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EPR Spectra of Five-coordinate Nitrosyl(protoporphyrin IX dimethyl ester)iron(II) in Toluene under Various Conditions

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Studies on five-coordinate nitrosyl complexes of iron(II) porphyrin have been carried out by the use of EPR, electronic, IR, and resonance Raman spectroscopies*. The results obtained by these studies have been utilized in elucidating the stereochemistry of the heme environment for nitrosylhemoproteins with a five-coordinate nitrosylheme such as NO derivatives of hemoglobins in the presence of IHP [1] or SDS [2], those of mutant hemoglobin M [3], and those of cytochrome c′ [4].

It has been demonstrated that, in the EPR spectra of nitrosylporphyrinatoiron(II) in non-polar solvents and of NOHb–salicylate system, the satellite absorptions as well as the main absorptions near $g = 2.0$ were observed below freezing point [2, 5, 6] and that the former and the latter absorptions were assigned to absorptions resulting from the dimer and the monomer of the nitrosyl complex, respectively [5]. However, the detailed steric arrangement of two nitrosylhemes in the dimer still remains ambiguous. On the other hand, nitrosyl(octaethylporphyrinato)iron(III) perchlorate in the solid state has been reported to form a π–π dimer in which both nitrosyl groups are oriented outwards [7].

In the course of the study on five-coordinate nitrosyl(protoporphyrin IX dimethyl ester)iron(II) complexes [4, 8, 9], it has been found that the EPR spectra of the toluene solution were sensitive to the frozen condition at 77 K. The EPR sample in a finely cracked opaque glass state was obtained on freezing the toluene solution very slowly, while the sample in an uncracked transparent glass state was obtained by freezing rapidly. The present paper reports that the EPR spectra of these two samples at 77 K differ from each other in line shape, depending on the concentration of Fe(PPIXDME)(NO) and the intensity of dimer absorption for the latter is higher than that for the former.

Fe(PPIXDME)(NO) was prepared as described previously [8] and EPR and electronic spectral measurements at 77 K were carried out [10]. Toluene was distilled prior to use. EPR and electronic spectral samples were prepared under N_2 atmosphere.

When the toluene solution of Fe(PPIXDME)(NO) was frozen very slowly by soaking gradually in liquid nitrogen, the frozen solution resulted in a finely cracked opaque glass state. On the other hand, when frozen rapidly in liquid nitrogen, the frozen solution resulted in an uncracked transparent glass state. The EPR spectra in these two states at 77 K are shown in Figs. 1–3 for the solution with higher concentration

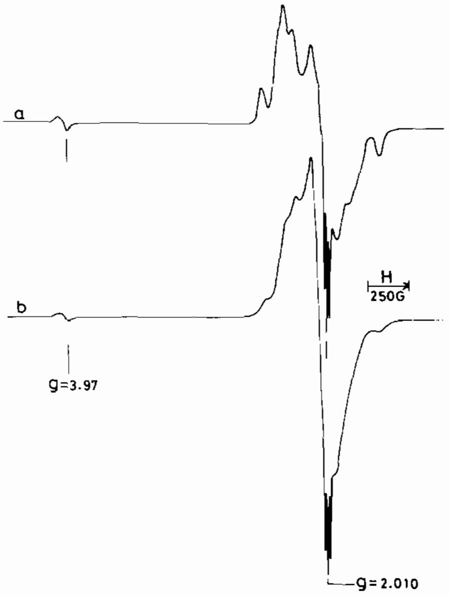

Fig. 1. The EPR spectra of Fe(PPIXDME)(NO) (7.34 mM) in frozen toluene glass at 77 K: (a) uncracked transparent glass state and (b) cracked opaque glass state. Instrument settings: modulation frequency and amplitude, 100 kHz and 2–4 G; microwave frequency and power, 9.177–9.185 GHz and 10 mW.

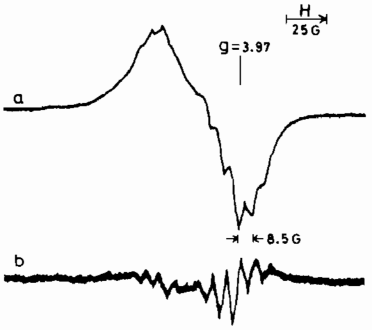

Fig. 2. The EPR spectra ($\Delta M = 2$) of Fe(PPIXDME)(NO) (7.34 mM in toluene) in uncracked transparent glass state at 77 K: (a) 1st- and 2nd-derivative displays. Instrument settings are given in legend of Fig. 1.

