Photosynth Res (2010) 104:163–176
DOI 10.1007/s11120-010-9531-2

REVIEW

Inorganic sulfur oxidizing system in green sulfur bacteria

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Received: 14 September 2009 / Accepted: 16 January 2010 / Published online: 9 February 2010

Abstract Green sulfur bacteria use various reduced sulfur compounds such as sulfide, elemental sulfur, and thiosulfate as electron donors for photoautotrophic growth. This article briefly summarizes what is known about the inorganic sulfur oxidizing systems of these bacteria with emphasis on the biochemical aspects. Enzymes that oxidize sulfide in green sulfur bacteria are membrane-bound sulfide-quinone oxidoreductase, periplasmic (sometimes membrane-bound) flavocytochrome *c* sulfide dehydrogenase, and monomeric flavocytochrome *c* (SoxF). Some green sulfur bacteria oxidize thiosulfate by the multienzyme system called either the TOMES (thiosulfate oxidizing multi-enzyme system) or Sox (sulfur oxidizing system) composed of the three periplasmic proteins: SoxB, SoxYZ, and SoxAXK with a soluble small molecule cytochrome *c* as the electron acceptor. The oxidation of sulfide and thiosulfate by these enzymes in vitro is assumed to yield two electrons and result in the transfer of a sulfur atom to persulfides, which are subsequently transformed to elemental sulfur. The elemental sulfur is temporarily stored in the form of globules attached to the extracellular surface of the outer membranes. The oxidation pathway of elemental sulfur to sulfate is currently unclear, although the participation of several proteins including those of the dissimilatory sulfite reductase system etc. is suggested from comparative genomic analyses.

Keywords Cytochrome · Green sulfur bacteria · Sox · Sulfide dehydrogenase · Thiosulfate

Abbreviations

APS	Adenosine phosphosulfate
cyt	Cytochrome
FCSD	Flavo-cytochrome *c* sulfide dehydrogenase
GSB	Green sulfur bacteria
ISP	Iron–sulfur protein
RC	Reaction center
S^0	Zero-valence sulfur
Sox	Sulfur oxidizing enzyme system
SQR	Sulfide-quinone reductase
TOMES	Thiosulfate oxidizing multi-enzyme system

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Utilization of reduced sulfur compounds by prokaryotes

In the global sulfur oxidation–reduction cycle, in addition to non-biological chemical reactions, biological oxidation to sulfate of inorganic sulfur compounds such as sulfide, persulfides, elemental sulfur, thiosulfate, polythionate etc. constitutes an important part of the half biogeochemical cycle, with biological reduction of sulfate to sulfide or elemental sulfur (sulfate respiration) contributing to the other half.

Monographs describing the sulfur metabolism in prokaryotes and phototrophic organisms including eukaryotes, respectively, have recently appeared (Dahl and Friedrich (2008) and Hell et al. (2008), respectively). Recent advances in various fields of research on phototrophic sulfur bacteria (phylogeny, ecology, genomics, metabolism etc.) were extensively reviewed (Frigaard and Dahl 2009). The organisms that use reduced sulfur compounds as electron donors and for respiration or photosynthesis are confined to prokaryotes. Some of the representative families are: *Chlorobiaceae* (Genus *Chlorobaculum*, *Chlorobium*, *Ancalochloris*, *Chloroherpeton*, *Pelodictyon*, *Prosthecochloris*), *Chromatiaceae* (*Chromatium*, *Allochromatium*, *Thiorhodococcus*), *Rhodobacteraceae* (*Paracoccus*, *Rhodobacter*, *Roseovarius*), *Bradyrhizobiaceae* (*Bradyrhizobium*), *Hyphomicrobiaceae* (*Starkeya*), *Burkholderiaceae* (*Ralstonia*), *Cyanobacteriaceae* (*Oscillatoria*) in the domain *Bacteria*, and *Sulfolobaceae* (Genus *Sulfolobus*, *Sulfurucoccus*) in the domain *Archaea*. The metabolic pathways of *Archaea* seem to be largely different from those of *Bacteria* although they have several components in common (Friedrich et al. 2005). Sulfur metabolism in the *Archaea* will not be discussed in this review.

Green sulfur bacteria (GSB) carry out anoxygenic photosynthesis with reduced sulfur compounds such as sulfide and elemental sulfur, and for some species thiosulfate as the electron donor for photoautotrophic growth (Brune 1989, 1995b; Overmann 2001; Imhoff 2008; Frigaard and Dahl 2009). The one known exception is *Chlorobium ferrooxidans*, assigned to the genus *Chlorobium* based on 16S-rRNA encoding DNA sequence analysis, which is unable to use reduced sulfur compounds as electron donors for growth and uses ferrous iron instead (Heising et al. 1999). Some GSB also use hydrogen and ferrous iron as the electron donors (Frigaard and Bryant 2008a, b).

In heliobacteria, photoautotrophic growth on reduced sulfur compounds and CO_2 has not been observed, although some of them use sulfide and/or thiosulfate as the sole source of sulfur in the biosynthesis of cysteine and methionine (Madigan 2001). Heliobacteria will not be discussed further in this review.

Outline of inorganic sulfur metabolism for electron donation to the reaction center (RC) in GSB

Studies that have led to a greater understanding of inorganic sulfur metabolism

The pathways of inorganic sulfur metabolism show significant similarities among various prokaryotic groups, with some notable differences among them. In studies where inorganic sulfur compounds were supplied as electron donors to phototrophic bacteria, several proteins involved in sulfur metabolism had been identified by the mid-1990s. These proteins include soluble flavocytochrome sulfide dehydrogenase (FCSD) (Kusai and Yamanaka 1973b), membrane-bound sulfide-quinone reductase (SQR) (Shahak et al. 1992), some components of the thiosulfate oxidizing multi-enzyme system (TOMES), etc. (extensively reviewed by Brune 1989, 1995b). Although there have subsequently been significant advances in studies of the inorganic sulfur metabolism by phototrophs, his reviews are still useful in providing a general view of relevant portions of the inorganic sulfur metabolism including the redox potentials of sulfur compounds.

Significant studies that have recently advanced our understanding of inorganic sulfur metabolism in GSB can be summarized as follows:

(1) Availability of the whole genome sequence of *Chlorobaculum tepidum* (formerly *Chlorobium tepidum*) (Eisen et al. 2002), followed by an ever-increasing number of additional sequences (currently, whole genome sequences from a total of 12 strains (11 complete sequences and one obtained by a whole genome shotgun sequencing project) are publicly available) (http://www.ncbi.nlm.nih.gov/, accessed January 14th, 2010).
(2) Studies advancing the understanding of the biochemical pathways as well as characterization of the genes of the proteins involved in sulfur metabolism in various inorganic sulfur-utilizing prokaryotes (Kelly et al. 1997; Friedrich et al. 2001, 2005).
(3) Comparative genomic studies and proposed models of putative metabolic pathways of inorganic sulfur compounds in GSB (Eisen et al. 2002; Frigaard and Bryant 2008a, b; Frigaard and Dahl 2009).
(4) Creation of mutants by targeted inactivation of the genes assumed to be involved in inorganic sulfur metabolism followed by analyses of the characteristics of the mutants (Chan et al. 2008c, 2009; Azai et al. 2009).
(5) Biochemical characterization of the proteins involved in thiosulfate oxidation in GSB (Ogawa et al. 2008, in press).

