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ORIGINAL PAPER

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Cysteine-mediated electron transfer in syntrophic acetate oxidation by cocultures of *Geobacter sulfurreducens* and *Wolinella succinogenes*

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Abstract Syntrophic cocultures of *Geobacter sulfurreducens* and *Wolinella succinogenes* oxidize acetate with nitrate as terminal electron acceptor. It has been postulated earlier that electrons are transferred in these cocultures not via hydrogen, but via a different carrier, e.g., a small *c*-type cytochrome that is detected in the supernatant of growing cultures. In the present study, L-cysteine, which was provided as a reducing agent, was found to mediate the electron transfer between the two partners. Low concentrations of L-cysteine or L-cystine (10–100 μM) supported syntrophic growth, and no acetate oxidation was observed in the absence of cysteine or cystine. Cell suspensions of *G. sulfurreducens* or coculture cell suspensions reduced cystine to cysteine, and suspensions of *W. succinogenes* or coculture suspensions oxidized cysteine with nitrate, as measured by the formation or depletion of free thiol groups. Added cysteine was rapidly oxidized by the coculture during growth, but the formed cystine was not entirely rereduced even under acceptor-limited conditions. The redox potential prevailing in acetate-oxidizing cocultures was –160 to –230 mV. Sulfide at low concentrations supported syntrophic growth as well and could replace cysteine. Neither growth nor acetate degradation was found with D-cysteine, homocysteine, cysteamine, 3-mercaptopropionate, dithiothreithol, thioglycolate, glutathione, coenzyme M, dimethylsulfoxide, trimethylamine-*N*-oxide, anthraquinone-2,6-disulfonate, or ascorbate.

Keywords Syntrophy · Acetate oxidation · *Geobacter sulfurreducens* · *Wolinella succinogenes* · Cysteine · Interspecies electron transfer

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Introduction

Geobacter sulfurreducens oxidizes acetate to CO_2 with fumarate, iron(III), elemental sulfur, or malate as terminal electron acceptor, while nitrate, sulfate, or thiosulfate are not reduced (Caccavo et al. 1994). In the absence of these electron acceptors, electrons can be transferred also to an anaerobic partner bacterium, e.g., *Wolinella succinogenes* (Cord-Ruwisch et al. 1998). This coculture oxidizes acetate to CO_2 with concomitant reduction of nitrate to ammonia.

Several observations indicated that in this coculture, different from other syntrophic cocultures (Schink 1997), electron transfer did not proceed via hydrogen as carrier. The cell mass produced was approximately 18 g per mol acetate oxidized, and only 20% of this cell mass was produced by *W. succinogenes* (Cord-Ruwisch et al. 1998). If electrons were transferred via interspecies hydrogen transfer, *W. succinogenes* should form 14–18 g dry mass per mol acetate [3–4 g per mol hydrogen, (Cord-Ruwisch et al. 1998)]. On the other hand, *G. sulfurreducens* should obtain –53 kJ per mol acetate oxidized with the observed hydrogen pressure of 0.02–0.04 Pa, enough to form 2/3 mol ATP. Even under optimal conditions, only 6.6 g dry cell mass could be formed with this amount of energy (Cord-Ruwisch et al. 1998). Moreover, the electron flux through the hydrogen pool at the observed low pressure could account for less than 0.01% of the total electron flux released in acetate oxidation (Cord-Ruwisch et al. 1998). Furthermore, hydrogenase activities, which were high in *G. sulfurreducens* pure cultures grown with acetate plus fumarate [450 $nmol \times min^{-1} \times (mg\ protein)^{-1}$], were not detectable in cocultures of *G. sulfurreducens* with *W. succinogenes* (Galushko and Schink 2000).

A small periplasmic cytochrome with a standard redox potential of –167 mV was reported to be released by *G. sulfurreducens* into the medium during growth, and has been discussed as a candidate to act as electron carrier in syntrophic growth (Seeliger et al. 1998). The purified cytochrome reduces ferrihydrite and is reduced by *G. sul-*

furreducens and reoxidized by *W. succinogenes* with nitrate as electron acceptor.

