on the chemical behavior of the metal-carbon bond. This seems to be the first observation of this type and calls for further studies.

Acknowledgment. We wish to thank Professor G. Closs for encouragement and helpful discussions. This study was supported in part by the donors of the Petroleum Research Fund, administered by the American Chemical Society, and the United States-Israel Binational Science Foundations (BSF), Jerusalem, Israel.

Registry No. $[Co^{II}(tspc)]Na_4$, 14586-48-2; $[(tspc)Co^{III}CH_2C(CH_3)_2OH]^{4-}$, 86766-36-1; $[(tspc)Co^{III}CH{=}C(CH_3)_2]^{4-}$, 86766-37-2; $[Co^{I}(tspc)]^{5-}$, 86766-38-3; $[Co^{III}(tspc)]^{3-}$, 69087-63-4; $\cdot CH_2C(CH_3)_2OH$, 5723-74-0; $Br_2^{-}\cdot$, 68565-50-4; $CO_2^{-}\cdot$, 34496-91-8; $CH_2{=}C(CH_3)_2$, 115-11-7; $OCHCH(CH_3)_2$, 78-84-2.

Contribution from the Department of Material Science, Technological University of Nagaoka, Nagaoka, Niigata 949-54, Japan

Reconstituted Myoglobins with Rhodium(III) Complexes of Meso- and Deuteroporphyrin IX

YASUHIRO AOYAMA, KATSUHIRO AOYAGI, HIROO TOI, and HISANOBU OGOSHI*

Received January 19, 1983

Reconstitution of sperm whale apomyoglobin with rhodium(III) complexes of meso- and deuteroporphyrin IX afforded stable rhodium(III) myoglobins (Meso-Rh(III)-Mb and Deut-Rh(III)-Mb) in a 1:1 (Rh to protein) stoichiometry. Inertness of Meso-Rh(III)-Mb toward external anions and reducing agents indicated that it is not a native ferrimyoglobin analogue but rather an analogue of an internal hemichrome. An organometallic Rh(III)-CH_3 derivative of mesoporphyrin IX was also successfully reconstituted into myoglobin (Meso-CH_3-Rh(III)-Mb). The ^1H NMR spectra indicated that the methyl group bonded to Rh(III) resonated at lower magnetic field by about 2 ppm upon incorporation into the protein, probably due to a compression effect of the amino acid residue(s) at the distal site. Prosthetic group exchange reactions between rhodium and iron myoglobins indicated a significant reduction in stability of Meso-CH_3-Rh(III)-Mb relative to Meso-Rh(III)-Mb.

Introduction

An interesting and important research area in the study of hemoproteins concerns metal substitution in the prosthetic heme.[1] The successful preparation of the oxygen-carrying cobalt(II)-reconstituted hemoglobin and myoglobin has provided valuable information as to the influence of an apoprotein on the reactivity of a prosthetic group and also as to the electronic structure, spin distribution in particular, in a prosthetic group.[1] Rhodium is a fifth-row transition metal in the homologous series with cobalt. The chemistry of rhodium porphyrins is rather well characterized.[2-4] The formation of dioxygen adducts of rhodium(II) porphyrins has also been reported.[3] Rhodium-reconstituted hemoproteins, however, have never been reported to our knowledge. In the present work, we have prepared myoglobins reconstituted with rhodium(III) meso- and deuteroporphyrin IX including a novel rhodium-methyl derivative as the first organometalloporphyrin incorporated into apohemoprotein. The physicochemical properties of rhodium myoglobins have been studied by spectroscopic means and exchange reactions with the protohemin.

Experimental Section

Electronic absorption, IR, mass, and routine ^1H NMR spectra were obtained with a Hitachi 320 spectrophotometer, a Hitachi 260-10 IR spectrometer, a Hitachi RMU-7M mass spectrometer, and a JEOL JNM-PMX 60 NMR spectrometer, respectively. For measurement of 270-MHz ^1H NMR spectra of rhodium myoglobins (Rh(III)-Mb), an aqueous solution of a Rh(III)-Mb was filtered with a millipore filter (Immersible CX-10) to give a concentrated solution (approximately 1 mL) containing 1-2 mM Rh(III)-Mb. Most of remaining water was further removed by centrifugal filtration (3000 rpm, 15 min) at 2 °C with a Hitachi 18PR-52 automatic high-speed refrigerated centrifuge equipped with membrane cones (Amicon, CF-25). ^2H_2O (≥1 mL) buffered at pD 7 with 50 mM phosphate was added, and the mixture was similarly filtered as described above. Addition-filtration procedures of ^2H_2O were repeated five times to remove traces of H_2O. The NMR spectrum of the final solution was measured with a JEOL FX 270 NMR spectrometer at 25 °C.

