Sulfite Reductase Activity in Extracts of Various Photosynthetic Bacteria

(*Rhodospirillaceae/Chromatiaceae/Chlorobiaceae/sulfate assimilation*)

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Contributed by Martin D. Kamen, April 2, 1974

ABSTRACT Extracts of representative bacterial strains from the various families of photosynthetic prokaryotes are demonstrated to possess significant levels of sulfite reductase [EC 1.8.99.1; hydrogen-sulfide:(acceptor)oxidoreductase] activity with reduced methyl viologen as electron donor, but not NADPH₂. The enzyme is localized primarily in the soluble fraction of the extracts, in contrast to adenylylsulfate reductase [EC 1.8.99.2; AMP, sulfite:(acceptor) oxidoreductase], which is bound normally in the membrane fractions of those bacteria in which it is found. Assignment of the sulfite reductase activities to the biosynthetic ("assimilatory") pathway is suggested by levels of specific activity noted and ready solubility.

Inorganic sulfur compounds serve as electron donors for the photosynthetic growth of the *Chromatiaceae* and *Chlorobiaceae* and as a source of sulfur for the biosynthesis of cysteine, methionine, and other sulfur-containing compounds in various photosynthetic bacteria (1). Surprisingly little is known about the pathways or enzymes involved either in oxidation or reduction of sulfur compounds by these bacteria. Even when an enzymatic activity is reported, it is often unclear whether it participates in the oxidative or reductive pathways of sulfur metabolism. During photosynthetic sulfur oxidation, customarily termed "dissimilatory sulfur metabolism" (2), relatively large amounts of sulfide are oxidized to sulfur and eventually to sulfate. One may expect that enzymes in this oxidative pathway would be present in relatively high specific activity, inducible by substrates, and possibly associated with chromatophores. During biosynthetic ("assimilatory") sulfate reduction, sulfate is reduced to sulfide only in amounts sufficient to meet nutritional needs for the biosynthesis of cell material. Enzymes involved in this process could be expected to be present in low specific activity, repressed by cysteine or methionine, and probably soluble.

Microorganisms belonging to the *Chromatiaceae* (*Thiorhodaceae*) and *Chlorobiaceae* possess adenylyl sulfate reductase (3) [EC 1.8.99.2; AMP, sulfite:(acceptor) oxidoreductase] in the chromatophore fraction which apparently catalyzes the oxidation of sulfite to sulfate during photosynthetic growth with reduced inorganic compounds of sulfur (4). When grown in the complete absence of reduced sulfur compounds, *Chromatium vinosum* SMG182 forms adenylyl sulfate from AMP and sulfite, although in reduced amounts (10% of levels found in autotrophically grown cells), so it has been suggested that adenylylsulfate may function in biosynthetic ("assimilatory") sulfate reduction in these microorganisms (4). The *Rhodospirillaceae* (*Athiorhodaceae*) lack adenylylsulfate reductase (4-6), even in the case of *Rhodospseudomonas palustris*, which is capable of oxidizing thiosulfate (7) or sulfide (8) to sulfate,

but nevertheless can reduce sulfate to acid-volatile sulfur compounds (9). As both the formation and reduction of 3'-phosphoadenylylsulfate have been reported (5, 9), these photosynthetic bacteria may reduce sulfate via 3'-phosphoadenylylsulfate reductase (10). Nutritional studies have shown that some *Chromatiaceae* are capable of utilizing sulfate as their sole sulfur source (11) and all *Rhodospirillaceae* can grow with sulfate (12). As for *Chlorobiaceae*, it has been reported, not surprisingly, that *Chlorobium thiosulfatophilum* is incapable of "assimilatory" sulfate reduction (13).

Sulfite reductase [EC 1.8.99.1; hydrogen-sulfide:(acceptor) oxidoreductase] activity has not been reported in extracts of any of the photosynthetic bacteria, although cells capable of reducing sulfate should possess a sulfite reductase (2). As the *Chromatiaceae* and *Chlorobiaceae* are also capable of oxidizing sulfide and sulfur to sulfate, some relationship may exist between sulfite reductase and this oxidative system. Further, the recent observation of a new type of heme (14), termed "siroheme," as well as non-heme iron associated with sulfite reductase and sulfite-reducing activities in the sulfate-reducing bacteria (15, 16), together with the demonstration that sulfite reductase is present in *Clostridium pasteurianum* (17), prompts initiation of studies designed to establish the presence or absence of sulfite reductase in the various groups of photosynthetic bacteria.

