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Oxidation of H_2, organic compounds and inorganic sulfur compounds coupled to reduction of O_2 or nitrate by sulfate-reducing bacteria

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Abstract. All of fourteen sulfate-reducing bacteria tested were able to carry out aerobic respiration with at least one of the following electron donors: H_2, lactate, pyruvate, formate, acetate, butyrate, ethanol, sulfide, thiosulfate, sulfite. Generally, we did not obtain growth with O_2 as electron acceptor. The bacteria were microaerophilic, since the respiration rates increased with decreasing O_2 concentrations or ceased after repeated O_2 additions. The amounts of O_2 consumed indicated that the organic substrates were oxidized incompletely to acetate; only *Desulfobacter postgatei* oxidized acetate with O_2 completely to CO_2. Many of the strains oxidized sulfite (completely to sulfate) or sulfide (incompletely, except *Desulfobulbus propionicus*); thiosulfate was oxidized only by strains of *Desulfovibrio desulfuricans*; trithionate and tetrathionate were not oxidized by any of the strains. With *Desulfovibrio desulfuricans* CSN and *Desulfobulbus propionicus* the oxidation of inorganic sulfur compounds was characterized in detail. *D. desulfuricans* formed sulfate during oxidation of sulfite, thiosulfate or elemental sulfur prepared from polysulfide. *D. propionicus* oxidized sulfite and sulfide to sulfate, and elemental sulfur mainly to thiosulfate. A novel pathway that couples the sulfur and nitrogen cycles was detected: *D. desulfuricans* and (only with nitrite) *D. propionicus* were able to completely oxidize sulfide coupled to the reduction of nitrate or nitrite to ammonia. Cell-free extracts of both strains did not oxidize sulfide or thiosulfate, but formed ATP during oxidation of sulfite (37 nmol per 100 nmol sulfite). This, and the effects of AMP, pyrophosphate and molybdate on sulfite oxidation, suggested that sulfate is formed via the (reversed) sulfate activation pathway (involving APS reductase and ATP sulfurylase). Thiosulfate oxidation with O_2 probably required a reductive first step, since it was obtained only with energized intact cells.

Key words: Sulfate reduction – Aerobic respiration – Nitrate ammonification – Oxidation of sulfur compounds – ATP formation – *Desulfobulbus propionicus* – *Desulfovibrio desulfuricans*

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Abbreviations: CCCP, carbonyl cyanide *m*-chlorophenylhydrazone; *APS*, adenosine phosphosulfate or adenylyl sulfate

During the last years evidence has been accumulating that the anaerobic sulfate-reducing bacteria are able to survive or even take advantage of the presence of molecular oxygen. Various observations can be classified into three categories:

(i) Sulfate-reducing bacteria are to some extent O_2-tolerant. Especially in the absence of H_2S (which forms toxic products with O_2) they remain viable for hours or even days when exposed to O_2 (Hardy und Hamilton 1981; Cypionka et al. 1985; Fukui and Takii 1990).

(ii) It has been suggested that sulfate-reducing bacteria are able to reduce sulfate in the presence of oxygen. Sulfate reducers are found in high numbers near or within the anoxic/oxic interfaces in sediments (Laanbroek and Pfennig 1981; Battersby et al. 1985; Jørgensen and Bak 1991). Sulfate reduction was even reported to go on in oxic zones of microbial mats at O_2 tensions near saturation (Cohen 1989; Canfield and DesMarais 1991). However, so far it has not been unequivocally demonstrated that a pure culture of sulfate-reducing bacteria carries out sulfate reduction in the presence of O_2.

(iii) Sulfate-reducing bacteria can respire with O_2 (Postgate 1979; Abdollahi und Wimpenny 1990; Dilling and Cypionka 1990). Some sulfate-reducing bacteria can utilize H_2, various organic compounds and even sulfur compounds as electron donors for microaerophilic respiration, with rates comparable to those of aerobic bacteria. The reduction of molecular O_2 by *Desulfovibrio desulfuricans* (up to 5 mM O_2 added stepwise to keep the concentration low) was coupled to the formation of ATP, but not to aerobic growth.

In the present work we compared the capacity of aerobic respiration with various substrates among freshwater and marine sulfate reducers. With two freshwater strains the oxidation of sulfur compounds was characterized in detail.

Materials and methods

Organisms and cultivation

The sulfate-reducing bacteria (Table 1) were cultivated in the medium described by Cypionka and Pfennig (1986) with H_2/CO_2 (80/20, v/v) or lactate (20 mM) as electron donor, and sulfate or nitrate (10 mM, each) as electron acceptor. Acetate (2 mM) was added as carbon source during growth with H_2 as electron donor. *Desulfovibrio desulfuricans* strain CSN and *Desulfobulbus propionicus* were cultivated in chemostats (Cypionka 1986) with H_2 and limiting concentrations of nitrate (10 mM) or sulfate (20 mM) at a dilution rate of 1 per day.

