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Dithionite Reduction in the Presence of a Tetrapyrrole-containing Fraction from the Desulfoviridin of *Desulfovibrio gigas*

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Abstract

Low-molecular-weight fractions obtained from the desulfoviridin of *D. gigas* and from the growth medium of *Desulfovibrio* sp. 10455 promoted the reduction of sodium dithionite to sulphide in the presence of reduced methylviologen. These fractions contained a tetrapyrrole of the isobacteriochlorin type which was not complexed with iron, nor was it complexed with protein. The observations are discussed in relation to the function of sulphite reductases in the sulphate-reducing bacteria.

Introduction

The dissimilatory sulphite reductase of *Desulfovibrio gigas* is a green protein (Lee and Peck 1971; Kobayashi *et al.* 1972; Jones and Skyring 1974) known as desulfoviridin. In addition, it has been shown that desulfoviridin and P582 (the dissimilatory sulphite reductase from *Desulfotomaculum nigrificans*) catalyse the reduction of sodium dithionite ($Na_2S_2O_4$) to hydrogen sulphide (Skyring and Trudinger 1972). Siegel *et al.* (1973) also record that a pronounced odour of H_2S could be detected following the addition of $Na_2S_2O_4$ to the sulphite reductase of *Echerichia coli* B. The chromophore of desulfoviridin from *Desulfovibrio* species has been extracted only as a metal-free tetrapyrrole (Postgate 1956; Murphy and Siegel 1973) which is known as sirohydrochlorin (Murphy and Siegel 1973) and in chemical studies no haem could be detected by the pyridine haemochrome technique (Postgate 1956; R. J. Porra, personal communication). However, e.p.r. spectra recorded at 8°K suggest that an appreciable amount of a haem may be present in desulfoviridin (Murphy *et al.* 1973*a*). Murphy and Siegel (1973) suggested that the prosthetic group may not be a haem but an intermediate iron–sirohydrochlorin complex with some properties characteristic of haems and others more typical of some metal-free tetrapyrroles. The role, if any, of the metal-free tetrapyrrole of desulfoviridin in sulphite reduction is not known. It has been shown that the assimilatory sulphite reductase of *E. coli* B has an iron–isobacteriochlorin prosthetic group, sirohaem (Murphy and Siegel 1973), which appears to be involved in enzymic activity (Siegel *et al.* 1974).

A strain of *Desulfovibrio* sp. [NCIB 10455 (Jones 1971)] was found to excrete an isobacteriochlorin-type pigment into the growth medium. Because of the apparent similarity between this pigment and the isobacteriochlorin from the desulfoviridin of *Desulfovibrio* species, a study of some of its properties was included in the present work. This isobacteriochlorin is spectrally different to the carboxylated porphyrin found intracellularly in cells of strain 10455 (Jones 1971).

The evidence presented here shows that a low-molecular-weight, water-soluble, isobacteriochlorin-containing fraction from the desulfoviridin of *D. gigas* and a similar compound from the spent growth medium of *Desulfovibrio* sp. 10455 promoted the reduction of $Na_2S_2O_4$ to sulphide in the presence of reduced methylviologen.

A preliminary report of this work has appeared elsewhere (Skyring 1975).

Materials and Methods

Pigments

D. gigas (National Collection of Industrial Bacteria, Torry Research Station, Aberdeen, Scotland, strain number 9332) was grown in the medium described by Jones and Skyring (1974) and *Desulfovibrio* sp. NCIB 10455 was grown in modified Starkey's medium (Jones 1971). *D. gigas* cells were disrupted by sonication, and partially purified 'G200' preparations of desulfoviridin were obtained as previously described (Jones and Skyring 1974). The pink-fluorescing compound which accumulated in the growth medium of strain 10455 was adsorbed onto Whatman DE52 anion-exchange cellulose, and equilibrated in 0·1 M KCl in 0·01 M tris–HCl buffer at pH 7·8. It was then washed with 0·3M and eluted with 0·6 M or 1·0 M KCl in 0·01 M tris–HCl buffer (pH 7·8), using a batchwise procedure.

Absorption Spectra

These were recorded using a Varian 635 spectrophotometer in normal (1-cm path) and gel-scanning modes.

Fluorescence Emission and Excitation Spectra

These were recorded on a fully corrected fluorescence spectrophotometer described by Boardman and Thorne (1968).

