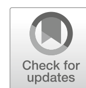
Bacterial Intracellular Sulphur Globules



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Abstract Reduced sulphur compounds such as sulphide, polysulphides, thiosulphate, and elemental sulphur are oxidized by a large and diverse group of prokaryotes. In many cases, intracellular globules of polymeric, water-insoluble sulphur are accumulated either as a transient product *en route* to sulphate or as the final product. Sulphur globule formation is especially widespread among sulphur-oxidizing Proteobacteria and occurs in purple sulphur bacteria of the family Chromatiaceae, in *Beggiatoa* species as well as in other "morphologically conspicuous" sulphur bacteria (e.g. *Thioploca*, *Achromatium, Thiovulum*). Sulphur globules are typically enclosed by a surface layer consisting of highly repetitive glycine-rich structural proteins (sulphur globule proteins, Sgps) and reside in the bacterial periplasm. Here, an overview of recent findings on the speciation of stored sulphur, the occurrence of Sgps and the enzymes involved in the formation and breakdown of bacterial sulphur globules is given.

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1 Introduction

Sulphur is the 16th element on the periodic table and the tenth most abundant element in the universe (Steudel and Chivers 2019). Sulphur serves essential functions in all living cells. In proteins it occurs not only in the form of cysteine and methionine, but also in iron-sulphur clusters, in several sulphur-containing cofactors like thiamine, biotin, coenzyme A and lipoic acid and is furthermore indispensable in tRNAs through a variety of modifications (Shigi 2014, 2018). Sulphur is a very versatile chemical element and undergoes permanent cycling in terrestrial as well as in marine environments. Dissimilatory sulphate reduction is the primary driver of the biogeochemical sulphur cycling. In this anaerobic respiratory process, sulphate is used as an electron acceptor instead of oxygen, nitrate or manganese [Mn(IV)] (Henkel et al 2019; Rabus et al 2015). In turn, hydrogen sulphide, polysulphides, thiosulphate, elemental sulphur and polythionates serve as electron donors for a huge array of chemo- and photolithotrophic bacteria and archaea such as *Acidithiobacillus* or *Acidianus* species (Dahl et al. 2008; Mangold et al. 2011; Kletzin et al. 2004; Frigaard and Dahl 2009; Dahl 2017). A large portion of these organisms forms sulphur globules both extracellularly and intracellularly (Dahl and Prange 2006; Dahl 2017; Maki 2013). Whether the sulphur accumulates as a transient or the final product varies depending on the species, the culture conditions and the reduced sulphur substrate.

Here, I attempt to give an update about the different sulphur-forming prokaryotes, the structure and chemical nature of bacterial sulphur inclusions and the metabolic pathways related to sulphur globule formation and degradation. An exclusive focus will be laid on sulphur globules deposited within the confines of the cell wall, i.e. sulphur present as a bacterial inclusion *senso strictu*. For further detailed information, the reader is referred to a number of reviews on oxidative sulphur metabolism (Frigaard and Dahl 2009; Dahl 2017; Wang et al. 2019; Friedrich et al. 2005; Dahl et al. 2008; Dahl and Prange 2006).

2 History

Internal sulphur globules are easily recognized even via light microscopy as they are highly light refractive (Fig. 1) and can reach diameters of several micrometres. Accordingly, the first mentioning of these conspicuous structures dates back to 1786, when Müller described intracellular spherical inclusions of unknown composition in ‘colourless’, egg-shaped algae (Müller 1786), later identified as *Thiovulum majus* (Rivière and Schmidt 2006). Over the following decades, several additional microorganisms were mentioned to contain similar inclusions (Ehrenberg 1838; Trevisan 1842; Perty 1852) and differentiated due to the presence of colour (the later *Thiospirillum*, *Chromatium*, *Lamprocystis*) and lack of colour (*Beggiatoa*, *Thiothrix* and *Thiovulum*). About a century after their discovery the cellular

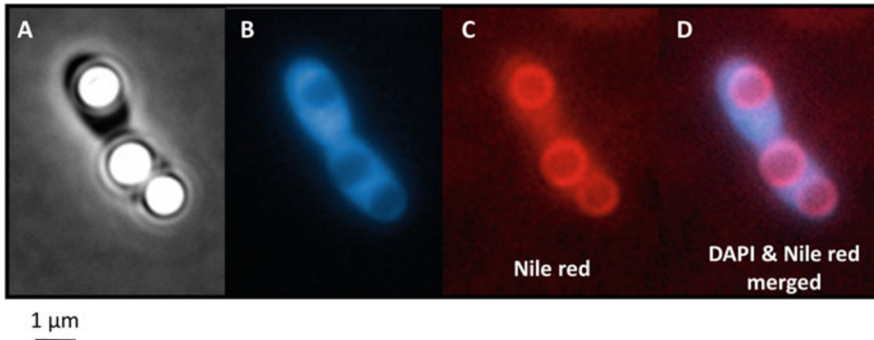

Fig. 1 *Allochromatium vinosum* DSM 180^T cells with sulphur globules visualized by light microscopy: **(a)** phase contrast **(b)** DNA stain with DAPI. In panel **(c)**, staining with Nile red highlights sulphur globules. **(d)**, DAPI and Nile red stain merged. Microscopy was carried out at room temperature using a Zeiss Axio Observer Z1 microscope (Zeiss, Jena, Germany) equipped with HXP 120 V light source and Axio Cam MR3 camera. Standard filter sets were used for DAPI (335–383 nm excitation and 420–470 nm emission) and Nile red (510–560 nm excitation and 590 nm long pass emission). Image acquisition and analysis were performed with Zen 2 software (Zeiss). Nile red is a useful probe of hydrophobic sites on proteins (Sackett and Wolff 1987) and probably interacts with the hydrophobic Sgps. The red halo in the Nile red image requires further investigation and currently remains unexplained. Photos courtesy of Fabian Grein

inclusions were proven to consist of elemental sulphur in *Beggiatoa* (C. Cramer in (Müller 1870); in *Allochromatium vinosum* (Cohn 1875); and in *Thiovulum muelleri* (Warming 1875)). A first systematic analysis of uncoloured and coloured 'sulphobacteria' and their sulphur globules was provided by Winogradsky (Winogradsky 1887), who also demonstrated the oxidation of hydrogen sulphide to stored sulphur under microaerophilic conditions in the chemotrophic *Beggiatoa* (Winogradsky 1889). Pioneering studies on the oxidation of sulphur in bacterial photosynthesis were done by van Niel whose classic studies about phototrophic sulphur bacteria and accumulation of elemental sulphur can be considered as milestones and provided the basis for further studies about sulphur compounds in photosynthesis (van Niel 1936; van Niel 1931). Those interested in the early research on sulphur bacteria are referred to discussions by (Waksman 1922; Waksman and Joffe 1922; Shively et al. 2006; Dahl and Prange 2006; Trüper 2008).

3 Elemental Sulphur

Naturally, sulphur occurs in a huge variety of environments (Cosmidis et al. 2019; Nims et al. 2019) such as volcanic areas including sulphidic springs (Macur et al. 2013; Kamyshny et al. 2014; Lau et al. 2017), deep-sea hydrothermal vents (Taylor et al. 1999), deep-sea hydrocarbon seeps (Eichinger et al. 2014) or marine sediments

and salt marshes (Kamyshny and Ferdelman 2010; Zopfi et al. 2004; Jørgensen and Nelson 2004; Taylor and Wirsen 1997).