Outline of the electron transport pathways leading to the reduction of the oxidized primary donor P840 in GSB

The RC of GSB is similar to photosystem I of oxygenic photosynthetic organisms, called Type I or iron-sulfur type RC with ferredoxin and flavodoxin as immediate electron acceptors (Sakurai et al. 1996; Hauska et al. 2001). The primary donor of the RC is a special pair of bacteriochlorophylls called P840 residing on the core homodimeric protein PscA, and its immediate electron donor is the RC-bound cytochrome (cyt) *c*-551 (PscC, *CT1639* gene product)

(Fig. 1). In the moderately thermophilic *C. tepidum* TLS, based on in vitro studies there seem to be at least two pathways for the reduction of the bound cyt *c*-551 (PscC). Itoh et al. (2002) showed that a soluble mono-heme cyt *c*-554 of about 10 kDa (CT0075) donates electrons to the bound cyt *c*-551 rather than directly to photooxidized P840. A similar small, mono-heme cyt *c*-555 is a good electron acceptor in thiosulfate oxidation in *C. limicola* f. *thiosulfatophilum* (Kusai and Yamanaka 1973a) as in *C. tepidum* (Ogawa et al. 2008). There is evidence that *C. tepidum* has an alternative electron transfer pathway, as a membrane preparation from this bacterium catalyzes efficient electron transfer from quinol to photooxidized P840 without addition of soluble cyt *c*-554 (Oh-oka et al. 1998). Based on these results, the authors proposed the electron transport pathway as: sulfide → membrane-bound SQR → membrane-bound quinol oxidoreductase (cyt *b*/Rieske-type iron–sulfur protein (ISP) complex) (Klughammer et al. 1995) → cyt *c*-556 bound to the complex → cyt *c*-551 bound to the RC → P840. The plausibility of this reaction scheme is supported by studies of mutant cells disrupted in the soluble cyt *c*-554 gene (*CT0075*). These mutant cells could still grow photoautotrophically in a medium containing sulfide and thiosulfate, although at a lower rate than the wild-type (Tsukatani et al. 2006).

Enzymes of inorganic sulfur oxidation

Enzymes of sulfide oxidation in GSB

There are four kinds of sulfide-oxidizing enzymes in GSB that have been characterized so far by in vitro biochemical assays (Table 1).

Sulfide-quinone reductase (SQR)

SQR (also called sulfide-quinone oxidoreductase) is distributed over various prokaryotes as well as eukaryotic

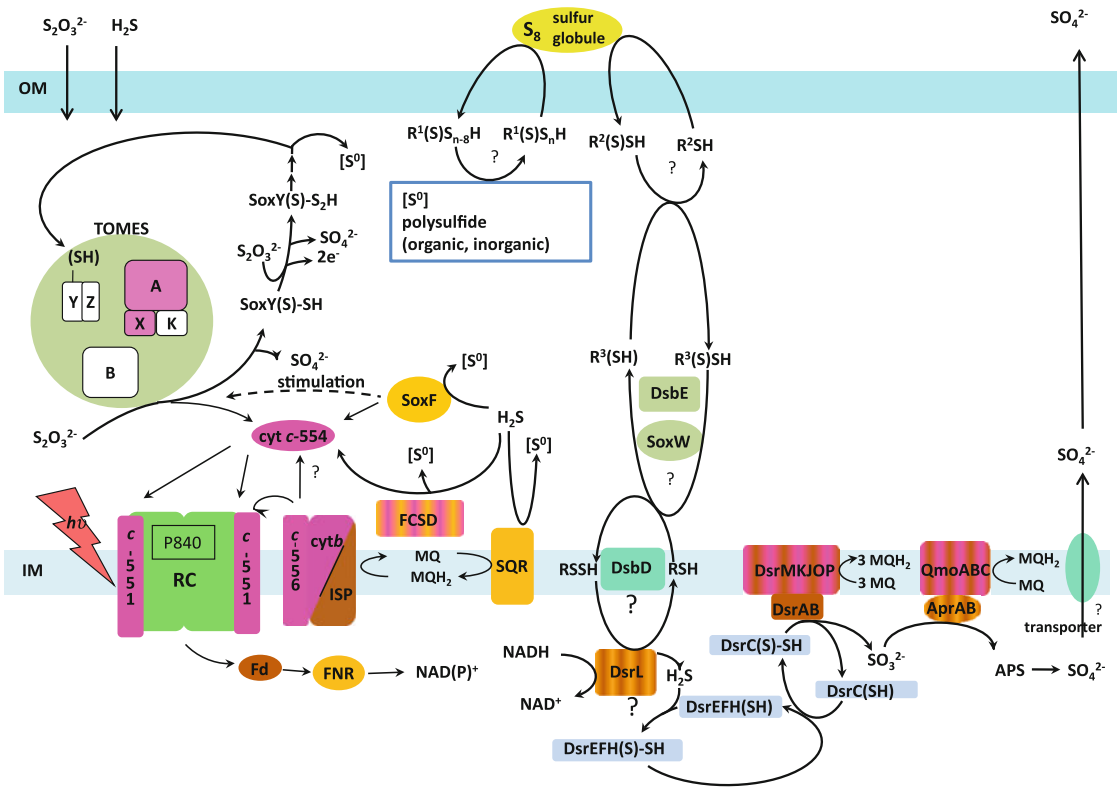

Fig. 1 Overview of known and hypothesized pathways of electron and inorganic sulfur compounds in *Chlorobaculum tepidum*. A part of the figure is derived from Eisen et al. (2002), Frigaard and Bryant (2008a, b), Dahl (2008), and Cort et al. (2008). Thin arrows indicate pathways of e^-, and thick ones, metabolic or transport pathways of substrates/products. *?* hypothetical, *Apr* adenosine 5′-phosphosulfate reductase, *APS* adenosine 5′-phosphosulfate, *c* cytochrome *c* with the wavelength (nm) of the α-band of the reduced form, *cyt b/ISP* cyt *b*/Rieske-type ISP complex, *Dsb* homologues encoded by genes in the *dsb* gene cluster encoding thiol:disulfide interchange proteins, *Dsr* proteins encoded by genes in the *dsr* gene cluster encoding dissimilatory sulfite reductase proteins, *FCSD* flavocytochrome *c* sulfide dehydrogenase, *Fd* ferredoxin, *FNR* ferrodoxin-NAD(P)$^+$/NAD(P)H oxidoreductase, *IM* inner membrane, *MQ* menaquinone, *OM* outer membrane, *Qmo* quinone oxidizing membrane protein, $R^{1,2,3}$ hypothetical thiol, *RC* reaction center, *[S^0]* the zero-valence sulfur or the equivalent (sulfur atom in organic and inorganic polysulfide, elemental sulfur), *Sox* proteins encoded by genes in the *sox* gene cluster, *SQR* sulfide-quinone reductase, *TOMES* thiosulfate oxidizing multi-enzyme system. For details, see text

