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Review

Sulfur oxidation by phototrophic bacteria

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Abbreviations: *Ab.*, *Amoebobacter*; APS, adenosine phosphosulfate; BChl, bacteriochlorophyll; BPheo, bacteriopheophytin; *Chl.*, *Chlorobium*; *Chr.*, *Chromatium*; *Ect.*, *Ectothiorhodospira*; EPR, electron paramagnetic resonance; FAD, flavin adenine dinucleotide; Fd, ferredoxin; GSH, glutathione; HiPIP, high-potential iron-sulfur protein; HOQNO, 2-heptyl-4-hydroxyquinoline-*N*-oxide; K_m, Michaelis constant; K_s, concentration of growth-limiting substrate at which the growth rate is half the extrapolated substrate-saturated rate; MQ, menoquinone; M_r, molecular weight; PEP, phospho*enol*pyruvate; 3-PGAL, 3-phosphoglyceraldehyde; P-840, photoactive reaction center bacteriochlorophyll in green sulfur bacteria; P-870, photoactive reaction center bacteriochlorophyll in purple bacteria; Q, quinone; QH_2, reduced quinone (quinol); *Rb.*, *Rhodobacter*; *Rc.*, *Rhodocyclus*; *Rm.*, *Rhodomicrobium*; *Rps.*, *Rhodopseudomonas*; *Rs.*, *Rhodospirillum*; SDS, sodium dodecyl sulfate; *Tb.*, *Thiobacillus*; *Tcp.*, *Thiocapsa*; TMPD, *N,N,N′,N′*-tetramethyl-*p*-phenylenediamine; UQ, ubiquinone; Δp, H^+ gradient (across a membrane); P_i, inorganic phosphate.

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I. Introduction

Most phototrophic bacteria can use reduced sulfur compounds as electron donors for photosynthetic CO_2 reduction. When H_2S is the electron donor, microscopically observable globules of elemental sulfur typically accumulate within or around the bacterial cells. There is a striking visual parallel between sulfur globule formation by phototrophic bacteria and the formation of bubbles of O_2 by submerged plants or algae during oxygenic photosynthesis, and the similarity between the overall equations for these processes is even more striking, i.e.:

bacteria: $2H_2S + CO_2 \xrightarrow{\text{light}} \{CH_2O\} + H_2O + 2S^0$

plants: $2H_2O + CO_2 \xrightarrow{\text{light}} \{CH_2O\} + H_2O + O_2$

where $\{CH_2O\}$ = intracellular organic material, e.g. carbohydrate. This led Van Niel to propose that the O_2 evolved in plant photosynthesis was derived from water rather than from CO_2 [203,204], a historically important insight into the redox nature of photosynthesis that has been amply confirmed. Unlike O_2, S^0 can be and usually is further oxidized, yielding SO_4^{2-} after H_2S has been consumed.

It is ironic that in spite of its early contributions to our understanding of the mechanism of photosynthesis, oxidative sulfur metabolism is rather poorly integrated into current schemes of photosynthetic electron transport. Contrary to what might be expected from the equations in the previous paragraph, the enzymology of photosynthetic sulfur oxidation has little in common with that used for O_2 evolution, making a direct evolutionary connection between the two processes unlikely. One purpose of this review is to introduce researchers not specializing in bacterial sulfur metabolism to current information about the sulfur-oxidizing capabilities of purple and green phototrophic bacteria and the enzymes mediating the remarkable variety of sulfur redox transformations that occur during oxidation of sulfide (and thiosulfate) to sulfate. Analytical methods that have been used to measure the sulfur compounds at different redox levels that are produced or consumed during sulfur oxidation are discussed briefly in an appendix. Possible sites of entry of electrons from sulfur into photosynthetic electron-transport pathways and the bioenergetics of photosynthetic sulfur oxidation will also be discussed. For additional information and perspectives, reviews on phototrophic bacterial sulfur metabolism by Trüper and Fischer [190], Trüper [189], and Fischer [47,48] may be consulted. Recent discussions of photosynthetic electron transport that consider pathways of electron flow from inorganic sulfur compounds have been written by Dutton [40], Knaff and Kämpf [91], and Pierson and Olson [130].

Dissimilatory sulfur metabolism (i.e., use of sulfur compounds as sources or sinks for electrons, as opposed to assimilatory sulfur metabolism which uses sulfur compounds as biosynthetic substrates) has been most

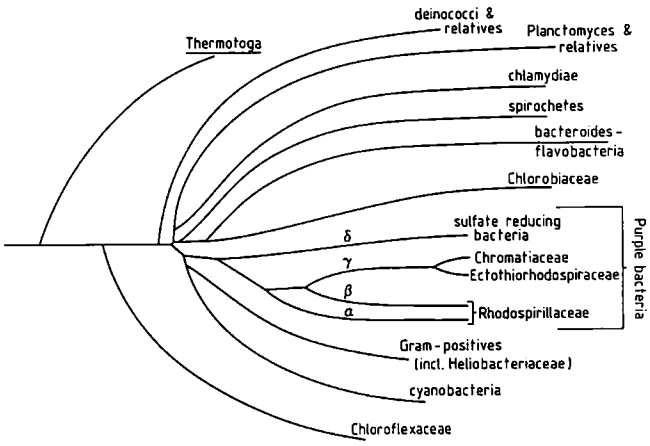

Fig. 1. Taxonomic scheme for the eubacteria based on 16S ribosomal RNA sequences, arranged to emphasize the phototrophic bacterial families. Note that the Rhodospirillaceae include species from both the α and β branches of the purple bactrial phylum, suggesting that future subdivisions of this family may be necessary. The best-studied species of the Rhodospirillaceae, including the the genera *Rhodospirillum, Rhodobacter* and *Rhodopseudomonas,* are members of the α subdivision, while the β subdivision includes the genus *Rhodocyclus.* The α, β and γ branches of the purple bacteria include many common nonphototrophic bacteria, in addition to the families shown here. This intermixing of phototrophic and nonphototrophic bacteria was not indicated in more classical taxonomic schemes and may necessitate further reorganization of some purple phototrophic bacterial families. Branch lengths are approximately proportional to evolutionary distance. (Redrawn and slightly modified from Woese [216].)

investigated in purple and in green sulfur bacteria. Purple phototrophic bacteria are currently placed in three families, namely Chromatiaceae, Ectothiorhodospiraceae, and Rhodospirillaceae [74,76,181,193]. The green sulfur bacteria constitute a single family, the Chlorobiaceae. According to taxonomic schemes based on 16S ribosomal RNA sequences, the purple bacterial families are all members of the same eubacterial phylum, while the Chlorobiaceae belong to a different phylum [216]. Fig. 1 shows a scheme that has been drawn to emphasize the positions of the phototrophic bacterial families. The close relationship of the purple bacterial families to each other is also apparent from their common pathways for photosynthetic electron transport and CO_2 fixation, which are different from those used by green sulfur bacteria. Some species of cyanobacteria [24,37] and a few species of Chloroflexaceae (green gliding bacteria) [58,109,188] are also able to photooxidize H_2S. However, because little is known about dissimilatory sulfide oxidation by either group, these organisms will not be discussed here.

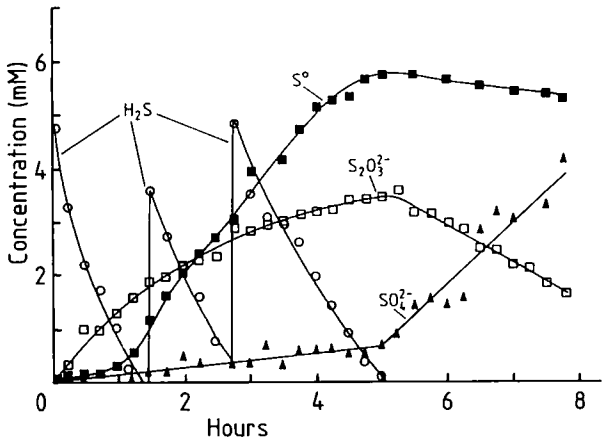

Fig. 2. Concentrations of H_2S (○), $S_2O_3^{2-}$ (□), S^0 (■), and SO_4^{2-} (▲) as a function of time in a *Chl. limicola f. thiosulfatophilum* culture fed initially and at two later times with sulfide. Note that $S_2O_3^{2-}$ appears sooner than S^0 after sulfide addition and that oxidation of S^0 is simultaneous with, rather than preceded by, $S_2O_3^{2-}$ oxidation after H_2S has been consumed. These data were originally presented by Schedel [144]. The figure has been redrawn from Fischer [47] with permission.

II. Patterns of sulfur oxidation by phototrophic bacteria

Although the overall equations for autotrophic bacterial photosynthesis were basically known by the end of the 1930's, the first quantitative measurements on sulfur oxidation kinetics began in the 1960's with experiments on sulfide oxidation by *Chromatium okenii* [185,195] and on thiosulfate oxidation by *Chromatium vinosum* [161]. Since then, several distinct patterns of reduced sulfur compound oxidation have emerged. Table I summarizes the sulfur-oxidizing capabilities of purple and of green sulfur bacteria. The bacterial species are grouped in their taxonomic families, and within each family according to their sulfur-oxidizing capabilities. Most of this information was tabulated earlier by Trüper [188]. The sulfur-oxidizing capabilities of the species in each family are briefly discussed below. As will become apparent, patterns of sulfur oxidation are rather complex, and tend to be different in different bacterial families. The Rhodospirillaceae exhibit several different patterns of sulfide oxidation products and intermediates formed during sulfide oxidation, almost as if the ability to photooxidize sulfide had originated independently several times within that family.

IIA. Chlorobiaceae

All of the Chlorobiaceae are obligate photoautotrophs able to use H_2S or S^0 as the electron donor. With most Chlorobiaceae, extracellular S^0 globules are the only detectable intermediate during oxidation of H_2S to SO_4^{2-}.

Two *Chlorobium* strains, namely *Chlorobium vibrioforme f. thiosulfatophilum* and *Chlorobium limicola f. thiosulfatophilum* are able to oxidize thiosulfate ($S_2O_3^{2-}$) to SO_4^{2-}. These two strains are unique among the phototrophic bacteria in several ways, including being the only ones so far known that can use tetrathionate ($S_4O_6^{2-}$) as an electron donor [87,102]. They are also the only phototrophic bacteria that perform a photochemical disproportionation of S^0 into H_2S and $S_2O_3^{2-}$ when illuminated in the absence of CO_2, the terminal electron acceptor [134]. Traces of SO_3^{2-} observed during this reaction suggest that H_2S and SO_3^{2-} may be the initial products, with $S_2O_3^{2-}$ being formed in a purely chemical reaction between SO_3^{2-} and S^0 [190]. The inability of thiosulfate-utilizing species of purple bacteria to carry out the sulfur disproportionation reaction suggests that ferredoxin, which is reduced during photosynthetic electron transport in Chlorobiacae but not in purple bacteria (see below), may donate electrons for reduction of S^0 to H_2S.

Besides being the only Chlorobiaceae able to oxidize $S_2O_3^{2-}$, *Chl. limicola f. thiosulfatophilum* and *Chl. vibrioforme f. thiosulfatophilum* differ from other Chlorobiaceae in accumulating $S_2O_3^{2-}$ as well as S^0 as an intermediate during H_2S oxidation in batch cultures [47,162] (see Fig. 2 for *Chl. limicola f. thiosulfatophilum*). ($S_2O_3^{2-}$ formation by *Chl. limicola f. thiosulfatophilum* did not occur during sulfide oxidation in continuous cultures, however [199].) With both *Chl. limicola f. thiosulfatophilum* and *Chl. vibrioforme f. thiosulfatophilum* formation of $S_2O_3^{2-}$ precedes S^0 formation during H_2S oxidation in batch cultures. The two thiosulfate-oxidizing strains differ from each other in that S^0 (extracellular globules) is formed as an intermediate during $S_2O_3^{2-}$ oxidation by *Chl. vibrioforme f. thiosulfatophilum* but not by *Chl. limicola f. thiosulfatophilum* [47].

TABLE I

Sulfur oxidizing abilities of phototrophic bacteria

The tabulated data were taken from Trüper [188], except where other references are given in superscripts next to the species to which they refer.

Organism	Donors used	Intermediates observed	Oxidation products
A. Chlorobiaceae			
(1) *Ancalochloris perfilievii*			
Chlorobium limicola			
Chl. chlorovibriodes			
Chl. phaeobacteriodes			
Chl. vibrioforme			
Chloroherpeton thalassium [57]	H_2S	S^0	SO_4^{2-}
Pelodictyon clathratiforme	S^0	–	SO_4^{2-}
Pd. luteolum			
Pd. phaeum			
Prosthecochloris aestuarii			
Pc. phaeoasteroides			
(2) *Chl. vibrioforme*	H_2S	$S_2O_3^{2-}$, S^0	SO_4^{2-}
f. thiosulfatophilum	S^0, $S_4O_6^{2-}$	–	SO_4^{2-}
	$S_2O_3^{2-}$	S^0	SO_4^{2-}
(3) *Chl. limicola*	H_2S	$S_2O_3^{2-}$, S^0	SO_4^{2-}
f. thiosulfatophilum	S^0, $S_2O_3^{2-}$, $S_4O_6^{2-}$	–	SO_4^{2-}
B. Chromatiaceae			
(1) *Chromatium buderi*			
Chr. okenii			
Chr. tepidum [108]			
Chr. warmingii			
Chr. weissei			
Lamprocystis roseopersicina [a]	H_2S	S^0	SO_4^{2-}
Thiocapsa pfennigii [b]	S^0	–	SO_4^{2-}
Thiocystis gelatinosa [b]			
Tcs. violaceae [c]			
Thiodictyon bacillosum [a]			
Td. elegans [a]			
Thiopedia rosea [a]			
Thiospirillum jenense			
(2) *Amoebobacter pedioformis* [b] [42]			
Ab. purpureus [b]			
Chr. gracile [b]			
Chr. minus [b]	H_2S	S^0	SO_4^{2-}
Chr. minutissimum [b]	$S_2O_3^{2-}$	S^0	SO_4^{2-}
Chr. purpuratum [b]	S^0	–	SO_4^{2-}
Chr. violascens [b]			
Thiocapsa roseopersicina			
(3) *Ab. pendens*	H_2S	S^0	SO_4^{2-}
Ab. roseus	$S_2O_3^{2-}$	S^0	SO_4^{2-}
Chr. vinosum	S^0, SO_4^{2-}	–	SO_4^{2-}
C. Ectothiorhodospiraceae			
(1) *Ectothiorhodospira abdelmalekii* [d] [178]	H_2S	polysulfides	S^0
Ect. halochloris [d] [179]			
(2) *Ect. halophila* [b]	H_2S	S^0	SO_4^{2-}
Ect. shaposhnikovii	S^0, $S_2O_3^{2-}$	–	SO_4^{2-}
Ect. vacuolata [b] [75]			
(3) *Ect. mobilis*	H_2S	S^0	SO_4^{2-}
	S^0, $S_2O_3^{2-}$, SO_3^{2-}	–	SO_4^{2-}

TABLE I (continued)

Organism	Donors used	Intermediates observed	Oxidation products
D. Rhodospirillaceae			
(1) *Rhodocyclus purpureus* [138]	none	–	–
(2) *Rhodobacter capsulatus* *Rb. sphaeroides* [e] *Rhodospirillum rubrum* [e]	H_2S	–	S^0
(3a) *Rhodopseudomonas marina* [f] [73]	H_2S	–	S^0, $S_2O_3^{2-}$
(3b) *Rhodomicrobium vannielii*	H_2S	–	$S_4O_6^{2-}$
(3c) *Rhodopila globiformis* [h]	$S_2O_3^{2-}$	–	$S_4O_6^{2-}$
(4) *Rhodopseudomonas palustris* [e]	H_2S, $S_2O_3^{2-}$	–	SO_4^{2-}
(5) *Rb. sulfidophilus* [122]	H_2S, $S_2O_3^{2-}$	SO_3^{2-}	SO_4^{2-}
(6) *Rhodopseudomonas sulfoviridis* [122]	H_2S	polysulfides or S^0	SO_4^{2-}
Rb. adriaticus [122]	S^0, $S_2O_3^{2-}$	–	SO_4^{2-}
Rb. veldkampii [122]			

[a] Not tested for ability to use $S_2O_3^{2-}$ or SO_3^{2-}.
[b] Not tested for ability to use SO_3^{2-}.
[c] Some strains oxidize $S_2O_3^{2-}$ and SO_3^{2-}.
[d] These species are incapable of photoautotrophic growth. Thus it is not clear that CO_2 is the terminal acceptor of electrons from H_2S.
[e] Tolerates only low sulfide concentrations.
[f] Poor photoautotrophic growth. H_2S oxidation was observed under photomixotrophic conditions.
[g] S^0 and $S_2O_3^{2-}$ are formed in side reactions between $S_4O_6^{2-}$ and H_2S in batch cultures.
[h] Sulfide inhibits growth, which may have prevented its oxidation from being observed. Although $S_2O_3^{2-}$ is oxidized to $S_4O_6^{2-}$ [177], photoautotrophic growth on $S_2O_3^{2-}$ has not been reported.

None of the Chlorobiaceae can use SO_3^{2-} as an electron donor.

IIB. Chromatiaceae

The Chromatiaceae can be divided into two groups on the basis of their sulfur-metabolizing capabilities [187]. One group, which includes the large-celled *Chromatium* species (*buderi, okenii, warmingii,* and *wessei*), *Thiospirillum jenense* and several others can only use H_2S or elemental sulfur as the electron donor. Organisms in this group are also incapable of assimilatory sulfate reduction and require H_2S or S^0 as a source of sulfur for biosynthesis. H_2S is oxidized to SO_4^{2-} with intracellular sulfur globules accumulating as an intermediate. Some of the species listed in this group in Table I (i.e., species of *Lamprocystis, Thiodictyon* and *Thiopedia*) appear not to have been tested for their ability to use $S_2O_3^{2-}$ or SO_3^{2-} as the electron donor and thus are tentatively assumed not to oxidize either compound.

Species of Chromatiaceae in the other group, which includes the small-celled *Chromatium* species, use $S_2O_3^{2-}$ as well as H_2S and S^0 as electron donors. Many of the species in this group are also capable of assimilatory sulfate reduction when grown photoheterotrophically, although *Chr. minus* and the *Amoebobacter* species are exceptions. Intracellular S^0 globules accumulate as an intermediate during $S_2O_3^{2-}$ oxidation. In a classic set of experiments using either sulfane-labeled thiosulfate (^{35}S-SO_3^{2-}) or sulfone-labeled thiosulfate (S-$^{35}SO_3^{2-}$) as the electron donor, Smith and Lascelles [161] demonstrated that the intracellular sulfur globules are derived entirely from the sulfane sulfur, while the sulfone sulfur is released as SO_4^{2-} at a rate equal to that of $S_2O_3^{2-}$ consumption (Fig. 3).

