PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

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Anaerobic oxidation of sulphur compounds as electron donors for bacterial photosynthesis

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Most phototrophic bacteria use reduced inorganic sulphur compounds as electron donors during anoxygenic photosynthesis. Principally, sulphide is oxidized via sulphite to sulphate. Elemental sulphur may appear as intermediary storage product (inside: *Chromatium*, *Thiocapsa*; outside: *Chlorobium*, *Ectothiorhodospira*; not in: *Rhodopseudomonas sulfidophila*). Adenosine phosphosulphate is an intermediate in sulphite oxidation by *Chromatium*, *Thiocapsa* and *Chlorobium*. Thiosulphate undergoes splitting to sulphide (or elemental sulphur) and sulphite, or is oxidized to tetrathionate. Sulphide may be oxidized to elemental sulphur by cytochrome *c* or to thiosulphate (perhaps sulphite?) by flavocytochrome *c*, or to sulphite by a reverse (sirohaem) sulphite reductase. The latter enzyme also oxidizes polysulphides and probably elemental sulphur. Sulphite is either oxidized by APS reductase to form adenosine phosphosulphate – from which sulphate is released by ADP sulphurylase – or by sulphite:acceptor oxidoreductase directly to sulphate. The electron acceptor of most of these oxidative enzymes are cytochromes or non-haem iron–sulphur proteins. The pathways of photolithotrophic sulphur oxidation in Chlorobiaceae, Chromatiaceae and Rhodospirillaceae are separately compiled under evaluation of the presently available data.

Introduction

Most phototrophic bacterial species depend on or are at least capable of utilizing reduced sulphur compounds as photosynthetic electron donors under anaerobic conditions. While sulphide is generally utilized by organisms with this capacity, the ability to use elemental sulphur is typical for the Chromatiaceae and Chlorobiaceae, not for the Rhodospirillaceae and the cyanobacteria. The utilization of thiosulphate is more common in Chromatiaceae and Rhodospirillaceae than in Chlorobiaceae. Very few species have been found to utilize tetrathionate or sulphite. A detailed list of such capabilities has been published elsewhere (Trüper 1981).

The photoautotrophic way of life requires reduced pyridine nucleotides and ATP for carbon dioxide fixation. Pyridine nucleotide photoreduction by phototrophic bacteria follows two principally different mechanisms (Knaff 1978): in the species of the Chromatiaceae and Rhodospirillaceae studied so far, the photosynthetic primary acceptor (supposedly a ubiquinone–iron complex) is considerably less electronegative than the NAD/NADH couple. Therefore an energy-dependent reverse electron flow mechanism was postulated for these, the purple bacteria. Evidence for this pathway was gained mainly from inhibitor and uncoupler studies of cyclic electron flow. When cyclic photophosphorylation is blocked, NAD is not reduced in the purple bacteria studied so far. Additional evidence for energy-dependent reverse electron flow was supplied by several studies on dark growth of Chromatiaceae at more or less reduced oxygen tensions. Especially the survey of Kämpf & Pfennig (1980) showed that the Chromatiaceae form two groups: one represented by the small-cell species (e.g. *C. vinosum*, *Thiocapsa roseopersicina*,

Thiocystis violacea), which turned out to be facultatively chemolithoautotrophic bacteria, and the other represented by the large-cell *Chromatium* species (e.g. *C. okenii*, *C. warmingii*), which proved to be strictly photolithotrophic bacteria like the Chlorobiaceae.

In the Chlorobiaceae the primary acceptor in photosynthesis, a membrane-bound iron–sulphur protein with a midpoint potential of −540 mV, has been proved to reduce NAD via ferredoxin without energy input from ATP or other high-energy intermediates. Here, NAD reduction is not blocked by uncouplers.

The ability to grow like a thiobacillus should be restricted to those phototrophic bacteria that can switch from cyclic photophosphorylation to ATP production by respiratory electron flow from reduced sulphur compounds to oxygen. Obviously the Chlorobiaceae and the large-cell Chromatiaceae are not geared for reverse electron flow and therefore unable to grow chemolithoautotrophically. There are, however, different opinions with respect to non-cyclic electron flow in *C. vinosum* (see, for example, Van Grondelle *et al.* 1977).

The two principally different methods of pyridine nucleotide reduction in phototrophic bacteria imply different pathways for the electrons derived from reduced inorganic sulphur compounds. In *C. vinosum* and some Rhodospirillaceae species, reduced sulphur compounds deliver their electrons to an electron transport chain (not via reaction centre bacteriochlorophyll and primary acceptor) as in chemolithotrophic bacteria of the *Thiobacillus* type, where they are 'pumped uphill' by high-energy phosphates (ATP). During anaerobic light metabolism these stem from cyclic photophosphorylation. During aerobic or microaerobic dark metabolism, however, part of the sulphur electrons are needed to 'go downhill', i.e. to produce ATP by respiratory phosphorylation. In the Chlorobiaceae the electrons from reduced sulphur compounds enter the non-cyclic photosynthetic electron flow chain, passing through the reaction centre bacteriochlorophyll and the primary acceptor and ending up in NAD(P)H.

