PHOSPHOLIPID-LIKE PORPHINATO IRON AND ZINC COMPLEXES: SYNTHESIS AND PROPERTIES

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Abstract - A new amphiphilic porphyrin having one hydrophilic group, 5,10,15-tri(α,α,α-o-pivalamidophenyl)-20-mono{α-o-[12-(2'-trimethylammonioethoxy)phosphinatoxy-2,2-dimethyldodecanamido]phenyl}porphine, and its zinc and iron complexes were prepared. These were efficiently embedded in a micelle or a liposome because of their amphiphilicity.

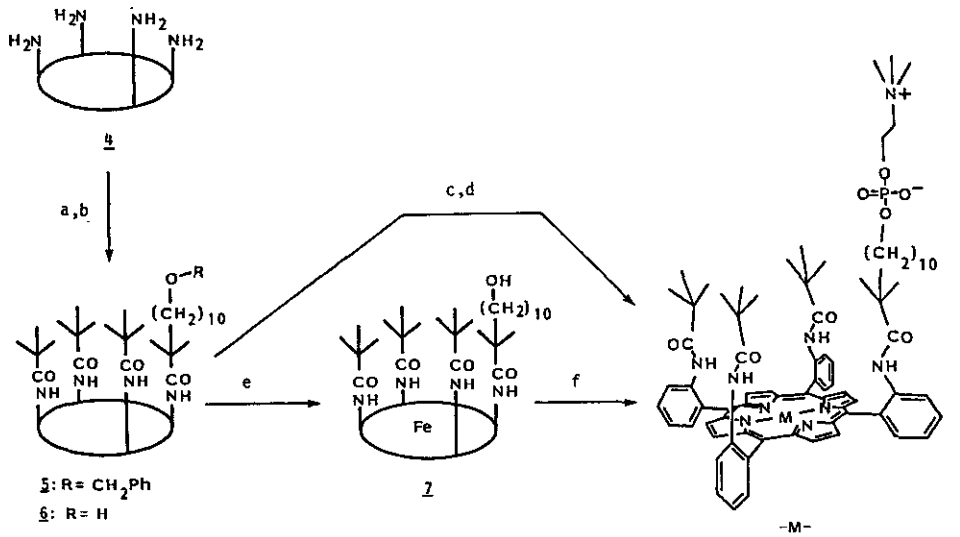
An interesting approach to mimic the reactions on biomembranes has been developed by using the liposome-bound metalloporphyrins: models for the photochemical reactions by chlorophylls,[1,2] for the redox reactions of cytochromes[3] and for respiratory hemoproteins.[4-7] We have recently synthesized the amphiphilic porphinato iron complexes having four carboxyl or four phosphocholine groups per a porphyrin as models for membrane-bound hemoproteins.[8,9] The control of the hydrophobic-hydrophilic balance of a metalloporphyrin seems to be the key to construct a better model. As a much easier way to control the hydrophobic-hydrophilic balance of porphyrins, we tried to develope a new synthetic process of amphiphilic porphyrins having only one hydrophilic group. The present report communicates the detailed synthesis and some properties of a new amphiphilic porphyrin, 5,10,15-tri(α,α,α-o-pivalamidophenyl)-20-mono{α-o-[12-(2'-trimethylammonioethoxy)phosphinatoxy-2,2-dimethyldodecanamido]phenyl}porphine, 1 , and its zinc 2 and iron 3 complexes.

12-Benzyloxy-2,2-dimethyldodecanoyl chloride was obtained by the reaction of 10-benzyloxydecanyl bromide with α-lithio isobutylate,[10] followed by the teatment with thionyl chloride (31%). This acid chloride (1.05 eq) was allowed to react with 5,10,15,20-tetra(α,α,α,α-o-aminophenyl)-

