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Dendrimer-Encapsulated Iron Porphyrin as a Novel Hemoprotein Mimic for Dioxygen Binding

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DENDRIMER-ENCAPSULATED IRON PORPHYRIN AS A NOVEL HEMOPROTEIN MIMIC FOR DIOXYGEN BINDING

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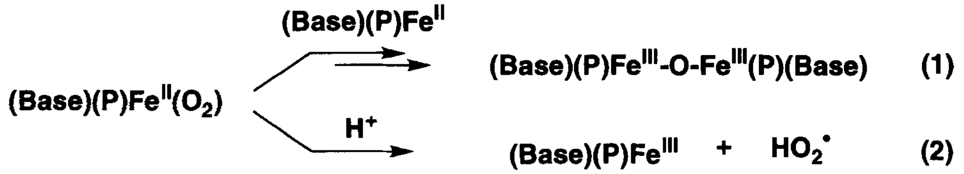
ABSTRACT

An iron(II) porphyrin/1-methylimidazole (Im) complex, covalently encapsulated within a large aryl ether dendrimer cage ($(Im)_2(L5P)Fe^{II}$), shows reversible dioxygen-binding activity, in which the dioxygen adduct ($(Im)(L5P)Fe^{II}O_2$) survives over a period of months even in the presence of water. $(Im)(L5P)Fe^{II}O_2$ was highly reluctant to undergo carbonylation upon exposure to a carbon monoxide atmosphere, where the half-life of $(Im)(L5P)Fe^{II}O_2$ was as long as 50 hours.

INTRODUCTION

In mimicking biological functions of hemoproteins, one of the attractive targets is to realize the reversible dioxygen-binding activity. However, (1) μ–oxo dimer formation and/or (2) H^+-driven autoxidation (Scheme 1) obstruct the reversibility of this process. In order to suppress the μ-oxo dimer formation, a variety of sterically hindered iron porphyrin complexes have been synthesized, some of



Scheme 1. Irreversible Oxidation Pathways for Iron Porphyrin-Dioxygen Adduct.

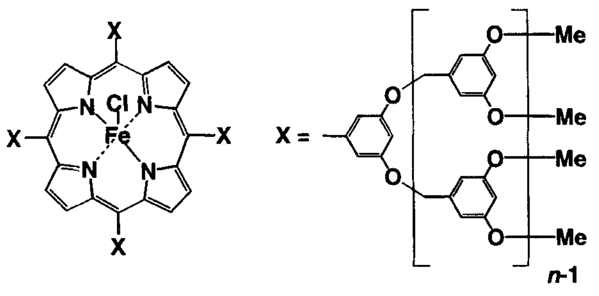


Figure 1. Dendrimer Iron Porphyrin Complexes ((L*n*P)FE^{III}Cl, *n* =1,3-5).

which form stable dioxygen adducts under anhydrous conditions, and show reversible dioxygen binding activities [1, 2]. On the other hand, in aqueous media, an iron complex of a picket-fence porphyrin embedded within a synthetic bilayer membrane is the only example that is capable of binding dioxygen in a reversible fashion [3]. In the present paper, we report that an iron porphyrin encapsulated within an aryl ether dendrimer box (Figure 1) is the first monomolecular model of hemoproteins, which realizes reversible binding of dioxygen even in the presence of water.

Synthesis of Dendrimer Iron Porphyrins ((L*n*P)Fe^{II}, *n* = 1, 3 - 5)