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*Abbreviations used: EPR, electron paramagnetic resonance; IR, infrared; IHP, inositol hexaphosphate; SDS, sodium dodecyl sulfate; NOHb, nitrosylhemoglobin; PPIX, dianion of protoporphyrin IX; PPIXDME, dianion of protoporphyrin IX dimethyl ester.

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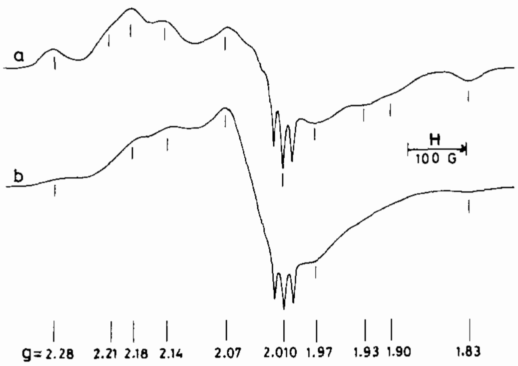

Fig. 3. The EPR spectra ($g = 2.3-1.8$ region) of Fe(PPIXDME)(NO) (7.34 mM) in frozen toluene glass at 77 K: (a) uncracked transparent glass state and (b) cracked opaque glass state. Instrument settings are given in legend of Fig. 1.

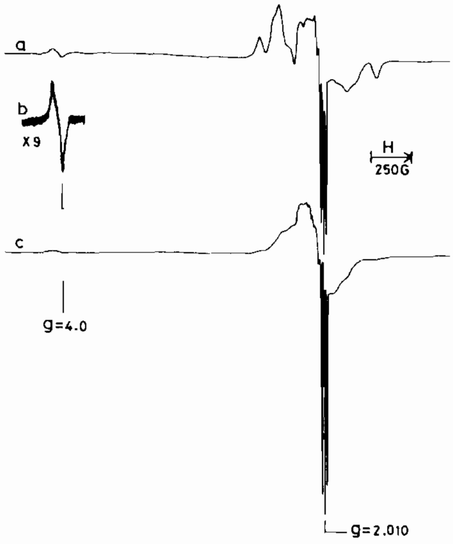

Fig. 4. The EPR spectra of Fe(PPIXDME)(NO) (0.59 mM) in frozen toluene glass at 77 K: (a) uncracked transparent glass state and (b) cracked opaque glass state. Instrument settings are given in legend of Fig. 1.

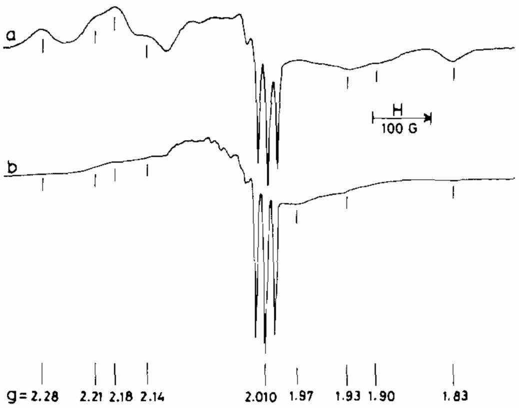

Fig. 5. The EPR spectra ($g = 2.3-1.8$ region) of Fe(PPIXDME)(NO) (0.59 mM) in frozen toluene glass at 77 K: (a) uncracked transparent glass state and (b) cracked opaque glass state. Instrument settings are given in legend of Fig. 1.

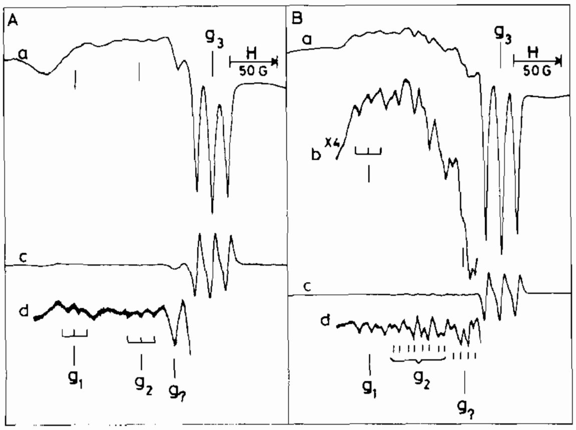

Fig. 6. The EPR spectra (monomer absorption) of Fe(PPIXDME)(NO) (0.59 mM) in frozen toluene glass at 77 K: (a, b) 1st- and (c, d) 2nd-derivative display, where (b) and (d) are the expansion of the ordinate of (a) and (c), respectively. (A) uncracked transparent glass state ($g_1 = 2.105, a_1 = 13$ G; $g_2 = 2.059$, $a_2 = 14$ G; $g_3 = 2.0104$, $a_3 = 16.5$ G; $g_? = 2.036$) and (B) cracked opaque glass state ($g_1 = 2.101, a_1 = 13.5$ G; $g_3 = 2.0103$, $a_3 = 16.5$ G; $g_? = 2.036$). Instrument settings are given in legend of Fig. 1.

