Spectral Properties of Carbon Monoxide or Cyanide Complexes of Cytochromes *c'* from Denitrifying Bacteria

SHINNICHIRO SUZUKI*, AKITSUGU NAKAHARA

Institute of Chemistry, College of General Education, Osaka University, Toyonaka, Osaka 560, Japan

TETSUHIKO YOSHIMURA

The Environmental Science Institute of Hyogo Prefecture, Yukihira-cho, Suma-ku, Kobe 654, Japan

HIDEKAZU IWASAKI

Department of Foreign Languages, Nagoya University of Foreign Studies, Nisshincho, Aichigun, Aichi 470-01, Japan

SOHSUKE SHIDARA

Department of Environmental Science, Faculty of Integrated Arts and Sciences, Hiroshima University, Naka-ku, Hiroshima 730, Japan

and TERUO MATSUBARA

Biological Laboratory, College of General Education, Nagoya University, Chikusa-ku, Nagoya 464, Japan

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Abstract

The CO and CN^- derivatives of cytochromes *c'* from three denitrifying bacteria (*Alcaligenes* sp. NCIB 11015 and *Achromobacter xylosoxidans* GIFU 543 and 1051) have been investigated by electronic absorption and magnetic circular dichroism (MCD) spectroscopy at room temperature. The spectral data have been characterized and compared with those of the corresponding derivatives of cytochromes *c'* from photosynthetic bacteria, cytochrome *c*, and hemoproteins containing b-type heme such as myoglobin, horseradish peroxidase, and indoleamine 2,3-dioxygenase. The absorption and MCD spectra of the CO–cytochromes *c'* complexes from the above denitrifying bacteria show low-spin ferrous chromophores with extra weak bands at around 435 nm, which are assigned to the unreacted ferrous species (high-spin). The yields of the unreacted species are 6–18%. Complete formation of the CO complex of *Alcaligenes* cytochrome *c'* takes place in the presence of n-propanol (50% n-propanol/50% 0.05 M phosphate buffer, pH 7.2). Since cytochromes *c'* from most photosynthetic bacteria give the 100% CO complexes, their CO affinities seem to be generally higher than those of cytochromes *c'* from denitrifying bacteria. The binding of cyanide to *Alcaligenes* ferric cytochrome *c'* is spectroscopically demonstrated as the second CN^- complex (low-spin) reported after the CN^-–cytochrome *c'* complex from *Chromatium vinosum* (a photosynthetic bacterium) reported already, although anionic ligands were earlier reported not to bind to the ferric cytochromes *c'*. The treatment of the CN^- complex with $Na_2S_2O_4$ results in formation of the ferrous CN^- complex (low-spin) with a small amount of the free ferrous species (high-spin).

Introduction

Cytochromes *c'* are a group of hemoproteins distributed in a wide variety of photosynthetic and chemoheterotrophic bacteria [1, 2]. These proteins are usually dimeric and are made up of identical polypeptide chains (*ca.* 14000 daltons), each incorporating a covalently bound protoheme IX as a prosthetic group. The cytochromes *c'*, however, have unusual spectroscopic and magnetic properties unlike those of other *c*-type cytochromes. Their visible absorption spectra at neutral pH are similar to those of myoglobin (Mb) and hemoglobin (Hb) rather than to those of cytochromes *c*. The unique magnetic properties of ferric cytochromes *c'* have been interpreted as originating from the heme-iron ground state which consists of a quantum mechanical admixture of an intermediate-spin ($S = 3/2$) and high-spin ($S = 5/2$) state coupled via the spin–orbit interaction [3]. Recent X-ray crystallographic studies of ferric cytochrome *c'* from *Rhodospirillum molischianum* demonstrated the penta-coordinate heme iron with a histidyl imidazole group providing the single axial ligand to the heme iron and also disclosed some features relating to the unusual mixed-spin state character [4, 5].

*Authors to whom correspondence should be addressed.

