CONVENIENT SYNTHESIS AND PROPERTIES OF WATER SOLUBLE CYCLODEXTRIN CAPPED MESOPORPHYRINATOIRON[1]

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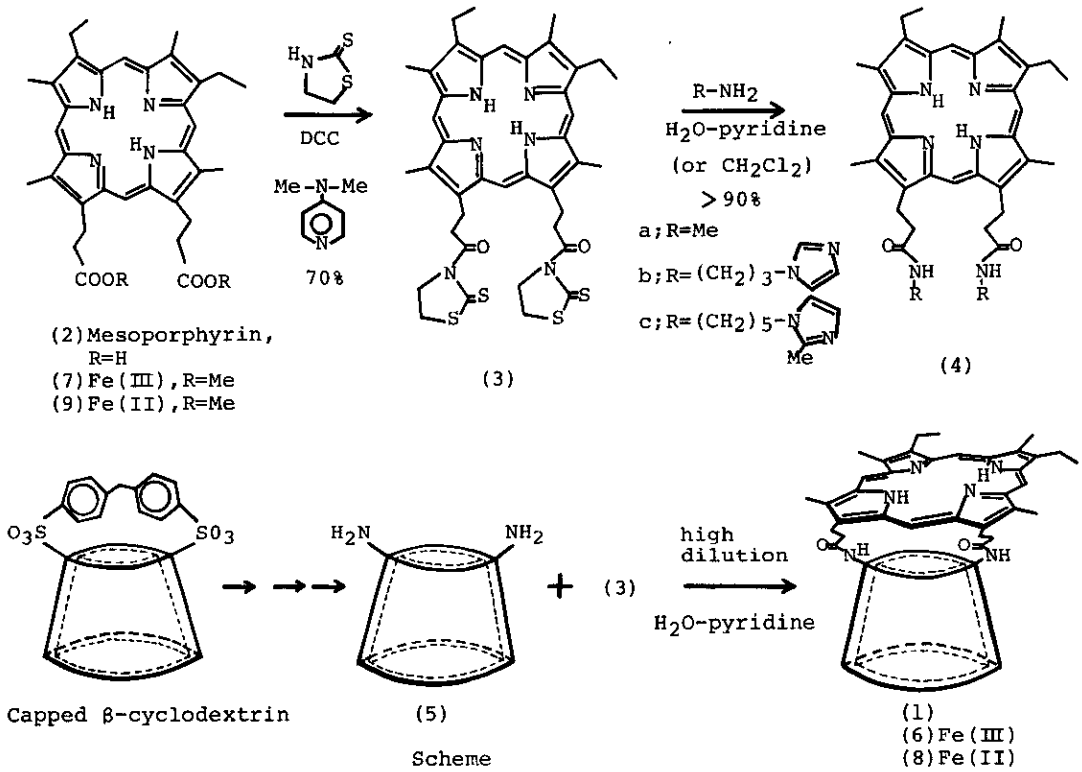
Abstract——The β-cyclodextrin-capped porphyrin was synthesized by a convenient procedure for a coupling reaction between diamino-β-cyclodextrin and mesoporphyrin dithiazolidine-2-thione in aqueous media. The thiazolidine compound was prepared by a condensation of mesoporphyrin and thiazolidine-2-thione in the presences of dicyclohexylcarbodiimide and N,N'-dimethylaminopyridine. The properties of the cyclodextrin capped heme were examined.

