April, 1987] © *1987 The Chemical Society of Japan* *Bull. Chem. Soc. Jpn.*, ***60***, *1375—1378 (1987)* 1375

Effect of Sodium Dodecyl Sulfate on Spectral Properties of Five- and Six-Coordinate Nitrosyl(protoporphyrin IX dimethyl esterato)iron(II) Complexes

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

(Received October 28, 1986)

EPR and electronic spectra of five- and six-coordinate nitrosyl(protoporphyrin IX dimethyl esterato)iron(II) (Fe(ppixdme)(NO)) complexes as model systems for nitrosylhemoproteins have been investigated in the absence and the presence of sodium dodecyl sulfate (SDS). Dimethyl sulfoxide, an O-donor ligand, is weakly coordinated to iron in an axial vacant position trans to a nitrosyl group in Fe(ppixdme)(NO). Upon addition of SDS to Fe(ppixdme)(NO) in dimethyl sulfoxide, EPR and electronic spectra were changed into five-coordinate type spectra. On the other hand, the addition of SDS to Fe(ppixdme)(NO)(NMeIm) in dimethyl sulfoxide resulted in EPR and electronic spectra arising from a mixture of five- and six-coordinate complexes. Accordingly, sodium dodecyl sulfate was found to hinder the interaction of dimethyl sulfoxide with the sixth axial vacant position of Fe(ppixdme)(NO), and to cleave only partly the iron to imidazole bond in Fe(ppixdme)(NO)(NMeIm) complex.

Sodium dodecyl sulfate (SDS), an anionic detergent, has been known to solubilize insoluble porphyrins and metalloporphyrins in aqueous solution in monomer form.[1,2] Effect of SDS on spectral properties of iron(II) and iron(III) porphyrin complexes with various axial ligands has been investigated.[2] Furthermore, it has been demonstrated that the hydrophobic part of SDS penetrates into the hydrophobic core of the protein to cause a considerable structural change of hemoglobin, myoglobin,[3] and cytochrome *c*′,[4] and that both the EPR and electronic spectra of NO-hemoglobin[5] and NO-cytochrome *c*′ modified by SDS[6] resemble those of five-coordinate model nitrosylheme in nonpolar organic solvents in spectral line shape.[7,8]

The clarification of the interaction of SDS and hydrophobic environments around heme in NO-hemoproteins seems to bear a significant implication in understanding the structure of the heme environments of the hemoproteins. In this paper, it is reported that EPR and electronic spectra of five- and six-coordinate nitrosyl(protoporphyrin IX dimethyl esterato)iron(II) (Fe(ppixdme)(NO)) complexes as model systems for NO-hemoproteins are changed upon addition of SDS.

Experimental

Materials and Methods. Fe(ppixdme)Cl and Fe(ppixdme)(NO) were prepared as described before.[7,9] Nitrogen monoxide (99.5% minimum) purchased from Seitetsu Kagaku Co. Ltd. was passed through a KOH column to remove higher nitrogen oxides. ^{15}NO gas was generated by the reaction of Na^{15}NO$_2$ (99.2% enrichment, Prochem) with an aqueous solution of ascorbic acid. 1-Methylimidazole (NMeIm) and dimethyl sulfoxide were distilled by flowing N$_2$ under reduced pressure and were deoxygenated by bubbling with pure N$_2$ prior to use. Sodium dodecyl sulfate (Wako chemicals) was purchased and used without further purification. All other chemicals used were obtained as the best available grade and were used without further purification.

EPR and electronic spectral measurements were carried out as described previously.[10]

Sample Preparation. EPR and electronic spectral samples were prepared by following two method. (a) Under N$_2$ atmosphere, Fe(ppixdme)(NO) was dissolved into dimethyl sulfoxide or NMeIm-dimethyl sulfoxide solution, in the absence or the presence of SDS. (b) The reaction of NO with Fe(ppixdme)Cl in dimethyl sulfoxide or NMeIm-dimethyl sulfoxide solution, in the absence or the presence of SDS was carried out in a Thunberg-type tube with an optical cuvette or with an EPR tube. The solutions were carefully degassed in the Thunberg tube by repeated freezing and thawing in vacuo and then were equilibrated with NO gas at about 1 atm.