One could speculate whether these basic differences in electron flow are also expressed by different enzymatic steps in sulphur metabolism. There are other basic differences between the purple and the green bacteria (Chlorobiaceae), not only in pigments and ultrastructure (see Pfennig & Trüper 1974). The Chlorobiaceae lack the Calvin cycle, they fix carbon dioxide via a reductive carboxylic acid cycle (Evans *et al.* 1966; Fuchs *et al.* 1980*a*, *b*; Ivanovsky *et al.* 1980), while the purple bacteria possess a regular Calvin cycle. Phylogenetic studies (Gibson *et al.* 1979; Fox *et al.* 1980), have shown that the green and purple bacteria are far more distantly related than it was generally believed and depicted by the hierarchy of higher taxa. Therefore it cannot be ruled out *a priori* that principal differences between these groups also exist in photolithotrophic sulphur metabolism.

In the following pages we summarize the present state of knowledge about the enzymology of photolithotrophic sulphur oxidation in Chlorobiaceae, Chromatiaceae and Rhodospirillaceae, no matter whether these will remain taxonomic families in the future. Owing to the few available data on Chloroflexaceae and cyanobacteria in this respect we shall not include these two groups in our considerations.

Chlorobiaceae (green sulphur bacteria)

All species in this family oxidize sulphide via elemental sulphur (outside the cells) to sulphate. Elemental sulphur added as sole sulphur source is oxidized to sulphate as well. Thiosulphate is only utilized by certain strains (*formae speciales*) of *Chlorobium limicola* and *Chl. vibrioforme*, that are

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

then called 'f. *thiosulfatophilum*'. The former species *Chl. thiosulfatophilum* had to be divided and associated with *Chl. limicola* and *Chl. vibrioforme*, respectively, owing to other properties (Pfennig & Trüper 1974).

Cell suspensions of *Chl. limicola* f. *thiosulfatophilum* oxidize sulphide (anaerobically in the light) forming first thiosulphate, which appears in the medium, then elemental sulphur; both are subsequently oxidized to sulphate (Schedel 1977). Sulphide oxidation in *Chl. vibrioforme* f. *thiosulfatophilum* follows the same pattern (Steinmetz & Fischer 1982). When elemental sulphur

TABLE 1. SEVERAL PROPERTIES OF CHLOROBIACEAE RELATED TO OXIDATIVE SULPHUR METABOLISM AND ELECTRON TRANSPORT

	Chlorobium limicola	*Chlorobium vibrioforme*	*Pelodictyon luteolum*	*Chlorobium limicola* f. *thiosulfatophilum*	*Chlorobium vibrioforme* f. *thiosulfatophilum*
thiosulphate utilized	−	−	−	+	+
sulphide oxidized to $S_2O_3^{2-}$ and S^0	−	−	−	+	+
thiosulphate oxidized to S^0 in the medium	−	−	−	−	+
S^0 disproportionation	−	−	−	+	+
cytochrome *c*-551	−		−	+	+
flavocytochrome *c*-553	+		−	+	+
cytochrome *c*-553	−		+	−	−
cytochrome *c*-555	+		+	+	+
rubredoxin	+		+	+	+
soluble sirohaem	−		−	−	−
rhodanese	−			+	+
thiosulphate reductase	−			+	+
thiosulphate:acceptor oxidoreductase				+	
sulphite:acceptor oxidoreductase					(−)
reverse sulphite reductase				(+)	
APS reductase				+	(−)
ADP sulphurylase					+

(+), (−), Preliminary results. Data were collected from Trüper (1981), Steinmetz & Fischer (1981, 1982), Lorenz (1975), Kirchhoff & Trüper (1974) and unpublished work of our department.

is given as the sole electron donor, *Chl. limicola* f. *thiosulfatophilum* does not form thiosulphate or sulphide as intermediate product during photooxidation to sulphate (Schedel 1977). The two thiosulphate-utilizing *formae* differ in thiosulphate oxidation in so far as *Chl. vibrioforme* f. *thiosulfatophilum* does form elemental sulphur during this process and *Chl. limicola* f. *thiosulfatophilum* does not.