5, 10, 15, 20-Tetrakis(3',5'-dimethoxyphenyl)porphine ($L1PH_2$) was synthesized from 3, 5-dimethoxybenzaldehyde and pyrrole in refluxing propionic acid, and the crude product was recrystallized from $CHCl_3$/MeOH. $L1PH_2$ was then converted into 5, 10, 15, 20-tetrakis(3, 5'-dihydroxyphenyl)porphine ($TP(OH)_8PH_2$) by the treatment with BBr_3 followed by water. Dendrimer porphyrin free bases ($LnPH_2$, *n* = 3-5; number of the aromatic layers) were synthesized by the alkaline-mediated coupling of $TP(OH)_8PH_2$ with aryl ether dendrimer bromides, [4] prepared according to the Fréchet's convergent method, and identified by ^1H NMR, UV/Vis, and MALDI-TOF-MS measurements. Insertion of iron to the porphyrin core was accomplished by refluxing a THF solution of a mixture of L*n*PH_2 (*n* = 1, 3-5) and $FeBr_2$ at 80°C for 18 hours. After the solvent was stripped off from the reaction mixture, the residue was washed several times with $CHCl_3$/water, and the organic layer separated was chromatographed on silica gel with $CHCl_3$ as eluent, where the second band collected was poured into MeOH containing HCl (0.1 M), whereupon (L*n*P)Fe^{III}Cl was precipitated as dark brown particles. Analytical data for (L*n*P)Fe^{III}Cl: *n* = 1: UV/Vis (toluene): λ_{max}/nm (log ε) 372.5 (4.75), 423.5 (5.45), 509.0 (4.62), 576.0 (4.12), and 660.0 (3.80); ^1H NMR (toluene-d_8, 25°C) 82.1 (pyrrole-β); *n* = 3: UV/Vis (toluene): λ_{max} /nm (log ε) 373.0 (4.62), 423.5 (5.19), 513.5 (4.33), 575.5 (3.90), and 662.5 (3.60); ^1H NMR (toluene-d_8, 25°C) 83.0 (pyrrole-ε); *n* = 4: UV/Vis (toluene): λ_{max} /nm (log ε) 378.0 (4.22), 424.0 (4.79), 511.0 (3.41), 583.5 (2.04), and 664.0 (3.04); ^1H NMR (toluene-d_8, 25°C) 82.4 (pyrrole-β); *n* = 5: UV/Vis (toluene): λ_{max} /nm (log ε) 378.5 (4.15), 425.0 (4.26), 510.0 (3.78), 581.5 (3.33), and 657.0 (3.29); ^1H NMR (toluene-d_8, 25°C) 82.3 (pyrrole-β). (L*n*P)Fe^{III}Cl was then reduced with sodium dithionite (1.5 equiv.) in toluene/water under argon, and the organic layer separated was repeatedly washed with deaerated water, dried over Na_2SO_4 overnight, and evaporated to dryness to leave (L*n*P)Fe^{II} in 95 % (*n* = 1) - 65 % (*n* = 5) yields based on L*n*PH_2. UV/Vis analytical data (λ_{max} /nm, toluene) of (L*n*P)Fe^{II} (*n* = 1, 3-5): *n* = 1: 417.0, 442.2, and 541.5; *n* = 3: 420.0, 445.5, and 543.0; *n* = 4: 421.5, 447.0, and 540.5; *n* = 5: 423.0, 447.5, and 537.0.

RESULTS AND DISCUSSION

Dendrimers are globular-shaped, hyper-branched macromolecules in nanoscopic size [5]. We have reported that the interior porphyrin functionality of the dendrimer porphyrin (L*n*PH_2) is almost encapsulated when the number of the aromatic layers in the dendrimer framework (*n*) is higher than 4.[4, 6-7] By molecular modeling studies, the diameters of L*4*PH_2 and L*5*PH_2 have been estimated to be 5 nm.

The dioxygen-binding activities of the (L*n*P)Fe^{II} family were investigated by means of electronic absorption spectroscopy in anhydrous or water-saturated toluene ($[(LnP)Fe^{II}]_0 = 2\times10^{-5}$ M) at 23°C in the presence of 30 equiv. of 1-methylimidazole (Im), where (L*n*P)Fe^{II} was in the form of bisimidazole complex

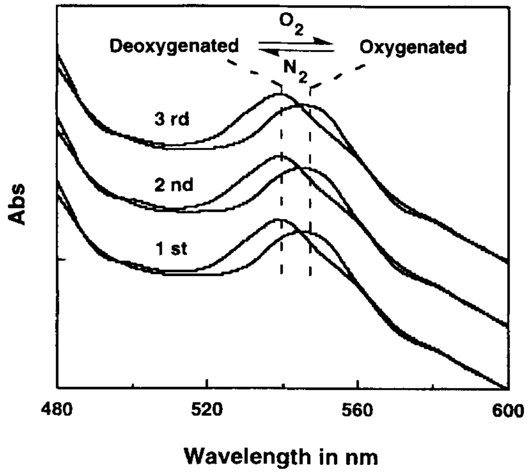


Figure 2. Reversible Dioxygen Binding Profiles of $(IM)_2(L5P)Fe^{II}$ in Dry Toluene.

