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Composition of the Sulfur Particle of *Chromatium vinosum* Strain D

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Sulfur particles were isolated from the purple sulfur photosynthetic bacterium, *Chromatium vinosum* strain D. The composition of these particles was determined to be 93% sulfur, 5% protein, and 0.6% lipid. Gel electrophoresis indicated the presence of a single protein species with a molecular weight of 13,500 daltons. From these results, the sulfur particle is postulated to be bounded by a membrane consisting entirely of protein.

Intracellular storage of sulfur by members of the family *Thiorhodaceae* was first reported by Winogradsky (21) and, later, elaborated upon by van Niel (17, 18). The molecular bases of the storage and subsequent metabolism of intracellular sulfur are unknown. Information regarding the structure of the intracellular sulfur particles has come from Hageage et al. (7), who presented X-ray diffraction evidence that the sulfur was present in a liquid form, and from Kran et al. (9) and Schmidt and Kamen (13), who noted a thin "membrane" surrounding the sulfur in sections of *Chromatium*. The electron microscopic evidence of Nicolson and Schmidt (12) greatly clarifies the nature of the sulfur particle and confirms the evidence presented here that the sulfur particle membrane consists entirely of protein.

MATERIALS AND METHODS

Chromatium vinosum strain D was grown autotrophically as previously described (13).

Sulfur particles. Cells were harvested by centrifugation for 10 min at 20,000 × *g* and suspended in potassium phosphate buffer (0.15 M, *p*H 7.5) in a ratio of 4 ml of buffer per g (wet weight) of cells. Cell disruption was carried out with a Ribi cell fractionator (Ivan Sorvall, Inc., Newton, Conn.), operating at 20,000 psi. Sulfur particles were isolated by centrifugation of the broken cell suspension (10 min at 1,000 × *g*). The pellet was freed of contaminating material by repeated suspension in distilled water and centrifugation.

Classical chromatophores. Chromatophores were prepared by the method of Cusanovich and Kamen (3).

Sulfur. Sulfur particles were suspended in 95% ethanol for 24 hr. Sulfur was determined from the absorbance at 264 nm.

Protein. Sulfur particles were freed of sulfur by extraction with chloroform. Protein was then determined by the biuret assay (6), modified to include a 4-hr reaction time. Samples were prepared for amino acid analysis by suspending dried sulfur particles in 6 N NCl, evacuating and sealing the hydrolysis tubes, and heating the tubes 24 hr at 110 C. Duplicate samples were run, one of which had been oxidized with performic acid to preserve cysteine as cysteic acid (8). Analyses were performed on a Beckman amino acid analyzer (5).

Bacteriochlorophyll. Bacteriochlorophyll was determined by the method of van Niel and Arnold (19).

Lipid. Dried sulfur particles (10 mg) were extracted twice with 1 ml of chloroform-methanol (2:1). Acetic acid (0.01 M, 0.8 ml) was added, and the aqueous phase was removed. The chloroform layer was washed twice with methanol-0.01 M acetic acid (1:1), dried with sodium sulfate, and evaporated under nitrogen. Sulfuric acid (2% in methanol, 0.5 ml) was added to the dried lipid fraction, and the methyl esters of the fatty acids were prepared by heating at 65 C for 5 hr. The residue was washed with methanol and extracted with hexane. The hexane was dried with sodium sulfate and evaporated under nitrogen. This fraction contained both fatty acid methyl esters and paraffins. These were separated by thin-layer chromatography on silica gel with a solvent system of benzene-hexane (1:1). Gas chromatography of the methyl esters was performed on a Varian Aerograph 200, by using a 6-ft (~1.8 m) column packed with 1% SE-30 on Gas-Chrom Q (100-200 mesh; Applied Science Laboratories, Inc., State College, Pa.). Nitrogen was employed as the carrier gas. The identity of the components was determined from the retention times of standard fatty acid methyl esters. Total weight of lipid was calculated from the detector response to known amounts of methyl stearate, based on the assumption that phosphatidyl glycerol with one 16-carbon and one 18-carbon fatty acid was the only lipid present.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Sodium dodecyl sulfate (SDS) gels were prepared by the procedure of Weber and Osburn (20). Protein samples were treated with 1% 2-mercaptoethanol, 4 M urea, and 1% SDS after the procedure of Dunker and Rueckert (4). Electrophoresis was carried out for 2.5 hr at 15 ma per gel. Gels were stained with Coomassie brilliant blue by the procedure of Chrambach et al. (2) and destained in 7% acetic acid. The gels were then

photographed by using Polaroid 55 P/N film, and densitometer traces of the negatives were made with a Joyce-Loebl microdensitometer. Horse heart cytochrome *c*, myoglobin, chymotrypsinogen, apoferritin, and ovalbumin were employed as molecular-weight standards.