Whether this cytochrome really is the only electron carrier in this coculture has been a matter of discussion ever after. First, the cytochrome is released into the medium also in pure cultures growing on acetate and fumarate when no electron transfer is necessary, and high concentrations of the cytochrome in the supernatant appear only towards the end of the exponential phase. Second, release of the cytochrome depends to a large extent on the growth conditions and is artificially stimulated, e.g., by increasing the ionic strength of the medium or by adding sulfate or nitrate (K.L. Straub, Konstanz, Germany, unpublished), neither of which is reduced by *G. sulfurreducens*. Third, there are at least two further cytochromes present in the culture supernatant as detected by SDS-PAGE and heme staining of concentrated supernatant (Lloyd et al. 1999; J. Kaden unpublished). Last, it was calculated that the shuttling activity of the small cytochrome can contribute to the overall electron-flow to insoluble iron(III) (ferrihydrite) only to a minor extent (Lloyd et al. 1999; K.L. Straub, Konstanz, Germany unpublished).

In the present study we show that L-cysteine, supplied in the cultures as reducing agent, acts as electron carrier in acetate-oxidizing syntrophic cocultures of *G. sulfurreducens* and *W. succinogenes.*

Materials and methods

Cultivation and growth conditions

Geobacter sulfurreducens strain PCA (DSM 12127, ATCC 51573) was obtained from R. Cord-Ruwisch (Perth, Australia), and *Wolinella succinogenes* (DSM 1740) was provided by A. Kröger (Frankfurt, Germany).

Bacteria were cultivated in a bicarbonate-buffered, cysteine-reduced anoxic freshwater medium (Cord-Ruwisch et al. 1998; Seeliger et al. 1998) under N_2/CO_2 (80/20 v/v) atmosphere. Medium for *G. sulfurreducens* did not contain vitamins, medium for *W. succinogenes* was free of ammonia, and the coculture medium contained neither vitamins nor ammonia. *G. sulfurreducens* was grown with 20 mM acetate plus 40 mM fumarate, *W. succinogenes* with 10 mM formate plus 10 mM nitrate and 5 mM acetate. Cocultures of the partners were cultivated with 20 mM acetate plus 10 mM nitrate.

Preparation of cell suspensions and growth experiments

For cell suspension experiments, bacteria were cultured in 1 l medium (1.2-l infusion bottles), harvested in the mid-exponential phase by centrifugation at 1,500×*g* for 20 min under anoxic conditions, and washed once with 50 mM potassium phosphate buffer, pH 7.0.

Cell suspension experiments were carried out in 15-ml tubes filled with 10 ml bicarbonate-buffered growth medium (Cord-Ruwisch et al. 1998; Seeliger et al. 1998). Substrates and carrier substances were added to the tubes from sterile, anoxic stock solutions by syringes. Experiments were started by adding the cells. The OD_{660} was adjusted to 1.5. Tubes were incubated at 30 °C in the dark. Samples were taken with syringes, centrifuged for 10 min at 20,000×*g*, and analyzed immediately for thiol group determination, or stored at –20 °C for acetate determination.

Cultures for growth experiments were grown in 100 ml medium in 120-ml infusion bottles to the late-exponential growth phase, and centrifuged at 1,500×*g* for 20 min. The supernatant was replaced under sterile, anoxic conditions by the same volume of fresh medium. Experiments were performed in 15-ml tubes as described above with 10 mM acetate and 10 mM nitrate as substrates. OD was measured at 660 nm wavelength directly in a tube photometer (Bausch and Lomb).

All growth and cell suspension experiments were carried out as sets of at least two parallels. Control experiments were conducted with pasteurized cell suspensions.

Analytical procedures

Acetate was determined by HPLC on a Biorad Aminex HPX-87H column, 300×7.8 mm. Samples were eluted with 5 mM sulfuric acid at 0.6 ml×min^{-1} flow rate and 40 °C. Compounds were detected by an ERC-7512 refraction index detector (Erma).

Thiol groups were quantified using a photometric test system based on Ellmann's reagent (Lange and Vejdelek 1980). Reaction of 5.5′-dithiobis(2-nitrobenzoic acid) with free thiols forms a light-absorbing component (ε=13,600 M^{-1}×cm^{-1}) which was measured in 1-ml cuvettes at 412 nm wavelength in a Uvikon spectrophotometer. Concentrations were calculated with L-cysteine as standard; the detection limit was at 10 μM cysteine.

Protein content was determined in cell suspensions using bicinchoninic acid according to the enhanced protocol of the BCA protein assay kit (Pierce), with bovine serum albumin fraction V (Pierce) as calibration standard.

