The Infrared Spectrum of Dioxygen Bound to the Synthetic Porphinatoiron in an Aqueous Medium

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Synopsis. A highly concentrated aqueous solution of heme ([heme]=25 mM) was prepared by embedding the 5,10,15,20-tetrakis(α,α,α,α-*o*-(2′,2′-dimethyl-20′-(2″-trimethylammonio)ethylphosphonatoxy)eicosanamido)phenyl)porphinatoiron(II) complex of 1-dodecylimidazole in bilayer of polymerized 1,2-bis(2,4-octadecadienoyl)-*sn*-glycero-3-phosphocholine liposome (poly-lipid liposome/heme). The dioxygen-binding mode of the poly-lipid liposome/oxy-heme in the aqueous medium was then measured by means of infrared difference spectroscopy: The ν_{O-O} value at 1161 cm^{-1} was found to correspond to those of the oxy picket-fence heme in Nujol mulls, oxy myoglobin, and oxy hemoglobin in an aqueous medium.

The present authors previously found and reported that the heme derivatives incorporated into a bilayer of natural, synthetic, or polymerized lipid liposome could bind dioxygen reversibly under physiological conditions.[1-3] We could also prepare a highly concentrated aqueous heme solution by embedding the 5,10,15,20-tetrakis(α,α,α,α-*o*-(2′,2′-dimethyl-20′-(2″-trimethylammonioethylphosphonatoxy)eicosanamido)phenyl)porphinatoiron(II) complex of 1-dodecylimidazole in bilayer of polymerized 1,2-bis(2,4-octadecadienoyl)-*sn*-glycero-3-phosphocholine liposome (abbreviated as poly-lipid liposome/heme). In this note, we described the dioxygen-binding mode of the poly-lipid liposome/oxy-heme in an aqueous medium, as measured by means of infrared difference spectroscopy and discussed it in comparison with those of the picket-fence oxy-heme[4] in Nujol mulls, and of oxy myoglobin (oxy-Mb) and oxy hemoglobin (oxy-Hb) in an aqueous medium.

Experimental

Meterials. The heme derivative and 1-dodecylimidazole were synthesized as previously reported.[2] The lipid derivative was prepared according to the procedure in the literature.[5] The Mb from Horse Skeletal Muscle was purchased from Sigma (special grade). The $^{18}O_2$ gas was purchased from the Takachiho Shouji Co., Ltd.

Preparation of samples. A mixture (methanol-benzene) of the heme (1.45 g), the imidazole (0.354 g), and the lipid (3.91 g) (molar ratio 1/3/10) was freeze-dried to give a reddish powder. Oxygen-free distilled water (pH 7, adjusted with a dil. NaOH solution, 10 ml) was then added, and the mixture was shaken by means of a Vortex mixer. It was ultrasonicated and homoginized in an ice-water bath under a nitrogen atmosphere (60 W×1 h, using a probe-type sonicator; Nihon-Seiki US-600) to yield a deep red-colored and concentrated liposome/heme solution ([heme]=25 mM†). The solution thus prepared was allowed to stand under ultraviolet (UV) irradiation (Rika-Seiki UVL-32LB, 64 W×3 day) at 40 °C under a nitrogen atmosphere in order to polymerize the lipid and to give a concd poly-lipid liposome/deoxy-heme solution. The pH and volume of the aqueous solution were adjusted to pH 7 and 20 ml with a dil. NaOH solution and oxygen-free distilled water. The reduction of the Fe(III)-heme derivative to the deoxy-heme spontaneously occurred during the polymerization. The complete polymerization and reduction were confirmed by means of UV and Vis absorption and ^{13}C NMR spectroscopical measurements, as has been reported previously.[3] The poly-lipid liposome/heme looked like a small unilammelar vesicle (SUV), and its diameter was estimated to be ca. 350 A by means of transmission electron microscopic measurements.