The two *formae* share a number of properties not owned by the rest of the Chlorobiaceae (see table 1). They are able to disproportionate elemental sulphur anaerobically in the light (Paschinger *et al.* 1974), as long as carbon dioxide is absent and the H_2S produced during the process is continuously removed by inert gas flushing; the oxidized product of this light-driven disproportionation is thiosulphate, although we have indications that sulphite is the oxidized

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

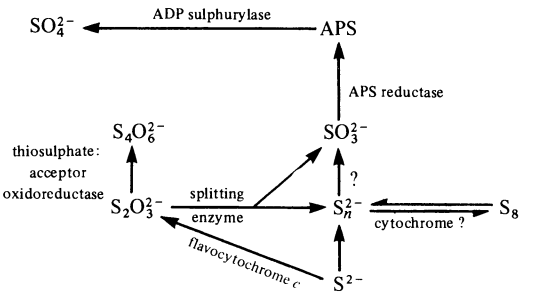
species that reacts non-enzymatically with elemental sulphur to form thiosulphate (Lorenz 1975). The stoichiometry of the disproportionation has been determined as

$$4S^0 + 3H_2O \rightarrow 2H_2S + H_2S_2O_3.$$

Obviously elemental sulphur reduction replaces carbon dioxide reduction during this process.

Kusai & Yamanaka (1973*a*, *c*) have reported that flavocytochrome *c*-553 of *Chl. limicola* f. *thiosulfatophilum* functions as a sulphide:cytochrome *c* reductase. The reaction is strongly inhibited by cyanide. The flavocytochrome is heat-labile and is rapidly reduced by sulphide, which is transformed into thiosulphate (Fischer 1977). Flavocytochrome *c*-553 has meanwhile also been found in the non-thiosulphate-utilizing *Chl. limicola* as well as in *Chl. vibrioforme* f. *thiosulfatophilum* (Steinmetz & Fischer 1981, 1982), but not in *Pelodictyon luteolum*, where the respective cytochrome *c*-553 turned out to lack a flavin moiety (M. Steinmetz, personal communication.

All green bacteria studied so far contain cytochrome *c*-555 (which is the cytochrome species feeding electrons directly to the reaction centre bacteriochlorophyll) and rubredoxin, the function of which is still unresolved.



SCHEME 1

It is not yet clear whether the sulphide oxidizing system in *Chl. limicola* f. *thiosulfatophilum* is membrane-bound *in vivo* (Evans & Buchanan 1965), although such a localization would be logical. From studies with membrane particle fractions ('chromatophores') from this organism, Knaff & Buchanan (1975) suggested that bound cytochrome *b* might be involved in non-cyclic electron flow from sulphide to NADP. In a scheme designed by Knaff (1978) cytochrome *b* has even been seen as the direct acceptor of electrons from sulphide.

The thiosulphate-utilizing *formae* of *Chlorobium* are apparently equipped with both thiosulphate-splitting enzyme (rhodanese, perhaps identical with thiosulphate reductase (Yoch & Lindstrom 1971; Prangenberg 1976)) and thiosulphate-combining enzyme (thiosulphate: acceptor oxidoreductase (Mathewson *et al.* 1968; Kusai & Yamanaka 1973*b*, *c*; Steinmetz & Fischer 1982)). *Chl. limicola* lacks these enzymes (Steinmetz & Fischer 1981). Thiosulphate: acceptor oxidoreductase was first reported from *Chl. limicola* f. *thiosulfatophilum* by Kusai & Yamanaka 1973*b*, *c*). This enzyme catalyses the reduction of *Chlorobium* cytochrome *c*-551 by thiosulphate. Reduced cytochrome *c*-551 then reduces cytochrome *c*-555, which again stimulates the former reaction (Kusai & Yamanaka 1973*c*). Steinmetz & Fischer (1982) proved that this cytochrome *c*-551 occurs in the other thiosulphate utilizing *forma* of *Chlorobium* as well. The non-thiosulphate-utilizing *Chl. limicola*, however, lacks cytochrome *c*-551, the endogenous electron acceptor of its thiosulphate-utilizing relative (Steinmetz & Fischer 1981).

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

These facts point towards a participation of both thiosulphate-splitting as well as thiosulphate-oxidizing processes during anaerobic photooxidation of thiosulphate by *Chlorobium*.

Adenosine phosphosulphate (APS) reductase has so far only been found in *Chl. limicola* f. *thiosulfatophilum* (Kirchhoff & Trüper 1974). We have not so far succeeded in measuring comparable amounts of this enzyme in the other species of the Chlorobiaceae. We have also not been able so far to prove the presence of sirohaem sulphite reductase in green sulphur bacteria.

At present, only a rather incomplete picture of sulphur metabolism in *Chlorobium* species may be drawn (scheme 1).