Under mildly acidic conditions (pH 6.25), *Chr. vinosum* oxidizes $S_2O_3^{2-}$ to $S_4O_6^{2-}$ instead of to $S^0 + SO_4^{2-}$ [160,161]. $S_4O_6^{2-}$ cannot be further metabolized by *Chr. vinosum.* Moreover, it inhibits oxidation of $S_2O_3^{2-}$ to $S^0 + SO_4^{2-}$ but not to $S_4O_6^{2-}$ when added to *Chr. vinosum* cultures growing at neutral pH. A possible explanation for this result might be that $S_4O_6^{2-}$ inhibits uptake of $S_2O_3^{2-}$ into the bacterial cytoplasm where it is converted to $S^0 + SO_4^{2-}$, while oxidation of $S_2O_3^{2-}$ to $S_4O_6^{2-}$ occurs periplasmically and thus is not affected. This explanation has not yet been tested experimentally. Although oxidation of $S_2O_3^{2-}$ is accompanied by CO_2 fixation [208], growth apparently does not occur in the presence of $S_4O_6^{2-}$ [160]. It is not known whether or not $S_4O_6^{2-}$ has a similar effect on other bacterial species. Trüper and Pfennig [192] found that a small *Chromatium* species that contains the carotenoid okenone (*Chr. minus*?) continued to oxidize $S_2O_3^{2-}$ under acidic

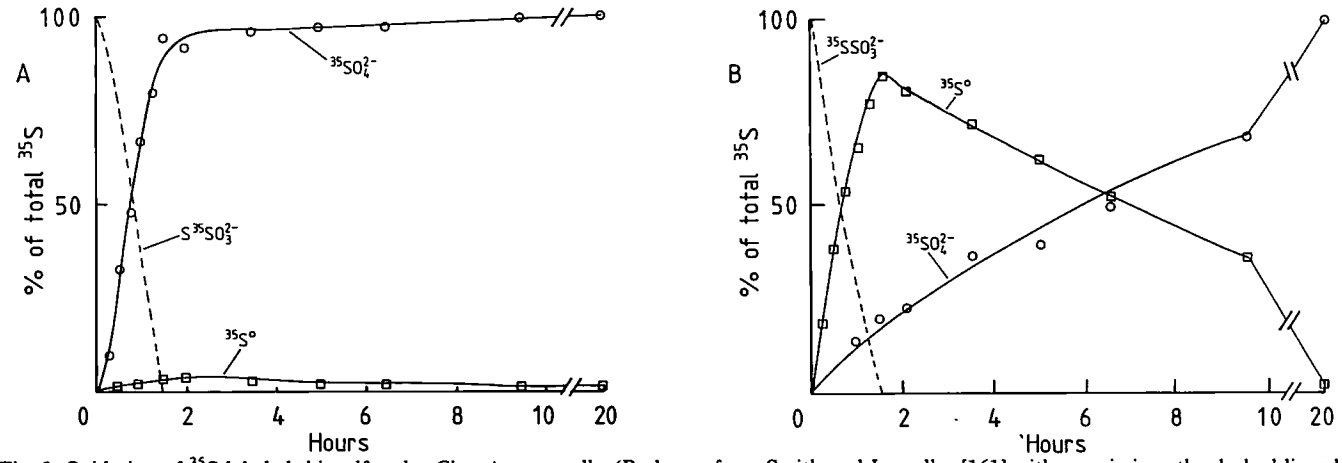

Fig. 3. Oxidation of ^{35}S-labeled thiosulfate by *Chr. vinosum* cells. (Redrawn from Smith and Lascelles [161] with permission; the dashed lines have been added to the original figures.) Symbols: dashed line, interpolated time course for thiosulfate consumption; squares, intracellular ^{35}S (i.e. $^{35}S^0$); circles, $^{35}SO_4^{2-}$. Cells were supplied initially with either 4 mM $S^{35}SO_3^{2-}$ (A) or 4 mM $^{35}SSO_3^{2-}$ (B).

conditions to $S^0 + SO_4^{2-}$ en route to SO_4^{2-}, but did not test for $S_4O_6^{2-}$ formation or for inhibition of $S_2O_3^{2-}$ oxidation by $S_4O_6^{2-}$.

Most of the small-celled Chromatiaceae have not been tested for their ability to use SO_3^{2-} as an electron donor. However *Thiocapsa roseopersicina* has been shown to use $S_2O_3^{2-}$ but not SO_3^{2-} as an electron donor. *Chr. vinosum Ab. pendens,* and *Ab. roseus* are known to use SO_3^{2-} as the electron donor.

IIC. Ectothiorhodospiraceae

Among the Ectothiorhodospiraceae, only the BChl *b*-containing extreme halophiles *Ectothiorhodospira halochloris* and *Ect. abdelmalekii* appear not to be true sulfur bacteria in that they cannot grow photoautotrophically on reduced sulfur compounds + bicarbonate [179]. Nevertheless, they tolerate relatively high sulfide concentrations in their culture medium and oxidize it to elemental sulfur, accumulating high concentrations of polysulfide as an intermediate, when grown photomixotrophically (i.e., with both an organic compound – acetate was used – and CO_2 as carbon sources) [178,179]. If sulfide oxidation is coupled to CO_2 fixation via the Calvin cycle as is the case with other purple bacteria that have been investigated [56,95], it is not clear why these species cannot grow photoautotrophically. Formation of polysulfides and H_2S by reduction of elemental sulfur was also observed in acetate-containing suspensions of these two species [179].

The photoautotrophic species of *Ectothiorhodospira* photooxidize sulfide to sulfate with intermediate accumulation of extracellular elemental sulfur globules (Fig. 4A). Transient formation of polysulfide during sulfide oxidation by these species has been reported and attributed to a chemical reaction between H_2S and elemental sulfur promoted by the alkalinity of the culture medium [187]. Elemental sulfur and $S_2O_3^{2-}$ (Fig. 4B) are both oxidized to SO_4^{2-} without observable intermediates. This is different from the situation in the thiosulfate-oxidizing Chromatiaceae, which produce S^0 as an intermediate during $S_2O_3^{2-}$ oxidation. *Ect. mobilis*

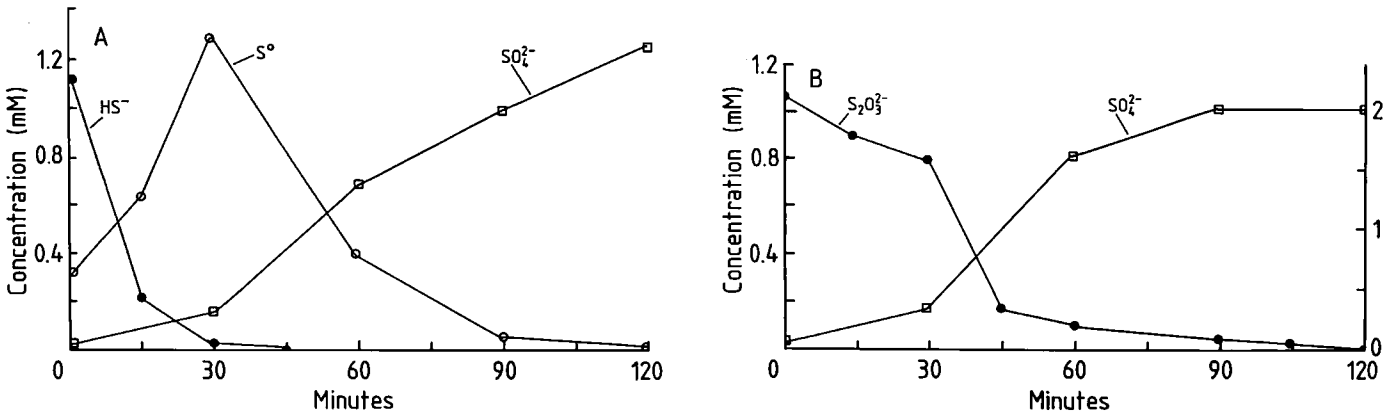

Fig. 4. Oxidation of sulfide (A) and thiosulfate (B) by *Ectothiorhodospira shaposhnikovii.* In (A) concentrations of sulfide (●), S^0 (○), and SO_4^{2-} (□) are shown as a function of time. In (B) it is shown that $S_2O_3^{2-}$ (●, concentration on left *y*-axis) is oxidized to SO_4^{2-} (□, concentration on right *y*-axis) without intermediate accumulation of S^0. (Redrawn from Kusche [100] with permission.)

was also reported to oxidize elemental selenium to SeO_4^{2-} by Shaposhnikov [154] in 1937 (cited by Trüper [186]).

SO_3^{2-} is used as an electron donor for photoautotrophic growth by *Ect. mobilis,* which oxidizes it to SO_4^{2-} [186]. A report that *Ect. shaposhnikovii* could also grow with SO_3^{2-} as the photosynthetic electron donor [96] could not be confirmed by Kusche [100]. One explanation for this discrepancy may be that the ability to oxidize SO_3^{2-} varies between strains of the same species. (A similar situation has been observed with *Thiocystis violacea* in the Chromatiaceae; see Table I.) *Ect. halophila* and *Ect. vacuolata* appear not to have been investigated with respect to their SO_3^{2-}-oxidizing abilities.

IID. Rhodospirillaceae

Until rather recently, the Rhodospirillaceae were considered to be generally incapable of photoautotrophic growth using H_2S as the electron donor. However, in several instances this has been shown to be due to the toxicity of H_2S to Rhodospirillaceae when present at concentrations used for cultivation of purple and green sulfur bacteria. Thus, Hansen and Van Gemerden [63] showed that *Rhodospirillum rubrum, Rhodopseudomonas palustris, Rhodobacter sphaeroides* and *Rb. capsulatus* could be grown photoautotrophically in a chemostat with sulfide supplied continuously at a low concentration. Growth of the first three of the above species was completely inhibited when the sulfide concentration in the culture medium exceeded 0.5 mM, while *Rb. capsulatus* tolerated up to 2 mM sulfide. In light of these results, earlier reports of the inability of Rhodospirillaceae to use sulfide as an electron donor are open to question. One species that appears to be truly unable to use sulfide as an electron donor is *Rhodocyclus purpureus.* Pfennig [138] reported that H_2S was not used by this organism, but that (in the concentrations tested) it also did not inhibit growth in a medium containing acetate and yeast extract. However, *Rc. purpureus* grows well as a photoautotroph with H_2 as the electron donor.

The Rhodospirillaceae known to utilize sulfide vary considerably in their oxidation capabilities. *Rs. rubrum, Rb. sphaeroides* and *Rb. capsulatus* can oxidize H_2S only to elemental sulfur, which accumulates extracellularly. The low redox potential of the H_2S/S^0 couple (Table II) suggests that this oxidation might be attributed to a nonspecific reaction between H_2S and electron carriers of higher redox potential (e.g., cytochrome *c*, cytochrome *c'*) which would be readily accessible to sulfide. However, *Rc. purpureus* (see above) provides an apparent counter example to the nonspecific oxidation hypothesis. Furthermore, *Rb. capsulatus* grows rapidly on sulfide and has a K_s for sulfide of 2 μM, indicating a sulfide affinity comparable to that of the Chlorobiaceae and Ectothiorhodospiraceae and higher than that of *Chr. vinosum* [198]. This suggests that *Rb. capsulatus* (and possibly most of the Rhodospirillaceae that use sulfide as a photosynthetic electron donor) have specific oxidoreductases for this purpose.

TABLE II

Redox potentials for sulfur compounds oxidized by phototrophic bacteria

All values except those of the $2S^0/H_2S_2$ and the $H_2S_2/2H_2S$ couples are taken directly from or are calculated from free-energy data tabulated by Thauer et al. [176]. Redox potentials for the couples $H_2S_2/2H_2S$ and $2S^0/H_2S_2$ were calculated from thermodynamic data for formation of H_2S_2 in the liquid phase tabulated by Mills [119]. These calculated redox potentials are in good agreement with previously measured values [109a,109b] after correcting to pH 7, assuming the pK values for H_2S_2 given in Ref. 8.

Redox couple	E_0' (mV)
SO_4^{2-}/HSO_3^-	−516
$S_2O_3^{2-}/HS^- + HSO_3^-$	−402
$2S^0/H_2S_2$	−340
S^0/HS^-	−270
$H_2S_2/2H_2S$	−200
HSO_3^-/HS^-	−116
$APS/AMP + HSO_3^-$	−60
HSO_3^-/S^0	−38
$S_4O_6^{2-}/S_2O_3^{2-}$	+24

Three species, namely *Rhodospeudomonas marina, Rhodomicrobium vannielii,* and *Rhodopila globiformis* produce $S_2O_3^{2-}$ or $S_4O_6^{2-}$ as end products of sulfur oxidation. Of these three, only *Rm. vannielii* grows well as a photoautotroph [205]. It oxidizes H_2S entirely to $S_4O_6^{2-}$ in sulfide-limited chemostat cultures [62]. Although $S_2O_3^{2-}$ and S^0 appear in batch cultures, neither is used as an electron donor, suggesting that they are side products resulting from a chemical reaction between H_2S and $S_4O_6^{2-}$ [188,189]. *Rps. marina* grows only poorly on H_2S, but oxidizes it to S^0 and $S_2O_3^{2-}$ when grown photomixotrophically [73]. *Rhodopila globiformis* has not been shown to grow photoautotrophically at all, but can oxidize $S_2O_3^{2-}$ to $S_4O_6^{2-}$ photomixotrophically [177].

Rps. sulfoviridis, Rb. adriaticus, Rb. veldkampii and *Rb. sulfidophilus* resemble the true sulfur bacteria in their ability to tolerate sulfide and to photooxidize it completely to SO_4^{2-} [122]. (The first three of these are even dependent on reduced sulfur compounds for growth, apparently because they lack assimilatory sulfate reduction.) Extracellular S^0 is an intermediate during sulfide oxidation by *Rb. adriaticus* and *Rb. veldkampii,* and extracellular polysulfide is also formed in *Rb. veldkampii* cultures. *Rps. sulfoviridis* accumulates an unidentified intermediate at approximately the redox level of elemental sulfur that may be intracellular poly-

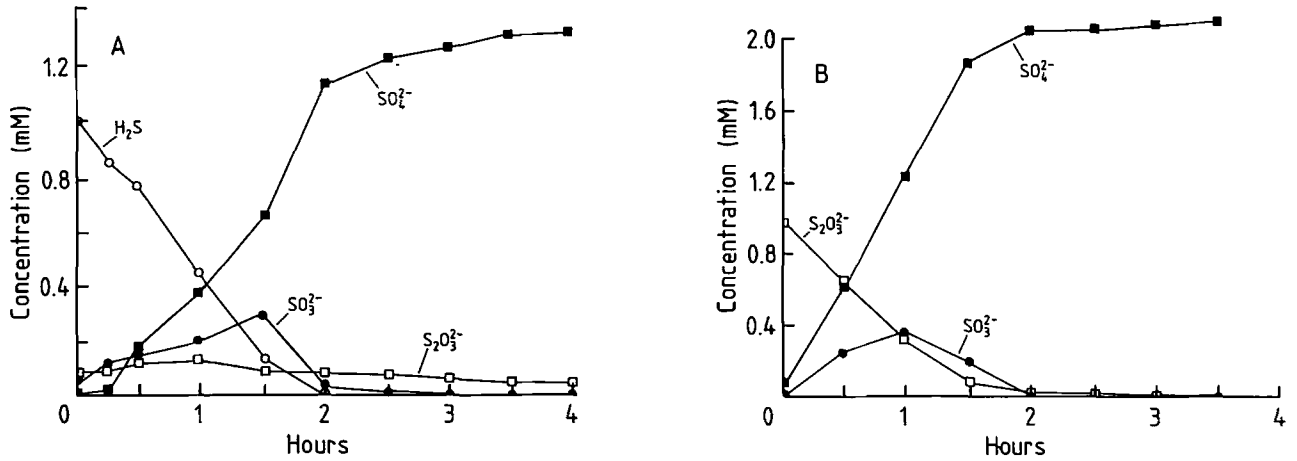

Fig. 5. Oxidation of sulfide (A) and thiosulfate (B) by *Rhodobacter sulfidophilus*. (A) Concentrations of sulfide (○), $S_2O_3^{2-}$ (□), SO_3^{2-} (●) and SO_4^{2-} (■) as a function of time after an initial addition of sulfide. (B) Concentrations of $S_2O_3^{2-}$ (□), SO_3^{2-} (●) and SO_4^{2-} (■) after an initial addition of $S_2O_3^{2-}$. Note that SO_3^{2-} is an intermediate during oxidation of both H_2S and $S_2O_3^{2-}$. (Redrawn from Neutzling et al. [122] with permission.)

sulfide. All three species oxidize $S_2O_3^{2-}$ to SO_4^{2-} without detectable intermediates [122].

Rb. sulfidophilus is unique among the phototrophic bacteria in transiently releasing SO_3^{2-} into the culture medium while oxidizing H_2S or $S_2O_3^{2-}$ to SO_4^{2-} (Fig. 5) [122]. No other intermediates were observed. Rather paradoxically, SO_3^{2-} by itself is not used as a photosynthetic electron donor by *Rb. sulfidophilus*, although it is consumed if H_2S or $S_2O_3^{2-}$ is also present.

Rps. palustris resembles the organisms described in the previous two paragraphs except for its extreme sensitivity to sulfide. It can oxidize H_2S (at low concentrations) and $S_2O_3^{2-}$ to SO_4^{2-} without observable intermediates. When the concentration of $S_2O_3^{2-}$ in the culture medium exceeds 10 mM, the preferential end product of its oxidation by *Rps. palustris* is $S_4O_6^{2-}$ rather than SO_4^{2-} [189].

IIE. The role of polysulfides in sulfide oxidation

Polysulfides have occasionally been observed during sulfide oxidation, particularly by the alkalophilic Ectothiorhodospiraceae and by some species of Rhodospirillaceae (see above). Logically, they might be expected to be intermediates in the oxidation of H_2S, with only one sulfur atom, to elemental sulfur, which is polyatomic (S_8 rings are the most stable form [166]). Furthermore, the oxidation level of polysulfides is intermediate between those of H_2S and elemental sulfur. Polysulfides of varying chain lengths between H_2S_2 and H_2S_8 and even longer have been synthesized [8]. They are thermodynamically unstable, decomposing to H_2S + elemental sulfur, but the activation energy for this is sufficiently high (about 25 kcal/mol) that the uncatalyzed reaction is negligibly slow.

Recently, Van Gemerden [199] investigated the oxidation of S_3^{2-} by *Chl. limicola f. thiosulfatophilum* and found that S_3^{2-} behaves more like a side product than an intermediate during H_2S oxidation. Although S_3^{2-} accumulated to a steady-state concentration of 70 μM in continuous cultures of *Chl. limicola f. thiosulfatophilum* growing on H_2S, there was a lag of about 40 min after sulfide in the medium disappeared before oxidation of S_3^{2-} began. S_3^{2-} oxidation was dependent on protein synthesis during the lag period and was prevented by adding chloramphenicol or puromycin when the supply of H_2S was cut off [207]. Furthermore, unlike the situation with H_2S oxidation, accumulation of S^0 during S_3^{2-} oxidation is negligible. On the other hand, sulfide-grown *Chr. vinosum* cells oxidized S_3^{2-} without any lag after it was added, indicating that S_3^{2-} oxidation is constitutive. It would be interesting to extend these observations to polysulfides of other chain lengths and to other bacterial species.

IIF. Sulfide toxicity

As indicated in the previous discussion, sulfide tolerance among the phototrophic bacteria is variable. A study of the rate of bacterial growth as a function of the sulfide concentration involving species of Chlorobiaceae, Chromatiaceae and Ectothiorhodospiraceae as well as *Rb. capsulatus* showed that even the most tolerant species are strongly inhibited when the sulfide concentration reaches 10 mM [198]. The reasons for sulfide toxicity and why it varies from one species to another are not known. This problem was recently discussed by Van Gemerden and De Wit [200] who made the following observations. (1) As noted previously by Van Niel, the most toxic form of sulfide is the fully protonated species, H_2S ($pK_1 = 7.04$). This is probably because cell membranes are freely permeable to H_2S but not to its charged dissociation products. (2) in *Chr. vinosum*, photosynthesis, as measured by CO_2 fixation and glycogen formation, was not inhibited even at a sulfide concentration (30 mM) that totally inhibits growth. This

would imply that high sulfide concentrations do not inhibit photosynthetic electron transport, photophosphorylation, or the Calvin cycle, at least in *Chr. vinosum*. They noted that the insolubility of sulfides of transition metals (e.g., Fe, Co, Zn) might interfere with their availability or metabolism and could be the factor responsible for growth inhibition. The effect of sulfide on photosynthesis is apparently modulated by the physiological state of the cells, however, because Morita et al. [121] observed that 1–2 mM sulfide inhibited CO_2 fixation in starved, but not actively growing *Chr. vinosum* cells. Montesinos [120] also found that CO_2 fixation by *Chr. minus* cells (collected from a bacterial plate in a stratified lake) was inhibited by sulfide concentrations above 1 mM, with 50% inhibition at 2.5 mM and total inhibition at 10 mM sulfide. Further work is needed to determine whether the site of inhibition under these conditions is in electron transport, photophosphorylation, or the CO_2 reduction cycle.