Elemental sulphur can be formed through abiogenic processes when sulphide is oxidized by molecular oxygen, possibly catalyzed by oxidized metals (Luther et al. 2011). Its presence in the environment is often associated with microbial oxidation of reduced sulphur compounds (Kleinjan et al. 2003).

Sulphur forms more than 30 solid allotropes, more than any other element. It exists in many forms, from cyclic octamers (S_8 rings) that crystallize in different structures to sulphur chains with varying numbers of S-S bonds, and polysulphides (Sn^{2-}) (Kamyshny and Ferdelman 2010; Meyer 1976; Trofimov et al. 2009). Only a few sulphur allotropes occur in biological systems. The thermodynamically most stable form at standard conditions is homocyclic, orthorhombic crystalline α-sulphur (α-S_8) (*cyclo*-octasulphur) (Roy and Trudinger 1970; Steudel 1996a, b). S_6, S_7 and S_{12} rings have also been detected in samples of biological origin, while bigger rings up to S_{20} were made accessible by chemical synthesis (Steudel 1987, 2000). Commercially available sulphur consists mainly of S_8 rings, traces of S_7 rings that are responsible for the bright yellow colour (Steudel and Holz 1988) and polymeric sulphur. Polymeric sulphur consists of very long helically wound chains of almost all sizes (Steudel 2000). Regardless of the molecular size, all sulphur allotropes are hydrophobic, are not wetted by water and have very low solubilities in water (Steudel and Eckert 2003).

4 Organisms Forming Intracellular Sulphur Globules

Sulphur can accumulate in the form of water-insoluble globules as a transient or the final product during the oxidation of reduced sulphur compounds (sulphide, polysulphides, thiosulphate, polythionates and elemental sulphur). Accordingly, sulphur-forming bacteria share environments characterized by elevated levels of hydrogen sulphide mainly produced by bacterial sulphate reduction in anoxic sediments rich in organic nutrients or originating from hydrothermal vents or cold seeps.

Concerning their physiology, two large groups of sulphur-storing bacteria can be differentiated: The first are phototrophic prokaryotes that use sulphur compounds as electron donors for CO_2 fixation in the light (Dahl 2017). Among these, purple sulphur bacteria of the family Chromatiaceae form intracellular sulphur deposits. On the other hand, chemotrophic (the classical "colourless") sulphur-oxidizing prokaryotes use the energy derived from the oxidation of sulphur compounds with either oxygen, nitrate or Mn(IV) oxide as electron acceptors to fix carbon dioxide (Henkel et al. 2019; Dahl et al. 2008; Friedrich 1998; Kletzin et al. 2004; Wang et al. 2019). Sulphur compounds are also an important energy source for symbiotic associations of chemoautotrophic sulphur bacteria with marine organisms from unicellular protists (Ott et al. 2004) to metazoans, such as meduzoans (Abouna et al. 2015), bivalves (Frenkiel et al. 1996) and nematodes (Himmel et al. 2009).

Although first discovered at hydrothermal vents, symbiosis with sulphur oxidizers is not limited to these highly specialized environments but has also been found in shallow subtidal sands, macrophyte debris, deep sea cold seeps, mangrove swamps, sea grass beds, anoxic marine basins, sewage outfalls and even rotting whale carcasses (Distel 1998; Kleiner et al. 2012; Petersen et al. 2016; Seah et al. 2019; Cavanaugh et al. 1981; Felbeck 1981; Nelson and Fisher 1995).

Concerning their systematic affiliation, the vast majority of organisms with reported capability for the formation of intracellular sulphur globules belong to the Proteobacteria (Table 1). Notable exceptions are the Gram-positives *Thermoanaerobacter sulfurigignens* and *Thermoanaerobacterium thermosulfurigienes* (Lee et al. 2007), which fall into the class Clostridia within the Firmicutes phylum. Sulphur globules within the confines of the cell have also been detected in *Thermus scotoductus* (Skirnisdottir et al. 2001) belonging to the class Deinococci within the phylum Thermus-Deinococcus.

Most reports about intracellular sulphur deposition are available for members of the α-, β-, γ- and ε-Proteobacteria (Table 1). The trait is widespread though not ubiquitous in alphaproteobacterial, microaerophilic, autotrophic magnetotactic bacteria which form the globules upon growth on sulphide and/or thiosulphate (Bazylinski et al. 2004, 2013; Bazylinski and Williams 2006; Keim et al. 2005; Williams et al. 2006; Lefevre et al. 2012; Spring and Bazylinski 2000). Another example among the Alphaproteobacteria is *Azospirillum thiophilum* for which intracellular sulphur globule formation has been described upon growth in the presence of sulphide (Lavrinenko et al. 2010). Within the β-Proteobacteria, we find the genus *Macromonas* (La Riviere and Schmidt 1999). The large cells of this genus are characterized by voluminous inclusions of calcium oxalate. In addition, sulphur globules may be present. *Macromonas bipunctata* can oxidize sulphide to sulphur by means of hydrogen peroxide; however, this process does not allow energy conservation (Willems 2014). Furthermore, the Betaproteobacterium *Thermothrix azorensis*, an aerobic, thermophilic, obligately chemolithoautotrophic sulphur oxidizer, appears to form inclusions of sulphur under certain growth conditions (incomplete thiosulphate oxidation, pH above 7.0) (Odintsova et al. 1996). *Thiovulum* is a spectacular genus belonging to the ε-branch of the Proteobacteria that has primarily been defined observationally by its large egg-shaped cells that can reach a length of 5–25μm. In the cells, sulphur globules are often concentrated at one cell pole (Marshall et al. 2012; La Riviere and Schmidt 1999). *Thiovulum* has so far evaded isolation in pure culture but appears to be a chemolithoautotrophic microaerophile. A single-cell genome is available (Marshall et al. 2012).

Among the Gammaproteobacteria, formation of intracellular sulphur globules is especially widespread (Table 1). Many of these bacteria belong to families within the order Chromatiales. Sulphur deposition is a characteristic trait of many purple sulphur bacteria of the family Chromatiaceae (Dahl 2017), while *Thiorhodospira sibirica* is the only phototrophic member of the family Ectothiorhodospiraceae that is capable of intracellular sulphur deposition (Bryantseva et al. 1999). In fact, this organism also forms extracellular sulphur deposits, the name-giving feature of the family. The Ectothiorhodospiraceae harbour additional species that store sulphur

Table 1 Systematic affiliation of selected proteobacteria with the capability of sulphur globule formation

Organism	Properties	(Predicted) *sgp* genes[a]	Molecular masses calculated without signal peptide [Da]	Enzyme (systems) for sulphur oxidation	References
Alphaproteobacteria					
Rhodospirillales					
Rhodospirillaceae					
Magnetovibrio blakemorei DSM 18854[T])	Chemolithoautotrophic growth on sulphide and thiosulphate, internal sulphur-rich globules upon growth on sulphide	BEN30_14055, 07955, 10335	13.253, 12.979, 12.497	SqrA, SqrC, SqrF, FccAB, SoxXYZAB, Dsr, Soe, Apr, Sat	Bazylinski et al. (2013), Trubitsyn et al. (2016)
Azospirillum thiophilum DSM 21654[T]	Simultaneous utilization of organic substrates and thiosulphate under microaerobic conditions, sulphur globules upon growth in the presence of sulphide	VY88_24200, 06955	9.610, 9.204	SqrA, SoxCDYZAXB	Kwak and Shin (2016), Lavrinenko et al. (2010), Frolov et al. (2013)
Betaproteobacteria					
Burkholderiales					
Comamonadaceae					
Macromonas sp. BK-30	Aerobic chemoorganotroph, oxidation of sulphide to sulphur does not provide useful energy	None identified		SoxY, partial SoxZ, SoxB	Willems (2014), Dubinina and Grabovich (1984) NZ_NWBQ01000022