Fluorescence Polarization Measurements

These were carried out with a Leitz polaroid-type polarizer–analyser set, and for excitation and emission wavelengths a grating correction factor was used (Henningsen *et al.* 1974). For fluorescence polarization measurements the desulfoviridin preparations were excited with light at 575 nm and the fluorescence emission recorded at 605 nm to avoid intramolecular distortion of the polarization of the emitted light.

Gel Electrophoresis and Reduction Reactions in Gels

The disc-electrophoretic methods used were similar to those previously described (Skyring and Trudinger 1972). A 7% polyacrylamide gel, pH 9·1, was used and the direction of separation was from cathode to anode. The gels, after electrophoresis of the samples, were placed in screw-cap test tubes (100 by 11 mm) filled with a solution containing 0·1 M tris–HCl (pH 6·8), 0·06% methylviologen, 0·1% sodium dithionite and 0·01% ferrous sulphate. The methylviologen was reduced by the $Na_2S_2O_4$ to its deep-blue reduced form. In some instances one or more of these components were omitted. The caps were screwed down and the tubes incubated at 50°C for 30–45 min. After incubation, the gels, impregnated with deep-blue reduced methylviologen, were removed from the tubes. The gels were cleared of the blue colour by immersion at room temperature in a shallow layer of distilled water; at this stage the reduced methylviologen is oxidized to the colourless leuco form yielding a clear gel in which the sites of sulphide formation are visible as black FeS bands. Proteins in the gels were stained with aniline blue black (Canalco).

Reduction Reactions in Cuvettes

The reduction of $Na_2S_2O_4$ was carried out in 3·8-ml capacity, 1-cm pathlength, glass Thunberg-type cuvettes in the following way:

(1) Desulfoviridin (470 μg/ml) was hydrolysed in 0·1 M tris–HCl, pH 7·0, by pronase (1·0 mg/ml) (Ca^{2+} activated) at 50°C; the rate of hydrolysis was monitored by recording the decrease in extinction at 627 nm and when the rate of change was zero, the release of chromophore was taken as completed; 0·1 ml of the contents of the cuvette was transferred to another cuvette and the volumes of both made up to 2·0 ml with 0·1 M tris–HCl buffer, pH 7·0.

(2) Methylviologen was added to both cuvettes and the spectrum of the hydrolysed desulfoviridin preparations recorded.

(3) 1·0 mg of $Na_2S_2O_4$ was added to the side arms and the cuvettes were gassed for 20 min with O_2-free He.

(4) The contents of the cuvettes were then mixed with the $Na_2S_2O_4$ to give, instantaneously, reduced methylviologen concentrations of about $1{\cdot}4\times10^{-4}$ M (Trudinger 1970*a*) and the subsequent reaction(s) in each cuvette were monitored at 605 nm, which is the absorption maximum for reduced methylviologen.

(5) When the reduced methylviologen was oxidized, the cuvettes were gassed with O_2-free He obtained by passage through zinc-reduced methylviologen and the H_2S evolved was estimated with 0·1 mM 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) in 0·1 M tris–HCl, pH 8·0 (Ellman 1959; Trudinger 1970*b*). Spectra of the hydrolysed desulfoviridin were again recorded.

(6) For the cuvette containing the higher concentration of chromophore the side bulb was replaced with another containing 1·0 mg $Na_2S_2O_4$ and the procedure from steps 4 to 5 was repeated.

Zinc and Iron Content of Desulfoviridin

Purified desulfoviridin was prepared from polyacrylamide gels (Jones and Skyring 1974) and the metal components of the major band assayed with a Varian atomic absorption spectrophotometer by the method of Donnelly *et al.* (1975).

Results and Discussion

Effects of Denaturing Desulfoviridin

The desulfoviridin preparation from the Sephadex G200 column had a typical absorption spectrum similar to that shown by Lee and Peck (1971) with absorption maxima at 627, 581, 410 and 390 nm. When run on a polyacrylamide gel, only bands identifiable as desulfoviridin showed reductase activity (with respect to $Na_2S_2O_4$) (Fig. 1*a*).