III. Electron transport and CO_2 fixation by phototrophic bacteria

Reduced sulfur compounds provide electrons for CO_2 fixation during photoautotrophic growth. In all cases, the electrons from sulfur are transferred via a photosynthetic electron-transport chain to electron acceptors (NAD^+ and ferredoxin) that are then used to reduce CO_2. Thus the pathways of photosynthetic electron transport and CO_2 fixation in green sulfur and purple bacteria are pertinent to the present discussion and will be briefly described.

IIIA. Green sulfur bacteria

Electron transport in green sulfur bacteria has recently been reviewed by Blankenship [9], and that review, as well as a review by Amesz [1] on primary photochemical processes in green bacteria, can be consulted for a more detailed discussion. Fig. 6 shows a scheme for electron transport in *Chl. limicola f. thiosulfatophilum* that is consistent with current knowledge about the electron carriers present in that organism. Electron-transport pathways in other Chlorobiaceae are thought to be similar.

Electron transport in green sulfur bacteria is initiated by photochemical electron transfer from P-840, the reaction center BChl *a*, to an initial electron acceptor now thought to be BChl *c* or a related compound [12,128]. (This electron acceptor would presumably be BChl *d* or *e* in species containing one of those pigments in place of BChl *c*.) The electron is rapidly transferred to a membrane-bound Fe-S protein with a redox potential of −540 mV [92,131,171] and then to ferredoxin (Fd), a soluble Fe-S protein. Fd reduces NAD^+ (and possibly also $NADP^+$) via ferredoxin-NAD^+ reductase,

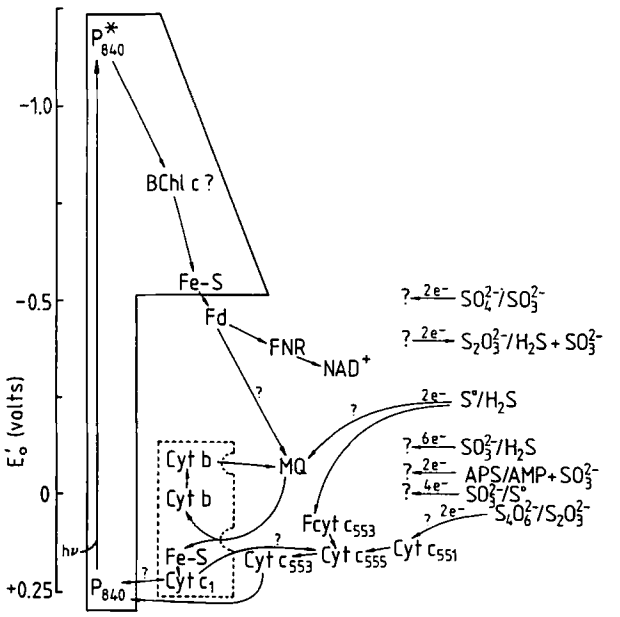

Fig. 6. Electron transport and sulfur redox reactions in the green sulfur bacterium *Chl. limicola f. thiosulfatophilum*. Vertical positions of electron carriers and sulfur redox couples correspond to their redox potentials (scale on left side of figure). Reaction center components are enclosed by a solid line. A dashed line encloses components of a putative cytochrome b/c_1 complex. Small question marks above arrows showing electron-transfer reactions indicate reactions that are not definitely established. Larger question marks next to arrows from sulfur redox couples indicate that the in vivo electron acceptors in these reactions have not yet been determined. The reductant for $S_2O_3^{2-}$ also has not been established, but is probably a thiol (see subsection IVE). Abbreviations: AMP, adenosine monophosphate; APS, adenosine phosphosulfate; BChl, bacteriochlorophyll; Cyt, cytochrome; Fcyt, flavocytochrome; Fd, ferredoxin; FNR, ferredoxin-NAD^+ reductase; $h\nu$, quantum of light; MQ, menaquinone; P_{840}, photoactive BChl *a* with an absorption maximum at 840 nm; P^*_{840}, excited singlet state of P-840.

a flavoprotein [16,98]. Reduced Fd, NADH and NADPH are subsequently used in CO_2 reduction.

Meanwhile, oxidized P-840 is reduced by a membrane-bound *c*-type cytochrome. Cytochrome *c*-553 reduces P^+-840 in reaction-center-enriched samples prepared without detergents [140,172], while cytochrome *c*-550.5 is the reductant in detergent-solubilized reaction centers from which cytochrome *c*-553 has been separated [71]. It is not clear whether both (or only one) of these cytochromes function(s) as the primary electron donor to P^+-840 in vivo. A portion of the cytochrome *c*-550.5 tends to copurify with cytochrome *b*-562 and it may be part of a cytochrome b/c_1 complex that catalyzes electron transport from menaquinone (MQ) ($E'_0 = -74$ mV) [176] to cytochrome *c*-555 (a soluble c_2-type cytochrome that could then reduce cytochrome *c*-553) or to cytochrome *c*-553 directly in the intact system. The main difficulty with this function for cytochrome *c*-550.5 is that its redox potential ($E_{m,7} = +220$ mV) is higher than that of either cytochrome *c*-555 ($E_{m,7} = +145$ mV) or cytochrome *c*-553 ($E_{m,7} = +165$ mV) [9].

Presumably, the detergent-solubilized cytochrome *b*-562 ($E_{m,7} = +8$ mV, but does not titrate as a single component) isolated by Hurt and Hauska [71] corresponds to or includes the cytochrome *b*-564 ($E_{m,7} = -90$ mV) observed previously in membrane preparations [90].

Electrons from H_2S can be transferred to the *c*-type cytochromes (and thus to P^+-840) either via flavocytochrome *c*-553 or via MQ and the presumed cytochrome b/c_1 complex. (In addition to MQ, green sulfur bacteria contain chlorobiumquinone ($E_0' = +39$ mV), the function of which is unknown [25,141]. It may replace MQ in some electron transport functions.) Evidence for transfer via flavocytochrome *c*-553 comes from experiments showing that flavocytochrome *c*-553 catalyzes cytochrome *c*-555 reduction [35,99]. Quinone-mediated electron transfer is suggested by observations that electron transport from H_2S is inhibited by antimycin A and 2-heptyl-4-hydroxyquinoline-*N*-oxide (HOQNO) [90,156] which inhibit quinone redox reactions mediated by cytochrome b/c_1 complexes. Electrons obtained by oxidizing $S_2O_3^{2-}$ to $S_4O_6^{2-}$ may be transferred to cytochrome *c*-551 (which is absent in green bacteria unable to use $S_2O_3^{2-}$ as an electron donor) and then to cytochrome *c*-555 [99]. However, there is some doubt that $S_4O_6^{2-}$ is an intermediate in $S_2O_3^{2-}$ oxidation in green sulfur bacteria [87]. Redox potentials of these and other relevant sulfur compounds are indicated by their positions on the right side of Fig. 6 and specified in Table II. Further discussion of sulfur redox reactions and the enzymes catalyzing them is presented in section IV.

Besides noncyclic electron transport from reduced sulfur compounds to NAD^+, Chlorobiaceae carry out cyclic electron transport. This is shown in Fig. 6 as involving electron transfer from reduced Fd to MQ and then via the cytochrome b/c_1 complex to the *c*-type cytochromes. This role of Fd in cyclic electron transport is speculative, but has been suggested previously [130], and is analogous to the role of Fd in cyclic electron transport around Photosystem I in higher plants [40]. Both photooxidation and photoreduction of cytochrome *b*-564 have been observed in isolated membrane preparations [50,90], indicating its participation in the cycle.

Cyclic electron transport generates a transmembranous gradient in the chemical potential of protons (Δp), composed of both a proton concentration gradient (ΔpH) and an electrical potential gradient ($\Delta\psi$), that is used to drive ATP synthesis. Both ATP synthesis [17] and generation of $\Delta\psi$ [159] were observed in illuminated *Chlorobium* cells in the absence of added electron donors and acceptors, indicating that they were due to cyclic rather than noncyclic electron transport.

Reduced Fd, NADH, NADPH and ATP are used for CO_2 fixation via a reductive carboxylic acid cycle [45,52,53,132] that is similar to the citric acid cycle operating in reverse. Starting with oxaloacetate, one turn of the cycle incorporates two molecules of CO_2 into acetate (as acetyl coenzyme A) and regenerates oxaloacetate. The net equation for this is as follows:

$$2CO_2 + \text{Co-A-SH} + 2Fd_{red} + 2NAD(P)H + 4H^+ + \text{flavin-}H_2 + 2ATP \rightarrow CH_3CO\text{-S-CoA} + 3H_2O + 2Fd_{ox} + 2NAD(P)^+ + \text{flavin} + 2(ADP + P_i)$$

To generate carbohydrates, green sulfur bacteria reductively carboxylate acetyl CoA to form pyruvate, using Fd_{red} as the reductant. Pyruvate is phosphorylated at the expense of two high-energy phosphate bonds (represented here as 2 ATP) to form phospho*enol*pyruvate (PEP). PEP can then be converted to 3-phosphoglyceraldehyde (3-PGAL) using an additional ATP and 1 NADH via a reversal of glycolysis, and 3-PGAL converted to glucose without further expenditure of ATP or reducing equivalents. The overall equation for reduction of three molecules of CO_2 to carbohydrate via this series of reactions is:

$$3CO_2 + 4Fd_{red} + 3NAD(P)H + 7H^+ + \text{flavin-}H_2 + 5ATP \rightarrow 3\{CH_2O\} + 3H_2O + 4Fd_{ox} + 3NAD(P)^+ + \text{flavin} + 5(ADP + P_i)$$

The significance of this pathway for the quantum requirement of green bacterial photosynthesis will be discussed in a later section.

IIIB. Purple bacteria

Photosynthetic electron transport in purple bacteria is basically cyclic. Fig. 7 shows the pathway in the purple sulfur bacterium *Chromatium vinosum*, and electron transport in other purple bacteria is thought to be similar. It is initiated by photochemical electron transfer from a BChl dimer (P-870 in the case of BChl *a*, and P-960 in the case of BChl *b*) to bacteriopheophytin (BPheo) and then to an Fe-associated quinone acceptor within the reaction center complex [89]. Oxidized P-870 is reduced by a cytochrome c_2 (cytochrome *c*-550 in Fig. 7), a small, soluble, monoheme protein with a redox potential typically in the range from +250 to +350 mV, that is located in the periplasmic space. (Reaction centers from *Chr. vinosum* [106,143,180], *Chr. tepidum* [127], *Thiocapsa pfennigii* [153], *Rhodopseudomonas viridis* [36,184,214], and two species of *Ectothiorhodospira* [44,104] contain a bound cytochrome subunit that mediates electron transfer from cytochrome c_2 to the BChl dimer [27,155].) Reduction of the quinone acceptor occurs on the cytoplasmic membrane surface and is accompanied by uptake of one proton from the cytoplasm per electron accepted. Upon 2-electron reduction to the quinol (QH_2) form, one of the quinones (Q_B) is displaced from the reaction center by

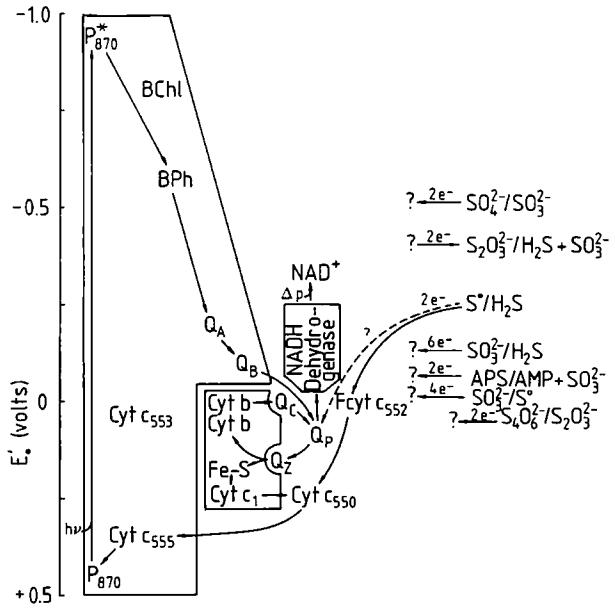

Fig. 7. Electron-transport and sulfur-redox reactions in the purple sulfur bacterium *Chr. vinosum*. Solid lines enclose the NADH dehydrogenase enzyme complex and components of the reaction center and cytochrome (Cyt) b/c_1 complexes. A dashed arrow indicates a suggested electron-transfer reaction that has not yet been shown to occur in *Chr. vinosum*. Question marks next to arrows from sulfur redox couples indicate that the in vivo acceptors of electrons in these sulfur redox reactions are not yet known. The electron donor for $S_2O_3^{2-}$ reduction (also indicated by a question mark) is probably a thiol. Abbreviations not already listed in the legend to Fig. 6; Δp, transmembranous H^+ gradient that drives reverse electron flow; BPh, bacteriopheophytin; P_{870} and P^*_{870}, ground state and excited singlet state of photoactive reaction center BChl; Q_A and Q_B, primary and secondary electron acceptor quinones associated with the reaction center; Q_c and Q_z, quinones bound at the reducing and oxidizing sites of the cytochrome b/c_1 complex; Q_p, pool of quinone in the photosynthetic membrane.

an oxidized quinone [29]. The quinol is then oxidized by a membrane-spanning cytochrome b/c_1 complex through a quinone cycle mechanism in which one electron is transferred to cytochrome c_2 (cytochrome *c*-550 in *Chr. vinosum*) while the other is transferred via a *b*-type cytochrome to another quinone molecule [67]. The cytochrome b/c_1 complex has a quinone-oxidizing (Q_z) and a quinone-reducing (Q_c) site arranged so that protons liberated during quinone oxidation are released into the periplasmic space while those taken up during quinone reduction are removed from the bacterial cytoplasm. The net result of the electron-transport reactions described is that 2 H^+ are transferred from the cytoplasm to the periplasm per electron that goes around the cycle [28].

The BChl *b*-containing extreme halophiles, *Ectothiorhodospira halochloris* and *Ect. abdelmalekii*, may be exceptions to the general pathway because their only soluble cytochromes have redox potentials near 0 V [100,177,178]. This low redox potential seems inconsistent with a function in quinone oxidation via a cytochrome b/c_1 complex. Although *Ect. halochloris* reaction centers, like those of *Rps. viridis*, contain a tightly bound cytochrome *c* subunit that acts as the initial electron donor to the photoactive BChl *b* dimer [44], a soluble carrier is still necessary to shuttle electrons from the cytochrome b/c_1 complex to the bound cytochrome *c*. Direct electron transfer from cytochrome c_1 to the reaction center can apparently occur in cytochrome c_2-less mutants of *Rb. capsulatus* [33], but it is unlikely to occur in *Ect. halochloris* because the reaction centers are completely surrounded by antenna complexes [44]. The absence of high-potential, soluble cytochromes is not universal among the Ectothiorhodospiraceae, however, since *Ect. sphaposhnikovii*, a moderately halophilic, BChl *a*-containing species, has a *c*-type cytochrome (cytochrome *c*-553(549), $E_{m,7} = +248$ mV) [101] that may function as a c_2-type cytochrome in that species.

The transmembranous proton potential gradient (Δp) generated during cyclic electron transport is used for two purposes, namely ATP synthesis and reverse electron flow from QH_2 to NAD^+. Although earlier reports indicated that 1 ATP was synthesized per 2.0–2.3 H^+ entering the cytoplasm via a membrane-spanning ATPase [133], a more recent measurement gave a value of 3.5 ± 1.3 H^+/ATP [23]. In fact, stoichiometries of H^+ translocated per ATP synthesized have tended to converge on a value of 3 H^+/ATP in mitochondria as well as in bacterial systems [64], and this has long been the preferred stoichiometry for chloroplasts [133]. Assuming that 3 H^+ cross the membrane per ATP synthesized, then 2 ATP will be synthesized per three electrons traversing the cyclic electron-transport chain.

Reduction of NAD^+ by QH_2 is also driven by Δp. This reaction is mediated by a membrane-spanning NADH : ubiquinone oxidoreductase (NADH dehydrogenase). Energy is required because the redox potential of the UQ/UQH_2 couple (+30 to +90 mV in bacterial membranes) [29,40,67] is much higher than that of the NAD^+/NADH couple (−320 mV). The stoichiometry between molecules of NAD^+ reduced and H^+ translocated through the membrane is uncertain. However, Scholes and Hinkle [151] obtained a stoichiometry of 4 H^+ per NAD^+ reduced via mitochondrial NADH : ubiquinone oxidoreductase, and this value also seems reasonable for purple phototrophic bacteria. Jones and Vernon [81] previously found that when the Δp used to drive NAD^+ reduction was supplied by ATP hydrolysis (the membrane-spanning ATPase responsible for ATP synthesis is reversible), 1.8 molecules of ATP were consumed per NAD^+ reduced by *Rhodospirillum rubrum* chromatophores. Using a stoichiometry of 3 H^+/ATP, an upper limit of 5.4 H ions translocated per NAD^+ reduced may be calculated.

The electrons used to reduce NAD^+ must be replenished with electrons from oxidizable substrates. The sulfur substrates used as electron donors by *Chr. vino-*

sum are arranged on the right side of Fig. 7 in positions corresponding to their redox potentials (Table II). As was the case with the Chlorobiaceae, electrons from sulfide may enter the electron-transport chain either via flavocytochrome *c*-552 and cytochrome c_2 or via quinone reduction. These reactions and the mechanisms of entry of electrons from other reduced sulfur compounds into the electron-transport chain are discussed further in the next section.

NADH and ATP produced during photosynthetic electron transport are used mainly for CO_2 fixation during photoautotrophic growth. In purple bacteria, CO_2-fixation occurs by the reductive pentose phosphate pathway (also called the Calvin cycle) [56,95,132], the overall equation for which is:

$$CO_2 + 2(NADH + H^+) + 3\,ATP$$

$$\rightarrow \{CH_2O\} + H_2O + 2NAD^+ + 3(ADP + P_i)$$

IV. Enzymology of sulfur oxidation

As discussed in the previous section, electrons released during sulfur oxidation reactions are thought to enter photosynthetic electron-transport chains of green and purple bacteria at the cytochrome *c* and/or the quinone level. A wide variety of enzymes catalyzing sulfur redox reactions have been isolated from phototrophic bacteria. Current information about these enzymes, their reaction mechanisms, and how they might transfer electrons to known components of photosynthetic electron-transport chains, are summarized below.

Fig. 8 shows redox transitions thought to occur during oxidation of sulfide and thiosulfate by phototrophic bacteria. The enzymes catalyzing these reactions are also indicated. Not shown are some reactions that seem to be unique to only a few species, such as oxidation of H_2S to $S_2O_3^{2-}$ by two species of *Chlorobium* and *Rps. marina* or to $S_4O_6^{2-}$ by *Rm. vannielii*. It is possible that these products are formed from reactions involving SO_3^{2-} and S^0. Polysulfides, which are observed during sulfide oxidation by some species and might be intermediates in oxidation of H_2S to S^0, are also not shown.

