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COMPARATIVE STUDIES OF MONOHEMIC BACTERIAL C-TYPE CYTOCHROMES. REDOX AND OPTICAL PROPERTIES OF DESULFOVIBRIO DESULFURICANS NORWAY CYTOCHROME $C_{553(550)}$ AND PSEUDOMONAS AERUGINOSA CYTOCHROME C_{551}

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Redox properties of cytochrome $c_{553(550)}$ from Desulfovibrio desulfuricans Norway ($E_o' = 0.04 + 0.02$ V/NHE) and cytochrome c_{551} from P. aeruginosa ($E_o' = 0.25 + 0.02$ V/NHE) are compared with those of some monohemic c-type cytochromes. The pK value for the equilibrium between the pH-dependent forms of cytochrome $c_{553(550)}$ (pK = 10.3 ± 0.1) has been also determined. It is to be noted that the difference between redox potentials can extend to nearly 250 mV, though the axial heme ligands are identical. Structural reasons have to be invoked to explain these variations.

C-type cytochromes are present in a variety of electron transport systems (1). Several mono- and polyhemic c-type cytochromes have been characterized, and the properties of the hemic group seem to be controlled by the iron ligands and by the immediate proteic environment. One of these properties, redox potential, constitutes a powerful basis for correlating redox behavior, structure and functions of these hemoproteins.

Intriguing features are observed when comparing the redox potentials of monohemic cytochromes c. It is well known that mitochondrial cytochrome c has a very positive potential (0.255 V/NHE (2)). On the other hand, in a recent work (3) we have obtained a redox potential value of 0.02 ± 0.01 V/NHE for the monohemic cytochrome c_{553} isolated from the anaerobic microorganism Desulfovibrio vulgaris Hildenborough extracted from sulfate reducing bacteria. This value is in agreement with EPR measurements (4). In these monohemic c-type cytochromes, the iron atom is linked to methionine and histidine residues at neutral pH ; when pH is raised, a transition between iron-methionine linked and unlinked forms can be observed.

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In the present work, the comparison has been extended to other monohemic bacterial cytochromes c : one isolated from another species of sulfate reducing bacteria (cytochrome $c_{553(550)}$ from Desulfovibrio desulfuricans Norway) and the other one from an aerobic bacteria (cytochrome c_{551} from Pseudomonas aeruginosa). The purification and the physicochemical properties of D. desulfuricans cytochrome $c_{553(550)}$ which presents a "split α" band in the reduced form have been reported in previous works (5,6). In the case of P. aeruginosa cytochrome c_{551}, it seems that the redox potential is very positive and depends on pH (7).

In the present work, voltammetry and spectrophotometry have been used to study redox and optical properties of cytochrome $c_{553(550)}$ and cytochrome c_{551} with the aim of comparing some properties of monohemic c-type cytochromes.

EXPERIMENTAL

Materials. Cytochrome $c_{553(550)}$ from D. desulfuricans Norway and cytochrome c_{551} from P. aeruginosa were prepared and purified as previously described (6,8). All chemicals used were reagent grade.

Methods and apparatus. The working electrode used for voltammetric measurements was the 4,4'-bipyridine activated gold electrode ; all experimental details are given in a previous work (3).