Gammaproteobacteria					
Chromatiales					
Chromatiaceae					
Allochromatium vinosum DSM 180^T	Phototrophic purple sulphur bacterium	**Alvin_0358 (SgpB), 1325 (SgpC), _1905 (SgpA), _2515 (SgpD)**	10.650, 8.482, 10.497, 21.033	SqrD, SqrF, FccAB, SoxBXYZA, Dsr, Soe, Apr, Sat	Weissgerber et al. (2014), Weissgerber et al. (2011), Brune (1995a)
Thiocapsa roseopersicina DSM 217^T	Phototrophic purple sulphur bacterium	SAMN05421783_111111 (SgpA), 1099 (SgpA), 113134 (SgpA), 101470 (SgpC), 11156 (SgpD)	11.158, 9.914, 10.380, 8.936, 24.160	SqrC, SqrD, SqrF, FccAB, SoxBXYZA, Dsr, Soe, Apr, Sat	Pfennig and Trüper (1971), Brune (1995a)
Ectothiorhodospiraceae					
Thioalkalivibrio paradoxus ARh 1 (DSM 13531^T)	Obligately aerobic chemolithoautotrophs, sulphur globules as obligate intermediates on thiosulphate and thiocyanate	THITH_04870, 06175, 07650, 08045 (SgpA)	10.045, 10.508, 10.537, 11,546	SqrF, FccAB, SoxYZ, SoxB, Dsr, Soe, Apr, Sat, TcDH	Berben et al. (2015)
Thiorhodospira sibirica ATCC 70058	Photolithoautotroph, sulphur deposition in periplasm and extracellularly, thiosulphate not oxidized	ThisiDRAFT_0642 (SgpA), 2422	11.560, 12.810	SqrB, SqrD, FccB, SoxYZ, sHdr, Soe	Bryantseva et al. (1999)
Thiotrichales					
Thiotrichaceae					
Achromatium sp. (*Candidatus* Achromatium palustre)	Single-cell genome, sulphur inclusions, presumably microaerophilic chemolithotrophs, oxidizes reduced sulphur compounds to sulphate	TI05_14580 (SgpA), 15635 (SgpA), 11440 (SgpA)	11.641, 10.438, 10.617	SqrA, SoxBYZXA, Dsr, Apr, Sat	Mansor et al. (2015), Salman et al. (2016)

(continued)

Table 1 (continued)

Organism	Properties	(Predicted) *sgp* genes[a]	Molecular masses calculated without signal peptide [Da]	Enzyme (systems) for sulphur oxidation	References
Beggiatoa leptomitoformis D-401	Chemolithoautotrophic growth with sulphide and thiosulphate, intracellular sulphur globules	BLE401_16800 (SgpA)	12.649	SqrA, SqrF, FccB, SoxYZ, SoxB	Dubinina et al. (2017)
Thiothrix caldifontis DSM 21228[T]	Capacity for chemolithoautotrophic growth with thiosulphate and sulphide, intracellular elemental sulphur and sulphate formed	SAMN05660964_00436, 01322, 01876, 01943, 00727, 02054	12.348, 13.846, 24.730, 23.558, 17.120, 17.382	SqrA, SqrF, FccA, FccB, SoxBZYAZAX, Dsr, Soe, Apr, Sat	Chernousova et al. (2009)
Candidatus Thiomargarita nelsonii	Chemolithotroph, oxidizes sulphide to sulphate, intracellular sulphur globules as intermediates	PN36_24595 (SgpA), 24600	11.726, 14.521	SqrF, FccB, SoxYZAXB, Dsr, Soe, Apr, Sat	Flood et al. (2016)
Thiolinaceae					
Thiolinea disciformis DSM 14473[T]	Thiosulphate and sulphide oxidized during chemoheterotrophic growth, sulphur within invaginated inner membrane, sulphate formation weak	A3IE_RS0105985	23.978	SqrA, SqrF, FccB, SoxB, SoxXA, SoxYZ, Dsr, Soe	Boden and Scott (2018)
Thiofilaceae					
Thiofilum flexile DSM 14609[T]	Thiosulphate and sulphide oxidized during chemoheterotrophic growth, sulphur within invaginated inner membrane	A3IK_RS21110	24.794	SqrA, SoxYZAXB, Dsr, Soe	Boden and Scott (2018)

Epsilonproteobacteria					
Campylobacteriales					
Thiovulaceae					
Thiovulum	Aerobic chemoautotroph, oxidizes hydrogen sulphide to elemental sulphur	None identified		SqrD, SqrF	Marshall et al. (2012)
Unclassified Gammaproteobacteria					
Sedimenticola selenatireducens DSM 17993^T	Chemolithoautotrophic growth with thiosulphate, sulphide, tetrathionate, under hypoxic or anaerobic conditions, sulphur globules formed as intermediates	FHP88_07550 (SgpA), FHP88_10320 (SgpA)	8.737, 19.305,	SqrA, SqrF, FccAB, SoxBYZAX, Dsr, Soe, Apr, Sat	Flood et al. (2015)
Thiolapillus brandeum Hiromi 1	Facultative chemolioautotroph, growth on sulphur, thiosulphate and tetrathionate with oxygen or nitrate	TBH_C1660 (SgpA), C0689 (SgpA), (SgpA)	10.985, 10.494, 10.265	SqrA, SqrF, FccAB, SoxXAZYB, Dsr, Soe, Apr, Sat	Nunoura et al. (2014)
Endosymbiont of *Riftia pachyptila*	Trophosome tissue of *Riftia pachyptila* (Annelida, Siboglinidae)	Rifp1Sym_bd00100 (SgpA), at00150 (SgpA), fc00030 (SgpD)	9.039, 7.598, 23.147	SqrA, FccAB, SoxB, SoxAX, SoxYZ, dsr, Soe, Apr, Sat	Gardebrecht et al. (2012), Markert et al. (2007)

(continued)

Table 1 (continued)

Organism	Properties	(Predicted) *sgp* genes[a]	Molecular masses calculated without signal peptide [Da]	Enzyme (systems) for sulphur oxidation	References
Solemya velesiana gill symbiont Sveles-Q1	Gill tissue from *Solemya velesiana* (Mollusca, Solemyidae)	BOW51_07440 (SgpA), 08055 (SgpA/B), 05805 (SgpA), 05810 (SgpA), 07780 (SgpA)	9.094, 9.232, 17.092, 9.741, 20.658	SqrA, SqrF, FccAB, SoxXAYZB, Dsr, Soe, Apr, Sat	Russell et al. (2017)