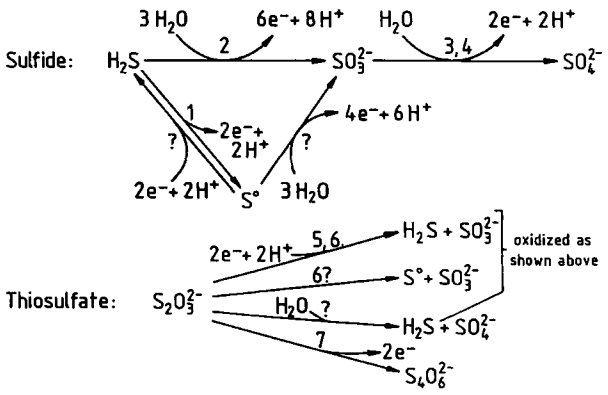

Fig. 8. Sulfur redox reactions and the enzymes catalyzing them arranged into metabolic pathways for sulfide and thiosulfate oxidation. (1) flavocytochrome *c*; (2) sulfite reductase; (3) APS reductase + ADP sulfurylase; (4) sulfite oxidoreductase; (5) thiosulfate reductase; (6) rhodanese; (7) thiosulfate oxidoreductase. Question marks indicate reactions for which a catalytic enzyme has not been found or (in the case of $S_2O_3^{2-}$ splitting to $S^0 + SO_3^{2-}$ by rhodanese) a reaction in which the function of the suggested catalytic enzyme is speculative (see text).

IVA. Oxidation of H_2S to S^0

IVA-1. The role of flavocytochrome c

Two possibilities are indicated in Fig. 8 for the initial step in sulfide oxidation, the first of which is oxidation to elemental sulfur catalyzed by a sulfide dehydrogenase. In several species of phototrophic bacteria, the sulfide dehydrogenase has been proposed to be flavocytochrome *c*. Flavocytochromes *c* have been isolated from *Chl. limicola, Chl. limicola f. thiosulfatophilum, Chl. phaeobacteriodes, Chl. vibrioforme f. thiosulfatophilum, Chr. vinosum, Chr. gracile* and *Rb. sulfidophilus* [47,116,123]. Bartsch [5] noted that flavocytochrome *c*-552 from *Chr. vinosum* becomes trapped inside chromatophores during cell disruption, suggesting a periplasmic location for this cytochrome in whole cells. No information has been presented about the intracellular locations of other flavocytochromes *c*. Several species of sulfur bacteria, however, have been examined with respect to their cytochrome content without finding a flavocytochrome *c*. These include *Chl. vibrioforme* (the non-thiosulfate-using form) [165], *Pelodictyon luteolum* [163], several species of *Ectothiorhodospira* [100,113], *Chr. warmingii* [211], *Thiocapsa pfennigii* [117] and *Thiocapsa roseopersicina* [49]. (Adenosine phosphosulfate reductase from *Tcp. roseopersicina* contains both flavin and heme *c* prosthetic groups (as well as Fe-S centers) [194], but it should not be confused with the flavocytochromes *c* thought to function as sulfide dehydrogenases being discussed here.) Furthermore, flavocytochrome *c* has not been found in *Rb. capsulatus, Rb. sphaeroides,* or *Rs. rubrum* [5,114], even though these species of purple 'nonsulfur' bacteria can oxidize H_2S to S^0. Thus it is clear that there is not a strict correlation between the ability to oxidize H_2S to elemental sulfur and the presence of an isolatable flavocytochrome *c*. Whether an unstable and/or membrane-bound flavocytochrome *c* might exist or whether another electron carrier substitutes for flavocytochrome *c* in these cases is unknown.

The best-studied of the flavocytochromes are flavocytochrome *c*-553, isolated from *Chl. limicola f. thiosulfatophilum,* and flavocytochrome *c*-552, from *Chr. vinosum.* The evidence that they catalyze sulfide oxidation in these organisms is fairly conclusive. Both proteins consist of two subunits in a one-to-one stoichiome-

try. The larger subunit (M_r = 46 000–47 000) contains a single FAD prosthetic group ($E_0' \simeq 0$ V) [32,183] bound by an 8-α-S-cysteinyl thioether linkage [84,115]. The smaller subunit contains a single heme *c* group (E_0' = +98 mV) [47,183] in *Chlorobium* and has an M_r of 11 000 [219], while in *Chr. vinosum* it has two heme *c* groups (E_0' = +32 mV) [115] and an M_r of about 20 000 [54]. With both flavocytochromes *c*, electron transfer to the heme *c* group(s) has been shown to occur via the flavin subunit [32].

Catalytic amounts of both flavocytochromes greatly accelerate the reduction of substrate levels of c_2-type cytochromes (including cytochrome *c*-555 from *Chlorobium* and cytochrome *c*-550 from *Chromatium*) by sulfide at micromolar concentrations, and the flavocytochrome from *Chlorobium* is itself reduced about 10 times as rapidly by sulfide at micromolar concentrations as are any of the other *c*-type cytochromes tested (Table III). Fukumori and Yamanaka [54] reported that flavocytochrome *c*-552 from *Chr. vinosum* has a K_m for sulfide of 12.5 μM, which agrees quite well with values of K_s for sulfide of 7–12 μM for whole cells of different *Chr. vinosum* strains found by Van Gemerden [198]. [*Chlorobium* cells have a K_s for sulfide of 2 μM, but the K_m for the isolated flavocytochrome *c* has not been reported.]

Both flavocytochromes can be separated into their flavin- and heme-containing subunits by exposure to trichloroacetic acid (*Chlorobium*) or urea (*Chromatium*) [54,115,218,219]. Neither subunit by itself catalyzes cytochrome *c* reduction, although the heme subunit binds to cytochrome *c* [34,35]. Attempts to reconstitute sulfide dehydrogenase activity by recombining the two subunits have not yet been successful.

The flavocytochrome *c*-catalyzed reduction of cytochrome *c* is inhibited by cyanide, with 1 μM CN^- causing 80% inhibition of the *Chlorobium* flavocytochrome *c*-553-catalyzed reaction [99]. A somewhat higher CN^- concentration is required with flavocytochrome *c*-552 (from *Chr. vinosum*) to produce the same degree of inhibition [54]. Inhibition by CN^- appears to be caused by a reaction between CN^- and the FAD group of the oxidized (but not the reduced) flavocytochrome to form an adduct in which the flavin absorption bands at 450 and 480 nm are bleached and a charge-transfer band at 670 nm is formed [30,31,99,116,219]. CN^- is tightly bound in the adduct, and cannot be removed by dialysis or gel filtration. Flavocytochrome *c* with bound CN^- is reduced slowly by H_2S, which causes CN^- to be released. CN^- is also removed from flavocytochrome *c*-553 by reaction with $HgCl_2$ followed by dialysis [99]. The flavocytochrome recovered after this treatment had the same absorption spectrum and enzymatic activity as the original protein. SO_3^{2-}, $S_2O_3^{2-}$ and mercaptans also form complexes with flavocytochrome *c* having spectral properties similar to those of the CN^- adduct [31], but the effects of these reagents on sulfide oxidation have not been thoroughly investigated. Recently, Meyer et al. [116] found that the absorption spectrum of the SO_3^{2-} adduct slowly returned to that of the original flavocytochrome under alkaline conditions, and suggested that the recovery might be due to a reaction between bound SO_3^{2-} and a nearby disulfide bond. Since inhibition of sulfide oxidation by CN^- indicates that adduct formation occurs at or near the catalytic center, the occurrence of a disulfide bond in this region of the flavocytochrome would be interesting.

Knaff and coworkers have demonstrated that both flavocytochromes form complexes with c_2-type cytochromes in solutions of low ionic strength and that this interaction involves the heme subunit of the flavocytochrome [34,35,59]. In the case of *Chromatium*

TABLE III

Data for flavocytochrome c catalyzed cytochrome c reduction

The tabulated values for the reactions showing catalysis by flavocytochrome *c*-553 were taken from Yamanaka and Kusai [219] while those for the reactions showing catalysis by flavocytochrome *c*-552 were taken from Fukumori and Yamanaka [54]. In both cases the sulfide concentration was 10 μM. The cytochrome *c* concentration was 50 μM in the flavocytochrome *c*-553 catalyzed reactions, but unspecified in the flavocytochrome *c*-552 catalyzed reactions. *N.* = *Nitrosomonas*, *Ps.* = *Pseudomonas*.

Electon acceptor	Uncatalyzed	+34 nM flavocytochrome *c*-553	+20 nM flavocytochrome *c*-552 [a]
Chl. cytochrome *c*-555	1.4–1.6	14.0 [b]	2.0 [d]
Chl. cytochrome *c*-551	16.2	16.2	–
Chl. flavocytochrome *c*-553	>190	–	
Yeast cytochrome *c*	7.28	21.8	
Horse cytochrome *c*	7.0	– [c]	37.2 [e]
Rs. rubrum cytochrome *c*-550	5.0	–	24.8
N. europaea cytochrome *c*-552	1.4	–	12.0
Ps. aeruginosa cytochrome *c*-554	5.7	–	6.1

[a] Flavocytochrome *c*-552 also catalyzes reduction of cytochrome *c*-550 from *Chr. vinosum*, cytochrome c_2 from *Rps. viridis*, and cytochrome *c* from yeast [34], but kinetic details have not been presented. It does not catalyze reduction of cytochrome *c'*, cytochrome *c*-553(550), or the soluble HiPIP from *Chr. vinosum* [34,54].

[b,c] Davidson et al. [35] found that flavocytochrome *c*-553 has a K_m for cytochrome *c*-555 of 14 μM and a V_{max} of 560 μmol cytochrome *c*-555 reduced per min per μmol flavocytochrome *c*-553 at low ionic strength (2.5 mM KCl) with 25 μM sulfide as the electron donor. For horse cytochrome *c*, these values (also measured at low ionic strength) are: K_m = 600 μM, V_{max} = 7.5 mmol cytochrome *c* reduced per min per μmol flavocytochrome *c*-553.

[d] In contrast to this observation, Davidson et al. [35] reported that flavocytochrome *c*-552 binds to and stimulates reduction of cytochrome *c*-555 by sulfide.

[e] Bosshard et al. [10] found that flavocytochrome *c*-552 has a K_m for horse cytochrome *c* of 16 μM and a V_{max} of 4.3 mmol cytochrome *c* reduced per min per μmol flavocytochrome *c*-552 with 15 μM sulfide as the electron donor at low ionic strength (30 mM KCl).

flavocytochrome *c*-552, the complex contains two cytochromes *c* per flavocytochrome *c*-552 and is held together so strongly that it passes through a gel filtration column as the undissociated complex. The *Chlorobium* flavocytochrome *c*-553-cytochrome *c* complex is less stable, and its stoichiometry has not been determined. In both cases, the complexes dissociate in high-ionic strength buffers with a concomitant decline in the rate of catalytic cytochrome *c* reduction with sulfide as the electron donor. Experiments on chemical modification of the cytochromes have shown that complex formation involves electrostatic interaction between positively charged lysine residues on cytochrome *c* and negatively charged carboxyl groups on flavocytochrome *c*-552 [10,206]. Although many of the experiments on complex formation and electron transfer have been performed using horse-heart cytochrome *c*, similar results have been obtained with cytochrome *c*-550 from *Chromatium* and cytochrome *c*-555 from *Chlorobium* (see Table III), and there is evidence that these cytochromes can donate electrons to the photosynthetic reaction centers of the organisms from which they are obtained.

The product of sulfide oxidation in the flavocytochrome *c*-catalyzed reaction is elemental sulfur (or an unstable precursor at approximately the same redox level – see below). Kusai and Yamanaka [99] found that two cytochrome *c* molecules were reduced per sulfide oxidized in the flavocytochrome *c*-553-catalyzed reaction. More recently, Gray and Knaff [59] analyzed the products of sulfide oxidation by cytochrome *c* in the flavocytochrome *c*-552-catalyzed reaction and found that a stoichiometric amount of elemental sulfur was formed, while no $S_2O_3^{2-}$ or SO_4^{2-} could be detected. An earlier report by Fischer [46] that $S_2O_3^{2-}$ was the product of H_2S oxidation by flavocytochrome *c*-553 may have been incorrect because only small amounts of flavocytochrome *c*-553 were available for those experiments and the $S_2O_3^{2-}$ assay could have given a positive reaction with S^0 under the conditions used (Fischer, U., personal communication).

Fig. 9 illustrates the postulated mode of action of flavocytochrome *c*-552 in H_2S oxidation based on the foregoing discussion. The reaction scheme for flavocytochrome *c*-553 from *Chlorobium* would be analogous.

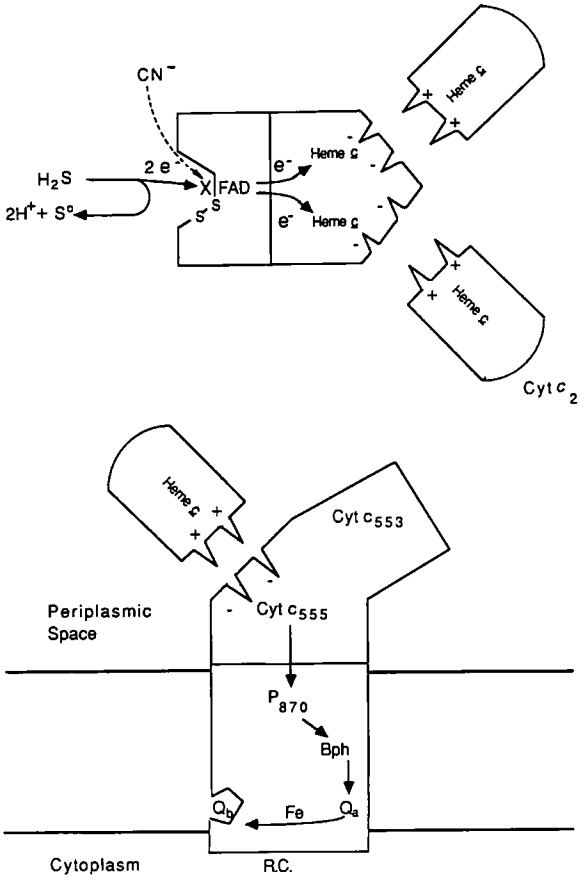

Fig. 9. Catalytic mechanism of flavocytochrome *c* as a sulfide dehydrogenase in *Chr. vinosum*. H_2S first reduces the flavocytochrome *c* FAD group, which then transfers electrons to the heme groups. Cytochrome (Cyt) c_2 (= cytochrome *c*-550 in vivo) binds specificaly to the heme subunit of flavocytochrome *c*-552 to accept electrons which it then shuttles to the reaction center. CN^-, a powerful inhibitor of flavocytochrome *c*-catalyzed cytochrome c_2 reduction, binds to FAD at the active site and blocks electron transfer from H_2S, as indicated by the dashed arrow. The mode of action of flavocytochrome *c*-553 in *Chl. limicola f. thiosulfatophilum* is similar except that the heme subunit contains only a single heme and probably binds only one cytochrome c_2 molecule (cytochrome c_2 = cytochrome *c*-555 in vivo).

IVA-2. Oxidation of sulfide by other cytochromes

As mentioned above, a flavocytochrome *c* has not been found in several species that oxidize H_2S to S^0. Several researchers have suggested that other cytochromes might substitute for flavocytochrome *c* as sulfide dehydrogenases in some of those species. Steinmetz et al. [165] speculated that *c*-type cytochromes without flavins substitute for flavocytochrome *c* in green bacteria lacking flavocytochrome *c*, but the only evidence in favor of this so far is the presence of the other cytochromes *c* and the absence of flavocytochrome *c* in bacteria able to oxidize H_2S to S^0. Similarly, Fischer and Trüper [48a,49] suggested that sulfide might be oxidized in *Tcp. roseopersicina* via either cytochrome *c*-550 or cytochrome *c'*, both of which can be reduced by sulfide. Then and Trüper [178] proposed that cytochrome *c*-551, a small, soluble periplasmic cytochrome ($E_{m,7} = -7$ mV), mediates sulfide oxidation in *Ect. abdelmalekii*. In the case of *Ect. shaposhnikovii*, Kusche and Trüper [101] found that photomixotrophic growth on sulfide + thiosulfate induces synthesis of cytochrome *b*-558 ($E_{m,7} = -210$ mV), a soluble protein that could be involved in sulfide oxidation. One difficulty with these suggestions is that sulfide oxidation by these monoheme cytochromes can occur only one electron at a time and thus would produce rather unstable sulfide radical intermediates (see Ref. 8 for a discussion of sulfide radicals).

There is kinetic evidence that sulfide oxidation by O_2 in aerobic solutions proceeds via a free-radical mechanism [21]. It may be that flavocytochrome *c* oxidizes sulfide more rapidly than the other cytochromes *c* (see Table III) because it can accept two electrons simultaneously and thus avoids sulfide radical intermediates.

It has also been suggested that sulfide is oxidized by electron transfer to the low-potential cytochrome bound to reaction centers of purple sulfur bacteria such as *Chr. vinosum* [68]. However, reduction of this cytochrome in whole cells by sulfide (or thiosulfate) takes several minutes [41,121] and may be simply a nonspecific reaction of the type that occurs between sulfide and a variety of other *c*-type cytochromes (see Table III). Reduction of the low-potential cytochrome in isolated reaction centers by sulfide has not been investigated.

IVA-3. Oxidation of sulfide by quinones

While it is clearly established that flavocytochrome *c* can catalyze sulfide oxidation in those species in which it is present, it is not established that this is the only or even the major pathway for sulfide oxidation. An alternative (or perhaps parallel) pathway is direct reduction of quinone mediated by a sulfide : quinone oxidoreductase. The redox potential of the H_2S/S^0 couple is substantially lower than that of the Q/QH_2 couple for either ubiquinone or menaquinone, so that spontaneous quinone reduction is thermodynamically favored. Brune and Trüper [15] recently prepared chromatophores from *Rb. sulfidophilus* that could photoreduce NAD^+ with sulfide as the electron donor, and found that sulfide could reduce UQ in these chromatophores in the dark. This argues against an obligatory participation of the reaction center (and of *c*-type cytochromes that donate electrons to it) in quinone reduction by sulfide. The product of sulfide oxidation by *Rb. sulfidophilus* chromatophores was not determined, and the enzyme(s) or electron carriers mediating electron transfer from H_2S to UQ remain to be investigated. Similar experiments need to be done with whole cells and with chromatophores from other bacterial species. As noted previously, antimycin A and HOQNO inhibit electron transport from sulfide in membranes from *Chl. limicola f. thiosulfatophilum* [90,156]. These results are consistent with an entry of electrons into the photosynthetic electron-transport chain via quinone in green, as well as purple, bacteria.

IVA-4. The elemental sulfur product

The initial product of sulfide oxidation is often written as 'S^0'. This should not be construed as literally meaning that atomic sulfur is first formed and that it then polymerizes to give stable products (e.g., S_8 rings). Atomic sulfur is an unstable, highly reactive species [111], and it is very unlikely that it could be an intermediate in sulfide oxidation. It is also unlikely that S_8 would be the initial product, as this would entail a concerted 16-electron oxidation of eight H_2S molecules. In spite of a lack of evidence on this subject, polysulfides (H_2S_n, where $n \geqq 2$) would seem to be logical intermediates. They are unstable with respect to disproportionation to $H_2S + \{(n-1)/8\}S_8$ [8], and the site of sulfur globule deposition (extracellular or intracellular) might be determined by the location of a biological catalyst for the disproportionation reaction. Polysulfides have also been suggested to be intermediates in sulfide oxidation by thiobacilli [129].

Redox potentials for the couples $2H_2S/H_2S_2$ and $H_2S_2/2S^0$ have been calculated and are presented in Table II. It is apparent that H_2S becomes a slightly weaker reductant when H_2S_2, rather than S^0, is its oxidation product. The lower potential for the H_2S_2/S^0 than for the $2H_2S/H_2S_2$ couple is consistent with the observed tendency of H_2S_2 to disproportionate to elemental sulfur + H_2S [8]. Both further oxidation and disproportionation reactions are presumably important for formation of long-chain polysulfides and ultimately S^0 (i.e., S_8) from H_2S_2 in phototrophic bacteria.