Modified porphyrins as artificial oxygen carriers have been extensively synthesized since the first 'picket fence' porphyrin reported by Collman.[2] For example, recently synthesized 'hanging imidazole' heme shows formation of a very stable oxygen adduct of which the life time is about one day in dry toluene.[3] However, those model hemes are mostly insoluble in aqueous media and the examinations of the oxygen or carbon monoxide binding affinities have been studied in anhydrous solvents. On the other hand, natural oxygen-carring enzymes, such as hemoglobin or myoglobin, behave in aqueous media. So far, few papers have been known on the syntheses of water soluble porphyrins. The cyclodextrin (CD) appended porphyrin, reported by Osa,[4] is composed of one point ester linkage between the CD and porphyrin, but the yield is low (6 %). On the other hand, the CD-capped porphyrins, prepared by Tsuchida,[5] are composed of two to four linkages with amide, urethane, or ester linkages between them, but not reported in detail for the syntheses. We here describe in detail a convenient procedure for the synthesis of β-cyclodextrin capped mesoporphyrin (1) which is composed of two amide linkages between the CD and porphyrin, and some properties of the present synthesized CD-capped heme.

The key step of the synthesis is of amide formation. The amide formation of porphyrin propionic acid sites is generally achieved by use of acid anhydrous,



(2) Mesoporphyrin, R=H
(7) Fe(III), R=Me
(9) Fe(II), R=Me

(3)

(4)

Capped β-cyclodextrin

(5)

(1)
(6) Fe(III)
(8) Fe(II)

Scheme

acid chloride, p-nitrophenyl ester or imidazolide in anhydrous media. However, the diamino-CD (vide infra) is only slightly soluble in organic media but becomes more soluble in water-contained solvent. We, therefore, selected the amide formation by use of an acyl-thiazolidine-2-thione which proceeds in water-contained solvents, reported by Fujita.[6] By this thiazolidine method, the amide formations of mesoporphyrin and various amines were examined. The reaction of mesoporphyrin (2) (0.18 mmol), 1,3-thiazolidine-2-thione(0.4 mmol), dicyclohexylcarbodiimide (DCC)(0.48 mmol) and N,N'-dimethylaminopyridin(DMAP)(0.2 mmol) in dry CH_2Cl_2 gave mesoporphyrin dithiazolidine-2-thione (3) in 70 % yield. The thiazolidine (3) was treated with amines in CH_2Cl_2 or water-contained pyridine to give amides (4) in quantitative yields (Scheme).

The diamino-CD (5), prepared in three steps from the 'capped cyclodextrin' by Tabushi's method,[7] was treated with the dithiazolidine (3) by high dilution method to give β-CD-capped mesoporphyrin (1) in 50 % yield. The CD porphyrin (1) exhibits a moleculer ion at m/z 1663 $(M+H)^+$ in the FAB mass spectrum and character-

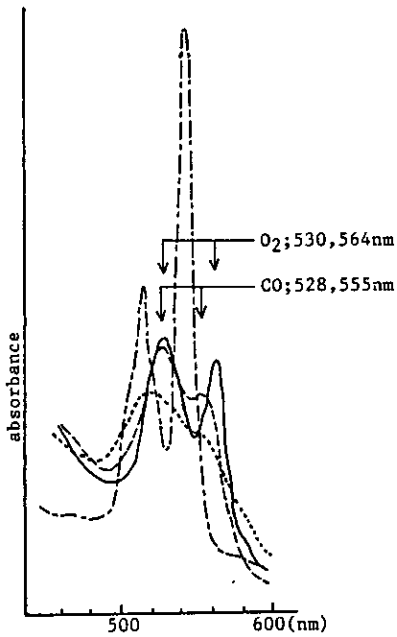


Fig.1 Visible spectra of (8) in 10%H_2O(pH7.0)-DMF at -45°C. (N-tritylimidazole) /(Heme)=50; a,O_2 free(-.-.-);b,O_2(———); c,CO(-----);d,Fe(III)(·····). (8);1x10^{-4}M.

