far that contains a gene encoding a periplasmic sulfite dehydrogenase of the SorA type. It is thus tempting to speculate that the periplasmic sulfite-binding SoxYZ is not necessary in the presence of a periplasmic sulfite-forming enzyme. Obviously, purple sulfur bacteria are equipped with different modules, and thereby means, to cope with externally available and internally generated sulfate.

**Conclusions**

The results presented in this study clearly show that the membrane-bound, cytoplasmically oriented iron–sulfur molybdoprotein SoeABC plays a major role in the oxidation of sulfite in the cytoplasm of *A. vinosum*, and very probably also in other phototrophic and chemotrophic bacteria. The periplasmic SoxYZ protein and the cytoplasmic sulfite-oxidizing enzymes are needed in parallel for effective sulfite oxidation in *A. vinosum*, suggesting some kind of interplay between these systems despite their localization in two different cellular compartments. While the exact function of SoxYZ in sulfite oxidation remains elusive at this point, it is tempting to speculate that it acts as a periplasmic sulfite-binding protein.

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**REFERENCES**


Isochromatium, Marichromatium, Thiococcus, Thiohalocapsa, and Allochromatium, Halochromatium, description of the new genera Thermochromatium.


Kojro, E. & Kroèger, A. (1992). A structural comparison of molybdenum Thiosulphate oxidation in the phototrophic sulphur bac-
(2006). Molecular basis of intramolecular


The membrane QmoABC complex interacts directly with the dissimilatory adenosine 5'-phosphosulfate reductase in sulfate reducing bacteria. *Front Microbiol* 3, 137.


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