Preparations of cells

In order to remove H_2S, cultures were sparged with CO_2 for 15 min. Then the cells were harvested by centrifugation (18000 · *g*, 10 min), washed once in N_2-saturated potassium phosphate buffer (50 mM, pH 7.0) or 30 mM KCl, resuspended under H_2 in the same solution to a concentration of 60 to 80 mg protein/ml.

For the preparation of cell-free extracts, washed cells were passed three times through a French press (Aminco, Silver Spring, Md., USA). In order to remove whole cells and cell debris, the lysate was centrifuged at 18000 · *g* for 10 min, and the supernatant used as cell-free extract.

Measurement of O_2 reduction and sulfide consumption

Respiration of washed cells was measured by means of O_2 electrodes (Bachofer, Reutlingen, FRG or Model 59, Yellow Springs Instruments Yellow Springs, Ohio, USA). Washed cells were incubated at 30 °C in 10 ml N_2-saturated potassium phosphate buffer (30 mM, pH 7.2). O_2 and H_2 were added as pulses of O_2- or H_2-saturated potassium phosphate buffer (30 mM, pH 7.2, assumed to contain 1.17 mM O_2 or 0.68 mM H_2 at atmospheric pressure and 25 °C). If sulfur compounds were to be analyzed, experiments were performed in 30 mM bicarbonate buffer, and O_2 was added as O_2-saturated distilled water. Sulfide oxidation was measured by means of an Ag/Ag_2S electrode coupled to a microcomputer (Cypionka 1989). Several experiments were performed with measuring pH, pS and pO_2 simultaneously by electrodes.

Chemical analyses

Thiosulfate was determined as described by Nor and Tabatabai (1975), sulfide as described by Cline (1969), sulfite as described by Pachmayr (1960), ammonia as described by Chaney and Marbach (1962), nitrite as described by Zimmermann (1979), and protein as described by Schmidt et al. (1963). Sulfate and nitrate were determined by ion chromatography using the columns LCA A01 or LCA A09 (Sykam, Gilching, FRG) as described by Bak et al. (1991). ATP was determined by the luciferin-luciferase method as described elsewhere (Stahlmann et al. 1991).

Results

Reduction of O_2 by freshwater and marine sulfate-reducing bacteria

The fourteen sulfate-reducing bacteria tested (Table 1) were all able to respire with O_2. Depending on the strain used, H_2, lactate, pyruvate, formate, ethanol, acetate, and butyrate, as well as sulfite, thiosulfate and sulfide served as electron donors for O_2 reduction. Freshly harvested cells reduced O_2 with endogenous substrates which usually were exhausted after several additions of O_2. The respiration rates with the different substrates added were

Table 1. Substrates oxidized with O_2 by sulfate-reducing bacteria[a]

Strain	H_2	Lactate	Pyruvate	SO_3^{2-}	$S_2O_3^{2-}$	H_2S	Others
Freshwater strains							Formate,
Desulfovibrio desulfuricans CSN[c]	+	+	+	+	+	+	ethanol
Desulfovibrio desulfuricans Essex DSM 642	+	+	+	+	+	(+)	
Desulfovibrio desulfuricans ATCC 27774	+	+	+	+	(+)	(+)	
Desulfovibrio vulgaris Marburg DSM 2119	+	+	+[b]	(+)	−	+	Formate
Desulfovibrio sulfodismutans DSM 3696	(−)	−	+[b]	(+)	−	+	
Desulfotomaculum orientis DSM 765	(−)	−	−	+	−	−	
Desulfobulbus propionicus DSM 2032	−	+[b]	−	+	−	+	Propionate
Marine strains							
Desulfovibrio salexigens NCIB 8403	−	−	−	(+)	−	−	
Desulfovibrio sapovorans DSM 2055	−	+	+	(+)	−	−	Butyrate
Desulfobacter postgatei DSM 2034	−	−	−	−	−	−	Acetate
Desulfobacter hydrogenophilus DSM 3380	−	−	−	(+)	−	−	
Desulfobacterium autotrophicum DSM 3382	+	+	−	(+)	−	−	Formate
Desulfococcus multivorans DSM 2059	−	+	+[b]	−	−	(+)	
Strain 6Ala[d]	−	−	−	−	−	+	

[a] Respiration was measured by means of an oxygen electrode. Washed cells (0.1 to 1.5 mg protein per ml) were incubated in N_2-saturated phosphate buffer (50 mM, pH 7.0) at 30 °C. Substrates (5 to 50 μM) and oxygen (0.1 to 2% O_2-saturated buffer) were added by means of Hamilton syringes. Experiments were carried in triplicate with different cell preparations. Respiration rates were corrected for endogenous respiration. There was no chemical O_2 uptake by autoxidation of sulfide or sulfite at the low concentrations tested.