[a]Genes predicted to encode sulphur globule proteins were identified by BLAST searches without compositional adjustments and without filtering for low complexity regions using the resources provided by Integrated Microbial Genomes (DOE Joint Genomes Institute, http://img.jgi.doe.gov) and GenBank (http://www.ncbi.nlm.nih.gov). The established sulphur globule proteins from *Allochromatium vinosum* (SgpA, Alvin_1905, SgpB, Alvin_0358, SgpC, Alvin_1325, SgpD, Alvin_2515) are printed in bold and were used as bait. For those predicted sulphur globule proteins, which clearly resemble one of the established *A. vinosum* Sgps more than the others, the most closely related *A.* vinosum protein is given in brackets. All listed predicted *sgp* genes encode Sec-dependent signal peptides. At this point, it cannot be excluded that the predicted *sgp* genes for the listed organisms are still incomplete. Baits for Blast searches for sulphur-oxidizing enzyme systems: FccAB from *Allochromatium vinosum* (AAA23316, AAB86576), SqrA from *Aquifex aeolicus* (NP_214500), SqrB from *Halorhodospira halophila* (WP_011814451), SqrC from *Chlorobaculum tepidum* (NP_661917), SqrD from *C. tepidum K(NP_661023), SqrE* from *C. tepidum* (NP_661769), SqrF from *A. aeolicus* (NP_213539), Dsr proteins (Alvin_1251 to Alvin_1262), AprBA (Alvin_1119–1120), Sat (Alvin_1118), SoeABC (Alvin_2492–2489) from *A. vinosum*, sHdr proteins from *Acidithiobacillus caldus* (Atc_2352–234

internally; these belong to the chemoautotrophic genus *Thioalkalivibrio* (Sorokin et al. 2003; Berben et al. 2015; Mu et al. 2016; Ahn et al. 2017). Recently, a member of the Thioalkalispiraceae, *Endothiovibriovibrio diazotrophicus*, was also described as containing intracellular sulphur globules (Bazylinski et al. 2017). The Thiotrichales are the second gammaproteobacterial order containing a variety of sulphur-storing chemotrophic sulphur oxidizers. Among these, the family Thiotrichaceae features some of the most conspicuous bacteria in nature. Species of the genera *Thiomargerita* and *Achromatium* as well as the filamentous sulphur-oxidizing bacteria of the genera *Beggiatoa*, *Thiothrix* and *Thioploca* are among the largest known prokaryotes (Mansor et al. 2015; Schulz and Jørgensen 2001; Schulz et al. 1999; Salman et al. 2016) and characterized by massive sulphur formation. The only representatives of the families Thiofilaceae and Thiolinaceae described so far also form intracellular sulphur globules (Boden and Scott 2018).

A vast majority of bacterial partners in thiotrophic symbioses with eukaryotes are taxonomically unclassified Gammaproteobacteria (Table 1). Regardless of whether the host is a protist or an invertebrate and whether the bacteria are associated as endo- or as ectosymbionts, formation of sulphur globules inside of their cells has often been noted (Grimonprez et al. 2018; Rinke et al. 2006, 2009; Bergin et al. 2018; Seah et al. 2019; Markert et al. 2011; Krieger et al. 2000; Frenkiel et al. 1996).

5 Subcellular Localization of Sulphur Globules

Internal sulphur globules are easily recognized even via light microscopy as they are highly light refractive (Fig. 1). Cultures and colonies of cells containing sulphur globules, therefore, exhibit a characteristic milky appearance (Fig. 2). Usually the diameter of sulphur globules is in the range of 1–3μm (Fig. 1), but sizes exceeding 15μm have also been reported (Williams et al. 1987; Head et al. 1996; Remsen 1978;

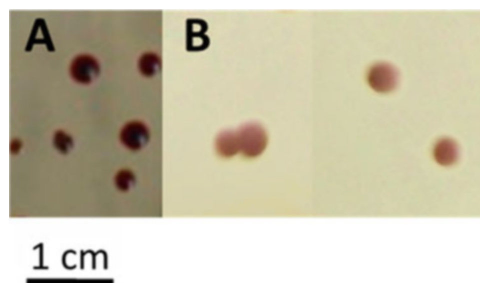

Fig. 2 Colonies *Allochromatium vinosum* DSM 180^{T} grown (**a**) on malate in the absence of reduced sulphur compounds (**b**) grown in the presence of sulphide and thiosulphate. Colonies appear milky-white due to massive accumulation of sulphur globules inside of the cells. *A. vinosum* was cultivated for 10 days on plates solidified with 1% (w/v) phytagel as described by (Pattaragulwanit and Dahl 1995)

Skirnisdottir et al. 2001). The sulphur can comprise 20–34% of the cell dry mass of *Beggiatoa* sp. and purple sulphur bacteria, respectively (Nelson and Castenholz 1981; Overmann 1997). While sulphur globules appear to be randomly localised in many bacterial species, specific cellular localizations have also been reported. In *Thiovulum* for example, the globules accumulate toward one cell pole (Marshall et al. 2012; La Riviere and Schmidt 1999).

A series of technical problems must be considered when it comes to elucidating the subcellular localization of sulphur globules using microscopic techniques. Sulphur dissolves during the preparation of biological samples for electron microscopy, and in addition, any remaining sulphur is subject to thermal degradation under the electron beam. Therefore, sulphur deposits appear as a conspicuous, empty, and electron-lucent space in electron micrographs (Strohl et al. 1981; Remsen and Trüper 1973; Vetter 1985; Pasteris et al. 2001). Nevertheless, microscopic evaluation has resolved the periplasm as the intracellular compartment harbouring sulphur globules in many cases. In studies with free-living filamentous sulphur bacteria, including *Thiothrix* (Bland and Staley 1978; Larkin and Shinabarger 1983; Williams et al. 1987), *Thioploca* (Maier and Murray 1965), *Thiofilum* and *Thiolinea* (Boden and Scott 2018) as well as *Beggiatoa* (de Albuquerque et al. 2010; Maier and Murray 1965; Larkin and Strohl 1983), sulphur inclusions were found to be located within invaginated pockets of the cytoplasmic membrane. In some cases, the sulphur globules appeared as a membrane-bound inclusion in the cytoplasm with no apparent connection to the cytoplasmic membrane (Strohl et al. 1981), which may be an effect of the specific sectioning plane (Shively et al. 1989). Other examples for which a periplasmic localization of sulphur globules has been settled are species of the genera *Thioalkalivibrio* (Sorokin et al. 2001) and *Thermus* (Skirnisdottir et al. 2001).

In some cases, it has been problematic to distinguish putative sulphur vesicles from other vesicle-like storage structures such as polyhydroxyalkanoate bodies. This applies especially to chemoautotrophic sulphur–oxidizing endosymbionts that reside in animal organs, e.g., in specialized gills of Vesicomyid clams (Goffredi and Barry 2002) or in so-called trophosomes in Vestimeniferan worms like *Riftia pachyptila* (Felbeck 1981; Cavanaugh 1983). Inside these organs, the symbiontic bacterial cells exhibit roundish to polymorphic electron-translucent vesicles whose membranes are infoldings of the cytoplasmic membrane, and the enclosed spaces are contiguous with the periplasmic space. Although these vesicles obviously share common ultrastructural characteristics with sulphur-containing globules of other organisms, it has been debated whether these structures are indeed related to sulphur storage (Bright and Sorgo 2003; Maina and Maloyi 1998; Vetter 1985). On the other hand, electron spectroscopic imaging pictures clearly identified sulphur in the globules of gutless oligochaete worm endosymbionts. A cytoplasmic localization was inferred for the globules without analysis via cryo-EM (Krieger et al. 2000).

