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Synthesis and Characterization of a Membrane-Spanning Porphinatoiron(II)

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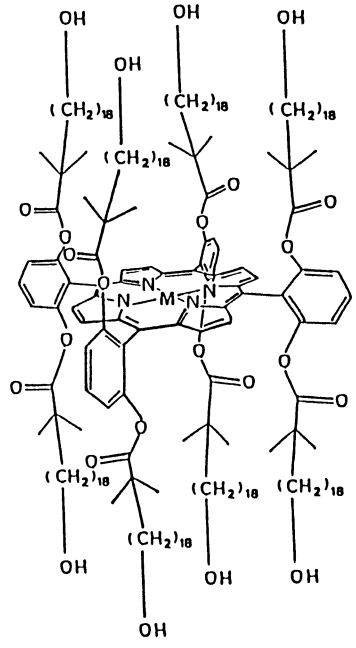
A new tetraphenylporphinatoiron(II) having a couple of four amphiphilic chains on both sides of the porphyrin ring plane, 5,10,15,20-tetra(2',6'-di(20"-hydroxy-2",2"-dimethylicosanoyloxy)-phenyl)porphinatoiron, was synthesized as a model for membrane intrinsic proteins. It was well incorporated in the liposomal bilayer and characterized. The complex with 1-dodecylimidazole embedded in phospholipid bilayer formed an oxygen adduct in water at 25 ℃.

Metalloporphyrins as models for water-soluble hemoglobin and myoglobin have been studied in detail as synthetic oxygen carriers.[1] A reversible oxygenation was often observed in aprotic organic solvents. The present authors have found that porphinatoiron(II) complexes having four amphiphilic chains on one side of a porphyrin ring plane can bind oxygen reversibly in water (pH 7.4) at 37 ℃, when they are embedded in the hydrophobic region of phospholipid bilayer of liposomes.[2] They could bind oxygen without irreversible oxidation only in the presence of an excess amount of an imidazole ligand because the non-fenced side was protected against bimolecular oxidation by the coordinated imidazole ligand.

But, less attention has been paid to the models for membrane intrinsic proteins such as Cytochromes from the standpoint of fixing a model active site into the hydrophobic region of lipid bilayer in aqueous solution. A model, which has amphiphilic residues with controlled chain-length on both sides of a porphyrin ring plane, should be molecularly designed for its spanning across lipid bilayer. Only Groves and Neuman reported the membrane-spanning metalloporphyrins for oxidation catalysis as models of bilayer-embedded enzymes, but the amphiphilic chains were not enough as the fence to prevent their irreversible oxidation by



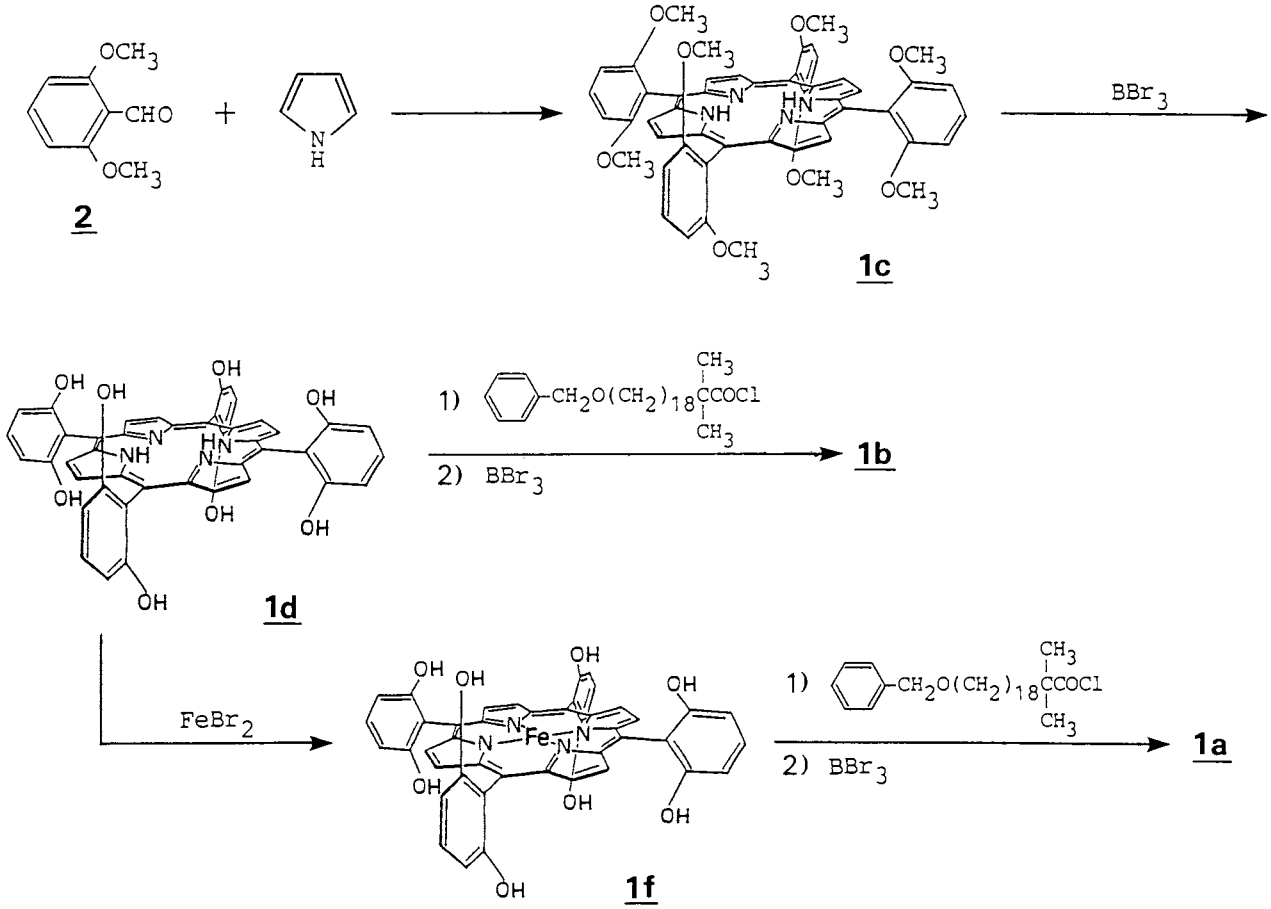
1a(M=Fe), 1b(M=-H H-)