Then, the solutions obtained by method (a) or (b) were transferred into an optical cuvette or an EPR tube (or flat cell). Both methods resulted in identical spectral results.

The concentrations of Fe(ppixdme)(NO), NMeIm, and SDS in EPR samples were 0.1—0.6 mM[†], 80 mM, and more than 80 mM, respectively; those in electronic spectral samples were 0.016 mM, 0.51—2.03, and 0.10—1.16 M, respectively.

Results and Discussion

It was shown that the N-donor ligands can be coordinated,[8,10–12] both at room temperature and at low temperature, to iron in the axial position trans to a nitrosyl group in Fe(ppixdme)(NO), while the weak ligands such as O- and S-donor ones can be, in general, coordinated only at low temperature.[13] However, the axial coordination of dimethyl sulfoxide, an O-donor ligand, to Fe(ppixdme)(NO) not only at low temperature but also at room temperature has been confirmed by the following findings: EPR *g* values (g_{iso}), the NO stretching frequencies, and the Soret band maxima of Fe(ppixdme)(NO) in various non-donor solvents at room temperature almost linearly decrease with an increase of a solvent polarity parameter (E_{T}), and on the other hand, those in dimethyl sulfoxide distinctly deviate from such linear relationships.[8,12]

Addition of Sodium Dodecyl Sulfate to Fe(ppixdme)(NO) in Dimethyl Sulfoxide. In the electronic spec-

---

[†] 1 M=1 mol dm^{-3}.

Table 1. Electronic Spectral Data of Nitrosyl(protoporphyrin IX dimethyl esterato)iron(II) Complexes and NO-Hemoproteins with and without Sodium Dodecyl Sulfate at Room Temperature[a)]

	Absorpn max/nm (ε/mM^{-1} cm^{-1})				
	γ(Soret)			β	α
Fe(ppixdme)(NO)[b)]	405(90.7)		480sh(11)	555(10.8)	
Fe(ppixdme)(NO) in toluene[b)]	401(81.2)		480sh(12)	550sh(10)	571(10.9)
Fe(ppixdme)(NO)+SDS[c)]	401.5(76.8)		478sh(10.4)	545(10.4)	563.5(10.6)
NO-cyt c′+SDS[d)]	395(76.9)		481.5(12.5)	545(9.7)	564(10.0)
Fe(ppixdme)(NO)(NMeIm)		419(115)		545.5(11.4)	576.5(10.7)
Fe(ppixdme)(NO)(NMeIm)+SDS[e)]	400sh(67)	417(91.2)	485sh(8.7)	545(11.1)	574(10.3)

a) Abbreviations used: Fe(ppixdme)(NO), nitrosyl(protoporphyrin IX dimethyl esterato)iron(II); NMeIm, 1-methylimidazole; SDS, sodium dodecyl sulfate; cyt c′, cytochrome c′ (*Alcaligenes* sp. NCIB 11015); sh, shoulder. Solvent used for model complexes is dimethyl sulfoxide unless otherwise stated. b) Ref. 8. c) [Fe(ppixdme)(NO)]=0.016 mM, [SDS]=0.1 M. d) Ref. 6. e) [Fe(ppixdme)(NO)]=0.016 mM, [NMeIm]=0.51 M, [SDS]=1.16 M.