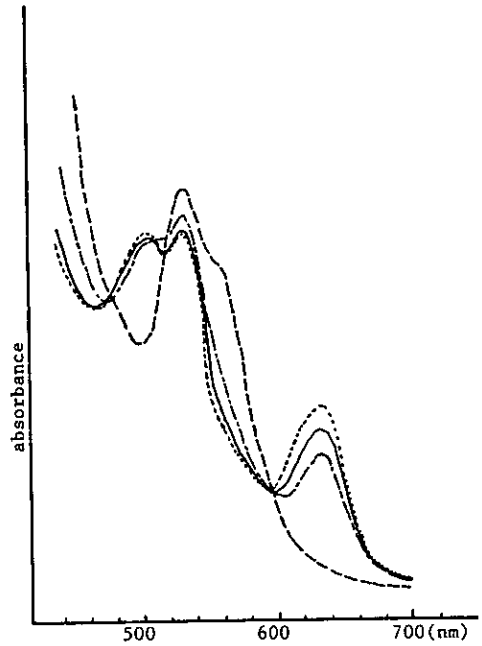


Fig.2 Visible spectra of (6) and (7) with imidazoles in DMF.
a,(·····)(6)1.1x10^{-5}M;
b,(———)(6)1.1x10^{-5}M,N-tritylimidazole 5.5x10^{-3}M;
c,(-·-·-)(6)1.1x10^{-5}M,imidazole 5.5x10^{-3}M;
d,(-----)(7)1.1x10^{-5}M, N-tritylimidazole 5.5x10^{-3}M.

istic absorption bands of porphyrin at 396(5.02), 496(4.68), 528(4.54), 566(4.39) and 618 nm (log ε=4.26)(in DMF) in the uv-visible spectrum.

The CD porphyrin (1) was treated with $FeCl_2$ in DMF to give the iron(III) complex (6).[8] The uv-visible spectrum of (6) exhibits characteristic of penta-coordinated high spin iron(III) complex[9] at 376(5.07), 501(3.97), 530(3.96) and 634nm(log ε =3.66). Figure 2 shows the visible spectra of (6) and the reference hemin (7) in the presence or absence of imidazoles. The absorption bands at 634 nm in the reference hemin (7) remarkably decreased with increasing amount of the imidazoles. In contrast, the decreasing in the CD hemin (6) was very little. The results of the spectra data indicate that the formation of the sixth ligand (low spin state) to penta-coordinated hemin (6) (high spin state) is effectively protected by the capping-CD moiety probably due to the steric hindrance.

Dithionite reduction of a solution of the CD hemin (6) in the presence of imidazoles in aqueous DMF (1:9 v/v) gave hexa-coordinated low spin iron(II) complex(8) (Fig. 1, curve a), the sixth ligand having been derived from solvent. The oxygenated CD heme, formed under oxygen, was generated to the CO-complex when carbon monoxide was passed into the solution of oxygen-adduct (Fig. 1, curve b and c).

Table. The life time of the oxygen-adduct[a].

No.	Solvent H_2O-DMF	Imidazole[b]	Conc. (M)	Temp. (°C)	Life time (min.)[e] CD heme (8)	Reference heme(9)
1	10% H_2O	Tri-IM	5×10^{-3}	-45	110	>300
2	10% H_2O	Tri-IM	5×10^{-3}	-25	21	24
3	2% H_2O	Tri-IM	5×10^{-3}	-45	120	>300
4	2% H_2O	Tri-IM	5×10^{-3}	-25	44	110
5	10% H_2O	Tri-IM(CD)[c]	5×10^{-3}	-45	113	119
6	10% H_2O	Tri-IM(CD)[c]	5×10^{-3}	-25	47	45
7	2% H_2O[d]	Tri-IM	5×10^{-3}	-45		>300
8	2% H_2O[d]	Tri-IM	5×10^{-3}	-25		89
9	10% H_2O	Me-IM	1×10^{-3}	-45	>300	>300
10	10% H_2O	Me-IM(CD)[c]	5×10^{-3}	-45	124	

a In all experiments, 2.5×10^{-2}M phosphate buffer (pH 7.0) and 1×10^{-4}M concentration of the hemes (8) and (9) were used.

b Tri-IM = N-tritylimidazole, Me-IM = N-methylimidazole.

c The β-cycoldextrin (5×10^{-3}M) was added.

d 10% MeOH was added.

e Half life time (τ); $\tau = t \ln 2/\ln[(A_0 - A_\infty)/(A_t - A_\infty)]$.

