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EXPERIMENTAL ARTICLES

The Functional Role of Reduced Inorganic Sulfur Compounds in the Metabolism of the Microaerophilic Bacterium *Spirillum winogradskii*

D. A. Podkopaeva*, M. Yu. Grabovich, and G. A. Dubinina***

**Winogradsky Institute of Microbiology, Russian Academy of Sciences, pr. 60-letiya Oktyabrya 7, k. 2, Moscow, 117312 Russia*
***Voronezh State University, Universitetskaya pl. 1, Voronezh, 394893 Russia*

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Abstract—Oxidation of reduced sulfur compounds by the microaerophilic sulfur bacterium *Spirillum winogradskii* was found to occur only concomitantly with consumption of an organic substrate and was not linked to their utilization as electron donors in energy metabolism. No enzymes of dissimilatory sulfur metabolism were found in the cells of the sulfur bacterium oxidizing thiosulfate to tetrathionate; oxidation of thiosulfate and sulfide was caused by their reaction with reactive oxygen species (ROSs), mostly H_2O_2 produced in the course of aerobic growth. A decreased lytic effect of ROSs in the presence of thiosulfate resulted in a twofold increase in the cell yield under aerobic conditions and more efficient substrate utilization. The latter effect was caused by decreased expenditure of energy for the biosynthesis of oxygen-protective polysaccharides. The stimulatory effect of thiosulfate on the growth processes was due to the activation of a number of TCA cycle enzymes producing the intermediates for constructive metabolism, especially of the NADP-dependent malic enzyme. As a result of thiosulfate-induced synthesis of SH-containing cell components, the integral antioxidative activity increased 1.5-fold.

Key words: microaerophily, *Spirillum winogradskii*, thiosulfate, H_2O_2, TCA cycle enzymes, antioxidative activity.

The problem of protection of living organisms from oxidative stress is among the important ones for modern biology. Reactive oxygen species (ROSs) are known to cause damage to the cells of organisms of different levels of organization, including microorganisms [1]. Microaerophilic microorganisms are especially sensitive to oxygen and its derivatives, ROSs. The causes of microaerophily may be due to the toxic effect of ROSs resulting from the absence or low activity of certain important links of the cellular antioxidative protective enzyme systems. For example, in the case of the microaerophilic sulfur bacterium *Spirillum winogradskii*, apart from low activity of the antioxidant enzymes, spatial disruption of the processes of H_2O_2 production and accumulation was shown [2].

Apart from the enzymatic systems of antioxidant protection, a number of organic and inorganic compounds can play the same role in microbial cells, including such cellular metabolites as pyruvate, thiols, and reduced sulfur compounds—sulfides, thiosulfate, metabisulfite, and other reducing agents [2–5]. Intracellular sulfur accumulation by aerobic organotrophic colorless sulfur bacteria was shown to result from the oxidation of such reduced sulfur compounds as sulfides by the hydrogen peroxide produced in the electron transport chain (ETC) [6].

Thiosulfate was shown to increase the cell yield of *Spirillum winogradskii* and to enhance its tolerance to aerobic growth conditions [2]. However, neither the mechanisms of metabolic action of reduced sulfur compounds nor their possible contribution to the energy metabolism have been studied in detail.

The goal of the present work was to determine the functional role of reduced sulfur compounds in the metabolism of carbon and energy (including their effect on some enzymatic systems) and in the antioxidant protection in the aerotolerant bacterium *Spirillum winogradskii.*

MATERIALS AND METHODS

Bacterial strains and growth conditions. The study was performed with the microaerophilic spirillum *Spirillum winogradskii* strain D-427 (DSMZ 12756) from the strain collection of the Laboratory of Microbial Ecology and Geochemical Activity at the Institute of Microbiology, Russian Academy of Sciences. For comparison, the oxygen-resistant cultures *Escherichia coli* VKM B-12 and *Rhodococcus erythro-*