The oxy- and carboxy-Mb solutions were prepared as reported in the literature.[6] ([Mb]=20 mM, pH 9, 0.1 M Tris buffer solution).

Measurements. The infrared difference spectra were measured for the poly-lipid liposome/heme and Mb in the aqueous media at 10 °C under $^{16}O_2$ vs. $^{18}O_2$, $^{16}O_2$ vs. $^{12}C^{16}O$, and $^{12}C^{16}O$ vs. a nitrogen atmosphere. The cells used were precisely matched in terms of path-length (25 μm) and calcium fluoride (CaF_2) window thickness. The spectra were recorded with a Japan Spectroscopic Co., ltd. Model IR-810-type infrared spectrophotometer in the absorbance mode, using a double beam, an expanded ordinate and abscissa, and a resolution of 2 cm^{-1}, with a data processor.

The UV and Vis absorption spectra were measured with a double- beam UV and Vis absorption spectroscophotometer (Shimazu MPS-2000). The cells with CaF_2 windows could be used for both the UV and Vis and infrared absorption spectroscopy, recorded in the same cell.

Results and Discussion

The values of the O-O and C-O stretching frequencies (ν_{O-O} and ν_{C-O}) of oxy- and carboxy-Mb were 1103 cm^{-1} (band width at 1/2 height=ca. 10 cm^{-1}) and 1944 cm^{-1} (ca. 16 cm^{-1}) respectively: these values were consistent with those reported

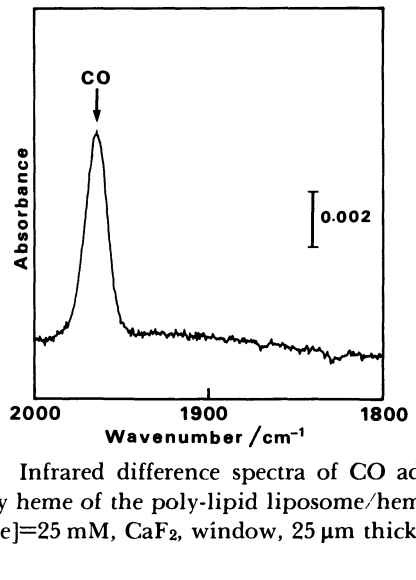

Fig. 1. Infrared difference spectra of CO adduct vs. deoxy heme of the poly-lipid liposome/heme. [heme]=25 mM, CaF_2, window, 25 μm thickness.

† 1 M=1 mol dm^{-3}.

previously.[6] The intensity relative to the CO band was also similar to the reference data. The UV and Vis absorption spectra of oxy- and carboxy-Mb did not change before and after infrared absorption spectroscopic measurements and agreed with those reported in the literature.[7] This result supports the validity of the measuring procedure used in the present experiment.

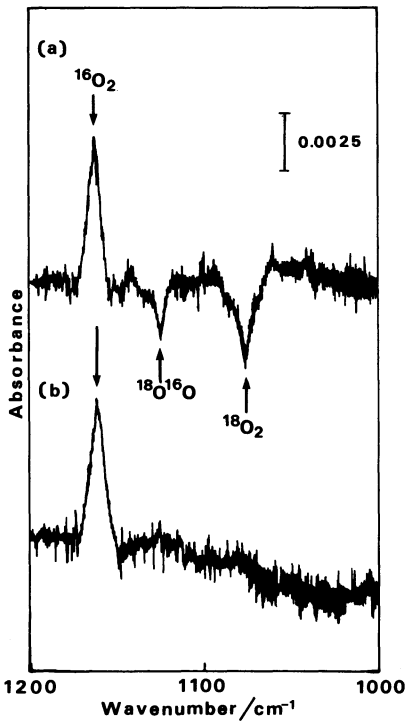

Fig. 2. Infrared difference spectra of $^{16}O_2$ vs. $^{18}O_2$ adducts (a) and $^{16}O_2$ vs. $^{12}C^{16}O$ adducts (b) of the poly-lipid liposome/heme. [heme]=25 mM, CaF_2 window, 25 μm thickness.

