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Thiosulfate sulfur transferases (Rhodaneses) of *Chlorobium vibrioforme* f. *thiosulfatophilum**

Manfred A. Steinmetz and Ulrich Fischer**

Arbeitsgruppe Geomikrobiologie, Fachbereich Biologie, Universität Oldenburg, Postfach 2503, D-2900 Oldenburg, Federal Republic of Germany

Abstract. Two enzymes containing thiosulfate sulfur transferase activity were purified from *Chlorobium vibrioforme* f. *thiosulfatophilum* by ion exchange chromatography, gel filtration and isoelectrofocusing. Enzyme I is a basic protein with an isoelectric point at pH 9.2 and has a molecular weight of 39,000. The K_m-values for thiosulfate and cyanide of the purified basic protein were 0.25 mM (thiosulfate) and 5 mM (cyanide). Enzyme II is an acidic protein. The enzyme has an isoelectric point at pH 4.6–4.7 and a molecular weight of 34,000. The K_m-values of the acidic protein were found to be 5 mM for thiosulfate and 125 mM for cyanide.

In addition to thiosulfate sulfur transferase activity, cellfree extracts of *Chlorobium vibrioforme* f. *thiosulfatophilum* also contained low thiosulfate oxidase activity and negligible thiosulfate reductase activity. The percent distribution of thiosulfate sulfur transferase and thiosulfate oxidase activities in the organism was independent of the offered sulfur compound (thiosulfate, sulfide or both) in the medium.

Key words: Green sulfur bacteria – Sulfur metabolism – Thiosulfate utilizing enzymes – *Chlorobium vibrioforme* f. *thiosulfatophilum*

Among the green sulfur bacteria (Chlorobiaceae) only strains of *Chlorobium limicola* f. *thiosulfatophilum* and *Chlorobium vibrioforme* f. *thiosulfatophilum* can utilize not only sulfide but also thiosulfate as electron donor during anoxygenic photosynthesis (Pfennig and Trüper 1974; Trüper 1978, 1981). They metabolize thiosulfate in different ways. Whole cells of *C. limicola* f. *thiosulfatophilum* converted thiosulfate directly to sulfate without measurable intermediates (Schedel 1978). To the contrary, *C. vibrioforme* f. *thiosulfatophilum* metabolized thiosulfate to sulfate via elemental sulfur (Steinmetz and Fischer 1982a). The latter finding indicated that thiosulfate was probably cleaved by enzymatic reactions. Different enzyme systems have been reported to be responsible for thiosulfate utilization by Chlorobiaceae (Mathewson et al. 1968; Kusai and Yamanaka 1973a, b; Prangenberg 1976; Schedel 1978). The following three enzymes can be considered to metabolize thiosulfate:

i) thiosulfate:acceptor oxidoreductase with ferricyanide or cytochrome as electron acceptor (Mathewson et al. 1968; Smith 1966; Kusai and Yamanaka 1973a, b)

ii) thiosulfate reductase with reduced methyl viologen, reduced glutathione or dihydrolipoic acid as electron donors (Hashwa and Pfennig 1972)

iii) thiosulfate sulfur transferase (EC 2.8.1.1) also termed rhodanese or thiosulfate:cyanide sulfur transferase (Sörbo 1955) with thiophilic anions (like CN^- or SO_3^{2-}) as acceptor substrates (Westley 1973).

Thiosulfate:acceptor oxidoreductase catalyzes a reaction where two thiosulfate molecules are oxidatively bound to form tetrathionate (Smith 1966; Rolls and Lindstrom 1967b). The other two enzymes mentioned above perform a cleavage of thiosulfate into sulfide and sulfite (Trüper and Pfennig 1966; Rolls and Lindstrom 1967a).

All three enzymes involved in thiosulfate metabolism have been found not only in phototrophic sulfur and non-sulfur bacteria (Trüper 1981; Knobloch et al. 1981) but also in chemolithoautotrophic bacteria like *Thiobacillus* species (Trudinger 1961a, b; Bowen et al. 1965; Lyric and Suzuki 1970). In this paper we describe the purification and some molecular properties of two thiosulfate sulfur transferases from *Chlorobium vibrioforme* f. *thiosulfatophilum*.