(7.34 mM) of Fe(PPIXDME)(NO) and are shown in Figs. 4–6 for the lower concentration (0.59 mM).

As shown in Figs. 1–5, EPR absorptions are observed at around $g = 2$ and 4. In the EPR absorptions at around $g = 2$, the absorptions with an intense triplet at $g = 2.010$ have been assigned to those resulting from a monomer and the absorptions at $g = 2.28$, 2.21, 2.18, 2.14, 1.97, 1.90, and 1.83 to those resulting from a dimer [5, 6]. It has been reported that two distinct groups were present in the latter absorptions and that the intensity of a group of absorptions at $g = 2.21$, 2.14, 1.97, and 1.90 was more strongly dependent on the Fe(PPIXDME)(NO) concentration than that of another group of absorptions at $g = 2.28$, 2.18, 1.93, and 1.83 [6]. The differences in frozen condition of Fe(PPIXDME)(NO) toluene solutions cause the difference in intensity of the two groups of absorptions, as is shown in Figs. 3 and 5. The latter group of absorptions alone has been found in the EPR spectra of NOHb–salicylate system [2] and in those of nitrosyl(octaethylnitroporphyrinato)iron(II) in toluene [5].

The EPR line shape and parameters of the absorption at around $g = 4$ (Fig. 2) resemble quite well those of nitrosyl(octaethylnitroporphyrinato)iron(II) in toluene at 9 K [5]. This absorption has been assigned to $\Delta M = 2$ dimer absorption which arises from the electron spin interaction of two NO groups.

The EPR intensity of dimer absorptions in opaque glass state is found to be much lower than that in transparent glass state, especially in a frozen solution with the lower concentration of Fe(PPIXDME)(NO) (Figs. 1, 3–5).

The monomer absorptions are relatively well resolved in the lower concentration of Fe(PPIXDME)(NO) (Fig. 6), suggesting the weakening of an intermolecular association [6]. Both the line shape and the parameters of the EPR spectrum of transparent toluene glass in Fig. 6A were quite similar to those of NOHb modified by SDS [2], of Fe(PPIX)(NO) intercalated into SDS micelles [11, 12], and of several nitrosylporphyrinatoiron(II) complexes in non-donor solvents [8, 11, 13]. The g_1, g_2, and g_3 absorptions split into a triplet, which is originated from the hyperfine interaction of the unpaired electron with the ^{14}N nucleus of the NO group, though the splitting of g_2 absorption was not well resolved in general. The g_3 absorption can be assigned to the g_z absorption. In the spectrum of opaque toluene glass in Fig. 6B, the additional splittings are observed in the region of g_2 and $g_?$ absorptions, in which the latter absorption remains unidentified. The pattern of these splittings slightly changes with rotation of a sample tube in a resonant cavity. On rotating a sample, such a change in the splitting pattern of the cracked glass sample has been observed also in cobalt(II) and copper(II) Schiff base complexes [14].

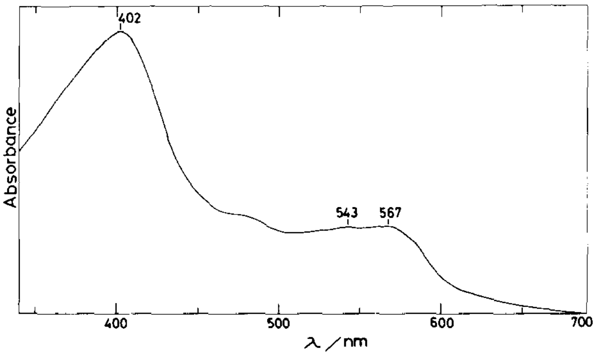

Fig. 7. The electronic absorption spectrum of Fe(PPIXDME)(NO) (0.021 mM) in frozen toluene glass at 77 K.

Figure 7 shows the electronic absorption spectrum of toluene glass of Fe(PPIXDME)(NO) (0.021 mM) at 77 K. The absorption maxima in wavelength at 77 K are similar to those of the toluene solution at room temperature [9]. Since an intermolecular association to form a dimer can hardly take place in this low concentration at room temperature, this result suggests that the electronic spectrum in Fig. 7 is not affected by a dimer formation on freezing.

Further investigations are in progress to elucidate the interpretation of the dimer absorptions and the origin in the monomer absorptions as well as the identification of $g_?$ absorption, in the EPR spectra of five-coordinate nitrosylporphyrinatoiron(II).

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