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Chromophores of dissimilatory sulphite reductase from wild strains *Desulfovibrio desulfuricans*

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Sulphate is the main sulphur source in the environment which can be reduced by two distinct enzymatic systems, a dissimilatory for energy production and an assimilatory one for biosynthetic purposes, respectively. The reduction of sulphite to sulphide is an intermediary step of sulphate reduction in the sulphate-asimilating and dissimilating organisms. Both the assimilatory and dissimilatory sulphite reductase catalyses the six-electron reduction of sulphite to sulphide.

Highly purified sulphite reductase has been isolated from assimilatory organisms (*E. coli*) [1] and from the sulphate reducing bacteria both of the *Desulfovibrio* and *Desulfotomaculum* species [2 - 5]. A variety of sulphite reductases have been purified from the latter organisms: desulfoviridin, desulforubidin, desulfofuscidin and P-582. These various dissimilatory sulphite reductases are identical with respect to tetrahydroporphyrin structure, but they differ with respect to the presence of iron atoms in porphyrin groups.

The chromophore of desulforubidin, desulfofuscidin and P-582 contains an iron atom siroheme [SIR(Fe)], whereas a metal-free siroheme called sirohydrochlorin [SIR] is the chromophore of desulfoviridin [6].

Although desulfoviridin is believed to be the sulphite reductase in *Desulfovibrio desulfuricans* species, there are some conflicting data concerning the presence of siroheme chromophore in this enzyme [6, 7]. The chromophores were extracted from the isolated and purified enzyme [7 - 10].

We have avoided the large scale cultivation of bacteria necessary for isolation of the enzyme and proved that it is possible to characterize the sulphite reductase chromophores in crude cell extracts from wild strains of *D. desulfuricans* [11], following extraction with acetone: 0.015 M HCl (9:2, v/v) [6].

Characteristics of absorption spectra of the acetone/HCl supernatants of the two *D. desulfuricans* strains are shown in Fig. 1. The results obtained confirmed the differences in the absorption spectra, both in optical spectra and the Soret band [6 - 8]. The siroheme shows a typical absorbance peak in the region of 584 - 590 nm and a single Soret band in the region of 375 - 380 nm, while sirohydrochlorin, an absorbance maximum at 626 - 630 nm and a split Soret band in the region of 370 - 410 nm, with a specific peak above 400 nm (404 - 410 nm).

Some strains, exemplified by DV-2/86, DV-5/86, DV-6/86 and DV-7/86, showed the maxima characteristic for siroheme, whereas in the strains DV-1/86, DV-3/86, DV-4/86 and DV-8/86, the mixture of both chromophores was found (Table 1). One should also note the quantitative differences in the absorbance maxima.

These observations are in good agreement with the data reported by Moura *et al.* [6]; according to those authors 75 - 80% of the tetrapyrolic chromophores in the purified desulfoviridin contain metal-free sirohydrochlorin, while 20 - 25% contain iron atoms.

Taking into account that sirohydrochlorin is a demetallized siroheme, we have made comparative studies with the bacteria collected at different growth stages.

In the strains DV-2/86 and DV-5/86, which at the early stage of growth (Table 1) and after 72 h showed exclusively the presence of siroheme, sirohydrochlorin was found to appear when cultivation of bacteria was prolonged to