Table 1 Some kinetic properties of inorganic sulfur oxidizing enzymes

		K_m (μM)	V_{max} (μmol e^-/μmol/min)	pH optimum	e^- acceptor used
H_2S ($pK_1 = 7.06$, $pK_2 = 12.44$)					
SQR	*Oscillatoria limnetica*	8	3.3–5		plastoquinone-1[b]
SQR	*Rhodobacter capsulatus*	2	3.5	6.3	dodecyl ubiquinone[c]
FCSD	*Thiobacillus* sp.	1.7	107[a]	8.6	hourse heart cyt *c*[d]
SoxF	*P. pantotrophus*	2.3	5.5	6	hourse heart cyt *c*[e]
SoxF	*C. tepidum*	2.1	100	>8	*C. t* cyt *c*-554[f]
SoxF	*C. tepidum*	2.1	0.2	>8	Hourse heart cyt *c*[f]
Core TOMES	*C. tepidum*	41	16	6–6.5	*C. t* cyt *c*-554[f]
$H_2S_2O_3$ ($pK_1 = 1.56$, $pK_2 = 3.73$)					
Core TOMES	*C. tepidum*	150	7.2	6–6.5	*C. t* cyt *c*-554[f]
Core TOMES + SoxF	*C. tepidum*	150	13.8	6–6.5	*C. t* cyt *c*-554[f]
H_2SO_3 ($pK_1 = 1.78$, $pK_2 = 6.99$)					
Core TOMES	*C. tepidum*	23	3.5		*C. t* cyt *c*-554[f]

[a] Calculated from :FCSD 51 kDa, $v = 2.1$ μmol cyt *c* reduced mg $protein^{-1}$ min^{-1}

[b] Arieli et al. 1994

[c] Schütz et al. 1997

[d] Visser et al. 1997

[e] Quentmeier et al. 2004

[f] Ogawa et al. in press

mitochondria (see Shahak and Hauska 2008). SQR from the cyanobacterium *Oscillatoria limnetica* is a membrane-bound protein that could be solubilized by mild detergent treatment. The purified protein was monomeric and shown to bind FAD as the prosthetic group (Arieli et al. 1994). SQR is assumed to supply electrons to the quinone pool presumably with persulfide, but not elemental sulfur as the product (Griesbeck et al. 2002) (see "Transient elemental sulfur formation" section, for further metabolism of persulfide). The presence of SQR activity in GSB was first demonstrated in *Chlorobium limicola* f. *thiosulfatophilum* (Shahak et al. 1992). A gene encoding SQR has been identified in all of the genomes of GSB sequenced thus far (Frigaard and Bryant 2008a, b). In GSB, the reduced menaquinone is then oxidized by cyt *b*/ISP complex accompanied by the generation of a proton-motive force (Hauska et al. 2001).

Flavocytochrome c-sulfide dehydrogenase (FCSD or FCC)

Flavocytochrome *c* is a relatively abundant soluble protein in *Chlorobium thiosulfatophilum* (Meyer et al. (1968). The protein was subsequently found to have sulfide dehydrogenase activity in vitro with soluble small molecule cyt *c* as the electron acceptor, and named flavocytochrome *c*-sulfide dehydrogenase (FCSD) (Kusai and Yamanaka 1973b). The protein is a tightly bound heterodimer composed of a *c*-type cytochrome subunit and a flavoprotein subunit encoded by the *fccA* and *fccB* genes respectively, in *Allochromatium vinosum* (Reinartz et al. 1998). FCSDs from GSB oxidize sulfide in vitro with various cyt *c* molecules as the electron acceptors, presumably with persulfide (H_2S_2) as the initial product and polysulfides (inorganic or organic) or elemental sulfur as the subsequent products. These products will be collectively referred to as the S^0 equivalent in this article. Purified FCSD from *Chlorobium limicola* f. *thiosulfatophilum* form a tight complex with the soluble small molecule cyt *c*-555 from this bacterium (Davidson et al. 1985), suggesting that the in vivo electron acceptor of FCSD is a soluble small molecule cyt *c*. In many microorganisms, FCSDs have been characterized as soluble proteins residing in the periplasmic space, but in some bacteria such as the colorless sulfur bacterium *Thiobacillus* sp. W5 (Visser et al. 1997), the purple bacteria *Ectothiorhodospira vacuolata* (Kostanjevecki et al. 2000) and the GSB *C. limicola* (Verté et al. 2002), FCSDs have been characterized as membrane-bound proteins.

Monomeric flavoprotein (SoxF)

Subsequent studies revealed that homologous genes encoding proteins with significant amino acid sequence identity with FCSD were frequently found in the thiosulfate oxidizing gene (*sox*) cluster of various bacteria (Friedrich et al. 2008), where the homologues of cyt *c* and flavoprotein genes are referred to as *soxE* and *soxF* (instead of *fccA* and *fccB)*, respectively. From the facultative lithotrophic bacterium *Paracoccus pantotrophus*, a monomeric flavoprotein

encoded by the *soxF* gene in the sulfur oxidizing *sox* gene cluster was prepared (Rother et al. 2001), and subsequently found to have sulfide dehydrogenase activity in vitro with externally added cyt *c* (from horse heart) as the electron acceptor (Quentmeier et al. 2004). Although the *sox* gene cluster of this bacterium contains *soxE* in tandem with *soxF* encoding the cyt *c* homologue of FCSD (Friedrich et al. 2000), SoxF was obtained as a monomeric flavoprotein devoid of bound cytochrome. For discussion of other possible physiological functions of SoxF, see below.

Sulfide dehydrogenase activity of thiosulfate oxidizing multi-enzyme system

The thiosulfate oxidizing enzyme system from *C. tepidum* (the core TOMES, see below) has low but measurable sulfide dehydrogenase activity (Ogawa et al. in press) although the affinity for sulfide is lower than the above three kinds of enzymes (Table 1).

Thiosulfate oxidizing activity of various *Bacteria*

Different pathways of thiosulfate oxidation: S4I pathway and TOMES (or Sox) pathway

Two different biochemical pathways for thiosulfate oxidation are distinguishable in the domain *Bacteria* (Kelly et al. 1997; Friedrich et al. 2001, 2005). In one pathway found in bacteria such as the lithoautotrophic proteobacterium *Acidiphilium acidophilum* (formerly *Thiobacillus acidophilus*), thiosulfate is first oxidized by a periplasmic thiosulfate dehydrogenase to tetrathionate (Okuzumi and Kita 1965; Meulenberg et al. 1993) either as the final or intermediary product (the tetrathionate (S4) intermediate pathway or S4I pathway (Kelly et al. 1997):

$$2S_2O_3^{2-}(\text{thiosulfate}) \rightarrow^{-} O_3SSSSO_3^{-}(\text{tetrathionate}) + 2e^{-}.$$

Briefly, in *A. acidophilum*, tetrathionate appears to be hydrolyzed by tetrathionate hydrolase to thiosulfate, sulfur, and sulfate (De Jong et al. 1997), and in *Acidithiobacillus ferrooxidans*, the electron acceptor of tetrathionate hydrolase seems to be ferric iron (Sugio et al. 2009). Although *Chlorobium vibrioforme* f. sp. *thiosulfatophilum* cells utilized tetrathionate as the electron donor for CO_2 assimilation, the thiosulfate oxidation in this bacterium did not seem to proceed through S4I pathway, because tetrathionate was not the oxidation product of thiosulfate (Khanna and Nicholas 1982).