The desulfoviridin did not fluoresce when irradiated with u.v. light (365 nm) until heated to 50°C. When heated desulfoviridin was separated by electrophoresis on a polyacrylamide gel, in addition to a band of desulfoviridin, a pink band (fluorescing red in 365-nm u.v. light) ran with the buffer front but it diffused rapidly through the gel after electrophoresis was stopped. This indicated that the fluorescent material was a water-soluble, negatively charged, low-molecular-weight compound. When this gel was incubated in the complete reaction mixture, containing $FeSO_4$, methylviologen and $Na_2S_2O_4$, black bands of FeS appeared coincident with the u.v.-fluorescent and the desulfoviridin bands (Fig. 1*b*). The spectrum of the fluorescent pigment in this band in the gel (pH 9·1) had absorption maxima at 585, 398 and 371 nm and shoulders at 625, 543 and 383 nm (Fig. 2*a*). This spectrum is similar but not identical to those of the chromophore of desulfoviridin (from *D. gigas*) in pyridine (Murphy and Siegel 1973) and of the *E. coli* sulphite reductase chromophore methyl ester in chloroform (Murphy *et al.* 1973*b*), the most marked difference being the absence of an absorption peak at 510 nm.

When desulfoviridin was hydrolysed at 50°C with pronase, and the products separated by electrophoresis on polyacrylamide gels, a band running with the buffer front and fluorescing pink in 365 nm u.v. light was free of aniline blue black-detectable protein, and it catalysed the reduction of $Na_2S_2O_4$ to sulphide (see Figs 1*c–g*). The absorption spectrum of the pronase-treated desulfoviridin (Fig. 2*b*) in 0·1 M phosphate buffer (pH 7·0) bore similarities to the spectrum of the heated material

in the fluorescent band in polyacrylamide gels but the major peak had shifted 6 nm towards the blue end of the spectrum (Fig. 2*a*). In 0·5 M NaOH the 620- and 400-nm peaks of spectrum (*b*) (Fig. 2) disappeared and the major absorption maxima were at 590, 550 and 372 nm with a shoulder at 385 nm (Fig. 2*d*). These various spectra are probably due to a mixture of cationic and neutral forms (Murphy *et al.* 1973*b*).

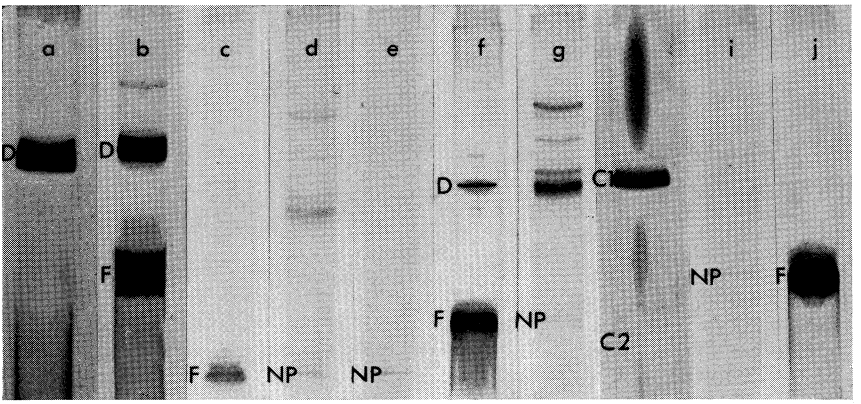

Fig. 1. Polyacrylamide disc gel electrophoresis of desulfoviridin preparations showing protein bands stained with aniline blue black (Canalco) (*d*, *e*, *g* and *i*) or sites of $Na_2S_2O_4$ reduction to sulfide (*a*, *b*, *c*, *f* and *h*). All separating gels were 7% polyacrylamide polymerized with ammonium persulphate and cross-linked with *N,N′*-methylenebisacrylamide. The direction of electrophoresis was from cathode to anode at 4 mA per gel.

(*a*) G200 desulfoviridin preparation; site of $Na_2S_2O_4$ reduction coincident with desulfoviridin (*D*).

(*b*) G200 desulfoviridin preparation heated at 50°C for 90 min; sites of $Na_2S_2O_4$ reduction coincident with desulfoviridin (*D*) and u.v.-fluorescent band (*F*).