Material and methods

Organism, growth and harvest of cells. Chlorobium vibrioforme f. *thiosulfatophilum* (DSM 263) was grown photolithoautotrophically in the medium described by Steinmetz and Fischer (1982a). Unless otherwise indicated, cells were harvested with a Christ Cryofuge (10,000 rpm) after elemental sulfur was consumed by the cells.

Preparation of crude cell extracts. 150 g wet cell material was suspended in 150 ml of 100 mM potassium phosphate buffer, pH 7.0, and homogenized. Cell disruption and removal of cell particles and chlorosomes were carried out as described by Steinmetz und Fischer (1981) except that the ultracentrifugation was done at 100,000 × *g* for 300 min. The 100,000 × *g* supernatant was desalted on a Sephadex G-25 column and adjusted to pH 7.8 with 1 N NaOH for DEAE-52 cellulose chromatography or to pH 6.5 with 1 N HCl for CM-52 cellulose chromatography. Further detailed purification steps are given in "Results".

* Dedicated to Prof. Dr. Norbert Pfennig on the occasion of his 60th birthday

** *Present address:* Syva-Merck GmbH, Alsfelder Str. 6, D-6100 Darmstadt 11, Federal Republic of Germany

Abbreviations. C, Chlorobium; SDS, sodium dodecylsulfate

Offprint requests to: U. Fischer

Protein determination. Protein was determined according to Lowry et al. (1951).

Molecular weight. The molecular weight of each thiosulfate sulfur transferase was determined by comparative gel filtration on Sephadex G-75 (equilibrated in 50 mM Tris-HCl, pH 7.8, containing 100 mM NaCl). Aldolase, bovine serum albumin, hen egg albumin and horse heart cytochrome *c* from Boehringer Combithek No. 104558 were used as marker proteins. The purity of thiosulfate sulfur transferase was controlled by SDS polyacrylamide gel electrophoresis (7.5% gel, 0.1% SDS). The gel was prepared as described by Steinmetz and Fischer (1982a, b).

Preparative isoelectrofocusing in granulated gel. Preparative isoelectrofocusing was carried out in a LKB Multiphor 2117 connected to the LKB Power Supply 2103 using the Ampholine electrofocusing kit for granulated gels (LKB 2117-501). The gel contained 4 ml of Ampholine, pH 3.0–9.5, and was prepared after the method given in the LKB instruction manual for electrofocusing (LKB Application Note 198). At the end of the operating time (16 h) the gel was cut into 30 pieces by a fractionating grid. In order to remove proteins from the gel and to measure the pH, each fraction was transferred into a small test tube containing 2 ml of distilled water. Enzymatic activity of thiosulfate sulfur transferase was measured after the samples were transferred into 50 mM Tris-acetate-buffer, pH 8.7, and centrifuged for 10 min at 4,500 rpm.

Isoelectric point. The isoelectric point of basic thiosulfate sulfur transferase was determined by flat bed electrofocusing in a LKB Multiphor 2117 on polyacrylamide gel (6% acrylamide) with a pH-gradient of 7.0–10.0. 14 ml of gel contained 1 ml of Ampholine, pH 9.0–11.0, and 0.4 ml of Ampholine, pH 7.0–9.0. Gels were fixed for 30–60 min in trichloro acetic acid (11.5%) and sulfosalicylic acid (3.5%), washed for 5 min in the destaining solution (500 ml ethanol, 160 ml glacial acidic acid and water to a total volume of 2,000 ml) and then stained for 10–15 min at 60° C (staining solution: 0.46 g Coomassie Brilliant Blue in 400 ml destaining solution). Destaining of the gels lasted 24 h by changing the destaining solution several times. The pH on the gel surface was determined as described by Steinmetz and Fischer (1982b).