In the other thiosulfate oxidation pathway found in bacteria such as the facultative lithotrophic *Paracoccus versutus* (formerly *Thiobacillus versutus)* (Kelly et al. 1997) and *P. pantotrophus* (Friedrich et al. 2001, 2005), the purple sulfur bacterium *Allochromatium vinosum* (Grimm et al. 2008) and the GSB *Chlorobaculum tepidum* (Ogawa et al. 2008), thiosulfate oxidation is catalyzed by the collaboration of several periplasmic proteins, referred to either as the TOMES (Kelly et al. 1997) or as the sulfur oxidizing system (Sox (Friedrich et al. 2000)).

Some bacteria such as the acidophilic sulfur oxidizing *Starkeya novella* (Kappler et al. 2001) and the purple sulfur bacterium *Allochromatium vinosum* (Hensen et al. 2006) appear to have both pathways.

Thiosulfate oxidizing multi-enzyme system (TOMES or Sox)

In bacteria that oxidize thiosulfate by TOMES, its components are found to be largely similar among different phyla, with some variations notably with respect to the presence or absence of sulfur dehydrogenase SoxCD. The TOMES components have been intensively studied in the facultative lithotrophic bacterium *P. versutus* (Kelly et al. 1997), *P. pantotrophus* (Friedrich et al. 2001, 2005), and in purple sulfur bacteria such as *Allochromatium vinosum* (Welte et al. 2009). At a minimum, three proteins (SoxAX, SoxB and SoxYZ) are indispensable to thiosulfate oxidation (Friedrich et al. 2001).

SoxAX is a heterodimeric (heterotrimeric in some bacteria, see below) protein, sometimes called cyt *c*-551 (Kusai and Yamanaka 1973c) which mediates electron transfer to external cytochrome and/or high-potential ISP (Meyer and Cusanovich 2003). In *P. pantotrophus*, SoxA is a di-heme (mono-heme in some bacteria, see below) and SoxX is a mono-heme subunit (Friedrich et al. 2000). SoxYZ is a colorless heterodimeric protein that binds the oxidized product of thiosulfate on the cysteinyl-SH group of SoxY as the intermediate to form a cysteinyl *S*-thiosulfonate (Quentmeier and Friedrich 2001). SoxB (also called Enzyme B (Kelly et al. 1997)) is a monomeric dimanganese protein (Cammack et al. 1989; Epel et al. 2005) that hydrolyzes the cysteinyl *S*-thiosulfonate to cysteinyl persulfide and sulfate (thiosulfohydrolase). Recently, the SoxB 3-D structure was determined by X-ray crystallography (Sauve et al. 2009). SoxCD is an $\alpha 2\beta 2$ heterotetramer (SoxC: binding molybdenum cofactor, SoxD: di-heme cyt *c*), which oxidatively hydrolyzes the cysteinyl persulfide.

In bacteria such as *P. pantotrophus* that have SoxCD, the following reaction scheme was proposed (Friedrich et al. 2001):

$$\text{SoxY}(-\text{SH}) +^{-} \text{SSO}_3^{-} \rightarrow \text{SoxY}(-\text{S}) - \text{SSO}_3^{-} + 2e^{-} + \text{H}^{+}, \quad (1)$$

catalyzed by SoxAX;

$$\text{SoxY}(-\text{S}) - \text{SSO}_3^{-} + \text{H}_2\text{O} \rightarrow \text{SoxY}(-\text{S}) - \text{SH} + \text{SO}_4^{2-} + \text{H}^{+}, \quad (2)$$

catalyzed by SoxB;

$$\mathrm{SoxY(-S)-SH+3H_2O \rightarrow SoxY(-S)SO_3^- + 6e^- + 7H^+}, \quad (3)$$

catalyzed by SoxCD; and

$$\mathrm{SoxY(-S)SO_3^- + H_2O \rightarrow SoxY(-SH) + SO_4^{2-} + H^+} \quad (4)$$

catalyzed by SoxB.

Experimental systems using reconstituted TOMES containing SoxCD yield eight electrons and two sulfate per thiosulfate.

In bacteria lacking SoxCD such as GSB, the reaction proceeds according to Eqs. 1 and 2 to yield SoxY(–S)–SH and sulfate yielding two electrons and one sulfate, and this reaction cycle is believed to be repeated multiple times to yield a poly-sulfide group (the S^0 equivalent) on SoxY.

Thiosulfate oxidizing multi-enzyme system (TOMES) of GSB

In 2002, the whole genome sequence of *C. tepidum* was determined (Eisen et al. 2002) and the sequence of the *sox* gene cluster of *C. limicola* was reported (Verté et al. 2002). It became apparent that the thiosulfate oxidizing proteins of GSB would be largely similar to those of *P. pantotrophus* (Friedrich et al. 2001). Genome sequencing of GSB continued, and Frigaard and Bryant (2008a, b) deduced the inorganic sulfur metabolic pathways from genome data of 10 strains available from NCBI GenBank and the two draft genome sequences. They concluded that most strains are able to oxidize sulfide, while oxidation of thiosulfate is less commonly encountered in cultivated strains.

In GSB, the biochemical studies of thiosulfate oxidation revealed several proteins by the mid 1970s. These include a multi-heme cyt *c*-551 (Meyer et al. 1968; Kusai and Yamanaka 1973c) and "thiosulfate-multiheme cyt *c* reductase" (Kusai and Yamanaka 1973c). Although these studies elucidated various important components of the thiosulfate oxidizing pathway of GSB (reviewed in Yamanaka 1996; Meyer and Cusanovich 2003), there was evidence that the biochemical pathway was far from complete.

The proteins essential for thiosulfate oxidation in *C. tepidum* were recently established by biochemical studies (Table 2) (Ogawa et al. 2008). These were composed of three periplasmic proteins (see below) very similar to those of *P. pantotrophus* except that SoxCD was absent. The three proteins will be referred to as the core TOMES, in order to distinguish it from the *P. pantotrophus*-type TOMES that contains SoxCD. Among the new findings was evidence of the presence of an additional subunit SoxK (SAXB) in the SoxAX complex. The presence of *soxK* in the *sox* gene cluster of various GSB had been noted in comparative genomic analyses (Frigaard and Bryant 2008a, b), but the function of the encoded protein was unknown (see below).

SoxB is an almost colorless monomeric protein with significant deduced amino acid sequence identity to *P. pantotrophus* SoxB and appears to be a di-manganese protein (Cammack et al. 1989).

SoxYZ is a colorless dimeric protein, and appears to be able to bind the thiosulfonyl residue as the oxidized product of thiosulfate on the conserved cysteinyl SH of SoxY (Quentmeier and Friedrich 2001).