(*c–g*) G200 desulfoviridin preparation heated to 50°C in the presence of pronase for 12 h. (*c*) Site of $Na_2S_2O_4$ reduction which was coincident with the u.v.-fluorescent band (*F*); 0·05 ml of sample was used. (*d*) Protein stain of a replicate gel for condition (*c*). (*e*) Protein stain of a gel devoid of sample; the band *NP* (non-protein), which was coincident with the black FeS band in (*c*), occurred in gel controls and was non-specific.

(*f* and *g*) Same as for (*c*) and (*d*) except that a 0·2-ml sample was used. (*f*) Sites of $Na_2S_2O_4$ reduction. (*g*) Protein stain of a replicate gel for condition (*f*)—note that the band *NP* is of the same intensity as the *NP* band in (*d*) and (*e*).

(*h*) G200 desulfoviridin preparation heated for 90 min at 50°C; gel incubated in the presence of reduced methylviologen and $Na_2S_2O_4$ only. The reduced methylviologen was cleared from the gel by inward diffusion of oxygen and areas *C1* and *C2* were cleared fastest; *C1* was coincident with desulfoviridin and *C2* was coincident with the u.v.-fluorescent pigment.

(*i* and *j*) u.v.-fluorescent pink pigment from *Desulfovibrio* sp. 10455. (*i*) Gel stained for protein and *NP* is similar to that in (*d*), (*e*) and (*g*) gels. (*j*) Replicate gel showing site of $Na_2S_2O_4$ reduction; this was coincident with the pink u.v.-fluorescent band (*F*).

The fluorescence polarization measurement for the pigment in this pronase-treated sample was zero, indicating that the low-molecular-weight, fluorescent material was not attached or orientated in any specific way to a high-molecular-weight (protein) moiety. When excited at 385 nm, the fluorescence emission spectrum of the pronase-treated desulfoviridin in 0·1 M phosphate buffer (pH 7·0) showed a major peak at 595 nm with a shoulder at 630–640 nm (Fig. 3*a*). The excitation spectrum (Fig. 3*b*)

obtained by recording the intensity of fluorescence emission at 630 nm showed peaks at 580 and 540 nm and a small shoulder at 490 nm. The emission spectrum was similar to that obtained by Murphy and Siegel (1973) for the methyl ester of the metal-free chromophore of desulfoviridin: the excitation spectrum suggests that it is the isobacteriochlorin which fluoresces.

Boiling desulfoviridin for 4 min released a u.v.-fluorescent pigment which had a spectrum in a gel (Fig. 2*c*) similar to the u.v.-fluorescent band of the desulfoviridin heated to 50°C (Fig. 2*a*). This boiled material, however, was unable to catalyse the