The elemental sulfur globules that form in various *Chromatium* species during sulfide oxidation can be isolated intact from osmotically disrupted spheroplasts and have been examined by X-ray diffraction [61]. Their diffraction pattern resembles that of liquid sulfur, and Hageage et al. [61] suggested that they consist of spherically symmetrical arrays of S_8 rings. The density of the sulfur globules (1.31 g/cm^3), however, is considerably lower than that of elemental sulfur (about 1.98 g/cm^3 in the solid state and 1.8 g/cm^3 in the molten state) [110]. Guerrero et al. [60] proposed that the sulfur globules consist of a hydrated form of elemental sulfur, and Mas and Van Gemerden [110] extended this proposal to suggest that they consist of long-chain polysulfides, with the charged end groups giving the globules a hydrophilic nature. For comparison, Steudel et al. [167] recently analyzed the sulfur globules formed extracellularly by *Thiobacillus ferrooxidans* grown aerobically on tetrathionate or pentathionate. They concluded that the globules consist of a nucleus, containing 96–98% S_8 with a 2–4% impurity of S_7 and S_6 molecules hindering crystallization, to which long-chain polythionates are attached by hydrophobic interaction along their midsections. The resulting globules thus have a hydrophobic core and a hydrophilic surface.

The sulfur globules of *Chr. vinosum* are surrounded by a monolayer of a single type of protein (M_r = 12000–14000) [125,148] and a similar protein (M_r = 18500) surrounds the sulfur globules of *Chr. buderi* [142]. This protein is soluble in 2 M urea + 10 mM mercaptoethanol, but aggregates into sheets when the urea is dialysed away [148]. It has no known function in sulfur metabolism, and tested negative for rhodanese activity (see below for a discussion of rhodanese). The

extracellular sulfur globules formed by the Chlorobiaceae and the Ectothiorhodospiraceae have not been examined in comparable detail. They appear not to be surrounded by a proteinaceous membrane [48].

IVB. Oxidation of H_2S to SO_3^{2-} – sulfite reductase

It has been proposed that H_2S might, at least in part, be oxidized directly to SO_3^{2-} by a sulfite reductase operating in reverse [47,189]. (Sulfite reductase, as the name implies, also catalyzes the reduction of sulfite to sulfide.) Schedel et al. [147] obtained a siroheme-containing sulfite reductase in approx. 80% purity from *Chr. vinosum*. The occurrence of this enzyme in cells grown photoautotrophically on sulfide + CO_2 but not in cells grown photoheterotrophically on malate + sulfate suggested that it functions in sulfide oxidation rather than in sulfate assimilation. They determined an M_r of 280000 for the enzyme by gel filtration and found it to contain two types of subunits, α and β, with M_r values of 37000 and 43000, respectively, from SDS polyacrylamide gel electrophoresis. They suggested an $\alpha_4\beta_4$ structure for the intact enzyme. In addition to an undetermined number of siroheme prosthetic groups (a likely number would be 4), they found 51 nonheme iron and 47 acid labile sulfide equivalents per enzyme molecule. Other dissimilatory and assimilatory sulfite reductases have siroheme and Fe_4S_4 centers as prosthetic groups [137,157]. The absorption spectrum of the isolated enzyme exhibits maxima at 392 nm, 595 nm and 724 nm due to the sirohemes. The absorbance at 724 nm is probably analogous to the 714 nm absorbance of the assimilatory sulfite reductase from *Escherichia coli* [158], and if so, is indicative of a high-spin state of the siroheme Fe^{3+}. During reduction of SO_3^{2-} to H_2S catalyzed by the isolated enzyme with reduced methyl viologen as the electron donor, $S_3O_6^{2-}$ and $S_2O_3^{2-}$ are released as side products, as is typical for dissimilatory sulfite reductases.

Kobayashi et al. [94] also reported the isolation of a sulfite reductase from photoautotrophically grown *Chr. vinosum*. Their enzyme is presumably the same as that isolated by Schedel et al. [147], although they reported an M_r of 180000 as determined by gel electrophoresis of the intact enzyme. The absorption spectrum of this enzyme was not measured, nor were its prosthetic groups determined. Enzymatic reduction of SO_3^{2-} was measured only in crude extracts, but SO_3^{2-} was eventually reduced completely to H_2S, with $S_2O_3^{2-}$ (but not $S_3O_6^{2-}$) accumulating as an intermediate.

Although Schedel et al. [147] suggested that their enzyme probably catalyzes oxidation of H_2S in vivo, they did not actually demonstrate that it could oxidize sulfide. In a report on a similar enzyme from *Thiobacillus denitrificans*, Schedel and Trüper [145] were able to detect radioactive $S_2O_3^{2-}$ as a product of reacting substrate levels of oxidized sulfite reductase with a slight excess of ^{35}S-labelled sulfide, but they were unsuccessful in finding electron acceptors that could be reduced in a sulfite reductase-catalyzed reaction. Possible electron acceptors in the sulfite reductase-catalyzed oxidation of H_2S in *Chr. vinosum* are also unknown.

The redox potentials of the siroheme and Fe-S prosthetic groups of the *Chr. vinosum* sulfite reductase have not yet been determined. If this enzyme indeed oxidizes H_2S to SO_3^{2-} ($E_0' = -116$ mV), then these prosthetic groups should have redox potentials considerably higher than the -340 mV (siroheme) and approx. -400 mV (Fe_4S_4) values found for these groups in the assimilatory *E. coli* enzyme [158].

Little is known about the mechanism of the sulfur redox transformations catalyzed by sulfite reductases. A recent crystal structure of the hemoprotein subunit of the assimilatory sulfite reductase from *E. coli* indicated that the siroheme iron and one iron of the Fe_4S_4 cluster are bridged by a cysteinyl sulfur [112] in agreement with earlier EPR and Mössbauer experiments showing these groups to be exchange coupled [22,77,78]. Similar results have been obtained with the assimilatory sulfite reductase from *Desulfovibrio vulgaris* [72]. The substrate apparently binds to the otherwise unoccupied 6th ligand position of the siroheme Fe atom. CN^- can also bind to form a stable complex that is inactive in sulfite reduction, unless CN^- is first removed by treatment with 3.3 M urea [158]. Very little is known about the catalytic site in dissimilatory sulfite reductases. There are no EPR data on the *Chr. vinosum* enzyme. Sulfite reductase has not yet been isolated from any other phototrophic bacteria.

IVC. Oxidation of elemental sulfur

Enzymes catalyzing the oxidation of elemental sulfur have not yet been isolated from any species of phototrophic bacteria. Although it has been suggested that sulfite reductase might be able to oxidize elemental sulfur to sulfite [189], there is no experimental evidence for this, at least in phototrophic bacteria.

Experiments on the non-photosynthetic sulfur oxidizing bacterium *Thiobacillus denitrificans* have yielded information about enzymatic oxidation of elemental sulfur that may be relevant to the present discussion. A glutathione-requiring enzyme that oxidizes elemental sulfur to SO_3^{2-} under anaerobic conditions using Fe^{3+} as the electron acceptor was recently isolated from this species [170]. The enzyme apparently occurs in the periplasmic space and is a dimer of identical 23 kDa subunits. It was not determined whether or not this enzyme has any redox-active prosthetic groups. Oh and Suzuki [129] have suggested that the function of glutathione in elemental sulfur oxidation is to attack nucleophilically elemental sulfur according to the equa-

tion: $GSH + S_8 \rightarrow GSS_8H$. The glutathione polysulfide product is then the substrate for further oxidation, eventually splitting off eight SO_3^{2-} ions and regenerating GSH. Although Fe^{3+} would not be a suitable electron acceptor for a phototrophic bacterial S^0 oxidoreductase, perhaps a periplasmic cytochrome or a high potential iron-sulfur protein could fulfill that function, particularly in the case of phototrophs that oxidize extracellularly accumulated elemental sulfur. The periplasmic electron carrier would then be oxidized by the photosynthetic reaction center.

A different view on S^0 oxidation by *Tb. ferrooxidans* was presented by Corbett and Ingeldew [26]. They found that S^0 oxidation by whole cells, using either Fe^{3+} or O_2 as the electron acceptor, was inhibited by HOQNO (20 μM HOQNO gave 50% inhibition). As HOQNO as a specific inhibitor of electron flow through the cytochrome b/c_1 segment of the electron-transport chain, this observation implies that electrons from S^0 oxidation enter the electron-transport chain at the quinone level, a possibility that is consistent with the redox potential of the S^0/SO_3^{2-} couple (see Table II) and should also be considered for phototrophic bacteria.

In view of the lack of any known phototrophic bacterial enzyme able to catalyze S^0 oxidation, the possibility that S^0 is formed on a side branch of the main sulfide oxidation pathway and must be reduced back to sulfide prior to further oxidation (perhaps via sulfite reductase) has also been considered [187]. Both purple [187,197,204] and green [95,134] sulfur bacteria can reduce S^0 to H_2S in the dark. The initial formation of measurable amounts of H_2S during photoautotrophic growth of *Rps. sulfoviridis* on elemental sulfur [122] is consistent with the idea that this species mobilizes S^0 by first reducing it to H_2S prior to further oxidation to SO_4^{2-}. Also consistent with this suggestion is the observation of Paschinger et al. [134] that suspensions of *Chl. limicola f. thiosulfatophilum* using S^0 as the electron donor for CO_2 reduction exhibit a burst of H_2S evolution followed by H_2S oxidation at the onset of illumination. Van Gemerden [197] showed that the dark reduction of S^0 to H_2S by *Chr. vinosum* was coupled to oxidation of glycogen to poly-β-hydroxybutyrate and suggested that S^0 reduction was used to dispose of excess NADH formed during anaerobic breakdown of glucose. This would allow generation of three molecules of ATP via substrate level phosphorylation per glucose monomer consumed.

Nothing is known about the enzymes catalyzing S^0 reduction in phototrophic bacteria. Fukumori and Yamanaka [54] reported that flavocytochrome *c*-552 from *Chr. vinosum* catalyzed reduction of S^0 to H_2S by dithionite-reduced methyl viologen, but Steinmetz and Fischer [162] observed that H_2S is produced in this assay even without flavocytochrome *c*. They also found that when the assay was modified by reducing methyl viologen with H_2 + hydrogenase instead of dithionite, flavocytochrome *c*-553 from *Chl. vibrioforme f. thiosulfatophilum* did not catalyze S^0 reduction. This observation is consistent with the fact that the redox potentials of the flavocytochrome *c* FAD and heme groups are considerably higher than that of the H_2S/S^0 couple, making it unlikely that any of the flavocytochromes *c* could catalyze S^0 reduction. Paulsen et al. [135], working with the nonphototrophic sulfur bacterium *Desulfuromonas acetoxidans,* found that isolated membranes catalyze S^0 reduction by NADH or, if ATP is supplied to drive reverse electron flow, by succinate. Analogous experiments on phototrophic bacterial preparations have not been reported.

IVD. Sulfite oxidation

SO_3^{2-} produced either by oxidation of H_2S via sulfite reductase or by reduction of $S_2O_3^{2-}$ to $H_2S + SO_3^{2-}$ (see below) is further oxidized to SO_4^{2-}, the final sulfur oxidation product in phototrophic bacteria. Two enzymes postulated to catalyze the oxidation are adenosine phosphosulfate (APS) reductase and sulfite: acceptor oxidoreductase.

TABLE IV

Characteristics of phototrophic bacterial APS reductases

ALS, acid labile sulfur; AMP, adenosine monophosphate; FAD, flavin adenine dinucleotide; M_r, molecular weight; NHI, nonheme iron.

Organism	M_r	Prosthetic groups	Optimal pH	K_m for SO_3^{2-} (mM)	K_m for AMP (μM)	Electron acceptor	Ref.
Tcp. roseopersicina	180000	1 flavin (FAD?), 2 heme *c*, 4 NHI, 6 ALS	8.0 9.0	1.5 0.093	73 50	$Fe(CN)_6^{3-}$ cytochrome *c* [a]	194
Chr. vinosum	–	–		1.7	10	$Fe(CN)_6^{3-}$	152
Chl. limicola f. thiosulfatophilum	210000	1 flavin, NHI also present	8.7	0.91	200	$Fe(CN)_6^{3-}$	88a
Chl. vibrioforme f. thiosulfatophilum	180000	1 FAD, 4–6 NHI and 6–8 ALS	8.0	0.17	130	$Fe(CN)_6^{3-}$	88

[a] From *Candida krusei*.

IVD-1. Adenosine phosphosulfate reductase

APS reductase catalyzes the reaction: SO_3^{2-} + AMP → APS + $2e^-$, where AMP is adenosine monophosphate. The in vivo acceptor of electrons in this reaction is not known. The enzyme is typically assayed in vitro by its ability to catalyze an AMP-dependent reduction of $Fe(CN)_6^{3-}$, although some enzymes can also use *c*-type cytochromes as electron acceptors (see Table IV). SO_4^{2-} is released from APS by reaction with P_i to form ADP + SO_4^{2-} in a reaction catalyzed by ADP sulfurylase. This conserves a part of the energy stored in the phosphosulfate bond ($\Delta G_0'$ of hydrolysis = −21 kcal/mol) [176] in the phosphoric anhydride linkage of ADP. (In some species, APS may be converted to ATP by reaction with pyrophosphate in an ATP-sulfurylase-catalyzed reaction, also conserving energy through formation of a phosphoric anhydride bond [7,48].)

APS reductase has been detected in several species of Chromatiaceae and Chlorobiaceae but not in species of Rhodospirillaceae that oxidize sulfide to sulfate [48,122,191]. Ectothiorhodospiraceae generally lack APS reductase, although low levels were reported for *Ect. mobilis* and *Ect. halophila.* Some thiobacilli also use APS reductase to oxidize SO_3^{2-} [85,129]. In sulfate-reducing bacteria, APS reductase catalyzes reduction of SO_4^{2-} to SO_3^{2-}, i.e., it operates in the direction suggested by its name [137].

Table IV summarizes the properties of the best-studied phototrophic bacterial APS reductases. All of the phototrophic bacterial enzymes examined are rather large proteins, with molecular weights of approx. 200 000 and contain both flavin (presumably FAD) and Fe-S centers as prosthetic groups. The enzyme from *Tcp. roseopersicina* has two hemes *c* in addition. *Thiocystis violaceae* has been reported to have an APS reductase (M_r = 190 000) that also contains heme *c* groups [47], but the kinetic properties of this enzyme have not been published. The enzyme from *Chr. vinosum* is membrane-bound, and has been investigated only in chromatophore suspensions [152]. Therefore, information about its molecular weight and prosthetic groups is lacking. None of the phototrophic bacterial APS reductases has been investigated systematically with regard to subunit composition, although Trüper and Rogers [194] reported that treatment of the *Tcp. roseopersicina* enzyme with saturated urea caused separation of the enzyme into heme-containing and flavin + FeS-containing subunits. In general, APS reductases from the phototrophic bacteria are similar to those from thiobacilli and sulfate reducing bacteria [136,169], although none of the enzymes from those organisms contain heme groups. Bramlett and Peck [11] reported that the enzyme from *Desulfovibrio vulgaris* contained subunits with M_r values of 70 000 and 20 000 and suggested that they occur in a 3 : 1 ratio in the intact enzyme. APS reductases are generally not well-characterized biophysically, and the redox potentials of the prosthetic groups have not been determined for any of the phototrophic bacterial enzymes.

Values of K_m for SO_3^{2-} are typically around 1 mM with $Fe(CN)_6^{3-}$ as the oxidant, although a 5-fold lower value was observed with the *Chl. vibrioforme f. thiosulfatophilum* enzyme (Table IV). When the *Tcp. roseopersicina* enzyme used cytochrome *c* from *Candida krusei* as the electron acceptor, the K_m for SO_3^{2-} was an order of magnitude lower (93 μM), and similar observations have been made for other APS reductases able to reduce both $Fe(CN)_6^{3-}$ and cytochrome *c* [169]. Trüper and Rogers [194] suggested on the basis of this evidence that a *c*-type cytochrome functions as the electron acceptor in vivo, and that the cytochrome in turn donates an electron to the reaction center BChl. However, it is now known that reduction of the oxidized reaction center by cytochrome *c* occurs in the periplasmic space, while APS reductase is almost certainly a cytoplasmic enzyme, given its requirement for AMP as a substrate and the fact that further metabolism of APS to ADP (and ATP) must involve intracellular enzymes. Thus electron transfer from APS reductase to cytochrome *c* and then to the reaction center now seems unlikely unless it occurs via a membrane-spanning carrier such as the cytochrome b/c_1 complex.

When APS reductase catalyses both cytochrome *c* and $Fe(CN)_6^{3-}$ reduction, the optimal pH for cytochrome *c* reduction is invariably at least 1 pH unit higher in the reaction with cytochrome *c*. Assuming that the values of K_m for sulfite with the two acceptors were determined at their pH optima, the lower K_m with cytochrome *c* may simply indicate that the dissociated form of an ionizable group with a pK_a intermediate between the different pH optima participates in SO_3^{2-} binding. One possibility is that this might be an SH group, since inhibition of APS reductases by sulfhydryl reagents has been observed and can be prevented or partly reversed by mercaptoethanol or glutathione [88,194]. The pK_a of HSO_3^{2-} is sufficiently low (7.2) that it will be mostly in the SO_3^{2-} form at the pH optima for both acceptors.

The K_m for AMP, the other substrate, varies from 50 to 200 μM with phototrophic bacterial APS reductases and is independent of the electron acceptor used. Similar results have been found with other APS reductases [169].

Peck and Bramlett [136] discussed the mechanism of APS reductase-catalyzed sulfite oxidation, and presented the following observations. (1) Sulfite first reacts with the flavin group to form an adduct, possibly with SO_3^{2-} bound at the N-5 position [118]. (2) Addition of AMP results in a $g = 1.94$ EPR signal. They suggested that reaction of AMP with the flavin adduct to form APS leaves behind a reduced flavin which in turn reduces the Fe-S center(s) responsible for the $g = 1.94$

signal. They also made the interesting observation that, at least with APS reductase from *Desulfovibrio vulgaris,* reduction of cytochrome *c* (but not $Fe(CN)_6^{3-}$) is strongly inhibited by superoxide dismutase and completely inhibited by anaerobic conditions [11]. Their explanation for these results is that the reduced flavin can react with O_2 to form a superoxide radical which in turn reduces cytochrome *c*. $Fe(CN)_6^{3-}$, on the other hand, is reduced preferentially by the Fe-S center. The greater stability of superoxide anions at high pH values might then account for the higher pH optimum with cytochrome *c*. However, O_2-mediated cytochrome *c* reduction seems inconsistent with the different cytochrome *c* specificities for different enzymes. For example, while the enzyme from *Tcp. roseopersicina* reduces cytochrome *c* from *Candida krusei* but not that from horse heart, the *Desulfobulbus propionicus* enzyme reduces horse heart but not *Candida krusei* cytochrome *c* [169]. The effects of superoxide dismutase and of anaerobic conditions on these reactions were not examined.

IVD-2. Sulfite : acceptor oxidoreductase

Sulfite may also be oxidized to sulfate in an AMP-independent reaction catalyzed by an enzyme called sulfite : acceptor oxidoreductase or sulfite dehydrogenase [47,107,122,189]. The term sulfite oxidoreductase will be used here as a shortened form of sulfite : acceptor oxidoreductase. As was the case with APS reductase, the in vivo acceptor of electrons from sulfite in the reaction catalyzed by this enzyme is unknown. $Fe(CN)_6^{3-}$ has been used to assay the enzyme in vitro. Various cytochromes *c* can accept electrons in reactions catalyzed by some sulfite oxidoreductases, but give reaction rates an order of magnitude lower than those observed using $Fe(CN)_6^{3-}$ with the phototrophic bacterial enzymes so far tested [122]. SO_3^{2-} is a strong reductant ($E_0' = -516$ mV). Consequently, reduction of most photosynthetic electron carriers is thermodynamically possible.