Interpretation of electron micrographs of phototrophic bacteria containing sulphur globules is complicated by the dense packing of these cells with intracytoplasmic membranes harbouring the photosynthetic apparatus. These so-called chromatophores are associated with sulphur globules in a highly organized

manner. Careful inspection of electron micrographs revealed that some chromatophores, the insides of which are extracytoplasmic or periplasmic (depending on whether the insides are continuous with the periplasm or not), open into the space enclosing the sulphur globules, thus implying an extracytoplasmic location for the globules themselves (Pattaragulwanit et al. 1998).

It is obvious from the last paragraphs that high-resolution microscopy has so far not provided assignment of the correct subcellular compartment for sulphur deposition in all cases. Fortunately, another very valuable information resource is available. As early as 1963, an envelope was reported for the sulphur globules of the purple sulphur bacterium *A. vinosum* (Kran et al. 1963) that was soon identified as a protein envelope (Nicolson and Schmidt 1971; Schmidt and Kamen 1970). In *A. vinosum*, the envelope consists of four different proteins of 8.5–20.8 kDa named SgpA, SgpB, SgpC and SgpD (Brune 1995a; Pattaragulwanit et al. 1998; Weissgerber et al. 2014). Relative transcript abundances for all four of the corresponding genes strongly increase upon the exposure of the cells to sulphide, thiosulphate and elemental sulphur compared to photoorganoheterotrophic growth on malate in the absence of reduced sulphur compounds (Weissgerber et al. 2013; Weissgerber et al. 2014). All four proteins are synthesized as precursors carrying amino-terminal signal peptides mediating Sec-dependent transport across the cytoplasmic membrane (Weissgerber et al. 2014; Pattaragulwanit et al. 1998). The proposed targeting process was experimentally confirmed with a *sgpA-phoA* fusion in *E. coli* (Pattaragulwanit et al. 1998) which finally resolved the subcellular localization of the globules in purple sulphur bacteria of the family Chromatiaceae. Single-layered electron-dense envelopes of 2–5 nm have also been observed for the sulphur globules of *Thioalkalivibrio paradoxus* (Berben et al. 2015) as well as for *Beggiatoa* and *Thiothrix* species (Strohl et al. 1981; Williams et al. 1987). In *Beggiatoa alba* BL15D, the envelope is pentalaminar, 12–14 nm thick and consists of three electron dense layers of 3.5, 2.1 and 3.5 nm thickness (Strohl et al. 1982). Sulphur inclusion envelopes have been described as being fragile in fixatives used for transmission electron microscopy (Strohl et al. 1981) which may explain why they are not always visible in electron micrographs of sulphur-depositing bacteria. In fact, recently performed BLAST searches revealed the presence of genes encoding putative sulphur globule proteins targeted to the periplasm in almost all genome-sequenced, globule-forming Proteobacteria analyzed (unpublished) with *Thiovulum* and *Macromonas* as the only notable exceptions. Table 1 provides an overview of selected species. The number of predicted *sgp* genes in a given organism can vary from only one, e.g., in *Beggiatoa alba* or in *Thiolinea disciformis* to six in *Thiothrix caldifontis* (Table 1) and even 15 in *Thiothrix lacustris* (not shown). Taken together, these observations provide strong indication that the general target compartment for sulphur storage is not the bacterial cytoplasm, but that deposited sulphur is separated from the cytoplasm by a unit membrane which may be continuous with the cytoplasmic membrane, depending on the organism.

6 Properties and Function of Sulphur Globule Proteins

Brune already noted in 1995 that SgpA, SgpB and SgpC from the purple sulphur bacteria *A. vinosum* and *Thiocapsa roseopersicina* exhibit sequence similarity with structural proteins containing repetitive amino acid sequences rich in regularly spaced glycine-like cytoskeletal keratins, insect and blood fluke egg-shell proteins and plant cell wall proteins (Brune 1995a). SgpC shows some sequence similarity to Gly and Trp-rich regions of prion proteins (Brune 1995a). SgpD from *A. vinosum* appears to be a coiled-coil protein (Weissgerber et al. 2014). The coiled coil is a protein motif characterized by superhelical twisting of two or more alpha helices around one another. They can form rod-like tertiary structures and include the intermediate filaments of the metazoan cytoskeleton as well as bacteria specific cytoskeletal proteins that typically assemble into stable macromolecular scaffolds (Lin and Thanbichler 2013; Rose and Meier 2004). In general, sulphur globule proteins appear to be rich in glycine, alanine and asparagine. Tyrosine and glutamine and proline can also be major constituents, depending on the protein (Brune 1995a).

In plant cell walls, glycine-rich proteins form an important group of structural protein components (Ringli et al. 2001). The primary sequences of these proteins contain more than 60% glycine, which is considerably higher than the glycine content in bacterial Sgps (Brune 1995a). Just as in glycine-rich proteins from plants, the sequences of Sgps often follow the motif $(Gly\text{-}X)_n$ in the glycine rich regions. In the proteins forming sulphur globule envelopes, Ala, Pro, Ser and Tyr are common at the X position. In some cases the motif varies, e.g. $(G\text{-}G\text{-}X)_n$, or is more complex. The structures proposed for the glycine-rich plant cell-wall proteins are antiparallel beta-pleated structures analogous to that of silk fibroin, in which the side chains of the X residues in the GXGX repeats all lie on one side of the sheet (Condit and Meagher 1986; Keller et al. 1988). If the sulphur globule proteins fold in a similar way, this may help to explain how they aid preserving the enclosed hydrophobic sulphur in a reactive state and at the same time impart hydrophilic properties to the globule surface (Steudel 1989). In the future, it will certainly be necessary to study secondary structure of Sgps in detail.

The presence of a protein envelope around the sulphur inclusions in sulphur-oxidizing bacteria suggests an important structure–function relationship. Indeed, mutants of *A. vinosum* lacking SgpB and SgpC are no longer able to oxidize sulphide and thiosulphate and to form sulphur inclusions from these sulphur compounds (Prange et al. 2004). In *A. vinosum* SgpA and SgpB can replace each other in the presence of SgpC. Still, SgpB and SgpA are not fully competent to replace each other as sulphur globule formation is not possible in mutants possessing soley SgpA or SgpB (Prange et al. 2004). A mutant containing SgpA and SgpB but lacking SgpC can grow on sulphide and thiosulphate. As this mutant forms significantly smaller sulphur globules, SgpC probably plays an important role in sulphur globule expansion. The construction of mutants lacking SgpA and SgpC or SgpA, SgpB and SgpC was not possible, leading to the conclusion that a basic level of Sgps is obligatory for cell survival even under conditions that do not allow sulphur globule formation

(Prange et al. 2004). SgpD appears to be the most abundant of the *A. vinosum* sulphur globule proteins (Weissgerber et al. 2014). Genetic information about its role is not available because mutants lacking the respective gene have not yet been analyzed. The analysis of the *A. vinsoum* sulphur globule proteome revealed SgpB as the second most abundant sulphur globule protein in this organism while peptides originating from SgpA and SgpC were less frequently detected (Weissgerber et al. 2014). In *A. vinosum*, all four *sgp* genes form separate transcriptional units (Pattaragulwanit et al. 1998; Prange et al. 2004; Weissgerber et al. 2013). All four genes are constitutively expressed and their expression is significantly enhanced in the presence of sulphide and thiosulphate (Weissgerber et al. 2013; Prange et al. 2004).