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polis were used; the latter strain was kindly provided by I.A. Borzenkov. *Spirillum winogradskii* and *E. coli* were grown on MPSS medium [7] with succinate (1 g/l) or acetate (1 g/l) as growth substrates. The medium for *Rh. erythropolis* was described in [8]. In certain experiments, the media were supplemented with filter-sterilized thiosulfate (1 g/l), FeS suspension (10 mg FeS per cultivation tube), CaS (0.2 ml of a 65 mg/l solution per 10 ml medium), or 0.1 ml of 0.05% polysulfide solution; the latter was introduced into the agar layer and overlaid with liquid medium. Medium of the same mineral composition (MPSS) supplemented with thiosulfate (1 g/l) was used to determine the capacity for autotrophic growth; for mixotrophic growth, it was supplemented with thiosulfate (1 g/l), acetate (50 or 100 mg/l), and yeast extract (100 mg/l). Experiments were performed at oxygen contents in the gas phase of 21 and 2%, corresponding to 8.5 and 0.6 mg/l O_2 in the liquid medium. Experiments on the effect of O_2 and thiosulfate on bacterial growth were performed using medium of the same composition but without peptone. Both whole-cell protein after centrifugation at 9000 *g* for 30 min and the supernatant soluble protein formed as the result of cell lysis were determined. The absence of cells in the supernatant was ascertained microscopically.

Analytical methods. Cell suspension, cell extracts (homogenates), and supernatants were obtained as described earlier [2]. Protein was determined by the Lowry method. Oxygen concentration in the gas phase was determined using an LKhM-80 gas chromatograph equipped with a katharometer; argon was used as the carrier gas at a flow rate of 40 ml/min; the filament current was 80 mA; the column was kept at room temperature. Oxygen concentration in the medium was determined by the Perfil'ev micromethod [9]. Acetate in the medium was determined by gas-adsorption chromatography on a Chrom-5 chromatograph (Czech Republic) equipped with a flame ionization detector. Total carbohydrate content was determined by the phenol method using an SF-26 spectrophotometer at 488 nm [10].

ATP determination in the cell suspension. ATP concentration in the cells was determined according to [11]. The ATPase inhibitor *N,N'*-dicyclohexylcarbodiimide (DCC) was introduced into the suspension to a concentration of 100 μM, and the measurement was performed after 10 min.

Analysis of inorganic sulfur compounds. The concentrations of S^{2-} and $S_2O_3^{2-}$ were determined by iodometric titration. When both $S_2O_3^{2-}$ and $S_4O_6^{2-}$ were present in the medium, their separate determination was performed by the cyanolytic method [12]. Intracellular elemental sulfur was identified by its characteristic refraction under a polarization microscope.

Determination of enzymatic activities. Enzymatic activities were determined in the supernatant of a two-day culture, in the middle of its exponential growth phase. The activity of the enzymes of the sulfur metabolism, the TCA cycle, gluconeogenesis, and the glutathione redox system was determined spectrophotometrically on the SF-26 by the standard methods as described previously [2, 13].

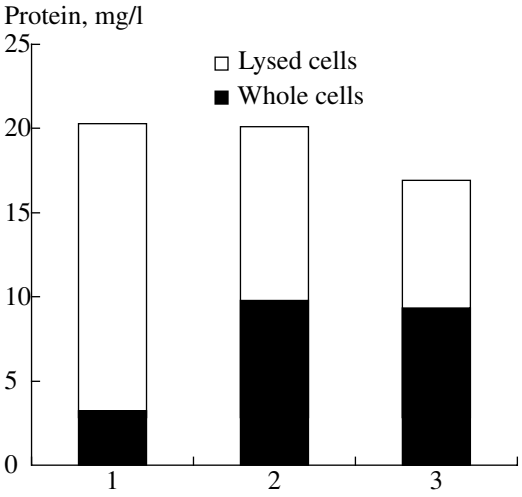

Fig. 1. Effect of thiosulfate and oxygen content in the gas phase on cell protein accumulation in cultures of *S. winogradskii* grown for 48 h (end of exponential growth) (1) aerobically without thiosulfate, (2) aerobically with thiosulfate, and (3) microaerobically without thiosulfate.

Determination of the antioxidative activity of the cells. The method was based on monitoring at λ = 600 nm the oxidation of reduced 2,6-dichlorophenolindophenol by oxygen dissolved in the reaction medium [14]. The reaction mixture without addition of cell homogenate was used as the control.

All the experiments were performed at least two to five times; the data presented in figures and tables are averaged values of representative experiments.

RESULTS

Effect of the Concentration of Oxygen and of Reduced Sulfur Compounds on Growth Processes

No growth of *S. winogradskii* was observed on mineral medium with thiosulfate no matter the concentration of oxygen in the gas phase. Growth occurred only when both succinate and thiosulfate were present and was accompanied by thiosulfate oxidation.