Redox potentials were measured in an anoxic 3-electrode system (Emde et al. 1989) using an Ag/AgCl reference electrode and a platinum mesh working electrode, with a potentiostat (type LB 81 M, Bank Elektronik) used as a potentiometer. Calculations refer to a redox potential of the Ag/AgCl electrode related to the standard hydrogen electrode of +203.5 mV (30 °C) (Kölling 2000). The reference electrode was calibrated with a 3 mM $K_3Fe(CN)_6$/3 mM $K_4Fe(CN)_6$ mixture in 100 mM KCl, with a known redox potential of +213.5 mV (30 °C, Ag/AgCl), corresponding to a redox potential of +417 mV compared to the standard hydrogen electrode at pH 7.0 (Kölling 2000).

Chemicals and gases

All chemicals were of analytical grade quality and were obtained from Sigma, Fluka, Aldrich, Pierce, or Riedel de Haen. Gases were purchased from Messer-Griesheim (Darmstadt, Germany), and Sauerstoffwerke Friedrichshafen (Friedrichshafen, Germany).

Results

Syntrophic acetate oxidation with cysteine as carrier

Geobacter sulfurreducens strain PCA grew in pure cultures with 20 mM acetate plus 40 mM fumarate at a doubling time of 7.7 h. Cysteine, ascorbate or dithiothreitol could serve as reducing agent. *W. succinogenes* grew in pure culture with 30 mM formate plus 10 mM nitrate and 5 mM acetate at 10 h doubling time, with cysteine or ascorbate as reducing agent. Growth data were similar to data described earlier (Cord-Ruwisch et al. 1998). Cocultures grew with 20 mM acetate plus 10 mM nitrate (doubling time 6.5 h) and were established as described (Cord-Ruwisch et al. 1998), with cysteine as reducing agent. After inoculation with 5% preculture, stationary phase was reached after 30 h. The ratio of *G. sulfurreducens* over *W. succinogenes* cells was in the range of 2:1 to 4.5:1 as described earlier (Cord-Ruwisch et al. 1998).

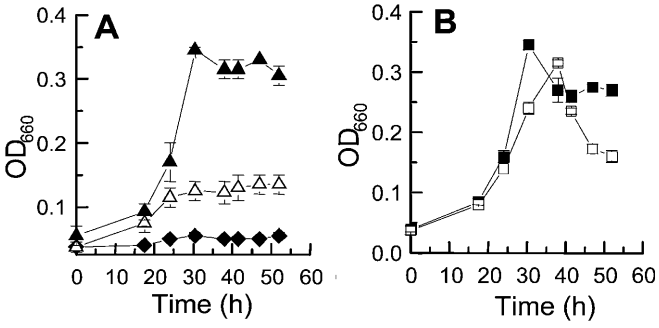

Fig. 1A, B Growth of a coculture of *Geobacter sulfurreducens* plus *Wolinella succinogenes* in the presence of L-cysteine or L-cystine at different concentrations. **A** Growth without addition of cysteine or cystine (◆), with 100 µM cysteine (▲), with 10 µM cysteine (△). **B** Growth with 100 µM cystine (■), with 10 µM cystine (❑). All values are means of two parallels. Cultures were inoculated with 5% washed preculture in a medium with 10 mM acetate plus 10 mM nitrate

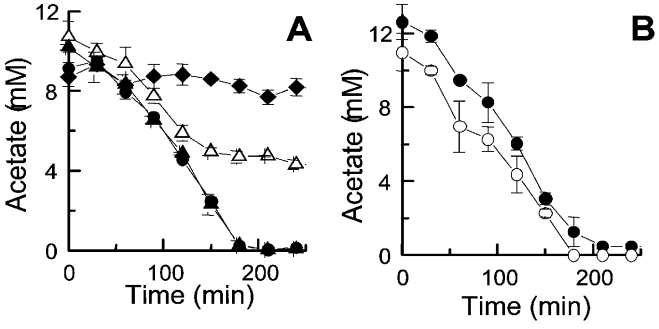

Fig. 2A, B Acetate oxidation in washed dense cell suspensions (OD_{660}=1.5) of *Geobacter sulfurreducens* plus *Wolinella succinogenes* cocultures. The experiment was carried out in bicarbonate-buffered mineral medium with 10 mM acetate plus 10 mM nitrate. **A** Acetate oxidation in the presence of cysteine at different concentrations: control without cysteine (◆), with 500 µM cysteine (●), with 100 µM cysteine (▲), with 10 µM cysteine (△). **B** Comparison of acetate oxidation in the presence of 500 µM cystine (○) or 500 µM cysteine (●)