SoxAXK is a heterotrimeric multi-heme protein, reddish brown in color. SoxA is an extremely low potential mono-heme cyt *c*-551 ($E_0' < -550$ mV) in *C. tepidum*. SoxA is a mono-heme protein in *S. novella* (Kappler et al. 2004, 2005) as in *C. tepidum*, but a di-heme protein in *P. panthotrophus* (Friedrich et al. 2000; Dambe et al. 2005) and *Rhodovulum sulfidophilum* (Appia-Ayme et al. 2001; Bamford et al. 2002). *C. tepidum* SoxX is a mono-heme cyt *c*-551 with a much higher E_0' value of +161 mV (Ogawa et al. 2008). SoxK is a newly identified subunit apparently devoid of prosthetic groups. The recombinant SoxK (rSoxK, over-expressed in *Escherichia coli* cells and purified) enhanced the binding of rSoxA and rSoxX as evidenced by gel-permeation chromatography, hence it is referred to as SAXB (SoxAX binding protein). In the presence of SoxB and SoxYZ, the mixture of rSoxA and rSoxX showed a low but detectable thiosulfate oxidation activity, which was significantly enhanced by the further addition of rSoxX to a level almost equal to the level of the

Table 2 Sox proteins of *C. tepidum*

Sox protein	Subunit	Prosthetic group etc.	Mr[b] (kDa)	Signal peptide
SoxAXK (cyt *c*-551)	SoxA	Mono-heme cyt *c*	30	+
	SoxX	Mono-heme cyt *c*	11	+
	SoxK		9	+
SoxB (thiosulfohydrolase)	Monomer	Di-manganese	62	+
SoxYZ (thiosulfonate and polysulfane-binding protein)	SoxY		13	+
	SoxZ		9	no
SoxF[a]	Monomer	FAD	44	+

[a] From Ogawa et al. in press. All other data are from Ogawa et al. (2008)

[b] Calculated from deduced mature polypeptides and prosthetic groups

mixture containing the native SoxAXK. The *soxK* homologues are found in the *sox* gene clusters of not only GSB, but also in those of a number of other families of bacteria including *Allochromatium vinosum*, *Bradyrhyzobium japonicum*, etc. (in about one-third of the *sox* gene clusters of the bacteria selected by Friedrich et al. (2008) for comparative studies) (Fig. 2). The unrooted phylogenetic tree indicated that the SoxKs of the GSB are congruent (Ogawa et al. 2008).

In the reconstituted in vitro reaction, the core TOMES of *C. tepidum* oxidized thiosulfate with various cyt *c* (including horse heart cyt *c*) as the electron acceptors (Ogawa et al. 2008) as reported with a partially purified enzyme system from *Chlorobium limicola* (Kusai and Yamanaka 1973c). The redox potentials of the core TOMES components and some inorganic sulfur compounds are listed in Table 3.

A reaction model of thiosulfate oxidation by TOMES was proposed by Friedrich et al. (2001) with the initial steps outlined as follows: in the presence of SoxYZ, SoxAX (the protein of this bacterium does not bind SoxK) initiates the oxidation resulting in covalent attachment of oxidized thiosulfate to a conserved cysteine of SoxY (Eq. 1 in "Thiosulfate oxidizing multienzyme system (TOMES or SOX)" section). SoxB would then hydrolytically release sulfate leaving the sulfane sulfur atom of thiosulfate on SoxY to yield persulfide (Eq. 2). In contrast to this scheme, we found that all three core TOMES components namely SoxAXK, SoxB, and SoxYZ were absolutely necessary for initiation of thiosulfate oxidation, and that no reduction of cytochromes (even as low as the stoichiometric amount of SoxYZ and SoxAXK) occurred in the absence of SoxB, indicating that participation of SoxB would also be required in the initial reaction step (Ogawa et al. in press).

The core TOMES also has sulfide dehydrogenase activity (see "Sulfide dehydrogenase activity of thiosulfate oxidizing multi-enzyme system" section), and the participation of all three core TOMES components was required for this activity (Ogawa et al. in press).

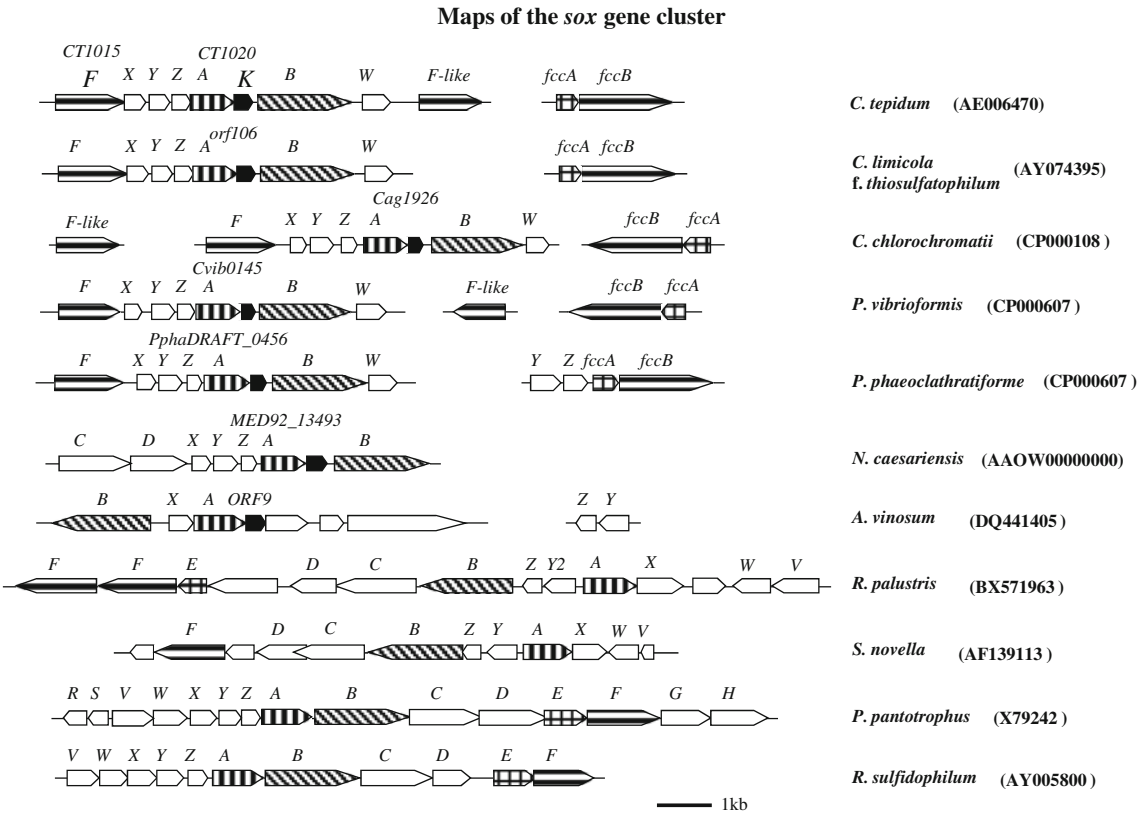

Fig. 2 Map of the *sox* gene cluster. *Black arrows*: SAXB gene (*soxK*) homologs. *Striped arrows* and genes are; *vertically*: *soxA*, *obliquely*: *soxB*, *horizontally*: *soxF*, *both vertically and horizontally*: *soxE*. Other hypothetical *sox* genes are shown in *light gray*. The sources used were (organism (GenBank nucleotide sequence accession number), accessed September 1, 2009): *Allochromatium vinosum* (DQ441405), *Chlorobium chlorochromatii* CaD3 (CP000108), *Chlorobium limicola* f. *thiosulfotophilum* (AY074395), *Chlorobium tepidum* TLS (AE006470), *N. caesariensis* (AAOW00000000), *Paracoccus pantotrophus* GB17 (X79242PDSOX (EMBL data library accession number), accessed 1 September 2009), *Pelodictyon phaeoclathratiforme* BU-1 (AAIK00000000), *Prosthecochloris vibrioformis* DSM 265 (CP000607), *Rhodopseudomonas palustris* CGA009 (BX571963), *Rhodovulum sulfidophilum* (AY005800), *Starkeya novella* DSMZ 506T (AF139113