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The investigations of the bindings of various ligands to the sixth vacant site of the heme iron in cytochromes *c'* are interesting for an understanding of the heme environment. In 1963, Taniguchi and Kamen studied the spectroscopic responses of *Rhodospirillum rubrum* cytochrome *c'* to exposure to a variety of ligands which can bind to the accessible heme group of Hbs and Mbs [6]. Only NO and CO have been reported to react with the cytochromes *c'*. Addition of ionic ligands (cyanide and azide) or bulky uncharged ligands (nitrosobenzene and 4-methylimidazole) caused no changes in the visible absorption spectra and these agents were believed to be unreactive with the heme group. On the other hand, Kassner and coworkers have recently presented evidence for the binding of ethylisocyanide to ferrous cytochromes *c'* from photosynthetic bacteria and compared the spectroscopic properties and equilibrium constants for the cytochromes from four bacteria [7, 8]. The differences in binding properties were discussed in terms of the different structures between amino acid residues associated with the distal heme pocket. As a matter of course, the equilibrium constants between the cytochromes *c'* and a bulky molecule, *i.e.* ethylisocyanide, are smaller than those between the cytochromes *c'* and CO. Thereafter, they also demonstrated the binding of cyanide ion to *Chromatium vinosum* ferric cytochrome *c'* and estimated the equilibrium constant (2.1×10^4 M^{-1}) for cyanide binding to ferric cytochrome *c'* [9], which is smaller than those (10^6–10^8 M^{-1}) of the cyanide derivatives of cytochrome *c*, Mb and Hb. These results indicate that the binding site of ferric cytochromes *c'* from photosynthetic bacteria may be significantly more accessible than previously reported by Taniguchi and Kamen [6].

We have recently investigated the detailed spectroscopic characterization of the cytochrome *c'* isolated from a denitrifying bacterium, *Alcaligenes* sp. (= *Achromobacter xylosoxidans* [10, 11]) NCIB 11015 [12]. Moreover, the NO derivative of the cytochrome *c'* was prepared in order to probe the heme environment. The electronic [13], EPR [13] and magnetic circular dichroism (MCD) [14] spectroscopic investigations of the nitrosylheme revealed that the heme-iron-to-axial-histidine bond is easily cleaved upon coordination of nitrosyl group to the vacant sixth coordination site of heme iron at neutral pH and that penta-coordinate nitrosylheme is formed.

In the present paper, carbon monoxide or cyanide complexes of the cytochromes *c'* from three denitrifying bacteria, *Alcaligenes* sp. NCIB 11015 and *Achromobacter xylosoxidans* GIFU 543 and GIFU 1051, have been characterized by electronic and MCD spectroscopy. The molecules of CO and CN^- as a probe may provide important information for structural studies of the heme environment and the properties of proteins. The CO-binding constant of *Alcaligenes* cytochrome *c'* has been reported to be lower than those of cytochromes *c'* from several photosynthetic bacteria [15]. The MCD spectra of the CO-binding cytochromes *c'* from the above denitrifying bacteria indicate the coexistence of unreacted ferrous cytochromes *c'* in the reaction mixtures, supporting the low CO-affinity of the cytochromes *c'*. We also present evidence for the binding of CN^- to the cytochromes *c'* from the denitrifying bacteria as well as *Chromatium vinosum* cytochrome *c'*.

Experimental

Cytochromes *c'* from *Alcaligenes* sp. NCIB 11015 and *Achromobacter xylosoxidans* GIFU 543 and GIFU 1051 were isolated and purified in a manner similar to the methods described already [12, 16, 17]. In the reaction with CO, a Thunberg-type cuvette (1-cm optical path length) adapted for gas change was used. The ferrous cytochromes reduced with a small amount of $Na_2S_2O_4$ in 50 mM sodium phosphate buffer (pH 7.2) were treated with CO gas of slightly below 1 atm. Treatments of the cytochromes with cyanide ion were carried out at high pH over long incubation times. Kassner *et al.* first observed the absorption spectrum of the CN^-–ferric cytochrome *c'* complex by the reaction of oxidized *Chromatium vinosum* cytochrome *c'* with KCN (0.1 M) in 0.1 M phosphate buffer (pH 7.0) [9]. However, the pH value of this reaction mixture actually increases from pH 7.0 to pH 9.3. Consequently, we incubated the cytochromes *c'* with KCN (3.5 M) in alkaline solution (0.1 M K_2HPO_4/KOH buffer, pH 11.0) for 8 days at room temperature and then measured the electronic absorption and MCD spectra of the CN^- complexes after diluting the mixture with the same buffer. The final CN^- concentrations were 0.2–0.4 M.

