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POLY(VINYLPYRROLIDONE)-BOUND DIPHENYLHEME

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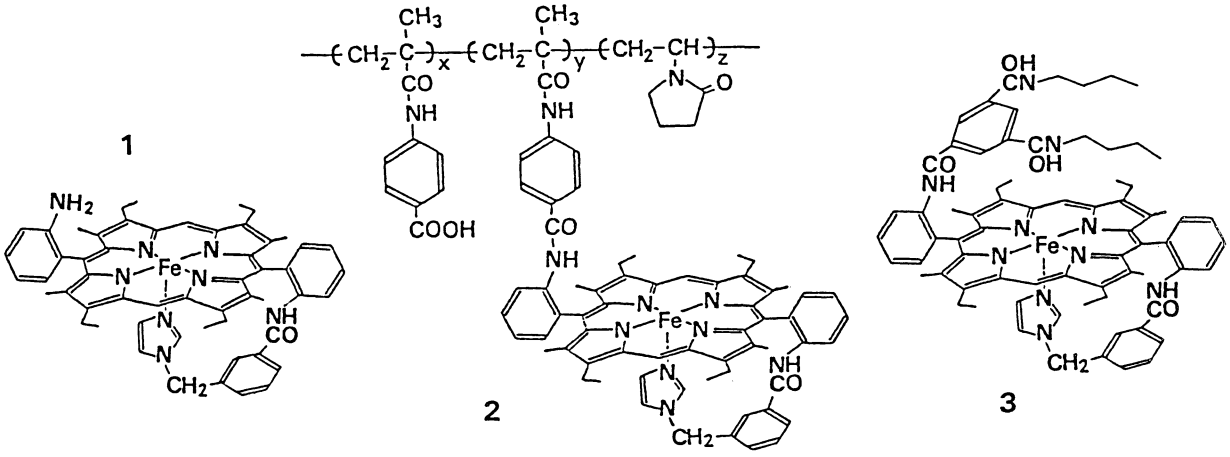
trans-5-(*o*-Aminophehyl)-15-{*o*-[*m*-(*N*-imidazolylmethyl)benzamido]phenyl}-2,8,12,18-tetraethyl-3,7,13,17-tetramethylporphinato iron(II) (diphenylheme) was covalently combined to poly(1-vinyl-2-pyrrolidone); it formed an oxygen adduct in cooled aqueous media.

Much effort has been expended to synthesize iron-porphyrin complexes that bind molecular oxygen reversibly like hemoglobin does.[1-4] In one approach, a series of heme derivatives with covalently bound imidazole-ligands have been synthesized and well studied. A typical example is mesoheme-mono-*N*-[3-(1-imidazolyl)propyl]amide monomethyl ester derivative[5] which forms an oxygen adduct in aprotic, organic solvents at low temperautre. When protoheme-mono-*N*-[3-(1-imidazolyl)propyl]amide is combined to a polymer,[6] the polymer-bound heme forms a semi-stable oxygen adduct with a life-time of *ca.* 1 h in an aqueous medium cooled to -30 °C.

In this communication we combined *trans*-5-(*o*-aminophenyl)-15-{*o*-[*m*-(*N*-imidazolylmethyl)benzamido]phenyl}-2,8,12,18-tetraethyl-3,7,13,17-tetramethyl-porphinato-iron(II) (abbreviated as diphenylheme) (1)[7] to water-soluble poly(1-vinyl-2-pyrrolidone) (2). For comparison, 1 was also combined with trimesic acid di(*n*-butylamide) (3). Diphenylheme has two *meso*-phenyl groups of which rotation



is restricted around the *meso* C-aryl C bond due to the 3,7,13,17-tetramethyl substituents on the porphyrin. The large rotation energy barrier ($\Delta G^{\ddagger} \geqslant 117$ kJ·mol^{-1})[7] should immobilize the imidazole ligand and strengthen the iron-imidazole bond. This also causes the restricted conformation of the group attached to the *meso*-phenyl group such as the trimesic acid residue in 3 and the polymer chain in 2: These groups are expected to cover the sixth coordination site, *i.e.* the oxygen-binding site of heme. Diphenylheme exhibits absorption spectral features very similar to protoheme. We report here the synthesis of 2 and its oxygen-binding ability in aqueous media.