Preparation of Rh(III) Complexes. Rhodium(III) complexes of meso- and deuteroporphyrin IX (Meso-Rh(III) and Deut-Rh(III)) were prepared according to the procedure of Grigg et al. for the preparation of Rh(III) etioporphyrin.[4] Thus, mesoporphyrin IX dimethyl ester[5] (300 mg) was dissolved in 30 mL of chloroform containing sodium acetate (1.5 g). A chloroform solution (30 mL) of $[Rh(CO)_2Cl]_2$ (300 mg) was added and the mixture stirred at room temperature for 2 h. Inorganic salts were removed by filtration, and the filtrate was stirred for 1 h after addition of iodine (200 mg). The course of the reaction was monitored by TLC, and when necessary, further iodine (100 mg) was added to complete the oxidation of the Rh(I) complex. The chloroform was evaporated in vacuo and the residue chromatographed on alumina (Brockman, activity grade II-III) with chloroform as eluant. The dimethyl ester of (mesoporphyrinato)rhodium(III) iodide (MesoDME-Rh(III)) thus obtained was further purified by preparative TLC (Merck Sharp and Dohme, Kieselgel 60 PF_{254}) with chloroform-acetone (95:5 v/v) as eluant, followed by recrystallization from methanol to give 130 mg (31.3%) of dark orange crystals. IR (KBr disk): 1725 cm^{-1} ($\nu_{C=O}$). ^1H NMR (in C^2HCl_3): δ 1.90 (t, 6 H, CH_2CH_3), 3.30 (t, 4 H, $CH_2CH_2CO_2CH_3$), 3.53 (s, 6 H, 1- and 3-CH_3), 3.67 (s, 12 H, 5- and 8-CH_3 and CO_2CH_3), 4.10 (q, 4 H, CH_2CH_3), 4.44 (t, 4 H, $CH_2CH_2CO_2CH_3$), 10.11 (s, 4 H, meso-H). Mass spectrum: *m/e*

(1) Hoffman, B. M. *Porphyrins* **1979**, *7*, 403-444 and references cited therein.
(2) (a) Ogoshi, H.; Setsune, J.; Omura, T.; Yoshida, Z. *J. Am. Chem. Soc.* **1975**, *97*, 6461. (b) Ogoshi, H.; Setsune, J.; Yoshida, Z. *J. Chem. Soc., Chem. Commun.* **1975**, 572. (c) Ogoshi, H.; Setsune, J.; Yoshida, Z. *J. Am. Chem. Soc.* **1977**, *99*, 3869. (d) Setsune, J.; Yoshida, Z.; Ogoshi, H. *J. Chem. Soc., Perkin Trans. 1* **1982**, 983.
(3) (a) Wayland, B. B.; Newman, A. R. *J. Am. Chem. Soc.* **1979**, *101*, 6472. (b) Wayland, B. B.; Newman, A. R. *Inorg. Chem.* **1981**, *20*, 3039. Also see: James, B. R.; Stynes, D. V. *J. Am. Chem. Soc.* **1972**, *94*, 6225.
(4) Abeysekera, A. M.; Grigg, R.; Trocha-Grimshaw, J.; Viswanatha, V. *J. Chem. Soc., Perkin Trans. 1* **1977**, 1395.
(5) Caughey, W. S.; Alben, J. O.; Fujimoto, W. Y.; York, J. L. *J. Org. Chem.* **1966**, *31*, 2631.

0020-1669/83/1322-3046$01.50/0

822 (M^+), 695 ($M^+ - I$). Anal. Calcd for $C_{36}H_{40}N_4O_4IRh \cdot 2H_2O$: C, 50.35; H, 5.13; N, 6.53. Found: C, 50.45; H, 5.24; N, 6.28. Similarly was prepared the dimethyl ester of (deuteroporphyrinato)rhodium(III) iodide (DeutDME-Rh(III)) (190 mg, 47.7%) from deuteroporphyrin IX dimethyl ester[5] (300 mg). IR (KBr disk): 1720 cm^{-1} ($\nu_{C=O}$). ^1H NMR (in C^2HCl_3): δ 3.15 (t, 4 H, $CH_2CH_2CO_2CH_3$), 3.51 (s, 6 H, 1- and 3-CH_3), 3.60 (s, 6 H, CO_2CH_3), 3.70 (s, 6 H, 5- and 8-CH_3), 4.27 (t, 4 H, $CH_2CH_2CO_2CH_3$), 9.07 (s, 2 H, 2- and 4-H), 10.00, 10.08, and 10.13 (each s, 4 H in a ratio of 1:2:1, meso-H). Mass spectrum: m/e 766 (M^+), 639 ($M^+ - I$). Anal. Calcd for $C_{32}H_{32}N_4O_4IRh \cdot H_2O$: C, 48.98; H, 4.34; N, 7.14. Found: C, 48.80; H, 4.43; N, 7.19.

To a methanol solution (30 mL) of MesoDME-Rh(III) (50 mg) was added sodium borohydride (10 mg) dissolved in 0.5 N NaOH (1 mL), and the mixture was stirred at 40 °C under nitrogen for 2 h. After the reaction mixture had cooled to room temperature, methyl iodide in excess amount was added. The mixture was stirred for 10 min, poured into water, and extracted with chloroform. Usual workup and chromatography on silica gel (Wakogel C-200) with chloroform as eluant, followed by recrystallization from methanol–chloroform, afforded 36 mg (83%) of the dimethyl ester of methylrhodium(III) mesoporphyrin (MesoDME-CH_3-Rh(III)). IR (KBr disk): 1735 cm^{-1} ($\nu_{C=O}$). ^1H NMR (in C^2HCl_3): δ −7.56 (s, 3 H, Rh(III)-CH_3), 1.57 and 1.66 (each t, 6 H, CH_2CH_3), 2.96 (m, 4 H, $CH_2CH_2CO_2CH_3$), 3.10, 3.13, 3.19, and 3.30 (each s, 12 H, 1-, 3-, 5-, and 8-CH_3), 3.51 and 3.57 (each s, 6 H, CO_2CH_3), 3.53 (m, 4 H, CH_2CH_3), 3.84 (m, 4 H, $CH_2CH_2CO_2CH_3$), 8.93 and 9.18 (each s, 4 H in a ratio of 3:1, meso-H). Mass spectrum: m/e 710 (M^+), 695 ($M^+ - CH_3$). Anal. Calcd for $C_{37}H_{43}N_4O_4Rh \cdot H_2O$: C, 60.99; H, 6.18; N, 7.69. Found: C, 61.49; H, 5.89; N, 7.67.