The results of investigation of the effect of thiosulfate on *S. winogradskii* growth under various oxygen concentrations are presented in Fig. 1. Under aerobic conditions, the cell yield was three times less compared to microaerobic conditions; cell lysis occurred. When thiosulfate was introduced into the medium, bacterial growth increased threefold. Compared to aerobic growth without thiosulfate, the amount of lysed cells decreased two- to threefold both for aerobic growth in the presence of thiosulfate and for microaerobic growth in the absence of thiosulfate; in all three cases, however,

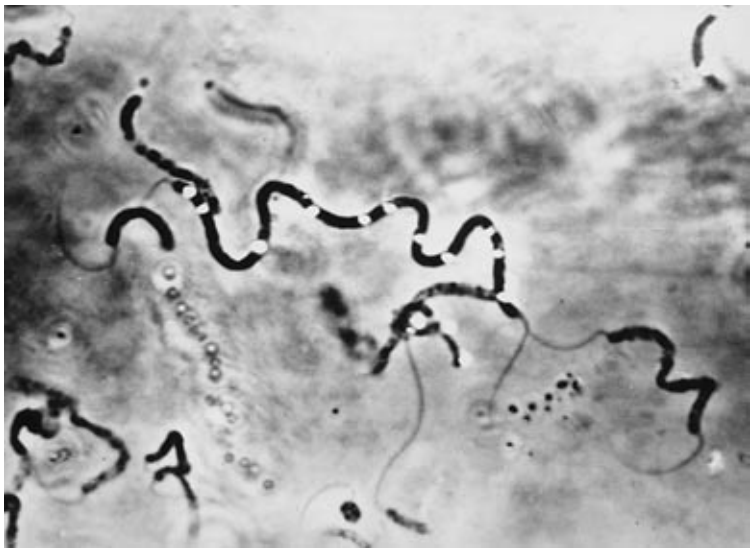

Fig. 2. Accumulation of elemental sulfur in the cells of *S. winogradskii*. Phase microscopy. Magnification, 1340×.

the total cell yield (whole and lysed cells) was about the same. Pronounced cell lysis both under aerobic conditions with thiosulfate and under microaerobic conditions is probably caused by the cellular mucous sheaths, preventing the drain of newly produced H_2O_2 into the culture medium.

The cells grown both under aerobic conditions with thiosulfate and under microaerobic conditions retained viability for up to a month and a half compared to 10 days for aerobic growth. Thiosulfate practically completely prevents the cell lysis that was shown [2] to result from H_2O_2 accumulation.

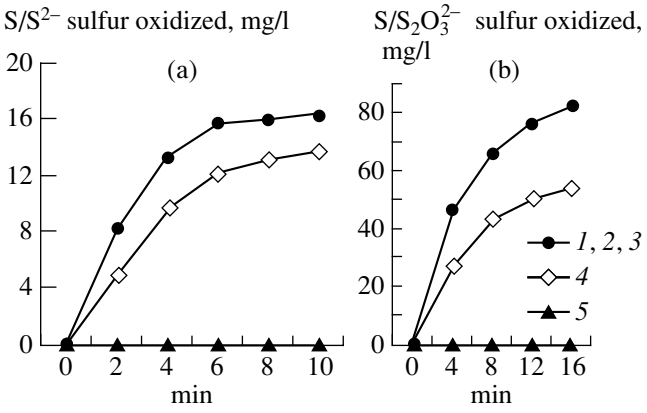

Fig. 3. Oxidation of (a) sulfide (initial concentration of sulfide sulfur, 8 mg/l; pH 7.8) and (b) thiosulfate (initial concentration of thiosulfate sulfur, 100 mg/l; pH 7.5) in constantly mixed cell suspensions of *S. winogradskii* (protein, 1 mg/ml): (*1*) chemical oxidation without bacteria; (*2*) heated cell suspension + catalase; (*3*) cell suspension + catalase; (*4*) cell suspension; (*5*) cell suspension + NaN_3 (10^{-5} M).