$((Im)_2(LnP)Fe^{II})$ [8]. As expected, the smallest $(Im)_2(L1P)Fe^{II}$ without any steric protection around the active site was instantly and irreversibly oxidized upon introduction of O_2 to give a μ-oxo dimer even in anhydrous toluene. In contrast, under such dry conditions, the larger $(Im)_2(LnP)Fe^{II}$ family (n = 3-) did not show any sign of μ-oxo dimer formation and exhibited reversible dioxygen-binding charac-teristics. An example is shown by the case of the largest $(Im)_2(L5P)Fe^{II}$ (Figure 2), where the absorbance at 538.5 nm, upon bubbling of O_2, was observed to shift to 548.5 nm characteristic of the dioxygen adduct $((Im)(L5P)Fe^{II}(O_2))$, but no further spectral changes associated with the μ–oxo dimer formation and/or H^+-driven oxidation to $(Im)(L5P)Fe^{III}X$ were observed over a period of 2 months in an atmospheric pressure of O_2. When O_2 was flushed out from the system by bubbling of N_2, $(Im)(L5P)Fe^{II}(O_2)$ revived to the deoxygenated form $((Im)_2(L5P)Fe^{II})$. Such an oxygenation - deoxygenation cycle could be repeated many times without any sign of irreversible oxidations at the active site.

Interestingly, $(Im)_2(LnP)Fe^{II}$ (n = 3-5) showed reversible dioxygen-binding characteristics even under wet conditions, but the lifetime of the dioxygen adduct depends upon the size of the dendrimer framework. In water-saturated toluene ($[H_2O]_0/[(LnP)Fe^{II}]_0$ = 1000: 1) [9], lower-generation $(Im)(L3P)Fe^{II}(O_2)$ and $(Im)(L4P)Fe^{II}(O_2)$ showed time-dependent spectral changes characteristic of the irreversible oxidation (Figure 3A). In sharp contrast, virtually no spectral

