Vol. 144, No. 1, 1987 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
April 14, 1987 Pages 224-231

SPECTRAL PROPERTIES OF CYTOCHROME c' FROM RHODOPSEUDOMONAS CAPSULATA B100 AND ITS CO COMPLEX

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Received February 18, 1987

Summary: The spectral properties of cytochrome c' from photosynthetic bacterium Rhodopseudomonas capsulata (= Rhodobacter capsulatus) B100 and its CO complex are reported. The electronic absorption, MCD, and EPR spectra have been compared with those of the other cytochromes c' and horse heart cytochrome c. EPR and electronic spectral results for the ferric cytochrome c' suggest that the ground state of heme-iron(III) at neutral pH consists of a quantum mechanical admixture of an intermediate-spin and a high-spin state and that at pH 11.0 is in a high-spin state. In the MCD spectrum of the CO-ferrous cytochrome c', the MCD intensity in the Soret band region was much higher than that of CO complexes of hemoproteins with a protoheme. The differences in a stereochemistry of the sixth-coordination position is discussed.

Cytochromes c', which belong to a unique class of c-type cytochromes, have been found in photosynthetic, denitrifying, and nitrogen-fixing bacteria (1). The chemical, physical, and structural properties of the cytochrome c' have been extensively investigated, but their physiological functions have not so far

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Abbreviations: Rps , Rhodopseudomonas, Rps. capsulata, Rhodopseudomonas capsulata B100; R , Rhodospirillum; Alcaligenes sp , Alcaligenes sp. NCIB 11015; Chromatium, Chromatium vinosum; Ach , Achromobacter; cyt, cytochrome; EPR, electron paramagnetic resonance; MCD, magnetic circular dichroism.

0006-291X/87 $1.50

been elucidated (1,2). The cytochromes c' have unusual magnetic and spectroscopic properties unlike those of other c-type cytochromes. The unusual magnetic properties of ferric cytochrome c' from Chromatium vinosum have been interpreted by Maltempo and Moss (3) as due to the heme-iron electronic configuration at the ground state, which consists of quantum mechanical admixture of an intermediate-spin (S = 3/2) and a high-spin (S = 5/2) state. The refined structure (1.67 Å resolution) of ferric cytochrome c' of Rhodospirillum molischianum has been recently reported (4). From the comparison of the amino acid sequence of the cytochrome c' from R. molischianum with that of the other cytochrome c' (5), it seems probable that the heme environment of the cytochrome c' quite resembles that of cytochrome c' from R. molischianum.

We have shown the spectral properties of cytochrome c' from non-photosynthetic bacteria such as Alcaligenes sp. NCIB 11015 (6,7,8) and two strains of Achromobacter xylosoxidans (9,10). In the present paper, the spectral properties of cytochrome c' from photosynthetic bacterium Rhodopseudomonas capsulata (= Rhodobacter capsulatus)(11) B100 are reported. Rps. capsulata B100 is a wild-type derivative of B10. Some properties for cytochrome c' from Rps. capsulata B10 have been recently reported by Serebryakova and Gogotov (12).

Materials and Methods

Rhodopseudomonas capsulata (= Rhodobacter capsulatus) B100 was cultured photoheterotrophically at 30 °C on RCV medium (13) modified to promote urease formation. The modified RCV medium contained 20 mM D-fructose and 7.5 mM arginine-HCl, in place of malate and ammonium sulfate, as C and N sources respectively, with 10 μM $NiCl_2 \cdot 6H_2O$ (Takakuwa, unpublished results). The potassium phosphate concentration was increased 2 folds of RCV medium. Harvested cells were washed with 0.9 % NaCl solution three times. Cell-free extracts were prepared by sonicating the cell suspension in 50 mM Tris-HCl buffer (pH 7.5) containing 1 mM EDTA and 10 mM β-mercaptoethanol at 0 °C for 15 min. The soluble fraction was separated from the cell debris by high-speed centrifugation (100,000xg, 90 min, 0 °C). The soluble fraction was fractionated with ammonium sulfate to get 60-100 % sat. fraction, which was used for purification of cytochrome c'.