Diphenylheme was combined to the polymer by reaction of 1 with poly(*N*-*p*-carboxyphenylmethacrylamide-*co*-1-vinyl-2-pyrrolidone) (4), which was obtained by copolymerization of *N*-*p*-carboxyphenylmethacrylamide (5) with 1-vinyl-2-pyrrolidone. 5 was prepared as follows. *p*-Aminobenzoic acid (25.5 mmol) was reacted with methacrylic acid chloride (76.5 mmol) in the presence of *N*,*N*-dimethylaniline (38.3 mmol) in THF (20 cm^3) at 0 °C for 1 h and at room temperature overnight. The product was chromatogrpahed on a silica-gel column with chloroform-methanol (10/1), and recrystallized from ethanol; white crystal (70%), mp 227-228 °C, Found: C, 64.1; H, 5.19; N, 6.83%, calcd for $C_{11}H_{11}NO_3$: C, 64.4; H, 5.40; N, 6.82; IR(KBr) 1660 cm^{-1}(-CONH-); ^{1}H-NMR(DMSO-d_6) δ = 10.97(s, 1H), 8.55 (s, 4H), 6.38(s, 1H), 6.00(s, 1H), 2.15(s, 3H).

5 (0.346 for 4a and 0.173 mmol for 4b) was copolymerized with 1-vinyl-2-pyrrolidone (34.6 mmol) in the presence of 2,2'-azobisisobutyronitrile (0.485 mmol) at 60 °C for 10 min. The obtained polymer was purified by precipitating from methanol to diethyl ether repeatedly. Yield; 61 (4a), 90% (4b). The 5 unit content in the copolymer was determined by UV absorption at 273 nm; 0.84 (4a), 0.47 mol% (4b). Molecular weight of the polymer was measured by vapor pressure osmometry in methanol; 160000 (4a), 280000 (4b).

The polymer 4 (5 unit 7.2 μmol) was reacted with 1 (10.8 μmol) in the presence of ethyl chloroformate (72 μmol) and triethylamine (72 μmol) at 0 °C for 2 h and at room temperature for 3 d. After concentration, the residue was dissolved in chloroform, and the insoluble part was removed by filtration, the solution was then poured into diethyl ether. The crude product was chromatographed on a bio-beads column with DMF. The first fraction was collected and brought to dryness. The product was precipitated in diethyl ether. The control experiment showed that noncovalently-bound heme was completely separated from the polymer by the purification procedure used. The degree of incorporation of the heme to the polymer was determined from the absorbance at 400 nm, referred to that of the diphenylheme. The bound heme content in 2 was x = 0.74, y = 0.10, z = 99.16 mol% using 4a and x = 0.41, y = 0.06 and z = 99.53 mol% using 4b. These polymers were soluble in alcohol, DMF, chloroform and also in water up to *ca.* 5 wt%.

3 was obtained by coupling 1 with trimesic acid di(*n*-butylamide) (benzene-1,3-di(*n*-butylamide)-5-carboxylic acid) in a similar manner.

The iron(III) derivatives were dissolved in either pH 10 buffer (0.2 M Na_2CO_3/$NaHCO_3$)-ethylene glycol (1/1) or DMF-H_2O (9/1) and reduced to the iron(II) derivatives with sodium dithionite ([$Na_2S_2O_4$]/(iron(III)] = 6) under a nitrogen

