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CORRELATIONS STUDIES BETWEEN STRUCTURAL AND REDOX PROPERTIES OF CYTOCHROMES c_3

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Individual redox potential values are determined for different cytochromes c_3. These polyhaemic cytochromes can be divided into two groups : one characterized by only one marked reduction step, the other one giving at least two well-marked reduction steps corresponding to redox potential values ranging from - 0.120 to - 0.400 V. Correlations between potential values and structural data are discussed.

Cytochrome c_3 can be defined as an original group of c-type cytochromes characteristic of all sulfate-reducing bacteria belonging to the genus Desulfovibrio. This cytochrome, not involved in oxygen respiration but more generally in electron transfer and storage functions is characterized by the presence of four haems per molecule with a molecular weight comparable to mitochondrial cytochrome c (about 14 000) (1). The iron axial ligands are two histidinyl side chains as with b-type cytochromes. The four haems exhibit non-identical and very low mid-point redox potentials with a lowest value of - 0.400 V (2) compared with + 0.290 V for mitochondrial cytochrome c (3,4).

The primary structures of cytochrome c_3 from 6 strains belonging to four species of Desulfovibrio (D. vulgaris Hildenborough strain (5) and Miyazaki strain (6), D. gigas (7), D. desulfuricans El Agheila Z (8) and Norway strain (1), and D. salexigens (9)) have been described. In addition, the three dimensional structure of the D. desulfuricans Norway strain cytochrome c_3 has been elucidated with a 2.5 Å resolution (10) and more recently the three dimensional structure of D. vulgaris Miyazaki strain has been published (11).

A trihaemic cytochrome c has been isolated from another group of bacteria Desulfuromonas acetoxidans (12) ; this cytochrome named $c_{551.5}$ (or c_7) is homologous to the sulfate-reducing bacterial cytochrome c_3 with one haem attachment missing (13). X-ray diffraction studies on this trihaemic cytochrome have been carried out by Haser et al. (14).

A number of spectroscopic NMR, EPR, Mössbauer, crystallographic and electrochemical studies (see for example 15-19) have been undertaken to gain infor-

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mation about the structure, function and organization of the haems in the same molecule. In an attempt to study correlations between amino acid sequence, haem iron coordination and haem exposure in cytochromes, extensive redox potential determinations of different cytochromes c_3 are described in this publication. In a previous work, a method for the analysis of cyclic and differential pulse polarography (DPP) curves was used for D. desulfuricans Norway cytochrome c_3 to determine individual redox potentials of polycentre molecules (2) ; it has been extended to other polyhaemic cytochromes c.

EXPERIMENTAL

Materials

Cytochromes c_3 from D. vulgaris Hildenborough and from D. desulfuricans El Agheila Z were respectively prepared according to (20) and (8). Isolation and growth of the sulfate reducing bacterium D. vulgaris strain "Zhilina" URSS have been previously described (21). The purification of cytochrome c_3 will be published elsewhere. D. desulfuricans (strain Berre S NCIB 8388) was previously isolated by J. Le Gall and the bacterium was harvested as earliest described (22). This cytochrome was purified by a method similar to that used for the other cytochrome c_3.

These cytochromes were judged to be pure by polyacrylamide gel electrophoresis performed according to Davis (23) and by amino-acid composition.

- Cyclic voltammetry and differential pulse polarography measurements

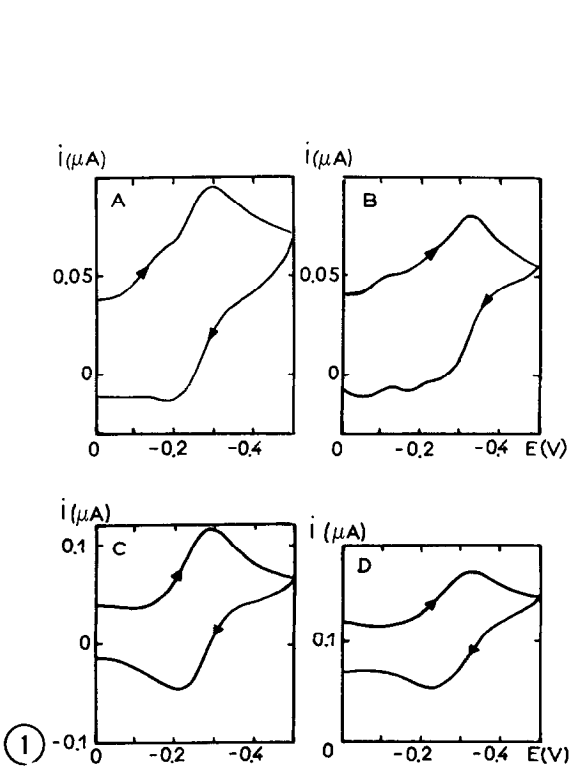
All cyclic voltammetry and DPP experiments were performed in 10 mM tris-HCl buffer pH 7.6 which served also as supporting electrolyte. Other experimental details are given in a previous paper (2). All potentials are referred to the normal hydrogen electrode (NHE).