All reagents used were of the highest grade commercially available. Carbon monoxide and horse heart cytochrome *c* (Type VI) were purchased from Seitetsu Kagaku Co. and Sigma Co., respectively.

The electronic spectra were recorded with a Shimadzu MPS-5000 or a Union SM 401 spectrophotometer. The MCD spectra were measured at room temperature with a JASCO J-500A spectropolarimeter attached to an electromagnet (1.3 T) and a JASCO DP-501 data processor.

Results and Discussion

Spectroscopic Characterization of CO Derivatives of Cytochromes c' from Denitrifying Bacteria

In Fig. 1 are depicted the visible absorption and MCD spectra of the CO–ferrous derivative of *Alcaligenes* cytochrome *c'* at pH 7.2. The absorption

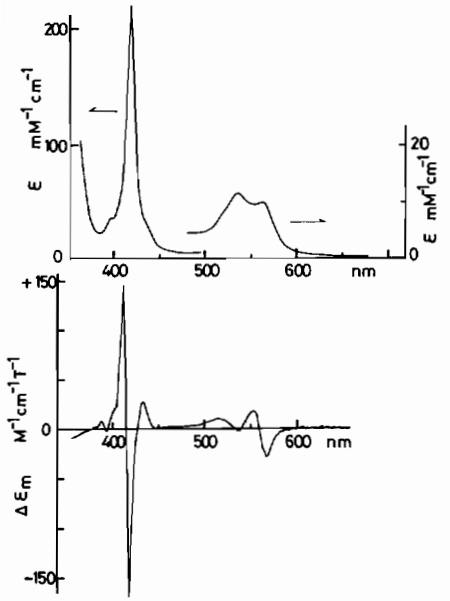

Fig. 1. Electronic (upper) and MCD (lower) spectra of *Alcaligenes* CO–cytochrome *c'* in 0.05 M phosphate buffer (pH 7.2) at room temperature.

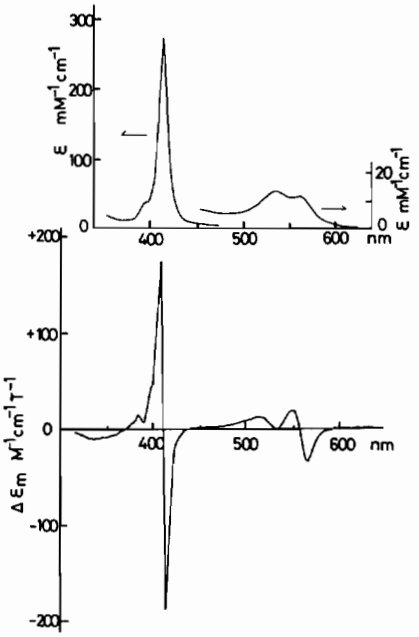

Fig. 2. Electronic (upper) and MCD (lower) spectra of *Alcaligenes* CO–cytochrome *c'* in the mixed solvent (50% n-propanol/50% 0.05 M phosphate buffer, pH 7.2) at room temperature.