Rh(III) porphyrin dimethyl esters thus obtained were hydrolyzed in methanol containing 1% KOH under reflux for 1 h to the corresponding dicarboxylic acids (Meso-Rh(III), Deut-Rh(III), and Meso-CH_3-Rh(III)).

Reconstitution of Rh(III)-Mb. Sperm whale apomyoglobin was prepared by the treatment of metmyoglobin with HCl–methyl ethyl ketone by modifying Teale's method.[6] A Rh(III) porphyrin was dissolved in a minimal volume of 0.1 N NaOH and diluted with deionized water to approximately 1 mM just before reconstitution. This solution was added dropwise into a stirred solution of apomyoglobin in phosphate buffer (pH 7.0). The reconstituted Rh(III)-Mb was separated from the excess Rh(III) porphyrin by gel filtration through a column of Sephadex G-25 with phosphate buffer (pH 6.0) and further purified on a CM-52 column with phosphate buffer (pH 7.0) as eluant. All procedures were manipulated in a cold room (0–5 °C).

Exchange of Prosthetic Groups. A mixture of 1 equiv of metmyoglobin (Fe(III)-Mb) and 4 equiv of a Rh(III) porphyrin (Meso-Rh(III) or Meso-CH_3-Rh(III)) in 50 mM phosphate buffer (pH 7.0) was left at 35 °C for 24 h. The mixture was applied on a column of CM-52, and the protein fraction containing Rh(III)-Mb and Fe(III)-Mb was eluted with 50 mM phosphate buffer (pH 7.0). Its composition was determined spectrophotometrically by taking advantage of the fact that Fe(III)-Mb, but not Rh(III)-Mb, underwent reduction with dithionite, leading to better spectral resolution. Thus, the absorbance at 404 nm before (A_0) and after dithionite treatment (A_∞) could be correlated with the concentrations of species involved as in the following equations where $\epsilon_{Rh(III)}$, $\epsilon_{Fe(III)}$, and $\epsilon_{Fe(II)}$ were the known molar extinction coefficients of Rh(III)-Mb, Fe(III)-Mb, and Fe(II)-Mb, respectively, at 404 nm and [Fe(III)-Mb] = [Fe(II)-Mb].

$$A_0 = \epsilon_{Rh(III)}[\text{Rh(III)-Mb}] + \epsilon_{Fe(III)}[\text{Fe(III)-Mb}]$$

$$A_\infty = \epsilon_{Rh(III)}[\text{Rh(III)-Mb}] + \epsilon_{Fe(II)}[\text{Fe(II)-Mb}]$$

Results

Preparation and Properties of Rhodium(III) Myoglobins. Rhodium(III) meso- and deuteroporphyrin IX (Meso-Rh(III) and Deut-Rh(III)) were prepared by the saponification of the precursor dimethyl esters (MesoDME-Rh(III) and DeutDME-Rh(III)), which in turn were obtained from the corresponding porphyrin-free bases via rhodium incorporation with $[Rh(CO)_2Cl]_2$ followed by oxidation with iodine. Reduction of MesoDME-Rh(III) with sodium borohydride and subsequent methylation of the resulting Rh(I) complex with methyl iodide afforded the rhodium–methyl derivative of mesoporphyrin IX dimethyl ester (MesoDME-CH_3-Rh(III)), which was saponified to give Meso-CH_3-Rh(III).

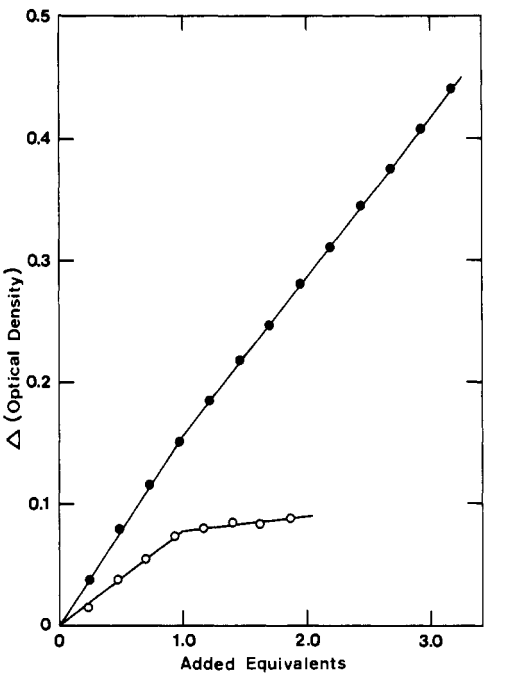
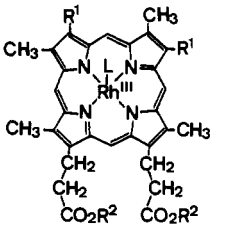


Figure 1. Change in optical density at 402 nm as apomyoglobin is added to Meso-Rh(III) (○) and at 401 nm as Meso-Rh(III) is added to apomyoglobin (●) in 50 mM phosphate buffer (pH 7.0).