Chromatiaceae (purple sulphur bacteria)

The Chromatiaceae – with the exception of the species of the genus *Ectothiorhodospira* – are capable of intracellular storage of elemental sulphur globules that are formed during the utilization of sulphide and thiosulphate in the light. *Ectothiorhodospira* species form sulphur globules outside the cells in the medium. Owing to their preference for alkaline media these organisms are excellent objects to demonstrate that the elemental sulphur is formed via polysulphides: when sulphide solution is fed to a sulphur-starved liquid culture the medium turns translucent yellow until it suddenly becomes opaque white and sulphur precipitates. This phenomenon is interpreted as a reaction of the sulphur formed first with the remaining sulphide to produce polysulphides; as soon as the free sulphide is consumed and the polysulphides are deprived of their free electrons they precipitate as elemental sulphur.

In general it can be said that if a phototrophic bacterium is able to utilize elemental sulphur as photosynthetic electron donor it will oxidize it to sulphate; under anaerobic conditions other free sulphur compounds have never been detected as intermediates. All Chromatiaceae species that store intracellular sulphur are also able to photooxidize extracellularly supplied elemental sulphur.

When growing with sulphide in batch culture such organisms start intracellular sulphur oxidation before the external sulphide is totally consumed (Trüper 1964, 1978); in *Ectothiorhodospira* elemental sulphur oxidation starts only after the complete consumption of sulphide (as in Chlorobiaceae). Obviously external sulphide inhibits sulphur oxidation more effectively in organisms that are unable to store intracellular sulphur. The site of sulphur oxidation must therefore be the cytoplasmic membrane. Only in sulphur-storing species must sulphur oxidation also be performed further inside the cell, probably at deep intrusions of the cytoplasmic membrane.

Sulphur disproportionation does not occur in Chromatiaceae.

The formation of elemental sulphur from sulphide in *Thiocapsa roseopersicina* is a heat-stable process and catalysed in the soluble fraction (Petushkova & Ivanovsky 1976*a*). A heat stable cytochrome *c*-550 was isolated from this organism by Fischer & Trüper (1977). This cytochrome is easily reduced by sulphide which is transformed into sulphur by this reaction, obviously a non-enzymatic one. Perhaps the reversible step $S^{2-} \rightleftharpoons S^0 + 2e^-$ mediated by cytochrome *c* could explain sulphur storage as a reversible process.

Also flavocytochrome *c*-552 of *C. vinosum* is a likely candidate to participate in sulphide oxidation *in vivo* forming thiosulphate (Fischer 1977). Fukumori & Yamanaka (1979*a*) identified flavocytochrome *c*-552 as a sulphide:cytochrome *c* reductase of reversible reactivity.

Knaff & Buchanan (1975) postulated the participation of cytochrome *b* in sulphide oxidation

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

by *C. vinosum*. For thiosulphate oxidation a participation of cytochrome *b* is denied by Van Grondelle *et al.* (1977).

Rhodanese and thiosulphate reductase as thiosulphate-splitting enzymes occur in most Chromatiaceae (see table 2) (Smith & Lascelles 1966; Yoch & Lindstrom 1971; Hashwa 1972, 1975; Prangenberg 1976). From comparative work with *Thiobacillus denitrificans* we expect that the thiosulphate splitting *in vivo* is due to the protein that exhibits rhodanese activity *in vitro* (Schedel 1977; Schedel & Trüper 1980).

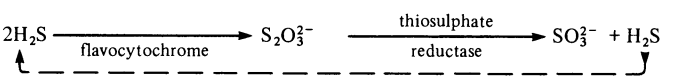
TABLE 2. SEVERAL PROPERTIES OF CHROMATIACEAE RELATED TO OXIDATIVE SULPHUR METABOLISM

	Thiocapsa roseopersicina	*Chromatium vinosum*	*Thiocystis violacea*	*Chromatium minutissimum*	*Chromatium gracile*	*Chromatium purpuratum*	*Chromatium minus*	*Chromatium warmingii*	*Thiocystis gelatinosa*	*Ectothiorhodospira mobilis*	*Ectothiorhodospira shaposhnikovii*	*Ectothiorhodospira vacuolata*	*Ectothiorhodospira halophila*	*Ectothiorhodospira halochloris*	*Ectothiorhodospira abdelmalekii*
S^0 inside (i) or outside (o) the cells	i	i	i	i	i	i	i	i	i	o	o	o	o	o	o
thiosulphate utilized	+	+	+	+	+	+	+	−	−	+	+	+	+	+	+
rhodanese	+	+					+	+	+	+		+			
thiosulphate reductase	+	+					+	+	+			+			
sirohaem sulphite reductase	−	+										−			
APS reductase	+	+	+	+	−	−				(−)	−	−	(−)	−	−
ADP sulphurylase	+	+	+	+	+	−				(−)	−	+	(−)		
sulphite:acceptor oxidoreductase	+	+	+	+	+	+				+	+	+	+		
thiosulphate:acceptor oxidoreductase		+													