Phenol-acetic acid-urea polyacrylamide gel electrophoresis. Gels were prepared by the method of Takayama et al. (16), and protein samples were prepared by the method of Baum et al. (1). Electrophoresis was carried out for 1.5 hr at 5 ma per gel. Gels were stained and traced as described above.

Electron microscopy. Negative staining of sulfur particle protein was performed on carbon-collodion-coated grids with 0.5% uranyl formate (*p*H 4.7). Electron microscopy was performed on a Phillips electron microscope (model EM-300) at 60 kv accelerating voltage.

RESULTS

Sulfur particle composition. The overall composition of the isolated sulfur particles is presented in Table 1. The high ratio of protein to lipid suggests that the presence of a protein-lipid membrane around the sulfur particle is unlikely and that the lipid probably results from contamination by chromatophores, which are known to associate strongly with the sulfur particle. Evidence for this view is presented in Tables 2 and 3. The similarity of bacteriochlorophyll-to-lipid ratios and fatty acid analyses indicates that intracellular sulfur is enveloped by sacks consisting entirely of protein. The ratio of bacteriochlorophyll to lipid in the sulfur particle preparation most closely resembles that of the light particles of Cusanovich and Kamen (3), a highly purified chromatophore fraction. This is in agreement with the results of Nicolson and Schmidt (12) which show that sulfur particle contamination consists primarily of isolated chromatophores rather than membrane fragments.

TABLE 1. *Per cent dry weight of the components of the sulfur particle*

Component	Dry wt (%)
Sulfur	93.5 ± 1.4
Protein	5.2 ± 0.3
Lipid	0.55
Bacteriochlorophyll	0.047

Protein characterization. Densitometric traces of the polyacrylamide gels are presented in Fig. 1. Protein solubilized from the sulfur particle migrated as a single band in the SDS gels. The

TABLE 2. *Ratio of bacteriochlorophyll to lipid in sulfur particles and subcellular preparations*

Cellular fraction	Bacteriochlorophyll/lipid
Sulfur particles	0.085
Classical chromatophores	0.20–0.33
Heavy particles[a]	0.15–0.22
Light particles[a]	0.11–0.14
Chromatophores[b]	0.034
Small particles[b]	0.035

[a] Calculated from the results of Cusanovich and Kamen (3).

[b] Calculated from the results of Newton and Newton (11).

TABLE 3. *Fatty acid analysis of sulfur particles and chromatophores*

Fatty acid	Per cent of total fatty acid	
	Sulfur particle	Chromatophore
14:0	2.4	1.3
14:1	0.8	0.6
16:0	24.3	23.1
16:1 (Palmitoleic)	29.1	26.8
18:0	4.3	5.5
18:1 (Vaccenic)	39.0	42.7

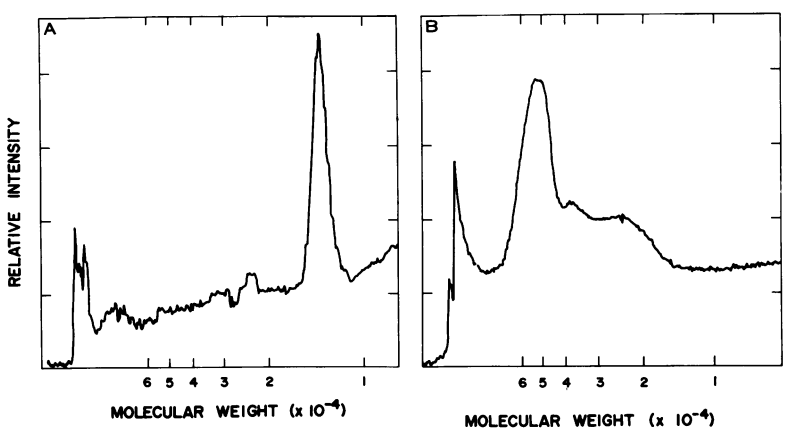

FIG. 1. *Polyacrylamide gel electrophoresis of sulfur particle protein. (A) SDS system. (B) Phenol-acetic acid-urea system. Identical results were obtained whether or not the protein was freed of sulfur by chloroform extraction.*

