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SYNTHESIS OF AMPHIPHILIC PORPHINATO IRON COMPLEXES HAVING PHOSPHORYLCHOLINE GROUPS

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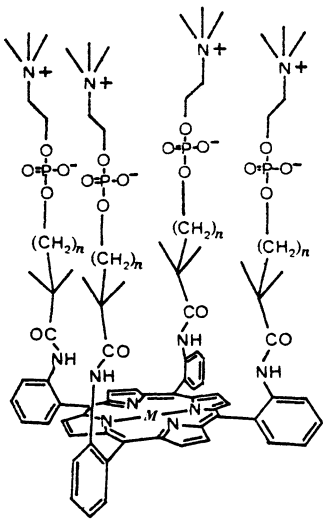
New porphyrin derivatives having alkyl phosphorylcholine groups as amphiphilic moieties and their iron complexes were prepared. Based on the hydrophobic-hydrophilic balance, some of the synthesized complexes were efficiently embedded in the bilayer of phospholipid liposomes.

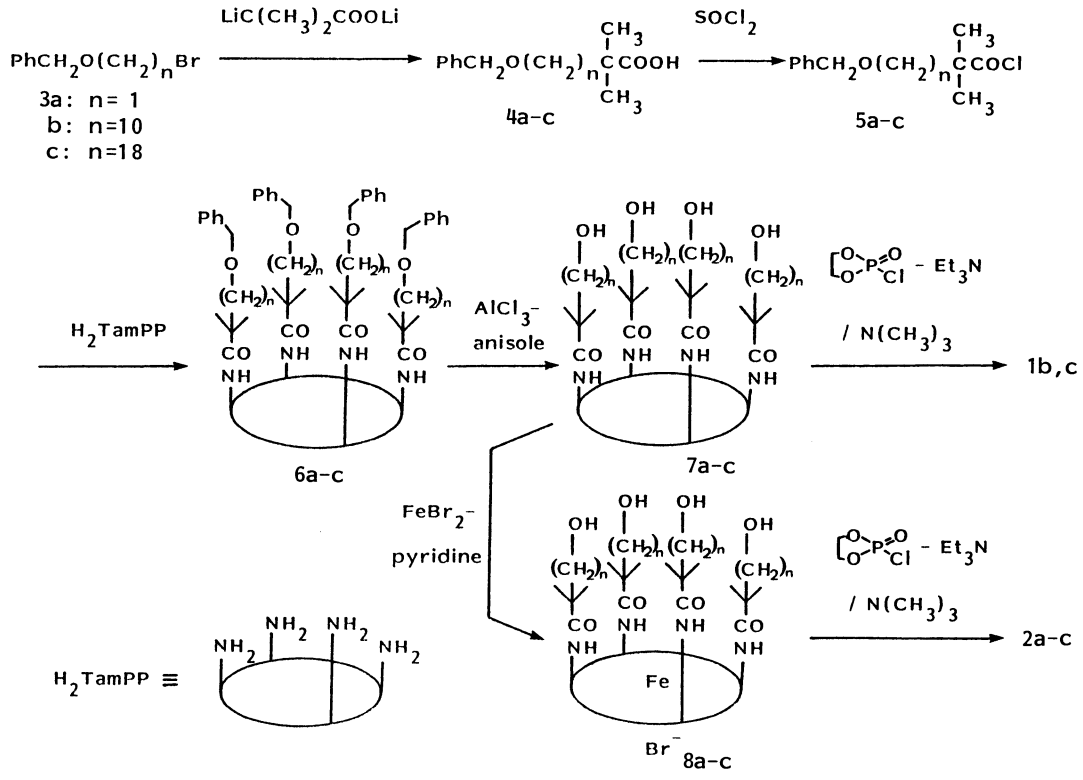
Various metalloporphyrin complexes have been synthesized as models of natural oxygen carriers like hemoglobin and myoglobin.[1] We have recently found that the *meso*-tetra(α,α,α,α-o-pivalamidophenyl)porphinato iron(II)[2] complex of 1-dodecyl-2-methylimidazole was embedded in the fatty region of liposome to give a stable oxygen adduct under semi physiological conditions.[3] It was considered that the hydrophobic environment of liposome protected the oxygen adduct from its irreversible oxidation.[4] In this communication we designed porphyrin derivatives (1b, c) which have both the hydrophobic surroundings upon the porphyrin ring and the hydrophilic groups apart from the porphyrin ring. These porphyrins are expected to be efficiently embedded in a bilayer of liposome based on their good hydrophobic-hydrophilic balance. Synthesis and property of the porphyrins (1b, c) and their iron complexes (2a-c) are reported.