\+ for rates >0.5 nmol O_2 per min and mg protein

(+) for rates <0.5 nmol O_2 per min and mg protein

+[b] activity observed after addition of increased substrate concentrations (1 mM)

(−) low activity detected only in part of the experiments

− no activity

[c] Cypionka 1989

[d] Alanine-degrading strain isolated from brackish Black Sea sediment (Cypionka, unpublished)

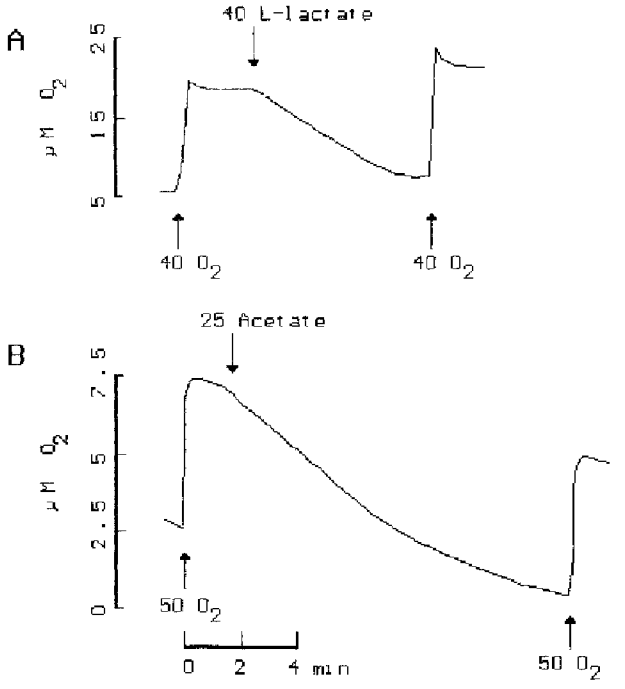

Fig. 1 A, B. Aerobic respiration of sulfate-reducing bacteria with lactate and acetate as electron donors. **A** *Desulfovibrio desulfuricans* CSN (washed cells, 0.32 mg protein/ml, assay volume 3 ml) oxidized L-lactate incompletely (probably to acetate) as indicated by an O_2/lactate ratio of about 1. **B** *Desulfobacter postgatei* (0.26 mg protein/ml, assay volume 10 ml) oxidized acetate with a stoichiometry of 2 O_2 per acetate that indicates complete oxidation to CO_2. The numbers at the arrows indicate nmol addition

between 1 and 25 nmol O_2 per min and mg protein. Rates comparable to those of aerobic bacteria (up to 670 nmol O_2 per min and mg protein) were found with strains of the genus *Desulfovibrio*. Generally, the sulfate reducers were microaerophilic; the respiration rates often increased with decreasing O_2 concentrations, and slowed down after repeated O_2 additions. Freshwater strains oxidized a greater range of substrates with O_2 than did marine strains.

Six strains were able to oxidize H_2 with O_2 (Tab. 1); the consumption of 0.5 O_2 per H_2 indicated the 'Knallgas reaction' with formation of water as end product. Most of the strains oxidized lactate; the disappearance of 1 O_2 per lactate (Fig. 1 A) indicated incomplete lactate oxidation to acetate in all cases, even though with sulfate as electron acceptor some of the strains (*Desulfobacterium autotrophicum, Desulfococcus multivorans, Desulfobacter hydrogenophilus*) are able to oxidize lactate completely to CO_2. Some strains consumed less O_2 with DL-lactate than with L-lactate, probably due to the lack of a racemase. *Desulfovibrio sulfodismutans, D. salexigens* and *Desulfotomaculum orientis* did not oxidize lactate with O_2, although this substrate is utilized with sulfate. *Desulfobacter postgatei* was the only strain which oxidized acetate; the disappearance of 2 O_2 per acetate indicated complete oxidation to CO_2 (Fig. 1 B).

Of the fourteen strains investigated all but one were able to oxidize inorganic sulfur compounds with O_2 (Tab. 1). While sulfide and sulfite were oxidized by most of the strains, O_2 uptake with thiosulfate was only observed with three strains of *Desulfovibrio desulfuricans*. Sulfite and thiosulfate were oxidized with uptake of 0.5 and 2 mol O_2, respectively, indicating complete oxidation to sulfate. With sulfide as electron donor, only *Desulfobulbus propionicus* consumed 2 O_2 per sulfide (see below); the other strains took up less O_2 during sulfide oxidation, indicating incomplete oxidation. While in freshwater strains the capacities of O_2 reduction with sulfite or with sulfide appeared always concomitant, marine strains oxidized only one of these sulfur compounds. Reduction of O_2 with trithionate or tetrathionate was not observed with any of the strains investigated.