Purification procedures were essentially the same as those described by Serebryakova and Gogotov (12), but the final step, the preparative electrophoresis in polyacrylamide gel, was replaced with adsorption and elution on hydroxylapatite column prepared by the method of Hirano et al (14). Cytochrome c' which was dialyzed against 0.01 M phosphate buffer, pH 7.0, was applied to hydroxylapatite column equilibrated with the same buffer. Cytochrome c' adsorbed on hydroxylapatite was washed with 0.02 M of phosphate buffer pH 7.0 and eluted with 0.05 M of the same buffer. Purity index, $A_{280\ nm}/A(ox.)_{400\ nm}$, of the hemoprotein thus obtained was 0.27.

Horse heart cytochrome c (Sigma, Type VI) was purchased and used without further purification. The reduced form of the protein was prepared by the addition of a minimum quantity of solid sodium dithionite to a solution of the ferric form under anaerobic conditions. The reaction of CO with ferrous form was carried out in a Thunberg-type tube with optical cuvette under anaerobic conditions. The EPR, electronic absorption, and MCD spectral measurements carried out as described previously (7). Heme concentrations were determined from electronic absorption measurements, using alkaline pyridine hemochrome (milli-molar extinction coefficient at 550 nm = 29.1 (15)) method. The buffers used here were as follows: pH 5.3, 6.2, and 7.2; 50 mM phosphate: pH 13.7; phosphate/hydroxide.

Results and Discussion

The split Soret band, which is characteristic of ferrous cytochrome c', was distinctly observed in the electronic spectrum of ferrous cytochrome c' from Rps. capsulata (Fig. 1), though the relative intensity ratio of peak to shoulder in the Soret band is larger in the ferrous cytochrome c' from Rps. capsulata than in that from Alcaligenes sp. (Table 1). In the spectrum of ferric

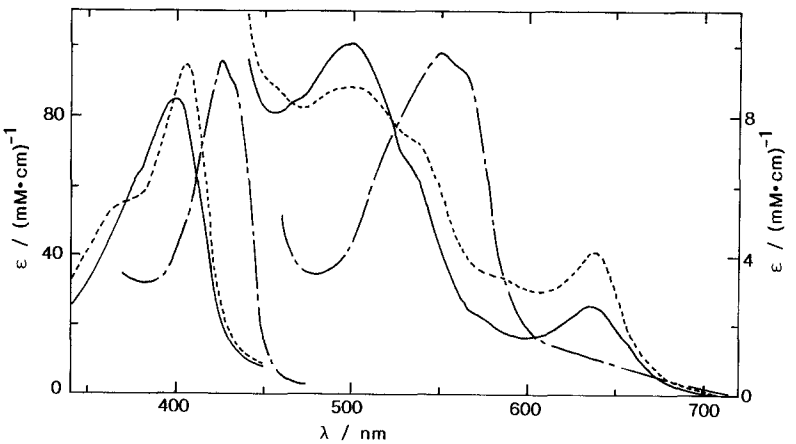

Fig. 1. Electronic spectra of cytochrome c' from Rhodopseudomonas capsulata B100 at room temperature: ferric forms at pH 7.2 (———) and at pH 11.0 (- - - - -); ferrous form at pH 7.2 (—·—·—).

Table I. Electronic Spectral Data for Ferric-, Ferrous- and CO-ferrous Cytochrome c' and CO-Ferrous Cytochrome c (Horse Heart) at Room Temperature[a]

	pH	λ_{max} / nm (ε / $mM^{-1}cm^{-1}$)[b]							Ref. No.
		Soret(γ)			β		α		
Fe(III) cyt c'									
Rps. capsulata B100	7.2	375sh (60)	400 (85.3)		465sh (8.4)	500 (10.1)	535sh (6.4)	635 (2.6)	
	11.0	370sh (55)	405.5 (94.4)		455sh (8.8)	500 (8.9)	535sh (7.3)	637.5 (4.1)	
Alcaligenes sp.	7.2	380sh (70)	401.5 (80.0)		460sh (8)	500 (10.0)	535sh (8)	643 (3.8)	7
	11.0	374sh (60)	404 (82.9)		460sh (9)	508 (9.1)	535sh (8)	643 (4.3)	7
Fe(II) cyt c'									
Rps. capsulata B100	7.2		426 (95.6)	435sh (84)	515sh (6.6)	550 (9.9)	565sh (9.1)		
Alcaligenes sp.	7.2		426.5 (91.5)	433sh (88)	520sh (7)	552.5 (10.7)	565sh (10)		7
CO-Fe(II) cyt c'									
Rps. capsulata B100	7.2	386 (33.7)	417 (255)			535 (12.9)	566.5 (10.5)		
R. rubrum[c]			416.5 (240)			534 (12.8)	564 (12)		24
Rps. palustris[c]			418 (262)			535 (12.6)	570 (11)		24
Alcaligenes sp.	7.2	397.5 (35)	419 (220)	435sh (30)		536.5 (11.5)	565 (10.2)		6
Ach. xylosoxidans GIFU 543	7.2	397 (37)	417.5 (219)	435sh (30)		535.5 (11.5)	565 (10.2)		10
CO-Fe(II) cyt c	13.7	394sh (39)	414 (257)			533 (12.5)	560 (10.1)		

