Isolation of a New Pigment, Desulforubidin, from Desulfovibrio desulfuricans (Norway Strain) and Its Role in Sulfite Reduction

JIN-PO LEE,¹ CHING-SUI YI, J. LEGALL, AND H. D. PECK, JR.
Department of Biochemistry, University of Georgia, Athens, Georgia 30602

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A new pigment, desulforubidin, that has sulfite reducing activity, has been purified from extracts of the Norway strain of Desulfovibrio desulfuricans, which lacks desulfoviridin.

Miller and Saleh (9) first reported the isolation of a strain of Desulfovibrio desulfuricans which lacked the green pigment, desulfoviridin (10), that is of taxonomic significance for the genus Desulfovibrio (11). This isolate, which reduced sulfate and sulfite to sulfide with molecular hydrogen at normal rates, was termed the Norway strain and was identical to D. desulfuricans in all other respects examined. The demonstration by Lee and Peck (6) that desulfoviridin is the enzyme responsible for the reduction of sulfite to trithionate raised the problem of the mechanism of sulfite reduction in the Norway strain of D. desulfuricans. In this investigation, we report the purification of a red pigment, which has been provisionally termed desulforubidin, from this anomalous strain of D. desulfuricans, and its identification as an enzyme that reduces sulfite mainly to trithionate, analogous to desulfoviridin.

D. desulfuricans (Norway strain) was kindly provided by J. R. Postgate and grown in a lactate-sulfate medium at 37 C as described previously (8). Except where noted, buffers were adjusted to pH 7.6 and contained 10 mM 2-mercaptoethanol. Extracts were prepared by means of a Gaulin homogenizer, and acidic proteins were removed by passing the extract through a short diethylaminoethyl (DEAE)-cellulose column (3.5 by 9 cm) equilibrated with 10 mM tris(hydroxymethyl)aminomethane (Tris)-hydrochloride buffer. The crude extract (Table 1) was then applied to an Amberlite CG-50, type II, column (4 by 10 cm) equilibrated with 10 mM phosphate buffer to remove most of the cytochrome; at this stage it is possible to detect the absorption peaks (Fig. 1, curve A) of desulforubidin in the eluate. More cytochrome was removed by passing the preparation through a silica gel column (4 by 15 cm) equilibrated with 1 mM Tris buffer, and then by passing the protein through a calcinated alumina column (4 by 15 cm) equilibrated with 0.1 M Tris buffer. During these procedures, the extent of purification could not be accurately assessed because the assay was not specific for the reduction of sulfite to trithionate (6).

The protein was applied to four DEAE-cellulose columns (2 by 43 cm), equilibrated with 0.05 M phosphate buffer, and the columns were washed with phosphate buffer in the following molarities and volumes: 0.05 M, 200 ml; 0.1 M, 750 ml; and 0.15 M, 270 ml. Desulforubidin was then eluted with a minimal volume of 0.3 M phosphate buffer and precipitated with ammonium sulfate, 0.5 saturation. The precipitate was dissolved in a minimal amount of 10 mM phosphate buffer, and dialyzed overnight against 2 liters of the same buffer. The desulforubidin in appropriate portions was passed through Sephadex G-200 columns (5 by 100 cm) equilibrated with 0.05 M Tris buffer by using ascending flow. The desulforubidin was precipitated with ammonium sulfate and dialyzed as described above. The protein was applied to another DEAE-cellulose column (3.9 by 30 cm) equilibrated with 0.1 M phosphate buffer, and washed with 500 ml of the same buffer and 500 ml of 0.15 M phosphate buffer. A linear gradient (500 ml) from 0.2 to 0.5 M phosphate buffer, pH 7.6, was used to elute the desulforubidin. Disk electrophoresis (1) of the purified protein (0.1 mg) indicated the presence of two closely migrating reddish-brown bands which, after staining with buffalo black, were revealed to be the only proteins in the preparation. This result is

¹ Present address: Department of Biochemistry, University of Iowa, Iowa City, Iowa 52240.
Table 1. Purification of desulfoviridin from Desulfovibrio desulfuricans (Norway strain)