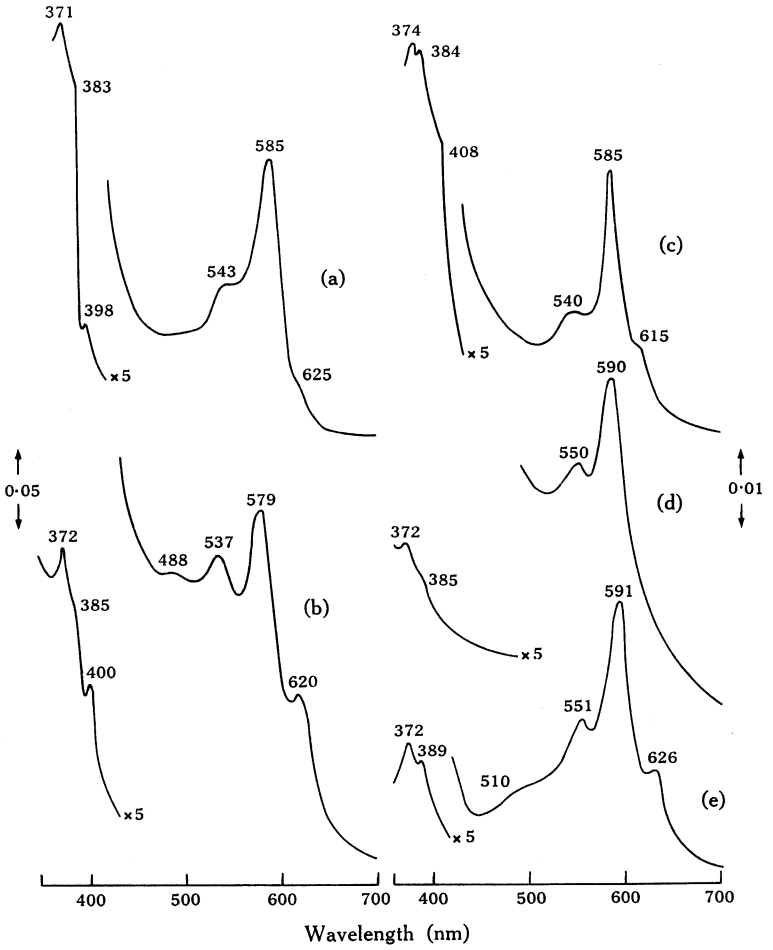

Fig. 2. (*a*) *In situ*, absolute absorption spectrum of the u.v.-fluorescent, pink band in a replicate of the gel shown in Fig. 1*b*; the desulfoviridin had been heated to 50°C. (*b*) Absolute absorption spectrum of desulfoviridin heated to 50°C for 12 h in the presence of pronase. (*c*) *In situ*, absolute absorption spectrum of the pink u.v.-fluorescent band in a gel after electrophoresis of boiled desulfoviridin. (*d*) Absolute absorption spectrum of the heated pronase-treated desulfoviridin in 0·05 M NaOH. (*e*) Absolute absorption spectrum of the freshly prepared pigment from *Desulfovibrio* sp. 10455, in 0·6 M KCl–0·01 M tris–HCl, pH 7·8. Each spectrum is given in two parts; the left-hand part was recorded at 1/5 the sensitivity of the right-hand part. The vertical lines indicate absorbance units.

reduction of $Na_2S_2O_4$. Since, from the experiments with pronase, the tetrapyrrole moiety from desulfoviridin did not appear to be attached to a protein, it is possible that boiling may have affected the side chains of the isobacteriochlorin macrocycle thus rendering the molecule inactive.

The foregoing experiments with polyacrylamide gels suggested that the isobacteriochlorin released by gentle denaturation of desulfoviridin catalysed the reduction of some sulphur species contained in a solution of $Na_2S_2O_4$. Figs 4*a–d* show that the desulfoviridin chromophore was released on treatment with pronase, that sulphide was produced during the reaction in cuvettes, that the rate of this reaction was increased by an increase in the chromophore concentration, and also that the chromophore was recovered after the reaction was completed; Fig. 4*c* shows that the reaction was repeatable on the same preparation. The reduced concentration of the chromophore (Fig. 4*f*) was at least partly due to its instability.

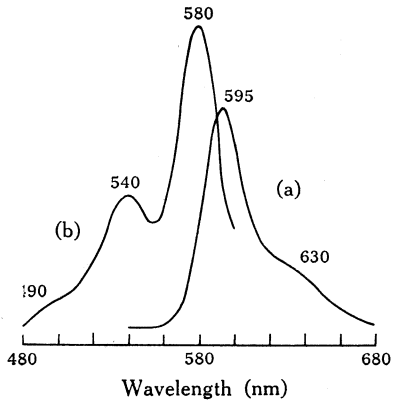

Fig. 3. The pronase-, heat-treated desulfoviridin, in 0·1 M phosphate buffer (pH 7·0), was used in these experiments. (*a*) Fluorescence emission spectrum when excited at 385 nm. (*b*) Excitation spectrum obtained by recording the fluorescence at an emission wavelength of 630 nm.

The molar extinction for the desulfoviridin chromophore is not yet available and it is unfortunate that a quantitative value cannot be given for the experiments shown in Fig. 4. However, for experiments I and II in Fig. 4*d* the chromophore was obtained from 446 and 24 μg of desulfoviridin respectively. The molecular weight of *D. gigas* desulfoviridin is 200000 (Lee and Peck 1971; Jones and Skyring 1974) and it is thought to contain 1–2 moles of chromophore per mole (Murphy *et al.* 1973). Therefore in experiments I and II the maximum concentration of the chromophore would be $2{\cdot}2 \times 10^{-3}$ and $1{\cdot}2 \times 10^{-4}$ mM respectively. In reality the concentrations would be less because the chromophore, under these experimental conditions, is unstable.