Interestingly, cells of *Beggiatoa alba* grown in the absence of sulphur compounds apparently contained small rudimentary sulphur inclusion envelopes. It was hypothesized that these envelopes were present in collapsed form until a reduced sulphur source became available (Strohl et al. 1982). A direct/covalent attachment of chains of stored sulphur to the proteins enclosing the globules does not appear to occur as a vast majority of studied or predicted Sgps do not contain any cysteine residues (Brune 1995a; Weissgerber et al. 2014). It has been speculated that protein envelope may provide binding sites for sulphur-metabolizing enzymes (Schmidt et al. 1971). To elucidate the possibility that enzymes taking part in sulphur globule formation and/or oxidation are bound to or interact with the envelope proteins, similar to the situation found for polyhydroxyalkanoate (PHA) granules (Jendrossek 2009), the sulphur globule proteome of *A. vinosum* was enriched and analyzed (Weissgerber et al. 2014). While this approach identified 78 proteins that occur exclusively in the sulphur globule proteome and were not detected in the soluble and membrane fractions, none of the established components of the periplasmic sulphide- and thiosulphate-metabolizing enzymes appeared to be enriched with the globules.

7 Speciation of Sulphur

The chemical nature of the sulphur in the globules has been the subject of intensive controversy (Pickering et al. 1998; Prange et al. 1999b, 2002; George et al. 2002, 2008; Berg et al. 2014; Pasteris et al. 2001). It has been recognized several decades ago that this sulphur has several properties that do not go along with those of elemental sulphur outside of biological systems. The first discrepancy relates to its low density of 1.2 (Guerrero et al. 1984), compared to 2.1 for the common α-sulphur (Meyer 1976). Moreover, globule sulphur has been described as ‘liquid’ and ‘hydrophilic’ (Steudel 1989; Hageage et al. 1970), while all allotropes of sulphur are solid and virtually water-insoluble at room temperature. In fact, analysis of sulphur in biological systems is generally hampered by the variety of possible reactions, the high reactivity and short lifetime of sulphur compounds with intermediate oxidation states that may be formed during these reactions, the allotropic enantiotropy of S_8 and its ability to catenate (Steudel 1982).

The first study focussing on the speciation of sulphur in sulphur globules dates back to 1970, applied polarizing microscopy and X-ray diffraction to a purple sulphur bacterium, A. *vinosum* (Hageage et al. 1970), and culminated in the conclusion that the sulphur is present in a 'liquid' or 'liquid-like' state. Later, this was questioned because Raman spectroscopy provided evidence for predominance of S_8 sulphur in the globules from *Beggiatoa* and *Thioploca* (Pasteris et al. 2001). Several other studies applied synchrotron-based X-ray absorption near-edge structure spectroscopy (XANES) at the sulphur K-edge to investigate the nature of intracellular sulphur globules (George et al. 2008; Pickering et al. 2001; Prange et al. 1999a, 2002; Lee et al. 2007), however, with contradicting results. Our own work indicated different speciation depending on metabolic properties of the organisms and the environmental conditions: long sulphur chains very probably terminated by organic residues (mono- or bisorganyl polysulphanes) in purple sulphur bacteria, *cyclo*-octasulphur in chemotrophic sulphur oxidizers like *Beggiatoa alba* and *Thiomargararita namibiensis* and long chain polythionates in the aerobically grown acidophilic sulphur oxidizer *Acidithiobacillus ferrooxidans* (Prange et al. 1999a, 2002). Others pointed out shortcomings of the detection method applied that may suffer from spectroscopic distortions dependent upon particle size and compositions. Experimental data was provided suggesting that the spectral differences observed for the sulphur globules from different organisms are not due to differences in sulphur speciation but are solely due to differences in the particle sizes of the sulphur globules (George et al. 2008). More recently, Raman spectroscopy has been applied to various sulphur globule-forming bacteria. This non-destructive analytical technique circumvents many of the problems associated with other characterization methods, as measurements can be collected on solid, liquid and live samples at room temperature and atmospheric pressure. Characteristic internal vibrational (molecular) spectra make elemental sulphur easy to detect and characterize (Eichinger et al. 2014; Pasteris et al. 2001; Berg et al. 2014; Oren et al. 2015; Maurin et al. 2010; Himmel et al. 2009). Additionally, Raman mapping produces high spatial (~1μm) and spectral resolution. A first Raman study on the globules of *Thioploca* and *Beggiatoa* indicated the presence of S_8 in a nano-crystalline form (Pasteris et al. 2001). Another study also identified S_8 as the main form of elemental sulphur in sulphur globules produced in *Beggiatoa* filaments, but only in subpopulations located at the sulphide–oxygen interface of gradient tubes or in early growth stage cultures (Berg et al. 2014). *Beggiatoa* mats in a deeper sulphide-rich, anoxic zone, and freshwater gradient cultures gave rise to Raman signals suggesting a mixture of S_8 rings and linear polysulphides (S_n^{2-}) within the globules. In vivo Raman spectra for *Thiothrix* presented the characteristic S_8 structure previously described for the crystalline S_8 standards and molten sulphur in the internal modes (Nims et al. 2019). The significance of the speciation of sulphur lies in the bioavailability of the different forms, i.e. amorphous polymeric or (nano)crystalline elemental sulphur. It has been shown, for example, that the purple sulphur bacterium *Allochromatium vinosum*, uses only the polymeric component and not the cyclo-octasulphur component when commercially available sulphur is provided as the substrate, i.e. the organism has difficulties to attack the comparatively stable S_8 rings

(Franz et al. 2007). It is possible that this also applies to sulphur stored inside of cells such that it is deposited in a chemical form that is readily available for further degradation.

8 Formation of Stored Sulphur

In general, sulphur stored in sulphur globules is formed by the oxidation of more reduced sulphur species. The location of the sulphur inclusions in the periplasmic space implies that globule formation from sulphide, polysulphides, thiosulphate, elemental sulphur and also the much less widely used substrate thiocyanate must occur in this cellular compartment (Fig. 3).

The main characterized enzymes for sulphide oxidation, FAD-containing flavocytochrome *c* (FccAB) and sulphide:quinone oxidoreductases (SQR) indeed

