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Oxygen Binding of Water-Soluble Cobalt Porphyrins in Aqueous Solution

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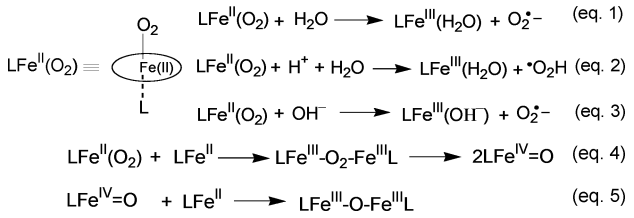
Water-soluble cobalt porphyrin **1**Co and imidazole ligand **2** were synthesized. **1**Co binds dioxygen in the presence of imidazole ligand **2** in aqueous solution. The formation of the oxygen adduct **2**-**1**Co(O_2) was studied using UV–vis and EPR spectroscopy. The impact of pH on the kinetic stability of the oxygen adduct was examined.

Hemoglobin (Hb) and myoglobin (Mb) are responsible for the storage and transport of molecular oxygen in biological systems. Using synthetic porphyrin models to mimic the functions of hemoprotein has been extensively explored.[1] Most reported synthetic O_2 binding systems have been studied in aprotic organic solvents such as toluene, benzene, and dichloromethane[1] because traces of water (H_2O, H^+, OH^-) can result in an irreversible decomposition of such oxygen adducts in seconds (eqs 1−3).[1c,2] In recent years,



(eq. 1) (eq. 2) (eq. 3) (eq. 4) (eq. 5)

water-soluble O_2 carriers have been sought because of proposed biomedical applications such as artificial red cells, photodynamic therapy (PDT) for tumor treatment, organ preservation, and DNA cleavage.[3] In contrast to the large amount of literature describing oxygen-carrying systems in aprotic organic solvents,[1] only a few examples of aqueous oxygen-carrying systems have been reported.[4,5] A major challenge in developing such aqueous systems is to build a relatively hydrophobic metalloporphyrin center within a water-soluble model system in order to prolong the lifetime of the oxygen adducts. Natural Hb and Mb create such a structure by a protein matrix around the heme center. The globin moiety provides a relatively hydrophobic microenvironment above each heme center, thus inhibiting water-promoted autoxidation. The isolation of heme centers in protein pockets also prevents the formation of μ-oxo dimers, which result from another important autoxidation path of synthetic oxygen carriers (eqs 4−5).[1c,2]

Among the rare oxygen-carrying models stable in aqueous media, Tsuchida et al. have used picket-fence porphyrins containing zwitterionic phospholipid substituents.[4] Such models are not truly soluble in water but form micelles dispersed in water. Other examples reported by Groves,[5a] Kano,[5b] and their co-workers employed a cyclodextrin-associated axial ligand to provide a hydrophobic environment encapsulating the iron(II) porphyrin center.

Cobalt porphyrins have also been investigated as oxygen carriers.[6] The cobalt analogues of Hb and Mb are referred as coboglobins (CoHb, CoMb).[7] Their oxygen-binding complexes ($LCo^{II}(O_2)$) are known to have greater kinetic stability toward decomposition compared with their heme counterparts. To the best of our knowledge, most oxygen-binding cobalt porphyrins have been studied in organic solvents.[8] Their potential as oxygen carriers in aqueous