patterns have definite low-spin character, as observed in the spectra of the CO complexes of many hemoproteins [18–20]. Since the stoichiometry of CO ligation to cytochromes *c'* has been found equal to one CO molecule per heme [21, 22], the axial ligands of the hexa-coordinate heme moiety are CO and imidazole. However, the electronic and MCD bands in Fig. 1 have a weak shoulder band at around 435 nm and an additional positive maximum at 434 nm, respectively. Similar shoulder bands have been observed in the spectra of CO–cytochrome *c'* from a few photosynthetic bacteria such as *Rhodospirillum rubrum* [6] and *Thiocapsa roseopersicina* [23] at around neutral pH, whereas they were not revealed in the spectra of CO complexes from *Rhodopseudomonas capsulata* (*Rhodobacter capsulatus*) B10 [24] or B100 [25], *Rhodopseudomonas palustris* [26], *Chromatium vinosum* [15], etc. These extra bands presumably result from the Soret band of unreacted ferrous cytochrome *c'*. The ferrous *Alcaligenes* cytochrome *c'* (high-spin state) gives an absorption spectrum with maxima at 426.5 ($\epsilon = 91.5$ mM^{-1} cm^{-1}) and 433 nm (shoulder, 88) and an MCD spectrum with an intense positive peak at 433 nm ($\Delta\epsilon_m$ = +158 M^{-1} cm^{-1} T^{-1}) in the Soret band region. The amount of unreacted species was estimated to be 18% at pH 7.2 from the MCD data, decreasing to 8% at pH 9.8 (see Table II).

Figure 2 shows the electronic and MCD spectra of the same CO complex in the mixture of 50% n-propanol and 50% 0.05 M phosphate buffer (pH 7.2). These are typical low-spin spectra where there are neither shoulder absorptions nor a positive MCD band in the Soret band region compared with the spectra in Fig. 1. Moreover, the increase of the Soret band in ϵ and $\Delta\epsilon_m$ values implies the complete formation of a CO species in the solvent mixture, although the peaks of the absorption and MCD bands are shifted to slightly lower wavelengths (Tables I and II).

The electronic absorption and MCD data of CO–cytochromes *c'* from three denitrifying bacteria are summarized in Tables I and II, respectively, together with those for *Rps. capsulata* and *Rps. palustris* cytochromes *c'*, horse cytochrome *c*, sperm whale myoglobin (Mb), horseradish peroxidase (HRP) and indoleamine 2,3-dioxygenase (IDO). The spectral data of cytochromes *c'* from *Achromobacter xylosoxidans* GIFU 543 and 1051 (abbreviated as GIFU 543 and GIFU 1051, respectively) at pH 7.2 also suggest the coexistence of unreacted ferrous species. The Soret MCD bands of the ferrous cytochromes *c'* from GIFU 543 and 1051 occur at 433 nm ($\Delta\epsilon_m$ = +157 and +190 M^{-1} cm^{-1} T^{-1}, respectively). Accordingly, on the basis of the $\Delta\epsilon_m$ values of the MCD bands at 433 nm, 15% and 6% of the free forms were calculated with respect to GIFU 543 and GIFU 1051 cytochromes *c'*, respectively.

The CO affinities at neutral pH of cytochromes *c'* from photosynthetic bacteria except *R. rubrum* and *T. roseopersicina* are generally higher than those from denitrifying bacteria. The smaller CO-formation constants of *Alcaligenes* and *R. rubrum* cytochromes *c'* than those of *Chromatium*, *Rps. capsulata* and

TABLE I. Electronic Spectral Data for CO–Ferrous Hemoproteins[a]

	Absorption maxima (nm) (ϵ (mM^{-1} cm^{-1}))				ϵ(Soret)/ϵ(α) ratio
	Soret(γ)		β	α	
Alc. sp. (pH 7.2)	418.5(220)	435(sh,30)	536.5(11.5)	565(9.8)	22
Alc. sp. (pH 9.8)	418.5(217)	435(sh,29)	536.5(11.5)	567.5(9.1)	24
Alc. sp. (n-PrOH)	415.5(270)		535(13.3)	562.5(11.3)	24
GIFU 543 (pH 7.2)	417.5(219)	435(sh,30)	535.5(11.5)	565(10.2)	22
GIFU 1051 (pH 7.2)	418.5(207)	435(sh,25)	535(12.7)	565(10.3)	20
Rps. capsulata[b] (pH 7.2)	417(255)		535(12.9)	566.5(10.5)	24
Rps. palustris[c]	418(262)		535(12.6)	570(11)	24
C. vinosum	418(282)		545(13.4)	565(11.5)	25
Horse *c* (pH 13.7)	414(254)		533(12.4)	560(10.0)	25
Mb[d, e]	423(187)		542(14)	579(12.2)	15
HRP[e]	422(137)		544(11.8)	575(11.4)	12
IDO[f]	420(200)		539(14.7)	570(15.6)	13