Meso-Rh(III) ; $R^1=C_2H_5$, $R^2=H$, L= I
Deut-Rh(III) ; $R^1=R^2=H$, L= I
Meso-CH_3-Rh(III) ; $R^1=C_2H_5$, $R^2=H$, L= CH_3
MesoDME-Rh(III) ; $R^1=C_2H_5$, $R^2=CH_3$, L= I
DeutDME-Rh(III) ; $R^1=H$, $R^2=CH_3$, L= I
MesoDME-CH_3-Rh(III) ; $R^1=C_2H_5$, R^2=L=CH_3

Stable rhodium myoglobins (Meso-Rh(III)-Mb and Deut-Rh(III)-Mb) are obtainable from combination of the sperm whale apomyoglobin with the complexes Meso-Rh(III) and Deut-Rh(III), respectively, followed by standard purification procedure. The stoichiometry of the combination of the precursors was confirmed by titrating the rhodium porphyrin with apomyoglobin or, inversely, apomyoglobin with the rhodium porphyrin. The results are shown in Figure 1. The sharp break point at addition of equimolar amounts indicates that the stability of the complex is quite large as in the cases of native[7] and iron-[8] and cobalt-reconstituted proteins.[9] Meso-CH_3-Rh(III) was also recombined with apomyoglobin. Gradual denaturation of the purified Meso-CH_3-Rh(III)-Mb at ambient temperature might indicate its

(6) Teale, F. W. J. *Biochim. Biophys. Acta* **1959**, *35*, 534.

(7) Rossi-Fanelli, A.; Antonini, E.; Caputo, A. *Biochim. Biophys. Acta* **1958**, *30*, 608.
(8) Asakura, T.; Sono, M. *J. Biol. Chem.* **1974**, *249*, 7087.
(9) Ridsdale, S.; Cassatt, J. C.; Steinhardt, J. *J. Biol. Chem.* **1973**, *248*, 771.

Table I. Absorption Maxima of Rh(III) Porphyrins and Rh(III) Myoglobins[a]

Rh(III) species	λ_{max}, nm (ϵ, mM^{-1} cm^{-1})				medium[b]
	UV	Soret	β	α	
Meso-Rh(III)-Mb	280 (24.7)	404 (121.1)	518 (14.2)	550 (13.2), 590 (1.6)	H_2O
Meso-Rh(III)		394 (99.3)	514 (12.7)	546 (23.8)	H_2O
MesoDME-Rh(III)		402 (134.2)	518 (16.6)	551 (29.6)	$CHCl_3$
MesoDME-Rh(III) + 1-MeIm (0.126 M)[c]		414 (137.8)	526 (20.8)	556 (24.7)	$CHCl_3$
Deut-Rh(III)-Mb	280 (25.0)	405 (110.3)	517 (12.7)	550 (11.2), 590 (1.5)	H_2O
Deut-Rh(III)		393 (112.9)	511 (12.6)	546 (18.8)	H_2O
DeutDME-Rh(III)		401 (127.2)	516 (16.2)	548 (24.5)	$CHCl_3$
Meso-CH_3-Rh(III)-Mb	280 (27.3)	404 (90.2)	519 (10.3)	551 (11.1), 590 (2.0)	H_2O
Meso-CH_3-Rh(III)		395 (58.7)	516 (7.9)	548 (16.9)	H_2O
MesoDME-CH_3-Rh(III)		393 (112.6)	508 (11.4)	542 (29.0)	$CHCl_3$
MesoDME-CH_3-Rh(III) + 1-MeIm (0.126 M)[c]		407 (131.3)	522 (13.9)	553 (16.1)	$CHCl_3$

[a] The ϵ values for Rh(III)-Mb are based on spectroscopic titration of Rh(III) porphyrins with increasing amounts of apomyoglobin. [b] H_2O contains 50 mM phosphate buffer (pH 7.0). [c] 1-MeIm stands for 1-methylimidazole.

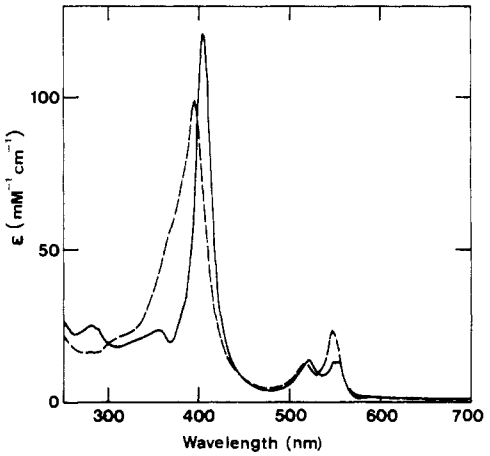


Figure 2. Electronic absorption spectra of Meso-Rh(III)-Mb (—) and Meso-Rh(III) (--) in 50 mM phosphate buffer (pH 7.0).

