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Cyclodextrin-Coupled Protoporphyrinatoiron Complex and Its Semi-Stable Oxygen Adduct Formation in an Aqueous Medium

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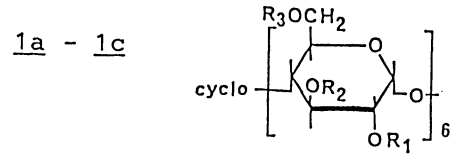
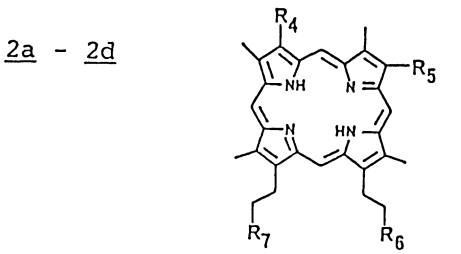
Protoporphinatoiron IX was coupled with α-cyclodextrin on its secondary hydroxy groups side with three urethane bonds. This cyclodextrin-coupled porphinatoiron shows reduced acute toxicity and forms an oxygen adduct in cooled aqueous medium.

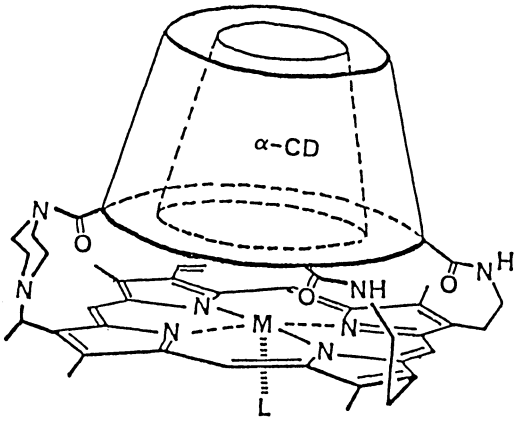
Much recent work has been aimed at synthesizing porphinatoiron derivatives with oxygen-binding ability as model compound of hemoproteins.[1] We have recently found that the porphinatoiron complexes combined with a water-soluble but hydrophobic polymer[2] or embedded in a phospholipid bilayer[3] bind molecular oxygen reversibly in aqueous media. We indicated that the reason for the oxygen-binding in aqueous solution was that the porphinatoiron complex takes a pentacoordinate structure which leaves the sixth coordination site vacant to bind molecular oxygen and that the oxygen adduct is surrounded with a hydrophobic environment of the polymer or the lipid bilayer. Cyclodextrins are water-soluble and non-toxic materials, and have a toroidal shape structure with a hydrophobic cavity. Some attempts to couple cyclodextrins with porphyrinatoirons by one or two covalent bonds have been reported.[4] In the present communication α-cyclodextrin was used as the sterically and hydrophobically protective group covering one face of a porphyrin plane and was coupled with a protoporphinatoiron IX derivative across its porphyrin plane by three covalent bonds, 1,3,5,7-tetramethyl-2(or 4)-vinyl-(α-cyclodextrin-2-yl)-O-tricarbonyl){4(or 2)-[1-(N-piperadino)ethyl]-6,7-bis-[2-aminoethyl]}porphinato iron(III) chloride (4a). Acute toxicity and oxygen-binding ability of the cyclodextrin-coupled porphinatoiron were measured and compared with the non coupled porphinatoiron.

The synthesis of 1,3,5,7-tetramethyl-2(or 4)-vinyl-(α-cyclodextrin-2-yl)-O-tricarbonyl){4(or 2)-[1-(N-piperadino)ethyl]-6,7-bis[2-aminoethyl]}porphine (3) is as follows. 6,6,6-Tritrityl-α-cyclodextrin (1b, R_1=R_2=OH, R_3=OH or O-trityl ([trityl]/[OH]=3)), which is a mixture of four possible isomers,[5] was prepared from α-cyclodextrin (1a, R_1=R_2=R_3=OH) according to literature procedure.[6] 1b was reacted with ethyl chloroformate to give carbonated cyclodextrin, the mixture of 6,6,6-tritrityl-poly(2,3-O-epicarbonyl)-α-cyclodextrin and 2,2'-O-carbonyl-bis[6,6,6-tritrityl-poly(2,3-O-epicarbonyl)-α-cyclodextrin] (1c, R_1, R_2=OH or $O(CO)_{1/2}$, R_3=OH or O-trityl). Protoporphyrin IX (2a, R_4=R_5=CH=CH_2, R_6=R_7=COOH)

