

# Utilization of solid ‘elemental’ sulfur by the phototrophic purple sulfur bacterium *Allochromatium vinosum*: a sulfur K-edge X-ray absorption spectroscopy study

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The purple sulfur bacterium *Allochromatium vinosum* can use elemental sulfur as an electron donor for anoxygenic photosynthesis. The elemental sulfur is taken up, transformed into intracellular sulfur globules and oxidized to sulfate. Commercially available ‘elemental’ sulfur usually consists of the two species *cyclo*-octasulfur and polymeric sulfur. The authors investigated whether only one sulfur species is used or at least preferred when *Alc. vinosum* takes up elemental sulfur and forms globules. To this end, *Alc. vinosum* was cultivated photolithoautotrophically with two types of elemental sulfur that differed in their *cyclo*-octasulfur : polymeric sulfur ratio, as well as with pure polymeric sulfur. Sulfur speciation was analysed using X-ray absorption spectroscopy, and sulfate contents were determined by HPLC to quantify the amount of elemental sulfur being taken up and oxidized by *Alc. vinosum*. The results show that *Alc. vinosum* uses only the polymeric sulfur (sulfur chain) fraction of elemental sulfur and is probably unable to take up and form sulfur globules from *cyclo*-octasulfur. Furthermore, direct cell–sulfur contact appears to be necessary for uptake of elemental sulfur by *Alc. vinosum*.

Received 31 October 2006

Revised 18 December 2006

Accepted 22 December 2006

## INTRODUCTION

It has been known for a long time that the anoxygenic phototrophic sulfur bacterium *Allochromatium vinosum* (formerly *Chromatium vinosum*) and other photo- and chemotrophic sulfur-oxidizing bacteria are able to take up externally added solid, virtually insoluble elemental sulfur (Thiele, 1968; Pfennig & Trüper, 1974). Although this step is very important in the global sulfur cycle (e.g. Brüser *et al.*, 2000), the process of mobilization and the utilization of different sulfur species is barely understood.

The formal valency of elemental sulfur is zero ( $S^0$ ). However, elemental sulfur tends to catenate and to form chains of various lengths ( $S_\infty$  or  $S_n$ ) and ring sizes ( $S_n$ ) (Steudel, 2000; Steudel & Eckert, 2003). Cyclic, orthorhombic  $\alpha$ -sulfur ( $\alpha$ - $S_8$ ) (*cyclo*-octasulfur or  $S_8$  rings) is the thermodynamically

most stable form of elemental sulfur at ambient temperature and pressure (e.g. Roy & Trudinger, 1970; Steudel, 2000). Accordingly, *cyclo*-octasulfur is one of the main components of typical commercially available elemental sulfur (‘flowers of sulfur’). In addition, elemental sulfur (flowers of sulfur) always contains some polymeric sulfur consisting of chain-like macromolecules, but the additional presence of large rings,  $S_n$  ( $n > 50$ ), is very likely (Steudel & Eckert, 2003). The bonding energy of the S–S bonds in polymeric sulfur is 2.4 kJ mol<sup>-1</sup> weaker than that in *cyclo*-octasulfur (Steudel *et al.*, 1985; Steudel, 1996; Steudel & Eckert, 2003). Accordingly, polymeric sulfur, i.e. chain-like sulfur, might be more easily accessible to sulfur-oxidizing bacteria and for elemental sulfur uptake.

Some studies of the use of solid elemental sulfur by different thiobacilli have been published in recent years (e.g. Silver, 1970; Baldensperger *et al.*, 1974; Espejo & Romero, 1987;

Abbreviation: XANES, X-ray absorption near-edge structure.

Pronk *et al.*, 1992). However, only one study, that of Laishley *et al.* (1986), has considered the speciation of the elemental sulfur. Those authors investigated the effect of the molecular composition of different forms of elemental sulfur (pure  $S_{\mu}$ , pure  $S_8$ , and a mixture of both and another sulfur species,  $S_x$ ) on the oxidation of sulfur by *Acidithiobacillus albertensis* (formerly *Thiobacillus albertis*; Kelly & Wood, 2000) under aerobic conditions. Those authors suggest that oxidation of elemental sulfur is more dependent on the crystal microstructure than on the specific molecular composition. However, chemical methods were used to produce and determine different elemental sulfur species, and the methods applied (heating, extraction) might have influenced the chemical structure.