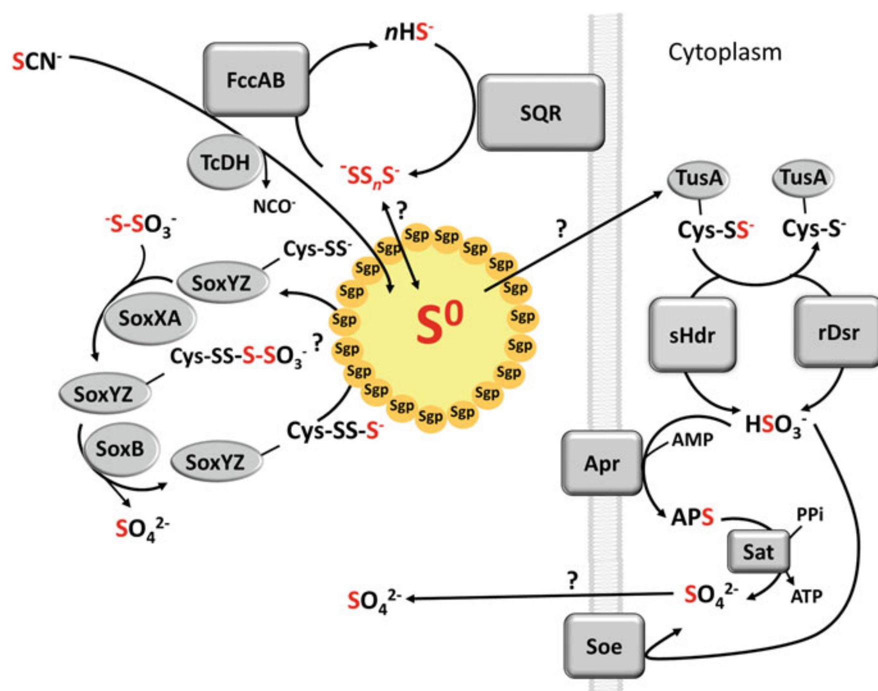

Fig. 3 Pathways of sulphur globule formation and degradation. A given organism may contain all or only a subset of the pathways depicted (cf. Table 1). For clarity, reactions are not given with exact stoichiometries. TcDh, thiocyanate dehydrogenase. SQR, sulphide:quinone oxidoreductase, FccAB, flavoctochrome *c* sulphide dehydrogenase, rDsr, sulphur oxidizing Dsr system, sHdr, sulphur-oxidizing heterodisulphide reductase-like system, Apr, APS reductase, Sat, ATP sulfurylase. Question marks indicate that neither the pathways of sulphane sulphur transport into the cytoplasm nor sulphate export from the cytoplasm have been experimentally clarified

reside in or are oriented towards the periplasm. All characterized SQRs are single-subunit flavoproteins asscociated with the cytoplasmic membrane. Based on protein structure, six distinct types of single subunit flavoprotein SQRs were identified (Marcia et al. 2009, 2010a, b; Shahak and Hauska 2008). Flavocytochrome is present as a soluble protein or as a membrane-bound enzyme and shows sulphide: cytochrome *c* oxidoreductase activity in vitro (Bosshard et al. 1986). Usually, the protein consists of a larger flavoprotein (FccB) and a smaller haemoprotein (FccA) (Castillo et al. 1994; Fukumori and Yamanaka 1979). All sulphur-globule-forming bacteria contain at least one and often several types of SQR. Flavocytochrome *c* can be present in addition (Table 1, Fig. 3). In *A. vinosum*, the gene for SqrF is followed in the same direction of transcription by two genes (Alvin_1196/97) each encoding a very short (32 amino acid) transmembrane protein. Deletion of the genes led to a ~ 60% reduced rate of sulphur formation from sulphide indicating a direct functional relation with SQR. Related genes occur in other purple sulphur bacteria, but nothing is known about their abundance and relevance in other sulphur-globule-forming bacteria (Weissgerber et al. 2013). In *A. vinosum*, mutants lacking flavocytochrome *c* sulphide oxidation proceeds with wild-type rates indicating that SQRs play a major role (Reinartz et al. 1998). Flavocytochrome *c* may represent a high affinity system for sulphide oxidation especially suited at very low sulphide concentration (Brune 1995b).

Polysulphides are the primary reaction products of SQR- and FccAB-catalyzed sulphide oxidation, and indeed, they are well-documented intermediates during the formation of sulphur globules from sulphide in *A. vinosum* (Prange et al. 2004). It is still unclear whether their conversion into sulphur stored in sulphur globules is a purely chemical process. Theoretically, this is possible because longer polysulphides are in equilibrium with elemental sulphur (Steudel et al. 1990). On the other hand, elevated protein and mRNA levels have been observed in *A. vinosum* for Alvin_1317–1319, constituting a putative sulphur or polysulphide reductase with highest similarity to archaeal SreABC (Laska et al. 2003). The active site molybdopterin-containing subunit PsrA is localized in the periplasm. This led to the proposal that this enzyme may be involved in the transformation of polysulphides to stored sulphur (Weissgerber et al. 2013; Weissgerber et al. 2014). However, only a minority of sulphur-globule-forming bacteria contains closely related genes shedding doubt on a general role of the encoded enzyme.

As apparent from Table 1, utilization of thiosulphate is very widespread among sulphur-globule-forming bacteria. When thiosulphate is oxidized, only its sulphane group is stored as sulphur; the sulphone sulphur is immediately excreted as sulphate. Thiosulphate oxidation is catalyzed by the periplasmic Sox system, consisting minimally of the proteins SoxXAK, SoxYZ and SoxB (Fig. 1). The heterodimeric SoxYZ protein acts as the central player and serves as a carrier of pathway intermediates (Sauvé et al. 2007). Recently, it has been shown that these intermediates are not simply bound to a cysteine residue located near the carboxy-terminus of the SoxY subunit as previously assumed but that the true carrier species is a SoxYZ-S-sulphane adduct (Grabarczyk and Berks 2017). The *c*-type cytochrome SoxXA (K) catalyzes the oxidative formation of a disulphide linkage between the sulphane

sulphur of thiosulphate and the persulphurated active site cysteine residue of SoxY (Bamford et al. 2002; Ogawa et al. 2008; Grabarczyk and Berks 2017). Then, the sulphone group is hydrolytically released as sulphate. This reaction is catalyzed by SoxB (Grabarczyk et al. 2015; Sauvé et al. 2009) and leaves the original sulphane sulphur of thiosulphate bound to SoxY (Fig. 3). From here, the sulphur is transferred to the sulphur globules by an unknown mechanism, possibly involving the rhodoanese-like protein SoxL (Welte et al. 2009). It may be important to note in this regard that polysulphurated SoxY($S_{3–4}$)Z species occur as intermediates of thiosulphate oxidation catalyzed by a reconstituted Sox system in vitro (Grabarczyk and Berks 2017). Such polysulphurated species could serve as direct donors for sulphur globule formation. In organisms that do not form sulphur globules from thiosulphate, the Sox pathway involves one further crucial enzyme, SoxCD. This hemomolybdoprotein acts as a sulphane dehydrogenase and oxidizes the SoxY-bound sulphane sulphur stemming from thiosulphate to the level of a sulphone which is finally hydrolytically released as sulphate in a reaction catalyzed by SoxB. Among the sulphur-storing organisms tabulated in Table 1, *Azospirillum thiophilum* is the only one containing *soxCD*-homologous genes. Notably, this organism forms sulphur globules only in the presence of sulphide but not on thiosulphate (Kwak and Shin 2016; Lavrinenko et al. 2010).

Many phototrophic and also chemotrophic sulphur oxidizers use external elemental sulphur as a substrate and transform it into intracellular sulphur deposits before further oxidation (Franz et al. 2007). How external elemental sulphur is transformed into internally stored sulphur is currently completely unclear. *A. vinosum* needs direct cell–sulphur contact for the uptake of elemental sulphur (Franz et al. 2007). Further details remain to be investigated.

Some *Thioalkalivibrio* species are able to form sulphur globules from thiocyanate (SCN^-) (Berben et al. 2017; Sorokin et al. 2002). Two different pathways for thiocyanate degradation have been described. In the first, a periplasmic cobalt-dependent enzyme, thiocyanate dehydrogenase, catalyzes direct oxidation of the sulphane atom, forming cyanate and sulphur (Berben et al. 2017; Tsallagov et al. 2019) (Fig. 3). The second pathway occurs in *Thioalkalivibrio thiocyanodenitrificans* (Berben et al. 2017) and involves hydrolysis of the $C \equiv N$ bond by thiocyanate hydrolase to form carbonyl sulphide (COS) and ammonia. The carbonyl sulphide is further hydrolyzed to CO_2 and sulphide by carbonyl sulphide hydrolase. *T. thiocyanodenitrificans* has not been reported to form sulphur globules from thiocyanate (Sorokin et al. 2004). In addition, none of the three genes for the subunits of its thiocyanate hydrolase encode signal peptides mediating transport into the periplasm (Berben et al. 2017). The carbonyl sulphide hydrolase from *Thiobacillus thioparus* also resides in the cytoplasm (Ogawa et al. 2013). It is therefore highly unlikely that the pathway is relevant for sulphur globule formation and it is not integrated into Fig. 3.