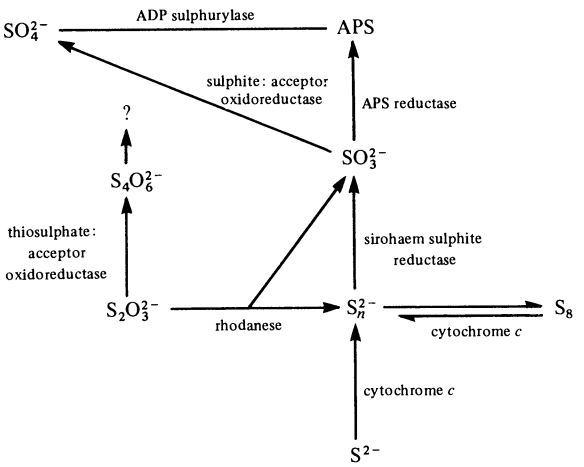
(−), Extremely low activities found recently. Data from Trüper (1978, 1981), Ulbricht (1981), Brückenhaus (1977), Prangenberg (1976), Schedel *et al.* (1979) and unpublished work from our department.

C. vinosum contains a sirohaem sulphite reductase (Schedel 1977; Schedel *et al.* 1979; Kobayashi *et al.* 1978; Seki *et al.* 1981) located in the soluble fraction. The enzyme is fully repressed under heterotrophic growth conditions (malate–sulphate, light, anaerobic). It contains sirohaem and non-haem iron–sulphur clusters. Its general properties render it rather similar to the respective enzymes from *Desulfovibrio vulgaris* (Lee *et al.* 1973; Kobayashi *et al.* 1972, 1974) and *Thiobacillus denitrificans* (Schedel & Trüper 1980). Its physiological role must be seen as that of a sulphide:acceptor oxidoreductase in *C. vinosum* as well as in the anaerobic *Thiobacillus denitrificans*. Because of the lack of a suitable artificial electron acceptor this hypothesis cannot be proved directly, but the following arguments support it indirectly: (1) it is present only in lithoautotrophic cells; (2) the reaction is reversible; (3) its high content in the cells is typical for dissimilatory enzymes, (4) it is analogous to the APS reductase that occurs in sulphate-reducing and in sulphur-oxidizing bacteria.

In *Thiocapsa roseopersicina* no sirohaem was found, although sulphite reductase activity could be affirmed (Jorzig 1979). If an organism contained a flavocytochrome *c* and a thiosulphate reductase, an explicit sulphite reductase would not be necessary, as sulphite production from sulphide could proceed as follows:



From earlier work it has been generalized that Chromatiaceae and Chlorobiaceae possess APS reductase while Rhodospirillaceae do not (Trüper & Peck 1970; Trüper 1975). From more recent studies (see Trüper 1981) it follows that the species of *Ectothiorhodospira* do not contain measurable APS reductase. This problem is, however, not yet clearly settled, because we have recently found low activities in *E. mobilis* and *E. halophila*. Here methodological difficulties have to be overcome. The APS reductase of *T. roseopersicina* is a remarkable enzyme because it is easily leached from the membranous fraction (Trüper & Rogers 1971), quite unlike that of *C. vinosum*, which could not be solubilized (Schwenn & Biere 1979). APS reductase activity in these bacteria is highest at the late exponential growth phase and decreases sharply in the stationary phase (Schwenn & Biere 1979; and own results). The enzyme from *T. roseopersicina* could be viewed as a flavocytochrome *c* as it contains haem groups in addition to the flavin and non-haem iron–sulphur clusters of other APS reductases (from *Desulfovibrio*, *Thiobacillus* and *Chlorobium*). No other flavocytochrome could be isolated from *T. roseopersicina* (Fischer & Trüper 1977). Also the APS reductase from *Thiocystis violacea* contains haem groups (Tatzki 1979).



SCHEME 2

The release of sulphate from APS is catalysed with the conservation of the energy-rich bond by ADP sulphurylase. This enzyme has been purified and characterized in our laboratory from three small-cell *Chromatium* species by Brückenhaus & Ulbricht (personal communication). As may be seen from table 2 there are inconsistencies with *C. gracile* and *E. vacuolata* that cannot be explained at present. The existence of *Chromatium* species possessing ADP sulphurylase but lacking APS reductase does not make sense and will therefore have to be studied in more detail.

The enzyme sulphite:acceptor oxidoreductase, which could be considered as a bypass of the APS reductase/ADP sulphurylase system, and therefore of less energy-conserving quality, turned out to be rather common in species of the Chromatiaceae. In *T. roseopersicina* it was first described by Petushkova & Ivanovsky (1976*a*, *b*). In this laboratory Brückenhaus (1977) and Ulbricht (1981) have isolated this enzyme from several *Chromatium* and *Ectothiorhodospira* species (cf. tables 2 and 4).