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(1) (a) Collman, J. P.; Boulatov, R.; Sunderland, C. J.; Fu, L. *Chem. Rev.* **2004**, *104*, 561. (b) Collman, J. P.; Fu, L. *Acc. Chem. Res.* **1999**, *32*, 455. (c) Momenteau, M.; Reed, C. A. *Chem. Rev.* **1994**, *94*, 659.
(2) Shikama, K. *Chem. Rev.* **1998**, *98*, 1357.
(3) (a) *Artificial Oxygen Carrier. Its Front Line*; Kobayashi, K.; Tsuchida, E., Hirohisa, H., Eds.; Springer-Verlag: Tokyo, 2005. (b) *The Porphyrin Handbook*; Kadish, K. M., Smith, K. M., Guilard, R., Eds.; Academic Press: San Diego, 2000, Vol. 6; Vol. 14.
(4) (a) Komatsu, T.; Moritake, M.; Nakagawa, A.; Tsuchida, E. *Chem. Eur. J.* **2002**, *8*, 5469. (b) Komatsu, T.; Hayakawa, S.; Yanagimoto, T.; Kobayakawa, M.; Nakagawa, A.; Tsuchida, E. *Bull. Chem. Soc. Jpn.* **2001**, *74*, 1703.
(5) (a) Zhou, H.; Groves, J. T. *Biophys. Chem.* **2003**, *105*, 639. (b) Kano, K.; Kitagishi, H.; Kodera, M.; Hirota, S. *Angew. Chem., Int. Ed.* **2005**, *44*, 435. (c) Komatsu, T.; Hayakawa, S.; Tsuchida, E.; Nishide, H. *Chem. Comm.* **2003**, 50.
(6) (a) Collman, J. P.; Zhang, X.; Wong, K.; Brauman, J. I. *J. Am. Chem. Soc.* **1994**, *116*, 6245. (b) Collman, J. P.; Brauman, J. I.; Doxsee, K. M.; Halbert, T. R.; Hayes, S. E.; Suslick, K. S. *J. Am. Chem. Soc.* **1978**, *100*, 2761. (c) Stynes, D. V.; Stynes, H. C.; James, B. R.; Ibers, J. A. *J. Am. Chem. Soc.* **1973**, *95*, 1796. (d) Stynes, D. V.; Stynes, H. C.; Ibers, J. A.; James, B. R. *J. Am. Chem. Soc.* **1973**, *95*, 1142. (e) Proniewicz, L. M.; Kincaid, J. R. *J. Am. Chem. Soc.* **1990**, *112*, 675.
(7) Jones, R. D.; Summerville, D. A.; Basolo, F. *Chem. Rev.* **1979**, *79*, 139.
(8) An aqueous oxygen-carrying cobalt porphyrin system was briefly mentioned in the literature, see: Fiammengo, R.; Wojciechowski, K.; Crego-Calama, M.; Timmerman, P.; Figoli, A.; Wessling, M.; Reinhoudt, D. N. *Org. Lett.* **2003**, *5*, 3367.

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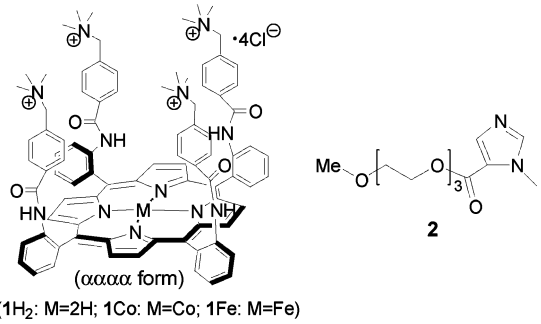

1 (1H_2: M=2H; 1Co: M=Co; 1Fe: M=Fe)

Figure 1. Design of model compounds.

solution has not been well explored. Thus, we planned to utilize the attenuated reactivity of cobalt porphyrins to develop aqueous oxygen-carrying systems. Herein, we report a new water-soluble cobalt porphyrin that binds dioxygen in aqueous media.

The water-soluble cobalt porphyrin **1**Co was prepared from αααα-TAPP (Figure 1).[9] The formation of a cage by the four carbamoylbenzyl quaternary amine groups is consistent with the symmetry manifest by the NMR spectra of the demetalated porphyrin **1**H_2 and its precursor.[9] Whereas the quaternary amine groups result in water solubility of **1**Co, the four bulky carbamoylbenzyl groups over the porphyrin ring are expected to establish a relatively congested and hydrophobic micro-environment over the metalloporphyrin center. This design is in accord with reports of native human Hb mutants:[10] Introduction of bulky or aromatic residues around heme centers markedly decreases the accessibility of this distal pocket to water and should inhibit the autoxidation of the oxygen complex. A water-soluble imidazole **2** was synthesized. Chelation of the imidazole **2** to **1**Co should mimic the proximal histidine residue in native hemoprotein.[1] The triethylene glycol moiety in the ligand **2** not only results in high water solubility but is also expected, because of the 1-methyl group, to provide steric hindrance preventing ligand **2** from fitting into the distal pocket of the porphyrin model **1**Co, thus leaving the sixth coordination site vacant for dioxygen binding. It seems reasonable that the four positively charged carbamoylbenzyl quaternary amine groups at the porphyrin periphery should prevent the μ-oxo dimerization by electrostatic and steric repulsion (Figure 2).