[a] Abbreviations used are described in the text. [b] Milli-molar extinction coefficients are expressed per heme. [c] At neutral pH.

cytochrome c', as the pH was increased from pH 7.2 to pH 11.0, a decrease in the intensity of the band at around 500 nm and an increase in the intensity of the bands at around 535 and 635 nm were observed, suggesting the slight increase of high-spin component. Such a pH dependence of the spectra of ferric cytochrome c' is similar to that of the other ferric cytochrome c'. However, the electronic spectra of ferric cytochromes c' from Rps. capsulata at pH 5.3 and 6.2 and from R. rubrum at pH 6.0 (16) were essentially identical with that at neutral pH, though those from denitrifying bacteria such as Alcaligenes sp. (7,17) and Ach. xylosoxidans GIFU strains (18) were successively changed from pH 7.2 to at least pH 5.0.

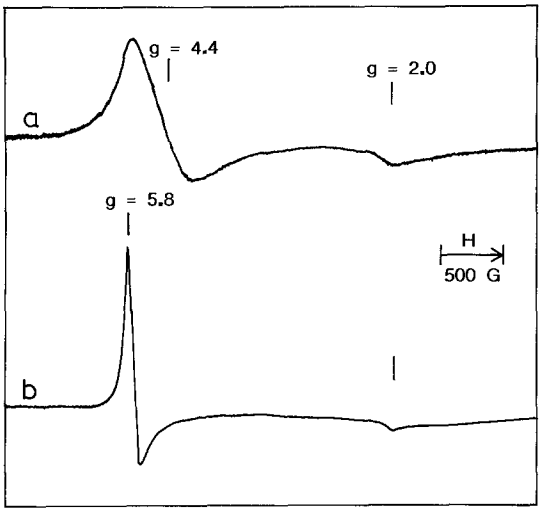

Fig. 2. EPR spectra of ferric cytochrome c' from Rhodopseudomonas capsulata B100 at 77 K (a) at pH 7.2 (b) at pH 11.0.

In the EPR spectra of ferric cytochrome c' from Rps. capsulata (Fig. 2), the absorption at around g = 5 was very broad (peak-to-peak width, 490 G) at pH 7.2 and narrow (120 G) at pH 11.0. The latter absorption can be assigned to high-spin $g_{\perp}$ absorption from analogy with ferric hemoproteins of high-spin state (19,20). The features of the former absorption (Table II) resemble those of absorption described in works of ferric cytochrome c' from Chromatium of Maltempo et al. (3,20), indicating that the ground state of heme iron(III) consists of a quantum

Table II. EPR Spectral Data of Ferric Cytochrome c' at 77 K and at pH 7.2[a]

	g values		ΔH^b/G	Ref. No.
	$g_{\perp}$	$g_{\parallel}$		
Fe(III) cyt c'				
Rps. capsulata B100	4.4	2.0	490	
Chromatium	4.77	1.99	620	21
Alcaligenes sp.	5.1	2.0	350	7
Ach. xylosoxidans GIFU 543	5.0	2.0	370	18

[a] Abbreviations used are described in the text.
[b] Peak-to-peak width of $g_{\perp}$ absorption.

mechanical admixture of an intermediate-spin and a high-spin state. Since the g value at zero-crossing of this absorption, which was tentatively assigned to $g_{\perp}$ value (21), is smaller than that of ferric cytochrome c' from Chromatium (Table II), the intermediate-spin component in the admixed state of the former may be slightly greater than that of the latter as has been predicted by the theory of Maltempo (3).

The MCD spectra for both ferric and ferrous cytochrome c' from Rps. capsulata were quite similar to those of cytochrome c' from Alcaligenes sp. (7).