Table 2. EPR Parameters of Nitrosyl(protoporphyrin IX dimethyl esterato)iron(II) Complexes and NO-Hemoproteins with and without Sodium Dodecyl Sulfate[a)]

	g values					Coupling const/G[b)]		
	g_{iso}	g_1	g_2'	g_2[c)]	g_3	a_1	a_2'	a_3
Fe(ppixdme)(^{14}NO)[d)]	2.048							
Fe(ppixdme)(^{14}NO) in toluene[e)]	2.052	2.102	2.058	2.038	2.010	13	16	16.7
Fe(ppixdme)(^{14}NO)+SDS[f)]	2.052	2.107	2.059	2.036	2.009_9	13	14	16.6
Fe(ppixdme)(^{15}NO)+SDS[f)]	2.052	2.106	2.059	2.032	2.009_7	21	21	22.8
^{14}NO-Hb+SDS[g)]		2.094	2.051		2.009			17
^{14}NO-cyt c′+SDS[h)]		2.105	2.054	2.035	2.010_2	14	14.2	16.5

a) See footnote a of Table 1 for abbreviations. Solvent used for model complexes is dimethyl sulfoxide unless otherwise stated. g_{iso} is a g values at room temperature and the other parameters are those at 77 K. b) 1T=10^{4}G. c) The g value in lowest field line of g_2 absorption which consists of three (^{14}NO) and two (^{15}NO) lines. d) Ref. 12. e) Ref. 7. f) [Fe(ppixdme)(NO)]=0.6 mM, [SDS]=0.8 M. g) Hb, hemoglobin. Ref. 5. h) Ref. 6.

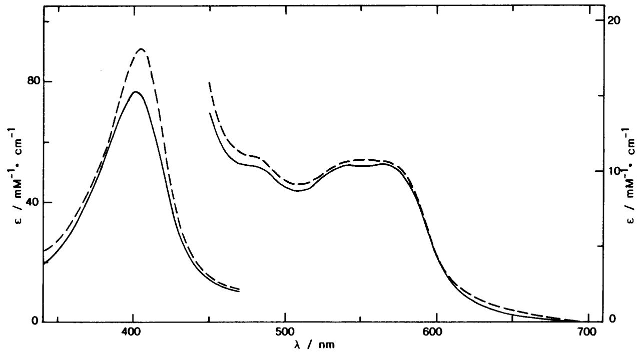

Fig. 1. Electronic spectra of Fe(ppixdme)(NO) (0.016 mM) in dimethyl sulfoxide in the presence (0.1 M) (——) and the absence (----) of sodium dodecyl sulfate at room temperature.

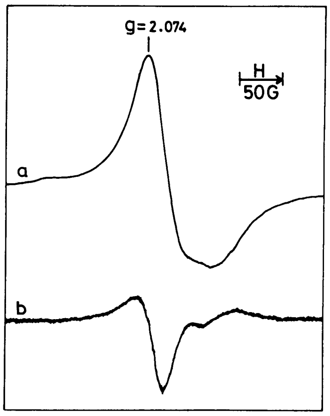

Fig. 2. EPR spectra of Fe(ppixdme)(NO) (0.4 mM) in dimethyl sulfoxide at 77 K; (a) first derivative, (b) second derivative. Instrument settings are given in caption of Fig. 3.

trum of Fe(ppixdme)(NO) in dimethyl sulfoxide (Table 1 and Fig. 1), the Soret band was revealed at 405 nm, which is located at longer wavelength than that (about 396 nm) predicted by λ_{max}(Soret)-E_T relationship,[8)] and α- and β-bands were not resolved, suggesting the coordination of a dimethyl sulfoxide to the sixth axial position. On the other hand, the addition of SDS to the dimethyl sulfoxide solution resulted in a spectral change. With an increase in SDS concentration, the Soret band was shifted to shorter wavelength side and the α- and β-bands were resolved. The apparent spectral pattern thus obtained (Fig. 1) resembles those of Fe(ppixdme)(NO) in non-donor solvents such as toluene and benzene[8)] and of NO-cytochrome c′ (*Alcaligenes* sp. NCIB 11015) with SDS.[6)]