Examination of oxygen binding of **1**Co was carried out in a pH 7.0 phosphate buffer solution at room temperature (22 °C). The concentration of **1**Co was adjusted to maintain a maximum absorption (A) at around 1.0 −1.2 in the Soret band (Figure 3). Under a nitrogen atmosphere, the **1**Co solution has a Soret peak at 420 nm (Figure 3, trace a). Upon addition of ligand **2** (∼100 equiv of **1**Co), the Soret peak shifts slightly to 422 nm and the absorption intensity decreases (Figure 3, trace b). This indicates the formation of a five-coordinate adduct **2**-**1**Co and is consistent with reports of five-coordinate cobalt porphyrins in organic solvents.[6c−d] In the presence of oxygen, the five-coordinate

(9) See Supporting Information for details.
(10) Mathews, A. J.; Rohlfs, R. J.; Olson, J. S.; Tame, J.; Renaud, J.-P.; Nagai, K. *J. Biol. Chem*. **1989**, *264*, 16573.

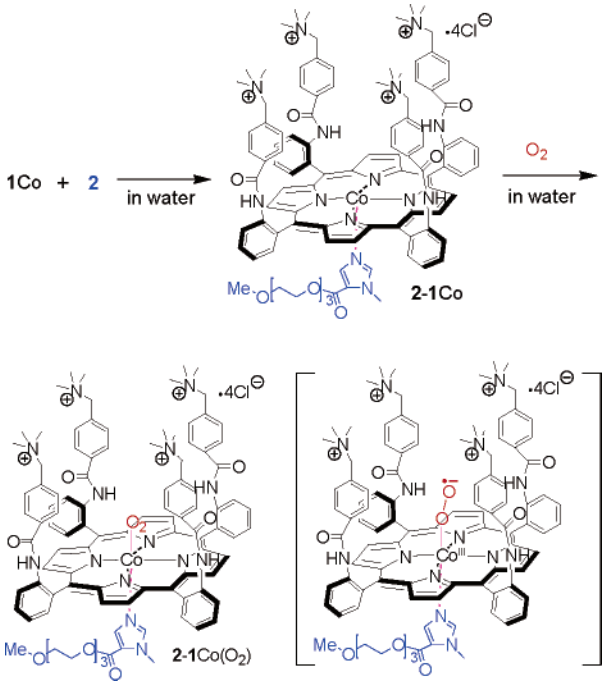

Figure 2. Oxygen binding of **1**Co in the presence of ligand **2**.

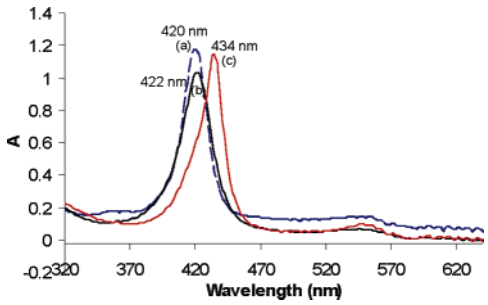

Figure 3. UV−vis spectra of **1**Co in PH 7.0 phosphate buffer solution (C = ∼ 5×10^{-6} M): (a) **1**Co under N_2; (b) **1**Co + ligand **2** (∼100 equiv) under N_2; (c) **1**Co + ligand **2** (∼100 equiv) under O_2.

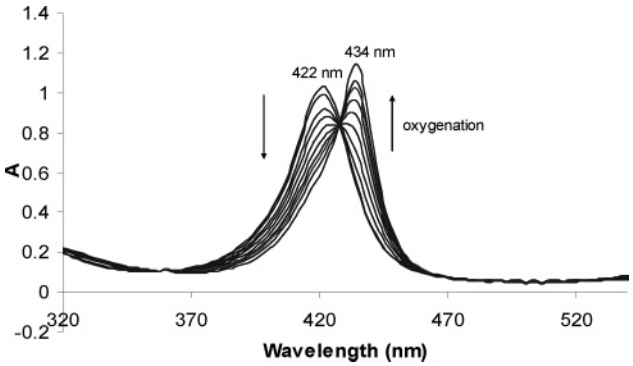

Figure 4. UV−vis absorption spectra of the oxygenation process (changing from **2**-**1**Co to **2**-**1**Co(O_2)).

complex immediately reacts, leading to a 12 nm shift of the Soret peak from 422 to 434 nm (Figure 3, trace c), which suggests the formation of the six-coordinate O_2 complex (**2**-**1**Co (O_2)).[6c−d] A good isosbestic point (428 nm) was observed in the UV−vis absorption spectra in the oxygenation process (Figure 4).