Table 3 Redox potentials of sulfur compounds and *C. tepidum* proteins

	E_0' (mV)
Inorganic sulfur compounds	
SO_4^{2-}/SO_3^-	−515[d]
$S + 2H^+/H_2S$	−274[e]
$2SO_4^{2-}/S_2O_3^{2-}$	−245[d]
SO_4^{2-}/S^0	−200[d]
Bacillus PS3 membrane	
Menaquinone	−60[f]
C. tepidum	
SoxAXK cyt *c*-551	+161 and lower than −550[g]
rSoxX[a]	+153[g]
rSoxA[b]	lower than −550[g]
cyt *c*-554	+148[h]
cyt *c*-551 (PsaC)	+180[i]
cyt *c*-551 (PsaC)	+177[c,j]
P840	+230[i]

[a] Recombinant SoxX

[b] Recombinant SoxA

[c] 53 mV lower than P840 (230 − 53 = 177 (mV))

[d] Brune 1995b

[e] Japanese Biochemical Society 1984

[f] Liebl et al. 1992

[g] Ogawa et al. 2008

[h] Itoh et al. 2002

[i] Okumura et al. 1994

[j] Kusumoto et al. 1999

The monomeric flavoprotein encoded by the soxF

In many thiosulfate oxidizing bacteria, the genes encoding TOMES proteins generally occur in either a single or multiple cluster(s) (the *sox* gene cluster(s)). The genes encoding SoxAX, SoxB, and SoxYZ (the core TOMES) are invariably present in these bacteria, and the encoded proteins share relatively high amino acid sequence identity with each other irrespective of the presence or absence of *soxCD* (Friedrich et al. 2008). However, there are some differences among thiosulfate oxidizing bacteria with respect to the presence or absence of the genes *soxE* and *soxF* in addition to *soxK*, *soxC*, and *soxD* as described above. In *P. pantotrophus*, the *soxF* and *soxE* genes (the homologues of *Allochromagtium vinosum fccB* and *fccA* genes, respectively (Reinartz et al. 1998)), are found in the sox gene cluster (Friedrich et al. 2000), where they encode a flavoprotein and cytochrome homologue, respectively, of the dimeric flavocytochrome *c* (FCSD) complex that acts as a sulfide dehydrogenase (see "Flavocytochrome *c*-sulfide dehydrogenase (FCSD or FCC)" section). In the thiosulfate oxidizing bacterial species selected by Friedrich et al. (2008) for comparative studies, the majority of species have one or sometimes two *soxF* homolog(s) within the *sox* gene cluster with roughly half of them devoid of *soxE*. In a few species, the *soxF* gene is absent from the *sox* gene cluster.

The bacterium *P. pantotrophus* has both *soxF* and *soxE* genes in the *sox* gene cluster, but SoxF protein "as isolated" from the cells occurred as a monomer (Rother et al. 2001) and catalyzed sulfide oxidation with horse heart cyt *c* in the absence of SoxE (Quentmeier et al. 2004). A *soxF* deletion mutant of *P. pantotrophus* was shown to have a slower growth rate than wild-type cells grown on thiosulfate and mutant cells oxidized thiosulfate at a rate of 40% of the wild-type cells (Bardischewsky et al. 2006). The rate of oxidation of thiosulfate in crude cell-free extracts prepared from the mutant cells using horse heart cyt *c* as the electron acceptor, was significantly increased when supplemented with SoxF isolated from wild-type cells. However, when SoxF was added to the reconstituted system composed of four purified *sox* proteins (the three core TOMES (SoxAX, SoxB, SoxYZ), and SoxCD), the thiosulfate oxidizing activity was not significantly increased. From these results, the authors speculated that SoxF acts on some component or under certain conditions present only in whole cells and crude cell-free extracts, but not in the purified reconstituted system. The biochemical basis of the stimulating effects of SoxF in the crude extract and the possible functions of SoxF in thiosulfate oxidation in *P. pantotrophus* remain to be investigated.

The *sox* gene cluster of *C. tepidum* contains *soxF* (also called *soxJ*; Frigaard and Bryant 2008a, b), *soxF2* in *C. limicola f. thiosulfatophilum* (Verté et al. 2002), and *fccB* in *Allochromatium vinosum* Reinartz et al. 1998), but *soxE* is absent (Fig. 2). In *C. tepidum*, *fccA* and *fccB* encoding FCSD are found in tandem about 1 Mb away from the *sox* gene cluster. From *C. tepidum* cells, a monomeric flavoprotein SoxF that has sulfide dehydrogenase activity was purified to homogeneity (Ogawa et al. in press). In the presence of the core TOMES, SoxF stimulated the reduction of cyt *c*-554 from this bacterium by about two-fold, indicating a possible physiological function of SoxF. The degree of stimulation by SoxF was low when horse heart cyt *c* and yeast cyt *c* were used as the electron acceptors (Ogawa et al. in press), indicating the importance of the species of electron acceptor used for the in vitro assays and a need for a reexamination of the SoxF assay system in other bacteria including *P. pantotrophus*.

In *P. pantotrophus*, SoxYZ has been isolated mostly in an inactivated state (Quentmeier et al. 2007; Quentmeier et al. 2008) leading to recent speculation that it is activated by SoxF in vivo suggesting an additional possible physiological function of SoxF (Friedrich et al. 2008).

Sulfite dehydrogenase activity

Although GSB are known to be unable to grow on sulfite as the electron donor (Brune 1989, 1995b), the purified periplasmic core TOMES from *C. tepidum* catalyzed thiosulfate oxidation with externally added cyt *c*-554 as the electron acceptor. However, this activity was rather quickly inactivated in a few minutes at sulfite concentrations higher than 0.5 mM in vitro (Ogawa et al. in press), and this may be the reason why GSB apparently can not grow on externally added sulfite as the sole electron donor. Theoretical calculations show that sulfite readily condense with some thiols (RSH) to form $RSSO_2^-$ (Steudel and Steudel 2010), and this may be the cause of the observed inactivation of TOMES by sulfite.

As will be described below ("Dissimilatory sulfite reductase (DSR)" section), elemental sulfur appears to be oxidized to sulfite in the cytoplasm by the dissimilatory sulfite reductase (DSR) system (Dahl 2008).