[a]Abbreviations: *Alc.* sp. = *Alcaligenes* sp. NCIB 11015 cytochrome *c'*; GIFU 543 = *Achromobacter xylosoxidans* GIFU 543 cytochrome *c'*; GIFU 1051 = *Achromobacter xylosoxidans* GIFU 1051 cytochrome *c'*; *Rps. capsulata* = *Rhodopseudomonas capsulata* B100 cytochrome *c'*; *Rps. palustris* = *Rhodopseudomonas palustris* cytochrome *c'*; *C. vinosum* = *Chromatium vinosum*; horse *c* = horse heart cytochrome *c*; Mb = sperm whale myoglobin; HRP = horseradish peroxidase; IDO = indoleamine 2,3-dioxygenase from rabbit small intestine; sh = shoulder band. Solvent: pH 7.2 = 0.05 M phosphate buffer, pH 7.2; pH 9.8 = 0.2 M $NaHCO_3$/0.2 M Na_2CO_3 buffer, pH 9.8; n-PrOH = 50% 0.05 M phosphate buffer (pH 7.2)/50% n-propanol; pH 13.7 = 0.1 M K_2HPO_4/KOH buffer, pH 13.7. [b]Ref. 25. [c]Ref. 27. [d]Ref. 28. [e]Ref. 29. [f]Ref. 18.

TABLE II. MCD Spectral Data for CO–Ferrous Hemoproteins at Room Temperature[a]

	λ (nm) ($\Delta\epsilon_m$ (M^{-1} cm^{-1} T^{-1}))				$\Delta\epsilon_m$(Soret)/$\Delta\epsilon_m$(α) ratio
	Soret (γ)		β	α	
Alc. sp. (pH 7.2)	413(+147)	434(+28)	515(+10)	554(+18)	
	415(0)		532(0)	560(0)	6.5
	418(−172)		535(−3.7)	568(−31)	
Alc. sp. (pH 9.8)	410(+36)	434(+13)	515(+7.4)	553(+15)	
	414(0)		530(0)	560(0)	2.4
	420(−58)		543(−3.3)	567(−25)	
Alc. sp. (n-PrOH)	408(+175)		513(+12)	550(+20)	
	411(0)		533(0)	558(0)	6.8
	414(−190)			565(−34)	
GIFU 543 (pH 7.2)	412(+144)	433(+28)	514(+10)	554(+19)	
	414(0)		530(0)	560(0)	6.2
	418(−167)		535(−2.1)	567(−31)	
GIFU 1051 (pH 7.2)	410(+48)	434(+9.4)	515(+8.5)	551(+17)	
	414(0)		530(0)	558(0)	2.8
	419(−65)		533(−0.9)	565(−23)	
Rps. capsulata[b] (pH 7.2)	410(+151)		515(+8.0)	553(+19)	
	412(0)		526(0)	559(0)	6.0
	416(−172)		534(−6.0)	566(−35)	
Horse *c* (pH 13.7)	406(+166)		510(+10)	548(+12)	
	410(0)		531(0)	554(0)	7.9
	413(−175)			563(−31)	
Mb[c]	417(+57)		525(+13)	565(+25)	
	422(0)			572(0)	1.9
	427(−63)		547(−4)	581(−40)	

(continued)

TABLE II. *(continued)*

HRP[d]	418(+53.0)	525(+10)	567(+26.4)	
	424(0)			1.6
	427(−51.5)	550(−5)	580(−38.5)	
IDO[e]	409(+66)	521(+)	559(+31)	
	416(0)		567(0)	1.8
	423(−70)	541(−)	575(−43.5)	

[a]For abbreviations and solvents, see the legend to Table I. [b]Ref. 25. [c]Ref. 28. [d]Ref. 29. [e]Ref. 18.

