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Spectral properties of *Achromobacter xylosoxidans* cytochromes *c′* and their NO complexes

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Cytochromes *c′* have been isolated from six strains of *Achromobacter xylosoxidans*: NCIB 11015 (formerly *Alcaligenes* sp. NCIB 11015), GIFU 543, 1048, 1051, 1055 and 1764. They are dimeric proteins with more positive redox potentials than those of cytochromes *c′* from phototrophic bacteria at neutral pH. The electronic absorption, EPR and MCD spectra on NO-ferrous cytochromes *c′* at physiological pH showed that the major part of the heme-iron of nitrosylheme was penta-coordinated. The EPR spectral results indicated that the ground state of the heme-iron of ferric cytochromes *c′* appears to be in an admixed spin states which consists of predominant high-spin with a slight intermediate-spin character at pH 7.2. These spectra were compared with those for cytochromes *c′* from phototrophic bacteria and the other hemoproteins.

1. Introduction

Cytochrome *c′* was found in some purple photosynthetic bacteria by Vernon and Kamen in 1954 [1]. In 1960 we found cytochrome *c′* (cryptocytochrome *c*) from a chemoheterotrophic denitrifying bacterium isolated from soil [2]. This bacterium was earlier known as *Pseudomonas denitrificans* and *Alcaligenes* sp. NCIB 11015, but was later definitely identified as a member of *Achromobacter xylosoxidans* [3,4].

Since 1986 we have isolated cytochromes *c′* from five strains of *Ach. xylosoxidans*, which were kindly supplied by Prof. E. Yabuuchi (School of Medicine, Gifu University): GIFU 543 (a type strain) [5], 1048 [6], 1051 [7], 1055 * [8] and 1764 * [6].

* These strains produce no gaseous products from nitrate and nitrite but have N_2O-reducing activity [5].

Abbreviations: EPR, electron paramagnetic resonance; CD, circular dichroism; MCD, magnetic circular dichroism; Fe(PPIXDME), dianion of protoporphyrin IX dimethyl ester; NMeIm, 1-methylimidazole; Mb, myoglobin; CCP, cytochrome *c* peroxidase; IDO, indoleamine dioxygenase; *Ach, Achromobacter*; *Rb, Rhodobacter*; *Rps, Rhodopseudomonas*; *Rsp, Rhodospirillum*; sh, shoulder.

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Achromobacter cytochromes are dimeric and made of identical subunits of about 14 kDa. They have more positive redox potentials (+108 to +132 mV) than those of cytochromes *c′* from phototrophs (−10 to +100 mV) at neutral pH [9].

In intact cells of *Ach. xylosoxidans* NCIB 11015 and GIFU 543, grown in the presence of nitrate, a large amount of cytochrome *c′* remains ligand-free but 10–15% of the total cytochrome *c′* is coordinated with NO [10].

Reaction of cytochrome c′ with NO

In 1969 we demonstrated that NO complex of ferrous cytochrome *c′* from *Achromobacter* has the characteristic Soret peak at 396 nm and pH 7.2 [11], while that of cytochrome *c′* from *Rsp. rubrum* has the Soret peak at 417 nm [12]. Since 1986 we have characterized the spectral properties of NO complexes of *Achromobacter* cytochromes *c′* by reference to the accumulated information on nitrosylcytochromes *c′* from various sources reported so far and models for nitrosylhemoproteins.

Table 1 shows the electronic spectra for NO-ferrous hemoproteins and their model complexes at room temperature. The NO-derivatives of ferrous cytochrome *c*, myoglobin, cytochrome *c* peroxidase and indoleamine 2,3-dioxygenase has a six-coordinate nitrosyl heme with

TABLE I

Electronic spectral data for NO-ferrous hemoproteins and their model complexes at room temperature

	pH	λ_{max} (nm) (ϵ_{mM}/heme)					Ref.
		Soret			β	α	
NO-cyt *c'*							
Ach. xylosoxidans	7.2	396.5	415 sh	485	541	565 sh	13
NCIB 11015		(78.9)		(9.8)	(10.4)	(10)	
Ach. xylosoxidans	7.2	398	413.5	483.5	539.5	566	5
GIFU 543		(73.8)	(70.3)	(8.9)	(10.4)	(9.7)	
Ach. xylosoxidans	7.2	398.5	413.5	483.5	541	566	7
GIFU 1051		(66.9)	(68.1)	(8.4)	(10.0)	(9.2)	
Ach. xylosoxidans	7.2	397.5	412.5	484	539	565	8
GIFU 1055		(79.8)	(79.1)	(9.6)	(11.4)	(10.6)	
Rb. capsulatus	7.2	395 sh	414	480 sh	540	570 sh	14
B100		(61)	(89)	(7.8)	(10.8)	(8.9)	
Rsp. rubrum	7.0	395 sh	417	482	530	560 sh	12
			(80)		(11)		
Rps. palustris	7.0		420		531	567	15
NO-cyt *c*	5.3		412		538.5	563.5	16
			(146)		(11.6)	(11.4)	
NO-Mb			420		548	579	17
			(127)		(11.3)	(10.1)	
NO-CCP			421		542	572	18
			(99)		(12.0)	(11.0)	
NO-IDO			418.5		544	574	19
			(127)		(12.2)	(12.5)	
Fe(PPIXDME)(NO)		400.5		480 sh	550 sh	569.5	20
(in benzene)		(81.6)		(11)	(10)	(10.5)	
Fe(PPIXDME)(NO)(NMeIm)			418.5		546.5	576.5	20
(in benzene)			(118)		(11.5)	(10.6)	

a histidine at the axial position *trans* to the nitrosyl group, which has been confirmed mainly by EPR spectral results [21]. Their electronic spectral data are similar to those of a six-coordinate model nitrosyl heme, Fe(PPIXDME)(NO)(N-base) as partly shown in Table I. The differences between six- and five-coordinate model nitrosylheme in electronic spectra can be distinctly found in the position of Soret band (six-coordinate, 412–419 nm, five-coordinate, 394–401 nm) and in the appearance of weak absorption at around 480 nm only in five-coordinates [20].