RESULTS

The comparison between the electrochemical behaviour and properties of polyhaemic cytochromes c (19) has been extended to different c_3 cytochromes.

In previous works, we have shown that D. vulgaris Hildenborough (24), D. desulfuricans Norway (24) cytochromes c_3 and Desulfuromonas acetoxidans cytochrome $c_{551.5}$ (25) can be considered as fast electrochemical systems at the mercury electrode. In the present paper, it has been established from cyclic voltammetry experiments (Fig. 1) that D. desulfuricans Berre, D. desulfuricans El Agheila Z, D. vulgaris Zhilina strain and T. commune cytochrome c_3 also constitute fast diffusion-controlled electrochemical systems when using the hanging mercury drop electrode.

Differential pulse polarograms have been analysed by using the Birke equation (26) and the subtracting method previously applied to D. desulfuricans Norway cytochrome c_3 (2). After refinement of the calculated values on the basis of the best agreement between the experimental and calculated DPP curves, the best calculated points are shown in Fig. 2 for the six cytochromes studied in the present paper. The best-fit values for the corresponding individual half-wave potentials are given in Table I. These potentials may be considered as individual redox potentials with a very good approximation. The



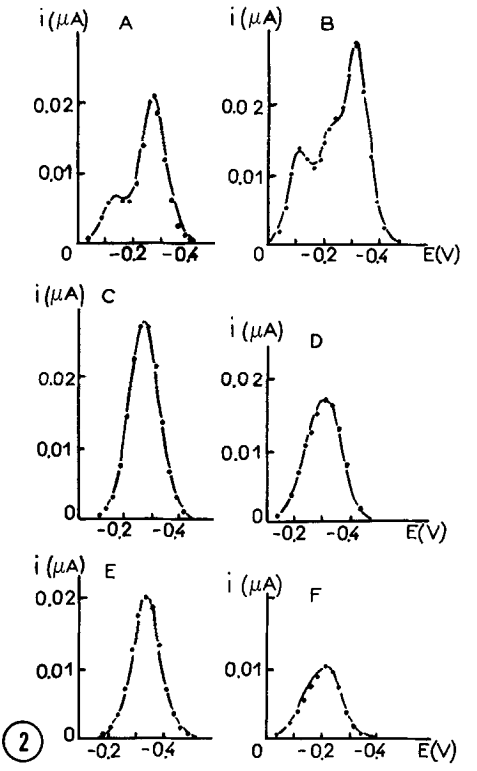


Fig. 1 - Cyclic voltammograms at the mercury electrode of cytochromes from : (A) T. commune (28 μM) ; (B) D. vulgaris Zhilina (55 μM) ; (C) D. desulfuricans El Agheila Z (38 μM) ; (D) D. desulfuricans Berre (30 μM) in 10 mM tris-HCl buffer pH 7.6. Scan rate : 10 mV s^{-1}.

Fig. 2 - Differential pulse polarograms of cytochromes from : (A) T. commune (28 μM) ; (B) D. vulgaris Zhilina (55 μM) ; (C) D. desulfuricans ET Agheila Z (38 μM) ; (D) D. desulfuricans Berre (30 μM) ; (E) D. vulgaris Hildenborough ; (F) Desulfuromonas acetoxidans (22 μM) in 10 mM tris-HCl buffer pH 7.6 at the dropping mercury electrode. Drop time : 5 s. (——) experimental curve ; (o) calculated points.

potential values obtained from other techniques and for other polyhaemic cytochromes are also included for comparison.

DISCUSSION

Table I shows that all the individual redox potential values determined for polyhaemic cytochromes c are negative. The most negative is that of D. desulfuricans Norway (E'_{04} = - 0.400 V) and the least negative, that of D. vulgaris Zhilina strain (E'_{01} = - 0.120 V). From the shape of DPP curves (Fig. 2) and from the redox potential values (Table I), it appears that the polyhaemic cytochromes studied can be divided into two groups : (I) cytochromes which are found to give DPP curve with only one reduction peak (or one peak and a more or less marked shoulder) i.e. D. desulfuricans Berre, D. desulfuricans El Agheila Z, D. vulgaris Hildenborough (16), D. vulgaris Miyazaki (27) and