The dependence of growth of the coculture on cysteine addition was further elucidated. As shown in Fig. 1, the coculture grew well with cysteine at 100 µM concentration, and the growth rate did not increase with higher cysteine concentrations. With 10 µM cysteine, growth ceased after 38 h, and only a rather low final OD_{660} was reached. No growth was observed in the absence of cysteine within the total experiment time (52 h). Also L-cystine supported growth of the coculture, and the growth rate with 100 µM cystine was equal to that with 100 µM cysteine (Fig. 1). With 10 µM cystine, the culture grew to the same final OD as with higher concentrations of cysteine or cystine, but slightly slower. These cocultures did not receive an additional reducing agent. The OD decrease of the cystine-containing culture in the stationary phase was due to aggregation and sedimentation.

As shown in Fig. 2A, cysteine stimulated acetate oxidation also in washed cell suspensions. In the presence of 100 µM cysteine, coculture suspensions degraded acetate to the detection limit within 150–180 min [0.16 µmol×min^{-1}×(mg protein)$^{-1}$]; only very slow degradation was observed in the absence of cysteine [0.002 µmol×min^{-1}×(mg protein)$^{-1}$]. Higher concentrations of cysteine did not increase the acetate oxidation rate [0.13–0.15 µmol×min^{-1}×(mg protein)$^{-1}$]. With 10 µM cysteine, acetate degradation ceased after about 5 mM acetate was oxidized, but the initial oxidation rate was nearly the same [0.12 µmol×min^{-1}×(mg protein)$^{-1}$] as with 100 µM cysteine. No acetate oxidation was observed in pasteurized controls. In Fig. 2B, the effect of cysteine addition was compared to that of cystine addition. Cysteine and cystine at 500 µM concentrations both caused the same acetate oxidation rate in coculture suspensions [0.13 and 0.10 µmol×min^{-1}×(mg protein)$^{-1}$, respectively].

Cysteine oxidation and cystine reduction rates

In order to examine whether the cysteine/cystine pair really acted as electron shuttle, cell suspensions of pure cultures of *G. sulfurreducens* and *W. succinogenes* as well as coculture cell suspensions were tested for their ability to reduce cystine or to oxidize cysteine, as measured via changes in the free thiol group content. *G. sulfurreducens* reduced cystine in pure culture at rates of 6.0–22 nmol×min^{-1}×(mg protein)$^{-1}$. With cells harvested in the exponential phase and tested directly, no difference in thiol formation rates in the presence or absence of acetate was observed (data not shown). If *G. sulfurreducens* cells were used from the stationary phase of pure culture, a clear difference in the cystine reduction rate with acetate (12.5 nmol×min^{-1}×(mg protein)$^{-1}$) vs without acetate (3.4 nmol×min^{-1}×(mg protein)$^{-1}$) was found (Fig. 3A). *W. succinogenes*, oxidized cysteine with nitrate in pure culture at a rate of 2.6 nmol×min^{-1}×(mg protein)$^{-1}$ (Fig. 3B). Coculture suspensions reduced cysteine at a rate of 1.3 nmol×min^{-1}×(mg protein)$^{-1}$ and oxidized cysteine

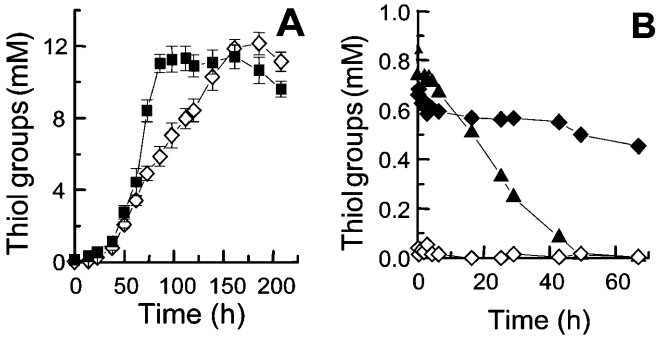

Fig. 3A, B Cystine reduction and cysteine oxidation by pure cultures of *G. sulfurreducens* or *Wolinella succinogenes* in dense cell suspensions (OD_{660}=1.5). **A** Formation of thiol groups from L-cystine in a cell suspension of *G. sulfurreducens* (from stationary phase) with 6 mM cystine as electron acceptor; with 2 mM acetate as electron donor (■), control without acetate (◊). **B** Oxidation of cysteine in a cell suspension of *W. succinogenes* with 10 mM nitrate as electron acceptor; with 1 mM cysteine (▲), control without cysteine (◊), control with 1 mM cysteine but without nitrate (◆)