porphine[11] 4 in dichloromethane (CH_2Cl_2) containing pyridine at room temperature and then excess moles of pivaloyl chloride were added to the stirred solution. The mixture was purified by column chromatography on silica gel to give the benzyl ether 5 (33%). Debenzylation of 5 was carried out by aluminium trichloride and anisole in CH_2Cl_2-nitromethane (1:1) to give the alcohol 6 (72%). The alcohol 6 was then phosphorylated with 2-chloro-2-oxo-1,3,2-dioxaphospholane in CH_2Cl_2, and the resultant phosphate triester was cleaved by excess moles of trimethylamine in acetonitrile[12] at 60°C to give the phosphocholine-bearing porphyrin 1 (97%). The zinc insertion of 1 by zinc(II) acetate in methanol gave the zinc complex 2 (81%) after purification on a gel column (Sephadex LH-60, methanol). On the other hand, the alcohol 6 was allowed to react with iron(II) bromide in the presence of pyridine in tetrahydrofuran (THF) to give the iron complex 7 (87%). The phosphocholination of 7 was carried out by the same method described above to afford the iron(III) complex 3 (55%). 1 , 2 and 3 were soluble in most organic solvents (chloroform, diethyl ether, benzene, methanol etc.) except hydrocarbon and water, while those having four phosphocholine groups[9] were soluble only in alcohols.

The ^{13}C nmr spectrum of the complex 2 is shown in Figure 1. The spectroscopic data and/or elemental analyses of the other compounds were consistent with the assigned structures.

The incorporation of 2 and 3 in a micelle or a liposome was studied by a gel permeation chromatography and a fluorescence spectral measurement. A mixture of 1 μmole of 2 and 50 μmole of L-α-dipalmitoylphosphatidylcholine (DPPC) in 5 ml of 0.05M phosphate buffer (pH7.0) was ultrasonicated at 60°C to give a liposome-dispersed solution. 2 was also dissolved in the same buffer



a, $PhCH_2O(CH_2)_{10}C(CH_3)_2COCl$, $(CH_3)_3CCOCl$, Py; b, $AlCl_3$, anisole;
c, $[O_2P(O)Cl]$, $N(CH_3)_3$; d, $Zn(OAc)_2$; e, $FeBr_2$; f, $[O_2P(O)Cl]$, $N(CH_3)_3$.

having 1 w/v% of Triton X-100 (poly(oxyethylene)-p-octylphenyl ether) to give a micelle solution. The incorporation of 2 in the micelle or the liposome was confirmed by a gel permeation chromatography on Sepharose 4B with the same buffer. The elution curves determined by monitoring the absorbance at 559 nm based on 2 were consistent with those of the liposome itself (detected at 300 nm) and the micelle (detected at 215 nm), respectively. The same result was obtained on 3. The fluorescence spectra of 2 (λ_{em}: 600 and 655 nm) were obtained by excitation at 425 nm. The fluorescence intensity in alcohols increased with the decrease of the solvent polarity (methanol>1-buthanol> 1-octanol) as expected.[13] The intensities of the liposome- and the micelle-embedded 2 in the aqueous medium were virtually equal to that in octanol, and were nearly twice as large as that in methanol. These results indicate that 2 and 3 are easily incorporated into the liposome or the micelle efficiently and the porphyrin moieties are situated in the hydrophobic region of those aggregates. These novel amphiphilic metalloporphyrins are expected to act as model compounds for the membrane-bound hemoproteins or chlorophylls.

EXPERIMENTAL SECTION

Apparatus Melting points were obtained on a Yanagimoto melting point apparatus and are not corrected. Ir spectra were recorded on a Hitachi 260-50 infrared spectrometer, uv-vis spectra on a Shimazu UV-240, and the fluorescence spectra on a JASCO FP-550. Nmr spectra were obtained by a JEOL JNM-FX100 spectrometer with tetramethylsilane as an internal standard. The field desorption mass specra (FDms) were obtained by a double focusing JEOL JMS-01SG-2 instrument and the fast atom bombardment mass spectra(FABms) by a JEOL JMS-DX300. Elemental analyses were performed with a