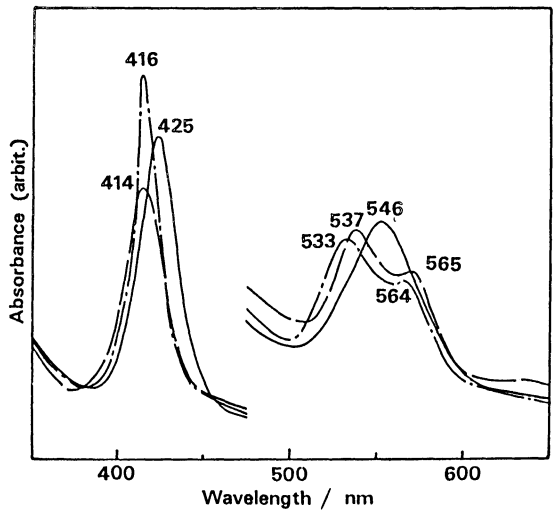


Fig. 1. UV and visible absorption spectra of 2 in pH 10 aqueous-ethylene glycol (1/1) solution at 15 °C. (——): deoxy, (— —): oxygen adduct, (—·—): CO adduct.

atmosphere.

The oxygen-binding ability of these heme compounds was first checked in cooled DMF-H_2O solution. 2 formed stable oxygen adduct (416, 540, and 567 nm) with the life-time (half-life period) of more than 2 h at 0 °C, which was comparable to that for 3 (108 min at 0 °C) but much longer than 1 (8 min).

The same oxygen adduct of 2 was observed even in pH 10 aqueous-ethylene glycol solution, in the temperature range: -30 °C-15 °C (Fig. 1). Absorption spectrum of the 2-oxygen adduct was in complete agreement with that in the DMF solution and similar to that of myoglobin. The spectrum of this oxygen adduct changed to that of the 2-CO adduct (413, 533, and 564 nm) by bubbling carbon monoxide through the solution, and returned to the deoxy complex by flushing carefully with nitrogen gas. This oxy-deoxy cycle was repeated several times at -15 °C.

That the reducing reagent can not participate in this reversible oxygenation is evidenced by the following results: (i) Stoichiometric amounts of sodium dithionite were used for the heme reduction. (ii) In case that dithionite was added again after the oxygenation, the oxy-heme was reduced directly to the deoxy-heme in the same manner as the oxy-hemoglobin. (iii) Heme reduction can also be effected by using organic reductants, such as ascorbic acid, glucose, or the reductase system.[8] In these systems, oxygen adducts with identical absorption spectra and life-times are observed.

In the aqueous-ethylene glycol system, the life-time of oxygen adduct of 2 was more than 2 h at -30 °C, *ca.* 10 min at 0 °C and 3 min at 15 °C. In contrast, 1 and 3 solubilized with surfactants in the same solvent was oxidized immediately even at -30 °C. In aqueous environment, oxyheme can be oxidized *via* protonation and subsequent cleavage of the Fe-O-O moiety, as well as the binuclear μ-oxo dimer mechanism. Reversible oxygen-binding in aqueous medium is efficient when heme is covalently bound to a water-soluble but hydrophobic polymer. The life-time of the 2-oxygen adduct was longer than that of the previously reported polymer-bound protoheme (life-time = 60 min at -30 °C, 1 min at -15 °C).[6] We can attribute this to at least two possibilities. First, the *m*-benzyl linked

imidazole in 2 is less floppy and has fewer degrees of rotation than a "tailed" alkyl-linked imidazole. Consequently the imidazole dissociation rate will be slower in 2. A more rigid and stable imidazole-heme coordination should result in a more stable oxygen adduct since the "base-off" mechanism is likely to be eliminated. Secondly, the presence of the phenylmethacrylamide spacing group near the oxygen binding site in 2 may stabilize the polar Fe-O_2 moiety *via* dipolar interactions. Our recent kinetic studies on oxygenation of a series of heme models equipped with polar groups near the coordination site clearly demonstrate the effectiveness of an *o*-anilido group in stabilizing the Fe-O_2 adduct. It seems, therefore, that the unique linkage and the polymer chain of 2 not only covers the sixth coordination site of heme but stabilize the oxygen adduct to allow the formation of stable oxygen adduct even in the aqueous media.

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