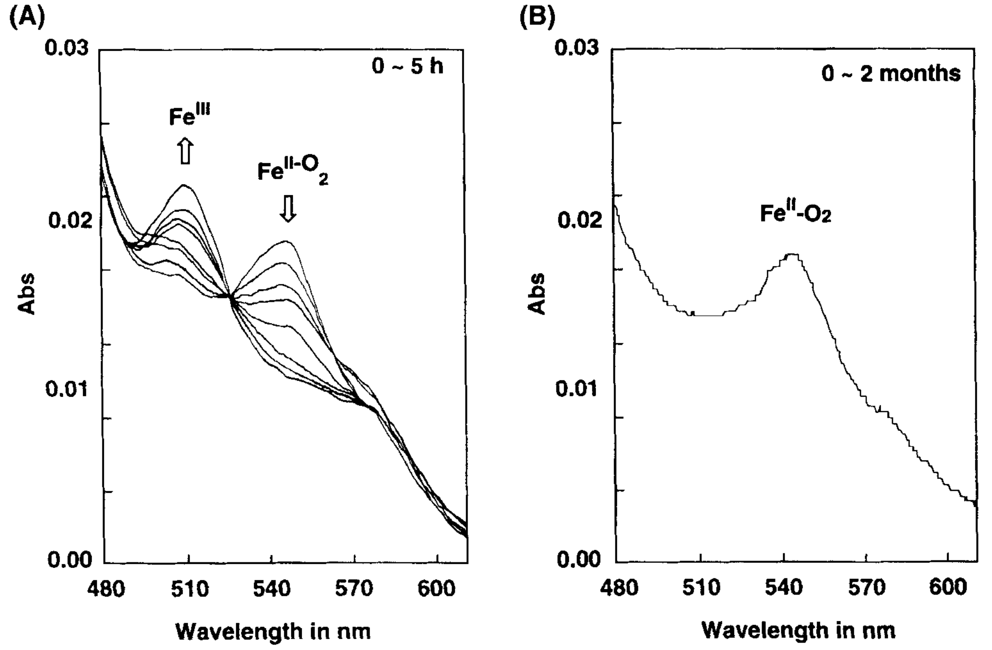


Figure 3. Changes in Electronic Absorption Bands of the Dioxyten Adducts (Im)(L*3*P)Fe^{II}(O$_2$) **(A)** and (Im)(L*5*P)Fe^{II}(O$_2$) **(B)** in Water-Saturated Toluene.

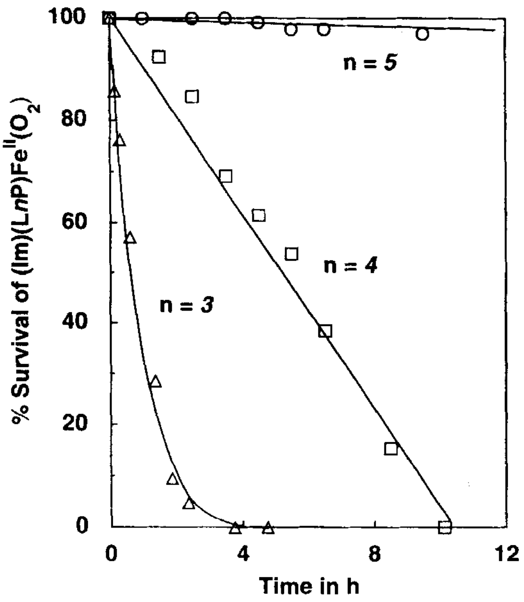


Figure 4. Stability of the Dioxygen Adducts (Im)(L*n*P)Fe^{II}(O$_2$).

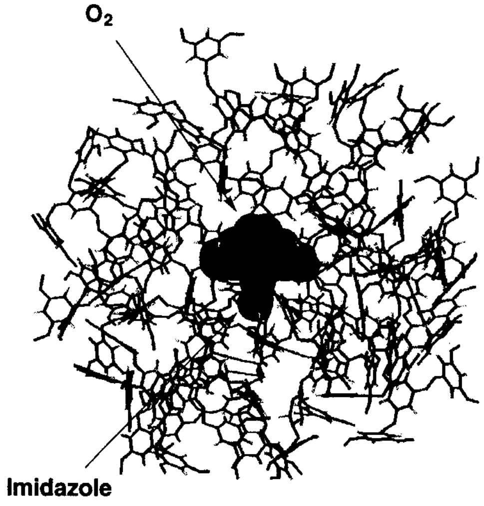


Figure 5. Schematic Representation of (Im)(L5P)Fe^{II}(O_2).

change was observed for the highest-generation (Im)(L5P)Fe^{II}(O_2) (Figure 3B). From the above spectral changes, the half-lives of (Im)(L3P)Fe^{II}(O_2) and (Im)(L4P)Fe^{II}(O_2) were evaluated to be 1.5 and 6 hours, respectively, while the largest (Im)(L5P)Fe^{II}(O_2) survived for more than 2 months (Figure 4). The first-order rate constants (k_{decay}) for this decaying process were 1.3 x 10^{-4}, 3.2 x 10^{-5}, and 7.4 x $10^{-13}s^{-1}$ for (Im)(L3P)Fe^{II}(O_2), (Im)(L4P)Fe^{II}(O_2), and (Im)(L5P)Fe^{II}(O_2), respectively, in which k_{decay} of (Im)(L5P)Fe^{II}(O_2) is about 7 orders of magnitude less than that of (Im)(L3P)Fe^{II}(O_2). Therefore, it is evident that the highest-generation (Im)(L5P)Fe^{II}(O_2) is sterically and hydrophobically protected by the large dendrimer framework (Figure 5), so that the irreversible oxidation pathways such as μ–oxo dimer formation and H^+-driven autoxidation (Scheme 1) are greatly suppressed.