Enzyme assays. Thiosulfate sulfur transferase activity was determined according to the method of Bowen et al. (1965) and modified by Schedel and Trüper (1980). One unit of enzyme was defined as that amount that converted 1 µmol $S_2O_3^{2-}$ per min at 30° C. The activity of the enzyme was also measured in a modified assay system according to Smith and Lascelles (1966): The reaction mixture contained 300 µl 1 M Tris-acetate, pH 8.7, 40 µl 5 mM 2,6-dichlorophenolindophenol (DCPIP), 10 µl 0.1 M $Na_2S_2O_3 \times 5H_2O$, 100 µl enzyme extract and filled up with distilled water to 2.8 ml. The mixture was preincubated at 25° C for 3 min and the reaction was started by the addition of 200 µl 0.2 M NaCN. The reduction of DCPIP at 600 nm against a blank (without DCPIP and extract) was followed with time in a registrating double beam spectrophotometer. One unit of enzyme was defined as that amount that reduced 1 µmol DCPIP per min at 25° C. Concentration of DCPIP was calculated using $E_{\text{mM 600 nm}}$ of 20.6.

Thiosulfate oxidase activity was photometrically determined according to Lyric and Suzuki (1970). One unit of enzyme was defined as that amount of enzyme which utilized 1 µmol $S_2O_3^{2-}$ per min at 30° C.

Thiosulfate reductase activity was measured with methyl viologen as electron donor in a modified assay according to Hashwa (1972): 50 µl 1 M Tris-acetate, pH 8.7, and 50–200 µl extract were given into a glas cuvette and filled up with distilled water to 400 µl. Then the cuvette was stoppered with a serum stopper and deaerated for 10 min. After regassing for the same time with oxygen-free nitrogen, 500 µl 2 mM reduced methyl viologen dissolved in 50 mM Tris-acetate, pH 8.7 was added by a Hamilton syringe. The reaction was started by the addition of 100 µl 50 mM $S_2O_3^{2-}$. The oxidation of reduced methyl viologen at 600 nm was photometrically followed with time. Calculations were done using $E_{\text{mM 600 nm}}$ of 113 for reduced methyl viologen (Thorneley 1974). Electrochemical preparation of reduced methyl viologen was carried out as described by Thorneley (1974).

Chemicals. All chemicals were purchased as described by Steinmetz and Fischer (1981, 1982a, b).

Results

Thiosulfate-utilizing enzymes in cell-free extracts. Crude extracts (100,000 × *g* supernatant) of *Chlorobium vibrioforme* f. *thiosulfatophilum* contained thiosulfate sulfur transferase, thiosulfate oxidase and thiosulfate reductase. Specific activities of these three enzymes and their percent distribution in thiosulfate turnover in the crude extract are listed in Table 1. Total amount of specific activities of all three enzymes was calculated as 100%. In comparison with the thiosulfate turnover catalyzed by thiosulfate sulfur transferase the activities of the other two enzymes were very low.

Ferricyanide served as electron acceptor in the thiosulfate oxidase system (Lyric and Suzuki 1970) and could not be replaced by cytochrome c-551 isolated from the same organism (Steinmetz and Fischer 1982a) as it was found by Kusai and Yamanaka (1973a, b) for cytochrome *c*-551 and the thiosulfate oxidizing enzyme from *C. limicola* f. *thiosulfatophilum*. The methyl viologen-dependent thiosulfate reductase assay was used, because Westley (1973) observed that thiosulfate sulfur transferase showed also thiosulfate reductase activity with dihydrolipoate as electron donor. This observation could also be confirmed for purified thiosulfate sulfur transferase from *C. vibrioforme* f. *thiosulfatophilum* (data not shown).

Influence of thiosulfate and sulfide on the activity of thiosulfate-utilizing enzymes. This experiment was performed to find out whether sulfide or thiosulfate had an influence on the formation of the enzymes involved in thiosulfate turnover. Therefore, cells of *C. vibrioforme* f. *thiosulfatophilum* were grown in culture media containing only sulfide or thiosulfate or both substances as electron donors. After complete consumption of the offered sulfur source the cultures with sulfide or thiosulfate in the medium were fed again with the same sulfur compound (4.4 g $Na_2S_2O_3 \times 5H_2O$ or 0.75 g $Na_2S \times 9H_2O$ per l) and harvested 4 h after feeding. The culture with both sulfur components was not fed again. The activities of thiosulfate sulfur transferase

Table 1. Specific activity and percent distribution of thiosulfate-utilizing enzymes in crude extracts from *Chlorobium vibrioforme* f. *thiosulfatophilum*