In control experiments without cells at low O_2 concentrations the various sulfide compounds were not chemically oxidized to sulfate, and did not cause O_2 uptake at detectable rates.

Table 2. Formation of sulfate by oxidation of morganic sulfur compounds with O_2

Sulfur compound	*Desulfovibrio desulfuricans* CSN[a]			*Desulfobulbus propionicus*[a]		
	Percent sulfate formed[b]	Respiration rate (nmol O_2 per min and mg protein)	Percent inhibition by CCCP[c]	Percent sulfate formed[b]	Respiration rate (nmol O_2 per min and mg protein)	Percent inhibition by CCCP[c]
Sulfite	103	3.8	37	107	3.8	60
Thiosulfate	194	1.5	100	—[d]	0	—[d]
Sulfide	0	<0.8	—[d]	108	18.5	0

[a] Washed cells (0.2 to 0.6 mg protein/ml) were incubated in N_2-saturated carbonate buffer (30 mM, pH 7.2). The sulfur compounds were added stepwise to final concentrations of 40 to 70 μM within 2 h, consumed O_2 was refilled to a concentration of 30 μM, each time. Respiration was measured by an O_2 electrode and sulfate by means of ion chromatography. Results are averaged from four experiments at two days

[b] Compared to the amount of sulfur compound added; corrected for the amount of sulfate formed in control experiments without added sulfur compounds

[c] The cells were preincubated with 50 μM CCCP for 30 min. Data corrected for eventual inhibition by 50 μM methanol as the solvent of CCCP

