Effect of Sodium Dodecyl Sulfate on Spectral Properties of the Nitric Oxide Complex of Ferrous Cytochrome *c'* from *Alcaligenes* sp. NCIB 11015

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Cytochromes *c'* are *c*-type cytochromes found in photosynthetic, denitrifying and nitrogen-fixing bacteria [1]. It has been demonstrated by X-ray crystallographic studies of the cytochrome *c'* from *Rhodospirillum molischianum* that the heme fifth ligand is a histidine residue and that the sixth coordination position is vacant and surrounded by hydrophobic amino acid residues [2]. From a comparison of the amino acid sequence of various cytochromes *c'* with the tertiary structure of *R. molischianum* cytochrome *c'*, the probable heme environments in cytochromes *c'* have been reasonably considered to resemble those in *R. molischianum* cytochrome *c'* [3]. However, the findings that the binding properties of CO and NO groups with the cytochromes *c'* differ depending on the origin of the cytochromes *c'* [4, 5] suggest that the heme environments also delicately differ for each cytochrome *c'*.

The *Alcaligenes* cytochrome *c'* was first purified from a denitrifying bacterium, *Alcaligenes* sp. NCIB 11015, and characterized by Iwasaki *et al.* [6, 7]. The cytochrome *c'* consists of a dimer with equivalent subunits (subunit molecular weight 14000) [8, 9]. We have investigated the spectral properties of *Alcaligenes* cytochrome *c'* and its NO complex under various conditions [5, 10]. In these reports, we have demonstrated that heme axial ligand(s) of the ferric cytochrome *c'* can be markedly affected by varying the pH of the medium and by the presence of sodium dodecyl sulfate (SDS), and that the heme-iron to histidine bond of the ferrous cytochrome *c'* is very weak and is cleaved upon coordination of the NO group. In the present work, the spectral properties of the NO-*Alcaligenes* cytochrome *c'* in the presence of SDS are investigated for the purpose of clarifying the stereochemistry of the heme environment.

Cytochrome *c'* from *Alcaligenes* sp. NCIB 11015 was isolated and purified by the method described previously [7]. The EPR and electronic absorption spectral measurements were carried out as described previously [5]. Heme concentrations were determined from electronic absorption measurements, using the alkaline pyridine hemochrome (ϵ_{mM} at 550 nm = 29.1 [11]) method. The buffer at pH 7.2 was 50 mM NaH_2PO_4/Na_2HPO_4.

Ferric cytochrome *c'*–SDS solution at pH 7.2 was reduced and reacted with NO under anaerobic conditions, as described previously [6]. The concentrations of cytochrome *c'* and SDS were 0.4 and 4.0 mM, respectively, for EPR measurements, and 0.025 and 0.9 mM, respectively, for electronic spectral measurements.

Both the line shape and the parameters of the EPR spectrum (at 77 K) for the NO–ferrous cytochrome *c'* with SDS were quite similar to those of the NO–hemoglobin modified by SDS [12] and of the five-coordinate model nitrosylhemes [13 - 15] (Table I, Fig. 1B). On the other hand, the EPR line shape of the NO–cytochrome *c'* without SDS was slightly different from that with SDS (Fig. 1A), although

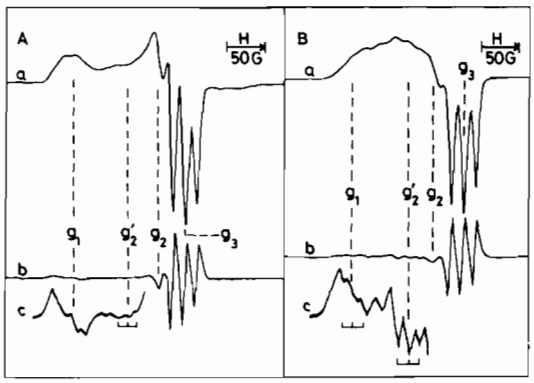

Fig. 1. EPR spectra of NO–cytochrome *c'* from *Alcaligenes* sp. NCIB 11015 at 77 K and at pH 7.2 (50 mM phosphate buffer): (A) in the absence and (B) in the presence of sodium dodecyl sulfate; (a) first-derivative, (b) second-derivative and (c) the expansion of the ordinate of (b). Instrument settings: modulation frequency and amplitude, 100 kHz and 2 G; microwave frequency and power, 9.174–9.176 GHz and 10 mW.

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TABLE I. EPR Parameters of NO–Hemoproteins and Model Complex at 77 K[a]

	g_1	g'_2	g_2[c]	g_3	Coupling constants[b] (G)			Reference
					a_1	a'_2	a_3	
NO–cyt c' (pH 7.2)	2.106	2.058	2.033	2.010(4)	~14	13	16.0	5
NO–cyt c' + SDS (pH 7.2)	2.105	2.054	2.035	2.010(2)	14	14.2	16.5	
NOHb + SDS (pH 7)	2.094	2.051		2.009			17	12
Fe(PPIXDME)(NO) in acetone	2.109	2.059	2.035	2.009(5)	13	16	16.5	16

[a]Abbreviations used: cyt c', cytochrome c' from *Alcaligenes* sp. NCIB 11015; Hb, hemoglobin; SDS, sodium dodecyl sulfate; PPIXDME, dianion of protoporphyrin IX dimethyl ester. [b]Originated from the hyperfine interactions of an unpaired electron with the ^{14}N nucleus of the NO group. [c]The g value in the lowest field line of the g_2 absorption which consists of three lines (triplet).

the former was similar to the latter in parameters (Table I).