Some irreversible reduction of methylviologen occurred during the reduction since on addition of the second lot of $Na_2S_2O_4$ the extinction at 605 nm was lower by 42%. The sulphide detected resulted from the reduction of $Na_2S_2O_4$ or from a rearrangement product (Schulek and Maros 1958) since no sulphide was detected in the control cuvette which did not contain desulfoviridin hydrolysate or in another control which contained zinc-reduced methylviologen but no $Na_2S_2O_4$. The former result for these control cuvettes also shows that the DTNB was reacting with sulphide and not SO_2, a component of some $Na_2S_2O_4$ preparations (Schulek and Maros 1958).

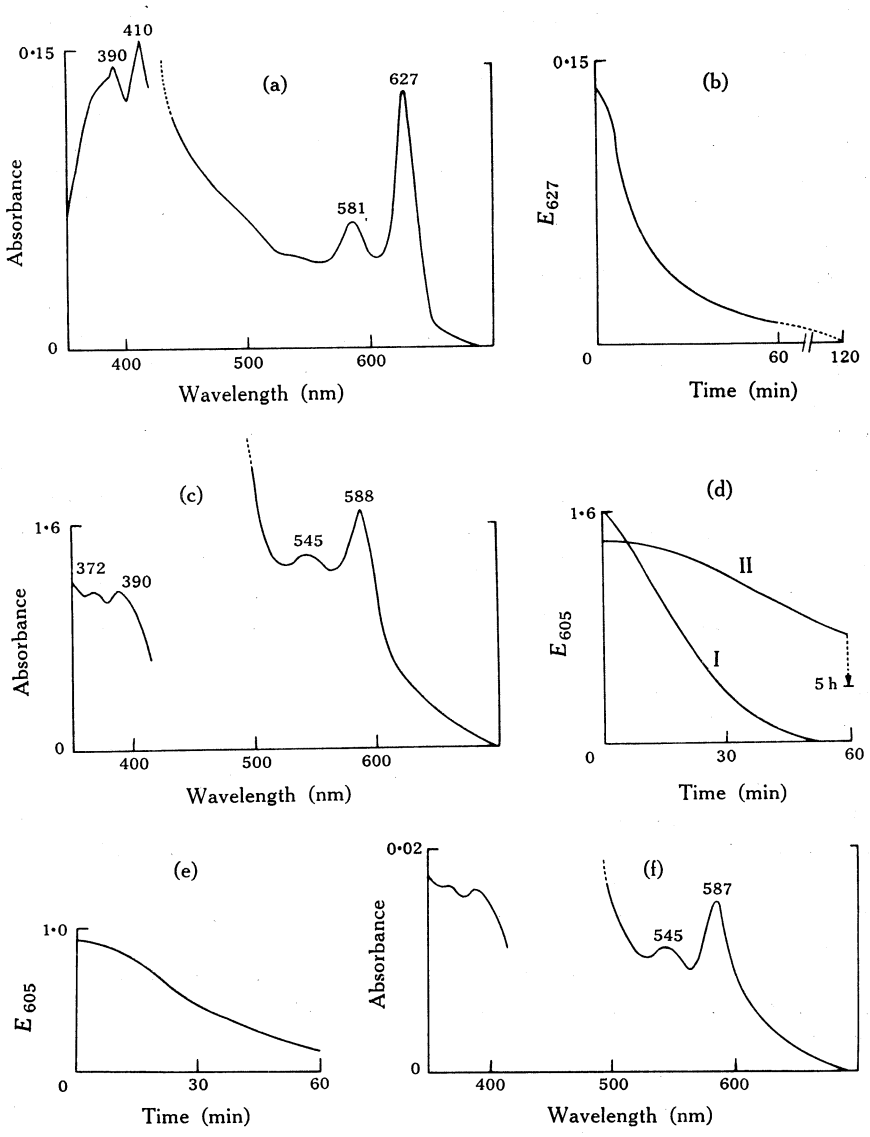

Fig. 4. (*a*) Spectrum of desulfoviridin (470 μg/ml) in 0·1 M tris–HCl, pH 7·0. (*b*) Proteolysis of preparation (*a*) in the presence of pronase (1 mg/ml) monitored at E_{627}. 0·1 ml of the hydrolysed desulfoviridin was transferred to another cuvette and the volumes of both adjusted to 2·0 ml with 0·1 M tris–HCl, pH 7·0. Methylviologen was added. (*c*) Spectrum of the desulfoviridin after proteolysis. (*d*) 1·0 mg of $Na_2S_2O_4$ was added to the side arms and the cuvettes gassed with O_2-free He for 20 min. The contents were mixed with the $Na_2S_2O_4$ in the side arms and the reoxidation of methylviologen monitored at E_{605}; I and II show the fall in E_{605} for cuvettes containing 1·9 and 0·1 mg of the hydrolysed desulfoviridin respectively. The sulphide produced was 35·0 and 17·0 μg in cuvettes I and II respectively. (*e*) The same reaction as (*d*) in cuvette I after another 1·0 mg $Na_2S_2O_4$ was added; 20 μg S^{2-} was produced from this reaction. (*f*) The spectrum of the contents of cuvette I after reaction (*e*).

The amount of sulphide formed with the most concentrated chromophore preparation (Fig. 4) was about 10% of the dithionite-S, indicating that not all of the dithionite-S was available for reduction.

Because this is a chemically complex system, interpretation of reaction rates (Fig. 4*b*) was difficult. The extinction at 605 nm at any time will depend on the relative rates of the oxidation of reduced methylviologen and the reduction of methylviologen. It does appear, however, from the shape of the curves in Fig. 4*d* that the reduction of methylviologen by $Na_2S_2O_4$ is rate limiting. If it were not, the extinction at 605 nm would have remained constant until the reductant was exhausted. That there was $Na_2S_2O_4$ (or some other reductant) still present after the methylviologen had been initially reduced was shown by a separate experiment. A cuvette having the same concentration of methylviologen and $Na_2S_2O_4$ was gassed vigorously with air until the reduced methylviologen became oxidized (colourless); almost instantaneously after bleaching the methylviologen was reduced (blue colour).