Enzyme	Specific activity (mU) (nmol $S_2O_3^{2-}$ utilized/min × mg protein)	Activity[a] (%)
Thiosulfate sulfur transferase	41	95
Thiosulfate oxidase	2	4.6
Thiosulfate reductase	0.17	0.4

[a] Total amount of specific activity of all enzymes was calculated as 100%

Table 2. Specific activity of thiosulfate sulfur transferase and thiosulfate oxidase in crude extracts from *C. vibrioforme* f. *thiosulfatophilum* grown on different sulfur compounds

Reduced sulfur compound in the medium	Specific activities (mU) (nmol $S_2O_3^{2-}$ utilized/min × mg protein)	
	Thiosulfate sulfur transferase	Thiosulfate oxidase
$Na_2S_2O_3 + Na_2S$	41 (94%)	2.7 (6%)
$Na_2S_2O_3$	65 (95%)	3.4 (5%)
Na_2S	34 (93%)	2.7 (7%)

Values in parentheses represent the percental part of the enzymes. Both enzyme activities obtained from the same culture conditions represent 100%

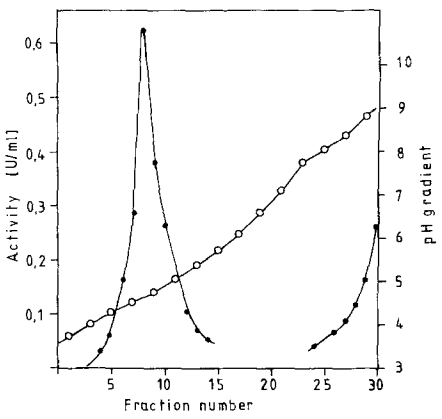

Fig. 1. Preparative isoelectric focusing of thiosulfate sulfur transferase of *Chlorobium vibrioforme* f. *thiosulfatophilum* on Ampholine gel, pH 3.0–9.5. The crude extract applied to the gel contained 150 mg desalted proteins. Electrical settings: 8 W constant power, 1,500 voltage. Temperature 5°C; operating time: 16 h. ○——○ pH gradient, ●——● thiosulfate sulfur transferase activity

and thiosulfate oxidase were examined in the crude extract and the specific activities are summarized in Table 2. Both enzymes were always present, independent of the offered sulfur compound in the medium. From the above-mentioned growth conditions one could also see that nearly 95% of thiosulfate was metabolized by thiosulfate sulfur transferase.

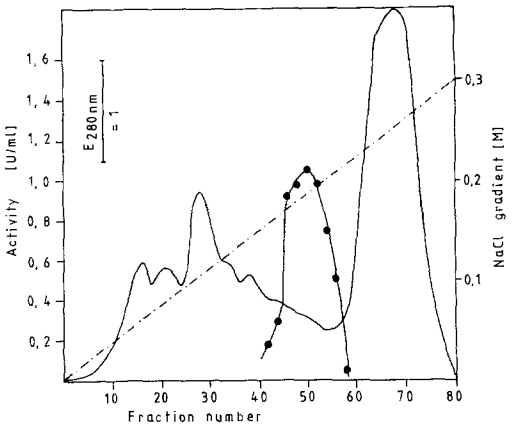

Fig. 2. Elution diagram of adsorbed proteins of a DEAE-52 cellulose chromatography of *C. vibrioforme* f. *thiosulfatophilum* 100,000 × *g* supernatant. Thiosulfate sulfur transferase activity was measured according to the method of Bowen et al. (1965). —— Protein distribution (280 nm), ●——● thiosulfate sulfur transferase activity, –·–·– sodium chloride gradient