During the oxidation of reduced inorganic sulfur compounds (sulfide, thiosulfate) by *Alc. vinosum* to the final product sulfate, sulfur globules are accumulated as an intermediate inside the cells (Brune, 1995; Dahl & Prange, 2006). The chemical nature of these globules has been determined by Prange *et al.* (2002a) as sulfur chains (most probably terminated by organic residues at one or both ends) by X-ray absorption near-edge structure (XANES) spectroscopy. This is also an excellent *in situ* technique to determine the sulfur speciation in biological samples (details can be found in e.g. Prange & Modrow, 2002). The method is non-destructive and a 'quantitative analysis' of XANES spectra can be performed (e.g. Modrow *et al.*, 2001; Prange *et al.*, 2002a, 2005). Furthermore, XANES can distinguish between sulfur rings and chain structures of zero valent sulfur (Prange *et al.*, 2006).

While Prange *et al.* (2002a) investigated the speciation of sulfur globules of *Alc. vinosum* and other sulfur oxidizers, we focused in this study on the question which sulfur species is preferred when *Alc. vinosum* takes up elemental sulfur. Furthermore, the aim was to analyse the sulfur speciation in the globules of *Alc. vinosum* after uptake of elemental sulfur and to investigate whether direct cell-sulfur contact is necessary for this process.

## METHODS

**Bacterial strain, medium and growth conditions.** *Alc. vinosum* DSMZ 180<sup>T</sup> was cultivated photolithoautotrophically in batch culture at 30 °C under anaerobic conditions for 5 days in a thermostatted glass fermenter (culture volume 1.6 l) containing modified Pfennig's medium (0 medium without sulfide) (Hensen *et al.*, 2006). Solid elemental sulfur ( $S^0$ ; 50 mM) was added as the sole sulfur source to the culture. To maintain pH  $7.0 \pm 0.1$ , sterile HCl and  $Na_2CO_3$  solutions were added by a pH-stat. As an inoculum for the growth experiments, cells of *Alc. vinosum* grown photoorganoheterotrophically on malate were used and added to the 0 medium. Protein content was determined using the Bradford assay (see below; Bradford, 1976). Two different commercially available forms of elemental sulfur were purchased and used as received from Riedel de Haen (sulfur 1; S1) and from Merck (sulfur 2; S2), respectively, in the fermenter experiments. Furthermore, small amounts of pure polymeric sulfur (50 mM), which were kindly provided by Professor Dr R. Steudel, TU Berlin, were used for culture volumes of 100 ml.

**Determination of sulfur compounds by HPLC.** Sulfur compounds (sulfide, thiosulfate, sulfite and sulfate) were determined by HPLC (Thermo Separation Products) using the method of Rethmeier *et al.* (1997). Sulfate was measured in culture supernatants.

**Determination of protein concentration.** The protein concentration of fermenter samples of *Alc. vinosum* was determined using Bradford reagent (Sigma). Liquid culture (1 ml) was centrifuged (15 700 g, 4 min) and the pellet was resuspended in 1 ml 1 M NaOH. The sample was incubated for 5 min at 95 °C and centrifuged again (15 700 g, 3 min). Supernatant (25  $\mu$ l) was mixed with 750  $\mu$ l Bradford reagent and incubated for 10 min. The absorbance was measured at 595 nm against a reagent blank. A calibration curve was recorded for the range 0–1.4 mg BSA ml<sup>-1</sup>.

**(XANES) spectroscopy – experimental.** XANES spectra were recorded at the Double Crystal Monochromator beamline of the Center for Advanced Microstructures and Devices (CAMD), Baton Rouge, LA (Hormes *et al.*, 2006). CAMD's storage ring was operated at an energy of 1.3 GeV with electron currents between 200 and 80 mA. The synchrotron radiation was monochromatized by a modified Lemonnier-type monochromator (Lemonnier *et al.*, 1978) equipped with InSb (111) crystals. The monochromatic flux rate per second on the sample was about  $5 \times 10^8$  photons (at 100 mA). Measurements (repeated twice) were performed in transmission mode with ionization chambers (60 mbar air pressure inside). For energy calibration, the spectrum of zinc sulfate was used as a 'secondary standard', setting the maximum of the first resonance (white line) to an energy of 2481.44 eV. (reproducibility  $\pm 0.1$  eV). Spectra were scanned with step widths of 0.5 eV between 2440 and 2468 eV, 0.1 eV between 2468 and 2485 eV, and 0.3 eV between 2490 and 2520 eV (integration time 1 s). Using the Origin program (OriginLab), a linear background determined in the pre-edge region was subtracted from the raw data to correct the spectra for contributions from higher shells and from supporting materials. Spectra were normalized at 2510 eV.