EXPERIMENTAL

Rhodospirillum rubrum was grown photosynthetically on a completely synthetic medium containing only sulfate and biotin (12); *Chromatium* strain D, was grown autotrophically with sulfide and thiosulfate as electron donors (12) and heterotrophically on acetate with sulfate and biotin as sulfur sources (12); *Chlorobium* PM and *Chlorobium* "Tassajara" were grown on a synthetic medium with thiosulfate as the main sulfur source (12); *Rps. gelatinosa*, *Rhodomicrobium vannielii*, *Rps. viridis* and *Rps. palustris* (strain 37) were grown on a modified Hunter medium (18) containing sulfate and biotin as sulfur sources. Extracts of each of these photosynthetic bacteria were prepared by suspending frozen cells in an equal volume of 0.2 M Tris (hydroxymethyl)-aminomethane (Tris) buffer (pH 8.0) and passing the cell suspension through a French pressure cell at 15,000 lbs./inch². Addition of a few crystals of DNase reduced the viscosity of the extract so that centrifugation at 30,000 × *g* in the Sorvall RC2B for 30 min was facilitated. The resultant supernatant fraction was termed "crude extract." An aliquot was reserved for assay, and the remainder was centrifuged at 160,000 × *g* for 2 hr in a Beckman centri-

TABLE 1. Distribution of sulfite reductase between chromatophores and soluble protein in extracts of *Chromatium* and *Chlorobium*

Extract	Activity (μ mole of H_2 per 10 min/ml)	
	minus sulfite	plus sulfite
<i>Chromatium</i> , strain D		
Crude	1.69	4.82
Soluble protein	0.89	4.46
Chromatophores	0.31	0.22
<i>Chlorobium</i> P.M.		
Crude	0.93	3.75
Soluble protein	0.70	3.34
Chromatophores	0.27	0.71

fuge (model L-2-50) to separate chromatophores from soluble protein. Sulfite reductase activity was determined by manometrically measuring hydrogen utilization in the presence of methyl viologen and hydrogenase from *Escherichia coli* as described (16). Sulfite reductase was also determined, with NADPH₂ as electron donor (15). Specific activities of sulfite reductase are reported as μ mole of H_2 utilized per min per mg of protein. Protein was determined by the biuret procedure (19).

RESULTS AND DISCUSSION

The distribution of sulfite reductase between the chromatophores and soluble protein of extracts of *Chromatium* and *Chlorobium* is shown in Table 1. Both crude extracts and soluble protein exhibited some hydrogen utilization in the absence of sulfite and the endogeneous substrate for this activity could not be eliminated by dialysis of the extracts. After removal of the chromatophores by centrifugation, the total sulfite reductase activity was not significantly decreased and clearly resided in the soluble protein. After resuspending the unwashed chromatophores in 1/2 volume of 0.2 M Tris (pH 8.0), we found negligible sulfite reductase activity in the preparation. We could conclude that, in contrast to adenylyl sulfate reductase (4), sulfite reductase was located in the soluble protein of these bacteria.

The specific activities of sulfite reductase in the soluble protein of various *Rhodospirillaceae*, *Chromatiaceae*, and *Chlorobiaceae* are shown in Table 2. In each assay, the formation of cadmium sulfide in the center wall of the Warburg vessels qualitatively paralleled hydrogen uptake in the presence of sulfite. The chromatophore fraction of each organism was also assayed for sulfite reductase, but in no case was significant activity found.

The specific activities of sulfite reductase range from less than 1×10^{-3} in *Rps. viridis* to 7×10^{-3} μ mole of H_2 per min mg of protein in the *Chlorobium* PM extract. The variation in activities may be owing to the fact that the cells were grown and stored for different lengths of time, and so reflect enzyme stability; however, extracts of freshly grown *R. rubrum* showed roughly the same specific activities as those from frozen cells. The efficacy of NADPH₂ as electron donor was also tested, but in no case was any sulfite-dependent oxidation of reduced NADPH₂ observed. Thus, the sulfite reductase in photosynthetic bacteria could be of the low-molecular-weight (60,000) plant type, which utilizes reduced methyl