	n=	-M-
1b:	10,	-H, H-
1c:	18,	-H, H-
2a:	1,	-Fe-
2b:	10,	-Fe-
2c:	18,	-Fe-

ω-Benzyloxyalkyl bromides 3a-c reacted with α-lithio isobutylate[5] in tetrahydrofuran (THF) and hexamethylphosphoric triamide at -60°C and then at room temperature, giving ω-benzyloxy-2,2-dimethylalkanoic acids 4a (53%), 4b (46%), and 4c (31%). The reaction of 4b (10 mmol) with thionyl chloride (16 mmol) gave the acid chloride 5b,

which then reacted with 5,10,15,20-tetra(α,α,α,α-o-aminophenyl)porphin (H_2TamPP)[2] (1.5 mmol) in THF containing pyridine (16 mmol) for 5 h at room temperature. The mixture was separated by column chromatography (silica gel, benzene-ether (10/1)), affording 6b (60%): IR ($CHCl_3$) 3440, 1680, 1510(-CONH-), 3330(pyrrole N-H), 3000, 2930, 2860(-CH_2- and -CH_3), 1580(aryl), and 1000 cm^{-1}(-CH_2OCH_2-); ^{1}H NMR($CDCl_3$) δ = -2.60 (2H, s, pyrrole NH), -0.23 (24H, s, -$C(CH_3)_2$CONH-), 3.46(8H, t, $PhCH_2OCH_2CH_2$-), 4.50(8H, s, $PhCH_2$-), 7.12(4H, s, amido NH), 7.32(20H, s) and 8.82 (8H, s). 6b (0.85 mmol) was debenzylated by aluminum trichloride (2.0 g) and anisole (2 ml) in dichloromethane-nitromethane (1/1) for 5 h at room temperature[6], and the mixture was cooled with ice-water, followed by extraction with dichloromethane. The extract was concentrated in vacuo and then recrystallized in benzene to give 7b (80%): mp 127-129.5°C, IR(KBr) 3600-3350(broad), and 1060 cm^{-1}(-CH_2OH); ^{1}H NMR($CDCl_3$) δ = 3.64(8H, s, -CH_2OH); UV_{max} and Vis_{max}($CHCl_3$) 418, 512, 545, 587, and 653 nm. 7b (0.35 mmol) was phosphorylated with 2-chloro-2-oxo-1,3,2-trioxaphospholane (3.5 mmol) in dichloromethane using triethylamine (3.5 mmol) at room temperature for 12 h, and the resultant phosphate triester was cleavaged by a large excess of anhydrous trimethylamine in acetonitrile for 24 h at 60°C.[7] The red-brownish precipitate was collected by filtration and then purified on a gel column (Sephadex LH-60, methanol). Pure 1b was obtained as amorphous solid (90%): mp 257-261°C, IR(KBr) 3600-3100(broad), 1240, 1060, 960(-OP(=O)(-O^-)$OCH_2CH_2N^+(CH_3)_3$) and 1690, 1510 cm^{-1}(-CONH-); UV_{max} and Vis_{max}(H_2O): 418, 512, 545, 586, and 650 nm; ^{1}H NMR(CD_3OD) δ = -0.23(24H, s), 3.23(36H, s, -$N^+(CH_3)_3$), 3.68(8H, m, -$CH_2N^+(CH_3)_3$), 3.85(8H, t, -$CH_2CH_2CH_2O$-), 4.25(8H, brs, -OCH_2CH_2N-), 7.43-8.40(16H, m, phenyl



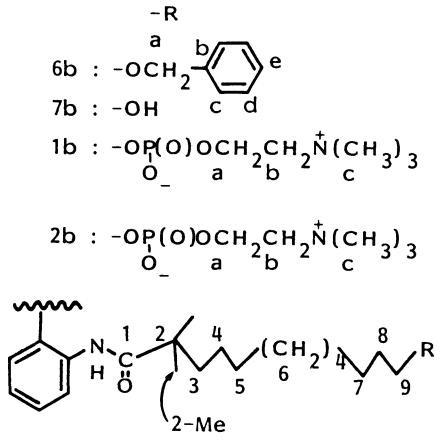


Table 1. ^{13}C NMR spectra data

carbon	δ_{TMS} (ppm) 6b	7b	1b	2b
1	174.7	174.9	175.3	176.6
2	42.5	42.3	41.5	45.4
3	41.1	41.0	40.3	43.6
4	24.9	24.9	24.2	27.6
5	30.0	29.9	30.2	30.5
6	29.5-29.7	29.3-29.9	28.8-29.9	29.5-30.5
7	26.2	25.7	25.1	27.6
8	29.7	32.7	30.2	31.7
9	70.4	62.8	64.9	66.6
2-Me	24.1	24.1	23.1	26.6
a	72.7		58.4	60.2
b	138.6		65.9	66.8
c	128.2		52.8	54.7
d	127.5			
e	127.8			

Solvent: 6b, 7b in $CDCl_3$, 1b in CD_3OD, and 2b in CD_3OD+NaCN/D_2O.

protons), and 8.80(8H, s, porphyrin ring β-protons). Similar procedure for 4c gave 1c.