The comparison of NO-cytochromes *c'* with model nitrosylheme in spectral data (Table I) indicated that NO-cytochromes *c'* from *Achromobacter, Rsp. rubrum* and *Rb. capsulatus* contain both five- and six-coordinate nitrosylheme, while that from *Rps. palustris* possibly contains only six-coordinate species. Further, it is evident that for NO-complex of cytochrome *c'* from *Achromobacter* NCIB strain the five coordinate species is much more than the six-coordinate species in relative amount.

The probable heme fifth ligand of cytochromes *c'* is a histidine residue and the vacant distal side of the heme can be surrounded by hydrophobic amino acid residues [22,23]. NO can be coordinated to the vacant axial position *trans* to the proximal histidine and the heme iron to histidine bond for the major part of cytochrome *c'* from *Achromobacter* NCIB 11015 is thought to be cleaved upon the coordination of NO.

These results have been clearly confirmed by EPR spectra [13,14] and MCD spectra [14,24] on model nitrosyl hemes and NO complexes of hemoproteins.

The quantitative ratio of five-coordinate nitrosyl heme to six-coordinate one among species is considered to be: *Ach. xylosoxidans* NCIB 11015 > *Ach. xylosoxidans* GIFU 543, 1051, 1055 > *Rb. capsulatus* B100, *Rsp. rubrum* > *Rps. palustris*.

The reaction of NO with ferric cytochrome *c'* from *Achromobacter* NCIB 11015 is interpreted on the basis of reductive nitrosylation. The reaction results in the formation of ferrous cytochrome *c'* in the first step. NO reacts with ferrous cytochrome *c'* in the second step [13], while the reaction of ferric cytochromes *c'* from photosynthetic bacteria with NO results in the formation of NO-ferric cytochromes *c'* [12,14].

EPR spectra of ferric cytochromes c' from *Achromobacter* at low temperature

Maltempo et al. [25] demonstrated that ferric cytochromes *c'* from photosynthetic bacteria at neutral pH have a heme-iron configulation corresponding to a

TABLE II

EPR spectral data of ferric cytochrome c′ and the other ferric hemoproteins [a]

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	pH	Temp. (K)	$g_\perp$			$g_\parallel$	ΔH[b] (G)	R[c] (%)	Ref.
			g_1	g_2	$(g_1+g_2)/2$	g_3			
Fe(III) cytochromes *c′*									
Ach. xylosoxidans NCIB 11015	7.2	6	6.18	5.34	5.76	1.99	210	5.3	6
Ach. xylosoxidans GIFU 543	7.2	6	6.23	5.36	5.80	1.99	220	5.4	6
Ach. xylosoxidans GIFU 1048	7.2	6	6.19	5.35	5.77	1.98	210	5.3	6
Ach. xylosoxidans GIFU 1051	7.2	6	6.18	5.45	5.82	1.99	200	4.6	6
Ach. xylosoxidans GIFU 1764	7.2	8	6.17	5.47	5.82	1.99	200	4.4	6
Chromatium vinosum	7.2	7		4.77	4.77	1.99	510		25
	10.8	17	6.14	5.68	5.91	2.00	50	2.9	25
Fe(III) hemoproteins									
Horseradish peroxidase	8.1	1.4	6.35	5.65	6.00	2.0		4.4	27
Cytochrome *c* peroxidase	5.0	1.5	6.4	5.6	6.0	2.0		5.0	28
Hemoglobin *M* Boston			6.30	5.71	6.01	2.00		3.7	29
Hemoglobin M Hyde Park			6.31	5.68	6.00	2.00		3.9	29
Catalase (bovine liver) I	6.0	1,4	6.56	5.42	5.99	2		7.1	30
II	6.0	1.4	6.90	5.04	5.97	2		11.6	30

[a] The accuracy of g values was $+0.01$.

[b] Peak(lower field)-to-trough(higher field) width of $g_\perp$ absorption.

[c] The percentage of rhombicity. $R = (\Delta g/16)\times 100$. Δg, the difference in g values between two components of $g_\perp$.

quantum mechanically mixed $S = 3/2$, $5/2$ state. Maltempo and Moss [26] pointed out that $g_\perp$ may be considered simply as a parameter indicating the percentage of spin state $S = 3/2$ in the admixed spin state. The $S = 3/2$ and $5/2$ components at the ground state of heme iron of *Chromatium* cytochrome c' were estimated to be 65% and 35%, respectively [25]. On the other hand, the heme iron of *Chromatium* cytochrome c' at pH 10.8, horseradish peroxidase, cytochrome *c* peroxidase, myoglobin M and catalase are in a pure high-spin state and these $g_\perp$ values ranged from 5.9 to 6.1 (Table II).

As shown in Table II, the $g_\perp$ values of *Achromobacter* ferric cytochromes c' at low temperature ranged from 5.76 to 5.82, slightly lower than those of pure high-spin hemoproteins. This result indicates that the ground state of the heme iron can appear to be in an admixed spin state which consists of predominant $S = 5/2$ (about 90%) with a slight $S = 3/2$ (about 10%).

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