AN IRON TETRAHYDROPORPHYRIN PROSTHETIC GROUP COMMON TO BOTH ASSIMILATORY AND DISSIMILATORY SULFITE REDUCTASES*

Matthew J. Murphy, Lewis M. Siegel, and Henry Kamin
Department of Biochemistry, Duke University Medical Center
and the Veterans Administration Hospital
Durham, North Carolina 27710

Daniel V. DerVartanian, Jin-Po Lee, Jean LeGall, and Harry D. Peck, Jr.
Department of Biochemistry, University of Georgia
Athens, Georgia 30601

Received July 2, 1973

SUMMARY

The heme† chromophore of the "assimilatory" *E. coli* sulfite reductase is an iron-octacarboxylic tetrahydroporphyrin of the isobacteriochlorin type (1). Although the two "dissimilatory" sulfite reductases, desulfoviridin and desulforubidin, from the sulfate reducing bacteria *Desulfovibrio gigas* and *Desulfovibrio desulfuricans* (Norway strain), have absorption spectra and reaction products which differ from those of *E. coli* sulfite reductase, the present studies indicate that they contain prosthetic groups with an organic structure closely similar or identical to that of the *E. coli* sulfite reductase heme. EPR spectra show high-spin ferriheme in all three enzymes. It is clear, however, that the prosthetic groups must reside in substantially different environments within their respective proteins.

INTRODUCTION

Assimilatory and dissimilatory sulfite reductases serve different roles in nature. While assimilatory sulfite reduction is a relatively minor pathway, primarily for the biosynthesis of sulfur amino acids, dissimilatory sulfite reduction is a large scale process linked, in sulfate-reducing bacteria, to cellular energy requirements (2). In addition, the products of sulfite reduction as catalyzed by assimilatory and dissimilatory enzymes appear to differ. Thus, while the assimilatory enzyme, *E. coli* sulfite reductase, catalyzes the stoichiometric conversion of sulfite to sulfide (3), the dissimilatory enzymes

---

*This work was supported by the following grants: AM-13460 (L.S.), AM-040662 (H.K.), GM-18895 and Res. Career Dev. Award 70010 (D.V.) from the N.I.H.; 7875 (L.S.) from the Vet. Admin.; GB-7905 (H.K.), GB-291618 (J.L.), and GB-36006X (H.P.) from the N.S.F.

†The terms "heme" and "porphin" are used as defined in Ref. 1 and 6.

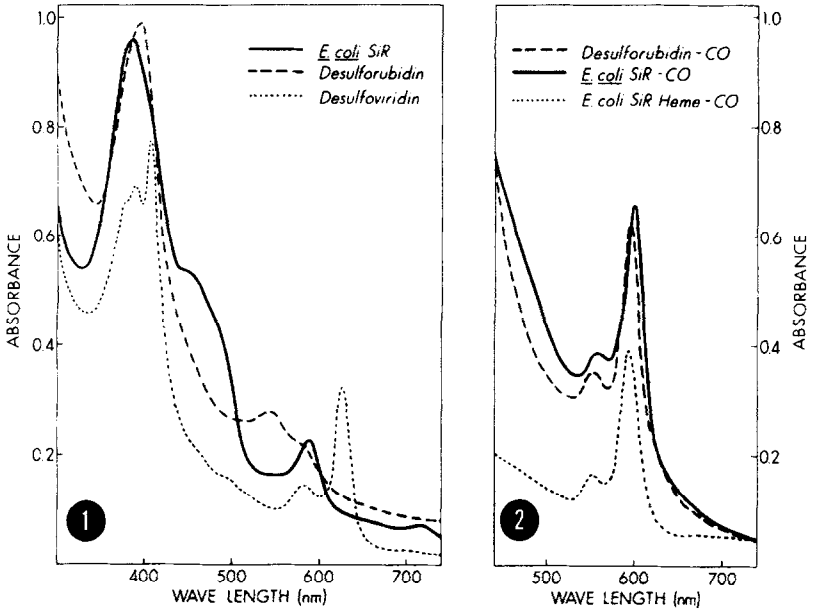

Fig. 1. Absorption spectra of assimilatory and dissimilatory sulfite reductases. Proteins were in std. buffer. *E. coli* sulfite reductase (SiR), 3.1 μM ——; desulforubidin, 5.0 μM ----; desulfoviridin, 3.9 μM ····.