A thiosulphate:acceptor oxidoreductase forming tetrathionate from thiosulphate was detected in *C. vinosum* by Smith (1966). The electron acceptor *in vivo* of this enzyme is in dispute as it is considered to be high-potential non-haem iron–sulphur protein (HiPIP) by Fukumori &

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Yamanaka (1979*b*) and membrane-bound flavocytochrome *c*-552 by Schmitt *et al.* (1981). Van Grondelle *et al.* (1977) suggested that a cytochrome *c*-551 acts as a mobile electron acceptor for thiosulphate residing in the periplasmic space. At present we cannot exclude the possibility that more than one such enzyme exists in *C. vinosum*: a thiosulphate:HiPIP oxidoreductase and a thiosulphate cytochrome *c* oxidoreductase.

At the present state of knowledge, a combined scheme of photolithotrophic sulphur metabolism in *C. vinosum* and *T. roseopersicina* may be drawn as in scheme 2.

Table 3. Several properties of Rhodospirillaceae related to oxidative sulphur metabolism

	Rhodopseudomonas palustris	*Rhodopseudomonas sulfoviridis*	*Rhodopseudomonas capsulata*	*Rhodopseudomonas sulfidophila*	*Rhodopseudomonas globiformis*	*Rhodomicrobium vannielii*	*Rhodospirillum rubrum*
sulphide utilized	(+)	+	(+)	+	−	(+)	(+)
thiosulphate utilized	+	+	−	+	+	−	−
rhodanese	+	+	+	+	+		+
thiosulphate reductase	+	+	+	+	+	+	+
thiosulphate:acceptor oxidoreductase	+	[+]		[+]	+		
reverse sulphite reductase			+		+		
sulphite:acceptor oxidoreductase		−		+			
APS reductase	−	−	−	−	−	−	−
ADP sulphurylase				−			

(+), In continuous culture at sulphide limitation; [+], low activity. Data from Trüper (1978, 1981), Keppen *et al.* (1980), Then & Trüper (1981), Hansen (1974), Prangenberg (1976), Schug (1979) and unpublished work from our department.

Rhodospirillaceae (purple 'non-sulphur' bacteria)

This family, which once bore the remarkable name 'Athiorhodaceae', has to be taken into considerations of photolithotrophic sulphur metabolism since the work of Hansen (1974; Hansen & van Gemerden 1972), who discovered that a number of species are very well capable of utilizing sulphide and thiosulphate as electron donors. The utilization of thiosulphate by *Rhodopseudomonas palustris* had been shown earlier by van Niel (1944). Hansen & Veldkamp (1973) described a new species, *Rhodopseudomonas sulfidophila*, as an organism that grows well at rather high sulphide concentrations, oxidizing it to sulphate without the production of elemental sulphur globules. Table 3 summarizes the enzymological results obtained so far for those species of the family that are able to make use of reduced sulphur compounds under photolithotrophic circumstances.

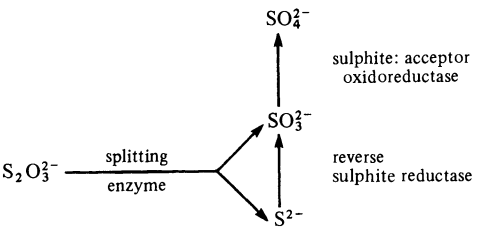
Within these species, *Rps. globiformis* is unable to oxidize sulphide. The organism possesses, however, a thiosulphate:cytochrome *c* oxidoreductase, which oxidizes thiosulphate to tetra-

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

thionate (Then & Trüper 1981). This reaction is of only limited value for this organism, as we have found that it cannot assimilate tetrathionate nor oxidize it further.

Thiosulphate utilization in *Rps. palustris* is considered inducible by Rolls & Lindstrom (1967) and Rodova & Pedan (1980) but constitutive by Hashwa (1972). Differences between strains cannot be excluded. When low thiosulphate concentrations (2–5 mM) are given, only sulphate is found; at higher concentrations (10–20 mM), tetrathionate appears in the medium (Rodova & Pedan 1980). The thiosulphate:cytochrome *c* oxidoreductase of this bacterium has been intensively studied (Appelt & Knobloch 1977; Appelt *et al.* 1979; Schleifer *et al.* 1981). The electron acceptor is cytochrome *c*-549.

Rhodomicrobium vannielii in anaerobic illuminated continuous culture under sulphide limitation oxidizes this to tetrathionate, while in batch cultures thiosulphate plus elemental sulphur appear, because of chemical reduction of tetrathionate by excess sulphide (Hansen 1974). *Rhodospirillum rubrum*, *Rps. capsulata* and *Rps. sphaeroides* oxidize sulphide only to elemental sulphur (Hansen 1974).