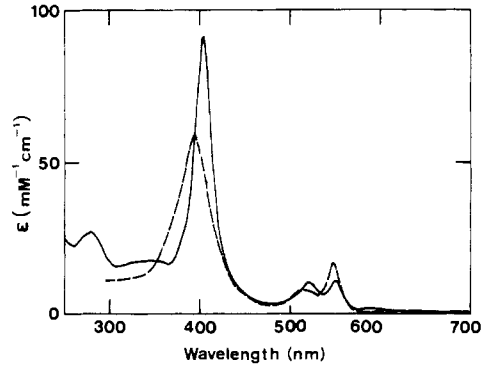


Figure 3. Electronic absorption spectra of Meso-CH_3-Rh(III)-Mb (—) and Meso-CH_3-Rh(III) (--) in 50 mM phosphate buffer (pH 7.0).

lower stability compared to those of Meso-Rh(III)-Mb and Deut-Rh(III)-Mb.

Figures 2 and 3 show the electronic absorption spectra of Meso-Rh(III)-Mb and Meso-Rh(III) and of Meso-CH_3-Rh(III)-Mb and Meso-CH_3-Rh(III), respectively, in buffer solution (pH 7.0). The protein spectra are characterized by the slight red-shifts of the Soret and visible bands, the significant reduction in the intensities of the α bands, and an appearance of new bands at 280 nm. These spectral features are consistent with the coordination of the histidyl imidazole with central Rh(III). Similar spectral changes in the Soret and visible spectra could be reproduced upon addition of 1-methylimidazole to the chloroform solutions of MesoDME-Rh(III) and MesoDME-CH_3-Rh(III) (Table I).

A 270-MHz ^1H NMR spectrum of Meso-Rh(III)-Mb in ^2H_2O at pD 7.0 was quite similar to that of oxymyoglobin[10]

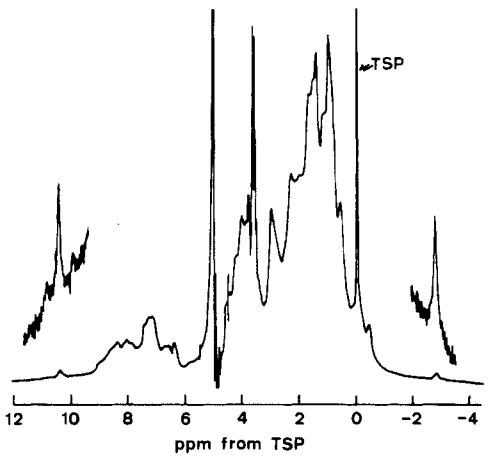


Figure 4. ^1H NMR spectrum at 270 MHz of Meso-Rh(III)-Mb in ^2H_2O buffered at pD 7.0 with 50 mM phosphate.

as is shown in Figure 4. In particular, the characteristic higher field signals at 2.75 and 0.41 ppm upfield from TSP (3-(trimethylsilyl)tetradeuteriopropionic acid, sodium salt) may correspond to the signals in oxymyoglobin spectrum at 2.8 and 0 ppm upfield from DSS (4,4-dimethyl-4-silapentanesulfonic acid).[10] The ^1H NMR spectrum of Meso-CH_3-Rh(III)-Mb showed resonances at 5.50 and 7.48 ppm upfield from TSP. These are assignable to the methyl protons axially bonded to Rh(III). Extreme upfield shifts are undoubtedly due to a porphyrin ring current effect.[1a] Meso-CH_3-Rh(III)-Mb was found to undergo gradual denaturation during measurements of NMR spectra, and the intensity ratio of the 7.48–5.50 ppm resonances increased with time course of measurement. MesoDME-CH_3-Rh(III) in C^2HCl_3 shows the Rh(III)–CH_3 resonance at 7.56 ppm upfield from Me_4Si (tetramethylsilane). These results may allow us to assign the signal at 5.50 ppm to the CH_3-Rh(III) in the normal protein and the signal at 7.48 ppm to that in the denatured protein.

Inertness of Meso-Rh(III)-Mb toward Anions and Reducing Agents. The iron(III) porphyrin and ferrimyoglobin are readily ligated with anions F^-, OCN^-, N_3^-, and CN^-.[11] However, the present Meso-Rh(III)-Mb in aqueous solutions at pH 7.0 showed no sign of binding with these anions; the electronic spectra remained essentially unchanged upon addition of 20–100-fold molar excess of these anions. The mesoporphyrin–Rh(III) complex itself, on the contrary, readily bound CN^- to give the cyano complex, causing the bathochromic shifts for the Soret (410 nm) and visible bands (523 and 555 nm).

(10) Shulman, R. G.; Wüthrich, K.; Yamane, T.; Patel, D. J.; Blumberg, W. E. *J. Mol. Biol.* **1970**, *53*, 143.
(11) Hanania, G. I. H.; Yeghiayan, A.; Cameron, B. F. *Biochem. J.* **1966**, *98*, 189.