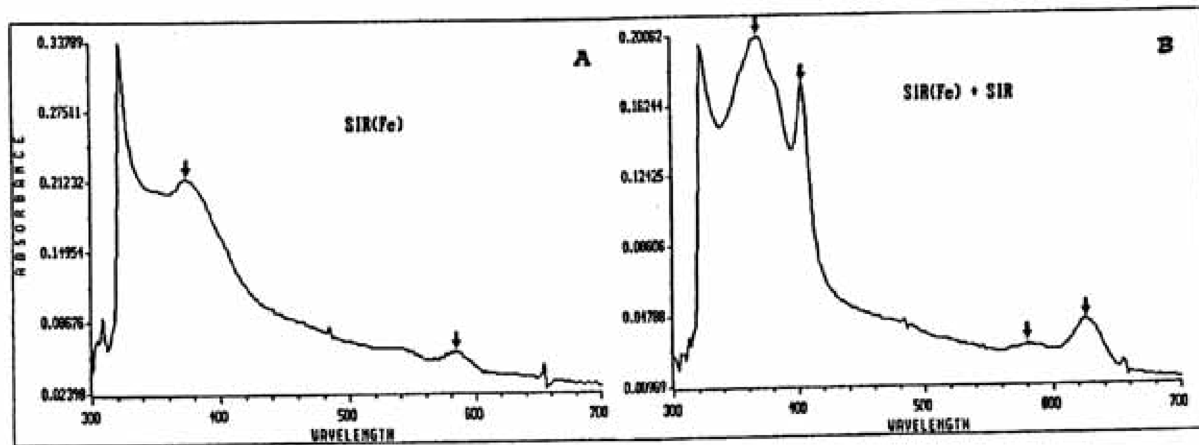

Fig. 1. The absorbance spectra of the acetone/HCl extracts from the cells of: D. desulfuricans DV-6/86 (A) and D. desulfuricans DV-1/86 (B).
SIR(Fe), siroheme; Sir(Fe) + SIR, siroheme and sirohydrochlorin mixture

Table 1
Absorption spectra of sulphite reductase chromophores of wild strains of D. desulfuricans, after 72 h of cultivation

D. desulfuricans strain	t_g^a (h)	A_{max} of the chromophore:		Soret band
		SIR(Fe)[b]	SIR[c]	
DV-1/86	9.7	368 nm 0.2006 580 nm 0.0314	404 nm 0.1738 626 nm 0.0400	split
DV-2/86	7.7	374 nm 0.2582 584 nm 0.0895	–[d]	single
DV-3/86	9.2	368 nm 0.2425 580 nm 0.0989	402 nm 0.2153 626 nm 0.0842	split
DV-4/86	10.2	374 nm 0.2582 584 nm 0.0133	402 nm 0.0841 626 nm 0.0143	split
DV-5/86	7.8	372 nm 0.3605 582 nm 0.1664	–[d]	single
DV-6/86	4.3	374 nm 0.2152 584 nm 0.0597	–[d]	single
DV-7/86	4.8	372 nm 0.2814 582 nm 0.1262	–[d]	single
DV-8/86	9.7	370 nm 0.4222 582 nm 0.2202	402 nm 0.3888 626 nm 0.1878	split

[a]Generation time
[b]SIR(Fe), siroheme
[c]SIR, sirohydrochlorin
[d]–, lack of absorbance

Table 2

Effect of prolonged cultivation of D. desulfuricans on the absorbance spectra of the sulphite reductase chromophores

D. desulfuricans strain	Culture age (h)	A_{max} of the chromophore		Soret band
		SIR(Fe)[a]	SIR[b]	
DV-2/86	72	374 nm 0.2582 584 nm 0.0895	–[c]	single
	144	374 nm 0.0675 –[c]	404 nm 0.0750 626 nm 0.0100	split
DV-5/86	72	372 nm 0.3605 582 nm 0.1664	–[c]	single
	144	372 nm 0.0750 –[c]	404 nm 0.0967 626 nm 0.0210	split

[a]SIR(Fe), siroheme
[b]SIR, sirohydrochlorin
[c]–, lack of absorbances

144 h (Table 2). This effect could be the reason for discrepancies in the observations on the type of chromophore. It is worth mentioning that the generation time of the strains DV-1/86, DV-3/86, DV-4/86 and DV-8/86 was longer than of those in which siroheme was the only chromophore (Table 1).

In conclusion, since desulfoviridin appears in *D. desulfuricans* by siroheme demetallization, its presence cannot be considered a characteristic, taxonomic criterion of this bacterial species.

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