Several attempts were made to determine if the chromophore of desulfoviridin could catalyse the reduction of sulphite. In the cuvette system, the methylviologen, reduced by zinc in a syringe, was injected into the cuvette solution (O_2-free) containing the tetrapyrrole fraction from desulfoviridin. The sulphite substrate was contained in the side arm. After a period of equilibrium the sulphite was tipped and mixed with the contents of the cuvette and the course of the reaction monitored at 605 nm wavelength. Reduction of sulphite to sulphide by the desulfoviridin chromophore was also examined in polyacrylamide gels. In this technique the methylviologen was reduced with iron (Skyring and Trudinger 1972). In neither system was sulphide detected, even after several repetitions.

Iron and the Activity of the Tetrapyrrole Fraction from Desulfoviridin

The spectral properties of the tetrapyrrole component obtained by heating desulfoviridin indicated that it was metal-free; however, it is possible that when (in a gel) incubated in the complete reaction mixture the tetrapyrrole may have reacted with iron to yield a catalytically active metallochromophore. To resolve this, gels containing the tetrapyrrole component were incubated in the complete reaction mixture minus the ferrous sulphate. When the gels were being cleared of reduced methylviologen the first areas to be completely cleared were those coincident with the intact desulfoviridin and the tetrapyrrole material; there was no FeS band coincident with the latter (see Fig. 1*h*, areas *C1* and *C2*). This result indicated that the tetrapyrrole material in the absence of added iron was involved in the methylviologen-linked reduction. Oxidation of reduced methylviologen by the tetrapyrrole material was not observed when a replicate gel was incubated in the reaction mixture minus $Na_2S_2O_4$. It was noted in this experiment that before the gel was incubated in the reaction mixture a band of apparently normal desulfoviridin was visible. After incubation and partial clearing of the reduced methylviologen a band of FeS was present in a position coincident with the desulfoviridin (Fig. 1*h*, region *C1*). Since this did not happen with unheated desulfoviridin in the absence of added iron it seemed that, in addition to removing the tetrapyrrole component, heating partially denatured the protein to expose iron atoms so that the protein reacted with sulphide produced by the proximal and still functional enzyme. Alternatively, the removal of some of the low-molecular-weight tetrapyrrole may have altered the structure of the protein so that non-haem iron was free to react with sulphide.

