Redox studies of the tetraheme cytochrome c_3 isolated from the propionate-oxidizing, sulfate-reducing bacterium *Desulfobulbus elongatus*

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A tetraheme cytochrome c_3 was purified from extracts of *Desulfobulbus elongatus*. Redox potentiometry studies indicated that one of the four hemes has a uniquely high E_m value (−30 mV), whereas the other three have a lower redox potential with E_m values of −165 mV. In the presence of a membrane preparation from *Db. elongatus* the cytochrome c_3 is partially reduced (23% at pH 7.6) by succinate. The physiological significance of the high redox potential of this cytochrome c_3 is discussed as a function of the propionate metabolism of *Desulfobulbus* which is known to proceed via the succinate pathway.

Cytochrome c_3 (Desulfobulbus) *Redox potentiometry* *Succinate pathway*

1. INTRODUCTION

Cytochromes c_3 constitute a class of multiple-heme cytochromes which are generally found in the sulfate-reducing bacteria belonging to the genus *Desulfovibrio*. They have low redox potentials and are considered as specific cofactors for the enzyme hydrogenase [1]. The two described species of *Desulfobulbus*, i.e. *Db. propionicus* and *Db. elongatus*, can be distinguished from the *Desulfovibrio* species by their ability to oxidize propionate into acetate and to ferment, in the absence of sulfate, lactate and pyruvate, into a mixture of acetate and propionate [2,3].

We found that lactate/sulfate-grown cells of *Db. elongatus* which contained extremely small amounts of hydrogenase activities [3] still contained high level of a multiple-heme cytochrome *c* apparently related to cytochrome c_3. We report here the purification, properties and possible physiological significance of this hemoprotein.

2. MATERIALS AND METHODS

Db. elongatus was grown on lactate/sulfate as described [3]. Wet cells (450 g) were resuspended in 0.01 M Tris-HCl buffer (pH 7.6) and broken by passing through a Manton-Gaulin homogenizer. Purification was performed from the soluble fraction obtained after high-speed centrifugation, by conventional chromatography on DEAE-Bio-Gel, CM-Bio-Gel and hydroxyapatite. Several cytochromes *c* were separated but only the major fraction was purified to homogeneity with a final yield of 9 mg of electrophoretically pure protein which presented a purity index (A_{551nm}(reduced)/A_{280nm}(oxidized)) of 3.7. The pellet from the high-speed centrifugation (140000 × *g* for 1 h) was utilized as a source of succinate dehydrogenase.

Protein concentration was determined by the method of Lowry et al. [4] with horse heart cytochrome *c* as standard.

The number of *c*-type heme groups per cytochrome molecule was estimated by the

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pyridine hemochromogen technique by using a millimolar extinction coefficient of 29.1 at 550 nm [5]. Hydrolysis of protein and analysis of amino acids were performed as in [6]. Cysteine was determined as cysteic acid by oxidation with performic acid prior to acid hydrolysis. Redox titration combined with spectrophotometry was carried out as in [7] in an anaerobic cuvette containing potassium phosphate buffer (0.1 M, pH 7.6), purified cytochrome c_3 and 10 μM of each of the following redox mediators: phenazine methosulfate, 2-hydroxy-1,4-naphthoquinone, 2-hydroxy-1,4-anthroquinone, methyl viologen.

3. RESULTS AND DISCUSSION

The amino acid composition of *Db. elongatus* cytochrome c_3 is show in table 1 and compared with three other cytochromes c_3 from different *Desulfovibrio* species. The results clearly indicate a close relationship between the four hemoproteins, in particular the presence of eight cysteinyl residues which are indicative of the presence of four hemes per molecule. This was confirmed by the pyridine hemochromogen method that gave a value of 3.9 hemes *c* per molecule of 13.4 kDa as determined by sedimentation equilibrium. Assuming a composition of four hemes and a total of 110 residues, a molecular mass of 13727 Da was calculated. The optical spectrum of the fully reduced cytochrome shows that the four hemes are in a low-spin complex (table 2). Since histidinyl residues are the only external heme iron ligands of all other cytochromes c_3 [8,9], the low value of the number of this residue (6 instead of 8) is unexplained and may be due to a low yield during protein hydrolysis. Methionine may be excluded as a possible ligand since the characteristic 690 nm band was absent as in all cytochromes c_3 [10].