Oxygen binding of the water-soluble iron porphyrin **1**Fe was also investigated in the presence of ligand **2** in an

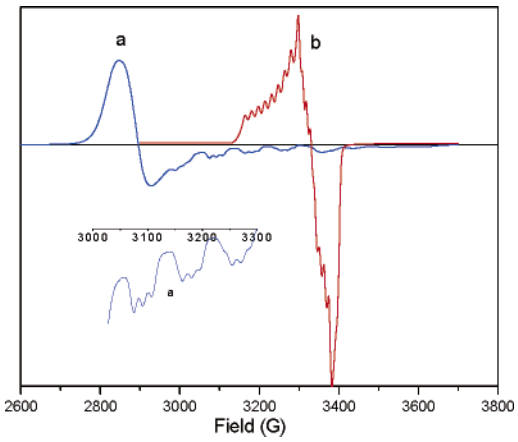

Figure 5. EPR Spectra of Complex **2**-**1**Co under PH 7.0 buffer solution at 77 K (**1**Co, C > 1mM; ligand **2**, 5−10 equiv): (a) under N_2; (b) after oxygenation.

aqueous solution, but the oxygenated species was found to be too transient to analyze.[4,5]

EPR spectroscopy is a definitive technique to examine oxygen binding of Co(II) porphyrins.[3b,7,11] The single electron in Co(II) (d7) is transferred to oxygen, forming the Co(III)($O_2^{\bullet-}$) adduct upon oxygenation (Figures 2, 5). Introduction of an excess of the imidazole ligand **2** to an aqueous solution of **1**Co (pH 7.0) led to the formation of the five-coordinate adduct **2**-**1**Co(II) (d_{z^2} ground state; $g_{||}$ = 2.02, $g_{\perp}$ = 2.31). Due to the interaction between ^{59}Co (I = 7/2) and ^{14}N (I = 1), the expected octet hyperfine structure with a triplet super-hyperfine coupling pattern was observed (Figure 5, trace a; $A_{||}$ (^{59}Co) = ∼86 G, $A_{||}$ (^{14}N) = ∼16 G). This observation rules out coordination of a second base even when ligand **2** was present in large excess. When the five-coordinate adduct **2**-**1**Co(II) is exposed to oxygen, a dramatic change in the EPR spectrum was observed ($g_{||}$ = 2.08, $g_{\perp}$ = 2.01; $A_{||}$ = 17 G, $A_{\perp}$ = 11 G), indicating the formation of the six-coordinate complex **2**-**1**Co(III) ($O_2^{\bullet-}$) (Figure 5, trace b).

The EPR signals of the oxygenated adduct lost intensity over time. After 1−2 h, only a weak EPR signal of **2**-**1**Co-(III) ($O_2^{\bullet-}$) was detected. This observation suggests decomposition of the oxygenation adduct **2**-**1**Co(III) ($O_2^{\bullet-}$) to an EPR-inactive Co(III)−X species (X denotes OH^-, $H_2PO_4^-$, or HPO_4^{2-} depending on the pH of the buffered solution). The impact of pH on the kinetic stability of the oxygenation adduct was examined. A very weak EPR signal of **2**-**1**Co-(III) ($O_2^{\bullet-}$) was detected at pH 5.0; in contrast, the oxygenated adduct was shown to be present in a pH 9.0 solution on the basis of the EPR spectrum.[9] This result implies that more-acidic conditions (H^+) accelerate the decomposition of cobalt superoxide ($O_2^{\bullet-}$ to $HOO^{\bullet}/H_2O_2$).[2]

In summary, using cobalt porphyrin **1**Co and imidazole ligand **2**, we have developed a new cobalt porphyrin system that carries oxygen in aqueous media. This metastable system demonstrates a new strategy for developing aqueous oxygen carriers and also provides an opportunity to probe the mechanisms related to the decomposition of hemoprotein oxygen adducts in aqueous media, as most reported models for the study of structure−function correlations can only be studied in organic solvents.[1]

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Supporting Information Available: Preparation, characterization, NMR spectra, and mass spectra of compound **1**Co, **1**H_2, and ligand **2**; EPR spectra of oxygen-binding system under different conditions. This material is available free of charge via the Internet at http://pubs.acs.org.

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(11) Collman, J. P.; Berg, K. E.; Sunderland, C. J.; Aukauloo, A.; Vance, M. A.; Solomon, E. I. *Inorg. Chem.* **2002**, *41*, 6583.