**Quantitative analysis of spectra.** The interactive fitting and plotting packages Mn-Fit 4.04/15 (available at [http://www-zeus.physik.uni-bonn.de/~brock/mn\\_fit.html](http://www-zeus.physik.uni-bonn.de/~brock/mn_fit.html)) and WINXAS (Ressler, 1998) were used. Both programs provide tools to find the minimum value of a multi-parameter function and to analyse the shape of the function around the minimum. Both packages yield comparable results (percentage contributions). A set of five reference spectra was used for fitting the bacterial spectra (reduced and oxidized glutathione, *cyclo*-octasulfur, polymeric sulfur and zinc sulfate), and a set of two reference spectra for fitting the elemental sulfur (*cyclo*-octasulfur, polymeric sulfur), respectively. A chi square criterion was applied to find the linear combination of these spectra that reproduced the XANES spectrum of interest with the highest probability. Further details concerning the quantitative analysis, especially its verification, potential and restrictions, have been published previously (Prange *et al.*, 2002a, 2003). The errors of the percentage contributions of sulfur species (Table 1) can be estimated to be <10% (absolute value) (Prange *et al.*, 2002a; 2003).

**Sample preparation.** The remaining sulfur platelets (see Results and Discussion) were separated from the culture by filtration. Sulfur platelets were placed homogeneously as a thin layer on a sulfur-free self-adhesive Kapton film (type 7010, CWC Klebetechnik), topped by a polypropylene film (SPEX Industries). Bacterial cells were prepared as described previously (Prange *et al.*, 1999, 2002a).

**Reference compounds.** Glutathione (oxidized and reduced) and zinc sulfate were used as reference compounds for fitting the spectra. These compounds were of reagent grade, purchased from Sigma and used as received. Furthermore, pure *cyclo*-octasulfur ( $S_8$  rings) and polymeric sulfur were used as reference compounds, both kindly

**Table 1.** Results of fitting the sulfur K-edge XANES spectra of sulfur 1 (S1), sulfur 2 (S2) and the remaining sulfur platelets to the sum of the reference spectra

Sample	Percentage contribution of sulfur species*	
	<i>cyclo</i> -octasulfur (S <sub>8</sub> rings)	Polymeric sulfur
S1 (added)	30	68
S1 (remaining)	95	3
S2 (added)	62	39
S2 (remaining)	84	12

Sample	Percentage contribution of sulfur species*				
	Gluta-thione (reduced)	Gluta-thione (oxidized)	<i>cyclo</i> -octasulfur	Polymeric sulfur	Zinc sulfate
<i>Alc. vinosum</i> cells with sulfur globules after uptake of elemental sulfur S1	8	15	–	78	–

\*Different sulfur species and their percentage contribution to sulfur speciation are shown. Error  $\leq 10\%$ ; –, contribution  $< 1\%$ .

provided by Professor Dr R. Steudel. The reference compounds were ground into fine powder and placed homogeneously on a Kapton film (see above).