The UV and Vis absorption spectrum (λ_{max}; 426, 535 and 562 nm) of the poly-lipid liposome/deoxy-heme changed to the spectrum that was assigned to the oxygen adduct (λ_{max}; 422 and 544 nm) upon exposure to oxygen. The spectrum of the oxygen adduct changed to that of the carbon monoxide (CO) adduct (423 and 540 nm) on bubbling through CO and then returned to that of the deoxy-heme on bubbling through nitrogen. The half-lifetime of the oxygen adduct was ca. 30 hr in the aqueous medium 10 °C.

Figure 1 shows the infrared spectrum of the CO adduct of the poly-lipid liposome/heme, as corrected with the spectrum of the corresponding deoxy-heme in the reference cell. The intense band with a maximum at 1966 cm^{-1} is similar to that of the picket-fence heme-CO complex of 1-methylimidazole in Nujol mulls (ν_{CO}=1969 cm^{-1}).[8] Similarly, the difference spectrum of the CO vs. the $^{16}O_2$ adducts of the poly-lipid liposome/heme showed an intense bond at 1966 cm^{-1}.

The difference spectrum (Fig. 2a) of the $^{16}O_2$ adduct vs. the CO adduct of the poly-lipid liposome/heme shows an intense band at 1161 cm^{-1} with a band-width of 14 cm^{-1} at 1/2 height, a value which agrees with those of the picket-fence heme complex in Nujol mulls and of oxy-Mb and oxy-Hb in aqueous media.[6,8,9] The intensity ralative to be CO band of the poly-lipid liposome/heme was similar to those of Mb and Hb. For the difference spectra of the $^{16}O_2$ vs. the $^{18}O_2$ adducts of the poly-lipid liposome/heme, the $^{16}O_2$ adduct shows an intense band at 1161 cm^{-1} while the $^{18}O_2$ adduct shows prominent bands at 1125 and 1076 cm^{-1} based on the frequency of ^{16}O–^{18}O and ^{18}O–^{18}O respectively (Fig. 2b). The UV and Vis absorption spectra supported the gaseous molecule-binding ability of the ploy-lipid liposome/heme after

Table 1. Infrared and Ultraviolet-Visible Absorption Spectral Data of the Poly-Lipid Liposome/Heme

Heme	Ligand	Solvent	O_2			
			$\nu_{16O-16O}$	$\Delta\nu_{1/2}$	$\nu_{18O-18O}$	λ_{max}
			cm^{-1}	cm^{-1}	cm^{-1}	nm
Poly-lipid liposome/heme	LIm	H_2O	1161	(11)	1076	544
Picket-fence heme	MIm	Nujol	1159	(—)	1075	548
Myoglobin	—	H_2O	1103	(8±1)	1065	542, 580
Hemoglobin	—	H_2O	1107	(9±1)	1065	541, 577
O_2 or CO gas	—	—	1556	(—)	—	—
O_2^-	—	—	1145	(—)	—	—

Heme	CO			Intensity ratio $A(O_2)/A(CO)$ (—)	Ref.
	ν_{C-O}	$\Delta\nu_{1/2}$	λ_{max}		
	cm^{-1}	cm^{-1}	nm		
Poly-lipid liposome/heme	1966	(14)	540	0.15	This work
Picket-fence heme	1969	(—)	542	—	8
Myoglobin	1944	(12)	540, 579	0.10	6
Hemoglobin	1951	(8)	540, 569	0.19	9
O_2 or CO gas	2143	(—)	—	—	10, 11
O_2^-	—	(—)	—	—	10

LIm; 1-laurylimidazole, MIm; 1-methylimidazole. $\Delta\nu_{1/2}$; band width ar 1/2 height, $A(O_2)/A(CO)$; intensity ratio between O_2 and CO bands.

the infrared-spectroscopic measurement.