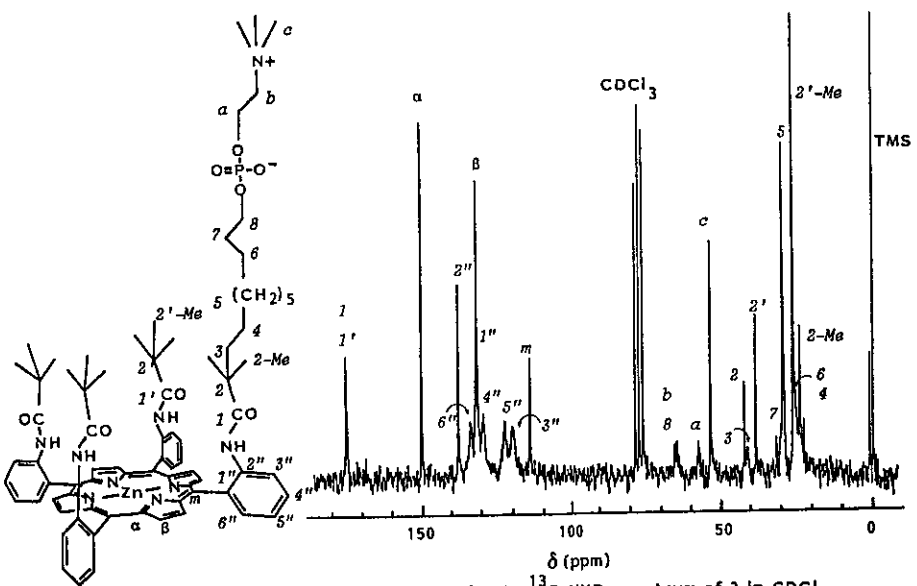


Figure 1 25.0 MHz ^{13}C-NMR spectrum of 2 in $CDCl_3$

Yanagimoto CHN corder MT-3 analyzer.

Reagents Tetrahydrofuran (THF) was dried over sodium and distilled from a ketyl solution (sodium and benzophenone) before use. Other solvents were dried and stored over molecular sieves 3A. L-α-Dipalmitoylphosphatidylcholine was purchased from Sigma Chemical Co. Silica gel 60 (Merck), neutral alumina (Merck), Toyopearl HW-40F (Toyo Soda Manufacturing Co.) and Sephadex LH-60 (Pharmacia Fine Chemicals) were used for the chromatographic separations. Tlc measurements were carried out on Silica Gel 60 (Merck).

12-Benzyloxy-2,2-dimethyldodecanoyl chloride A solution containing 330 mmol of sodium benzyloxide in benzyl alcohol (70 ml) was added under reflux to a solution of 1,10-dibromodecane (100 g, 330 mmol) in THF (250 ml) during 2 h. After refluxing for 5 h, the precipitate was filtered off and the filtrate was concentrated. The oily residue was distilled under reduced pressure to give 46 g (43%) of 10-benzyloxydecanyl bromide: bp 185-189°C/3 mmHg; pmr($CDCl_3$): δ 3.40(2H,t), 3.43 (2H,t), 4.50(2H,s), 7.35(5H,s), and 1.25(12H,brs). 2-Methylpropionic acid(6.5 ml, 70 mmol) was addded to the THF-hexane(100 ml/70 ml) solution of lithium diisopropylamide (140 mmol) under nitrogen gas at -20°C, and then hexamethylphosphoric triamide (8 ml) was added. The mixture was allowed to be heated to 50°C for 1 h and cooled to -20°C again. To this was added 10-benzyloxydodecanyl bromide (18 g, 55 mmol). After heating at 45°C for 2 h, the reaction mixture was poured into ice-cold 10% hydrochloric acid (250 ml) and then extracted with diethyl ether (250 ml x 2). The combined ether layer were washed with diluted hydrochloric acid (100 ml x 2) and water (50 ml x 2), and then dried over anhydrous sodium sulfate. After the removal of the solvent, the crude product was recrystallized from hexane to afford 12-benzyloxy-2,2-dimethyldodecanoic acid (8.37 g, 46%): mp 53.5-54.5°C; ir(KBr) 1703 cm^{-1}; pmr($CDCl_3$) δ 1.18(6H,s), 1.26(16H,s), 3.46(2H,t), 4.51 (2H,s), and 7.33(5H,s). Anal. Calcd. for $C_{21}H_{34}O_3$: C, 75.40; H, 10.25%. Found: C, 75.64; H, 10.09%. The obtained acid was treated with thionyl chloride (1.2 ml, 17 mmol) in benzene (20 ml) at room temperature for 5 h. After the concentration in vacuo, 12-benzyloxy-2,2-dimethyldodecanoyl chloride was obtained as a colorless oil (100%): ir(CCl_4) 1790 cm^{-1}.

