

Biochemistry of Sulfur Oxidation: News, Views and Omics Approaches

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Among the sulfur-oxidizing anoxygenic phototrophic bacteria, the Gammaproteobacterium *Allochrochromatium vinosum* has been developed into a model organism not only for biochemical and structural analysis of enzymes involved in oxidative sulfur metabolism but also for a systems biology approach including comparative genome analysis, genome-wide transcriptional profiling, differential expression proteomics and metabolomic profiling (1, 2). Thus, a rather comprehensive and coherent picture of bioenergetic processes and sulfur oxidation via the Dsr (dissimilatory sulfite reductase) pathway can now be provided. Classical reverse genetics and *in vitro* analyses of sulfur transfer reactions via tandem mass spectrometry further aided the detection of new genes/proteins participating in oxidative sulfur metabolism. The identification of the cytoplasmically oriented sulfite-oxidizing iron-sulfur molybdoprotein SoeABC as a major player in the oxidation of sulfite to sulfate and the detection of extensive sulfur trafficking networks involving rhodanese, TusA, DsrE-like proteins, DsrEFH and DsrC in the cytoplasm *A. vinosum* (3) may serve as examples.

An *rhd-tusA-dsrE2* or at least a *tusA-dsrE2* arrangement also occurs in many photo- and chemotrophic sulfur oxidizers that do not contain DsrC and the Dsr pathway. Those sulfur oxidizers include archaea such as *Metallosphaera cuprina* and *Acidianus hospitalis* as well as bacteria such as members of the family Aquificaceae and the genera *Acidithiobacillus* and *Thioalkalivibrio*. Here, the *tusA-dsrE2* genes are linked not only with genes encoding a possible heterodisulfide reductase complex but also with genes for (a) liponamide-binding protein(s), proteins involved in biosynthesis of the latter and in some cases for liponamide dehydrogenase. Archaeal TusA and DsrE3A react with tetrathionate yielding protein-Cys-thiosulfonates and thiosulfate is transferred from DsrE3A to TusA *in vitro* (4). These findings provide the basis for discussing new pathways of sulfur oxidation involving not only trafficking of sulfur atoms but also of thiosulfate molecules. Thiosulfonated proteins could serve as substrates for the HdrC1B1AHypHdrC2B2 complex that might interact with a liponamide-binding protein and liponamide dehydrogenase thus even opening the possibility of transfer of some of the electrons arising from sulfane sulfur oxidation to NAD^+ .

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Colorless Sulfur Bacteria

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Until recently colorless Sulfur Bacteria were among the last bacterial groups, which were allocated to a genus purely on the base of morphological characteristics. One of the major reasons for this outdated practice was, that their 16S rRNA gene, which is generally used for phylogenetic classification, often contains large introns, which hinders its amplification (Salman et al, 2012). Therefore only few sequences of this gene could be retrieved. Now the phylogeny of this group could finally be revised to fulfill modern standards and several new genera had to be introduced sharing a similar morphology but showing too much difference in their phylogeny to remain members of one genus (Salman et al., 2011). Hopefully, this progress will also help to clarify the physiological properties of the different lineages of Sulfur Bacteria, especially of those, which share a similar morphology but differ in their physiology.

Colorless Sulfur Bacteria are mainly known for their ability to oxidize sulfide with oxygen or nitrate, but in addition to this, also other electron acceptors or donors may be used. To a large extent these alternative energy sources define the ecological niches of the different genera and may help to explain their respective distributions. For example, in a marine *Beggiatoa* culture, which cannot use nitrate as electron acceptor, steep sulfide gradients induce a movement of filaments to greater depths in older cultures. Sulfur is respired and empty filaments return to the surface. Apparently, the rate of oxidation of sulfide to sulfur cannot be regulated by this strain and the filaments prevent bursting by respiring internal sulfur (Schwedt et al., 2012). This physiological reaction to steep sulfide gradients is probably also important under natural conditions, as frequently filaments with hardly any sulfur inclusions are observed side by side with filaments, which are completely filled with sulfur.

The steepness of sulfide gradient seems to be an important factor shaping the ecological niches of the different genera of colorless Sulfur Bacteria. The growth rate usually depends on the flux of sulfide and will increase linearly with increasing sulfide fluxes as long as an electron acceptor is available. Nevertheless, sulfide becomes toxic as soon as its accumulation cannot be prevented by a high oxidation rate. Apparently, in this situation Sulfur Bacteria can use internally stored polyphosphate as a last resort to survive high sulfide and anoxia, but the principal behind this reaction is not yet understood (Brock & Schulz-Vogt, 2011). In general, polyphosphate accumulation is observed in many sulfide oxidizing bacteria and may fulfill a very basic function in this physiological group of bacteria.

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Pathways of Sulfur disproportionation deduced from enzyme studies and genomic data and their implications for the possible role of sulfur disproportionation in the evolution of sulfur metabolisms.

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The disproportionation of elemental sulfur in which elemental sulfur serves both as electron donor and acceptor and generates simultaneously hydrogen sulfide and sulfate (Bak & Cypionka, 1987; Thamdrup et al., 1993; Finster, 2008) is a fascinating microbiological process at the borderline in the transition zone between biology and geochemistry. Recently, the genomes of the two isolated representatives of the genus *Desulfocapsa* were sequenced and the preliminary inspections provide evidence for the presence of the complete pathways of sulfate reduction, CO₂ fixation via the CODH-pathway thus confirming physiological and isotopic studies. Also the genes behind nitrogen fixation are present. In addition, an operon was identified, which is made responsible for elemental sulfur reduction in representatives of the genus *Chlorobium* and relatives.

The organisms make a living out of this process from a minimum amount of energy and completely rely scavenging of hydrogen sulfide. The process is endergonic under standard condition with a ΔG^0 of +10 KJ per mol of elemental sulfur. Only at hydrogen sulfide concentrations less than 0.1 mM elemental sulfur disproportionation becomes sufficiently exergonic to support microbial growth. In natural environments this may be achieved by removal/scavenging of hydrogen sulfide by FeIII or FeII. The process is carried out by microorganisms affiliated with the metabolic guild of sulfate-reducing bacteria within the genus *Desulfocapsa* (Janssen et al., 1996; Finster et al., 1998; Finster, 2008). Studies of the biochemistry of a few isolates using enzyme assays and sulfur isotope discrimination indicate that disproportionating microbes reverse the sulfate reduction pathway during disproportionation. In addition, it has been demonstrated that *Desulfocapsa sulfexigens* shifts to sulfur reduction instead of disproportionation when hydrogen is supplied as electron donor. Carbon isotope signatures indicate that CO₂ is fixed via the carbon monoxide dehydrogenase (CODH) pathway (Frederiksen & Finster, 2003, 2004). Further on the disproportionation of elemental sulfur is accompanied by accelerated pyrite formation, partly through the hydrogen generating “Wächtershäuser –reaction” (Canfield et al., 1998). Studies of the isotopic signatures of 3.5 billion year old barium sulfate deposits provided strong evidence for biological elemental sulfur disproportionation. Thus elemental sulfur disproportionation would be one of the oldest documented biological processes on Earth (Philippot et al., 2007). Based on the available geological, physiological, biochemical and genetic information the hypothesis is put forward that sulfur disproportionating members of the genus *Desulfocapsa* could be studied as models of a chemolithoautotrophic version of “LUCA”, which meets the complex demands of the early Earth environment. I will present ideas obtained from recent genomic studies on the pathways of sulfur transformations and invite for the discussion of future investigations.

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