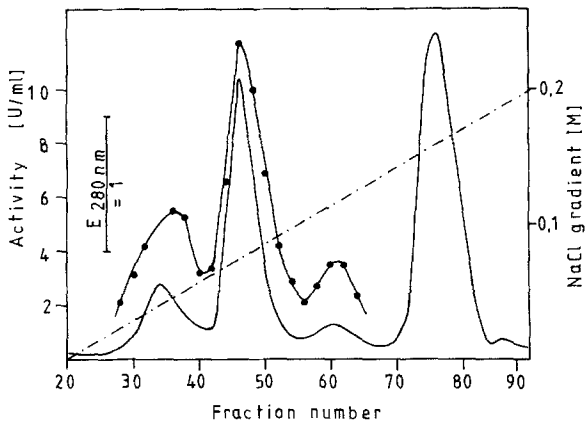

Fig. 3. Elution diagram of adsorbed proteins of a CM-52 cellulose chromatography of *C. vibrioforme* f. *thiosulfatophilum* 100,000 × *g* supernatant. Thiosulfate sulfur transferase activity was measured according to the method of Smith and Lascelles (1966). —— Protein distribution (280 nm), ●——● thiosulfate sulfur transferase activity, –·–·– sodium chloride gradient

Purification and separation of basic and acidic thiosulfate sulfur transferases. Preparative isoelectric focusing with desalted 100,000 × *g* supernatant of *C. vibrioforme* f. *thiosulfatophilum* was performed to find out how thiosulfate sulfur transferase could be enriched as quickly as possible in large quantities. By this procedure we found two distinctly separated thiosulfate sulfur transferase activities, one at acidic pH values, the other in basic pH ranges, as clearly demonstrated in Fig. 1. This result caused us to apply desalted supernatant of the 100,000 × *g* ultracentrifugation onto DEAE-52 cellulose (for the acidic enzyme) and onto CM-52 cellulose (for the basic enzyme) for further enrichment steps.

From the DEAE-52 cellulose column (equilibrated in 2 mM Tris-HCl, pH 7.8, bed volume 10 ml) adsorbed proteins were eluted with 200 ml of 20 mM Tris-HCl, pH 7.8, with a linear NaCl gradient of 0–0.2 M. Protein distribution and activity of the acidic thiosulfate sulfur transferase (determined after Bowen et al. 1965) are shown in Fig. 2. Fractions containing this activity were pooled and concen-

Table 3. Comparison of the molecular properties of basic and acidic thiosulfate sulfur transferase from *C. vibrioforme* f. *thiosulfatophilum*

Properties	Acidic enzyme	Basic enzyme
Isoelectric point	4.7	9.2
Molecular weight	34,000	39,000
K_m value (thiosulfate)	5 mM	0.25 mM
K_m value (cyanide)	125 mM	5 mM

trated to a few ml by ultrafiltration using a YM 5 membrane (Amicon Corporation). The concentrated ultrafiltrate was passed through a Sephadex G-75 (equilibrated in 50 mM Tris-HCl, pH 7.8, containing 100 mM NaCl; bed volume 180 ml) and eluted with the same buffer. The fraction containing thiosulfate sulfur transferase activity was then used for molecular and kinetic investigations. Basic thiosulfate sulfur transferase was eluted with 200 ml of 20 mM Tris-HCl, pH 7.2, and a linear NaCl gradient of 0–0.3 M from a small CM-52 cellulose column (equilibrated in 2 mM potassium-phosphate buffer, pH 6.5; bed volume 5 ml) (Fig. 3). The last protein band (fraction no. 70–85) of Fig. 3 was red coloured and could be identified by its spectrum as the small cytochrome *c*-555 described by Steinmetz and Fischer (1982a). The further purification steps for the basic enzyme were the same as described above for the acidic enzyme.

Properties of the acidic and basic enzyme. The molecular weights of both enzymes were determined by comparative gel filtration through Sephadex G-75. The basic thiosulfate sulfur transferase had a molecular weight of 39,000. SDS polyacrylamide gel electrophoresis (7.5% acrylamide; 0.1% SDS) with the eluate of the Sephadex G-75 filtration revealed only a single band. This indicated that the enzyme was purified to homogeneity. To the contrary, the acidic enzyme was not pure. Several but distinct separated protein bands were eluted from the Sephadex G-75 column. But the only activity maximum of the basic enzyme was clearly found at a molecular weight of 34,000.

The isoelectric point of the acidic enzyme was at pH 4.6–4.7 (Fig. 1). Since the activity maximum for the basic enzyme was at the rim of the gel, the isoelectric point of this enzyme was determined on a polyacrylamide gel with a pH gradient of 7.0–10.0. By this procedure the isoelectric point was detected at pH 9.2. Kinetic studies with both enriched enzymes were made to determine their K_m-values for both substrates, cyanide and thiosulfate.