[d] Not tested due to no or very low activity

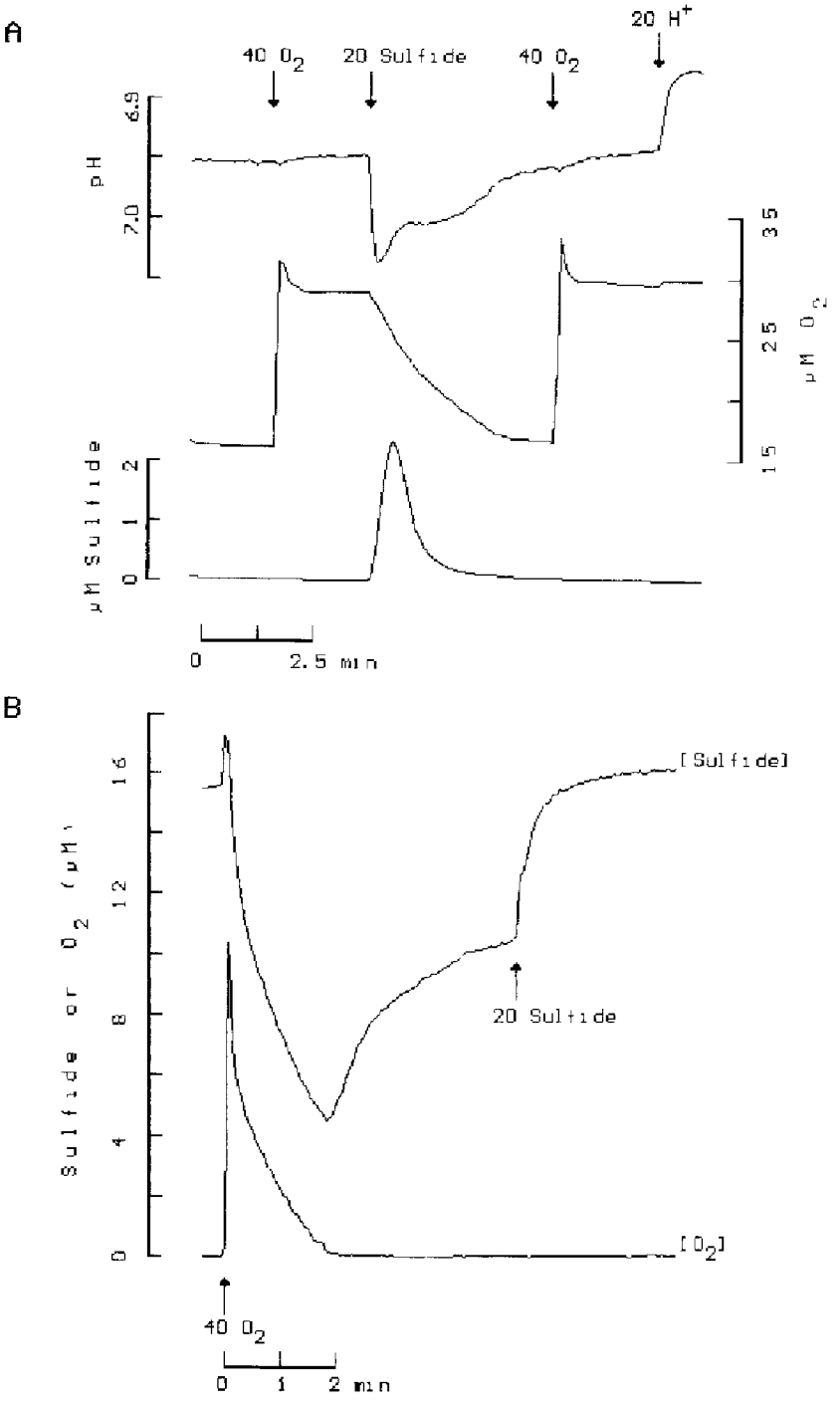

Fig. 2A, B. Oxidation of sulfide with O_2 by *Desulfobulbus propionicus*. **A** After addition of sulfide to cells incubated in the presence O_2 at low concentration; **B** after addition of O_2 to cells incubated in the presence of sulfide at low concentration. The concentrations of washed cells (in mg protein per ml) were 0.4 in **A** and 0.15 in **B**. Numbers indicate nmol addition to 3 ml suspension

Aerobic respiration with inorganic sulfur compounds in Desulfovibrio desulfuricans and Desulfobulbus propionicus

With two freshwater strains, *Desulfovibrio desulfuricans* strain CSN and *Desulfobulbus propionicus*, the oxidation of inorganic sulfur compounds was characterized in detail. As already indicated by the O_2 uptake stoichiometry, washed cells of *D. desulfuricans* formed stoichiometric amounts of sulfate from sulfite or thiosulfate (Table 2). This strain oxidized sulfide only slowly with O_2, and did not produce sulfate (see, however, sulfide oxidation with nitrate, below).

D. propionicus oxidized sulfide and sulfite completely to sulfate. Sulfide oxidation was biphasic indicating the formation of intermediates (Fig. 2). If sulfide was added to cells incubated in the presence of O_2 (Fig. 2A), biphasic curves of oxygen uptake and acidification were observed. If O_2 was added to cells incubated in the presence of sulfide (Fig. 2B), the disappearance of 1 sulfide per O_2 was observed, half of which was recovered, however, after complete O_2 consumption. This could be explained by oxidation of sulfide to thiosulfate in the first, and disproportionation of thiosulfate in the second step. However, separately added thiosulfate was not consumed by *D. propionicus* as long as oxygen was present (Table 3).

Table 3. Oxidation of sulfide with nitrate or nitrite as electron acceptor

	Desulfovibrio desulfuricans CSN[a]				*Desulfobulbus propionicus*[a]			
Electron acceptor	Percent[b] ammonia formed	Percent[c] sulfate formed	Rate (nmol sulfide per min and mg protein)	Percent inhibition by CCCP[d]	Percent[b] ammonia formed	Percent[c] sulfate formed	Rate (nmol sulfide per min and mg protein)	Percent inhibition by CCCP[e] protein)
Nitrate	83	95	20	100	0[e]	0[e]	0	–
Nitrite	85	65	18	100	81	70	19	0

[a] The electron acceptor was added stepwise to a final concentration of 105 µM; consumed sulfide was refilled after each pulse. Sulfide utilization was measured by means of a sulfide electrode, sulfate by ion chromatography and ammonia by a photometrical test. Data averaged from two experiments at different days. For further conditions see Table 2

[b] Compared to the amount of added nitrate or nitrite; corrected fo the amount of ammonia formed in control experiments without nitrate or nitrite added. In control experiments without sulfide added, cells of both strains converted about 35% of the added nitrate or nitrite to ammonia without sulfate production

[c] Compared to the amount of sulfide disappeared; corrected for the amount of sulfate formed in control experiments without sulfide added

[d] The cells were preincubated with 50 µM CCCP for 30 min. Data corrected for eventual inhibition by 50 µM methanol as the solvent of CCCP

[e] Cells of *Desulfobulbus propionicus* grew very slowly with nitrate. With washed cells, no nitrate-dependent oxidation of sulfide was observed with the sulfide electrode

Oxidation of elemental sulfur and polysulfide

Polysulfide or fresh sulfur suspensions (prepared after Pfennig and Biebl 1976, by dilution of polysulfide and sparging with CO_2 in order to remove free H_2S) were oxidized with O_2 with rates higher than those of sulfide oxidation. As end products, *Desulfovibrio desulfuricans* produced mainly sulfate, and *Desulfobulbus propionicus* mainly thiosulfate. No aerobic respiration was observed with sulfur flower or sulfur suspensions prepared according to Janek (1933).