Because SO_3^{2-} is such a strong reductant, nonspecific reduction of intermediate electron carriers that in turn react with $Fe(CN)_6^{3-}$ is a potential hazard in assaying enzyme activity. For example, SO_3^{2-} reduces 2,6-dichlorophenolindophenol in a phenazine methosulfate-stimulated reaction and can also reduce Fe^{3+} in an uncatalyzed reaction [26,161]. Neutzling et al. [122] noted some heat-stable catalytic activity that passed through an $M_r = 2000$ cut-off ultrafiltration membrane. Nevertheless, the same authors noted that most of the sulfite oxidoreductase activity was membrane-bound in the four species of Rhodospirillaceae they examined. The enzyme could be solubilized with Triton X-100, but, except in the case of *Rb. adriaticus,* precipitated and tended to lose activity on removal of the detergent. The enzymes from *Rb. adriaticus* and *Rb. sulfidophilus* exhibited K_m's for SO_3^{2-} of 750 μM and 700 μM, respectively, while that from *Rps. sulfoviridis* had a substantially lower K_m (40 μM).

According to surveys presented by Trüper and Fischer [47,48,189,190], sulfite oxidoreductase has been detected in all of the Chromatiaceae and Ectothiorhodaceae examined, as well as in the sulfur-oxidizing Rhodospirillaceae discussed above. In contrast, it appears to be absent, or present only at very low levels in the Chlorobiaceae. Among the purple sulfur bacterial enzymes, only that from *Chr. vinosum* appears to have been investigated in detail. Unlike the enzymes from Rhodospirillaceae, that enzyme is a soluble protein. It has an M_r of 68000 and a K_m for SO_3^{2-} of 380 μM (Ref. 196; cited in Ref. 122), but little else has been published about it. Nothing is known about prosthetic groups of phototrophic bacterial sulfite oxidoreductases. Fischer [47] remarked that the absorption spectrum of the *Chr. vinosum* enzyme resembled that of cytochrome *c'*, but noted that cytochrome *c'* may have been present as a contaminant in the preparation.

The occurrence of both APS reductase and sulfite oxidoreductase in some species of Chromatiaceae suggests the possibility of parallel pathways for SO_3^{2-} oxidation. Evidence for this was found by Fry et al. [51] who examined sulfur isotope fractionation during SO_3^{2-} photooxidation by *Chr. vinosum.* They found that the SO_4^{2-} formed in this process was initially enriched in ^{34}S (relative to ^{32}S) (an inverse isotope effect) but that this was followed, after about 20% of the SO_3^{2-} was oxidized, by a preferential formation of $^{32}SO_4^{2-}$ (a normal isotope effect). They speculated that the change might have been due to competition between sulfite oxidoreductase and APS reductase for SO_3^{2-} during the oxidation process.

It seems unlikely, however, that the sole function of sulfite oxidoreductase would be to compete with APS reductase for SO_3^{2-} on the main sulfur oxidation pathway and (as the sulfite oxidoreductase reaction does not conserve energy through an associated phosphorylation) to thus lower the energetic efficiency of the process. An alternative possibility (at least in the case of bacteria like *Chr. vinosum* that grow well on SO_3^{2-} and possess both enzymes) might be that sulfite oxidoreductase is a periplasmic enzyme with the specific function of oxidizing externally supplied SO_3^{2-}, while APS reductase is a cytoplasmic enzyme on the main pathway of sulfur oxidation. This role for sulfite oxidoreductase might be correlated with protection against high concentrations of SO_3^{2-} in the external medium, since SO_3^{2-} is known to be toxic to other organisms [80,215]. This could explain the rather unusual ability of species like *Chr. vinosum* to grow on SO_3^{2-}. In this case, the isotopic fractionation experiments may have detected competition between sulfite oxidoreductase and a permease allowing SO_3^{2-} to enter the bacterial cells. The intracell-

ular location of the *Chr. vinosum* enzyme should be determined to see if it is consistent with this suggestion.

Thiobacilli contain sulfite : cytochrome *c* oxidoreductases that may be analogous to the phototrophic bacterial sulfite oxidoreductases. However, the preferred electron acceptor for this enzyme is clearly cytochrome *c*, and rates with $Fe(CN)_6^{3-}$ are about 20-fold lower [182]. The enzymes from *Thiobacillus novellus* [182] and *Tb. versutus* [107] both have molybdenum-containing cofactors. In addition, the *Tb. novellus* enzyme contains a heme *c* group absorbing at 550 nm, while that from *Tb. versutus* is tightly bound to a cytochrome *c*-551 and irreversibly loses activity when cytochrome *c*-551 is dissociated from it. Both the molybdenum and heme groups are reduced by added SO_3^{2-} [182], and both enzymes (as well as one isolated previously from *Tb. thioparus*) have similar values of K_m for SO_3^{2-} (14–40 μM) [107]. The sulfite oxidoreductases from thiobacilli are soluble proteins with molecular weights of about 40 000. The use of cytochromes *c* as electron acceptors suggests that they are located in the periplasmic space in bacterial cells. The *Tb. versutus* enzyme appears to be part of a larger complex that oxidizes $S_2O_3^{2-}$ to SO_4^{2-} without releasing SO_3^{2-} as a free intermediate [107].

Mitochondria from animal liver cells also contain a sulfite : cytochrome *c* oxidoreuctase. This enzyme (a dimer with an M_r of 100 000–120 000) has molybdenum and heme *b* as cofactors and is located in the intermembrane space [80,124]. Its function is removal of SO_3^{2-} produced during cysteine and methionine catabolism, which is toxic at high levels.

IVE. Thiosulfate oxidation

IVE-1. Thiosulfate : acceptor oxidoreductase

Four different possibilities for the initial step of $S_2O_3^{2-}$ metabolism are shown in Fig. 8. The simplest of these is that $S_2O_3^{2-}$ is oxidized directly to tetrathionate ($S_4O_6^{2-}$) in an enzyme-catalyzed reaction. Enzymes catalyzing oxidation of $S_2O_3^{2-}$ to $S_4O_6^{2-}$ coupled to reduction of various electron acceptors have been isolated from *Chr. vinosum* [55,93,150,160], *Chl. limicola f. thiosulfatophilum* [99], and *Rps. palustris* [2,93] – see Table V. The first thiosulfate : acceptor oxidoreductase (hereafter called thiosulfate oxidoreductase) was isolated from *Chr. vinosum* by Smith [160], who used $Fe(CN)_6^{3-}$ as the electron acceptor. This enzyme (for $S_2O_3^{2-}$ $K_m = 1.5$ mM) had a pH optimum of 5.0 and was inactive at pH 7.0. Smith suggested that it catalyzed $S_2O_3^{2-}$ oxidation only when cells were suspended in a slightly acidic medium, in agreement with the observation that *Chr. vinosum* formed $S_4O_6^{2-}$ as the product of $S_2O_3^{2-}$ oxidation only under acidic conditions. Fukumori and Yamanaka [55] isolated what was probably the same enzyme and found that a high potential iron-sulfur protein (HiPIP) isolated from *Chr. vinosum* was an efficient electron acceptor (for HiPIP $K_m = 130$ μM). The enzyme was moderately sensitive to inhibition by SO_3^{2-} and CN^-, with 50% inhibition observed at millimolar concentrations. Both the amino acid sequence [39] and the crystal structure [18] of the *Chr. vinosum* HiPIP had previously been determined, although no function in electron transport had been assigned to it. Fukumori and Yamanaka suggested that the thiosulfate-reduced HiPIP was photooxidized by the reaction center. Knobloch and coworkers [93,150] isolated a seemingly different thiosulfate oxidoreductase from *Chr. vinosum* that catalyzed cytochrome *c* reduction at pH 8. They found the best acceptor to be a flavocytochrome *c*-552 obtained from chromatophores after treatment with cold acetone. This enzyme was reported to have a K_m for $S_2O_3^{2-}$ of 2 μM and a K_m for the acceptor flavocytochrome *c*-552 of 0.2 μM.

Van Grondelle et al. [202] found that cytochrome *c*-550 (also referred to as cytochrome *c*-551) in *Chr. vinosum* cells is reduced by $S_2O_3^{2-}$, probably via an intermediate enzyme system. Fischer's review from 1984 [47] suggests that the intermediate enzyme system is a periplasmic thiosulfate oxidoreductase. While this is possible, the data of Van Grondelle et al. [202] are equally consistent with other pathways for $S_2O_3^{2-}$

TABLE V

Characteristics of isolated phototrophic bacterial thiosulfate oxidoreductases

Organism	M_r	Optimal pH	K_m for $S_2O_3^{2-}$	Electron acceptor	Ref.
Chr. vinosum	?	5.0	1.5 mM	$Fe(CN)_6^{3-}$	160
Chr. vinosum	35 000	?	6 μM	flavocytochrome *c*-552 (membrane-bound); horse cytochrome *c*	93, 150
Chl. limicola f. thiosulfatophilum	80 000	6.0	1.7 mM	cytochrome *c*-551, yeast cytochrome *c*	99
Rps. palustris	93 000	?	2 μM	cytochrome *c* (*Rps. palustris*)	2, 93

oxidation. For example, $S_2O_3^{2-}$ may be taken up initially into the cellular cytoplasm and reductively cleaved to form H_2S and SO_3^{2-} which then reduce cytochrome *c*-550 via the membrane-spanning cytochrome b/c_1 complex. Although Van Grondelle et al. were unable to detect a cytochrome b/c_1 complex in *Chr. vinosum,* subsequent work has demonstrated that one is present and that it reduces cytochrome *c*-550 during cyclic electron transport [27]. In fact, while the experimental evidence for this was not presented, Fig. 9 in the paper of Van Grondelle et al. [202] indicates that reduction of cytochrome *c*-550 by $S_2O_3^{2-}$ is inhibited by HOQNO, which would be expected if this reaction is mediated by a cytochrome b/c_1 complex.

The thiosulfate oxidoreductase isolated from *Chl. limicola f. thiosulfatophilum* resembles the *Chr. vinosum* enzyme first isolated by Smith [160] in having a rather high K_m for $S_2O_3^{2-}$ and an acidic pH optimum (6.0) [99]. The electron acceptor for that enzyme was cytochrome *c*-551 from *Chlorobium,* and its rate of reduction was increased 4-fold by adding a stoichiometric amount of cytochrome *c*-555 from the same organism. It has since been shown that cytochrome *c*-551 and cytochrome *c*-555 from *Chlorobium* form a complex that might be the preferred electron acceptor in this reaction [34]. The enzyme is inhibited by SO_3^{2-} (80% at 0.1 mM) and by CN^- (47% at 0.1 mM). It is presumed that electrons from cytochrome *c*-551 ($E_{m,7} = +135$ mV) are transferred via cytochrome *c*-555 ($E_{m,7} = +145$ mV) to P-840, the reaction center BChl *a* (Fig. 6). The occurrence of a similar cytochrome *c*-551 in *Chl. vibrioforme f. thiosulfatophilum* [162], the only other thiosulfate-utilizing green bacterium, along with its absence in the non-thiosulfate-utilizing strains of these species [47] is often cited as evidence for a specific function of cytochrome *c*-551 in $S_2O_3^{2-}$ oxidation. However, Steinmetz and Fischer [164] were unable to detect an enzyme catalyzing reduction cytochrome *c*-551 by $S_2O_3^{2-}$ in *Chl. vibrioforme f. thiosulfatophilum.* Kusai and Yamanaka [99] did not identify the product of S_2O_3 oxidation, but stated that a test for $S_4O_6^{2-}$ gave negative results.

When *Rps. palustris* is grown with high concentrations of $S_2O_3^{2-}$ (more than 10 mM) in the culture medium, $S_4O_6^{2-}$ is the oxidation product. Knobloch and coworkers [2,93,149] reported isolating an enzyme from *Rps. palustris* that catalyzes reduction of cytochrome *c*-549.5 from the same organism by $S_2O_3^{2-}$ with K_m's of 2 μM for both $S_2O_3^{2-}$ and cytochrome *c*-549.5. The pH optimum of this enzyme was not given, but the assay was conducted at pH 8. Although the acceptor cytochrome *c* was apparently different from cytochrome c_2, it presumably transfers electrons to P-870 in *Rps. palustris.*

In spite of the evidence just summarized for thiosulfate : acceptor oxidoreductases in several bacterial species, it seems unlikely that they play a significant role in oxidizing $S_2O_3^{2-}$ to SO_4^{2-}. Experiments on the oxidation of either $^{35}SSO_3^{2-}$ or $S^{35}SO_3^{2-}$ by different species of Chromatiaceae showed that a very early step in $S_2O_3^{2-}$ oxidation involved splitting of the $S_2O_3^{2-}$ molecule, with the sulfane sulfur being stored intracellularly as S^0 and the sulfone sulfur being released as SO_4^{2-} [161, 192]; see Fig. 3. Analogous experiments on *Chl. vibrioforme f. thiosulfatophilum* also showed that the sulfone sulfur was quickly released into the medium as SO_4^{2-} while the sulfane sulfur was first converted to a form (probably elemental sulfur or intracellular polysulfide) that was trapped with the cells on a membrane filter [87]. Thus in those species so far examined, the initial step in $S_2O_3^{2-}$ oxidation is a separation of the two sulfur atoms of $S_2O_3^{2-}$ which are then processed differently, rather than an oxidative combination of 2 $S_2O_3^{2-}$ to form $S_4O_6^{2-}$. A second argument against an initial oxidation of $S_2O_3^{2-}$ to $S_4O_6^{2-}$ en route to SO_4^{2-} is that $S_4O_6^{2-}$ is not further metabolized by any phototrophic bacterial species other than *Chl. limicola f. thiosulfatophilum* and *Chl. vibrioforme f. thiosulfatophilum.* In fact, $S_4O_6^{2-}$ inhibits oxidation of $S_2O_3^{2-}$ to $SO_4^{2-} + S^0$ by *Chr. vinosum* [160,161], perhaps by interfering with transport of $S_2O_3^{2-}$ into the bacterial cells.

IVE-2. Rhodanese and thiosulfate reductase

The initial reaction for thiosulfate utilization that seems to be most popular in the current literature on phototrophic bacterial sulfur metabolism is reduction to $H_2S + SO_3^{2-}$. Two distinct but similar enzymes, namely rhodanese and thiosulfate reductase, have been proposed as catalysts, and thiols, such as lipoic acid and glutathione, are the best electron donors for this reduction [65,66,161]. (Rhodanese apparently is not involved in thiosulfate oxidation by *Thiobacillus* A_2, however [107a]). Methyl viologen (reduced by H_2 + hydrogenase) is also effective in the thiosulfate reductase-catalyzed reaction [65]. Both enzymes function as thiosulfate : sulfur transferases, releasing SO_3^{2-} from $S_2O_3^{2-}$ and producing persulfides (RSSH) from thiol acceptors. The persulfide product reacts with a second –SH group (located in the same molecule in lipoic acid and in a separate molecule in glutathione) to form a disulfide and release H_2S.

Rhodanese occurs in plants and animals as well as in bacteria, and has been suggested to function in cyanide detoxification and formation of sulfide for Fe-S clusters in iron-sulfur proteins [19,212]. A 0.21 nm resolution X-ray crystal structure for the enzyme from bovine liver mitochondria has been obtained and used to interpret its catalytic function [69,139]. This 33 kDa enzyme reacts initially with $S_2O_3^{2-}$ to form a cysteine persulfide at the active site with SO_3^{2-} being released into solution. The enzyme-bound sulfur is then transferred to a thiophilic acceptor (e.g., CN^-, SO_3^{2-} or a thiol) in a second reaction step. As the name rhodanese implies,

the preferred acceptor is CN^-, forming SCN^-, which is called 'rhodanid' in German. It is this reaction that makes rhodanese effective in cyanide detoxification, but other functions may be more important in the absence of CN^-.

Rhodanese was first isolated from *Chr. vinosum* by Smith and Lascelles [161] and found to have a pH optimum of 8.7, a K_m for $S_2O_3^{2-}$ of 0.6 mM and a K_m for CN^- of 20 mM. Hashwa [65] subsequently reported an M_r of 45 000 for the *Chr. vinosum* enzyme. Rhodanese activity is widely distributed among the phototrophic bacteria [161,190,220], including species such as *Rs. rubrum* and *Rb. sphaeroides* that do not oxidize $S_2O_3^{2-}$. However, Trüper and Fischer [190] reported that neither rhodanese nor thiosulfate reductase was detected in the non-thiosulfate-utilizing from of *Chl. limicola*, while both enzymes occur in *Chl. limicola f. thiosulfatophilum* and *Chl. vibrioforme f. thiosulfatophilum.* Steinmetz and Fischer [164] were able to isolate two rhodaneses from *Chl. limicola f. thiosulfatophilum.* The most active and abundant of these is a basic protein ($M_r = 39\,000$) with a K_m for $S_2O_3^{2-}$ of 0.25 mM and a K_m for CN^- of 5 mM.

Thiosulfate reductase differs from rhodanese in that CN^- does not work well as a thiophilic acceptor. Some researchers, e.g., Hashwa [65,66], have tried to distinguish between thiosulfate reductase and rhodanese on the basis of the ability of the former, but not the latter, to use reduced methyl viologen as the electron donor for $S_2O_3^{2-}$ reduction. However, as Hashwa acknowledged, it is not clear that this distinction is valid. Thiosulfate reductases are generally rather unstable and thus have not been well studied. An exception is the yeast enzyme ($M_r = 17\,000$) [20]. Unlike rhodanese, it first binds glutathione (GSH), the thiophilic acceptor, followed by reaction with $S_2O_3^{2-}$ to release SO_3^{2-} + GSSH. The latter then spontaneously reacts with a second GSH molecule to form GSSG + H_2S. Presumably lipoic acid and other thiols react analogously with some thiosulfate reductases. Thiosulfate reductases from phototropic bacteria are less well studied and seem to be less stringent about not using CN^- as a thiophilic acceptor; in fact they may be simply different varieties of rhodanese. Hashwa [65] was able to isolate a thiosulfate reductase ($M_r = 90\,000$) with relatively low rhodanese activity from *Chr. vinosum.* It had a K_m for lipoic acid of 1.25 mM, but also reacted with CN^- with a K_m of 3.3 mM. Enzyme fractions selectively enriched in rhodanese or thiosulfate reductase activity were also obtained from *Rps. palustris* [65].

Trüper and Pfennig [192] demonstrated that suspensions of *Thiocapsa roseopersicina* cells produced H_2S from $S_2O_3^{2-}$ in the dark by sweeping H_2S from the cell suspension into a zinc acetate trap. H_2S production did not occur with broken cells, presumably because of a need to generate a reductant (e.g., lipoic acid) via cellular metabolism. Attempts to detect SO_3^{2-}, the other product of $S_2O_3^{2-}$ reduction, were unsuccessful. More recently, however, Khanna and Nicholas [87] reported that SO_3^{2-} produced from $S_2O_3^{2-}$ in *Chl. limicola f. thiosulfatophilum* can be trapped as a complex with *N*-ethylmaleimide.

One difficulty with the proposed reduction of $S_2O_3^{2-}$ by lipoic acid or glutathione is the fact that the redox potential for the $(H_2S + SO_3^{2-})/S_2O_3^{2-}$ couple (−402 mV) is considerably lower than that of either (reduced lipoate)/(oxidized lipoate) (−320 mV) or 2 GSH/GSSG (−230 mV) [79], so that the equilibrium for the reaction opposes $S_2O_3^{2-}$ reduction. However, efficient oxidation of the reaction products (H_2S and SO_3^{2-}) by reaction with photosynthetic electron carriers could overcome this unfavorable equilibrium. Furthermore, a diverse assortment of nonphototrophic bacterial species have developed similar dissimilatory $S_2O_3^{2-}$ reduction reactions [4], indicating that the low redox potential for $S_2O_3^{2-}$ reduction is not an insurmountable obstacle.