For the basic enzyme the K_m for thiosulfate was determined as 0.25 mM and for cyanide as 5 mM. The K_m-values of the acidic protein were 5 mM for thiosulfate and 125 mM for cyanide. Molecular and kinetic properties of both enzymes are listed in Table 3.

Discussion

The present work has revealed that the thiosulfate-utilizing green sulfur bacterium *Chlorobium vibrioforme* f. *thiosulfatophilum* possesses two thiosulfate sulfur transferases. Both enzymes differ clearly in their isoelectric points and in their affinity towards thiosulfate and cyanide, while there is no great difference in their molecular weights (see Table 3). The molecular weight and K_m-values obtained for thiosulfate sulfur transferase of *C. vibrioforme* f. *thiosulfatophilum* lie in the order of magnitude found for other thiosulfate-utilizing bacteria (see Trüper 1981, Hashwa 1972, Bowen et al. 1965). Regarding the specific activity of all thiosulfate-utilizing enzymes found in *C. vibrioforme* f. *thiosulfatophilum* (Tables 1 and 2) it seems that thiosulfate sulfur transferase is the dominant enzyme initiating an enzymatic cleavage of thiosulfate. It seems that the further oxidation of the sulfane sulfur from the thiosulfate cleavage is differently regulated by the thiosulfate-utilizing species of the green sulfur bacteria. Studies with whole cells of *C. vibrioforme* f. *thiosulfatophilum* have shown that thiosulfate was converted via elemental sulfur to sulfate (Steinmetz und Fischer 1982a). When Khanna and Nicholas (1981, 1982) fed the same organism with ^{35}S radioactively labelled thiosulfate they could demonstrate that radioactivity occurred rapidly in the sulfate molecule when $S^{35}SO_3^{2-}$ was used. On the contrary, if they offered $^{35}SSO_3^{2-}$ to the cells, the isotope accumulated in the elemental sulfur deposited outside the cells. Elemental sulfur was slowly further oxidized to sulfate. This experiment clearly indicated that *C. vibrioforme* f. *thiosulfatophilum* did not oxidize thiosulfate to tetrathionate. Consequently, thiosulfate was cleaved enzymatically forming elemental sulfur and probably sulfite which was rapidly further oxidized to sulfate.

In contrast to the organism described above, *C. limicola* f. *thiosulfatophilum* oxidized extracellularly offered elemental sulfur or thiosulfate directly to sulfate without formation of any intermediates (Schedel 1978). Since the sulfane sulfur of the enzymatic thiosulfate cleavage did not appear as elemental sulfur in the medium, Schedel (1978) postulated that a high transportation rate of elemental sulfur into the cells and an elemental sulfur oxidizing enzyme were responsible for the rapid further oxidation to sulfate.

Mathewson and coworkers (1968) and Kusai and Yamanaka (1973a, b) found that cytochrome *c*-551 was the electron acceptor of the thiosulfate oxidizing system in *C. limicola* f. *thiosulfatophilum*. Low activity of thiosulfate oxidase was also found in *C. vibrioforme* f. *thiosulfatophilum*, but the cells' own cytochrome *c*-551 could not replace ferricyanide as electron acceptor as it was described by Kusai and Yamanaka (1973a, b) for the thiosulfate-utilizing subspecies of *C. limicola*. Yoch and Lindstrom (1971) found thiosulfate sulfur transferase activity in *C. limicola* f. *thiosulfatophilum* and Schedel (1978) could demonstrate that this enzyme is the protein responsible for thiosulfate splitting. Therefore, thiosulfate sulfur transferase seems to initiate enzymatic thiosulfate metabolism in thiosulfate-utilizing green sulfur bacteria. The *in vitro* results confirmed the observation obtained with whole cells where an enzymatic cleavage of thiosulfate was assumed (Schedel 1978; Steinmetz and Fischer 1982a). From *C. vibrioforme* f. *thiosulfatophilum* we could isolate two thiosulfate sulfur transferases. Until now it remains unclear whether these enzymes can be regarded as isoenzymes or whether they catalyze different reactions *in vivo*. Thiosulfate sulfur transferase is a widely distributed enzyme and occurs in liver tissue, plant roots and bacteria (Roy and Trudinger 1970). The enzyme participates in sulfur metabolism of *Thiobacillus denitrificans* (Bowen et al. 1965; Schedel and Trüper 1980) and besides thiosulfate reductase occurs in *Thiocapsa roseopersicina* (Petushkova and Ivanovskii 1976). Thiosulfate sulfur transferase (Smith and Lascelles 1966) and thio-