5,10,15-Tri(α,α,α-o-pivalamidophenyl)-20-mono[α-o-(12-benzyloxy-2,2-dimethyldodecanamido)phenyl]-porphine (5) To a stirred solution containing 2.1 g(3.1 mmol) of 5,10,15,20-tetra(α,α,α,α-o-aminophenyl)porphine[11] 4 and 1 ml of pyridine in CH_2Cl_2 (50 ml) was added 1.09 g (3.27 mmol) of 12-benzyloxy-2,2-dimethyldodecanoyl chloride. After 3 h, pivaloyl chloride (5 ml) and pyridine (5 ml) were added. Then the mixture was stirred for 2 h. The reaction mixture was poured into saturated aqueous sodium hydrogen carbonate (200 ml), and the separated organic layer was washed with 4% sodium hydrogen carbonate (200 ml x 2) and water (200 ml). After the removal of the solvent, the residue was purified by a silica gel column chromatography with a solvent of benzene-ether (7/1). The third eluate was collected and recrystallized from ether-methanol to give 1.26 g

(33%) of the benzyl ether 5: mp 87.5-89.0°C; ir(KBr) 3440, 1690 (amide) and 3330 cm^{-1}(pyrrole NH); pmr($CDCl_3$) δ -2.60(4H,s, pyrrole NH), -0.21(6H,s,-$CH_2C(CH_3)_2CO$-), 0.09 and 0.05(27H, each s,-$C(CH_3)_3$), 3.46(2H,t,J=6.4Hz,$PhCH_2OCH_2CH_2$-), 4.50(2H,s,$PhCH_2O$-), 7.4-8.4(21H,m,phenyl), and 8.82(8H,s,pyrrole β-protons); FDms: 1242(M^+). Anal. Calcd. for $C_{80}H_{90}N_8O_5$: C, 77.26; H, 7.30; N, 9.01%. Found: C, 76.89; H, 7.31; N, 8.88%. Rf of tlc (benzene/ether=4/1): 0.28.

5,10,15-Tri(α,α,α-o-pivalamidophenyl)-20-mono[α-o-(12-hydroxy-2,2-dimethyldodecanamido)phenyl]-porphine (6) A solution of the benzyl ether 5 (0.65 g, 0.52 mmol) and anisole (1 ml) in CH_2Cl_2-nitromethane (15 ml/15 ml) was treated with 2.0 g of aluminium trichloride at room temperature for 3 h, and then the mixture was poured into 200 ml of crushed ice, extracted with 50 ml of CH_2Cl_2. The organic layer was washed with water (100 ml) and 4% sodium hydrogen carbonate (100 ml x 2), dried over anhydrous sodium sulfate, and evaporated. The resultant residue was chromatographed on silica gel with a solvent of chloroform-ether (10/1). The second eluate was collected and concentrated. The crude product was recrystallized from benzene-hexane to give 0.43 g (72%) of the alcohol 6: mp 224-227°C; Rf of tlc (chloroform/ether=3/1): 0.23; ir(KBr) 3450 cm^{-1}(OH); pmr($CDCl_3$) δ 3.79(2H,t,J=6.5Hz,$HOCH_2CH_2$-); FDms: 1153(M+1)$^+$. Anal. Calcd. for $C_{73}H_{84}N_8O_5$: C, 76.01; H, 7.34; N, 9.72%. Found: C, 76.17; H, 7.46; N, 9.43%.

5,10,15-Tri(α,α,α-o-pivalamidophenyl)-20-mono{α-o-[12-(2'-trimethylammonioethoxy)phosphinatoxy-2,2-dimethyldodecanamido]phenyl}porphine (1) 2-Chloro-2-oxo-1,3,2-dioxaphospholane (0.2 g, 1.45 mmol) was added to a solution containing 0.325 g (0.28 mmol) of the alcohol 6 and 0.2 ml of triethylamine in CH_2Cl_2 (20 ml), and the mixture was stirred at room temperature for 16 h. After the solvent was removed by evaporation, the resultant phosphate triester was allowed to react with 10 ml of trimethylamine in acetonitrile (25 ml) with a stainless steel pressure tube at 60°C for 16 h. After the concentration under the reduced pressure, the resultant residue was dissolved in 100 ml of chloroform. After being washed with water (100 ml x 2), the extract was concentrated by evaporation and the product was purified by a column chromatography on Toyopearl HW-40 with methanol. The main fraction gave 0.357 g (97%) of the phosphocholine-bearing porphine 1: mp 120-124°C; ir(KBr) 1230, 1060(phosphocholine) and 3330 cm^{-1}(pyrrole NH); uv-vis(benzene) 420,513,548,588 and 643 nm; pmr ($CDCl_3$) δ 3.42(9H,s,-$\overset{+}{N}(CH_3)_3$);FABms: 1380(M+1)$^+$; ^{13}C-nmr ($CDCl_3$) δ 130.9(1"),138.4(2"),120.9(3"), 130.0(4"),123.1(5"),134.2(6"),147.0(α),131.7(β),114.9(*m*),175.4,175.5(1'),38.9(2'),26.4(2'-Me),174.9 (1),42.3(2),41.0(3),24.8(4),29.5-29.9(5),25.8(6), 29.7(7),66.1(8),24.2(2-Me),66.1(a),59.5(b),54.4 (c). The numbers and letters in parentheses represent the carbon positions in Figure 1.