Utilization of elemental sulfur

Transient elemental sulfur formation

Many GSB can use elemental sulfur, and they transiently deposit elemental sulfur outside the cells in the course of oxidation of sulfide and thiosulfate, which is finally oxidized to sulfate. Oxidation of the latters in the periplasmic space would yield polysulfides (including persulfide HSSH, the S^0 equivalent, see Fig. 1), which are then converted to elemental surfur. In contrast, purple sulfur bacteria transiently deposit elemental sulfur (or organic polysulfides) in the periplasmic space as globules surrounded by the proteins called Spgs (Pattaragulwanit et al. 1998). Three Spg proteins were identified from *Allochromatium vinosum* and two from *T. roseopersicina* (Brune 1995a; Grimm et al. 2008).

Results of in vitro experiments with the reconstituted core TOMES from *C. tepidum* showed that the stoichiometric amounts of cytochrome reduced were about two for each molecule of thiosulfate, sulfide or sulfite oxidized (Ogawa et al. in press). The ratio was also about two with SoxF-catalyzed sulfide oxidation. In these experiments, the amount of cytochrome reduced usually far exceeded (by at least eight-fold) the concentrations of the core TOMES components or SoxF2. This was also true with the sulfide dehydrogenase catalyzed by FCSD (Kusai and Yamanaka 1973b).

These results indicate that in GSB, the reconstituted TOMES yields two electrons and one sulfate per thiosulfate leaving a sulfane sulfur on SoxYZ in the periplasmic space, and this reaction cycle is repeated multiple times to yield the S^0 equivalent in a polysulfide group. The sulfide oxidation catalyzed by the monomeric SoxF and the dimeric FCSD appears to initially yield the S^0 equivalent in a polysulfide, which appears to be subsequently converted to elemental sulfur (S^0).

In inorganic chemistry, sulfanes (H_2S_n) (polysulfide) are known to be rather unstable compounds, and freshly prepared sulfane oil is a mixture of H_2S_4, H_2S_5, H_2S_6, H_2S_3 (in this order with decreasing concentrations) with the higher sulfanes up to H_2S_{25} and trace amounts of di- (H_2S_2), mono-sulfane (H_2S), etc. (Steudel 2003). Upon aging of the sulfane oil, in addition to the interchange of S^0, the following reactions take place:

$$2H_2S_n \rightarrow H_2S_{n+x} + H_2S_{n-x},$$

and

$$H_2S_n \rightarrow H_2S_{n-8} + S_8(\text{elemental sulfur}).$$

Because of this property of inorganic sulfane, it is conceivable that the polysulfide group bound to SoxY(SH) (or subsequently transferred to some thiol, RSH) will spontaneously liberate elemental sulfur in GSB:

$$\text{SoxY(S)} - S_nH \rightarrow \text{SoxY(S)} - S_{n-8}H + S_8$$

or

$$\text{SoxY(S)} - S_nH + RSH \rightarrow \text{SoxY(S)} - S_{n-m}H + RS_{m+1}H.$$

Alternatively, an as of yet unidentified factor(s) (either protein(s) or small molecule thiol(s)) may participate in the reaction.

The reactions of subsequent sulfur globule utilization

As in other bacteria, the biochemistry of the metabolic pathways of elemental sulfur is one of the least understood parts of inorganic sulfur metabolism in GSB, although several models with involvement of possible protein (s) have been proposed based on comparative genomics and mutant analyses (in GSB: Eisen et al. 2002; Chan et al. 2008a, c; Frigaard and Bryant 2008a, b, and in purple sulfur bacteria: Sander and Dahl 2009).

In the following discussion, we consider some of the possible candidate proteins in GSB.

Dissimilatory sulfite reductase (DSR)

The reverse dissimilatory sulfite reductase (DsrAB) of the purple sulfur bacterium *Allochromatium vinosum* (formerly *Chromatium vinosum*) is encoded together with 13 other proteins in the *dsr* operon (Dahl et al. 2005; Sander and Dahl 2009). Polar insertional mutations immediately upstream of *dsrA*, and in *dsrB*, *dsrH* and *dsrM*, were found to lead to an inability of the mutants to oxidize stored

sulfur S^0 (Pott and Dahl 1998). These genes were found to be not essential for sulfide or thiosulfate oxidation, implying that the enzyme works in the reverse direction in the oxidation of S^0 to sulfite. The Dsr proteins are either cytoplasmic or inner membrane-bound, indicating that the extracellular or periplasmic S^0 equivalent has to be transported across the cytoplasmic membrane. Reaction models of cytoplasmic sulfide oxidation involving a dissimilatory sulfite reductase DsrAB, a membrane-bound quinol oxidase-like DsrM, and ISP DsrK were presented (Pott and Dahl 1998), followed by more refined models (Grimm et al. 2008; Dahl 2008; Sander and Dahl 2009).

Similar *dsr* genes were found in the genome of *C. tepidum* (Eisen et al. 2002), and subsequently in all the genomes of GSB opened to the public thus far except *C. ferrooxidans* (Frigaard and Bryant 2008a, b), strongly suggesting that the Dsr system also works in GSB by oxidizing the S^0 equivalent to sulfite. A model of complete S^0 oxidation in GSB with participation of several Dsr proteins and several other proteins including some hypothetical ones has been proposed (Frigaard and Bryant 2008a).

The proposed biochemical pathway suggested by comparative genomic analyses leading to complete oxidation of intracellular sulfite to sulfate (in the reverse direction for those of *Archaea*) for *C. tepidum* is: adenosine phosphosulfate (APS) formation by APS reductase coupled to quinone reduction by Qmo (see below), and formation of sulfate from APS (possibly: APS + pyrophosphate → ATP + sulfate). For a further discussion of the possible metabolic pathways of sulfur oxidation in the cytoplasm involving Dsr, see Sander and Dahl (2009) (see also Fig. 1). The cytoplasmic sulfite oxidation pathway appears to slightly differ among GSB strains because the *qmo* genes are apparently absent in several GSB strains (Frigaard and Bryant 2008a; Frigaard and Dahl 2009).

Transmembrane transfers of the S^0 equivalents: several candidates and SoxVW homologues

Given that the Dsr system also functions in GSB, it follows that for intracellular oxidation of the extracellular elemental sulfur, the S^0 equivalent must be supplied to the cytoplasmic space by some mechanism. It is unclear in what form(s) the elemental sulfur is transformed, and how it crosses the outer membrane, the periplasmic space, and the inner membrane in GSB. From comparative genomic analyses, Frigaard and Bryant (2008a, b) proposed the following candidates for the transmembrane transport of S^0: polysulfide-reductase-like complexes PsrABC (a molybdopterin-containing PsrA, a [4Fe-4S]-cluster-binding PsrB, and a membrane-anchoring PsrC), sulfhydrogenase-like complex (CT0494, CT0495, and CT0496) and heterodisulfide-reductase-like complexes (encoded by CT1891-CY1894).

In the following, we discuss some additional candidates for the transmembrane transport of S^0. In *E. coli*, the protein DsbD (thiol:disulfide interchange protein) transfers disulfide across the inner membrane to the periplasmic space and is required for maturation of periplasmic cytochromes (thioether bond formation) (Missiakas et al. 1995; for a review, see Messens and Collet 2006).