was treated with hydrogen bromide to give 2,4-di(1-bromoethyl)deuteroporphyrin IX (2b, R_4=R_5=CH(Br)CH_3, R_6=R_7=COOH).[7] Reaction of 2b with piperazine followed by esterification to give 2(or4)-[1-(N-piperadino)ethyl]-4(or2)-vinyldeuteroporphyrin IX diethyl ester (2c, R_4(or R_5)=CH(R_8)CH_3, R_5(or R_4)=CH=CH_2, R_6=R_7=$COOCH_2CH_3$).[8] Treatment of this with 1c, and subsequent hydrolysis of the porphyrin's two esters and of the cyclodextrin's residual carbonates to give the cyclodextrin-coupled porphyrin with single urethane bond, 2(or 4)-{1-[4-(6,6,6-tritrityl- α -cyclodextrin-2-yl)-O-carbonyl(piperadin-1-yl)]ethyl}-4(or 2)-vinyldeuteroporphyrin IX diethylester (2d, R_4(or R_5)=CH(R_9)CH_3, R_5(or R_4)=CH=CH_2, R_6=R_7=COOH). Purification of 2d from 1b and 2c was accomplished by gel permeation chromatography on Biobeads eluted with N,N-dimethylformamide (DMF), which also supports that 2d has molecular weight of 2400 approximately corresponding to that of a 1/1 couple of the cyclodextrin and the porphyrin.[9] The carboxylic acid groups of 2d were reacted with diphenylphosphoryl azide to give the acyl azide groups which were then converted to the isocyanate groups by heating. The resulting isocyanate groups of 2d were coupled with the secondary hydroxy groups of 2d intramolecularly in a dilute solution. Following detritylation with hydrochloric acid to give the cyclodextrin-coupled porphyrin which was isolated as one main band by the above mentioned GPC on Biobeads. The coupling between 1c and 2c and the following duplicated cyclizations were confirmed by the GPC mentioned above and by IR and UV-visible spectroscopies.[10] The iron insertion to 3 was achieved using ferrous chloride and the following purification with the GPC gave 4a. The complete iron insertion was confirmed by disappearance of the fluorescent spectrum of 3 and by UV-visible spectroscopy.[11] 4a was water-soluble up to 15 mM. The LD_{50} for intravenously administered 4a in male Sprague-Dawley rats was >500 mg/kg (no





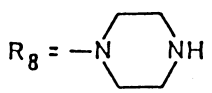


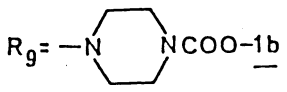
3 M=2H L=none

4a M=Fe^{III} L=Cl

4b M=Fe^{II} L=Sterically Bulky Imidazole

5 M=Zn^{II} L=none





toxic effect was observed at 500 mg/kg dose level). LD_{50} for intravenously administered protoporphinatoiron IX in same animals was 90 mg/kg (43mg/kg in the literature[12]). Toxicity of porphinatoiron is reduced by the coupled cyclodextrin.

The oxygen-binding to 1,3,5,7-tetramethyl-2(or 4)-vinyl-(α -cyclodextrin-2-yl)-O-carbonyl){4(or 2)-[1-(N-piperadino)ethyl]-6,7-bis[2-aminoethyl] } porphinato iron(II)-bulky imidazole complex (4b) was carried out as follows. The aqueous medium was an oxygen-free mixture of pH 7.4 phosphate buffer solution and ethylene glycol (an antifreeze agent) (vol. 1/1). The cyclodextrin-coupled porphinatoiron complex (4b) was prepared by reducing 4a with a small excess of sodium dithionite under nitrogen atmosphere in the presence of an axial, sterically bulky and water-soluble ligand (L of 4b) such as poly(1-vinyl-2-methylimidazole). The reduction of 4a to 4b was also carried out with ascorbic acid or met-hemoglobin reductase[13] to contraindicate any contribution of the reducing agent to the following oxygen adduct observation. The cyclodextrin-coupled porphinatoiron complex 4b showed the visible absortion spectrum (λ_{max} 434, 553 nm ; ref. hemoglobin λ_{max} 430, 556 nm) which has been assigned to a pentacoordinate porphinatoiron complex. On exposure to oxygen the corresponding protoporphinatoiron IX 2a complex was irreversibly oxidized at 30 to -30 °C even in the presence of 1 w/v% of α-cyclodextrin. However, when the dark red solution of deoxy-4b was cooled to -30 °C and then exposed to oxygen, it became brilliant red and produced a spectrum (λ_{max} 412, 541, and 572 nm), which resembles that of oxy-hemoglobin (λ_{max} 414, 542, and 578 nm). The spectrum of deoxy-4b reappeared when nitrogen gas was carefully introduced in the solution. The spectrum of oxy-4b could be also changed to that of the carboxy-4b (λ_{max} 418, 537, and 566 nm) by bubbling carbon monoxide. These indicated that the ferrous state of the central iron of 4b did not change during the exposure to oxygen and that the reversible oxygen-binding was occurring in the aqueous medium.