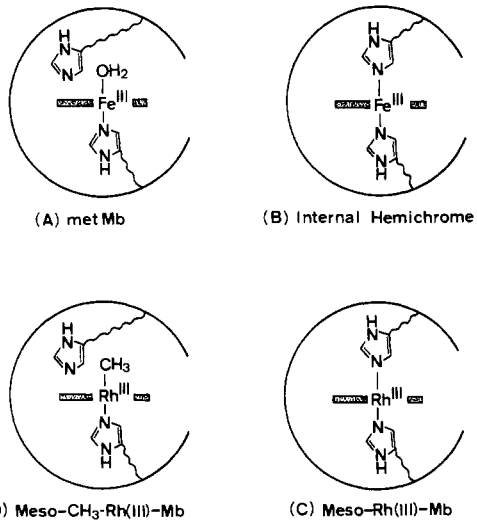


Figure 5. Schematic representation of axial coordination structures in metMb, internal hemichrome, Meso-Rh(III)-Mb, and Meso-CH_3-Rh(III)-Mb.

Another characteristic feature of Meso-Rh(III)-Mb is its resistance to reduction. Chemical reduction of Meso-Rh(III)-Mb was attempted anaerobically with conventional reducing agents such as dithionite, borohydride, and hydrogen gas, but in neither case did the reduction of the central rhodium take place.

Exchange of Rh(III) and Fe(III) Prosthetic Groups. In order to evaluate the "stability" of Rh(III)-Mb, exchange reactions of Rh(III) and Fe(III) (protohemin) prosthetic groups were carried out. Incubation of 1 equiv of Fe(III)-Mb (metMb) and 4 equiv of Meso-Rh(III) in 50 mM phosphate buffer (pH 7.0) at 35 °C for 24 h resulted in replacement of most of the prosthetic protohemin by Meso-Rh(III); the recovered myoglobin mixture consisted of Meso-Rh(III)-Mb (90%) and Fe(III)-Mb (10%). A similar experiment with Meso-CH_3-Rh(III) instead of Meso-Rh(III) led to a myoglobin mixture of Meso-CH_3-Rh(III)-Mb (15%) and Fe(III)-Mb (85%).

Discussion

Axial Coordination. Meso-Rh(III)-Mb resembles the native myoglobin in many respects: tight binding of apoprotein and prosthetic group, involvement of metal–imidazole bonding, and protein conformation as evaluated from ^1H NMR spectra. The central Fe(III) in ferrimyoglobin (metMb) is coordinated with the imidazole group of the proximal His at the fifth coordination site, whereas the sixth coordination site is occupied by an aquo ligand that interacts with another imidazole group of the distal His (Figure 5A). In this respect, a striking difference between Rh(III)- and Fe(III)-Mb is that the former does not bind anions, indicating nonexistence of an exchangeable aquo ligand. These observations lead us to suggest that the Rh(III) binds both proximal and distal imidazole (Figure 5C) as in internal hemichrome (Figure 5B). Formation of internal hemichromes has been proposed as an intermediate in the acid denaturation of oxymyoglobin.[12,13] It is also found in abnormal hemoglobins. The resistance of Meso-Rh(III)-Mb to reduction of the central Rh(III) to Rh(II)-Mb may also arise from the internal hemichrome structure.[14] An internal hemichrome formulation has been suggested for Co(III)-globin on the basis of observations that it does not bind anions, except for a very slow binding of CN^-, and that there is no ionizable water ligand coordinated to the metal.[9] However, Co(III)-globin can be reduced by dithionite, although slowly.[15-18] Thus, at least in some circumstances, hemichrome formation in Co(III)-globin can be reversed.[1]

On the basis of spectroscopic evidence (Figure 3), Meso-CH_3-Rh(III)-Mb must essentially have the structure represented by Im-Rh(III)-CH_3 (Figure 5D). It is generally accepted that the imidazole group of the distal His in hemoglobin and myoglobin interacts with the ligand atom bonded to the iron.[19,20] The importance of the O_2–distal imidazole interaction has been suggested by several workers,[21] and its validity has been confirmed by a recent neutron diffraction study.[22] The significance of Meso-CH_3-Rh(III)-Mb lies in the fact that it is the first reconstituted hemoprotein with an organometalloporphyrin, in which such a hydrophobic group as the σ-bonded methyl is directed to the distal His. An X-ray diffraction study of an analogous Rh(III)-CH_3 derivative of octaethylporphyrin indicates that the rhodium atom lies in the porphyrin plane with the Rh(III)-CH_3 bond distance of approximately 1.9 Å.[23] Thus, the methyl group in Meso-CH_3-Rh(III)-Mb may have nonbonding interaction with the distal imidazole. The ^1H NMR spectrum of MesoDME-CH_3-Rh(III) in C^2HCl_3 indicates that the signal of the methyl bonded to Rh(III) appears at extremely higher field (−7.56 ppm) due to a diamagnetic ring-current effect of the porphyrin. The corresponding resonance in Meso-CH_3-Rh(III)-Mb occurs at −5.50 ppm. The downfield shift by approximately 2 ppm upon incorporation into protein might originate from a steric repulsion between the methyl group and an amino acid residue of the protein, possibly the imidazole group of distal His. Such a steric repulsion may decrease the electron density of the methyl hydrogens by the so-called compression effect in organic chemistry.[24]

Stability of Rh(III)-Mb. Prosthetic group exchange reactions provide an indication of the stability of the reconstituted myoglobin. Most (90%) of the prosthetic protohemin in native myoglobin is replaced by Meso-Rh(III) in 4-fold molar excess, while only a small fraction (15%) is replaced by Meso-CH_3-Rh(III) under similar conditions. The stability of Meso-Rh(III)-Mb and a significant reduction in stability of Meso-CH_3-Rh(III)-Mb may be explained on the basis of different structures for their axial coordination at the distal site (Figure 5C, D): an enhancement of stability due to interaction between Rh(III) and distal imidazole (Figure 5C) and a destabilization due to a rather repulsive, steric interaction between the axial methyl group and amino acid residues, especially distal imidazole (Figure 5D).