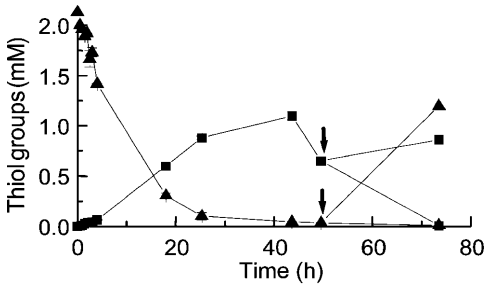

Fig. 4 Cystine reduction and cysteine oxidation in dense cell suspensions (OD_{660}=1.5) of *G. sulfurreducens* plus *W. succinogenes* cocultures. Cystine reduction (■) was performed with 4 mM acetate as electron donor and 1 mM cystine as electron acceptor. The *arrow* indicates the addition of 4 mM nitrate to one of the parallels. Cysteine oxidation (▲) was performed with 4 mM nitrate as electron acceptor and 2 mM cysteine as electron donor. The *arrow* indicates the addition of 4 mM acetate to one of the two parallels

with nitrate at a rate of 3.5 nmol×min^{-1}×(mg protein)$^{-1}$, respectively (Fig. 4). Addition of nitrate to a suspension oxidizing acetate with cystine and generating free thiols resulted in an immediate decrease of free thiol groups (Fig. 4). Addition of acetate to a coculture suspension that had been oxidizing cysteine with nitrate before again caused formation of free thiol groups.

Steady-state conditions in the coculture

The concentration of thiol groups in the coculture during growth with 10 mM acetate plus 10 mM nitrate was determined after addition of cysteine or cystine to various concentrations (100–400 µM cysteine, 50–200 µM cystine). Free thiol groups were formed in the cystine-treated assays up to 25–65 µM during growth, proportional to the concentration of added cystine. In the cysteine-treated assays the thiol concentration decreased at rates of 4 µM×h^{-1} (100 µM cysteine) to 9.1 µM×h^{-1} (400 µM cysteine) during growth. Independent of the cysteine or cystine concentrations supplied, the thiol group concentration decreased to <10 µM at the end of the growth phase, and the resazurin in the medium turned pink.

The apparent redox potential during acetate oxidation was measured in the growing coculture with a redox electrode. At steady state during exponential growth, the redox potential was –169 mV to –235 mV. With limiting nitrate, the value shifted to –315 mV at the end of growth; with limiting acetate, it shifted to –130 mV at the end of growth (Fig. 5).

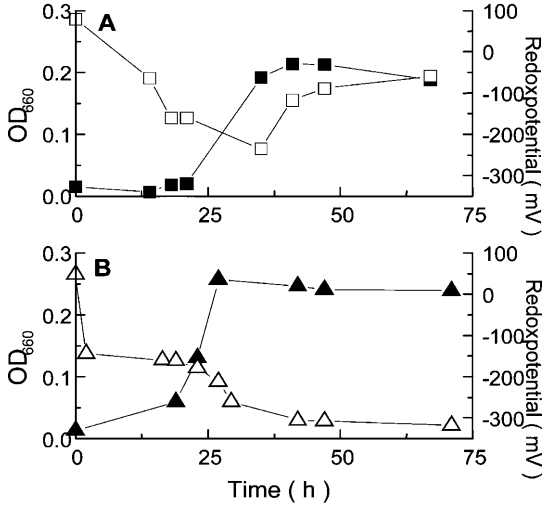

Fig. 5A, B Redox potential measurements in growing cocultures. **A** Growth with limiting acetate; (■) OD_{660}, (❑) redox potential in the culture related to the standard hydrogen electrode. **B** Growth with limiting nitrate; (▲) OD_{660}, (△) redox potential in the culture related to the standard hydrogen electrode

Alternative carriers

The coculture was found to be rather sensitive towards sulfide as reducing agent; no growth was observed in the presence of 1 mM sulfide. With 200 µM or less sulfide, the culture grew even without cysteine or cystine addition. Growth rate and final optical density were similar to those of cocultures growing with cysteine or cystine. A visible turbidity formed in the culture 2 h after inoculation. Microscopy revealed that this turbidity was due to colloidal sulfur globules which disappeared during further growth.