dioxygen.[3]

In this work, a new membrane-spanning and both-faces hindered porphinatoiron (5,10,15,20-tetra(2',6'-di(20"-hydroxy-2",2"-dimethylicosanoyloxy)phenyl)porphinatoiron, 1a) was designed and its incorporation into lipid bilayers was confirmed by various physicochemical methods. The oxygen adduct formation was studied in water (pH 7.4) at 25 ℃.

The synthetic route for the membrane-spanning heme 1a is as follows. First, 2,6-dimethoxybenzaldehyde (2) was synthesized by the following process. To one mol of 2,6-dimethoxybenzoic acid in dry acetone were added 2.5 mol of potassium carbonate and 1.2 mol of dimethyl sulfate, and then the mixture was refluxed under argon atmosphere for 23 h. Recrystallization from ethyl acetate-hexane gave methyl 2,6-dimethoxybenzoate (3; yield, 75%; mp 88-89 ℃). 3 was reduced under argon atmosphere by lithium aluminium hydride in dry tetrahydrofuran for 1 h. Recrystallization from ethyl acetate-n-hexane gave 2,6-dimethoxybenzylalcohol (4; yield, 69%; mp 55-56 ℃). 4 was oxidized by manganese oxide in refluxing tetrahydrofuran under argon atmosphere for 24 h. Recrystallization from chloroform-hexane gave 2 (yield, 65%; mp 95-96 ℃ (lit. 96-97 ℃)[4]).

2,6-Dimethoxybenzaldehyde in propionic acid was warmed to 100 ℃ and to this was added an equimolar amount of pyrrole dropwise. It was then heated for 7 h. After cooling to room temperature, the precipitates were collected on a glass filter by suction filtration and purified by column chromatography on silica gel as eluted with dichloromethane to give 5,10,15,20-tetra(2',6'-dimethoxyphenyl)-