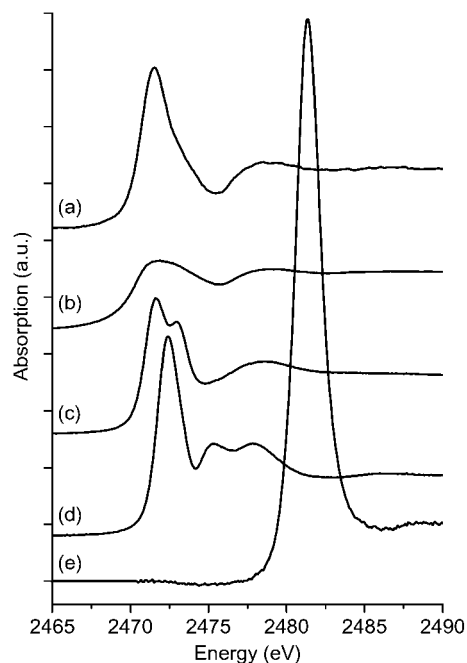
## RESULTS AND DISCUSSION

The aim of this investigation was to clarify whether *Alc. vinosum* is able to use both S<sub>8</sub> rings and polymeric sulfur chains, or whether it prefers one of those species. For this purpose, *Alc. vinosum* was cultivated photolithoautotrophically with 50 mM elemental sulfur as sole sulfur source. Immediately after addition, the sulfur formed hydrophobic clumps on the surface of the medium, but under continuous stirring it was completely dispersed into fine particles after 3 h. This dispersion into the medium was simply a result of stirring and was independent of the presence of the cells. The presence of particulate sulfur in the medium caused problems with the quantification of sulfur during the experiments. *Alc. vinosum* accumulates sulfur globules as an obligate intermediate of solid elemental sulfur oxidation, which, to the best of our knowledge, has never been described before. The sulfur-containing cells were completely surrounded by the finely dispersed solid elemental sulfur particles supplied as a substrate. Thus, in the samples taken during the fermentations, a quantitative separation of cells and elemental sulfur was not possible. A quantitative assessment of the amount of sulfur outside versus inside the cells was therefore not possible. Instead, the formation and subsequent degradation of intracellular sulfur globules

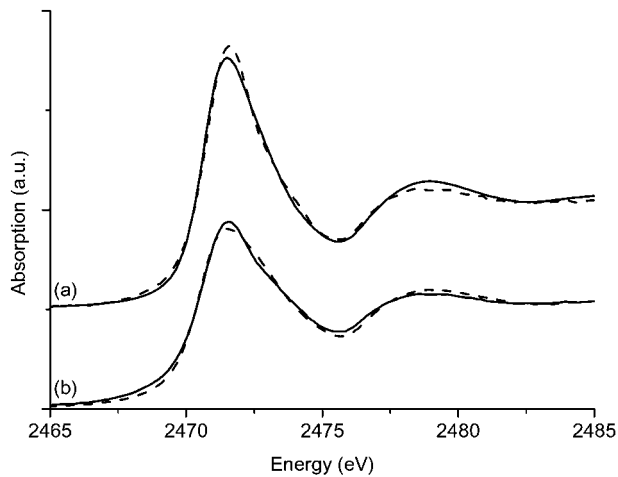
following the uptake of externally added elemental sulfur was observed microscopically, and quantified indirectly by determining the final oxidation product sulfate. Also, the concentration of protein in the cultures was determined as an indicator of growth of the cells.

Firstly, the exact chemical speciation of the two forms of elemental sulfur (S1 and S2) that were used for the growth experiments was analysed by XANES spectroscopy to determine the ratios of S<sub>8</sub> rings to polymeric sulfur. Fig. 1(a, b) shows the sulfur K-edge XANES spectra of pure *cyclo*-octasulfur and pure polymeric sulfur, respectively. Fig. 2 shows the sulfur K-edge XANES spectra of the two different batches of elemental sulfur and the corresponding fits. The quantitative analyses of the spectra showed that the two forms of elemental sulfur differed significantly in the ratio of S<sub>8</sub> rings and polymeric sulfur (Table 1).

In the first set of growth experiments, S1 was added to the culture. Three hours after the addition of S1, *Alc. vinosum* started to accumulate sulfur globules. An increase in the amount of intracellular sulfur globules was observed microscopically during the following 9 h. Oxidation of intracellular stored sulfur to sulfate started after 12 h. The sulfur globules were completely degraded and oxidized to sulfate after 132 h (Table 2). During that time, protein concentration increased from 0.106 to 0.738 mg ml<sup>-1</sup> (Table 3), indicating growth of the culture with elemental



**Fig. 1.** Sulfur K-edge XANES spectra of the reference compounds which were used for fitting bacterial and sulfur spectra: (a) polymeric sulfur, (b) *cyclo*-octasulfur (S<sub>8</sub> rings), (c) oxidized glutathione, (d) reduced glutathione and (e) zinc sulfate. a.u., Arbitrary units.