Metal Components of Desulfoviridin

The major band from two G200 preparations of desulfoviridin contained iron and zinc and if these metals were derived from the desulfoviridin alone, then each molecule of the latter contained 17–18 atoms of iron and 1–2 atoms of zinc. Trudinger (1970) also found that iron and zinc were associated with a partially purified preparation of P582, the dissimilatory sulphite reductase from *Desulfotomaculum nigrificans*; if these metals were associated with the P582 pigment only, then the concentration was 16 atoms of iron and 2 atoms of zinc per molecule of enzyme. Siegel *et al.* (1973) found that *E. coli* B sulphite reductase contained 20–21 atoms of iron per molecule (molecular weight 670 000). The significance of zinc as a component of these enzymes has not yet been elucidated.

Desulfovibrio *sp. 10455 Pigment*

The spectrum (Fig. 2*e*) of the pigment from *Desulfovibrio* sp. 10455 is similar but not identical to that of the chromophore from *D. gigas*. This pigment also caused the reduction of $Na_2S_2O_4$ in the presence of reduced methylviologen and it is apparently not associated with a protein (Figs 1*j* and *k*). Physiological reasons for the excretion of this tetrapyrrole by strain 10455 are not known. Because of its peculiarly unique properties, however, it is possible that it may be an excessively produced intermediate in the synthesis of the tetrapyrrole component for the desulfoviridin which this strain of *Desulfovibrio* produced. This strain should be useful in subsequent studies requiring a protein-free tetrapyrrole of the isobacteriochlorin kind.

General Discussion

It has been shown that cobalt and rhodium complexes of sulphonated tetraphenyl porphyrins catalyse the reduction of acetylene and nitrogen (Fleisher and Krishnamurthy 1973). It has also been shown that other tetrapyrroles such as haems, when unassociated with protein, will catalyse peroxidase-type reactions (Lemberg and Legge 1949). The present observation is novel in that absorption and fluorescence spectral studies indicated that the active tetrapyrrole derived from the functional sulphite reductase, desulfoviridin, was metal-free.

The biochemistry of sulphite reduction in the sulphate-reducing bacteria is not well understood. On the basis of work by Kobayashi *et al.* (1969) and Lee and Peck (1971) it was thought until recently that desulfoviridin catalysed the reduction of sulphite to trithionate only and that trithionate and thiosulphate reductases were necessary *in vivo* to complete the reduction through to sulphide. However, Kobayashi *et al.* (1974) and Jones and Skyring (1974, 1975) have shown that *in vitro*, desulfoviridin can indeed catalyse the reduction of sulphite to sulphide. Further, they have shown that various sulphite assay procedures affect the kinds and quantities of the end products of sulphite reduction catalysed by desulfoviridin. To explain this Kobayashi *et al.* (1974) suggested that during the desulfoviridin-catalysed reduction of sulphite a reactive intermediate, sulphoxylate (SO_2^{2-}), reacted with sulphite to form trithionate. Recently, Chambers and Trudinger (1975) have shown that thiosulphate and trithionate are unlikely intermediates in the reduction of sulphite in living cells of *D. desulfuricans* and *D. gigas*. In the present work, therefore, the reduction of $Na_2S_2O_4$ is thought to be significant since dithionite is in equilibrium with the sulphoxylate radical ($SO_2^{-\bullet}$) which may be a reactive intermediate in the reduction of sulphite by sulphate-

reducing bacteria. On the other hand, solutions of $Na_2S_2O_4$ are chemically complex, possibly containing several inorganic sulphur compounds. In the presence of oxygen, $Na_2S_2O_4$ is oxidized to sulphate and bisulphate, whilst in anaerobic solutions it rearranges to thiosulphate and bisulphite or metabisulphite (Krachmer 1970); the form of the ion at the sulphite state of oxidation would depend on the pH. At least with the presently used techniques, catalytic reduction of sulphite promoted by the desulfoviridin chromophore could not be demonstrated and on previous occasions thiosulphate reduction was not catalysed by desulfoviridin. The dithionite ion, or some other species in equilibrium with it, appears the more likely substrate. Thus, studies of the isobacteriochlorin-promoted reduction of $Na_2S_2O_4$ may assist in understanding the mechanism of enzymic reaction in the reduction of sulphite by the sulphate-reducing bacteria. These observations may also be important in the understanding of the function and evolution of biologically active enzyme molecules containing metalloporphyrin prosthetic groups.

Acknowledgments

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