The electronic spectrum of CO-ferrous cytochrome c' from Rps. capsulata B100 at pH 7.2 (Fig. 3) resembles those of analogues from the other photosynthetic bacteria such as Rps. capsulata B10 (12), R. rubrum, and Rps. palustris at neutral pH (Table I). As is shown in Table I, the electronic spectra of CO-cytochrome c' from denitrifying bacteria such as Alcaligenes sp. and Ach. xylosoxidans GIFU 543 at pH 7.2 have a shoulder absorption at around 435 nm. This shoulder absorption has been observed also in the spectrum of CO-cytochrome c' from a photosynthetic bacterium R. rubrum at around pH 6, which may result from Soret band of unreacted ferrous cytochrome c' (22). This suggests that the CO affinity at neutral pH of cytochrome c' from photosynthetic bacteria is higher than that from denitrifying bacteria.

In the MCD spectrum of CO-cytochrome c' from Rps. capsulata at pH 7.2 (Fig. 3), the MCD intensity in the Soret band region is, by about twice, higher than those (23) of CO complexes of indoleamine 2,3-dioxygenase, myoglobin, and horseraddish peroxidase containing a protoheme, while that of the former in the α- and β-band region is similar to those of the latter. Since the overall MCD spectral pattern of the CO-cytochrome c' at pH 7.2 quite resembles that of the CO-cytochrome c (horse heart) at pH

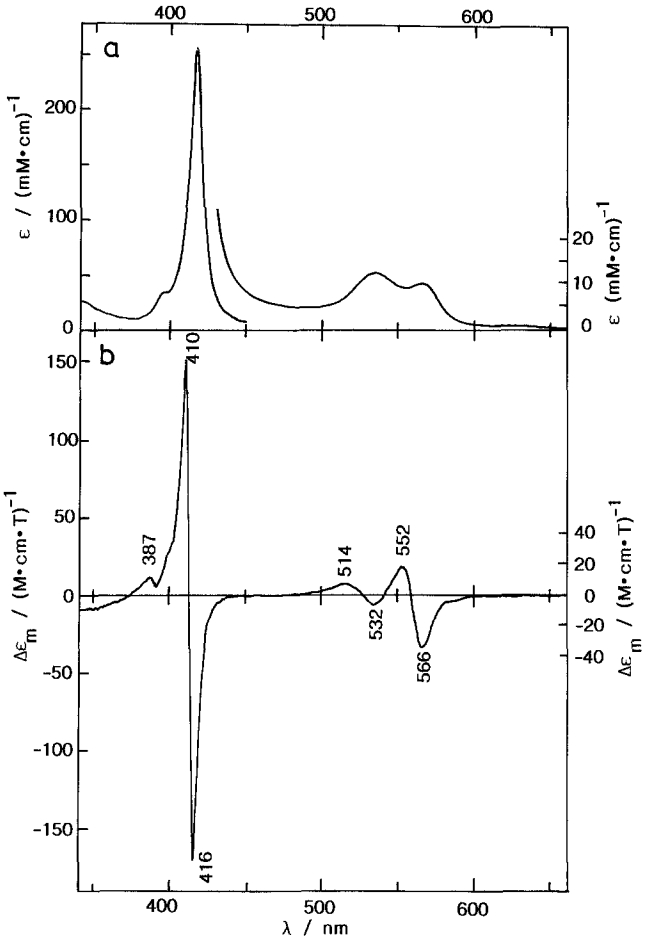

Fig. 3. Electronic (a) and MCD spectra (b) of CO-ferrous cytochrome c' from Rhodopseudomonas capsulata B100 at room temperature and at pH 7.2.

13.7, such a spectrum may be characteristic of CO complex of c-type cytochromes. However, the electronic spectral bands of CO-cytochromes c' at neutral pH locate at longer wavelength side compared with those of CO-cytochrome c (horse heart) at pH 13.7 (Table I). In the case of cytochrome c', accordingly, CO coordination to the heme iron would be affected not only by the fact that they have a c-type heme, but also by the fact that the sixth-coordination position is closely surrounded by hydrophobic and aromatic amino acid residues (4).

Our further interest is focused on the differences between cytochrome c' from photosynthetic and denitrifying bacteria in

the spectral properties under various conditions and the physiological functions. Details will be reported in the future.

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