With other reducing agents such as ascorbate, dithiothreithol, iron(II)chloride (pH adjusted to 7.0 and controlled during the experiment), or without any reducing agent, only very slow growth after a long lag phase of several days was observed in the coculture. When these reagents were used at 500 µM concentration, only the cysteine-reduced control showed growth within the test time of 50 h, and completed growth after 30 h. The reduction state of the medium was checked by added resazurin, which stayed colorless throughout the entire growth phase, and turned pink in cysteine-reduced cultures at the end of growth when acetate became limiting.

Several other electron carriers were tested as possible electron carriers in coculture cell suspensions and in growth experiments. No acetate oxidation was observed with anthraquinone-2,6-disulfonate (AQDS). A rapid color change indicated that AQDS was reduced to anthrahydroquinone-2,6-disulfonate (AHQDS) in cell suspensions, but neither significant acetate degradation nor growth was observed. No growth or acetate degradation was observed either with dimethylsulfoxide, trimethylamine-*N*-oxide, ethanethiol, propanethiol, ethanedithiol or mercaptoethanol each at 100 µM concentration. Possible toxic effects of these compounds were checked for in growth experiments with 1 mM cysteine as carrier and 100 µM of the respective compound. Only the control assays with cysteine showed normal growth, whereas cultures with ethanethiol, propanethiol, ethanedithiol, mercaptoethanol or dithiothreithol were strongly inhibited. Growth started only after a lag phase of 2 weeks. D-Cysteine, homocysteine,

cysteamine, 3-mercaptopropionate, thioglycolate, glutathione, and coenzyme M at 100 μM concentration did not support growth. With 100 μM mercaptoethanol, sometimes a slight decrease of acetate concentration was observed in cell suspensions.

Discussion

In the present report, we identified the cystine/cysteine redox couple as the electron carrier used in syntrophic acetate oxidation by *G. sulfurreducens*/*W. succinogenes* cocultures. Cysteine fulfills all requirements of an electron carrier in this coculture: it is available in the medium at rather high concentrations, it is easily diffusible across the outer membrane of gram-negative bacteria due to its small size, and it is oxidized by one partner and re- reduced by the other one, as shown.

Energetic and kinetic aspects

With cysteine as electron carrier, acetate oxidation by *G. sulfurreducens* in the coculture would follow the reaction:

$$CH_3COO^- + H^+ + 2\ H_2O + 4\ \text{cystine} \rightarrow 2\ CO_2 + 8\ \text{cysteine}$$

Under standard conditions, the redox potential of the cystine/cysteine couple is –348 mV, and therefore more negative than the standard potential of the CO_2/acetate couple at –290 mV (calculated from the free energies of formation, values taken from Thauer et al. 1977). Thus, the overall reaction under standard conditions is endergonic ($\Delta G^{\circ\prime}$=+44.3 kJ×mol^{-1}). However, the redox potential of the cystine/cysteine couple depends strongly on the concentration (Fig. 6) because two molecules of cysteine are formed by reduction of one molecule of cystine. With cysteine and cystine both at 50 μM concentration as supplied in our culture medium, the equilibrium redox potential is –219 mV. On the other hand, the standard redox potential of the CO_2/acetate couple is shifted only slightly to –283 mV under the conditions prevailing in our culture (10 mM acetate; 0.3 atm CO_2). Under these conditions, acetate oxidation with cystine is exergonic with $\Delta G'$=–49 kJ×mol^{-1}, providing just enough energy to form 2/3 mol ATP per mol acetate. The redox potential of –219 mV is in the range of the redox potentials measured in our acetate-oxidizing cocultures, i. e., –170 to –230 mV, and supports the hypothesis that this couple acts as electron acceptor in acetate oxidation by *G. sulfurreducens*.