SCHEME 3

In an investigation into the sulphur metabolism of *Rps. sulfidophila* we have recently found that during both sulphide and thiosulphate oxidation sulphite is transiently excreted into the medium and further oxidized to sulphate only after sulphide or thiosulphate are fully consumed (Pfleiderer & Trüper, unpublished). This is the first time that sulphite has been found as a free intermediate in the sulphur metabolism of a phototrophic bacterium. The cytoplasm of sulphide-grown cells was analysed for sulphur compounds and found to contain sulphate and thiosulphate as well as certain organic sulphur compounds. Enzyme studies revealed that sulphide-grown cells of *Rps. sulfidophila* contain sulphite reductase, sulphite:acceptor oxidoreductase, thiosulphate reductase, rhodanese, and a low activity of thiosulphate:acceptor oxidoreductase, while APS reductase and ADP sulphurylase were lacking. When the cells were grown with thiosulphate, enzyme activities were similar with the exception of sulphite reductase, which was not found. These results, although preliminary, allow us to draw a still speculative view about photolithotrophic sulphide and thiosulphate oxidation in *Rps. sulfidophila* (scheme 3). This sequence appears to be the simplest type of sulphide oxidation, requiring (perhaps besides transport mechanisms) just two enzymes. The formation of sulphite as intermediate could be explained by different reaction velocities of the enzymes or by certain regulatory phenomena.

Rps. sulfoviridis is able to grow photolithotrophically with sulphide or thiosulphate. We have studied the concentration changes of sulphur compounds in the media of batch cultures and found that sulphide is rapidly consumed while a yet unidentified sulphur compound (not elemental sulphur) is accumulating in the cells. After a lag phase this intracellular 'sulphur' is transformed into sulphate, appearing in the medium. Thiosulphate, however, is oxidized at a constant rate, with sulphate as the only product (Neutzling 1980).

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Enzymes and electron transport proteins: conclusions

Table 4 presents a list of purified and partly characterized enzymes involved in the photolithotrophic sulphur metabolism of phototrophic bacteria. As can be seen from this list, our knowledge is still rather incomplete. There is not one organism from which the enzymes of a complete pathway from sulphide or thiosulphate to sulphate have been purified and characterized. In many cases the findings with whole cells seem to contradict enzyme studies.

Table 4. Purified and partly characterized enzymes of photolithotrophic sulphur metabolism

enzyme	organism	molecular mass/kDa	other properties	references
sulphide:cytochrome *c* reductase	*Chlorobium limicola* f. *thiosulfatophilum*	50	flavocytochrome *c*-553	(1), (2)
	Chlorobium vibrioforme f. *thiosulfatophilum*	63	flavocytochrome *c*-553	(3)
	Chlorobium limicola	56	flavocytochrome *c*-553	(4)
	Chromatium vinosum	72	flavocytochrome *c*-552	(5)
rhodanese	*Chromatium vinosum*	10 37 170	different aggregations	(6)
sirohaem sulphite reductase	*Chromatium vinosum*	280 180	$\alpha_4\beta_4$ subunit structure	(7) (8)
APS reductase	*Thiocapsa roseopersicina*	180	contains haem	(9)
	Chlorobium limicola f. *thiosulfatophilum*	210	no haem	(10)
	Thiocystis violacea	190	contains haem	(11)
ADP sulphurylase	*Chromatium vinosum*	180		(12)
	Chromatium gracile	200		(13)
	Chromatium minutissimum	160	constitutive	(13)
sulphite:acceptor oxidoreductase	*Chromatium vinosum*	70	perhaps cytochrome *c'*	(12)
	Chromatium purpuratum	87		(13)
	Chromatium minutissium	130		(13)
thiosulphate:acceptor oxidoreductase	*Chromatium vinosum*	36	acceptor:cytochrome *c*-552 or HiPIP	(14),(15)
	Rhodopseudomonas palustris	93	acceptor:cytochrome *c*-549	(16)
	Rhodopseudomonas globiformis	180	acceptor:cytochrome *c*	(17)
	Chlorobium limicola f. *thiosulfatophilum*	80	acceptor:cytochrome *c* 551	(2)

References: (1) Meyer *et al.* (1968); (2) Kusai & Yamanaka (1973); (3) Steinmetz & Fischer (1981); (4) Steinmetz & Fischer (1982); (5) Fukumori & Yamanaka (1979*a*); (6) Giani (1976); (7) Schedel (1977), Schedel *et al.* (1979); (8) Kobayashi *et al.* (1978); (9) Trüper & Rogers (1971); (10) Kirchhoff & Trüper (1974); (11) Tatzki (1979); (12) Brückenhaus & Trüper (unpublished); (13) Ulbricht & Trüper (unpublished); (14) Schmitt *et al.* (1981); (15) Fukumori & Yamanaka (1979*b*); (16) Schleifer *et al.* (1981); (17) Then & Trüper (1981); (18) Fischer & Trüper (1977); (19) Bartsch *et al.* (1968); (20) Bartsch (1978).