TABLE 2. Specific activity of sulfite reductase in extracts of various photosynthetic bacteria

Microorganism	Sulfite reductase (μ mole of H_2 per min/mg of protein $\times 10^{-3}$)*
<i>Rhodospseudomonas viridis</i>	0.74
<i>Rhodomicrobium vannielii</i>	6.5
<i>Rhodospirillum rubrum</i>	4.7
<i>Rhodospseudomonas gelatinosa</i>	3.7
<i>Rhodospseudomonas palustris</i>	3.74
<i>Chromatium</i> strain D (autotrophically grown)	4.2
<i>Chromatium</i> strain D (heterotrophically grown)	2.1
<i>Chlorobium</i> P.M.	7.0
<i>Chlorobium</i> T	2.6

* Values should be multiplied by 10^{-3} as indicated.

viologen (probably ferredoxin physiologically) but not NADPH₂ as electron donor and which lacks FAD and FMN, rather than the high molecular weight (685,000) enterobacterial type, which utilizes both NADPH₂ and reduced methyl viologen as electron donors and contains equimolar amounts of FAD and FMN (15).

The levels of sulfite reductase found in these photosynthetic bacteria are comparable to the specific activities found in extracts of most organisms that carry out biosynthetic ("assimilatory") sulfate reduction, e.g., *E. coli* (15), *Aspergillus nidulans* (20), *Porphyra yezoensis* (21), and *C. pasteurianum* (17), but generally higher than the specific activities of sulfite reductase found in green plants (22). The specific activity of sulfite reductase (desulfovireidin) from the sulfate-reducing bacteria (16) wherein the enzyme serves a respiratory ("dissimilatory") function is 10-fold higher than those found in the photosynthetic bacteria. In addition, adenylyl sulfate reductase, which appears to be involved in photosynthetic energy metabolism, is reported to have a specific activity of 100–500 (same units as in Table 2) in *Chlorobium* and *Chromatium* and to occur in the chromatophore fraction (4). ADP sulfurylase, which is also involved in energy metabolism has a specific activity of 80 (6). Two other enzymes of uncertain involvement in sulfur metabolism, thiosulfate reductase and rhodanese (23), have been studied in photosynthetic bacteria in some detail. Both are found in the soluble protein fraction and exhibit about the same specific activities as sulfite reductase. These comparisons, as well as nutritional studies, suggest that sulfite reductase is involved physiologically in the assimilation of sulfate and is not responsible for the oxidation of sulfide to sulfate. This conclusion is also supported by the fact that sulfite reductase is present in *Rhodospirillaceae*, which are unable to oxidize sulfide further than to elemental sulfur (8). The presence of sulfite reductase in autotrophically grown *Chromatium* indicates that it is not subject to feedback control, as appears to be the case for this enzyme from *C. pasteurianum* (17). It has been reported that *Chlorobium* is unable to grow on sulfate and does not catalyze "assimilatory" sulfate reduction (10). The presence of both adenylylsulfate reductase and sulfite reductase in the *Chlorobiaceae* indicates that the ability to reduce sulfate should be reinvestigated in these bacteria.

It has often been suggested that sulfate reduction is a very ancient process, particularly based on the fractionation of sulfur isotopes (24). This is consistent from a biochemical point of view with the demonstration (14) that the siroheme of sulfite reductase is a derivative of uroporphyrin III, the first porphyrin in the heme biosynthetic pathway, rather than protoporphyrin IX. If one accepts the inference (14) that all sulfite reductases contain this newly described siroheme, then it seems reasonable to conclude that the ability to biosynthesize porphyrins was acquired at a very early stage of evolution. While the presence of sulfite reductase in the photosynthetic green and purple sulfur bacteria, as well as in the clostridia and sulfate-reducing bacteria, accords with such a notion, there is an interesting complication introduced, however, in the case of the *Rhodospirillaceae*, which exhibit a *c*-type cytochrome complement strongly homologous with mitochondrial cytochrome *c* (25, 26). The further characterization of the sulfite reductase enzymes in the various photosynthetic bacterial groups and comparative studies of their structural and functional properties should provide new insights into the evolution of these microorganisms.

We gratefully acknowledge financial support of these studies by grants from the National Institutes of Health (GM-18528 to M.D.K.) and the National Science Foundation (GB-36006 to H.D.P. and GB-36019X to M.D.K.).

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