It is also possible that rhodanese (or a similar enzyme) might transfer the sulfane sulfur of $S_2O_3^{2-}$ to a sulfur globule without prior reduction to H_2S, perhaps by using a growing polysulfide chain as a thiophilic acceptor. In this case, the initial splitting of $S_2O_3^{2-}$ would yield $S^0 + SO_3^{2-}$. However, there is no experimental evidence supporting this possiiblity over the initial reduction possibility.

IVE-3. Hydrolytic cleavage of thiosulfate

A final possibility for the $S_2O_3^{2-}$ splitting reaction, suggested originally by Trüper and Pfennig [192] is hydrolytic cleavage to yield $H_2S + SO_4^{2-}$ directly. The $\Delta G_0'$ for the reaction (−5.2 kcal/mol) [3] is highly favorable. Bak and Pfennig [3] recently isolated a new bacterial species, *Desulfovibrio sulfodismutans,* that derives energy for growth from this reaction, although they assume that it occurs in two steps, namely an initial reduction of $S_2O_3^{2-}$ to $H_2S + SO_3^{2-}$ followed by oxidation of SO_3^{2-} to SO_4^{2-} in an APS reductase + ADP sulfurylase catalyzed reaction that yields energy by substrate level phosphorylation. Although a one-step hydrolysis of $S_2O_3^{2-}$ to $H_2S + SO_4^{2-}$ would be an elegant and simple way to make $S_2O_3^{2-}$ available to phototrophic bacteria, an enzyme catalyzing this reaction has not yet been found. It is apparent that the breakdown of $S_2O_3^{2-}$ via this reaction could produce toxic levels of H_2S unless the enzyme catalyzing it were well regulated.

V. Energetics of sulfur oxidation

Unlike the sulfate reducing bacteria [137,175] and the thiobacilli [85], which derive energy for ATP synthesis from dissimilatory sulfur metabolism, the phototrophic bacteria use sulfur compounds primarily as a source of electrons and derive energy from light. It is probably for

this reason that little attention has been given to the possibility of energy conservation during noncyclic electron transport or to the intracellular location of phototrophic bacterial sulfur oxidizing enzymes, which can have important consequences for energy conservation during noncyclic electron transport.

It is apparent from Fig. 8 that oxidation of reduced sulfur compounds results in the release of a considerable number of H^+ that might contribute to the development of a transmembranous H^+ gradient. For example, oxidation of reduced sulfur compounds in the periplasmic space followed by photochemical electron transfer via a membrane-spanning reaction center could contribute to the transmembranous Δp that drives ATP synthesis and reverse electron flow. This advantage of periplasmic oxidation of inorganic sulfur compounds was extensively discussed by Hooper and DiSpirito [70] who proposed that both phototrophic and nonphototrophic bacteria using simple reductants as electron donors oxidize them on the extracytoplasmic side of the cell membrane. In the case of phototrophic bacteria, H_2S, $S_2O_3^{2-}$ and other reduced sulfur compounds might reduce a periplasmic cytochrome *c* that in turn is oxidized by the reaction center. Electrons are transferred from the reaction center to UQ (purple bacteria) or to NAD^+ (green sulfur bacteria). The net result is release of 1 H^+ in the periplasm and uptake of 1 H^+ from the cytoplasm per electron transported (Fig. 10). This scheme for energy conservation would be analogous to, but reversed from, those proposed by Thauer and Badziong [175] and by Peck and LeGall [137] for the sulfate-reducing bacteria, in which cytoplasmic reduction of oxidized sulfur compounds to H_2S coupled to periplasmic oxidation of H_2 produces a transmembranous Δp used for ATP synthesis.

Fig. 10 shows a scheme for oxidation of H_2S to SO_4^{2-} via periplasmic enzymes, namely flavocytochrome *c*, a hypothetical sulfur : cytochrome *c* oxidoreductase, and a sulfite : acceptor oxidoreductase. Of these enzymes, only flavocytochrome *c* has been reported to be periplasmic [5]. The cellular location of sulfite oxidoreductase has not yet been studied in any phototrophic bacteria. Thiosulfate oxidoreductase is included as a periplasmic enzyme because it reduces either a cytochrome *c* or a high potential iron-sulfur protein that can then be oxidized by the reaction center.

An alternative possibility is that electrons from sulfur might be transferred initially to quinones within the photosynthetic membrane [15,40]. The reduced quinones would then be oxidized either by the cytochrome b/c_1 complex or by Δp-driven reverse electron flow through NADH dehydrogenase. This alternative takes advantage of the low redox potentials of sulfur compounds, and, in the case of green sulfur bacteria, inserts an additional site of energy conservation (the cytochrome b/c_1 complex) that is missing in electron-transport pathways involving an initial reduction of cytochrome *c* (see Fig. 6). Because quinones can accept both the protons and electrons released during sulfur oxidation reactions, it is somewhat arbitrary from a bioenergetic viewpoint whether these reactions occur on the cytoplasmic or the periplasmic membrane surface.

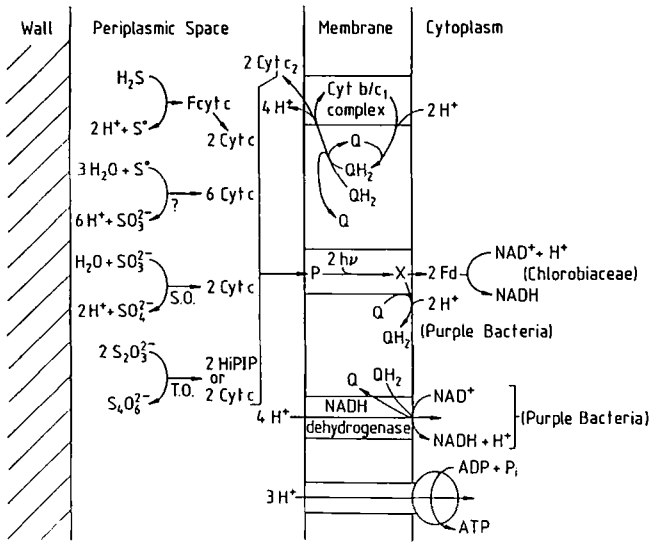

Fig. 10. Hypothetical scheme for sulfur oxidation with periplasmic cytochromes accepting electrons in the initial oxidation reactions. H^+ released in these reactions contribute to acidification of the extracytoplasmic medium, while reaction-center-driven quinone and NAD^+ reduction reactions lead to uptake of cytoplasmic protons (i.e., alkalinization of the cytoplasm). The transmembranous Δp generated in these reactions, as well as during cyclic electron flow via the cytochrome b/c_1 complex, provides energy for ATP synthesis and, in purple bacteria, reverse electron flow from QH_2 to NAD^+. Abbreviations: Cyt, cytochrome; Fd, ferredoxin; P, photoactive reaction center bacteriochlorophyll; Q, quinone; QH_2, reduced quinone; S.O., sulfite : acceptor oxidoreductase; T.O., thiosulfate : acceptor oxidoreductase; X, primary electron acceptor in the reaction center (see section III). A question mark denotes a hypothetical sulfur : cytochrome *c* oxidoreductase.

Fig. 11 shows a scheme in which sulfide is oxidized to sulfate intracellularly via sulfite reductase and APS reductase. The requirement for AMP as a substrate for APS reductase makes a cytoplasmic location likely for that enzyme. Sulfite reductase is known to be cytoplasmic in sulfate reducing bacteria [128a] and is also shown here as being cytoplasmic, although Hooper and DiSpirito [70] have suggested that this enzyme should be periplasmic. Sulfite reductase has so far been found only in *Chr. vinosum,* and might be replaced by a sulfide : quinone oxidoreductase that oxidizes H_2S to S^0 + a sulfur : quinone oxidoreductase in most other species. Although neither enzyme has yet been isolated from any phototrophic bacteria, there is evidence for H_2S oxidation by UQ in *Rb. sulfidophilus* [15], and for S^0 oxidation via quinone and a cytochrome b/c_1 complex in the nonphototrophic, sulfur-oxidizing bacterium *Thiobacillus ferrooxidans* [26]. Intracellular thiosulfate is assumed to be oxidized by the same enzymes that

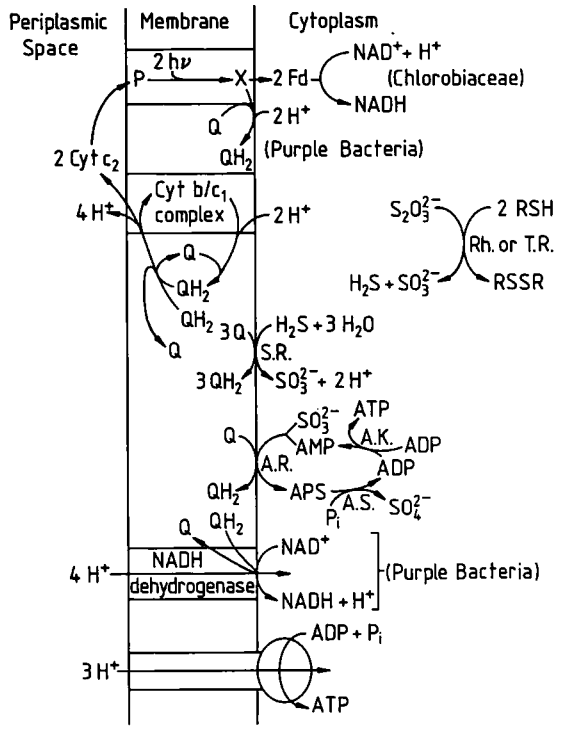

Fig. 11. Hypothetical scheme for sulfur oxidation reactions with quinones in the membrane acting as acceptors for both electrons and protons in the initial oxidation reactions. In purple bacteria, the reduced quinone is oxidized by Δp-driven reverse electron flow to NAD^+ through NADH dehydrogenase, while in Chlorobiaceae, electrons from QH_2 are transferred via a cytochrome b/c_1 complex and cytochrome c_2 to the reaction center, which then reduces NAD^+ photochemically. The transmembraneous Δp that drives ATP synthesis and NAD^+ reduction in purple bacteria is generated by cyclic electron transport through the reaction center and the cytochrome b/c_1 complex. Cyclic electron transport can also occur in Chlorobiaceae, with a portion of the electrons from reduced Fd being used for quinone, rather than NAD^+, reduction (not shown). Abbreviations: A.K., adenylate kinase; A.R., APS reductase; A.S., ADP sulfurylase; Cyt, cytochrome; Fd, ferredoxin; P, photoactive reaction center bacteriochlorophyll; Q, quinone; QH_2, reduced quinone; Rh., rhodanese; S.R., sulfite reductase; T.R., thiosulfate reductase; X, primary electron acceptor in the reaction center.

oxidize sulfide, after an initial reduction to $H_2S + SO_3^{2-}$ via rhodanese or thiosulfate reductase. Oxidation of $S_2O_3^{2-}$ and SO_3^{2-} within the cytoplasm in this scheme and a requirement for specific permeases to catalyze their uptake could explain the inability of many phototrophic bacteria to use these compounds as electron donors.

Both schemes can be criticized. For example, periplasmic SO_3^{2-} oxidation as shown in Fig. 10 seems inconsistent with the inefficiency of *c*-type cytochromes as electron acceptors for the few phototrophic bacterial sulfite oxidoreductases so far examined, and with the inability of most phototrophic bacteria to grow using SO_3^{2-} as the electron donor, since externally added SO_3^{2-} should have access to its oxidation site. However, SO_3^{2-} is known to be toxic to many organisms [80,215] and it may be that its toxic effects at substrate levels, rather than an inability to oxidize it, are responsible for the inability of most phototrophic bacteria to grow on SO_3^{2-}. A problem with the scheme shown in Fig. 11 is that neither sulfite reductase nor APS reductase has been shown to use a quinone as an electron acceptor, although that possibility has not been excluded and is consistent with the redox potentials of the sulfur compounds being oxidized. Furthermore, the observed inhibition of noncyclic electron transport by HOQNO and antimycin A in green sulfur bacterial membrane preparations [90,156] is consistent with the participation of a quinone and a cytochrome b/c_1 complex in sulfide oxidation.

The main reason for presenting the schemes shown in Figs. 10 and 11 as alternatives is that they imply different energetic requirements for photosynthetic electron transport. Because the scheme in Fig. 11 supplies electrons from sulfur to the electron-transport chain at a lower redox potential than that in Fig. 10, it should also be more efficient. In the following sections, the actual efficiencies of the two schemes will be calculated and compared. It is important to realize, however, that various hybrid schemes combining features from both Figs. 10 and 11 are also possible. For example, other alternatives might incorporate both H_2S oxidation via a periplasmic flavocytochrome *c* and SO_3^{2-} oxidation via a cytoplasmic APS reductase. Such intermediate schemes would have energetic efficiencies intermediate between those calculated for Figs. 10 and 11.

VA. Quantum requirement for photosynthesis in purple sulfur bacteria

Given the information presented previously about electron transport and CO_2 fixation in purple bacteria (subsection IIIB) it is possible to calculate minimum quantum requirements for CO_2 fixation on the basis of the schemes shown in Figs. 10 and 11.

In Fig. 10, the reactions and their energy requirements are as follows:

(1) 4 e^- are transferred photochemically to UQ; 4 H^+ are released outside the cell and 4 H^+ taken up inside . . . . . 4 *hν*

(2) 4 e^- are transferred from 2 UQH_2 to 2 NAD^+. This is driven by approx. 8 H^+ crossing the membrane. 4 H^+ come from step (1), 4 are provided by cyclic electron transport at 2 H^+ per e^- transported . . . . . 2 *hν*

(3) 3 ATP are synthesized via ATP-ase, requiring 3 H^+ crossing the membrane per ATP, or 9 H^+ total. These are provided by cyclic electron transport . . . . . 4.5 *hν*

Total for 2 NADH and 3 ATP per CO_2 reduced in Calvin cycle . . . . . 10.5 *hν*

For Fig. 11, the energy requirements are:
(1) 4 e^- and 4 H^+ are transferred to UQ ..... 0 *hν*
(2) 4 e^- are transferred from 2 UQH_2 to 2 NAD^+, driven by approx. 8 H^+ crossing the membrane. All 8 H^+ are provided by proton pumping accompanying cyclic electron transport ..... 4 *hν*
(3) 3 ATP are synthesized as described above .. 4.5 *hν*

Total for 2 NADH and 3 ATP per CO_2 reduced in Calvin cycle ..... 8.5 *hν*

These calculations have not considered the formation of ATP from APS resulting from the activities of ADP sulfurylase and adenylate kinase (Fig. 11). These reactions would contribute 1 ATP per H_2S undergoing an 8-electron oxidation to SO_4^{2-}, or 0.5 ATP per CO_2 reduced. For the 8-electron oxidation of $S_2O_3^{2-}$ to 2 SO_4^{2-}, 1 ATP would be formed per CO_2 reduced, assuming that both sulfur atoms of $S_2O_3^{2-}$ were oxidized to SO_4^{2-} via APS. These contributions would lower the requirement for CO_2 photoreduction by 2/3 of a quantum with sulfide as the electron donor and by $1\frac{1}{3}$ quanta with $S_2O_3^{2-}$ as the electron donor.

Energy costs for transporting sulfur compounds into the bacterial cytoplasm are also not considered due to lack of information about this subject. Cypionka [32a] presented evidence that uptake of $S_2O_3^{2-}$, SO_3^{2-} and SO_4^{2-} into cells of the sulfate reducing bacterium *Desulfovibrio desulfuricans* is driven by the concomitant uptake of 2 H^+ per sulfur anion, while H_2S diffuses freely through the membrane in its undissociated form. If sulfate efflux from phototrophic bacteria also occurs via a proton symport mechanism, it could actually contribute to the transmembranous Δp, thus providing energy for uptake of $S_2O_3^{2-}$ and possibly SO_3^{2-}. Unfortunately, transport of inorganic sulfur compounds into and out of phototrophic bacterial cells appears never to have been investigated.

The calculated values may be compared to an actual quantum requirement for CO_2 fixation of 12 ± 1.5 (mean and standard deviation) measured manometrically by Wassink et al. [209] using *Chr. vinosum* suspensions with $S_2O_3^{2-}$ as the electron donor at pH 6.3. This pH was selected for several reasons, including the linearity of rate of CO_2 uptake as a function of light intensity (curves obtained at higher pH values had a pronounced sigmoid shape) and the fact that the light-saturated rate of CO_2 uptake with $S_2O_3^{2-}$ was maximal at this pH value. As noted previously, $S_2O_3^{2-}$ oxidation by *Chr. vinosum* is abnormal at pH 6.3 in that the oxidation product is $S_4O_6^{2-}$, which inhibits oxidation of $S_2O_3^{2-}$ to $S^0 + SO_4^{2-}$ as well as growth of *Chr. vinosum* [160,208]. $S_2O_3^{2-}$ is thought to be oxidized to $S_4O_6^{2-}$ in the periplasmic space as shown in Fig. 10, and the measured quantum requirement is consistent with this possibility. This suggests that a lower quantum requirement might be found at a higher pH – i.e., conditions under which $S_2O_3^{2-}$ could enter the cytoplasm and be oxidized completely to SO_4^{2-}. Nevertheless, measured quantum requirements for CO_2 fixation with H_2 as the electron donor averaged 11.4 at pH 6.3 and 12.6 at pH 7.6, in reasonable agreement with the values determined for $S_2O_3^{2-}$ at pH 6.3 [209].

It is apparent from the foregoing discussion that the difference between the minimal quantum requirements predicted from the schemes in Figs. 10 and 11 is relatively small. Both values are slightly lower than the measured quantum requirements.

VB. Energetics of chemoautotrophy in purple sulfur bacteria

Several species of purple sulfur bacteria, including *Amoebobacter roseus* [58a], *Chr. gracile, Chr. vinosum, Thiocystis violaceae* [83,83], and *Tcp. roseopersicina* [38,97,201] can grow chemoautotrophically on H_2S or $S_2O_3^{2-}$ by electron transfer to O_2 under semiaerobic to aerobic conditions. Importantly for the present discussion, the site of entry of electrons into the electron-transport chains of *Chr. vinosum* and *Tcp. roseopersicina* can be evaluated from the energetics of their chemoautotrophic growth on $S_2O_3^{2-}$ and suggests that at least some of the electrons obtained from $S_2O_3^{2-}$ oxidation must enter the electron-transport chain at the quinone level, as shown in Fig. 11. $S_2O_3^{2-}$ is the most useful and best-studied chemolithotrophic electron donor because, unlike H_2S [21,168], it is not oxidized spontaneously by O_2 at an appreciable rate. As is the case during phototrophic growth, chemotrophically growing purple sulfur bacteria accumulate intracellular globules of elemental sulfur as an intermediate during $S_2O_3^{2-}$ oxidation to SO_4^{2-}. Phototrophically grown *Tcp. roseopersicina* cultures can oxidize $S_2O_3^{2-}$ aerobically without an adaptation period [97,201], suggesting that the terminal oxidase is constitutive and that the same enzymes that catalyze photosynthetic $S_2O_3^{2-}$ oxidation also catalyze its aerobic oxidation. Thus consideration of the energetics of chemoautotrophic growth can provide insights into the site of entry of electrons into the photosynthetic electron-transport chain.

De Wit and Van Gemerden [38] found that during growth of *Tcp. roseopersicina* on $S_2O_3^{2-}$, CO_2 and O_2, 24–32% of the electrons from $S_2O_3^{2-}$ were used for CO_2 reduction while the remainder were transferred to O_2. In agreement with these results, the yield of *Tcp. roseopersicina* cells during chemoautotrophic growth is about 1/3 of that obtained during photoautotrophic growth, in which all of the electrons from $S_2O_3^{2-}$ are used for CO_2 reduction [201]. Analogous experiments on chemoautotrophically grown *Chr. vinosum* indicated that an average of 25% of the electrons from $S_2O_3^{2-}$ were used

for CO_2 reduction while the other 75% were transferred to O_2 [83].