molecular weight was determined as 13,500 ± 1,000 daltons. In the phenol-acetic acid-urea system, the protein band migrated much more slowly. Although this system did not provide accurate molecular-weight data, the mobility relative to standards suggested a species with a molecular weight of 40,000 to 60,000 daltons.

The results of the amino acid analysis of the sulfur particle protein are presented in Table 4. The minimum molecular weight calculated from these results, minus the contributions of tryptophan, methionine, and the ammonia lost from glutamine and asparagine, is 6,130 daltons. The gel electrophoresis results indicate that the actual molecular weight is 12,260 daltons.

Electron microscopy. Protein solubilized from sulfur particles with 2 M urea and 10 mM 2-mercaptoethanol was observed to aggregate into sheets when the urea was dialyzed out. Electron micrographs of these sheets are presented in Fig. 2 and 3. The sheet shown in Fig. 2 is much larger than the size necessary to surround a sulfur particle (12).

TABLE 4. *Amino acid analysis of the sulfur particle protein*[a]

Amino acid	Molar ratio
Cysteic acid	0.55
Aspartic acid	3.53
Threonine	1.49
Serine	2.06
Glutamic acid	7.80
Proline	3.15
Glycine	6.20
Alanine	4.50
Valine	1.67
Isoleucine	1.00
Leucine	2.36
Tyrosine	3.17
Phenylalanine	1.92
Histidine	0.81
Lysine	1.29
Arginine	2.38

[a] Molar ratios, based on isoleucine, are the average of the analyses of three preparations.

DISCUSSION

The *Chromatium* sulfur particle shares a unique position with the gas vacuoles of *Halobacterium halobium* (15) and *Anabaena flos-aquae* (14) in having a "membrane" which consists solely of protein. The results presented here suggest that the *Chromatium* membrane consists of a single protein with a molecular weight of approximately 13,500 daltons, which is apparently capable of self-aggregation. Although the exact function of this protein sack remains unknown, some reasonable speculation seems justified. The storage and mobilization of sulfur by *Chromatium* requires enzymatic systems capable of handling a relatively inaccessible substrate. These could presumably be present in the membrane surrounding the sulfur, in analogy to the poly-β-hydroxybutyrate granule (10), or in the photosynthetically active chromatophore membrane. In a test of these alternatives (G. L. Schmidt, Ph.D. Thesis, 1970), the reaction of sulfur in the sulfur particle with a nucleophilic agent, cyanide ion, was found to be nonenzymatic. Consequently, the sulfur particle protein is presumed to have a passive role in sulfur metabolism. In view of the conclusion of Nicolson and Schmidt (12) that the membrane is a unimolecular protein layer, it is unlikely that the protein provides a hydrophobic boundary which facilitates the synthesis and degradation of the sulfur polymers presumed to be intermediates in sulfur metabolism. It is more reasonable to assume that the protein acts primarily as a barrier to separate the sulfur from the interior of the cell and, perhaps, to provide binding sites for the enzymes responsible for sulfur metabolism.