**Fig. 2.** Sulfur K-edge XANES spectra (solid lines) of (a) the elemental sulfur sulfur 1 (S1) and (b) sulfur 2 (S2) used in the growth experiments, and accompanying fits (dashed lines). a.u., Arbitrary units.

sulfur as the single sulfur source. Further uptake and oxidation of elemental sulfur did not occur in the following 24 h, and small sulfur platelets remained in the culture (see below). Only 37 mM sulfate was formed from 50 mM sulfur, showing that only 74 % of the added elemental sulfur was taken up and oxidized to sulfate. Taking an error of  $\pm 10$  % of the quantitative analysis into account, this result fits quite well to the 68 % polymeric sulfur fraction in S1 (Table 1). The protein concentration of the culture increased only marginally after oxidation of the intracellular stored sulfur, also indicating that further sulfur oxidation did not occur in that time. The occurrence of sulfur platelets was observed about 30 h after the addition of elemental sulfur; they remained in the culture and were not taken up by *Alc. vinosum*. A corresponding formation of sulfur platelets was never observed in control experiments without cells. XANES analyses of these remaining sulfur platelets

showed that they consisted almost completely ( $\sim 95$  %) of  $S_8$  rings (Fig. 3a, Table 1). The level of polymeric chains in the remaining sulfur platelets was only  $\sim 3$  %, compared to  $\sim 68$  % at the beginning of the experiment.

In a second set of growth experiments, cultures of *Alc. vinosum* were fed with elemental sulfur with a different  $S_8$  ring : polymeric chain ratio (S2). Quantitative analysis of the XANES spectra (Fig. 2b) showed that the polymeric chain fraction was about 39 % (Table 1) and therefore significantly lower than that of S1. Dispersion of the added elemental sulfur, formation of the sulfur globules, generation of sulfur platelets and the beginning of sulfate production were comparable to those of the first experiment. Sulfur platelets also remained in the culture and were not taken up and oxidized by *Alc. vinosum*. A constant amount of 22.6 mM sulfate was determined for 153 h, corresponding to 45 % of the expected amount of sulfate. The added elemental sulfur contained  $\sim 39$  % polymeric sulfur (compared to 45 % sulfate) and the remaining sulfur platelets mostly consisted of *cyclo*-octasulfur (Tables 1 and 2).

Both sets of growth experiments yielded evidence that *Alc. vinosum* can use only polymeric sulfur, or at least strongly prefers this sulfur species when growing on elemental sulfur. This interpretation was supported by the observation that cultures of *Alc. vinosum* fed with pure polymeric sulfur (50 mM) took up the added sulfur completely, formed intracellular sulfur globules, and completely oxidized the stored sulfur to 49 mM sulfate in the medium. Sulfur platelets did not appear after degradation of the sulfur globules. To make sure that the speciation in the sulfur globules of *Alc. vinosum* grown on elemental sulfur was comparable to that of *Alc. vinosum* grown on sulfide, we investigated *Alc. vinosum* cells (with internal sulfur globules) by XANES spectroscopy (Fig. 3c). Fitting the spectrum with five reference compounds (Fig. 1, Table 1) yielded a sulfur speciation very similar to that obtained for *Alc. vinosum* cells grown on sulfide (Prange *et al.*, 2002a). Only the

**Table 2.** Sulfate determination and observation by light microscopy of sulfur globule formation in cultures fed with sulfur 1 (S1), sulfur 2 (S2), as well as S1 from the cells separated by a dialysis membrane

The percentage values in parentheses show the relative amounts of sulfur recovered as sulfate, compared to a total of 50 mM in the culture. –, Sulfur globules were not observed; ND, not determined.

Fed with		Time (h)							
		0	3	12	84	114	132	156	267
S1	Sulfate concn (mM)	0.0 (0 %)	0.0 (0 %)	0.2 (0.4 %)	18.2 (36 %)	27.9 (56 %)	37.3 (74 %)	37.3 (74 %)	ND
	Sulfur globules	–	Yes	Yes	Yes	Yes	–	–	ND
S2	Sulfate concn (mM)	0.0 (0 %)	0.0 (0 %)	0.1 (0.2 %)	16.2 (32 %)	22.6 (45 %)	22.6 (45 %)	22.6 (45 %)	22.6 (45 %)
	Sulfur globules	–	Yes	Yes	Yes	–	–	–	–
S1 in dialysis membrane	Sulfate concn (mM)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Sulfur globules	–	–	–	–	–	–	–	–