The situation is worse when it comes to the genuine cellular electron acceptors of the oxidative enzyme steps. Table 5 gives a list of electron transport proteins that have been purified from phototrophic bacteria and are thought to be involved in sulphur metabolism. Here, too, misinterpretation of results *in vitro* is easy, as redox reactions will often proceed with any electron acceptor of a suitable redox potential. For many of the purified enzymes the electron acceptors *in vivo* are still unknown. An especial difficulty is the impossibility of studying the oxidation of

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

sulphide to sulphite by (sirohaem) sulphite reductases because the substrate sulphide itself will immediately reduce chemically any reasonable electron acceptor in the test system. Here, only the reduction of the enzyme itself by sulphide could be demonstrated (Schedel 1977).

TABLE 5. PURIFIED ELECTRON TRANSPORT PROTEINS WITH FUNCTIONS IN SULPHUR METABOLISM

electron transport component	molecular mass/kDa	organism	function	references (see table 4)
cytochrome *c*-550	34	*Thiocapsa roseopersicina*	chemical oxidation of S^{2-} to S^0	(18)
flavocytochrome *c*-552	72	*Chromatium vinosum*	enzymatic oxidation of sulphide	(19)
flavocytochrome *c*-553	50	*Chlorobium limicola* f. *thiosulfatophilum*	enzymatic oxidation of sulphide	(1), (2)
flavocytochrome *c*-553	63	*Chlorobium vibrioforme* f. *thiosulfatophilum*	enzymatic oxidation of sulphide	(4)
flavocytochrome *c*-553	56	*Chlorobium limicola*	enzymatic oxidation of sulphide	(3)
cytochrome *c*-551	45	*Chlorobium limicola* f. *thiosulfatophilum*	acceptor for thiosulphate: oxidoreductase	(2)
cytochrome *c*-551	32	*Chlorobium vibrioforme* f. *thiosulfactophilum*	acceptor for thiosulphate: oxidoreductase	(4)
cytochrome *c*-555	10	*Chlorobium limicola* f. *thiosulfatophilum*	stimulation of cytochrome *c*-551 reduction during thiosulphate oxidation	(1), (2)
cytochrome *c*-555	12.5	*Chlorobium vibrioforme* f. *thiosulfatophilum*	stimulation of cytochrome *c*-551 reduction during thiosulphate oxidation	(4)
cytochrome *c*-549	17	*Rhodopseudomonas palustris*	acceptor for thiosulphate: oxidoreductase	(16)
cytochrome *c*-552 (membrane-bound)	71.5	*Chromatium vinosum*	acceptor for thiosulphate: oxidoreductase	(14)
high-potential iron–sulphur protein	9.3	*Chromatium vinosum*	acceptor for thiosulphate: oxidoreductase	(20), (15)

A comparison of pathways in sulphur photooxidation by phototrophic bacteria shows that there are a number of basic similarities in the different families:

(1) electrons from sulphur compounds are in most cases accepted by cytochromes, no matter whether they are then directly led to the photosynthetic reaction centre or are used for energy-dependent pyridine nucleotide reduction;

(2) several enzymes occur in all the families discussed here, especially those for thiosulphate utilization;

(3) several enzymes occur in only two families, e.g. APS reductase, ADP sulphurylase, sulphite:acceptor oxidoreductase;

(4) none of the families are uniformly equipped with sulphur enzymes;

(5) the present state of knowledge is rather incomplete within each of the families.

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Discussion

D. P. Kelly (*Department of Environmental Sciences, University of Warwick, U.K.*). I am rather unhappy at the observation of thiosulphate as a product of sulphide oxidation by flavocytochrome *c* and the related failure to observe any sulphite reductase activity. It is still necessary to explain a six-electron oxidation of one S^{2-} per $S_2O_3^{2-}$ formed. Is it possible that a 'reverse sulphite reductase' is normally functioning in intact cells, but is labile in extracts and results only in the observation of a partial reaction? I am thinking that thiosulphate can be accumulated during sulphite reduction by the enzyme from sulphate-reducing bacteria, but is not the normal product. Perhaps the flavocytochrome is a component of the reductase.

H. G. Trüper. In *Chromatium vinosum* the reverse sirohaem sulphite reductase has been isolated by Schedel in our laboratory. The enzyme did not contain a flavocytochrome besides the sirohaem. On the other hand, *C. vinosum* is known to contain an easily extractable flavocytochrome *c*. Therefore I do not think that the latter is a constituent of the sulphite reductase.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES