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STRUCTURAL HOMOLOGY OF TETRAHEME CYTOCHROME c_3

Low potential tetraheme cytochromes c_3 (molecular mass 13 KDa) are found in sulfate reducing bacteria belonging to the genus *Desulfovibrio*. They seem to play an important role in electron transfer processes, but at the present moment their physiological role is still controversial. Cytochrome c_3 can act as an electron carrier for hydrogenase (although recently direct electron transfer was shown to occur between some of the electron carrier proteins, *e.g.*, *D. gigas* FdII and flavodoxin, and hydrogenase) and in some species involved in the reduction of elemental sulfur. Each heme, in this class of cytochromes, is bound to the protein by two thioether linkages involving cysteine residues, and the fifth and sixth ligands of each heme iron are histidinyl residues. Table I indicates all the tetraheme cytochromes c_3 that have been isolated until now as well as some of their physicochemical properties. The amino acid compositions are quite different from cytochrome to cytochrome originating very different isoelectric points. Tetraheme cytochromes are conserved in all the *Desulfovibrio* species analysed so far. It is interesting to note that even when the terminal acceptor is modified (*i.e.* nitrate by sulfate in *D. desulfuricans* (strain 27774) this multiheme cytochrome is still conserved. Cytochrome $c_{551.5}$ (c_7), a three heme containing cytochrome isolated from the sulfur reducing bacterium *Desulfuromonas acetoxidans*, is a close relative to cytochrome c_3. The four hemes in cytochrome c_3, are localized in nonequivalent protein environments (see below the comparison of the NMR and EPR spectral data) and each heme exhibits different redox midpoint potentials. The midpoint redox potentials of all the hemes are negative but the span in redox potential between the lowest and the highest one varies in this class of homologous proteins. As an example, in *D. vulgaris* cytochrome c_3 this difference is 80 mV, in *D. gigas* cytochrome c_3 100

Table I *

Desulfovibrio species	State of physico-chemical characterization	Isoelectric point	Number of residues	Molecular mass
D. gigas	P,A,S,NMR, EPR,MB	5.2	111	14400
D. vulgaris (Hildenborough)	P,A,S,NMR, EPR,MB	10.2	107	14100
D. vulgaris (Miyazaki)	P,A,S,X-ray, NMR,MB	10.6	107	14000
D. desulfuricans (Norway 4)	P,A,S,X-ray, NMR,EPR	7	118	15100
D. baculatus (strain 9974)	P,A,NMR, EPR	7	(118)	15100
D. desulfuricans (strain 27774)	P,A,NMR,EPR	n.d.	(103)	13500
D. desulfuricans (Berre eau)	P,NMR,EPR	8.6	n.d.	14000
D. desulfuricans (El Algheila Z)	P,A,S,NMR,EPR	10.0	102	13400
D. salexigens (British Guiana)	P,A,S,NMR	10.8	106	14000
D. desulfuricans (Cholinicus)	P,A	8.0	(108)	14300
D. africanus (Benghazi)	P,A	8.5	(109)	14900

P — Purified
A — Amino acid analysis
S — Sequence
NMR — Nuclear Magnetic Resonance
EPR — Electron Paramagnetic Resonance
MB — Mössbauer Spectroscopy
* Table composed from references [1-5] and references therein.

mV, and in *D. desulfuricans* (Norway strain) cytochrome c_3 this value is 200 mV (using the microscopic redox potentials determinated by EPR) [6-8].

A comparison of the NMR and EPR characteristics of this class of homologous proteins is presented in order to better understand the structure — function relationships.

Fig. 1 shows the low field region of the NMR spectra of several cytochromes c_3 isolated from different *Desulfovibriones*. An obvious common feature is the low downfield chemical shifts experienced by the heme methyl groups in this low spin paramagnetic state of the protein. The differences observed in the distribution of resonances are also striking. There is a wide variation in the distribution of heme methyl resonances between the different cytochromes c_3. These differences fall largely into three regions: the region downfield of 25 ppm, the region between 15 ppm and 25 ppm and the region upfield of 15 ppm. The presence of resonances downfield of 25 ppm is common to all cytochromes c_3. The fact that there are not many resonances in any of the spectra of Fig. 1 downfield of 25 ppm suggests that these proteins have similar structures. However, there is a striking difference: *D. gigas* cytochrome c_3 has three resonances in this region while the remaining five proteins have only two resonances. Similar differences can be seen in the regions between 15 ppm and 25 ppm. The heme methyl resonances in these regions of the spectra of some of the proteins, such as that from *D. desulfuricans* (Norway strain) are bunched together and cover a small range of chemical shift values whilst for other proteins, such as that from *D. salexigens*, they are better resolved and cover a wider range of chemical shift values. All of these differences may result from two causes: differences in the relative spatial orientations of the four heme groups, and differences in the electronic properties of the four heme groups.

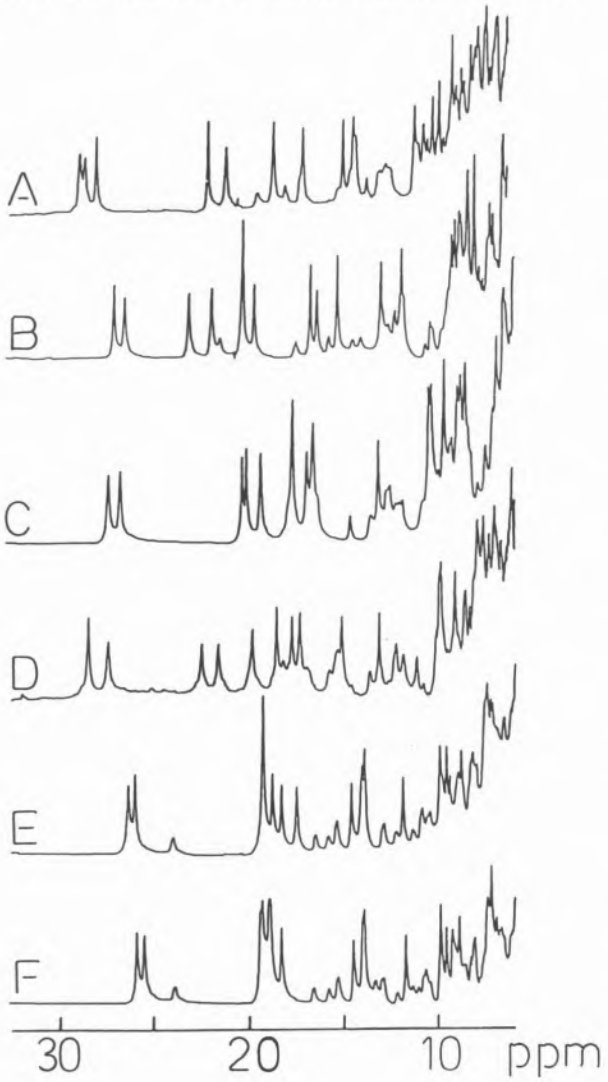

Fig. 1

Low-field NMR spectra of several ferricytochromes c_3 *at 313 K. A* — D. gigas; *B* — D. salexigens; *C* — D. vulgaris *(strain Hildenborough); D* — D. desulfuricans *(El Algheila Z); E* — D. desulfuricans *(Norway 4); F* — D. baculatus *(strain 9974)*

Despite of the emphasis upon the differences between the spectra of cytochromes c_3 it is relevant to notice that there is a high degree of similarity between them. For instance, in all cases there are 10 to 12 heme methyl resonances with chemical shift values >12 ppm.

Fig. 2 compares the EPR spectra of several cytochromes c_3 from different *Desulfovibrio* species. The cytochromes c_3 exhibit quite different EPR characteristics. Cytochrome c_3 from *D. desulfuricans* (Norway strain), *D. baculatus* (strain 9974) and *D. desulfuricans* (strain Berre eau) show quite similar characteristics. They all have broad features at $g \simeq 3.3$, a resonance around $g = 3.0$ and a shoulder on this peak to lower g-values. For other cytochromes, like *D. gigas* and *D. desulfuricans* (El Algheila Z) cytochromes c_3, the broad peak around $g \simeq 3.30$ is missing and only one prominent EPR signal is observed with a g_{max} around

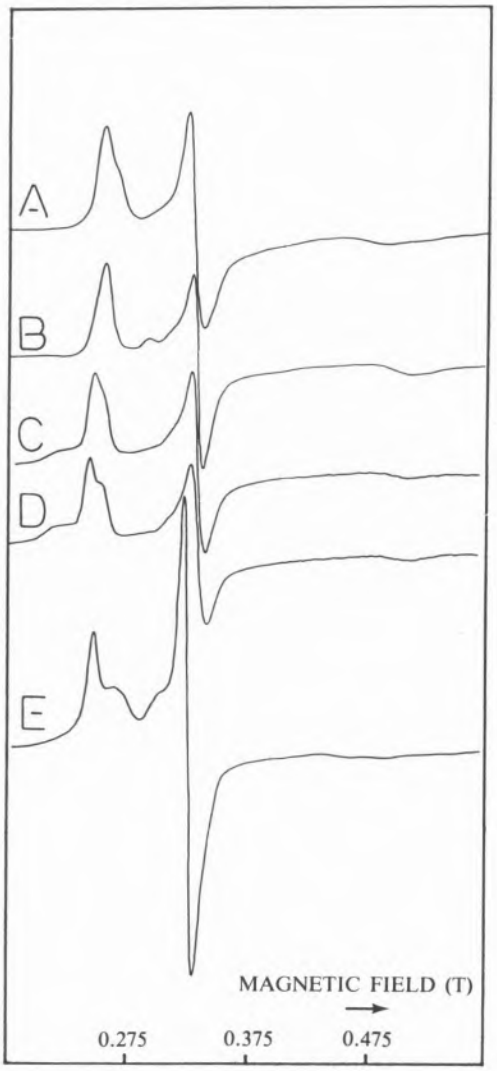

Fig. 2

EPR spectra of several cytochromes c_3 *at 10 K, microwave power 0.63 mW and modulation amplitude 0.5 mT.*
A — D. gigas; *B* — D. desulfuricans *(El Algheila); C* — D. desulfuricans *(Norway 4); D* — D. baculatus *(strain 9974); E* — D. vulgaris *(strain Hildenborough)*

$g \simeq 3.0$-2.9, showing in some cases a shoulder. *D. vulgaris* cytochrome c_3 is still different since three g_{max} values are quite discernible at g-values 3.12, 2.97 and 2.87. The g_{med} is sharper when compared to the g_{med} signals from other cytochromes c_3. It was recently shown that in heme model compounds where the two imidazoles are perpendicular to each other, the EPR signals are extremely anisotropic with g_{max} values of the order of 3.4 [9,10]. The X-ray structure of cytochromes c_3 from *D. vulgaris* (strain Miyazaki) and *D. desulfuricans* (strain Norway) show that three of the heme groups have the two axial histidines in the same plane. Only one heme in both these cytochromes has the two axial histidines perpendicular to each other. This heme is also the one most exposed to the solvent [10].

In this context, re-examination of the EPR data indicates that the heme originating g-features above 3.0 in *D. vulgaris* (Hildenborough) and *D. desulfuricans* (Norway strain) should be assigned to the heme having two axial histidinyl residues perpendicular to each other. Also this heme has the lowest redox potential (−325 mV) in *D. desulfuricans* (strain Norway 4). However, in *D. gigas* and *D. desulfuricans* (El Algheila Z), the EPR characteristics are different and the signal with high g_{max} is not present. It is possible that in these proteins all the histidines are coplanar. The X-ray structures have not yet been determined.

Preliminar Mössbauer studies indicate that in the native state there is a weak magnetic interaction between the different hemes at 4.2 K in the absence of an external magnetic field. Also, measurable spectral differences that correlate with the EPR data are observed within this group of multiheme proteins. Comparison of Mössbauer spectra of *D. gigas* cytochrome c_3 (without high g_{max} features) and *D. vulgaris* (Hildenborough) cytochrome c_3 (having a g_{max} feature greater than 3.0) show that the magnetic component with the largest magnetic hyperfine constant is present in *D. vulgaris* cytochrome c_3 and absent in the *D. gigas* protein.

The screening of the EPR and NMR characteristics of this class of cytochromes would probably permit a division of this type of proteins into sub-groups with more similar properties, using structural criteria.

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PS1.46 — TH

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CHARACTERIZATION OF TWO LOW-SPIN BACTERIAL SIROHEME PROTEINS

Sulfite reductase catalyses the rather unusual six-electron reduction of $SO_3^=$ to $S^=$.

Two low molecular weight proteins with sulfite reductase activity have been isolated from *M. barkeri* (DSM 800) [1,2], 23 KD and *Drm. acetoxidans* (strain 5071) [2], 23.5 KD. The enzymes contain one siroheme (iron-tetrahydroporphyrin prosthetic group) and one $[Fe_4S_4]$ cluster per minimal molecular weight.

The visible spectrum characteristics of both enzymes are very similar to those of siroheme containing enzymes; however, no band at 715 nm, characteristic of high-spin Fe^{3+} complexes of isobacteriochlorins is observed [3]. Low temperature EPR studies show that as isolated the proteins siroheme is in a low-spin ferric state (S = 1/2) with g-values at 2.40, 2.30 and 1.88 for the *M. barkeri* enzyme and g-values at 2.44, 2.33 and 1.81 for the *Drm. acetoxidans* enzyme.

EPR studies on model complexes have shown that ferric isobacteriochlorins with a single axial ligand are always high-spin while ferric isobacteriochlorins with two axial ligands are low-spin. The fact that in these sulfite reductases the siroheme is low-spin ferric suggests that it is six-coordinated. The siroheme of all the other sulfite reductases characterized so far has been shown to be in a high-spin ferric state with EPR features at 6.63, 5.24 and 1.98 [4].

The sulfite reductase from *M. barkeri* and *Drm. acetoxidans* together with the assimilatory sulfite reductase from *D. vulgaris* (strain Hildenborough) [5] which also shows a siroheme in the low-spin state belong to a new class of sulfite reductases. They are small molecular weight proteins with one siroheme and a $[Fe_4S_4]$ center per polypeptide chain. In the native state their siroheme is low-spin ferric. The physiological significance of this observation is not known and deserves further investigation.

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