Rps. palustris cytochromes *c'* [15] support the above spectroscopic results. The chromophores of CO–cytochromes *c'* from three denitrifying bacteria, however, are more similar to those from photosynthetic bacteria (*Rps. capsulata, Rps. palustris* and *C. vinosum*) and horse cytochrome *c* than those from hemoproteins containing b-type heme, as shown in Table I. The Soret bands (414–419 nm) of the CO complexes of cytochromes *c'* and *c* appear at the shorter wavelength region than those (420–423 nm) for Mb, HRP and IDO and the intensity ratios (20–25) of the Soret band/α band are different from those (12–15) for the b-type hemoproteins. The MCD bands associated with the Soret and α bands of CO–ferrous hemoproteins (low-spin) are assigned to the Faraday A term [20]. The ratios of the Soret-to-α-band in Table II are 6.0–7.9 for c-type hemoproteins (except *Alcaligenes* cytochrome *c'* at pH 9.8 (2.4) and GIFU 1051 cytochrome *c'* (2.8)) and 1.6–1.9 for b-type hemoproteins. The anomalously small ratios of the CO–cytochromes *c'* from *Alcaligenes* sp. at pH 9.8 and GIFU 1051 at pH 7.2 might imply a similarity of their CO-binding heme environments to those of three b-type hemoproteins such as Mb, HRP and IDO.

Some factors have been proposed to account for the differences between the affinities of CO for cytochromes *c'* and those for high-spin b-type hemoproteins [8, 30]. The X-ray crystallographic analysis of *R. molischianum* cytochrome *c'* showed that amino acid residues in the ligand binding pocket of the cytochrome *c'* are more tightly packed and more hydrophobic than those in the globin ligand pocket and thus restrict access of exogenous ligands to the sixth iron coordination site [4, 5]. It seems likely that the restriction of the ligand binding pocket for CO decreases generally in the following order: globins > cytochromes *c'* from photosynthetic bacteria > cytochromes *c'* from denitrifying bacteria. This steric hindrance at the sixth axial site could be somewhat relaxed by the interaction between the protein and a hydrophobic alcohol, n-propanol, because 100% formation of the CO derivative of *Alcaligenes* cytochrome *c'* was observed in the mixture of 50% phosphate buffer (pH 7.2) and 50% n-propanol (Fig. 2). In cytochromes *c'* the lack of a hydrogen bonding group capable of forming a stabilizing interaction with the exogenous ligands such as that found in Mb and Hb [31] also may be unfavorable for the formation of a hexa-coordinate heme.

Spectroscopic Characterization of the CN^- Derivative of Alcaligenes sp. Cytochrome c'

Figure 3 shows the electronic absorption spectra of CN^- complexes of ferric and ferrous cytochromes *c'* from *Alcaligenes* sp. in 0.1 M phosphate buffer solutions (pH 11.0). The MCD spectra of these derivatives at room temperature are represented in Fig. 4. These spectra of the ferric CN^-–cytochrome *c'* are essentially similar to those of hexa-coordinate ferric hemoproteins (low-spin) containing cyanide ion (Table III), although the absorption maximum of the Soret band is a little different from those for the CN^- complexes of *Chromatium* cytochrome *c'* and horse cytochrome *c*. These findings imply that the low-spin heme in the cytochrome *c'* from a denitrifying bacterium, *Alcaligenes* sp., also binds one histidyl imidazole group and one cyanide ion as the axial ligands of the heme chromophore. The absorption spectrum of the *Alcaligenes* ferric cytochrome *c'* in the absence of CN^- ion at pH 11.0 exhibits a feature of quantum mechanical admixture of an intermediate-spin state and a high-spin state [12].