Fig. 2. Absorption spectra of CO complexes. Desulforubidin was diluted into a CO-saturated solution of std. buffer to a final concentration of 0.93 μM and $Na_2S_2O_4$ was added. The absorbance of the desulforubidin-CO complex is multiplied by a factor of 10. *E. coli* sulfite reductase (SiR)-CO complex was prepared as described previously (6) using NADPH as reductant. The CO complex of the extracted *E. coli* sulfite reductase heme was prepared as described previously (6) using $Na_2S_2O_4$ as reductant.

of *Desulfovibrio* appear to yield a variety of products, predominately trithionate (4,5). The assimilatory sulfite reductases purified to date have exhibited a prominent absorption maximum in the 580-90 nm region (6), but the dissimilatory sulfite reductases have yielded a more diverse set of spectra. Thus, the absorption spectrum of *E. coli* sulfite reductase (Fig. 1) has maxima at 386 and 587 nm due to the heme component, plus a shoulder at 455 nm due to flavin. In contrast, desulforubidin and desulfoviridin, dissimilatory sulfite reductases from two different strains of *Desulfovibrio* [*D. desulfuricans*, Norway strain (8), and *D. gigas* (7), respectively] have spectra (Fig. 1) with the wavelength maxima: desulforubidin-392, 545, and 580 (shoulder) nm; desulfoviridin-380 (shoulder), 390, 408, 583, and 628 nm. Furthermore, desulfoviridin yields a highly fluorescent chromophore upon extraction (5,9).

Thus, the three sulfite reductases have diverse spectral properties, suggesting a diversity of prosthetic groups; Postgate (9) has postulated that the desulfoviridin prosthetic group is a highly carboxylated chlorin.

This paper will demonstrate that, despite these differences, desulfoviridin and desulforubidin bear tetrapyrrolic prosthetic groups which are similar or identical in their organic moiety to that of the *E. coli* sulfite reductase heme. The latter has recently been identified as a novel iron-containing octacarboxylic tetrahydroporphyrin of the isobacteriochlorin type (1).

MATERIALS AND METHODS

Chemicals (1), *E. coli* sulfite reductase (6), desulfoviridin (7), and desulforubidin (8) were obtained as described previously. Enzymes were extensively dialyzed *vs.* 0.05 M potassium phosphate-0.1 mM EDTA, pH 7.7 (std. buffer), before use. Absorption spectra were measured in a Cary model 14 spectrophotometer at room temperature in 1 cm light path cuvettes against appropriate solvent blanks. Demetallization, esterification, and chromatographic procedures have been described elsewhere (1, 10). ε_{386} for *E. coli* sulfite reductase is 3.1 x 10^5 $M^{-1}cm^{-1}$ (6). ε_{408} for desulfoviridin and ε_{392} for desulforubidin both had the value 2.0 x 10^5 $M^{-1}cm^{-1}$, based on protein content measured by a modified biuret method (6) and published molecular weights (7,8).

RESULTS AND DISCUSSION

Complex formation: Although absorption spectra of desulforubidin and *E. coli* sulfite reductase differ, both enzymes yield CO complexes with similar spectra (Fig. 2). The *E. coli* enzyme as reported (6) has bands at 600 and 556 nm; desulforubidin has bands at 593 and 550 nm. The desulforubidin-CO spectrum, although shifted slightly from that of the *E. coli* holoenzyme-CO complex, is quite similar to that of the CO complex of the free heme derived from the latter enzyme. These observations suggest that the desulforubidin prosthetic group is similar to that of *E. coli* sulfite reductase; this will also be apparent from the data to follow. Treatment of desulfoviridin with a number of reducing agents and CO has not yielded a CO complex.

Extraction of enzyme chromophores: Desulforubidin was extracted with acetone-0.015 N HCl. The spectrum of the extract (Fig. 3) has maxima at 375