The life times of the oxygen-adduct of the CD heme (8) and the reference heme (9) were examined in aqueous DMF cooled at -25° and -45°C. The results (Table) indicate that the life time of the oxygen-adduct of the CD heme (8) was shorter than that of (9) (No. 1-4). The unexpected but very interesting results of destabilization of the oxygen-adduct owing to the CD-binding could be explained by means of a rapid protonation on the oxygen due to the CD-included water molecule, called 'high energy water,'[10] rather than due to bulk water or hydroxyl groups of the CD (see Fig. 3). This explanation is supported by following phenomena: i) at -25°C, the effect of bulk water to the life time of the (8)-oxygen was less than that of the (9)-oxygen (No. 2 and 4); ii) in the CD heme (8), addition of smaller base ligand, N-methylimidazole, gave more stable oxygen-adduct than that of larger N-tritylimidazole (No. 1 and 9)[11]; iii) addition of β-CD in the media including N-methylimidazole decreased the life time of the (8)-oxygen (No. 10), whereas the effect in the N-tritylimidazole was not observed in the CD heme (8)(No. 5); iv) addition of methanol in the reference heme (8) gave a little effect to the life time, that is, the effect of hydroxyl groups of the CD should be much less than that of water. These phenomena suggest that the CD-included water molecule is

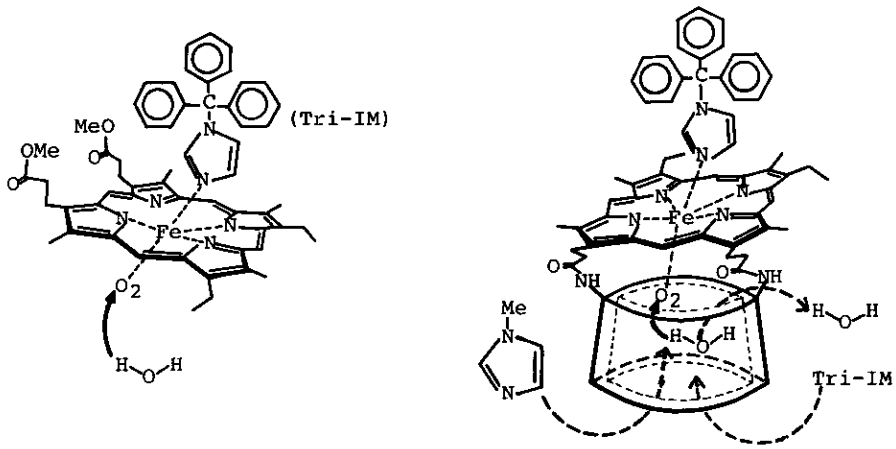


Figure 3

removed from the CD cavity by addition of N-methyl imidazole which is capable of incorporating deeply into the cavity, whereas N-trityl imidazole does not give such effect because of the steric factor (see Figure 3).

It should be pointed out that i) amide formation of porphyrin propionic acid sites with various amines, even which are slightly soluble in organic media, was utilized by means of the thiazolidine procedure in aqueous media; ii) by use of the thiazolidine method, water soluble CD-capped porphyrin (1) was synthesized in good yield; iii) the life time of the oxygen-adduct of the CD heme (8) was influenced by the 'high energy water' included in the CD cavity and/or various base lignds.

EXPERIMENTAL

Proton nmr spectra were recorded with JEOL FX-100 and GX-270 , ^{13}C nmr spectra with with a GX-270, uv-visible spectra with a Hitachi 557, ir spectra with a Hitachi 215, FAB ms spectra with a JEOL HX-100 spectrometers. All purified solvents were used for reactions of the syntheses and measurements of uv-visible spectra.