Similar to the situation with the proton/hydrogen couple in interspecies hydrogen transfer (Schink 1997), the redox potential of the cysteine/cystine couple can be influenced further by removal of the reduced form by *W. succinogenes.* At E_h=–200 mV, the calculated concentration ratio would be 99.64 μM cystine to 0.34 μM cysteine. Obviously, cysteine was oxidized very efficiently in the coculture, and no free thiol groups were detected even under conditions of electron acceptor limitation. The free energy of the reaction under these conditions is shifted further to –66 kJ×mol^{-1}.

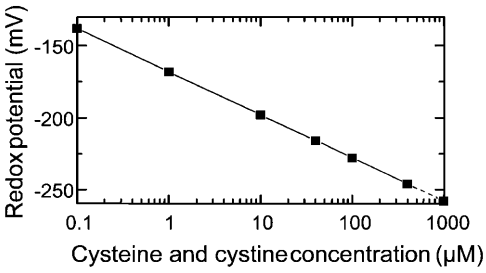

Fig. 6 Midpoint redox potentials of the cystine/cysteine couple at equal concentrations of cystine and cysteine . Values were calculated for equimolar concentrations of cysteine and cystine at pH 7.0; the standard redox potential is –348 mV. Potentials for concentrations higher than 400 μM are only of theoretical value because of the limited solubility of cystine in aqueous solutions

One important argument against an essential role of hydrogen as electron carrier in this coculture was that the electron flow through the extremely small hydrogen pool measured could account only for less than 0.01% of the total electron flux (Cord-Ruwisch et al. 1998). Cysteine at 100 μM concentration at the same cell density has an electron transport capacity of 123 μM×min^{-1} (diffusion coefficient 7.962×10^{-6} cm^2×s^{-1}, as calculated from Schwarzenbach et al. 1993), which secures approximately 70% of the total electron transfer between the partners.

The reduction of cysteine by *G. sulfurreducens* was stimulated by acetate if the cells had previously been starved for their electron donor. Unfortunately, we observed a high background reduction rate, perhaps due to storage material in the cells, that precluded an exact determination of the stoichiometry of acetate-dependent cystine reduction. In coculture experiments, a dependency of cystine reduction on acetate supply was clearly shown. After cells were starved for acetate in the presence of nitrate, only the addition of acetate caused a reformation of cysteine. This cystine reduction was about twice as fast as in the absence of nitrate, indicating that the biologically formed cystine was better available to the cells than the externally provided cysteine.

The low solubility of cystine could be the reason why the supply of cysteine or cystine at higher concentration did not increase the reaction rate, and why fast recycling of the carrier is necessary in this syntrophic interaction. In pure culture, cystine reduction is slow because it is hardly accessible to the cells. Nevertheless, the use of disulfides including cystine or thiols as electron acceptor or donor has been described for several Eubacteria and Archaea (Hedderich et al. 1999), especially for *Desulfuromonas acetoxidans* (Pfennig and Biebl 1976), a close relative of *G. sulfurreducens* (Caccavo et al. 1994).

Cysteine supply at low concentration supported growth or acetate oxidation only at decreasing rate, probably due to cysteine assimilation. Assuming that 1% of the total cell mass is sulfur (Schlegel 1992) and that the total cell yield of the coculture is 18 g×(mol acetate)$^{-1}$ (Cord-Ruwisch et al. 1998), about 60 μM cysteine would be con-

sumed for cell matter synthesis in our experiments, causing a severe limitation of the carrier.

Ecological aspects

One may argue that the system examined here is rather artificial: nitrate-dependent acetate oxidation usually does not require syntrophic cooperation of two different bacteria, and cysteine will hardly be found in natural habitats at >10 μM concentration. Nonetheless, this cooperation may serve as a model for syntrophic interactions using electron transfer through carriers different from hydrogen.

Although acetate oxidation in our cocultures depended on cysteine, acetate was oxidized at a low rate also in cysteine-free cell suspensions. The cooperation appears to be rather specific for L-cysteine since many other carrier substances tested did not support growth or acetate oxidation, not even compounds structurally closely related to cysteine such as D-cysteine, homocysteine, cysteamine or 3-mercaptopropionate. The only alternative to cysteine found to operate as carrier was sulfide.