Complete oxidation of sulfide with nitrate or nitrite

Desulfovibrio desulfuricans and *Desulfobulbus propionicus* are able to grow with nitrate as electron acceptor which is reduced to ammonia (Cypionka 1989; Widdel and Pfennig 1982). These strains oxidized sulfide coupled to the reduction of oxidized nitrogen compounds to ammonia (Table 3). *D. desulfuricans* consumed 1 sulfide per nitrate and 0.75 sulfide per nitrite (Fig. 3); sulfate and ammonia were formed as end products in stoichiometric amounts and with higher rates than with O_2 as electron acceptor. The capacity of nitrate reduction in *D. desulfuricans* was induced only after growth with nitrate, while the capacity of nitrite reduction was present constitutively. *D. propionicus* grew slowly with nitrate in our hands, and produced only small amounts of ammonia. Oxidation of sulfide by washed cell of this strain was observed with nitrite, but not with nitrate.

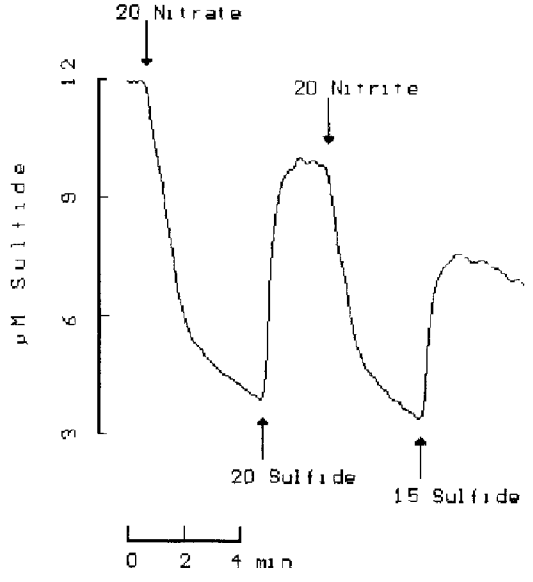

Fig. 3. Oxidation of sulfide with nitrate or nitrite by *Desulfovibrio desulfuricans* CSN. Washed cells (0.16 mg protein per ml) were incubated in N_2-saturated KCl. Numbers indicate nmol addition to 3 ml cell suspension

Mechanism of sulfate formation

Cell-free extracts of *Desulfovibrio desulfuricans* and *Desulfobulbus propionicus* oxidized sulfite, but not sulfide or thiosulfate with O_2 as electron acceptor. The oxidation of sulfur compounds was coupled to ATP formation (Table 4). With cell-free extracts higher amounts of ATP were detected after sulfite oxidation than with whole cells, presumably due to rapid ATP consumption by whole cells. Addition of 100 nmol sulfite caused formation of 37 nmol ATP with cell-free extract of *D. desulfuricans*, and 29 nmol with *D. propionicus*. The sulfite-dependent O_2 reduction by cell-free extracts of *D. desulfuricans* was accelerated by AMP (1 mM) and pyrophosphate (5 mM), whereas molybdate (0.1 mM) caused inhibition. These results indicated that sulfate was formed via the reversed sulfate activation pathway, i.e. APS reductase and ATP sulfurylase.

The uncoupler CCCP inhibited the reduction of O_2 with thiosulfate and sulfur or polysulfide by washed cells of *D. desulfuricans* irreversibly (Table 2). O_2 reduction with sulfide by *D. propionicus* was not affected. Aerobic respiration with sulfite was partially inhibited by CCCP in both of the strains (Table 2). The oxidation of sulfide with nitrate or nitrite was completely inhibited by CCCP in *D. desulfuricans* (Table 3).

Table 4. ATP formation coupled to the oxidation of sulfur compounds with O_2 by washed cells and cell-free extracts[a]

Sulfur compound	ATP formed (nmol per 100 nmol sulfur compound added)			
	Desulfovibrio desulfuricans		*Desulfobulbus propionicus*	
	Whole cells	Cell-free extract	Whole cells	Cell-free extract
Sulfite	19	37	14	29
Thiosulfate	16	3	nt[b]	nt[b]
Sulfide	nt[b]	nt[b]	19	nt[b]

[a] Washed cells (1.0 mg protein/ml) or cell-free extracts (0.2 mg protein/ml) were incubated in N_2-saturated phosphate buffer (50 mM, pH 7.0). 2 min after addition of the sulfur compound, a sample of 50 µl was taken for ATP determination. Data corrected for ATP at zero-time and averaged over two experiments at different days. Control experiments revealed that cell-free extracts did not form ATP by chemiosmotic mechanisms during oxidation of H_2

[b] Not tested due to no or very low activity

Discussion

Reduction of O_2 by sulfate-reducing bacteria

Our study demonstrates that the capacity of aerobic respiration is widespread among sulfate-reducing bacteria. Obviously, these classically anaerobic bacteria know to handle the strong oxidizing agent O_2, and even form ATP coupled to aerobic respiration (Dilling and Cypionka 1990). In an undisturbed sediment as their typical natural environment, they might be able to shift the oxic/anoxic interface upwards, and thus get closer to their organic substrates that originate from the oxic milieu. That the capacity of aerobic respiration was more pronounced in freshwater than in marine strains might be an adaptation to sulfate limitation, which typically occurs in freshwater but not in marine sediments.