Upon growth in the presence of FeS, CaS, or polysulfides, elemental sulfur accumulates in the cells (Fig. 2). Immersion of washed cells grown in the absence of reduced sulfur compounds into a polysulfide-containing medium resulted in a similar microscopic picture; after 20-min exposure, the sulfur globules were already visible. Sulfides (FeS, CaS) were shown to extend the duration of the logarithmic growth phase and, as shown above, the viability and storage duration of the cultures.

The Mechanisms of the Oxidation of Reduced Sulfur Compounds

The rates of thiosulfate and sulfide oxidation and the analysis of decomposition products were investigated using a suspension of aerobically grown cells. The effect of catalase and therefore of intracellular H_2O_2 accumulation on the rates of sulfide and thiosulfate oxidation was considered. The results of these experiments are presented in Fig. 3. First of all, they indicate high rates of the oxidation of sulfur compounds in the cell suspension. Both sulfide and thiosulfate oxidation increased somewhat on addition of sodium azide (1×10^{-5} M) due to the inhibition of H_2O_2-decomposing catalase (the catalase activity of the cells is insignificant [2]).

Experimental results demonstrated a direct relationship between sulfide or thiosulfate oxidation and H_2O_2 accumulation in the cell suspension. It was confirmed by control experiments; no sulfide or thiosulfate oxidation occurred when catalase was added to cells killed by heating at 100°C.

Insignificant oxidation of the introduced sulfur compounds occurred in the heated cell suspension without catalase. In these cases, any enzymatic effect on sulfur compounds was excluded. Since the oxidation

observed in the heated suspension during the first several minutes of the experiment was terminated by catalase treatment, it was evidently caused by H_2O_2.

The dynamics of thiosulfate oxidation to tetrathionate in the culture is presented in Fig. 4. Thiosulfate was quantitatively oxidized to tetrathionate.

ATP Production in the Cell Suspension

The experimental results presented in Table 1 indicate that thiosulfate addition to the washed cell suspension resulted only in a slight, 10% increase of the ATP production rate. In the presence of acetate in the reaction mixture, however, addition of catalase or thiosulfate resulted in a pronounced, 1.6-fold increase of the intracellular ATP synthesis. It should be noted that simultaneous application of thiosulfate and catalase did not result in an additional increase in ATP synthesis; the 10% ATP increase after thiosulfate introduction was probably caused by the rapid "amelioration" of the toxic effect of H_2O_2 on cellular metabolism.

Preincubation of the cell suspension with DCC, an inhibitor of membrane-bound ATPase, decreased ATP synthesis by an average of 55–60%, thus indicating the relation of ATP synthesis to ATPase.

For the microaerobically grown cell suspension, similar rates of ATP production were found (data not shown).

The results of these experiments provide indirect evidence against the use of sulfur compounds in energy metabolism.

Activity of the Enzymes of Sulfur Metabolism

The activity of the specific enzymes of sulfur metabolism was determined in order to obtain direct evidence of utilization of reduced sulfur compounds in the energy metabolism of the spirilla when grown in the presence of an organic substrate. The results are presented in Table 2. No activity of specific dissimilation-type oxidases, sulfite oxidase and thiosulfate oxidase, was found when *S. winogradskii* was grown in polysulfide- or thiosulfate-containing media. Of the enzyme systems, only certain reductases participating in assimilatory processes of sulfur metabolism were found. The presence of thiosulfate in the medium mildly stimulated the activity of the thiosulfate-decomposing complex, a sulfur cycle enzyme of assimilatory type.

Thus, in spite of 2.5- to 3-fold increase in cell yield in the succinate medium caused by thiosulfate oxidation, no dissimilation-type enzymes of the sulfur metabolism were found. This is a direct proof of the inability of these spirilla to utilize sulfur compounds in their energy metabolism and to grow lithotrophically.

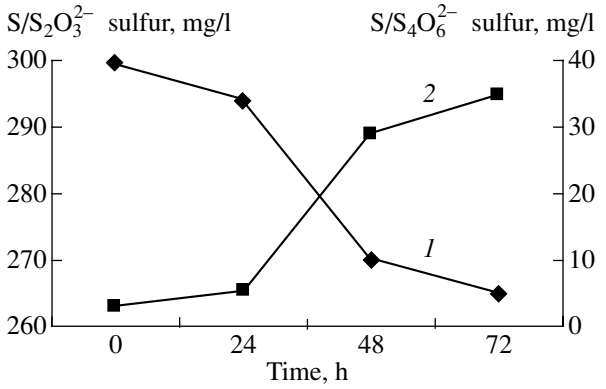

Fig. 4. Thiosulfate consumption and tetrathionate accumulation by *S. winogradskii* in the medium with thiosulfate and succinate: (*1*) thiosulfate, (*2*) tetrathionate.