5,10,15-Tri(α,α,α-o-pivalamidophenyl)-20-mono{α-o-[12-(2'-trimethylammonioethoxy)phosphinatoxy-2,2-dimethyldodecanamido]phenyl}porphinato zinc(II) (2) A solution of 53 mg (0.04 mmol) of the porphyrin 1 in methanol (50 ml) was treated under reflux with 100 mg of zinc acetate dihydrate under a nitrogen gas atmosphere for 2 h. After evaporation, the residue was dissolved in 40 ml of chloroform. The solution was washed twice with water and then the solvent was distilled off under

the reduced pressure. The residue was chromatographed on Sephadex LH-60 with methanol to give 45 mg (81%) of the zinc complex: mp 224-227°C; ir(KBr) 1260 and 1070 cm^{-1}(phosphocholine) and the disappearence of 3330 cm^{-1}(pyrrole NH); uv-vis(benzene) 320,433,522(shoulder),565 and 605 nm; pmr($CDCl_3$) δ 3.14(9H,s,$-\overset{+}{N}(\underline{CH_3})_3$), 3.53(2H,t,$-CH_2\underline{CH_2}\overset{+}{N}-$), 3.79(2H,m,$-(CH_2)_9\underline{CH_2}O-$) and 4.13(2H,brs, $-O\underline{CH_2}CH_2\overset{+}{N}-$); FABms: 1380$(M+1)^+$. Anal. Calcd. for $C_{78}H_{94}N_9O_8PZn\cdot 5H_2O$: C, 63.64; H, 7.12; N, 8.56%. Found: C, 63.29; H, 7.56; N, 8.73%.

5,10,15-Tri(α,α,α-o-pivalamidophenyl)-20-mono[α-o-(12-hydroxy-2,2-dimethyldodecanamido)phenyl]-porphinato iron(III) bromide (7) Ferrous bromide tetrahydrate (0.6 g) was added to a solution of 0.575 g (0.5 mmol) of the porphine 6 and 0.3 ml of pyridine in THF (40 ml) under nitrogen gas atmosphere, and the mixture was stirred for 4 h under reflux. After the removal of the solvent, the residue was purified on an alumina column with chloroform-methanol (50/1). To the first eluate was added 1 ml of 48% hydrobromic acid, and the mixture was concentrated under the reduced pressure. The residue was recrystallized from methanol-CH_2Cl_2 to give 0.56 g (87%) of the iron complex 7: mp 235-237°C; Rf of tlc (chloroform/methanol=9/1): 0.57; ir(KBr) 3450 cm^{-1}(OH) and the disappearence of 3330 cm^{-1}(pyrrole NH); FDms: 1287$(M+1)^+$. Anal. Calcd. for $C_{73}H_{82}N_8O_5FeBr$: C, 68.11; H, 6.42; N, 8.70%. Found: C, 67.84; H, 6.46; N, 8.58%.

5,10,15-Tri(α,α,α-o-pivalamidophenyl)-20-mono{α-o-[12-(2'-trimethylammonioethoxy)phosphinatoxy-2,2-dimethyldodecanamido]phenyl}porphinato iron(III) (3) A mixture of the alcohol 7 (0.345 g, 0.27 mmol), triethylamine (0.15 g, 1.45 mmol) and 2-chloro-2-oxo-1,3,2-dioxaphospholane (0.20 g, 1.4 mmol) in 20 ml of CH_2Cl_2 was stirred for 12 h at room temperature, and then the resultant triester was cleaved by the same procedure as described in the case of the compound 1 to afford 0.202 g (55%) of the complex 3: mp 178-180°C; ir(KBr) 1230 and 1080 cm^{-1}(phosphocholine); uv-vis(benzene) 421,510(shoulder) and 563 nm; FABms: 1372$(M+1)^+$. Anal. Calcd. for $C_{78}H_{94}N_9O_8PFe\cdot 3H_2O$: C, 65.67; H, 7.07; N, 8.83%. Found: C, 65.34; H, 7.48; N, 8.80%.

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