Another interesting aspect is the gas permeability through the dendrimer framework. As shown in Figure 6, when O_2 was bubbled at a flow rate of 25 ml min^{-1} into a toluene solution of lower generation (Im)(L3P)Fe^{II}, the dioxygen uptake was completed only within 2 minutes. Bubbling of N_2 into this system at the same flow rate resulted in complete deoxygenation of (Im)(L3P)Fe^{II}O_2 to give $(Im)_2$(L3P)Fe^{II} in 30 minutes. On the other hand, in the case of the highest-generation $(Im)_2$(L5P)Fe^{II} under the same conditions, the complete oxygenation and deoxygenation required such longer reaction times as 12 and 180 minutes,

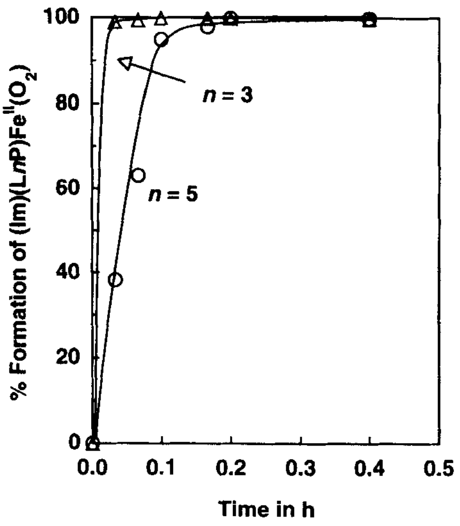


Figure 6. Formation of the Dioxygen Adducts $(Im)(LnP)Fe^{II}(O_2)$ upon Exposure of $(IM)_2(LnP)Fe^{II}$ to O_2 in Dry Toluene.

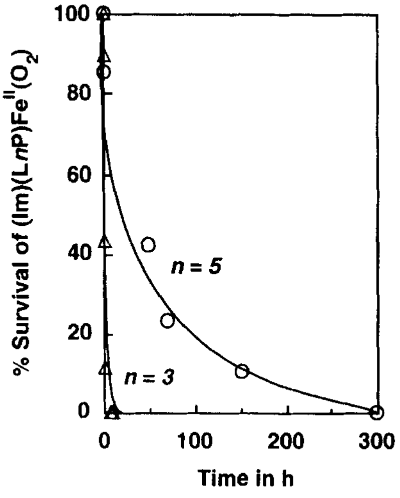


Figure 7. Durability of the Dioxygen Adduct $(Im)(LnP)Fe^{II}(O_2)$ Toward Carbonylation upon Exposure to CO in Dry Toluene.

respectively. From these observations, the dendrimer framework seems to affect the permeabilities of O_2 and N_2 from the exterior to the interior of (L*n*P)Fe^{II}.

In general, synthetic hemes, in a carbon monoxide (CO) atmosphere, are known to be more easily carbonylated than natural hemoproteins. Very interestingly, we found that the highest-generation (Im)(L5P)$Fe^{II}(O_2)$ shows an unusually high durability to carbonylation in a CO atmosphere (Figure 7). Upon bubbling CO at a flow rate of 25 ml min^{-1} into a toluene solution of (Im)(L5P)$Fe^{II}(O_2)$, the dioxygen adduct was transformed only very slowly to the corresponding CO adduct (426, 548 nm),[10] where the half-life of the dioxygen adduct was as long as 50 hours. This is in sharp contrast with the case of lower-generation (Im)(L3P)Fe^{II}, where the dioxygen adduct (Im)(L3P)$Fe^{II}(O_2)$ was completely converted to the corresponding CO adduct in only 30 minutes (half-life; 3 minutes). The present finding may indicate a new potential of dendritic structures as gas separators.

CONCLUSION

Through studies on the dioxygen binding profiles of the dendrimer iron porphyrin family ((L*n*P)Fe^{II}, $n = 1, 3\text{-}5$), we have demonstrated the highest-generation (L5P)Fe^{II} as the first monomolecular model of oxygen-carrying hemoproteins. Studies with a water-soluble dendrimer iron porphyrin will be of further interest from both fundamental and practical viewpoints.

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