Thiosulfate Effect on the Biosynthetic Processes and on Enzyme Systems

The results of investigation of the effect of thiosulfate on accumulation of cell protein and polysaccharides and on the efficiency of growth substrate utilization are presented in Table 3. Upon growth on acetate as a substrate, the net cell yield of *S. winogradskii*, including both whole and lysed cell protein, was about the same independent of the presence of thiosulfate (see also Fig. 1). Extracellular polysaccharides were produced during aerobic growth; in the presence of thiosulfate, their production decreased 1.5- to 2-fold. The calculated fraction of acetate used for polysaccharide synthesis was over 50% under aerobic conditions without thiosulfate and not more than 30% in the presence of thiosulfate. The economic coefficient of acetate consumption as calculated using the data of one of the experiments cited in Table 3 increased 1.5-fold under aerobic conditions with thiosulfate. These results indicate that, in the presence of thiosulfate, growth sub-

Table 1. ATP production rate in the cell suspension of *S. winogradskii*, nmol ATP/(min mg protein)

Experimental setup	ATP production rate	%
Endogenous respiration	3.4	100
+ DCC	1.5	44
+ $S_2O_3^{2-}$	3.7	109
+ catalase	4.5	132
+ acetate	4.0	118
+ acetate + DCC	1.5	44
+ acetate + catalase	5.5	162
+ acetate + $S_2O_3^{2-}$	5.6	165
+ $S_2O_3^{2-}$ + DCC	1.5	44
+ $S_2O_3^{2-}$ + catalase	4.8	141

Table 2. Effect of the oxygen regime of cultivation and of reduced sulfur compounds on the activity of the main enzymes of sulfur metabolism of *S. winogradskii*, nmol/(min mg protein)

Enzymes	Growth conditions		
	Aerobic		Microaerobic
	$-S_2O_3^{2-}$	$+S_2O_3^{2-}$	$-S_2O_3^{2-}$
Sulfite–cytochrome *c* oxidoreductase	0	0*	0
Sulfite–ferricyanide oxidoreductase	0	0*	0
Thiosulfate–cytochrome *c* oxidoreductase	0	0*	0
Thiosulfate–ferricyanide oxidoreductase	0	0*	0
Sulfur reductase	ND	3.5	ND
Thiosulfate-decomposing complex	17.0	25.0	12.8
Rhodanese	46.3	46.3	16.9

Note: ND, not determined.
* No activity regardless of the sulfur source (polysulfide or thiosulfate).

Table 3. Effect of thiosulfate on acetate consumption, polysaccharide synthesis, and growth yield coefficient of *S. winogradskii*

Growth conditions	Acetate consumption		Polysaccharide synthesis		Protein synthesis**		*K*
	mmol/l	C, mg/l*	mg/l	C, mg/l*	mg/l	cell protein C, mg/l***	
Aerobic – $S_2O_3^{2-}$	0.68	16.3	5.6	2.2	2.5	3.0	3.7
Aerobic + $S_2O_3^{2-}$	0.51	12.2	3.5	1.4	2.9	3.5	5.7

Note: Cells were taken from the midexponential growth phase (24 h). *K* is the growth yield coefficient, mg protein/mmol acetate.
* Polysaccharide carbon was calculated as glucose carbon.
** Cell protein (whole cells + lysed cells).
*** Cell carbon was calculated according to [17].

Table 4. Effect of thiosulfate on the activity of the enzymes of *S. winogradskii* involved in phosphoenolpyruvate and carbohydrate synthesis, nmol/(min mg protein)

Enzymes	Growth conditions		
	Aerobic – $S_2O_3^{2-}$ (A)	Aerobic + $S_2O_3^{2-}$ (B)	A/B ratio
Phosphoenolpyruvate synthase	210.0	175.0	1.2
Phosphoglucoisomerase	48.9	22.4	2.2
Fructose-bis-phosphatase	48.9	20.9	2.3

strate consumption was more efficient due to its decreased expenditure in polysaccharide biosynthesis.