RESULTS

Redox properties. The differential pulse voltammograms of a 79 μM cytochrome $c_{553(550)}$ solution pH 7.0 are shown in Fig. 1a. In the absence of 4,4'-bipyridine (curve 1), no well-developed peak can be detected. Likewise, cathodic and anodic cyclic voltammetry peaks in Fig. 1b (curve 1) are very slight. In the presence of 0.01 M 4,4'-bipyridine, a well-shaped differential pulse voltammetry peak is observed at E_p = - 0.15 V/Ag-AgCl (i.e. 0.03 ± 0.01 V/NHE after conversion (3)) in Fig. 1a (curve 2). Well-developed cathodic and anodic peaks at E_{pc} = - 0.19 V and E_{pa} = - 0.13 V/Ag-AgCl (after correction for background current) are detected by cyclic voltammetry in Fig. 1b (curve 2). The separation $\Delta E_p = E_{pa} - E_{pc} \sim 60$ mV is consistent with the theoretical value (9) for a reversible diffusion-controlled monoelectronic process. Moreover, peaks currents i_{pa} and i_{pc} were found to increase linearly with $v^{1/2}$ (v = scan rate) as expected for a diffusion controlled process (9). The midpoint potential between E_{pa} and E_{pc}, which for a reversible process corresponds to the redox potential (10) is found at - 0.16 V/Ag - AgCl (i.e. 0.05 ± 0.02 V/NHE). Thus, an average value of 0.04 ± 0.02 V/NHE resulting from differential pulse and cyclic voltammetry measurements is estimated for the redox potential of cytochrome $c_{553(550)}$.

Similar curves have been obtained for P. aeruginosa cytochrome c_{551} ; a value of E'_o = 0.25 ± 0.02 V/NHE has been determined, in agreement with previous results (7).

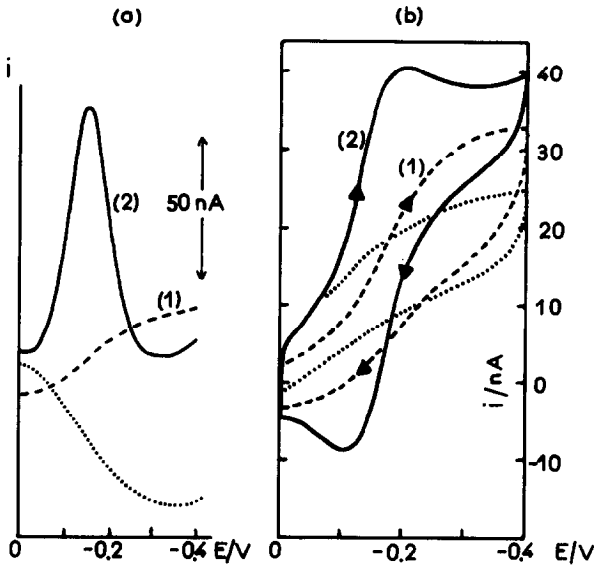

Fig. 1 - Effect of 4,4'-bipyridine on the electrochemical reactions at the gold electrode of 79 µM cytochrome $c_{553(550)}$ in 0.05 M sodium phosphate buffer at pH 7.0 :
(a) differential pulse voltammograms, scan rate 2 mV s^{-1}
(b) cyclic voltammograms, scan rate 1 mV s^{-1} (1) in the absence (2) in the presence of 0.01 M 4,4'-bipyridine ; (....) background solutions.

Effect of pH. The spectrum of cytochrome $c_{553(550)}$ in the oxidized form shows an absorption band at 690 nm attributed to the interaction of methionine ligand with the heme iron (1,11). When pH is raised, it is observed in Fig. 2 (curve 1) that the absorbance of the 690 nm band decreases above pH $\sim$ 9.5 ; a simultaneous decrease is noted for the absorbance of the 529 nm band (curve 2) (3). From the disappearance of the 690 nm band, it may be assumed that a new conformational ("alkaline") state appears, in equilibrium with the native ("neutral") form as follows

"neutral" form $\overset{-H^+}{\rightleftharpoons}$ "alkaline" form

A pK value of 10.3 $\pm$ 0.1 has been determined for this transition (Fig. 2). It will be noted that the 690 nm band is restored when pH goes back to its initial value of 7. It is not the case with P. aeruginosa cytochrome c_{551} : the 690 nm band disappears above pH $\sim$ 9.7, it is absent at pH $\sim$ 11 but does not totally reappear when pH decreases. New bands are also observed with decreasing pH in P. aeruginosa cytochrome c_{551} spectrum. Thus, no pK value can be given for this cytochrome.