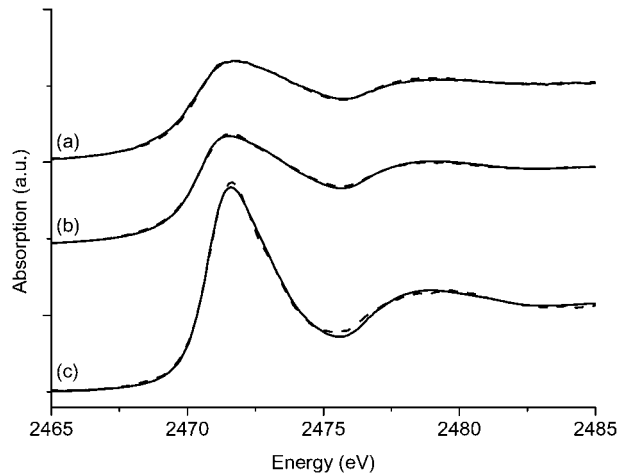
**Table 3.** Protein determination of cultures fed with sulfur 1 (S1), sulfur 2 (S2), as well as S1 from the cells separated by a dialysis membrane

Values show protein concentration ( $\text{mg ml}^{-1}$ ). ND, Not determined.

Fed with:	Time (h)							
	0	3	12	84	114	132	156	267
S1	0.106	0.141	0.202	0.469	0.561	0.702	0.738	ND
S2	0.048	0.059	0.062	0.263	0.265	0.245	0.252	0.229
S1 in dialysis membrane	0.078	0.078	0.077	0.074	ND	ND	ND	ND

monosulfane:disulfane ratio differed, and this can be attributed to physiological variability (cf. Prange *et al.*, 2002b).

Our observation that *Alc. vinosum* only uses, or at least strongly prefers, polymeric sulfur (sulfur chains) is in contrast to the findings of Laishley *et al.* (1986) for *Atb. albertensis*, which has the ability to oxidize *cyclo*-octasulfur as well as polymeric sulfur. However, it has to be kept in mind that *Atb. albertensis* is an aerobic, chemotrophic sulfur-oxidizing bacterium that grows at low pH, and therefore differences in elemental sulfur use can be expected. While *Atb. albertensis* is supposed to be more affected by the sulfur crystal microstructure than by its specific molecular composition (Laishley *et al.*, 1986), the ability to take up elemental sulfur in the phototrophic bacterium *Alc. vinosum* seems to be completely dependent on the specific molecular composition of the elemental sulfur. On the basis of the results presented in this study for *Alc. vinosum*, one might speculate that sulfur chains are the microbiologically preferred form of elemental, zero valent sulfur for other sulfur-oxidizing bacteria also. Our hypothesis gains some support from the recent study of Urich *et al.* (2006). They investigated the influence of different sulfur species on enzyme functions in the sulfur oxygenase reductase from the thermoacidophilic archaeon *Aquifex aeolicus*. Theoretical considerations on the basis of the crystal structure of this enzyme led to the hypothesis that linear, but not cyclic, sulfur species can serve as a substrate for this enzyme. Furthermore, sulfur globules of *Alc. vinosum* consist exclusively of sulfur chains (mono-bis-organyl sulfanes) that are gradually shortened during oxidation of the sulfur stored in the sulfur globules (Prange *et al.*, 2002a, b). Therefore, it appears that the organism is completely unable to deal with sulfur rings, the chemically more stable *cyclo*-octasulfur, outside or inside the cell. A different mechanism (instead of direct uptake of elemental sulfur) has been hypothesized for the green sulfur bacterium *Prosthecochloris vibrioformis*. In this bacterium, sulfide appears as an intermediate during elemental sulfur oxidation (Borkenstein & Fischer, 2006), which corresponds to the earlier findings of Paschinger *et al.* (1974) for *Chlorobium limicola*. In *Alc. vinosum*, however, we did not find evidence

**Fig. 3.** Sulfur K-edge XANES spectra (solid lines) of remaining sulfur platelets in the cultures and accompanying fits (dashed lines): (a) sulfur 1 (S1), (b) sulfur 2 (S2) and (c) *Alc. vinosum* cells containing sulfur globules after uptake of elemental sulfur. a.u., Arbitrary units.

in the XANES spectra for the formation of intermediates such as sulfide or polysulfides during uptake of elemental sulfur. Furthermore, sulfide was not detectable by the very sensitive detection technique as a bismane derivative. Finally, to the best of our knowledge, no enzymes have been reported which can perform a sulfur ring cleavage or sulfur ring activation.