Carbohydrate synthesis is known to start with the synthesis of the phosphoenolpyruvate precursor in the course of gluconeogenesis. The data on the effect of thiosulfate on the enzymatic activity of phosphoenolpyruvate synthase and of certain enzymes of gluconeogenesis are listed in Table 4. As evident from these results, the activity of all these enzymes decreased 1.2- to 2-fold in the presence of thiosulfate.

In Table 5 the results of the investigation of the thiosulfate effect on the activity of the TCA cycle enzymes in *S. winogradskii* under unimpeded oxygen supply are listed. A cell suspension of the aerobic heterotrophic bacterium *E. coli* strain VKM B-12 was used for comparison. The average activity of all the enzymes studied except for NADP-dependent isocitrate dehydrogenase (IDH) was found to be lower by an order of magnitude in spirilla. Thus, the TCA cycle of the spirilla had a low rate of functioning. Unlike NAD-dependent malate dehydrogenase (MDH), NADP-IDH is known to participate in constructive metabolic reactions and is not affected by ROSs [2]. Low activity of the former enzyme was characteristic for *S. winogradskii* independently of growth conditions; this enzyme may possibly limit the TCA cycle rate. The activity of NADP-dependent MDH and malic enzyme (ME), which directly participate in intermediate supply for constructive metabolism, was substantially higher than that of the NAD-dependent MDH. These data indicate that, in the case of growth with thiosulfate, the activity of the enzymes studied increased 1.5-fold and that of the NADP-dependent ME, 4-fold.

Table 5. Comparison of the activities (nmol/(min mg protein)) of TCA cycle enzymes in *S. winogradskii* and *E. coli*

Enzymes	Microorganisms and growth conditions		
	S. winogradskii		*E. coli*, strain VKM B-12
	Aerobic	Aerobic + $S_2O_3^{2-}$	Aerobic
Aconitate hydratase	35.5	47.0	160.0
Fumarate hydratase	20.0	37.5	220.0
Isocitrate dehydrogenase (NADP-dependent)	118.0	135.0	80.0
Succinate dehydrogenase	78.2	ND	340.0
Citrate synthase	17.8	29.3	130.0
Malate dehydrogenase (NADP-dependent)	60.3	86.8	ND
Malic enzyme (NADP-dependent)	39.5	153.0	ND
Malate dehydrogenase (NAD-dependent)	12.8	10.5	210.0

Note: ND, not determined; cell suspensions were obtained by culturing both strains on PSS medium with succinate.

These results indicate very low activity of the TCA cycle enzymes in the spirilla, five- to tenfold less compared to *E. coli*, except for the NADP-dependent IDH. Low activity of these enzymes, especially of the NAD-dependent MDH, implies low rates of the cellular TCA cycle and, consequently, low cell yield. Thiosulfate enhanced the activity of these enzymes by an average of 1.5-fold. The activity of one of the key enzymes of the anaplerotic pathway of cell biosynthesis, NADP-dependent ME, increased fourfold in the presence of thiosulfate. It may be therefore concluded that the beneficial effect of thiosulfate was most pronounced in the case of the TCA cycle enzymes involved in constructive metabolism.

Thiosulfate also has a beneficial effect on the enzymes of the glutathione redox system. The activity of glutathione peroxidase increased twofold, from 177 to 349 nmol/(min mg protein).

For the sake of comparison, antioxidative activity (AOA) of cells was determined for a number of bacterial species varying in their resistance to oxygen (Table 6). These results demonstrate that the integral AOA value, reflecting resistance to oxidative stress, was most affected by thiosulfate in the case of the microaerophilic heterotrophic strains *Beggiatoa leptomitiformis* D-405 and D-402, lacking catalase. It is less pronounced in other sulfur bacteria—*S. winogradskii, M. bipunctata* strain D-405, and *A. bipunctata* strain D-411. Thiosulfate had no effect on AOA values of the control strain *Rh. erythropolis*, which is extremely resistant to O_2 and H_2O_2.

DISCUSSION

No attempt to cultivate *S. winogradskii* on mineral media with thiosulfate without organic substrates (acetate or succinate) was successful. It was found that the stimulatory effect of thiosulfate on cell yield in the presence of organic matter was not related to utilization of sulfur compounds for energetic purposes. This is evident from the absence of activity of the enzymes of the dissimilation-type sulfur metabolism and from the data on ATP synthesis by the cell suspension in the presence of reduced sulfur compounds.