The use of nearly 1/3 of the electrons from $S_2O_3^{2-}$ for CO_2 reduction found for *Tcp. roseopersicina* is clearly inconsistent with cytochrome *c* being the sole point of entry for electrons from $S_2O_3^{2-}$ into the respiratory electron-transport chain. Two electrons passing from cytochrome *c* to O_2 via the cytochrome oxidase coupling site would yield roughly enough energy to drive one electron to NAD^+ via the cytochrome b/c_1 and NADH dehydrogenase coupling sites, assuming that the contribution to Δp from electron transport through each of the sites is the same and that electron transport through the cytochrome b/c_1 and NADH dehydrogenase coupling sites is reversible. This would leave no additional electrons to be transported to O_2 to supply the ATP needed for CO_2 fixation and for other biosynthetic processes that occur during bacterial growth. Although the stoichiometry between electrons transported to CO_2 and to O_2 in *Chr. vinosum* is less obviously inconsistent with cytochrome *c* being the initial acceptor of electrons from $S_2O_3^{2-}$, the portion of the electrons used for CO_2 reduction is still rather high.

Experimental observations suggest that reverse electron flow through two coupling sites in series requires more than twice as much energy as reverse electron flow through a single coupling site. Jones and Vernon [81] found that while 1.8 ATP molecules were hydrolyzed per NAD^+ reduced by succinate (which reduces UQ directly), 5.2 ATP's were required per NAD^+ reduced with ascorbate + TMPD (which reduces cytochrome c_2) in unilluminated suspensions of *Rhodospirillum rubrum* chromatophores. About the same number of ATP molecules appears to be required for reverse electron flow from cytochrome *c* to NAD^+ in the thiobacilli [85]. Thus the energy requirement for reverse electron flow from cytochrome *c* may be even higher than suggested in the preceding paragraph.

A more detailed analysis of the energetics of chemoautotrophic metabolism in thiobacilli that reached similar conclusions was presented by Kelly [85], who also discussed bacterial growth yields per mole of $S_2O_3^{2-}$ as a function of the site of entry of electrons into the electron-transport chain. Interestingly, different species of thiobacilli differ in their pathways of electron transport from $S_2O_3^{2-}$. In the aerobic thiobacilli, $S_2O_3^{2-}$ is oxidized by electron transfer to cytochrome *c* and about 13% of the electrons from $S_2O_3^{2-}$ are used for CO_2 reduction. In contrast, electrons from $S_2O_3^{2-}$ enter the electron transport chain at the 'flavin or cytochrome *b*' (i.e., quinone) level in the facultative anaerobe *Thiobacillus denitrificans*. Up to 29% of the electrons from $S_2O_3^{2-}$ can be used for CO_2 reduction by aerobically grown *Tb. denitrificans*, a value comparable to those found for *Chr. vinosum* and *Tcp. roseopersicina*. Different extents of CO_2 fixation by these bacteria are readily apparent from differences in the maximal dry-weight cell yields observed during chemoautotrophic growth on $S_2O_3^{2-}$. Thus, the extrapolated maximal growth yields for the aerobic thiobacilli (average approx. 6.7 g/mol $S_2O_3^{2-}$) are about 1/2 that for *Tb. denitrificans* (approx. 14.7 g/mol $S_2O_3^{2-}$). The observed growth yields for *Tb. denitrificans* (11.7 g and 13.2 g/mol $S_2O_3^{2-}$ in two different studies cited by Kelly [85]) are very close to the average value of 12 g/mol $S_2O_3^{2-}$ found by Kämpf and Pfennig [83] for *Chr. vinosum*. In conclusion, it now seems very likely that a substantial fraction of the electrons obtained by oxidation of $S_2O_3^{2-}$ must enter the electron-transport chain at the quinone level during chemoautotrophic growth (and probably also photoautotrophic growth) of purple sulfur bacteria.

Oxidation of $S_2O_3^{2-}$ via UQ during chemoautotrophic growth of purple sulfur bacteria suggests that inhibitors of quinone redox reactions in the cytochrome b/c_1 complex should inhibit O_2 consumption. Kämpf and Pfennig [83] reported that antimycin A did not inhibit electron transport from $S_2O_3^{2-}$ to O_2 in suspensions of *Chr. vinosum* cells. However, that inhibitor is also ineffective against cyclic electron transport in *Chr. vinosum* cells, suggesting that it does not penetrate the cells to reach its site of action [173,202]. Other inhibitors, such as HOQNO, should also be tested.

VC. Quantum requirement for photosynthesis in green sulfur bacteria

Evaluating the energetics of green bacterial sulfur oxidation is complicated by the lack of information about the number of H ions crossing the membrane per ATP synthesized and per electron transferred through the putative cytochrome b/c_1 complex. Also it is not known whether or not light is required to reduce the flavin that in turn reduces fumarate to succinate during the reductive carboxylic acid cycle used for CO_2 fixation. (The observation of Paulsen et al. [135] that membranes from the sulfur-reducing bacterium *Desulfuromonas acetoxidans* catalyze an MQ-mediated reduction of fumarate to succinate by H_2S shows that a similar reaction is at least possible in the green sulfur bacteria.) In spite of these uncertainties, it is instructive to calculate upper and lower limits for the quantum requirement for CO_2 fixation using different assumptions.

The upper limit may be estimated by assuming that reduced sulfur compounds are oxidized periplasmically by *c*-type cytochromes (Fig. 10) and that flavin, ferredoxin, and pyridine nucleotides are all reduced photochemically via the reaction center. Photochemical transfer of 12 electrons through the membrane to reduce the 1 flavin, 4 Fd, and 3 $NAD(P)^+$ molecules required to reduce 3 CO_2 molecules (see section III) will cause 12 H ions to be released in the periplasmic space and an

equal number to be taken up from the cytoplasm. (Although Fd accepts only electrons, its reduction via the membrane-spanning reaction center complex makes a contribution to the $\Delta\psi$ portion of the transmembranous Δp. Furthermore, H^+ is actually taken up in subsequent CO_2 reduction reactions involving Fd.) Assuming that $3H^+$ enter the cell per ATP synthesized via the membrane-spanning ATP-ase (i.e., assuming the same stoichiometry chosen for purple bacteria (section III)), a quantum requirement for CO_2 reduction may be calculated as follows:

(1) 12 e^- are transferred photochemically to flavin, Fd, and $NAD(P)^+$; 12 H^+ are released outside and taken up inside the cell ..... 12 $h\nu$
(2) 5 ATP are synthesized with 3 H^+ entering the cell per ATP. 12 of the 15 required H^+ come from step (1), 3 come from cyclic electron transport at a ratio of 2 H^+ per e^- cycled ..... 1.5 $h\nu$

Total for 4 Fd_{red} + 3 NAD(P)H + 1 flavin H_2 + 5 ATP needed to reduce 3 CO_2 ..... 13.5 $h\nu$
or 4.5 $h\nu$ per CO_2

The lower limit may be calculated by assuming that cytochrome *c* reduction occurs via a cytochrome b/c_1 complex (Fig. 11). Making the reasonable assumption that this doubles the magnitude of the transmembranous Δp generated during photoreduction of Fd and $NAD(P)^+$ and also assuming that flavin reduction does not require light, the minimum quantum requirement can be calculated as follows:

(1) 10 e^- are transferred to 4 Fd and 3 $NAD(P)^+$ via a cytochrome b/c_1 complex and the reaction center; 20 H^+ are pumped out of the cell (flavin reduction is nonphotochemical) ..... 10 $h\nu$
(2) 5 ATP are synthesized with 3 H^+ entering the cell per ATP. 15 of the 20 H^+ from step (1) are used; 5 remain for other biosynthetic reactions ..... 0 $h\nu$

Total for reduction of 3 CO_2 ..... 10 $h\nu$
or $3\frac{1}{3} h\nu$ per CO_2

The calculated quantum requirement in this case is quite remarkable in that less than one photon is needed per electron used in carbon reduction. Furthermore, this calculation implies that cyclic photophosphorylation is unnecessary for CO_2 fixation and that noncyclic photophosphorylation produces more ATP than is required for CO_2 fixation.

Measured quantum requirements for CO_2 fixation, however, are higher than would be expected from these values. Larsen et al. [103] found that 9–10 quanta were required per CO_2 reduced by *Chl. limicola f. thiosulfatophilum* using H_2, $S_2O_3^{2-}$ or $S_4O_6^{2-}$ as the electron donor. These carefully determined experimental values are twice those estimated here. The size of this discrepancy is not easily explained, and suggests that a reinvestigation of the quantum requirement for CO_2 fixation might be appropriate. If the calculated quantum requirements eventually prove to be correct, this suggests that the low quantum requirement for CO_2 fixation as well as the large photosynthetic antenna may be an adaptation that allows green sulfur bacteria to grow in weakly illuminated environments.

VI. Summary and Conclusions

A wide variety of green and purple phototrophic bacteria are able to use inorganic sulfur compounds as electron donors for photosynthetic CO_2 reduction. The inorganic sulfur compounds are usually oxidized to SO_4^{2-}, although a few species produce S^0, $S_2O_3^{2-}$ or $S_4O_6^{2-}$ as the final oxidation product. H_2S is toxic at high concentrations, but most species can use it as an electron donor. S^0 is also widely used, while $S_2O_3^{2-}$ is less commonly and SO_3^{2-} is rarely used. Elemental sulfur is nearly always produced during oxidation of H_2S, and accumulated intracellularly in Chromatiaceae and extracellularly in Ectothiorhodospiraceae and Chlorobiaceae. Polysulfides are logical intermediates during oxidation of H_2S to S^0, but this possibility has not yet been systematically investigated. Elemental sulfur is also an intermediate during $S_2O_3^{2-}$ oxidation by Chromatiacae, but not by Ectothiorhodospiraceae.

A variety of enzymes catalyzing sulfur redox reactions have been isolated, and the reactions they catalyze have been arranged into likely pathways for sulfur oxidation. However, the lack of a phototrophic bacterial enzyme known to catalyze S^0 oxidation leaves a gap in this metabolic pathway. Almost nothing is known about the intracellular locations of most of the sulfur-oxidizing enzymes. The related problem of transport of inorganic sulfur compounds into and out of phototrophic bacterial cells is also largely unexplored.

In order to understand how the sulfur oxidation reactions interface with photosynthetic electron transport, it is necessary to know which photosynthetic electron carriers are reduced during the sulfur oxidation reactions. Of the isolated enzymes, only flavocytochrome *c*, which oxidizes H_2S to S^0, has been well studied as to its catalytic mechanism and the identity of its in vivo electron acceptor, which is a c_2-type cytochrome. Two of the enzymes, APS reductase and sulfite : acceptor oxidoreductase have typically been assayed by measuring $Fe(CN)_6^{3-}$ reduction, and the in vivo electron acceptors are not known. No electron acceptor has yet been found for the proposed sulfite reductase-catalyzed oxidation of H_2S to SO_3^{2-}, although there is evidence that this reaction occurs during sulfide oxidation in *Chr. vinosum*. Examination of the

energetics of chemoautotrophic growth by 2 species of Chromatiaceae on $S_2O_3^{2-} + O_2$ indicates that at least some of the electrons from $S_2O_3^{2-}$ must enter the electron-transport chain at the quinone level, and quinones may function as electron acceptors in some of the reactions for which the in vivo acceptor has not yet been found. There is also some evidence that H_2S may be oxidized by quinones, although nothing is known about the enzyme catalyzing this reaction or its importance relative to flavocytochrome *c*-catalyzed H_2S oxidation.

Likely in vivo electron acceptors have been found for thiosulfate : acceptor oxidoreductases, but these enzymes are probably not important for thiosulfate oxidation under normal growth conditions. Instead, thiosulfate oxidation is probably initiated by a rhodanese- or thiosulfate reductase-catalyzed reduction to H_2S and SO_3^{2-}, which are then processed by enzymes of the sulfide oxidation pathway. An alternative possibility might be that $S_2O_3^{2-}$ is initially hydrolyzed to produce $H_2S + SO_4^{2-}$.

The purple bacteria and green sulfur bacteria use very different pathways for photosynthetic electron transport and CO_2 fixation. Minimum quantum requirements for photoautotrophic CO_2 fixation were calculated on the basis of these pathways, assuming different possible sites for entry of electrons from sulfur compounds into the electron-transport chain. These calculations gave requirements of 8.5–10.5 quanta per CO_2 fixed by purple bacteria and 3.33–4.5 quanta per CO_2 fixed by green bacteria, assuming carbohydrate to be the product of CO_2 fixation in both cases. The green bacterial result is remarkably low, and in contrast to older measured values, which indicated that both green and purple bacteria have similar quantum requirements (8–12 quanta per CO_2 reduced).

Appendix. Analytical methods

Measurement of sulfur redox changes mediated by phototrophic bacteria requires analytical methods to determine the concentrations of different sulfur compounds as a function of time under various experimental conditions. Therefore methods that have been used for quantitative analysis of the various sulfur compounds consumed or produced by illuminated phototrophic bacteria will be briefly described and references given in which detailed procedures can be found. Most of these methods are spectrophotometric. Typically, changes in sulfur compound concentration with time are obtained by taking aliquots of the experimental sample at different times and assaying for the sulfur compounds of interest.

Anionic sulfur compounds at different redox levels can also be separated by ion chromatography [174]. This method is sufficiently sensitive to measure micromolar concentrations of these compounds and samples can be chromatographed reasonably rapidly. Because the ions of interest are separated during analysis, ion chromatography should be less sensitive to interferences than are spectrophotometric methods. Applications of ion chromatography to the study of phototrophic bacterial sulfur oxidation reactions have been reported by Gray and Knaff [59] and by Eichler and Pfennig [43].

A-1. Sulfide (H_2S)

Sulfide is usually determined using its quantitative reaction with *N,N*-dimethyl-*p*-phenylenediamine to form methylene blue as described by Trüper and Schlegel [195]. $FeNH_4(SO_4)_2$ is included in the reaction mixture to oxidize leuco-methylene blue to its colored form, the amount of which is determined from its absorbance at 670 nm. This method is useful in the micro- to millimolar concentration range.

Sulfide in samples taken from a bacterial suspension can also be measured with a sulfide ion-selective electrode [199]. Adding an anaerobic, alkaline ascorbate buffer partially converts H_2S and HS^- to S^{2-} while avoiding oxidation. Because the electrode actually detects S^{2-} rather than H_2S or HS^-, this increases the sensitivity of the measurement, which is accurate for sulfide concentrations between 0.1 μM and 1 mM [199]. A sulfide electrode can also be used to monitor continuously sulfide concentrations in bacterial suspensions at neutral pH [13,14]. This method works best for measuring light-induced changes in sulfide concentration in the range between 1 μM and 100 μM.

A-2. Elemental sulfur (S^0)

Two methods have been used for elemental sulfur determination. The simplest is to extract the elemental sulfur from bacterial cells collected by centrifugation using an organic solvent (e.g., methanol or ethanol) and to determine the concentration of sulfur from its absorbance at 260 nm [199]. When methanol extracts containing S^0 are shaken with hexane, the elemental sulfur is transferred quantitatively to the hexane phase, a procedure that decreases the level of interference by extracted pigments. Van Gemerden [199] reported the following extinction coefficients (in $g^{-1} \cdot l \cdot cm$) for S^0 at 260 nm: methanol, 23.9; ethanol or hexane, 25.4. Correction for photosynthetic pigment absorbance can be made by measuring absorbances in extracts from sulfur-free cells.

The alternative method is to convert S^0 to SCN^- by hot cyanolysis and then determine the amount of SCN^- from the absorbance at 460 nm of its Fe^{3+} complex [146,161b]. In this procedure, bacterial cells and extracellular or intracellular sulfur globules are collected

by ultrafiltration (or centrifugation) and washed to remove $S_2O_3^{2-}$, polythionates, and polysulfides, which also react in this assay if present. The sulfur-containing sample is then incubated at 90–100 °C for 10–15 min in a 0.1 M NaCN solution, followed by addition of $Fe(NO_3)_3$ in HNO_3. The amount of S^0 present is determined from the absorbance at 460 nm using a standard curve prepared by the reaction of $Fe(NO_3)_3$ with known amounts of NaSCN.

A-3. Polythionates, polysulfide and thiosulfate

Thiosulfate ($S_2O_3^{2-}$) and tetrathionate ($S_4O_6^{2-}$) have also typically been measured by cyanolysis [86,213,217] followed by formation of the ferric thiocyanate complex. $S_4O_6^{2-}$ reacts with cold NaCN according to the equation:

$$S_4O_6^{2-} + CN^- + H_2O \rightarrow S_2O_3^{2-} + SO_4^{2-} + 2H^+ + SCN^-$$

After 20 min at 5 °C [86] or 5 min at 18 °C [123], ferric thiocyanate is formed and determined from its absorbance at 460 nm as described above. This method does not distinguish between $S_4O_6^{2-}$ and higher polythionates ($S_nO_6^{2-}$) which also react with CN^- to form thiocyanate.

$S_2O_3^{2-}$ is relatively inert to cyanolysis, but reacts rapidly in the presence of Cu^{2+} as follows [86,161b]: $S_2O_3^{2-} + CN^- \rightarrow SCN^- + SO_3^{2-}$. This method must be corrected for more reactive sulfur compounds (e.g., polythionates and polysulfides) if they are also present in the reaction mixture, taking into account that polythionates yield approx. one more SCN ion in the presence than in the absence of Cu^{2+} [126,217]. Cu^{2+} also catalyzes cyanolysis of S^0, which should be removed by filtration prior to the analysis [199].

Polysulfides are somewhat less reactive than polythionates but more reactive than $S_2O_3^{2-}$ toward CN^- (see Schedel and Trüper [146] for activation energies for cyanolysis of various reduced sulfur compounds). Polysulfides with the formula S_n^{2-} react with CN^- to yield $n-1$ SCN ions per molecule, even in the absence of a Cu^{2+} catalyst, on incubating at 30 °C for 30 min [123,178]. Polysulfide determinations must of course be corrected for any polythionates present in the medium. In practice, this is not a serious limitation because polythionates are not usually produced during sulfur oxidation by phototrophic bacteria.

A-4. Sulfite (SO_3^{2-})

SO_3^{2-} can be specifically and quantitatively detected in an assay based on its sulfite oxidase-catalyzed reaction with O_2 to form $H_2O_2 + SO_4^{2-}$ [6]. The H_2O_2 formed in turn oxidizes NADH to NAD^+ in the presence of NADH peroxidase, and the amount of SO_3^{2-} initially present is calculated from the decrease in absorbance by NADH at 340 nm. SO_3^{2-} has also been determined from its reaction with basic fuchsin (pararosaniline) and formaldehyde to form an adduct that absorbs maximally at 580 nm. A recent version of this method is given by Leinweber and Monty [105].

A-5. Sulfate (SO_4^{2-})

SO_4^{2-}, the usual end product of sulfur photooxidation, has been determined from the turbidity produced from its quantitative and rather specific reaction with $BaCl_2$ under acidic conditions to produce a $BaSO_4$ precipitate. This method was recently described in detail by Sörbo [161a].

A-6. ^{35}S-labeling

The radioactive isotope ^{35}S decays with a half-life of 88 days by emission of a β-particle intermediate in energy between those emitted by ^3H and ^{32}P [210]. Thus it has been useful for following redox changes of sulfur in labeled substrates during oxidation by sulfur bacteria. A particularly elegant use of ^{35}S-labeling involved supplying bacterial cultures with either $^{35}SSO_3^{2-}$ (sulfane-labeled thiosulfate) or $S^{35}SO_3^{2-}$ (sulfone-labeled thiosulfate) to demonstrate that the elemental sulfur that accumulates as an intermediate during $S_2O_3^{2-}$ oxidation by purple sulfur bacteria is derived entirely from the sulfane sulfur of thiosulfate [161,192] (see Fig. 3).

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