In spite of their capacity of aerobic respiration the sulfate reducers have to be classified as anaerobic bacteria furtheron. So far, we did not observe unequivocally aerobic growth in homogenously shaken cultures. Furthermore, some substrates utilized with sulfate as electron acceptor were not oxidized with O_2. This is presumably caused by the O_2 sensitivity of some enzymes involved in substrate oxidation (e.g., hydrogenase in *Desulfovibrio vulgaris*, Fitz and Cypionka 1991).

Oxidation of sulfur compounds by sulfate-reducing bacteria

Oxygen enables sulfate-reducing bacteria to oxidize inorganic sulfur compounds. In several strains complete oxidation to sulfate was obtained. This exceeds the partial sulfur oxidation observed during disproportionation of thiosulfate or sulfite to sulfide and sulfate (Bak and Cypionka 1987). The capacity of both, oxidation and reduction of sulfur compounds depending on the available electron acceptor, has been described in eubacterial and archaebacterial sulfur bacteria like *Wolinella* (Macy et al. 1986) or *Acidianus* (Segerer et al. 1986). However, those bacteria are unable to reduce sulfate and thus to catalyze the reactions of the whole sulfur cycle in both directions. Complete oxidation of sulfide coupled to the ammonification of nitrate or nitrite demonstrates that the oxidation of sulfur compounds by the sulfate-reducing bacteria is not purely chemical or O_2-dependent. Furthermore, this is a novel process coupling the sulfur and nitrogen cycles: the denitrifying sulfur-oxidizing bacteria like *Thiobacillus denitrificans* form N_2 but not ammonia as the completely reduced form of nitrogen.

Our experiments on the mechanisms of sulfur oxidation are not in favor of the still controversely discussed trithionate pathway of sulfite reduction (Kobayashi et al. 1969; Fitz and Cypionka 1989, 1990). Most of the strains were able to oxidize sulfide (or sulfite) with O_2. But only with three strains oxidation of thiosulfate was observed, and no strain oxidized trithionate with O_2. Thus, if sulfite reduction includes the trithionate pathway, the latter must be irreversible or sensitive to O_2.

The first step of thiosulfate oxidation is most probably the reductive cleavage to sulfide and sulfite by thiosulfate reductase, like already proposed for thiosulfate disproportionation (Krämer and Cypionka 1989). The uncoupler sensitivity and the observation that thiosulfate oxidation was not catalyzed by cell-free extracts suggest that an energy-dependent reversed electron transport is involved, which can be carried out by energized whole cells, only.

Sulfate formation from sulfite obviously includes the reaction of sulfate activation, APS reductase and ATP sulfurylase, as proposed also for the disproportionation of sulfite. This was demonstrated by ATP formation in cell-free extracts (that cannot carry out ATP regeneration by chemiosmotic mechanisms), as well as by the action patterns of AMP, pyrophosphate, and molybdate.

In summary, we have learned that the sulfur metabolism of the dissimilatory sulfate-reducing bacteria is no one-way street. Besides reduction and disproportionation of sulfur compounds (Bak and Cypionka 1987; Krämer and Cypionka 1989), their complete oxidation is possible with oxygen or even with nitrate as electron acceptor.

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References

Abdollahi H, Wimpenny JWT (1990) Effects of oxygen on the growth of *Desulfovibrio desulfuricans*. J Gen Microbiol 136: 1025–1030

Bak F, Cypionka H (1987) A novel type of energy conservation involving fermentation of inorganic sulphur compounds. Nature 326: 891–892

Bak F, Scheff G, Jansen K-H (1991) A rapid and sensitive ion chromatographic technique for the determination of sulfate and sulfate reduction rates in freshwater lake sediments. FEMS Microbiol Ecol 85: 23–30

Battersby NS, Malcolm SJ, Brown CM, Stanley SO (1985) Sulphate reduction in oxic and suboxic North East Atlantic sediments. FEMS Microbiol Ecol 31: 225–228

Canfield DE, DesMarais DJ (1991) Aerobic sulfate reduction in microbial mats. Science 251: 1471–1473

Chaney AL, Marbach EP (1962) Modified reagents for the determination of urea and ammonia. Clin Chem 8: 130–132

Cline JD (1969) Spectrophotometric determination of hydrogen sulfide in natural waters. Limnol Oceanogr 14: 454–458