At room temperature, the EPR spectra of Fe(ppixdme)(NO) in organic solvents exhibit a well-resolved triplet.[7)] The g_{iso} values at central signal of a triplet in non-donor solvents were in the range of 2.053 to 2.050,[12)] while that in dimethyl sulfoxide was 2.048 (Table 2). At 77 K, Fe(ppixdme)(NO) in non-donor

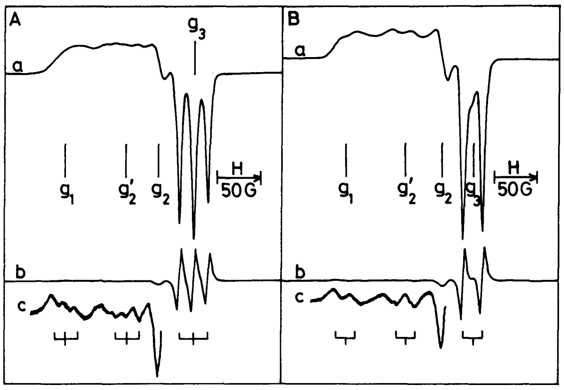

Fig. 3. EPR spectra of (A) Fe(ppixmde)(^{14}NO) and (B) Fe(ppixdme)(^{15}NO) (0.6 mM) in dimethyl sulfoxide in the presence of sodium dodecyl sulfate (0.8 M) at 77 K; (a) first derivative, (b) second derivative, and (c) expansion of the ordinate of (b). Instrument settings: modulation frequency and amplitude, 100 kHz and 2 G; microwave frequency and power, 9.166—9.171 GHz and 10 mW.

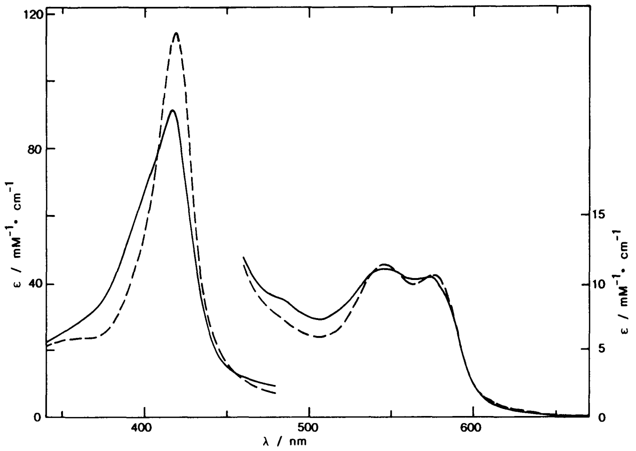

Fig. 4. Electronic spectra of Fe(ppixdme)(NO)(0.016 mM)-NMeIm(0.51 M) in dimethyl sulfoxide in the presence (1.16 M) (——) and the absence (----) of sodium dodecyl sulfate at room temperature.

solvents exhibits characteristic EPR spectra with an intense triplet in g_3 (or g_z) absorption,[7,14] while the spectral line shape in dimethyl sulfoxide was markedly different from such usual ones as shown in Fig. 2. Thus, the EPR spectral results of Fe(ppixdme)(NO) in dimethyl sulfoxide both at room temperature and at 77 K also suggest the pronounced interaction of a dimethyl sulfoxide with the vacant axial position of Fe(ppixdme)(NO).

The EPR spectrum of Fe(ppixdme)(NO) in dimethyl sulfoxide was markedly changed upon addition of SDS. Both the line shape and the parameters of the spectra thus obtained were quite similar to those of five-coordinate model nitrosylheme,[9,14] of NO-hemoglobin modified by SDS,[5] and of NO-cytochrome c′ (*Alcaligenes* sp. NCIB 11015) with SDS[6] (Table 2 and Fig. 3). Although nitrosylporphyrinatoiron(II) in nonpolar solvents occasionally exhibits complicated EPR spectra arising from a mixture of monomer and dimer below frozen temperature,[12,15,16] the satellite absorptions which are attributable to a dimer were not observed at all in the EPR spectrum of Fe(ppixdme)(NO) with SDS in dimethyl sulfoxide (Fig. 3).