Cyanide ion serves generally as a high-affinity ligand for the ferric state of most hemoproteins and the resulting complexes are invariably in the low-spin state [31], but the equilibrium constants for cyanide

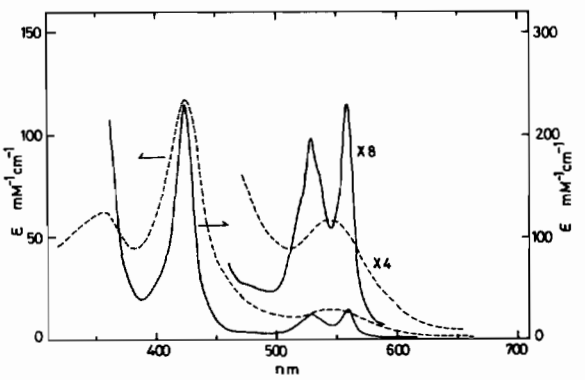

Fig. 3. Electronic spectra of *Alcaligenes* ferrous (solid line) and ferric (broken line) CN^-–cytochrome *c'* at pH 11.0.

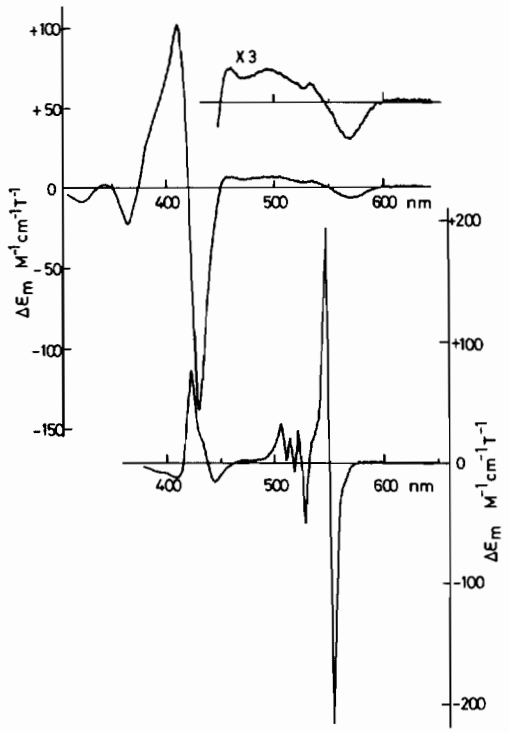

Fig. 4. MCD spectra of *Alcaligenes* ferric (upper) and ferrous (lower) CN^-–cytochrome *c'* at pH 11.0 and room temperature.

TABLE III. Absorption and MCD Spectral Data for CN^-–Ferric Hemoproteins[a]

	Absorption maxima (nm) (ϵ (mM^{-1} cm^{-1}))	MCD (Soret) (nm) ($\Delta\epsilon_m$ (M^{-1} cm^{-1} T^{-1}))
Alc. sp. (pH 11)	424.5(114) 545(14)	410(+102) 420(0) 430(−139)
C. vinosum[b]	417(121) 538(10.1)	
Horse *c*[c] (pH 14)	414(105) 539(9.7)	402(+90) 410(0) 418(−119)
Mb[d] (pH 6.4–6.8)	422(116) 540(11.3)	415(+72) 423(0) 431(−91)
IDO[e] (pH 6.0)	419(115) 540(14)	410(+91) 418(0) 427(−116)

[a]For abbreviations, see the legend to Table I. [b]Ref. 9. [c]Ref. 33 and this work. [d]Refs. 18 and 29. [e]Refs. 18 and 34.