Mesoporphyrin di-1,3-thiazolidine-2-thione (3)

Mesoporphyrin (2)(100 mg, 0.177 mmol), 1,3-thiazolidine-2-thione (48 mg, 0.4 mmol), DCC (100 mg, 0.48 mmol) and DMAP (24 mg, 0.2 mmol) in dry CH_2Cl_2(10 ml) were stirred under an argon atmosphere at room temperature for 15 h. The solvent was evaporated in vacuo and the residue chromatographed on the SiO_2 column to give 95 mg of (3) in 70 % yield: ^1H nmr (100 MHz, $CDCl_3$, ppm), 10.08 and 10.19(4H,

meso-H), 4.19-4.5(12H), 4.08(4H, $C\underline{H}_2CH_3$), 3.62-3.67(12H, $CH_2C\underline{H}_3$), 2.66-2.87(m, 4H, S-$C\underline{H}_2$), 1.86(t, J=8 Hz, 6H, $CH_2C\underline{H}_3$); visible(Zn complex)(pyridine)λ_{max}nm(log ε); 414(5.84), 542(4.52), 578(4.44).

Mesoporphyrin diamide (4)

A typical reaction in water-contained pyridine was carried out in the following conditions. To a solution of (3) (40 mg, 0.052 mmol) in H_2O-pyridine (1/9 v/v, 10ml), 40 % aq. methylamine (10 ml) was added and stirred at room temperature for 30 min. The solvent was evaporated in vacuo and the residue was taken on the SiO_2 preparative tlc to give 29 mg of (3a) in 94 % yield. (3b)(99 %), (3c)(91 %).

β-Cyclodextrin-capped mesoporphyrin (1)

A solution of the diamino-β-cyclodextrin(110 mg, 0.097 mmol), prepared in the three steps from the capped cyclodextrin according to the method of Tabushi[7], in H_2O (30 ml) and a solution of (3)(75 mg, 0.097 mmol) in pyridine(30 ml) were dropwise during 2 h to a solution of 50 % aq. pyridine(200 ml) by use of syringes under stirring and an argon atmosphere at room temperature. After stirring for 12 h, the solvent was evaporated in vacuo and the residue was taken on the ptlc (using cellulose, BuOH-AcOH-H_2O=4:1:5 upper layer added 10 % MeOH) to give 80 mg of (1) in 50 % yield: FAB ms, m/z 1663$(M+H)^+$ corresponding to $C_{76}H_{106}O_{35}N_6$; ^{1}H nmr spectrum(100 MHz, in pridine-d_5-D_2O, 50°C, ppm), 9.94, 9.65 and 9.57(4H, meso-H), 4.82(7H, anomeric H), 1.2(6H, $CH_2C\underline{H}_3$), 2.7 - 4.4(others); ^{13}C nmr spectrum (in pyridine-d_5-D_2O, 25°C), 173.5 and 173.1(2xNH$\underline{C}$O), 102.7-103.7(CD-$\underline{C}$1), 96.5 and 86.3(meso-$\underline{C}$), 82.2-83.0(CD-$\underline{C}$2), 73.4-74.0(CD-$\underline{C}$3,4,5), 60.9-61.2(CD-$\underline{C}$6), 41.6 (CD-$\underline{C}$6-NH), 39.2-39.8 and 21.4-22.6($\underline{C}H_2$), 19.7, 17.6 and 11.1($\underline{C}H_3$); visible spectra, in DMF λ_{max}nm (log ε), 396(5.02), 496(4.68), 528(4.54), 566(4.39), 6.18 (4.26), 8.7 x 10^{-6}M in carbonate buffer pH 10.49 (λ_{max}), 390, 504, 536,572 and 624; 1.7 x 10^{-5}M in carbonate buffer pH 10.49 (λ_{max}), 380, 504, 536, 572 and 624; 1.7 x 10^{-5}M in tris buffer pH 7.3 (λ_{max}), 374, 504, 536, 572 and 624.

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11. Tsuchida has also observed that addition of a drop of benzene prolonged the life time of the oxygen-adduct of the CD-capped heme to about 1 h in the aq. medium at -10°C.[5b)]

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