Interspecies electron transfer through a sulfur/sulfide cycle has been described for *D. acetoxidans* (Pfennig and Biebl 1976; Biebl and Pfennig 1978), which couples acetate oxidation with electron transfer to the phototrophic green sulfur bacterium *Chlorobium limicola* (Biebl and Pfennig 1978). This electron transfer operates at 53–92 μM sulfide concentration (Biebl and Pfennig 1978). Acetate oxidation in *G. sulfurreducens* proceeds through the citric acid cycle (Mikoulinskaia et al. 1999; Galushko and Schink 2000) as is true also for acetate oxidation by *D. acetoxidans* (Thauer 1988). Sulfide oxidation by *W. succinogenes* has been reported in the past (Macy et al. 1986), and the sulfide dehydrogenase enzyme that reversibly catalyzes sulfide oxidation and sulfur reduction was isolated (Schröder et al. 1988). The S^0/H_2S couple with a standard redox potential of –240 mV represents a suitable alternative to cystine/cysteine for our coculture, also from an energetic point of view. Future work will have to elucidate whether the enzymes catalyzing cystine reduction and cysteine oxidation in our coculture are related to those involved in electron transfer via the sulfur/sulfide cycle.

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References

Biebl H, Pfennig N (1978) Growth yields of green sulfur bacteria in mixed cultures with sulfur and sulfate reducing bacteria. Arch Microbiol 117:9–16

Caccavo F, Jr., Lonergan DJ, Lovley DR, Davis M, Stolz JF, McInerney MJ (1994) *Geobacter sulfurreducens* sp. nov., a hydrogen- and acetate-oxidizing dissimilatory metal-reducing microorganism. Appl Environ Microbiol 60:3752–3759

Cord-Ruwisch R, Lovley DR, Schink B (1998) Growth of *Geobacter sulfurreducens* with acetate in syntrophic cooperation with hydrogen-oxidizing anaerobic partners. Appl Environ Microbiol 64:2232–2236

Emde R, Swain A, Schink B (1989) Anaerobic oxidation of glycerol by *Escherichia coli* in an amperometric poised-potential culture system. Appl Microbiol Biotechnol 32:170–175

Galushko AS, Schink B (2000) Oxidation of acetate through reactions of the citric acid cycle by *Geobacter sulfurreducens* in pure culture and in syntrophic coculture. Arch Microbiol 174:314–321

Hedderich R, Klimmek O, Kröger A, Dirmeier R, Keller M, Stetter KO (1999) Anaerobic respiration with elemental sulfur and with disulfides. FEMS Microbiol Rev 22:353–381

Kölling M (2000) Comparison of different methods for redox potential determinations in natural waters. In: Schüring J, Schulz HD, Fischer WR, Böttcher J, Duijnisveld WHM (eds) Redox: fundamentals, processes and applications. Springer, Berlin Heidelberg New York

Lange B, Vejdelek ZJ (1980) Photometrische Analyse. VCH Verlagsgesellschaft, Weinheim

Lloyd JR, Blunt-Harris EL, Lovley DR (1999) The periplasmic 9.6-kilodalton *c*-type cytochrome of *Geobacter sulfurreducens* is not an electron shuttle to Fe(III). J Bacteriol 181:7647–7649

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 source. Arch Microbiol 144:147–150

Mikoulinskaia O, Akimenko V, Galouchko A, Thauer RK, Hedderich R (1999) Cytochrome c-dependent methacrylate reductase from *Geobacter sulfurreducens* AM-1. Eur J Biochem 263: 346–352

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

Schink B (1997) Energetics of syntrophic cooperation in methanogenic degradation. Microbiol Mol Biol Rev 61:262–280

Schlegel HG (1992) Allgemeine Mikrobiologie. Thieme, Stuttgart

Schröder I, Kröger A, Macy JM (1988) Isolation of the sulphur reductase and reconstitution of the sulphur respiration of *Wolinella succionogenes*. Arch Microbiol 149:572–579

Schwarzenbach RP, Gschwend PM, Imboden DM (1993) Environmental organic chemistry. Wiley Interscience, New York

Seeliger S, Cord-Ruwisch R, Schink B (1998) A periplasmic and extracellular *c*-type cytochrome of *Geobacter sulfurreducens* acts as a ferric iron reductase and as an electron carrier to other acceptors or to partner bacteria. J Bacteriol 180:3686–3691

Thauer RK (1988) Citric-acid cycle, 50 years on. Modifications and an alternative pathway in anaerobic bacteria. Eur J Biochem 176:497–508

Thauer RK, Jungermann K, Decker K (1977) Energy conservation in chemotrophic anaerobic bacteria. Bacteriol Rev 41:100–180