Cohen Y (1989) Photosynthesis in cyanobacterial mats and its relation to the sulfur cycle: a model for microbial sulfur interactions. In: Cohen Y, Rosenberg E (eds). Microbial mats. Physiological ecology of benthic microbial communities. Am Soc Microbiol, Washington, pp 22–36

Cypionka H (1986) Sulfide-controlled continuous culture of sulfate-reducing bacteria. J Microbiol Methods 5: 1–9

Cypionka H (1989) Characterization of sulfate transport in *Desulfovibrio desulfuricans*. Arch Microbiol 152: 237–243

Cypionka H, Widdel F, Pfennig N (1985) Survival of sulfate-reducing bacteria after oxygen stress, and growth in sulfate-free oxygen-sulfide gradients. FEMS Microbiol Ecol 31: 39–45

Cypionka H, Pfennig N (1986) Growth yields of *Desulfotomaculum orientis* with hydrogen in chemostat culture. Arch Microbiol 143: 366–369

Dilling W, Cypionka H (1990) Aerobic respiration in sulfate-reducing bacteria. FEMS Microbiol Lett 71: 123–128

Fitz RM, Cypionka H (1989) A study on electron transport-driven proton translocation in *Desulfovibrio desulfuricans*. Arch Microbiol 152: 369–376

Fitz RM, Cypionka H (1990) Formation of thiosulfate and trithionate during sulfite reduction by washed cells of *Desulfovibrio desulfuricans*. Arch Microbiol 154: 400–406

Fitz RM, Cypionka H (1991) Generation of a proton gradient in *Desulfovibrio vulgaris*. Arch Microbiol 155: 444–448

Fukui M, Takii S (1990) Survival of sulfate-reducing bacteria in oxic surface sediment of a seawater lake. FEMS Microbiol Ecol 73: 317–322

Hardy JA, Hamilton WA (1981) The oxygen tolerance of sulphate-reducing bacteria isolated from North Sea waters. Current Microbiol 6: 259–262

Janek A (1933) Ein neues Verfahren zur Herstellung von Schwefelsolen. Kolloid Z 64: 31–32

Jørgensen BB, Bak F (1991) Pathways and microbiology of thiosulfate transformations and sulfate reduction in a marine sediment (Kattegat, Denmark). Appl Environ Microbiol 57: 847–856

Kobayashi K, Tachibana S, Ishimoto M (1969) Intermediary formation of trithionate in sulfite reduction by sulfate-reducing bacteria. J Biochem 65: 155–157

Krämer M, Cypionka H (1989) Sulfate formation *via* ATP sulfurylase in thiosulfate- and sulfite-disproportionating bacteria. Arch Microbiol 151: 232–237

Laanbroek HJ, Pfennig N (1981) Oxidation of short-chain fatty acids by sulfate-reducing bacteria in freshwater and marine sediments. Arch Microbiol 128: 330–335

Macy JM, Schröder I, Thauer RK, Kröger A (1986) Growth of *Wolinella succinogenes* on H_2S plus fumarate and on formate plus sulfur as energy sources. Arch Microbiol 144: 147–150

Nor YM, Tabatabai MA (1975) Colorimetric determinations of microgram quantities of thiosulfate and tetrathionate. Anal Lett 8: 537–547

Pachmayr F (1960) Vorkommen und Bestimmung von Schwefelverbindungen in Mineralwasser. Ph D Thesis, University of München, FRG

Pfennig N, Biebl H (1976) *Desulfuromonas acetoxidans* gen. nov. and sp. nov., a new anaerobic, sulfur-reducing, acetate-oxidizing bacterium. Arch Microbiol 110: 3–12

Postgate JR (1979) The sulphate-reducing bacteria. University Press, Cambridge, UK

Schmidt K, Liaaen-Jensen S, Schlegel HG (1963) Die Carotinoide der Thiorhodaceae. I. Okenon als Hauptcarotinoid von *Chromatium okenii* Perty. Arch Mikrobiol 46: 117–126

Segerer A, Neuner A, Kristjansson JK, Stetter KO (1986) *Acidianus infernus* gen. nov., sp. nov., and *Acidianus brierleyi* comb. nov.: Facultatively aerobic, extremely acidophilic thermophilic sulfur-metabolizing archaebacteria. Int J System Bacteriol 36: 559–564

Stahlmann J, Warthmann R, Cypionka H (1991) Na^+-dependent accumulation of sulfate and thiosulfate in marine sulfate-reducing bacteria. Arch Microbiol 155: 554–558

Widdel F, Pfennig N (1982) Studies on dissimilatory sulfate-reducing bacteria that decompose fatty acids. II. Incomplete oxidation of propionate by *Desulfobulbus propionicus* gen. nov., sp. nov. Arch Microbiol 131: 360–365

Zimmermann M (1979) Photometrische Metall- und Wasseranalysen. Wissenschaftliche Verlagsanstalt, Stuttgart