These results indicate that sodium dodecyl sulfate hinders the interaction of dimethyl sulfoxide, O-donor ligand, with the axial vacant position in Fe(ppixdme)(NO) and the dimerization of Fe(ppixdme)(NO) molecule.

Both the Soret band maximum and g_{iso} value of Fe(ppixdme)(NO) with SDS in dimethyl sulfoxide were quite similar to those in toluene, a nonpolar solvent (Tables 1 and 2). This suggests that Fe(ppixdme)(NO) molecule is surrounded by hydrophobic part of SDS and consequently the polarity of molecular environment is decreased locally.

Thus, the nitrosylheme in both NO-hemoproteins modified by SDS and model system with SDS is surrounded by the hydrophobic part of SDS or the environment with a very small polarity.

It has been recently demonstrated from the EPR study on the single crystal of Fe(tpp)(NO) doped in Zn(tpp) (tpp, *m*-tetraphenylporphyrinato) that g_2 (or g_y) value is 2.028.[17] Thus, the 'g_2 absorption' of Fig. 3 and Table 2 can be identified as a lowest field line of triplet or doublet in such a g_2 absorption, which is derived from an ^{14}N nucleus of ^{14}NO or an ^{15}N nucleus of ^{15}NO, respectively. Accordingly, the g_2' absorption of Fig. 3 and Table 2 remains unidentified.

Addition of Sodium Dodecyl Sulfate to Fe(ppixdme)(NO)(NMeIm) in Dimethyl Sulfoxide. The Soret band in the electronic spectrum of six-coordinate Fe(ppixdme)(NO)(NMeIm) in dimethyl sulfoxide (Fig. 4) was narrower and more intense and was located at longer wavelength side than that of five-coordinate Fe(ppixdme)(NO) in non-donor solvents.[8] Upon addition of SDS to the solution, the Soret band was weakened, the shoulder absorptions was revealed at around 400 and 485 nm, and α- and β-bands were broadened (Fig. 4), which are compatible with the appearance of five-coordinate complex and concomitant decrease in concentration of six-coordinate complex. From the Soret band intensity in the solution with SDS (Fig. 4), the contents of the six- and five-coordinate complexes are estimated to be 60 and 40%, respectively. On freezing the solution with SDS at 77 K, the positions of the band maxima were slightly varied to 400sh, 419.5, 485sh, 542, and 575 nm and the absorptions derived from five-coordinate complex were decreased in intensity. However, the spectrum could not be completely changed to that of five-coordinate complex even in much higher concentration of SDS.

The EPR spectrum of Fe(ppixdme)(NO)(NMeIm) in dimethyl sulfoxide at 77 K exhibited the line shape characteristic of six-coordinate nitrosylheme with

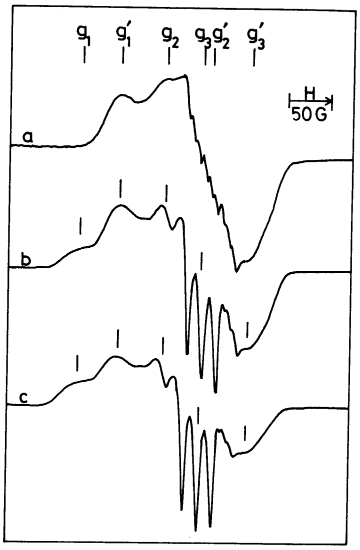

Fig. 5. EPR spectra of Fe(ppixdme)(NO)(0.4 mM)-NMeIm(80 mM) in dimethyl sulfoxide (a) in the absence and (b,c) in the presence of sodium dodecyl sulfate (b, 0.32 M; c, 4 M-almost saturated) at 77 K (g_1=2.10, g_2=2.035, g_3=2.010; g_1'=2.070, g_2'=2.004, g_3'=1.97). Instrument settings are given in caption of Fig. 3.

three g values (g_1', g_2', and g_3' in Fig. 5). Upon addition of SDS, the absorptions (g_1, g_2, and g_3) being attributable to five-coordinate complex were appeared and intensified (Figs. 5-b,c). Even trace amounts of five-coordinate complex were found to be detectable in the EPR spectrum of the sample containing nitrosylheme because the triplet in g_3 absorption of the five-coordinate complex is extremely intense.