The stimulatory effect of thiosulfate on *S. winogradskii* growth is therefore not related to the use of sulfur compounds as electron donors for mixotrophic growth.

Revealing the mechanisms of the effect of sulfur compounds on the processes of cell growth and metabolism was the second goal. High rates of H_2O_2 production were previously shown in spirilla cells during growth on media containing organic substrates [2]. Reaction of H_2O_2 with reduced sulfur compounds is known to result in formation of different products, mostly tetrathionate in the course of oxidation of thiosulfate and elemental sulfur in the presence of sulfide (FeS, CaS) [3, 4]. Experiments on the mechanisms of

Table 6. Effect of thiosulfate on the antioxidative activity of sulfur bacteria (AOA)

Bacteria	AOA, units/(min mg protein)		
	Growth conditions		
	$-S_2O_3^{2-}$ (A)	$+S_2O_3^{2-}$ (B)	B/A, %
Beggiatoa leptomitiformis D-402	2.8	3.9	139
Beggiatoa leptomitiformis D-405	1.5	2.9	193
Macromonas bipunctata D-405	0.9	1.6	178
Aquaspirillum bipunctata D-411	0.9	1.4	156
Spirillum winogradskii D-427	1.6	2.4	150
Rhodococcus erythropolis	0.8	0.8	100

oxidation of reduced sulfur compounds by cell suspensions using inhibitor analysis revealed that spirilla, like other colorless sulfur bacteria [3, 4], oxidize sulfur compounds via reaction with the toxic products of oxygen metabolism, primarily H_2O_2.

Apart from its beneficial effect on cell viability caused by removal of H_2O_2 and probably other toxic oxygen species, thiosulfate affects cellular metabolism as a whole. The effect of sulfur compounds is revealed in decreased expenditures on the biosynthetic and energetic processes of polysaccharide synthesis and cell protection from toxic oxygen species under aerobic conditions. Increased efficiency of the growth substrate utilization, economic coefficient, and cell yield are the final results (Table 3).

Removal of H_2O_2 by sulfur compounds directly decreases its toxic effect on cell components and on a number of enzymatic systems of carbon metabolism, the TCA cycle in particular. As we demonstrated previously for the NAD-dependent MDH [2] and for the enzymes containing iron–sulfur clusters (e.g., aconitate and fumarate hydratases), for sulfur-containing proteins and amino acids [16–18], low activity of the TCA cycle enzymes under aerobic conditions may be to some extent the result of their inhibition by toxic oxygen species. The results obtained enable the suggestion that low activity of the TCA cycle enzymes, especially the NADP-dependent MDH of the obligate microaerophile *Spirillum volutans* [19], is the result of the same causes. The results presented above demonstrated that cultivation of spirilla in the presence of thiosulfate promoted enhanced activity of all the TCA cycle enzymes, especially of the NADP-dependent ME, involved in the anaplerotic reaction producing pyruvate, the substrate for amino acid synthesis.

Under the influence of thiosulfate on spirilla, the integral AOA value, reflecting the net antioxidative capability of the cell, increases 1.5-fold, probably due to induced synthesis of sulfur-containing cell components capable of binding ROSs, primarily the compounds containing an SH group. It is worth mentioning that the original AOA value for the catalase-negative *Beggiatoa leptomitiformis* strain D-405 was higher than for the other strains studied. The response to thiosulfate introduction was also the highest for *Beggiatoa leptomitiformis*. Meanwhile, the low AOA value of *Rh. erythropolis,* resistant to high concentrations of O_2 and H_2O_2 (I.A. Borzenkov, personal communication), reflects the high degree of cell protection against oxidative stress; this is probably the reason why thiosulfate does not affect this factor.

These results therefore indicate a polyfunctional role of inorganic reduced sulfur compounds in the activities of *S. winogradskii*. The positive effect of thiosulfate reveals itself in optimization of enzymatic systems and of the constructive processes of carbon metabolism and, finally, in stabilization of the growth of the microaerophilic sulfur spirillum under aerobic conditions.

Similar mechanisms probably lay at the root of the stimulatory effect of reduced sulfur compounds both for sulfur-oxidizing heterotrophs and for a number of other aerobic heterotrophic microorganisms abounding in microaerobic habitats with a supply of hydrogen sulfide.

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