The data of the infrared and UV and Vis absorption spectra are summarized in Table 1. The O–O stretching frequency of the poly-lipid liposome/oxy-heme differs from that of the gaseous oxygen molecule (ν_{O-O}=1556 cm^{-1}),[10] but is similar to that of superoxide (O_2^-, 1145 cm^{-1}).[10] Metal dioxygen complexes with both oxygen atoms bound to a metal (side-on type) have an O–O stretch between 800 and 900 cm^{-1} [12,13] and an O–O bond order of about 1.0. On the other hand, both cobalt dioxygen complexes with a configuration such as the bent end-on type[14] and an O–O bond order of 1.5 exhibit an O–O stretching frequency between 1000 and 1500 cm^{-1}.[15,16] Thus, it may be concluded that molecular dioxygen obviously coordinates to the synthetic lipid-heme, even in an aqueous medium, and that its coordination structure is a bent end-on type one similar to oxy-Mb, oxy-Hb, and the oxygen adduct of the picket-fence heme.

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References

1) E. Tsuchida, H. Nishide, M. Yuasa, E. Hasegawa, and Y. Matsushita, *J. Chem. Soc., Dalton Trans.*, **1984**, 1147.

2) E. Tsuchida, H. Nishide, M. Yuasa, E. Hasegawa, Y. Matsushita, and K. Eshima, *J. Chem. Soc., Dalton Trans.*, **1985**, 275.

3) E. Tsuchida, E. Hasegawa, Y. Matsushita, K. Eshima, M. Yuasa, and H. Nishide, *Chem. Lett.*, **1985**, 969.

4) J. P. Collman, R. R. Gagne, C. A. Reed, T. R. Halbert, G. Lang, and W. T. Robinson, *J. Am. Chem. Soc.*, **97**, 1427 (1975).

5) B. Hupfer, H. Ringsdorf, and H. Schupp, *Makromol. Chem.*, **182**, 247 (1981); B. Hupfer, H. Ringsdorf, and H. Schupp, *Chem. Phys. Lipids*, **33**, 355 (1983).

6) J. C. Maxwell, J. A. Volpe, C. H. Barlow, and W. S. Caughey, *Biochem. Biophys. Res. Commun.*, **58**, 166 (1974).

7) E. Antonini and M. Brunori, "Hemoglobin and Myoglobin in their Reaction with Ligands," Nor-Holland Pub., Amsterdam (1970).

8) J. P. Collman, J. I. Brauman, T. R. Halbert, and K. S. Suslick, *Proc. Natl. Acad. Sci. U.S.A.*, **73**, 3333 (1976).

9) C. H. Barlow, J. C. Maxwell, W. J. Wallace, and W. S. Caughey, *Biochem. Biophys. Res. Commun.*, **55**, 91 (1973).

10) G. Herzberg, "Molecular Spectra and Molecular Structure I. Spectra of Diatomic Molecules," Van Nostrand Co., New York (1950), p. 560.

11) K. Nakamoto, "Infrared Spectra of Inorganic and Coordination Compounds," Wiley-Interscience, New York (1970), p. 78.

12) J. A. McGinnety, R. J. Doedens, and J. A. Ibers, *Inorg. Chem.*, **6**, 2243 (1967).

13) A. Nakamura, Y. Tatsuno, M. Yamamoto, and S. Otsuka, *J. Am. Chem. Soc.*, **93**, 6052 (1971).

14) L. Pauling, "Hemoglobin," ed by F. J. W. Roughton and J. C. Kendrew, Butterworths, London (1949), pp. 57—65.

15) A. L. Crumbliss and F. Basolo, *J. Am. Chem. Soc.*, **92**, 55 (1970).

16) J. D. Landels and G. A. Rodley, *Syn. Inorg. Metal-Org. Chem.*, **2**, 65 (1972).