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Vol (ml)</th>
<th>Protein (mg/ml)</th>
<th>Sp act (μ mole of H₂ per min per mg of protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude extract</td>
<td>2,100</td>
<td>29.5</td>
<td>0.16</td>
</tr>
<tr>
<td>Amberlite eluate</td>
<td>1,780</td>
<td>11.0</td>
<td>0.32</td>
</tr>
<tr>
<td>Silica gel eluate</td>
<td>1,875</td>
<td>6.0</td>
<td>0.53</td>
</tr>
<tr>
<td>Alumina eluate</td>
<td>2,100</td>
<td>2.7</td>
<td>0.154</td>
</tr>
<tr>
<td>First DEAE-cellulose</td>
<td>72</td>
<td>20</td>
<td>0.248</td>
</tr>
<tr>
<td>Sephadex (G-200)</td>
<td>560</td>
<td>0.6</td>
<td>0.346</td>
</tr>
<tr>
<td>Second DEAE-cellulose</td>
<td>25</td>
<td>4.4</td>
<td>0.410</td>
</tr>
</tbody>
</table>

![Absorption spectra of desulforubidin, desulfoviridin, and the pyridine hemochromogen of desulfuribidin](image)

**FIG. 1.** Absorption spectra of desulforubidin, desulfoviridin, and the pyridine hemochromogen of desulfuribidin. Curve A, desulforubidin (2.2 mg/ml), absorption maxima at 392, 545 and 580 nm; curve B, desulfoviridin from Desulfovibrio gigas (1.13 mg/ml), absorption maxima at 390, 408, 580 and 628 nm; curve C, desulforubidin (2.2 mg/ml) plus 0.2 N NaOH, 5% pyridine, and several crystals of dithionite, absorption maxima at 398 and 558 nm.

Desulforubidin also catalyzed the sulfite-dependent oxidation of methyl viologen reduced either chemically or by hydrogenase, and the purified desulforubidin had a specific activity of 0.41 μmol of H₂ utilized per min per mg of protein. This value compares well with the specific activity (0.63 μmol per min per mg of protein) of purified desulfoviridin (6). Both reddish-brown protein bands, observed with disk electrophoresis, catalyzed the sulfite-dependent oxidation of reduced methyl viologen when activity was determined in situ anaerobically on poliacrylamide gels. By using paper (4) and ion-exchange chromatography (3), the main product of the reaction was quantitatively determined to be trithionate with a ratio of H₂ to sulfite utilized of 1:3. As reported for purified desulfoviridin from D. vulgaris (5) and with crude extracts of several sulfate-reducing bacteria (15), desulforubidin also forms sulfide, and the amount produced with this enzyme varies from 2 to 8% of the total sulfur metabolized.

Desulforubidin has major absorption peaks at 392, 545, and 580 nm, with a weak absorption maximum at 720 nm (Fig. 1, curve A). It lacks the 628-nm absorption and split Soret peaks (5, 6) shown by desulfoviridin (Fig. 1, curve B). The absorption spectrum of desulforubidin also differs from that of the CO-reacting enzyme from Desulfitococcus heauton (16) and the assimilatory sulfite reductase (12) in that its major long-wavelength absorption is at 545 rather than 580 nm. Furthermore, the molecular weight (225,000) of desulforubidin, estimated from the S₉ₑₑ₉ₑ value of 9.8, is considerably larger than that of either of the latter two enzymes (13, 16), and it is not bleached by dithionite. We concluded that this absorption spectrum is due to a single protein having sulfite-reducing activity, rather than a mixture of proteins, and therefore it represents another type of sulfite reductase. However, because desulforubidin does exhibit a Soret peak and forms an alkaline pyridine hemochromogen (Fig. 1, curve C) similar to that described for the sulfite reductase from Escherichia coli (14), it must be tentatively regarded as a hemoprotein. The replacement of desulfoviridin by desulforubidin in the genus Desulfovibrio may be of widespread occurrence, because this replacement has also been demonstrated in extracts of a sulfate-reducing bacterium isolated by N. Pfenning from established cultures (2) of Chloropseudomonas ethylica (J. LeGall, unpublished data).

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**LITERATURE CITED**


4. Kobayashi, K., S. Tachibana, and M. Ishimoto. 1969. Intermediary formation of trithionate in sulfite reduc-