sulfate oxidase activity (Smith 1966) were found in *Chromatium vinosum*. High potential iron sulfur protein (HIPIP) of *Chromatium vinosum* was found to be a potent electron acceptor of the thiosulfate-oxidizing enzyme and tetrathionate was assumed to be the reaction product formed (Fukumori and Yamanaka 1979). On the other hand, Schmitt et al. (1981) found that a membrane-bound flavocytochrome *c*-552 served as electron acceptor for the thiosulfate-oxidizing enzyme of that organism.

Thiosulfate sulfur transferase functioned not only in dissimilatory thiosulfate metabolism but catalyzes also other reactions. The enzyme reconstituted ferredoxin, free of nonheme iron and acid-labile sulfide in the presence of $FeCl_3$, cysteine and thiosulfate (Finazzi-Agrò et al. 1971; Tomati et al. 1974). With dihydrolipoate as electron donor the enzyme showed also thiosulfate reductase activity (Westley 1973) by forming SO_3^{2-} and HS^-. A distinct difference between thiosulfate sulfur transferase and thiosulfate reductase activity can be made with regard to their substrate specifity. The latter enzyme is unable to transfer the sulfane group of thiosulfate onto cyanide (Westley 1973). Cyanide, sulfite, organic sulfinates, thiols or dithiols are suitable acceptor substrates for thiosulfate sulfur transferase while thiosulfate, thiosulfonates, persulfides and polysulfides serve as good sulfur-donor substrates (Westley 1973). With persulfides or polysulfides as sulfur donors and sulfite as acceptor, the enzyme catalyzed also the formation of thiosulfate (Westley 1973). The nature of the *in vivo* sulfur acceptor of thiosulfate sulfur transferase in *C. vibrioforme* f. *thiosulfatophilum* remains unknown. With cyanide as acceptor the enzyme can only be regarded with respect to a cyanide-detoxifying function which may certainly be of minor importance for the organism. Certain thiol or dithiol compounds like glutathione or dihydrolipoate can also function as sulfane sulfur acceptors of the enzyme (Westley 1973; Sörbo 1975). In this case the sulfane sulfur is transferred to the acceptor thiol to form a hydropolysulfide (Siegel 1975). Such a reaction, with a probably particle-bound thiol as sulfur acceptor could be catalyzed by thiosulfate sulfur transferase found in extracts of thiobacilli (see Siegel 1975). Whether such a particle-bound thiol or glutathione of *C. vibrioforme* f. *thiosulfatophilum* will take over an acceptor function of thiosulfate sulfur transferase during thiosulfate cleavage to form hydropolysulfides (RS_nSH) is unknown but worth to be elucidated.

The thiosulfate-utilizing strains of the green sulfur bacteria initiate anaerobic sulfide oxidation by the formation of thiosulfate (Schedel 1978, Steinmetz and Fischer 1982a). This behaviour was not observed with the nonthiosulfate-utilizing strains (Steinmetz and Fischer 1981, 1982b). The rapid formation of thiosulfate from sulfide might be a certain advantage in the struggle for sulfide as substrate for the thiosulfate-utilizing Chlorobiaceae over those which cannot use thiosulfate. According to its manifold catalytic activities it could be possible that one of the thiosulfate sulfur transferases found in *C. vibrioforme* f. *thiosulfatophilum* will catalyze thiosulfate formation to provide the organism with the advantage mentioned.

Further experiments on the coexistence of thiosulfate-utilizing and non-utilizing green sulfur bacteria in a sulfide limited culture could perhaps help to clarify the above assumption.

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