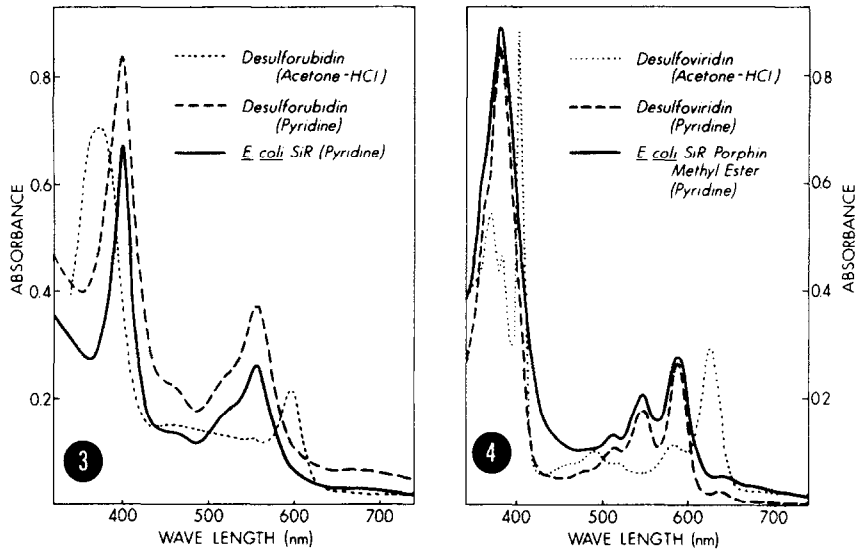

Fig. 3. 0.5 ml of 54 μM desulforubidin in std. buffer was added to 4.5 ml of 0.015 N HCl in acetone at 0°. The absorption spectrum of the supernatant fluid after centrifugation was recorded ····. The desulforubidin chromophore was transferred to pyridine and chromatographed as described previously for the *E. coli* sulfite reductase chromophore (6) and the absorption spectrum recorded ----. The absorption spectrum of the extracted heme of *E. coli* sulfite reductase in pyridine is shown for comparison ——.

Fig. 4. 0.5 ml of 44 μM desulfoviridin was added to 4.5 ml of 0.015 N HCl in acetone at 0°. The absorption spectrum of the supernatant fluid after centrifugation was recorded ·····. The desulfoviridin chromophore was transferred to pyridine and chromatographed as described for the *E. coli* sulfite reductase heme (6) ----. An absorption spectrum of the *E. coli* sulfite reductase porphin methyl ester in pyridine is shown for comparison ——.

and 594 nm, with a ratio of 3.4. Extraction of *E. coli* sulfite reductase yields a heme with a virtually identical spectrum (6). The extracted desulforubidin chromophore upon transfer to pyridine by methods outlined previously (1) yielded a spectrum (Fig. 3) with maxima at 401 and 557 nm, with a ratio of 2.3, and a shoulder at 520 nm. This spectrum is strikingly similar to that of the *E. coli* sulfite reductase heme (Fig. 3). We conclude from these data, as well as from the CO spectra, that the prosthetic group of desulforubidin is a heme compound of the type found in the *E. coli* sulfite reductase.

When desulfoviridin was extracted with acetone-0.015 N HCl, a highly fluorescent supernatant was obtained; an absorption spectrum of the extract is shown in Fig. 4. Upon transfer of the chromophore to pyridine, a spectrum was obtained with the following maxima: 378, 510, 545, 588, and 638 nm

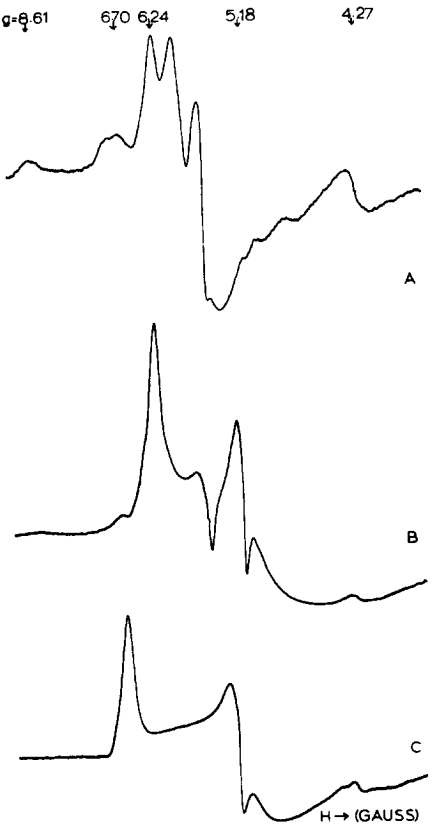

Fig. 5. EPR spectra of sulfite reductases. A, desulfoviridin (166 μM), gain = 300. B, desulforubidin (142 μM), gain = 100. C, *E. coli* sulfite reductase (66 μM), gain = 100. EPR conditions and instrumentation as in Ref. 11 and as follows: temperature, 8.5°K; frequency, 9.163 GHz; scanning rate, 400 gauss/min; microwave power, 10 mW; modulation amplitude, 5.9 G; time constant, 0.3 sec.