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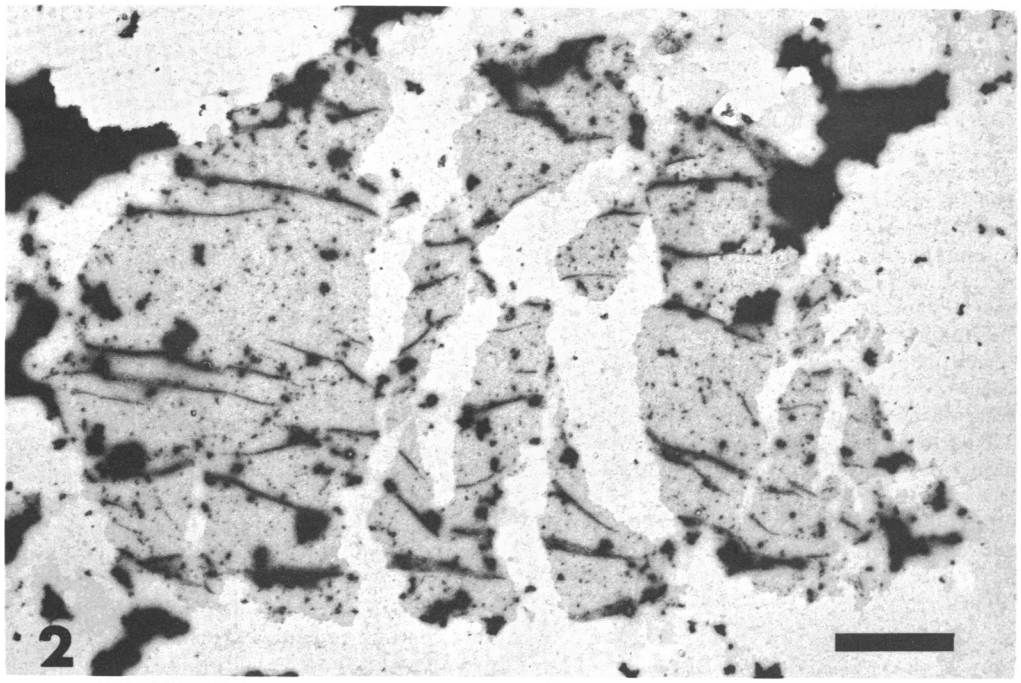

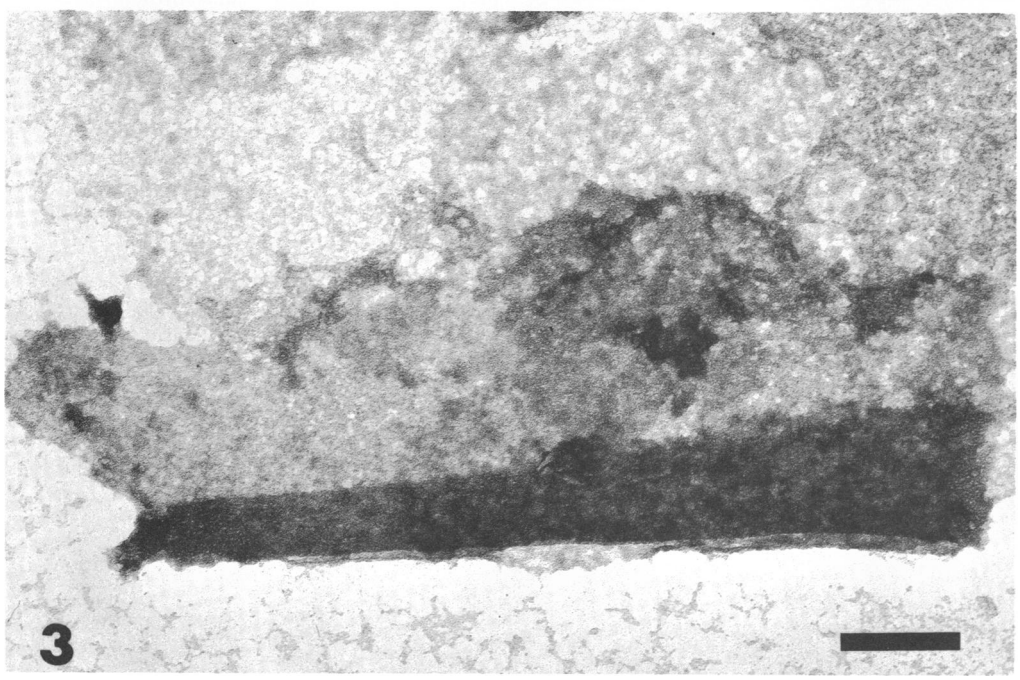

FIG. 2. *Chromatium sulfur particle protein solubilized in 2 M urea-10 mM 2-mercaptoethanol and dialyzed against several changes of 20 milliosmol phosphate buffer. The dialysate was dried down on a carbon-collodion film in the presence of 0.5% uranyl formate. Bar equals 1 μm.*