TABLE I - Redox potentials of cytochromes c_3

Cytochromes	E'_{o_1}	E'_{o_2}	E'_{o_3}	E'_{o_4}	Other data
Group I					
D. desulfuricans Berre S	- 0.225	- 0.305	- 0.335	- 0.375	
D. desulfuricans El Agheila Z	- 0.235	- 0.265	- 0.290	- 0.320	
D. vulgaris Hildenborough	- 0.290	- 0.335	- 0.345	- 0.375	- 0.284 - 0.310 - 0.319 - 0.324 (16)
Desulfuromonas acetoxidans	- 0.140	- 0.210	- 0.240		- 0.102 - 0.177 - 0.177 (29)
D. vulgaris Miyazaki					- 0.222 - 0.274 - 0.294 - 0.335 (27)
D. gigas					- 0.235 - 0.235 - 0.306 - 0.315 (28)
Group II					
D. desulfuricans Norway					- 0.165 - 0.305 - 0.365 - 0.400 (2)
D. vulgaris Zhilina	- 0.120	- 0.230	- 0.305	- 0.325	
T. commune*	- 0.140	- 0.280	- 0.280	- 0.280	

For all the calculations the common value of $D = 0.8 \times 10^{-6}$ cm^2 s^{-1} has been used for the diffusion coefficient of polyhaemic c-type cytochromes (2). The results are obtained with a precision of + 0.010 V. *Manuscript in preparation : Hatchikian, E.C., Papavassiliou, P., Bianco, P. and Haladjian J.

D. gigas cytochromes c_3 (28) and D. acetoxidans cytochrome $c_{551.5}$ (29) ; (II) cytochromes which are found to exhibit at least two well-separated reduction peaks corresponding to well-separated reduction steps, i.e. D. desulfuricans Norway (2), D. vulgaris Zhilina strain and T. commune cytochromes c_3. D. vulgaris Zhilina strain cytochrome c_3 gave the most striking result because its DPP curve exhibits a marked shoulder and two well-separated peaks.

In the case of D. vulgaris Hildenborough, a comparison between our values (Table I) resulting from electrochemical measurements and the data obtained from EPR determination (16) shows a relatively good agreement for E'_{01} and E'_{02}, but discrepancies are noted for E'_{03} and E'_{04} values. We think that the oxidation-reduction potentiometry where reduction is attained by adding sodium dithionite to the protein solution is not suitable for determining very low negative redox potentials. The titration curves obtained (III and IV) do not

present a plateau corresponding to total reduction of haem, due to the limited reducing power of dithionite. Thus, it is likely that the values - 0.319 and - 0.324 V (16) are less negative than the true midpoint potential values. Differences in experimental conditions can also explain the discrepancies observed for Desulfuromonas acetoxidans cytochrome $c_{551.5}$ (29).

It would be interesting to correlate the potential values in Table I with structural data. Only D. desulfuricans Norway and D. vulgaris Miyazaki cytochrome c_3 have been studied from a structural point of view (10,11) but fortunately they are representative of the two different groups defined in Table I.

Theoretical calculations made by Kassner (30) have shown that the redox potentials should be related to the hydrophobicity of the haem environment. Small perturbations in the number, position or nature of hydrophobic side chains close to the haem may be a more important determinant of redox potential than the overall dielectric constant inside the protein. For Stellwagen (31), the redox potential is inversely dependent on the exposure of the haem to an aqueous solvent and predictions on the relative exposure of haems could be attempted, it appears that the calculated percent exposures of haems in the cytochromes studied are included within the range 31 % (- 0.120 V) - 50 % (- 0.400 V). A comparison between the two three dimensional structures of D. vulgaris Miyazaki and D. desulfuricans Norway cytochromes c_3 has been reported (32). In spite of their poor homology in amino acid sequences, the relative orientation of the four haems are very similar. Deletion and insertion of peptide fragments occur only in the outer loop regions and seem to have no influence on the relative haem orientations.

In D. desulfuricans Norway, haem 3 is partly shielded from tne external environment and Tyr 8 is close to the plane of porphyrin. The redox potential of - 0.165 V could be attributed to this haem. By comparison the corresponding haem (haem 3) in the structure of D. vulgaris Miyazaki does not seem to be more exposed than in D. desulfuricans Norway and Tyr 43 has the same place as Tyr 8. No important differences appear to exist when the hydrophobic cavities of this two related haems are compared. On the basis of these examples, other factors might be postulated to link protein conformation with redox potential or reactivity of the iron atom. For example, Valentine et al. (33) have noticed that the N_δ of the histidine residue bonded to the haem iron, is bonded to a carbonyl oxygen of the peptide backbone by a hydrogen bond. Their conclusions are that the strength and the geometry of this hydrogen bond might contribute to the modulation of redox potentials in cytochromes c. In cytochrome c_3 in which two histidine residues are implicated as axial ligands of the iron the role of this hydrogen bond geometry would result, according to

Valentine, in a greater stabilization of the oxidized form which can be correlated with the autooxidability of cytochrome c_3.

When a refined high resolution three dimensional structure of cytochromes c_3 from D. vulgaris Miyazaki and D. desulfuricans Norway is available the role of this hydrogen bond should be investigated.

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