For some thiobacilli able to use elemental sulfur, direct cell-sulfur contact is necessary for its oxidation (Schaeffer *et al.*, 1963; Bryant *et al.*, 1984; Espejo & Romero, 1987). Therefore, it was investigated whether *Alc. vinosum* also needs direct cell-sulfur contact for elemental sulfur oxidation. The elemental sulfur (S1) was separated from the cells by a dialysis membrane with a 100 kDa cut-off. This completely prevented the formation of sulfur globules. Sulfate was not detected during 84 h, and the protein concentration did not increase, showing that the culture did not grow during that time. This indicates that it is necessary for *Alc. vinosum* to be attached to elemental sulfur particles, and that direct cell-sulfur contact is a prerequisite for the uptake of elemental sulfur (see Note Added in Proof). The mechanism of uptake, however, is completely unknown. Only a few studies of the elemental sulfur adhesion mechanisms of chemotrophic sulfur oxidizers are available. Takakuwa *et al.* (1979) have shown for *Acidithiobacillus thiooxidans* that thiol groups are probably involved in the process. Also, the adhesion ability seems to be energy dependent. For *Atb. albertensis*, Bryant *et al.* (1984) have shown that adhesion to elemental sulfur is due to an interaction between the glycocalyx of the organism and the sulfur surface. In other studies, Ohmura *et al.* (1996) and Ramírez *et al.* (2004) have found evidence for an outer-membrane protein in *Acidithiobacillus ferrooxidans* (for-

merly *Thiobacillus ferrooxidans*) which is probably involved in cell-sulfur adhesion. Extracellular polymeric substances (LPSs), which have been found in *Atb. ferrooxidans* and *Atb. thiooxidans* when attached to pyrite (Gehrke *et al.*, 1998; Harneit *et al.*, 2006), might have a similar function. Experiments to investigate the adhesion of elemental sulfur to phototrophic bacteria, however, have been not carried out so far, but will be performed in future in *Alc. vinosum*.

## Conclusions

Our results show that the molecular composition of elemental sulfur is essential for its uptake and oxidation in *Alc. vinosum*. This organism can only use, or at least strongly prefers, the polymeric sulfur fraction (sulfur chains) when taking up elemental sulfur. Furthermore, direct cell-sulfur contact is needed for the uptake of elemental sulfur. Probably, speciation of elemental sulfur plays an important role in a more general way and we hypothesize that sulfur chains are also the microbiologically preferred form when elemental sulfur is taken up and oxidized by other micro-organisms. In our ongoing research, the utilization, mobilization and oxidation of elemental sulfur by other sulfur-oxidizing bacteria will be investigated.

## NOTE ADDED IN PROOF

In parallel to our work on the purple sulfur bacterium *Allochromatium vinosum*, similar experiments were performed by Clemens Borkenstein and Ulrich Fischer, University of Bremen, Germany, on the green sulfur bacterium *P. vibrioformis* (C. Borkenstein & U. Fischer, publication in preparation). Separation of elemental sulfur and *P. vibrioformis* cells by dialysis tubes resulted in no growth, which strengthens the necessity of a close contact between *P. vibrioformis* cells and elemental sulfur (U. Fischer, personal communication) and which is in good accordance with our findings for *Alc. vinosum*.

## ACKNOWLEDGEMENTS

The State of Louisiana is acknowledged for providing the operating budget of CAMD. A.P. thanks the Heinrich-Hertz Foundation for support of two research visits at CAMD. This study was partly supported by the German Research Council (DFG Da351/3-3, 3-4, C. D.) and the Fonds der Chemischen Industrie (grant 661209, A. P.). Excellent technical assistance by Chris M. Bianchetti, Russell 'Rusty' Louis and Gregory Merchan is gratefully acknowledged.

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Edited by: H. L. Drake