9 Degradation of Stored Sulphur

9.1 Oxidative Degradation

The majority of sulphur-storing organisms has the capacity to completely oxidize sulphur to sulphate (Table 1, Fig. 3). The enzyme systems involved reside in the cytoplasm necessitating sulphur transfer from the periplasm as the storage compartment to the cytoplasm as the compartment for further oxidation. How this transfer is achieved has not been not clarified. Low-molecular-weight organic persulphide such as glutathione amide persulphide has been proposed as a carrier molecule; however although potential transporters for such molecules are encoded in the genome of *A. vinosum,* they have not been genetically or biochemically characterized from this or any other sulphur-oxidizing prokaryote (Weissgerber et al. 2014).

Sulphur is never processed in a free form in the cytoplasm but rather in a protein-bound persulphidic state (Dahl 2015; Tanabe et al. 2019). A cascade of sulphur transfer reactions usually involving a rhodanese-like protein, a protein of the DsrE family and a TusA homolog delivers the sulphur to an oxidizing enzyme machinery that generates sulphite (Venceslau et al. 2014; Liu et al. 2014; Tanabe et al. 2019; Dahl 2015; Stockdreher et al. 2014). The sulphur carrier protein TusA has been recognized as a central element in these reactions (Tanabe et al. 2019). For better clarity, Fig. 3 shows only this central sulphur carrier protein instead of each single sulphur transferase. The pathway employed for the oxidation of protein-bound sulphane sulphur to sulphite can vary (Fig. 3).

The best-characterized cytoplasmic sulphite-generating pathway involves reverse-acting dissimilatory sulphite reductase rDsrAB as a central player (Pott and Dahl 1998; Dahl et al. 2005; Stockdreher et al. 2012). This pathway occurs in a majority of sulphur-globule-forming organisms (Table 1). The protein DsrC serves as the substrate-binding entity (Cort et al. 2008; Stockdreher et al. 2012). Presumably, the membrane-bound DsrMKJOP electron-transporting complex oxidizes persulphurated DsrC, thus generating a DsrC trisulphide, in which a sulphur atom is bridging two strictly conserved cysteine residues. As DsrC trisulphide has been identified as the reaction product of DsrAB in a sulphate reducer (Santos et al. 2015) and very probably serves as the substrate for oxidation catalysed by rDsrAB which releases sulphite and the reduced DsrC protein as products. The two released electrons are used to generate NADH. This reaction is catalyzed by the iron-sulphur flavoprotein DsrL, an intimate interaction partner of rDsrAB (Löffler et al. 2020).

The second sulphite-generating pathway, the so-called sulphur-oxidizing heterodisulphide reductase-like (sHdr) pathway (Cao et al. 2018; Koch and Dahl 2018), is much less studied and occurs in only a few organisms forming intracellular sulphur globules like *Thiorhodospira sibirica* (Table 1) and several *Thioalkalivibrio* species (Berben et al. 2019). The central element of this pathway is an enzyme complex resembling heterodisulphide reductase HdrABC from methanogenic archaea (Kaster et al. 2011; Wagner et al. 2017). The other crucial component of the pathway is a novel lipoate-binding protein (Cao et al. 2018). Both the sHdr

complex and the lipoate-binding protein have been identified as indispensable for sulphur compound oxidation in the Alphaproteobacterium *Hyphomicrobium denitrificans* (Cao et al. 2018; Koch and Dahl 2018). The reaction mechanism of the Hdr-LbpA-based sulphur oxidation system is currently unclear although an experimentally testable hypothesis has recently been put forward (Tanabe et al. 2019).

Neither the Dsr nor the sHdr pathway is confined to sulphur oxidizers with the capacity for depositing intracellular sulphur globules. Both pathways also occur in sulphur oxidizers that do not form sulphur deposits, e.g. species of the genera *Thiobacillus* or *Acidithiobacillus* (Quatrini et al. 2009; Beller et al. 2006a, b). The Dsr and sHdr pathways occur virtually exclusively. Only very few organisms bear the genetic potential for both oxidation routes (Berben et al. 2019; Koch and Dahl 2018).

Sulphite is usually oxidized to the final product sulphate. All the sulphur-storing organisms tabulated in Table 1 that contain sulphite-generating enzyme systems in the cytoplasm also have the ability to oxidize sulphite in this compartment. Again, two pathways exist (Fig. 3) that can either occur individually or in parallel. The first pathway involves direct oxidation of sulphite to sulphate via the cytoplasm-oriented membrane-bound iron-sulphur molybdoenzyme SoeABC (Dahl et al. 2013). The second pathway proceeds via formation of the intermediate adenosine-5-′-phosphosulphate (APS) and is catalyzed by APS reductase and ATP sulphurylase (Sat) (Dahl 1996; Parey et al. 2013). In *A. vinosum*, the periplasmic substrate-binding protein SoxYZ is needed in parallel to the cytoplasmic enzymes for effective sulphite oxidation (Dahl et al. 2013). Whether this also applies to other sulphur-oxidizing bacteria has not been elucidated.

9.2 *Reductive Degradation*

In purple sulphur bacteria, sulphur globules serve as an electron acceptor reserve that allow rudimentary anaerobic respiration under anoxic conditions leading to production of sulphide (van Gemerden 1968). *Beggiatoa* OH-75-2a used sulphur globules that were accumulated during aerobic thiosulphate oxidation to sustain anaerobic metabolism and several days of anoxia. Reduction of stored sulphur to sulphide with concomitant de novo synthesis of cell material was also found during anoxic incubation of *Beggiatoa alba* BL18LD. Furthermore, elemental sulphur stored as globules in thioautotrophic symbionts may serve as an electron sink, leading to production of sulphide during temporary anoxia (Gardebrecht et al. 2012; Arndt et al. 2001; Duplessis et al. 2004). Similar processes have been suggested for the sulphur globules in those organisms that lack enzymes to further oxidize stored sulphur, i.e. Dsr or sHdr systems, as is the case for *Thiovulum* for example (Table 1). *Thiovulum* may have to oscillate between an aerobic mode of energy conservation in which elemental sulphur accumulates in the cell and an anaerobic mode of energy conservation in which intracellular sulphur serves as an electron acceptor, perhaps

with formate acting as an electron donor or via anaerobic sulphur disproportionation (Marshall et al. 2012). The mechanisms underlying reductive degradation of stored sulphur are unresolved.

10 Outlook

Much remains to be learned on bacterial sulphur globules. This especially applies to the abundance, function and structure of the proteins in the globule envelopes. As evident from Table 1, most—if not all—organisms depositing intracellular sulphur encode periplasmic sulphur globule proteins; however, the only organisms for which the proteins have been unambiguously identified are *A. vinosum* and *Thiocapsa roseopersicina* (Brune 1995a). None of the proteins have been structurally characterized nor have their interactions been analysed. Further research should also finally clarify the question whether any other proteins involved in formation or degradation of the globules may be specifically attached to the protein envelope.

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