(ratio of absorbances: 1.00, 0.12, 0.21, 0.31 and 0.03). These are characteristic of the spectrum of a tetrahydroporphyrin of the isobacteriochlorin type (adjacent pyrrole rings reduced) and the spectrum is virtually identical to that reported previously (1), and reproduced in Fig. 4, for the pyridine solution of the porphin obtained by demetallization of the *E. coli* sulfite reductase heme. Desulfoviridin chromophore methyl ester was also prepared; its absorption spectrum in the basic solvent piperidine is identical to that of the non-esterified porphin.

Comparison of porphin methyl esters: A porphin methyl ester was also prepared from desulforubidin heme, by demetallization and esterification. The

desulforubidin and desulfoviridin porphin methyl esters were found essentially identical to the porphin methyl ester derived from *E. coli* sulfite reductase heme with respect to the following properties (described in Ref. 1): absorption spectra in acidic (methanol-5% H_2SO_4) and basic (piperidine) solvents; fluorescence spectra in acidic (2 N HCl) and basic (piperidine) solvents; and chromatographic behavior on two different thin layer systems.

EPR: Extraction of a porphin from desulfoviridin, under conditions in which a corresponding heme is extracted from desulforubidin and *E. coli* sulfite reductase, raises the question as to whether the tetrapyrrole prosthetic group of desulfoviridin contains a metal when bound to the enzyme. To obtain information on this question, EPR spectra were measured at 8.5°K for each of the three sulfite reductases. As shown in Fig. 5, each enzyme exhibits complex "split" g = 6 type signals, characteristic of high-spin ferriheme in differing rhombic field strengths. Predominant signals and their tentatively assigned g-values were: 6.70, 5.24, and ca. 4.9 (broad) for *E. coli* sulfite reductase; 6.24, 5.60, 5.18, and ca. 4.6 (broad) for desulforubidin; and 6.24, 5.76, 5.62, and 5.44 for desulfoviridin. One of many possible explanations for the complex and varying degrees of rhombic splitting indicated by the spectra may be found in differences in local environmental orientations greatly affecting the aplanarity of the heme systems. It is interesting to note that desulforubidin and *E. coli* sulfite reductase, which show similarities in ligand reactivity (e.g., reactivity with CO) reveal considerable similarity in general EPR features. Preliminary determination of the extractable chromophore content of desulfoviridin has yielded 1-2 moles per mole enzyme [based on ε_{588} = 24,000 for the porphin methyl ester in pyridine, established by the insertion of iron into the compound to form the corresponding heme (10, and Murphy and Siegel, unpublished data)], while quantitation of the desulfoviridin EPR signal with respect to Cu-EDTA, yielded 0.8 moles heme per mole enzyme. Thus, the heme content of desulfoviridin must be considered significant with respect to its content of extractable tetrapyrrole prosthetic group.

We conclude then that the three sulfite reductases examined contain similar types of iron tetrahydroporphyrin prosthetic group. It is evident from the diverse EPR and absorption spectra of the three enzymes and the fact that the chromophore from desulfoviridin is extracted as a porphin that these prosthetic groups must exist in very different environments within their respective proteins.

REFERENCES

1. Murphy, M. J., Siegel, L. M., Kamin, H., and Rosenthal, D. (1973) J. Biol. Chem., 248, 2801-2814.
2. Peck, H. D., Jr., (1966) Biochem. Biophys. Res. Commun., 22, 112-118.
3. Siegel, L. M., and Kamin, H., (1968) "Flavins and Flavoproteins" (K. Yagi, ed.), p. 15-40, University Park Press, Baltimore.
4. Lee, J. P., and Peck, H. D., Jr. (1971) Biochem. Biophys. Res. Commun., 45, 583-589.
5. Kobayashi, K., Takahashi, E., and Ishimoto, M. (1972) J. Biochem. (Tokyo), 72, 879-887.
6. Siegel, L. M., Murphy, M. J., and Kamin, H. (1973) J. Biol. Chem., 248, 251-264.
7. Lee, J. P., Yi, C. S., LeGall, J., and Peck, H. D., Jr. (1973) J. Bacteriol., 115, 453-455.
8. Lee, J. P., LeGall, J., and Peck, H. D., Jr. (1973) J. Bacteriol., 115 529-542.
9. Postgate, J. R. (1956) J. Gen. Microbiol., 14, 545-572.
10. Murphy, M. J., and Siegel, L. M., (1973) J. Biol. Chem. in press.
11. DerVartanian, D. V., Iburg, L. K., and Morgan, T. V. (1973) Biochim. Biophys. Acta, 305, 173-178.