porphyrin (1c) in 15% yield. 1c was dissolved in dry dichloromethane and cooled in an ice-water bath. To this was added boron tribromide and the solution was stirred for 2 h with ice-cooling and then for 20 h at room temperature. It was poured into ice-water and the product was extracted with ethyl acetate. Column chromatography on silica gel as eluted by ethyl acetate-methanol (20/1 (v/v)) gave 5,10,15,20-tetra(2',6'-dihydroxyphenyl)porphine (1d) in 92% yield. To the ice-cooled solution of 1d in dry tetrahydrofuran was added dropwise 20-fold mol of 20-benzyloxy-2,2-dimethylicosanoyl chloride[5] dissolved in tetrahydrofuran for 1 h. After adding equivalent mol of N,N-dimethylaminopyridine, the reaction mixture was refluxed for 12 h. The residues obtained by removing the solvent under reduced pressure were taken in chloroform and the organic layer was washed with dilute HCl and then 5wt% $NaHCO_3$. Chromatographic purification on silica gel as eluted with dichloromethane gave 5,10,15,20-tetra(2',6'-di(2",2"-dimethyl-20"-benzyloxyicosanoyloxy)phenyl)porphine (1e) in 55% yield.[6] To 1e dissolved in dry dichloromethane and cooled in an ice-water bath was added an excess amount of boron tribromide and the mixture was stirred for 2 h and then for 3 h at room temperature. The reaction mixture was treated as in the case of 1d and the mixture was purified by silica gel column chromatography (chloroform/methanol (20/1(v/v)) to give the membrane-spanning porphyrin, 5,10,15,20-tetra(2',6'-di(20"-hydroxy-2",2"-dimethylicosanoyloxy)phenyl)porphine (1b) in 80% yield.[7]

5,10,15,20-Tetra(2',6'-dihydroxyphenyl)porphinatoiron(III) bromide[8] 1f was prepared by the reaction of 1d with iron(II) bromide in refluxing tetrahydrofuran for 6 h under argon atmosphere. The membrane-spanning porphinatoiron 1a[9] was synthesized by the same manner as 1b described above, by using 1f in place of 1d. The yield based on 1d was 68%.

The liposome composed of egg yolk phosphatidylcholine (5), 1-dodecylimidazole and 1b (molar ratio, 200/100/1) was prepared in 1/30 mol cm^{-3} phosphate buffer (pH 7.4) according to the usual method[2] by sonication. The average diameter of the liposomes was determined by a quasi elastic light scattering measurement to be 32 ± 7 nm. The electron microphotographic measurement with staining by uranyl acetate showed the formation of single unilamellar vesicles (SUV) with the same diameter. The incorporation of 1a or 1b into liposomes was confirmed by the gel permeation chromatography on Sepharose CL-4B in water, which indicated that 1a was eluted with liposomes, and by fluorescence measurements[10] of 1b (excitation wavelength: 411 nm; emission maximum: 650 nm), which indicated that 1b was fixed in the hydrophobic region of lipid bilayer.

The liposome-embedded 1a/1-dodecyl-imidazole complex (λ_{max}: 563, 537, 431 nm under nitrogen atmosphere), of which central iron had been reduced by a small excess amount of ascorbic acid under nitrogen atmosphere to the ferrous ion, gave the oxygen adduct under oxygen atmosphere in water (pH 7.4) at 25 ℃ (λ_{max}: 548, 423 nm). The oxygen complex formed the carbon monoxide adduct (λ_{max}: 544, 425 nm) by bubbling carbon monoxide gas through the solution, indicating no irreversible oxidation of the ferrous 1a.

Thus, it was elucidated that a new porphinatoiron having amphiphilic fences on both sides of the porphyrin ring plane was well incorporated into a lipid

bilayer of liposomes as membrane intrinsic proteins do and could bind dioxygen reversibly in water (pH 7.4) at 25 ℃.

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6) 1e: IR(KBr) 1760 cm^{-1}($\nu_{C=O}$). λ_{max} (chloroform) 651, 584, 540, 510, 416 nm. ^1H NMR ($CDCl_3$, TMS) δ -2.8(2H, s, NH), -0.7(72H, s, pivaloyl), 3.4(16H, t, $-C\underline{H}_2OCH_2$), 4.5(16H, s, $-OC\underline{H}_2$), 7.2-7.9(64H, m, phenyl), 8.8(8H, s, pyrrole).
7) 1b: IR(KBr) 1760 cm^{-1}($\nu_{C=O}$). Anal. Found. C, 73.64; H, 9.82, N, 1.8%. Calcd for $C_{220}H_{366}N_4O_{24}$ 1.5$CHCl_3$: C, 73.28; H, 10.20; N, 1.5%. λ_{max} (chloroform) 651, 584, 540, 510, 416 nm. TLC(silica gel, chloroform/methanol(15/1(v/v)) R_f=0.31
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9) 1a: IR(KBr) 1760 cm^{-1}($\nu_{C=O}$). Anal. Found: C, 73.71; H, 10.45; N, 1.3%. Calcd for $C_{220}H_{364}N_4O_{24}FeBr$: C, 73.71; H, 10.23; N, 1.6%. λ_{max} (chloroform) 680, 650, 578, 508, 417 nm. TLC(silica gel, chloroform/methanol(7/1(v/v)) R_f=0.66.
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