Table 1

Amino acid composition of *Db. elongatus* cytochrome c_3 and comparison with other cytochromes c_3[a]

Amino acid	*Db.elongatus*	*D.vulgaris*	*D.desulfuricans* El Agheila
Lys	10	20	15
His	6	9	8
Arg	2	1	1
Trp	0	0	1
Asp + Asn	8	12	8
Thr	8	5	5
Ser	7	6	8
Glu + Gln	10	5	6
Pro	10	4	6
Gly	10	9	8
Ala	14	10	13
Cys	8	8	8
Val	5	8	5
Met	1	3	4
Ile	3	0	2
Leu	5	2	0
Tyr	1	3	1
Phe	2	2	3
Total	110	107	102

[a] From [14]

The analogy with other cytochromes c_3 is further substantiated by the fact that, in contrast to mitochondrial cytochrome *c*, *Db. elongatus* cytochrome c_3 is fully reduced by molecular hydrogen in the presence of pure periplasmic hydrogenase from *D. vulgaris*. Redox potentiometry combined with optical spectroscopy (fig.2) showed that *Db. elongatus* cytochrome c_3 had two completely distinct classes of hemes with E_m values of -30 mV (~25% of reduction) and -165 mV (~75% of reduction). It thus appears that one of the four hemes has a uniquely high redox potential for a cytochrome c_3.

The presence of a high succinate dehydrogenase activity has been detected in *Db. propionicus* [11] and is compatible with the proposed succinate pathway for propionate oxidation. Since the redox potential of the couple succinate/fumarate is close to that of the high-potential heme of *Db. elongatus* cytochrome c_3, the reduction of this protein by succinate in the presence of a particulate fraction was

Table 2

Absorption maxima and molar extinction coefficients of *Db. elongatus* cytochrome c_3

Oxidized		Dithionite-reduced	
λ_{max} (nm)	ϵ ($\times 10^{-3}$)	λ_{max} (nm)	ϵ ($\times 10^{-3}$)
531	35.6	551	116.5
408	417.0	522	61.4
280	31.4	418	717.9

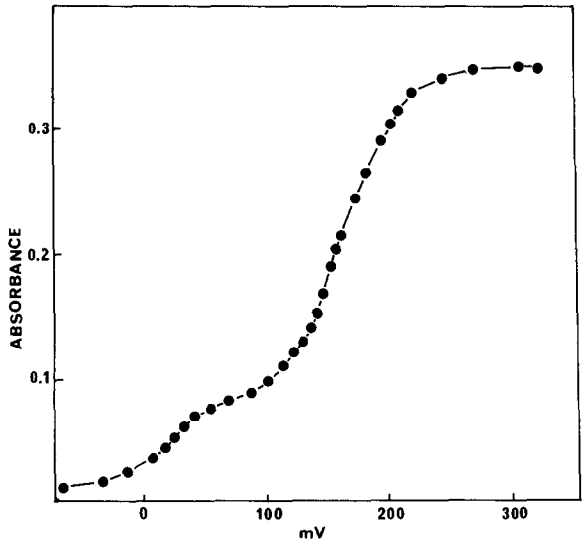

Fig.1. Redox titration of *Db. elongatus* cytochrome c_3. Absorbance was measured at 553 nm from 50 to −140 mV and at 551 nm from −140 to −350 mV.

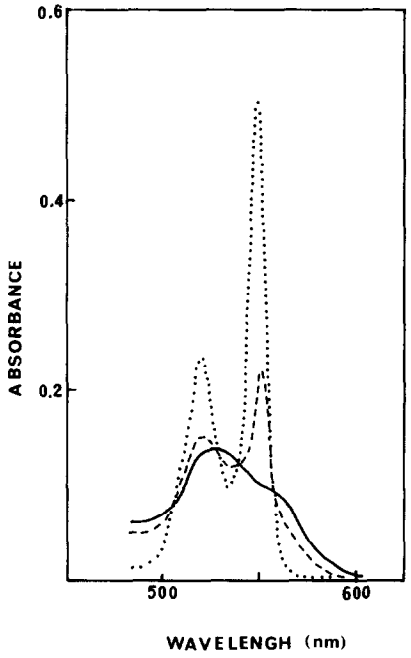

Fig.2. Absorption spectra of *Db. elongatus* cytochrome c_3: (——) oxidized; (– – –) reduced by succinate in the presence of a membrane preparation (protein concentration, 50 μg/ml); (···) reduced by sodium dithionite. To prevent reoxidation by oxygen these experiments were performed in an anaerobic cuvette.

assayed. The result, shown in fig.1, indicates that only one heme is reduced (23% of the total α-band at pH 7.6).

It thus appears that, in *Db. elongatus*, the synthesis of tetraheme cytochrome c_3 is not necessarily linked to a high specific activity hydrogenase. Furthermore, this hemoprotein is adapted to the presence of a succinate dehydrogenase within the cell since one of its hemes has uniquely acquired a redox potential which is compatible with the succinate/fumarate redox couple. Some *Desulfovibrio* species can utilize fumarate as a terminal electron acceptor [12,13] and *D. multispirans* was shown to contain a unidirectional fumarate reductase [12]. However, the presence of a high-potential cytochrome c_3 in these organisms has not been reported. Further studies will involve the cellular localisation of both succinate dehydrogenase and cytochrome c_3 as well as the search for intermediary electron transfer proteins. Preliminary experiments have shown that solubilized preparations of succinate dehydrogenase are unable to reduce the cytochrome c_3 with succinate. This suggests that other redox components, possibly quinones, are necessary for electron transfer between succinate dehydrogenase and cytochrome c_3.

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