DISCUSSION

By comparison with mitochondrial c-type cytochrome, some bacterial c-type cytochromes have a significantly lower molecular weight (Mr 9 000) but

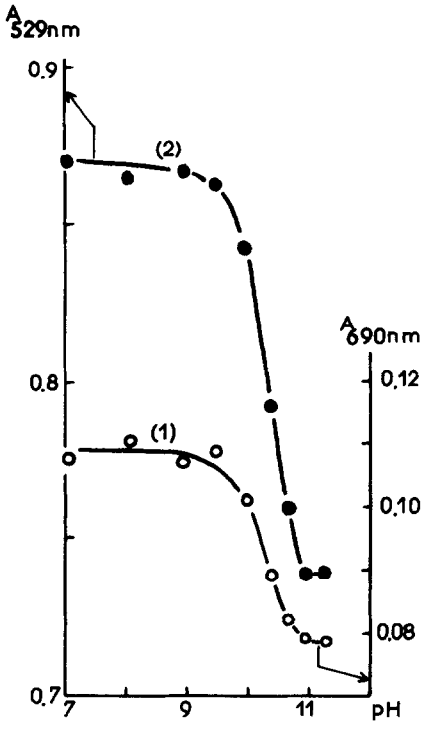

Fig. 2 - Effect of pH on the absorbance of the 690 nm band (1) (right-hand scale) and of the 529 nm band (2) (left-hand scale) of 35 µM cytochrome $c_{553(550)}$ in 0.05 M sodium phosphate + 0.05 M sodium borate buffer.

in the case of Pseudomonas cytochrome c_{551} the midpoint potential is similar to eucaryotic cytochromes.

Up to now, only two monohemic c-type cytochromes have been isolated from sulfate reducing bacteria, D. vulgaris cytochrome c_{553} and D. desulfuricans cytochrome $c_{553(550)}$. A comparison between some properties of monohemic c-type cytochromes is given in Table I. It is worth noting that the redox potentials are separated by about 230 mV, though the axial heme ligands are identical. Moreover, it appears that D. desulfuricans and D. vulgaris cytochromes c_{553} present a strong resistance to the effect of increasing pH. In fact, pK value appears as a measure of the stability of the Fe-S band. It has been shown (13,14) that a correlation can be directly related to the donor power of the methionine sulfur atom ; a decrase in the Fe-S bond length is linked to an increase in the ability of sulfur atom to donate electrons to the iron atom, with a stabilization of the Fe(III) state and a decrease in redox potential. This correlation seems to agree with the results given in Table I, but it is not excluded that other factors can govern the redox potential values of c-type cytochromes.

Recently high resolution ^1H NMR studies on these bacterial monohemic cytochromes c (Senn, H., Guerlesquin, F., Bruschi, M. and Wüthrich, K., per-

TABLE I - Comparison between some properties of monohemic c-type cytochromes

	D. desulfuricans Norway cytochrome $c_{553(550)}$ (6, this work)	D. vulgaris Hildenborough cytochrome c_{553} (3,4)	P. aeruginosa cytochrome c_{551} (7,8, this work)	Horse heart cytochrome c (1, 12)
Molecular weight	9 200	9 100	9 000	12 318
E'_o (V)/NHE at pH 7	0.04 ± 0.02	0.02 ± 0.01	0.25 ± 0.02	0.25 ± 0.01
pK	10.3 ± 0.1	10.9 ± 0.1		9.3
Isoelectric point	6.6	8.0	4.7	10.05
"split α" band (reduced form)	yes	no	no	no

sonal communication) has indicated a different chirality at the sulfur atom of methionine between the oxidized and the reduced form. It seems that these "small" bacterial cytochromes c which probably correspond to an early evolutionary stage do not show the same type of methionine coordination as the mitochondrial cytochrome c.

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