FIG. 3. *Legend is the same as for Fig. 2. Bar equals 0.1 μm.*

LITERATURE CITED

1. Baum, H., H. I. Silman, J. S. Rieske, and S. H. Lipton. 1967. On the composition and structural organization of complex III of the mitochondrial electron transport chain. J. Biol. Chem. **242**:4876–4887.
2. Chrambach, A., R. A. Reisfeld, M. Wyckoff, and J. Zaccari. 1967. A procedure for rapid and sensitive staining of protein fractionated by polyacrylamide gel electrophoresis. Anal. Biochem. **20**:150–154.
3. Cusanovich, M. A., and M. D. Kamen. 1968. Light-induced electron transport in *Chromatium* strain D. I. Isolation and characterization of *Chromatium* chromatophores. Biochim. Biophys. Acta **153**:376–396.
4. Dunker, A. K., and R. R. Rueckert. 1969. Observations on molecular weight determinations on polyacrylamide gel. J. Biol. Chem. **244**:5074–5080.
5. Dus, K., S. Lindroth, R. Pabst, and R. M. Smith. 1966. Continuous amino acid analysis: elution programming and automatic column selection by means of a rotating valve. Anal. Biochem. **14**:41–52.
6. Gornall, A. G., C. J. Bardawill, and M. M. David. 1949. Determination of serum proteins by means of the biuret reaction. J. Biol. Chem. **177**:751–766.
7. Hageage, G. J., E. D. Eanes, and R. L. Gherna. 1970. X-ray diffraction studies of the sulfur globules accumulated by *Chromatium* species. J. Bacteriol. **101**:464–469.
8. Hirs, C. H. W. 1967. Determination of cysteine as cysteic acid, p. 59–62. *In* S. P. Colowick and N. O. Kaplan (ed.), Methods in enzymology, vol. 11. Academic Press Inc., New York.
9. Kran, G., F. W. Schlote, and H. G. Schlegel. 1963. Cytologische Untersuchungungen an *Chromatium okenii* Perty. Naturwissenschaften **50**:728–730.
10. Merrick, J., and M. Douderoff. 1964. Depolymerization of poly-β-hydroxybutyrate by an intracellular enzyme system. J. Bacteriol. **88**:60–71.
11. Newton, J. W., and G. A. Newton. 1957. Composition of the photoreactive subcellular particles from *Chromatium*. Arch. Biochem. Biophys. **71**:250–265.
12. Nicolson, G. L., and G. L. Schmidt. 1971. Structure of the Bacteriol. **105**:1142–1148.
13. Schmidt, G. L., and M. D. Kamen. 1970. Variable cellular composition of *Chromatium* in growing cultures. Arch. Mikrobiol. **73**:1–18.
14. Smith, R. V., A. Peat, and C. J. Bailey. 1969. The isolation and characterization of gas cylinder membranes and α-granules from *Anabaena flos-aquae* D124. Arch. Mikrobiol. **65**:87–97.
15. Stoeckenius, W., and W. H. Kunau. 1968. Further characterization of particulate fractions from lysed cell envelopes of *Halobacterium halobium* and isolation of gas vacuole membranes. J. Cell Biol. **38**:337–357.
16. Takayama, K., D. H. MacLennan, A. Tzagoloff, and C. D. Stoner. 1964. Studies on the electron transfer system. LXVII. Polyacrylamide gel electrophoresis of the mitochondrial electron transfer complexes. Arch. Biochem. Biophys. **114**:223–230.
17. van Niel, C. B. 1932. On the morphology and physiology of the purple and green sulfur bacteria. Arch. Mikrobiol. **3**: 1–112.
18. van Niel, C. B. 1936. On the metabolism of the *Thiorhodaceae*. Arch. Mikrobiol. **7**:323–358.
19. van Niel, C. B., and W. Arnold. 1938. The quantitative estimation of bacteriochlorophyll. Enzymologia **5**:244–250.
20. Weber, K., and M. Osburn. 1969. The reliability of molecular weight determinations by dodecyl sulfate-polyacrylamide gel electrophoresis. J. Biol. Chem. **244**:4406–4412.
21. Winogradsky, S. N. 1887. Über Schwefelbakterien. Bot. Ztg. **45**:489–508.