binding to *Chromatium* ferric cytochrome *c'* decreases about two or four orders of magnitude compared to those for horse cytochrome *c* or horse and human hemoglobins, respectively [9]. In the case of *Alcaligenes* cytochrome *c'*, the equilibrium constant could not be obtained, since the rate of complex formation was extremely slow. The yield of the CN^- complex of *Alcaligenes* cytochrome *c'* was estimated to be 55% in 0.2 M phosphate buffer (pH 10) containing 0.3 M KCN after 7 days, whereas *Chromatium* cytochrome *c'* perfectly binds cyanide ion in 0.1 M phosphate buffer containing 0.1 M KCN after several hours [9]. The cytochrome *c'* from *Achromobacter* GIFU 543 exhibited lower CN^- reactivity (35% yield) than that from *Alcaligenes* sp. under the same conditions. A previous report by Taniguchi and Kamen indicated that no anionic ligands bind to the ferric cytochrome *c'* from a photosynthetic bacterium, *Rhodospirillum rubrum* [6], over a wide pH range. On the other hand, Kassner *et al.* demonstrated that ferric *Chromatium* cytochrome *c'* binds cyanide ion, so that the heme-binding site of the cytochrome *c'* is significantly more accessible to ligands than previously suggested [9]. The heme-binding sites of cytochromes *c'* from denitrifying bacteria such as *Alcaligenes* sp. and *Achromobacter* GIFU 543 are also accessible to cyanide ion, though their reactivities are considerably lower than that of *Chromatium* cytochrome *c'*. It is probable that the ligand-binding pockets of the cytochromes *c'* from two denitrifying bacteria are smaller than that from a photosynthetic bacterium, *C. vinosum*. Moreover, azide ion did not bind to ferric *Alcaligenes* cytochrome *c'* in 0.1 M phosphate buffer (pH 11) containing 5 M NaN_3 even after standing for 6 days at room temperature, which supports the low reactivity of the cytochrome *c'* to anionic ligands.

The absorption and MCD spectra of CN^-–*Alcaligenes* cytochrome *c'* reduced with sodium dithionate are exhibited in Figs. 3 and 4, respectively. These spectra indicate the characteristic features of the ferrous low-spin hemochrome of the CN^- derivative. Especially, the MCD spectrum (Fig. 4) is similar to that of ferrous cytochrome *c* in that it shows a distinct Faraday A term in the α-band region [32]. The shoulder of the Soret band at around 432 nm is probably due to the free form of unreacted ferrous cytochrome *c'*, which gives an intense positive MCD band at 433 nm [12]. On reduction, cyanide derivatives of Mb and Hb generally tend to dissociate spontaneously [31]. Consequently, the reduced sample of the CN^-–cytochrome *c'* also contains the unreacted form (high-spin) as a minor species.

The absorption and MCD spectral data for CN^-–ferrous hemoproteins are enumerated in Table IV. The absorption peaks of the electronic and MCD spectra of the CN^-–ferrous *Alcaligenes* cytochrome *c'* complex are more similar to those for horse cytochrome *c* than those for Mb and IDO. On the contrary, the spectral data for the CN^-–ferric *Alcaligenes* cytochrome *c'* complex resemble those for Mb

TABLE IV. Absorption and MCD Spectral Data for CN^-–Ferrous Hemoproteins[a]

	Absorption maxima (nm) (ϵ (mM^{-1} cm^{-1}))	MCD (Soret) (nm) ($\Delta\epsilon_m$ (M^{-1} cm^{-1} T^{-1}))
Alc. sp. (pH 11)	424(222) 529(23) 559(28)	422(+77) 432(sh, +21) 437(0) 444(−16)
Horse *c*[b] (pH 14)	421(164) 525(16.8) 554(20.4)	418(+71) 424(0) 430(−22)
Mb[c] (pH 9.1)	433(160) 535(15) 566(20)	429(+20) 435(0) 441(−8.4)
IDO[c] (pH 6.0)	428(182) 532(17.3) 562(26.1)	425(+43) 433(0) 438(−10)

[a]For abbreviations, see the legend to Table I. [b]Ref. 33 and this work. [c]Ref. 18.

and IDO more closely than those for *Chromatium* cytochrome *c'* and horse cytochrome *c* (Table III). Thus it is presumed that the heme environment of *Alcaligenes* cytochrome *c'* binding a cyanide ion bears a resemblance to those of c-type hemoproteins in the ferrous form and to those of b-type hemoproteins in the ferric form. A distinction between the heme environments might result from the Fe–C–N bond angles and the possible influence of variable hydrogen bonds to the coordinated cyanide ion and imidazole group.

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