The *sox* gene cluster of *P. pantotrophus* contains *soxVW*, which if mutated renders the cells unable to grow on thiosulfate (Bardischewsky et al. 2006). SoxW is a periplasmic protein containing a thioredoxin motif. SoxV is a homologue of DsbD, and is deduced to be a cytoplasmic membrane protein that has a cysteinyl group which transfers reducing equivalents from cytoplasmic thioredoxin to the periplasm, reducing target proteins such as SoxW in the periplasm (heterodisulfide reductase). From analyses of the *soxV* and *soxW* mutants of the purple bacterium *R. sulfidophilum*, it was found that SoxV, but not SoxW was essential to lithoautotrophic growth of this bacterium with thiosulfate (Appia-Ayme and Berks 2002). Furthermore, it was found that SoxV was required for maintaining SoxW in the reduced state in the presence of thiosulfate. Similar results were obtained with *sox* mutants of *P. pantotrophus*, and in addition, the TOMES protein levels of the mutants were found to be unaffected by the *soxV* mutation, indicating that the *soxV*-minus phenotype is not due to a defect in the maturation of cytochromes (Bardischewsky et al. 2006).

In GSB that use thiosulfate, *soxW* is invariably present in the *sox* gene cluster, but *soxV* is apparently absent from the cluster. With *E. coli* DsbD [AP_004637] as the query protein sequence, we found CT1075 from the *C. tepidum* genome with an *E*-value of 9e−66 from a BLASTP search result and that the gene was annotated as "thiol:disulfide interchange protein DsbD" by the KEGG internet site (http://www.kegg.jp/ja/, accessed January 14, 2010). All 12 of the publicly available whole genome sequences from GSB strains have a gene encoding a CT1075 homologue (at a minimum of 57% amino acid sequence identity). In GSB, the involvement of SoxW in the periplasm and DsbD in transferring the S^0 equivalent across the inner membrane from periplasm to cytosol (in the reverse direction that occurs in *E. coli* cells) seems to be a possibility (Fig. 1). Another possibility is that CT1075 carries S^0 (in the form of di-thiol) outside as in *E. coli*, because *Chlorobium ferrooxidans* has a *CT1075* homologue in its genome and is unable to grow when inorganic sulfur compounds are the electron donors.

In the vicinity of *CT1075*, there are several genes encoding putative proteins related to sulfur metabolism; CT1072; thiol-disulfide interchange protein DsbE (also called CcmG), CT1074; ApbE family protein, and CT1078 (the gene, in the reverse orientation); lipoic acid synthetase

LipA. In *P. pantotophus*, CcmG (DsbE) is a periplasmic thioredoxin required for *c*- and aa_3-type cytochrome biogenesis (Page and Ferguson 1997; Fabianek et al. 1998). In *Salmonella*, ApbE is reported to be a lipoprotein involved in thiamine synthesis, and the lack of ApbC or ApbE results in a defect in the Fe–S cluster metabolism (Beck and Downs 1998). LipA is assumed to be involved in sulfide donation in lipoate biosynthesis (Miller et al. 2000).

Qmo, a putative quinone-interacting membrane-bound heterodisulfide reductase complex

The outcome of S^0 oxidation seems to differ among GSB. In several strains including *C. tepidum*, the genes encoding the homolog of Qmo of the sulfate reducing *Archaea* were found from comparative genome analyses (Frigaard and Bryant 2008a, b). Chan et al. (2008c) created a *C. tepidum* mutant by deleting the region *CT868-876* including one of the putative *qmo* genes, and found that the mutant was completely defective for growth on thiosulfate as the sole electron donor, but only slightly defective for growth on sulfide or thiosulfate plus sulfide. Based on their results, they suggested that *CT0872* of previously unknown function is the most likely candidate for the thiosulfate oxidation phenotype observed in their mutant strain. Lending credence to this hypothesis is the conservation of *CT0872* in the chemolithotrophic sulfur oxidizing bacteria and *Archaea.*

Miscellaneous proteins possibly involved in inorganic sulfur metabolism

From comparative genome sequence analyses, in addition to the above described proteins, potential involvement of the following proteins in some part of sulfur metabolism have been discussed; rhodanese-like protein, SoyYZ (SoxYZ-related protein), Rubisco-like protein, etc. (for details, see Frigaard and Bryant 2008a, b; Frigaard and Dahl 2009)).

Creation of mutants by targeted inactivation of genes involved in sulfur metabolism

Chan et al. (2008a, b) pointed out the value of targeted mutant studies, particularly when there appears to be genetically redundant enzymes encoded within the genome. Chan et al. (2009) created a *C. tepidum* mutant by disrupting two putative SQR genes, and found that its SQR activity was completely abolished and that the mutant could still grow, although more slowly than the wild-type on sulfide as the sole electron donor. They found that transcripts of *CT2081* (*fccB*) encoding the flavoprotein of FCSD and of *soxF* (*CT1015* or *soxJ*) were detected at all stages of growth on sulfide, but failed to detect any sulfide dehydrogenase activity in vitro in the soluble or membrane fraction with horse heart cyt *c* as the electron acceptor. They speculated that the failure to detect any sulfide dehydrogenase activity might be ascribed to the fact that horse heart cyt *c* is not a suitable acceptor for FCSD of this bacterium. This might be the case as monomeric SoxF has sulfide dehydrogenase activity with a high affinity for sulfide, and cyt *c*-554 from this bacterium is by far a better electron acceptor than horse heart cyt *c* (Ogawa et al. in press).

Azai et al. (2009) reported that a mutant of *C. tepidum* devoid of the gene encoding cyt *c*-554 (a good electron acceptor in the reconstituted TOMES (Ogawa et al. 2008)) could grow on thiosulfate at slower rates than the wild type and that a double mutant devoid of both cyt *c*-554 and SoxB could not grow on thiosulfate. Their results indicate that SoxB is indispensable to in vivo oxidation of thiosulfate, and that in the absence of cyt *c*-554, some factor(s) mediates electron transport, although at much slower rate, between the core TOMES and RC-bound cyt *c*-551 that serves as an immediate electron donor to the oxidized primary donor $P840^+$. Because a variety of *c*-type cytochromes can accept electrons from the core TOMES (Kusai and Yamanaka 1973a, c, Ogawa et al. 2008, in press), it seems likely that some cytochrome(s) could mediate the electron transport in the mutant cells.

Future studies

There are various observations that have yet to be elucidated biochemically, and some of them are as follows.

- The reviews of Brune (1989, 1995b) inferred from reports that some GCB could use tetrathionate and perform a photochemical disproponation of S^0 into H_2S and $S_2O_3^{2-}$ probably by the reaction scheme: $3S + 3H_2O \rightarrow H_2S + S_2O_3^{2-} + 4H^+$.
- Photosynthetically growing cells of *Chlorobium limicola* f. *thiosulfatophylum* (DSM 249) were reported to transiently form thiosulfate from sulfide oxidation and to have disproponation activity of S^0 into H_2S and $S_2O_3^{2-}$ (Trüper et al. 1988).
- The mutants of *C. tepidum* created by disrupting one or both of the two putative SQR genes showed differences in susceptibility to high concentrations of sulfide (Chan et al. 2009).

Acknowledgment We thank M. Kitashima for comparative genomic analyses and preparation of the figures. This work was supported in part by the Global COE Program (Integrative Life Science Based

on the Study of Biosignaling Mechanisms) and by the High-tech Research Center project, MEXT, Japan to KI.

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