The present study indicates that an organometalloporphyrin may be utilized as a unique probe for the characterization of

(12) Gibson, Q. H.; Antonini, E. *J. Biol. Chem.* **1963**, *238*, 1384.
(13) Rossi-Fanelli, A.; Antonini, E.; Caputo, A. *Arch. Biochem. Biophys.* **1959**, *85*, 37.
(14) It should also be noted that the Rh(II) porphyrin exists as a metal–metal-bonded dimer[2c] and the monomeric deoxy Rh(II) porphyrin has never been demonstrated unambiguously.
(15) Holden, H. F. *Aust. J. Exp. Biol. Med. Sci.* **1941**, *19*, 1, 89.
(16) Hsu, G. C.; Spilburg, C. A.; Bull, C.; Hoffman, B. M. *Proc. Natl. Acad. Sci. U.S.A.* **1972**, *69*, 2122.
(17) Yonetani, T.; Yamamoto, H.; Woodraw, G. V., III. *J. Biol. Chem.* **1974**, *249*, 682.
(18) Padlan, E. A.; Eaton, W. A.; Yonetani, T. *J. Biol. Chem.* **1975**, *250*, 7069.
(19) Perutz, M. F. *Proc. R. Soc. London, Ser. B* **1969**, *173*, 113.
(20) Novel, J. C.; Nunes, A. C.; Shoenborn, B. P. *Science (Washington, D.C.)* **1975**, *190*, 568.
(21) (a) Reference 19. (b) Yonetani, T.; Yamamoto, H.; Iizuka, T. *J. Biol. Chem.* **1974**, *244*, 6696. (c) Caughey, W. S.; Barlow, C. H.; Maxwell, J. C.; Volpe, J. A.; Wallace, W. J. *Ann. N.Y. Acad. Sci.* **1975**, *244*, 1. (d) Ikeda-Saito, M.; Iizuka, T.; Yamamoto, H.; Kayne, F. J.; Yonetani, T. *J. Biol. Chem.* **1977**, *252*, 4882. (e) Tucker, P. W.; Phillips, S. E. V.; Perutz, M. F.; Houchens, R.; Caughey, W. S. *Proc. Natl. Acad. Sci. U.S.A.* **1978**, *75*, 1076.
(22) Phillips, S. E. V.; Shoenborn, B. P. *Nature (London)* **1981**, *292*, 81.
(23) Takenaka, A.; Syal, S. K.; Sasada, Y.; Omura, T.; Ogoshi, H.; Yoshida, Z. *Acta Crystallogr., Sect. B* **1976**, *B32*, 62.
(24) Winstein, S.; Carter, P.; Anet, F. A. L.; Bourn, A. J. R. *J. Am. Chem. Soc.* **1965**, *87*, 5247.

the distal site in a hemoprotein.

Acknowledgment. We are indebted to Dr. A. Kato and Dr. Y. Ohgo (Niigata College of Pharmacy) for measurements of mass spectra, to JEOL Ltd. for measurements of 270-MHz ^{1}H NMR spectra, and to Dr. Y. Sugiura (Kyoto University) for elemental analyses. This work was supported by a Grant-in-Aid for Special Project Research from the Ministry of Education, Science, and Culture (Subject No. 511311) and in part by a Grant-in-Aid of New Drug Development from the Ministry of Health and Welfare.

Registry No. Meso-Rh(III), 86863-10-7; Deut-Rh(III), 86863-11-8; Meso-CH_3-Rh(III), 86863-12-9; MesoDME, 1263-63-4; $[Rh(CO)_2Cl]_2$, 14523-22-9; MesoDME-Rh(III), 86863-13-0; DeutDME-Rh(III), 86884-69-7; MesoDME-CH_3-Rh(III), 86863-14-1.

Contribution from the Department of Chemistry, University of Notre Dame, Notre Dame, Indiana 46556

Complexation of Zinc Tetraphenylporphyrin by Adsorbed Poly(4-vinylpyridine): Equilibrium Studies

D. SCOTT BECKER and R. G. HAYES*

Received February 4, 1983

We have measured equilibrium constants for the reversible complexation of zinc tetraphenylporphyrin with poly(4-vinylpyridine) both in solution and in a system in which the polymer had been adsorbed onto fused silica. The complexation of the porphyrin with adsorbed polymer follows the behavior expected for reversible complexation at independent sites (so-called Langmuir-type isotherm). The equilibrium constant for complexation to the polymer in solution is somewhat smaller than that for complexation to pyridine, but the equivalent quantity for complexation to the polymer adsorbed onto silica is very large.