The electronic spectrum (at 20 °C) for the NO-ferrous cytochrome c' with SDS (Fig. 2) resembled that for a five-coordinate model, nitrosyl(protoporphyrin IX dimethyl ester)iron(II), in organic solvents (Table II) [17]. The relative intensity ratio of the α- to β-band (α/β ratio) was greater than one in the spectra for the NO–cytochrome c' with SDS and for the five-coordinate model, while it was below one in that for the NO–cytochrome c' without SDS.

The hydrophobic part of SDS, an anionic detergent, is known to penetrate into the hydrophobic core of the protein and to cause a considerable structural change in the heme pocket of hemoglobin, myoglobin [18, 19] and *Alcaligenes* cytochrome c' [10].

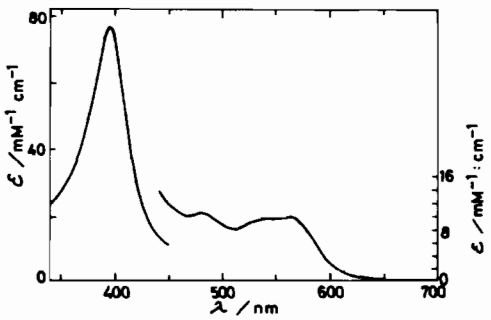

Fig. 2. Electronic spectrum of NO–cytochrome c' from *Alcaligenes* sp. NCIB 11015 in the presence of sodium dodecyl sulfate at 20 °C and at pH 7.2 (50 mM phosphate buffer).

On addition of SDS to both ferric and ferrous cytochrome c' from *Alcaligenes*, the amino acid residues around the heme group are displaced and the cytochrome c' exhibits the low-spin type spectra which originated from the heme-iron with two axial ligands. If a nitrosyl group is coordinated to such a heme-iron, one of two axial ligands may be replaced by the nitrosyl group and the nitrosylheme thus obtained may be six-coordinated, as has been reported for the coordination of the nitrosyl group to cytochrome c [20]. However, both the EPR and electronic spectra for the NO–ferrous cytochrome c' with SDS exhibited the spectral features characteristic of five-coordinate nitrosylheme. These results suggest that coordination of the nitrosyl group leads to further structural changes in the heme pocket. The nitrosylheme is probably surrounded by the hydrophobic part of SDS because the EPR and electronic spectral features apparently resemble those of nitrosylhemoglobin modified by SDS [12] and of the five-coordinate model nitrosylheme in non-polar organic solvents [14, 17].

The EPR and electronic spectral features for the NO–cytochrome c' without SDS are slightly different from those with SDS, as mentioned above. This suggests that there are some electronic and steric interactions between the nitrosyl group and hydro-

TABLE II. Electronic Spectral Data of NO–Cytochrome c' from *Alcaligenes* sp. NCIB 11015 and Model Complex at 20 °C[a]

	Absorption maxima (nm) (extinction coefficient ($mM^{-1} cm^{-1}$))				Reference
	Soret (γ)		β	α	
NO–cyt c' (pH 7.2)	396.5 (78.9)	415sh	485 (9.8)	541 (10.4)	565sh (10)	5
NO–cyt c' + SDS (pH 7.2)	395 (76.9)		481.5 (12.5)	545 (9.7)	564 (10.0)	
Fe(PPIXDME)(NO) in acetone	396 (91.2)		480.5 (12.1)	542.5 (11.7)	566 (12.2)	17

[a]Abbreviations used: cyt c', cytochrome c'; SDS, sodium dodecyl sulfate; PPIXDME, dianion of protoporphyrin IX dimethyl ester; sh, shoulder.

phobic amino acid residues in the NO–cytochrome c' without SDS, as is inferred from analogy with the heme environment of *R. molischianum* cytochrome c' with the close packing of the residues about the sixth axial coordination position [2]. Details of the effect of SDS and the other reagents on cytochrome c' and its NO derivative will be published in the future.

The g_2 absorption in the EPR spectrum of five-coordinate nitrosylheme has been so far assigned to the absorption at g'_2 in Fig. 1 [14, 15, 21]. However, it seems unreasonable that this g'_2 absorption is assigned to the g_2 absorption, because the intensity of the g'_2 absorption in EPR spectra of the NO–cytochrome c' varies appreciably on addition of SDS. On the other hand, the intensity and the position of an unidentified absorption, which has been called a $g_?$ absorption [15] and is represented as g_2 absorption in Fig. 1, does not vary with addition of SDS and this absorption has always been observed in the spectra of five-coordinate nitrosylheme [12–15, 21]. Thus, this absorption can be assigned to a g_2 absorption. Since the position of the g_2 absorption in NO–cytochrome c' shifts to the higher field side by about 4G from the ^{14}NO- to the ^{15}NO-derivative [5], the g_2 absorption in Fig. 1 can be identified as a lowest field line of a triplet derived from a ^{14}N nucleus of ^{14}NO, and the other two lines are hidden in intense g_3 absorption. Recently, the EPR spectrum of the single crystal of Fe(TTP)(NO) doped in Zn(TPP) (TPP = *meso*-tetraphenylporphyrinato) has been precisely analyzed [22]. From this study, it has been demonstrated that principal values of g and A tensors for the Fe(TPP)(NO) single crystal are dependent upon temperature and g_{xx}, g_{yy} and g_{zz} values at 27 K are 2.106, 2.028 and 2.010, respectively. Accordingly, it seems probable that, in the EPR spectrum of five-coordinate nitrosylheme, the absorptions which have so far been called g_2 and $g_?$ are respectively ascribed to an unidentified absorption and a part of a triplet of the g_2 absorption.

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