7b reacted with ferrous bromide($FeBr_2$) in THF under nitrogen atmosphere[2] to afford 8b (91%): mp 76.0-79.0°C, UV_{max} and Vis_{max} ($CHCl_3$) 417, 505 nm. This was phosphocholinated by a method similar to that described above, to give 2b(90%): mp 145-150°C, IR(KBr) 3600-3100(broad), 1240, 1090, 970 cm^{-1}(-OP(=O)(O^-)-$OCH_2CH_2N^+(CH_3)_3$), UV_{max} and Vis_{max}(H_2O): 415, 510(shoulder), and 568 nm. In the similar way, 2a and 2c were obtained from 7a and 7c, respectively.

The ^{13}C NMR spectrum data are shown in Table 1. The spectroscopic data of the other compounds were consistent with the assigned structures.

The porphyrins (1b, c) and their iron complexes (2a-c) were insoluble in aprotic solvents, e.g. chloroform, THF, DMF, and DMSO, but were well soluble in protic ones, e.g. methanol, ethanol, and acetic acid. 1b, 2a, and 2b were also soluble in water at pH 7.0. Molecular weight was estimated with gel permeation chromatography (Sephadex G-200 for 2b and Sephadex G-75 for 2a, 0.1 M

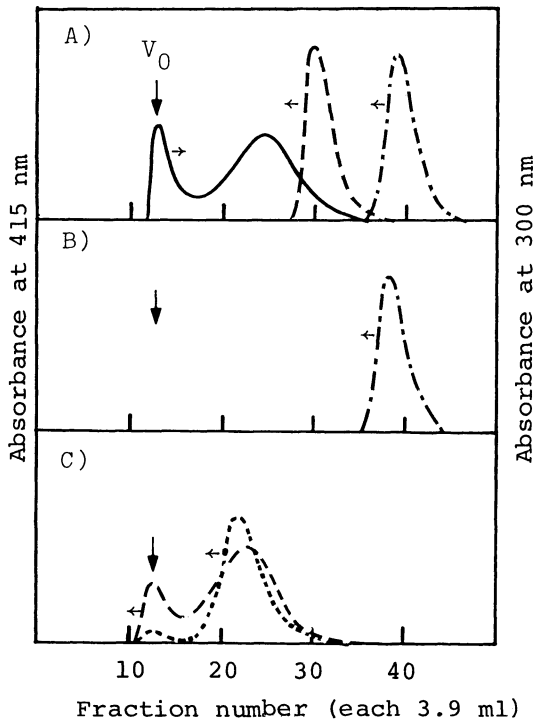


Fig. 1. Elution Curves of Liposome and 2 on Sepharose 4B.
A)——: liposome of PC (detected with 300 nm), ---: 2b (415 nm), -·-: 2a (415 nm), B)-·-: the mixture of 2a and PC, C)---: the mixture of 2b and PC, ···: the mixture of 2c and PC, V_0: void volume determined by blue dextran-2000.

(pH 7.0) phosphate buffer)[8] by monitoring with the wavelength of 415 nm. It was found that 2b forms large aggregates (about 40 molecules) due to its surfactive property, but 2a only a dimer.

In order to study the incorporation of 2a-c in the liposome, the following experiment was carried out. The methanol solution of 2 (0.2 μmol) and egg yolk phosphatidylcholine (PC) (20 μmol) was evaporated in vacuo. 0.1 M/pH 7.0 phosphate buffer (5 ml) was added, and this mixture was ultrasonicated for 30 min in an ice-water bath. The resultant solution was examined by gel permeation on Sepharose 4B (2 x 50 cm) with the same buffer, and the elution curves are shown in Fig. 1. The elution pattern indicates that 2b and 2c are embedded in the liposome but 2a not. The hydrophobic long alkyl chain is necessary for 2b and 2c to be embedded. It is concluded that new amphiphilic iron porphyrin complexes are embedded in a bilayer of liposome because their hydrophobic-hydrophilic balance is controlled to increase the compatibility with the phospholipid liposome.

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