The oxygen adduct of 4b is not stable and slowly degrades to the iron (III) derivative through isosbestic points in the change of the visible absorption spectrum. Its degradation obeys first order kinetics from which the lifetime (τ: half-life period) of the oxygen adduct of 4b was calculated. τ 110 min at -30 °C, 16 min at -15 °C, and 1 min at 0 °C. The lifetime is independent of the 4b concentration ranging from 0.15 to 0.01 mM. This suggests that the irreversible oxidation to Fe(III) of oxy-4b proceeds mainly via a unimolecular process.

A hydrophobic effect of the coupled cyclodextrin was suggested by a fluorescence spectrum of the zinc complex (5) of 3 in aqueous medium. Fluorescence spectrum of 5 (λ_{max} 592 nm) in the presence of L was red shifted in comparison with that of the zinc complex of 2c (λ_{max} 588 nm). It was considered that a toroidal shape structure with a hydrophobic inside moiety of the coupled cyclodextrin keeps the sixth coordinate site of porphinatoiron vacant for oxygen-binding, prevents the irreversible oxidation via a μ-dioxodimer formation, and provides a hydrophobic cavity for the bound oxygen to retard a water molecule-driven oxidation.

The semi-stable oxygen-adduct formation ability and the reduced acute toxicity of 4b suggest a medical activity of 4b with oxygen in vivo.

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8) 2c was a 1/1 mixture of 2-(1-(N-piperadino)ethyl)-4-vinyl- and 2-vinyl-4-(1-(N-piperadino)ethyl)-isomers which were derived from 2b by its partial N-alkylation and following thermal elimination of hydrogen bromide. PMR spectrum of 2c:δ_H (100 MHz, $CDCl_3$, $SiMe_4$ standard) -3.76(s ,2H, pyrrole-NH), 1.07 (m, 6H, $COOCH_2CH_3$), 1.95 (d, 3H, $>CHCH_3$), 3.12 (m, 12H, piperazine-H, CH_2CH_2COO), 3.44-3.58 (m, 12H, pyrrolic $-CH_3$), 4.09 (m, 4H, CH_2CH_2COO), 6.05 (m, 1H, $>CHCH_3$), 6.25 (m, 2H, $-CH=CH_2$), 8.23 (m, 1H, $-CH=CH_2$), and 9.95-10.78 (m, 4H, meso-H); m/z 704 (M^+).
9) Analytical data of 2d: GPC: m.w. 2400; IR: 3400, 1720, 1710 and 1100-1000 cm^{-1} attributed to the cyclodextrin, the porphyrin, and the urethane bonding, respectively; $_{max}$ in DMF: 398, 498, 532, 562, and 622 nm due to the porphyrin.
10) IR spectrum of 3: 3400 (ν(O-H)), 1100-1000 cm^{-1}(υ(C-O), α-cyclodextrin), 1720, 1700, and 1610 cm^{-1}(two types of urethane bonds). The UV-visible spectrum of 3 in DMF(λ_{max}: 398, 498, 532, 562, and 622 nm) was broadened to some extent. FT-CMR spectrum of 3 in d_6-DMSO was considerably broadened probably because 3 was the mixture of a few isomers. The datum of elemental analysis was in accord with the indicated structure. Anal. Calcd for $C_{75}H_{101}N_8O_{33}$: C/N 8.04, Found: C/N 8.00. The purity of 3 was confirmed by a single elution peak in GPC measurement on Biobeads SX-2 (solvent: DMF) and therefore physico-chemical properties of the above mentioned isomers of 3 were thought of very similar. The separation of the isomers was examined by HPLC on silica gel, but it was unsuccessful because of their strong absorption. There is no substances with free carboxylic acid on account of the aqueous solution of 3 being neutral.
11) The yield for each substance was as follows. Yield (compd.): 63% (1b), 87% (1c), 92%(2b), 31% (2c), 76% (2d), 71% (3), 80% (4a), 85% (5).
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