Accordingly, the clevage of iron to 1-methylimidazole bond probably results from the interaction of the hydrophobic part of SDS with 6th coordination position or, possibly, from the intercalation of the five-coordinate complex into the micelles of SDS in dimethyl sulfoxide. However, since unhindered N-donor ligands such as imidazoles and pyridines are strongly coordinated to iron in the axial position trans to a nitrosyl group in nitrosylporphyrinatoiron(II),[12] all the iron to nitrogen bond can not be cleaved even in almost saturated solution of SDS.

References

1) J. E. Falk, "Porphyrins and Metalloporphyrins," Elsevier, Amsterdam (1964), p. 117; P. Hambright, "Porphyrins and Metalloporphyrins," ed by K. M. Smith, Elsevier, Amsterdam (1975), p. 233.

2) J. Simplicio, *Biochemistry*, **11**, 2525 (1972); J. Simplicio, *ibid.*, **11**, 2529 (1972); J. Simplicio, K. Schwenzer, and F. Maenpa, *J. Am. Chem. Soc.*, **97**, 7319 (1975).

3) K. Gersonde and H. Netter, *J. Mol. Biol.*, **10**, 475 (1964); K. Kasuga, S. Nakao, T. Kamezawa, K. Tsukahara, and Y. Yamamoto, *Biochim. Biophys. Acta*, **788**, 313 (1984).

4) H. Akutsu, Y. Kyogoku, and T. Horio, *Biochemistry*, **22**, 2055 (1983); T. Yoshimura, S. Suzuki, A. Nakahara, H. Iwasaki, M. Masuko, and T. Matsubara, *Biochim. Biophys. Acta*, **831**, 267 (1985).

5) H. Kon, *J. Biol. Chem.*, **379**, 103 (1975).

6) T. Yoshimura, S. Suzuki, A. Nakahara, H. Iwasaki, T. Matsubara, and M. Iwaizumi, *Inorg. Chim. Acta*, **136**, L17 (1987).

7) T. Yoshimura, *Bull. Chem. Soc. Jpn.*, **51**, 1237 (1978).

8) T. Yoshimura and T. Ozaki, *Arch. Biochem. Biophys.*, **229**, 126 (1984).

9) T. Yoshimura and T. Ozaki, *Bull. Chem. Soc. Jpn.*, **52**, 2268 (1979).

10) T. Yoshimura, *Inorg. Chem.*, **25**, 688 (1986).

11) H. Kon and N. Kataoka, *Biochemistry*, **8**, 4757 (1969); T. Yoshimura, T. Ozaki, Y. Shintani, and H. Watanabe, *Arch. Biochem. Biophys.*, **193**, 301 (1979); T. Yoshimura, *Inorg. Chim. Acta*, **46**, 69 (1980).

12) T. Yoshimura, *Arch. Biochem. Biophys.*, **220**, 167 (1983).

13) T. Yoshimura, *Inorg. Chim. Acta*, **57**, 99 (1982).

14) H. Kon, Biochim. *Biophys. Acta*, **379**, 103 (1975).

15) H. Kon, M. Chikira, and K. M. Smith, *J. Chem. Soc., Dalton Trans.*, **1981**, 1726.

16) T. Yoshimura, *Inorg. Chim. Acta*, **125**, L27 (1986).

17) Y. Ohba, Thesis, Tohoku University, Sendai (1984).