Introduction

Porphyrin compounds have elicited considerable interest for a long time as potential sensitizers for photochemical and photoelectrochemical processes, in particular for processes involving energy storage.[1-7] Among these interests has been an interest in attaching porphyrin compounds to electrodes or to materials that might serve as electrodes.[8-14] One method of attachment available is the ligation of a metalloporphyrin through its axial position(s) to groups that are naturally found on a surface or that have been introduced onto a surface by chemical modification of the surface. In the work we report herein, we have measured the equilibrium properties of zinc tetraphenylporphyrin with poly(4-vinylpyridine), the latter in solution or as a surface modifier on fused silica. Poly(4-vinylpyridine) as a surface-modifying agent, especially for graphite electrodes, has been studied by Anson's group.[15-18] The polymer proves to adsorb to surfaces of many kinds and could, thus, serve as a modifying agent for various electrodes or as a part of a heterogeneous catalytic system.

Experimental Section

Zinc tetraphenylporphyrin (ZnTPP) was synthesized and purified by using the methods of Adler et al.[19] Poly(4-vinylpyridine) was purchased from Aldrich Chemical Co. Inc. and used as received. The product used is a 4-vinylpyridine/styrene copolymer with 10% styrene. It has a weight-average molecular weight, from viscosity, of 3.2×10^5. We abbreviate it henceforth as PVP. Spectral grade benzene was used without further purification. Pyridine and methylene chloride were purified by standard procedures.[20] The quartz substrates were flat rectangles with dimensions of 5.0 cm × 1.0 cm × 0.1 cm and were of Suprasil grade quartz.

Prior to exposure to PVP, the quartz flats were cleaned by boiling in dilute HNO_3 and rinsed thoroughly with deionized water. They were then rinsed heavily with CH_3OH and oven-dried for 1 h at 110 °C. Then the samples were cleaned with CH_2Cl_2 and placed into a 0.5% solution of PVP in CH_2Cl_2 for 30 min. Afterward the samples were rinsed with CH_2Cl_2 and analyzed. PVP samples were coordinated with ZnTPP by being placed into a fresh 10^{-3} M solution of ZnTPP in benzene for 1 h. The samples were rinsed with benzene, analyzed, and treated appropriately for further reaction.

All UV and vis spectra were recorded on a Varian Cary 219 spectrophotometer or a Perkin-Elmer 552 spectrophotometer. The PVP coverages were based on pyridine units as determined by the difference in absorption between 257 and 280 nm. The difference of the extinction coefficients was obtained from PVP in CH_2Cl_2 at the same wavelengths. This corrected for any background scattering due to surface roughness of the quartz substrates. The extinction

(1) Harriman, A.; Porter, G.; Richoux, M.-L. *J. Chem. Soc., Faraday Trans. 2* **1981**, (10), 1939.
(2) Okura, I.; Takeuchi, M.; Kim-Thuon, N. *Photochem. Photobiol.* **1980**, *31* (5), 423.
(3) Pileni, M.-P.; Braun, H. M.; Graetzel, M. *Photochem. Photobiol.* **1980**, *31* (5), 423.
(4) Kampa, F. J.; Yamashita, K.; Fajer, J. *Nature (London)* **1980**, *284* (No. 5751), 40.
(5) Harel, Y.; Manassen, J. *Photochem. Photobiol.* **1980**, *31* (5), 451.
(6) Potter, W.; Young, R. N.; Levin, G. *J. Am. Chem. Soc.* **1980**, *102* (7), 2471.
(7) McLendon, G.; Miller, D. S. *J. Chem. Soc., Chem. Commun.* **1980**, 533.
(8) Mercer-Smith, J. A.; Whitten, D. *J. Am. Chem. Soc.* **1979**, *101*, 6620.
(9) Umezawa, Y.; Yamamura, T. *J. Electroanal. Chem. Interfacial Electrochem.* **1979**, *95*, 113.
(10) Chandrosekaren, K.; Giannotti, C.; Monserrat, K.; Otruba, J. P.; Whitten, D. *J. Am. Chem. Soc.* **1982**, *104*, 6200.
(11) Tanimura, K.; Kawai, T.; Sakata, T. *J. Phys. Chem.* **1980**, *84*, 751.
(12) Lennox, J. C.; Murray, R. W. *J. Electroanal. Chem. Interfacial Electrochem.* **1977**, *78*, 751.
(13) Davis, D. G.; Murray, R. W. *J. Electroanal. Chem. Interfacial Electrochem.* **1977**, *78*, 395.
(14) Kawai, T.; Tanimura, K.; Sakata, T. *Chem. Phys. Lett.* **1978**, *56*, 541.
(15) Oyama, N.; Anson, F. C. *J. Am. Chem. Soc.* **1979**, *101*, 739.
(16) Oyama, N.; Anson, F. C. *J. Am. Chem. Soc.* **1979**, *101*, 3450.
(17) Shigehara, K.; Oyama, N.; Anson, F. C. *J. Am. Chem. Soc.* **1981**, *103*, 2552.
(18) Oyama, N.; Anson, F. C. *J. Electrochem. Soc.* **1980**, *247*, 249, 640.
(19) Adler, L. F.; Kampas, K. J. *Inorg. Nucl. Chem. Lett.* **1970**, *32*, 2443.
(20) Gordon, A. J.; Ford, R. A. "The Chemist's Companion"; Wiley: New York, 1972.

0020-1669/83/1322-3050$01.50/0