Photoreduction of Self-Assembled Lipidporphyrinato-iron(III) Chloride with Hyaluronic Acid under Semi-Physiological Conditions

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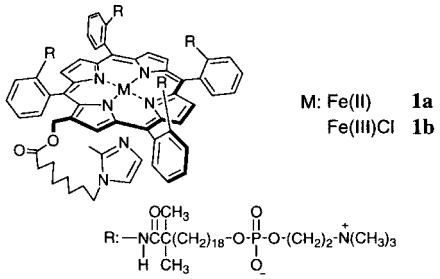
(Received October 18, 1999; CL-990884)

Photoirradiation into the LMCT band (λ_{max}: 362 nm) of self-assembled amphiphilic tetraphenylporphyrinato-iron(III) (lipidporphyrinato-iron(III)) chloride with hyaluronic acid leads to reduction of the central ferric ion in saline solution (pH 7.4, 25 °C); the obtained lipidporphyrinato-iron(II) can reversibly bind and release dioxygen.

If the redox behavior of the iron center of the heme complex, which plays crucial roles in biological systems, can be controlled by light-irradiation, this chemistry allows the photomanipulation and/or photo-resuscitation of the activities of natural hemoproteins. From this point of view, the photochemistry of ferric porphyrins, especially the photoreduction of the central Fe(III) ion in aqueous media is of current interest.[1,2] The Fe(III)porphyrins generally coordinate a counter anion and show a ligand-to-metal charge-transfer (LMCT) transition in the near UV region. It has been known that photoirradiation into this LMCT band leads to reduction of the metal center involving radical dissociation.[2-5] Hendrickson and co-workers revealed the photoreduction of the tetraphenylporphyrinato-iron(III) halide ([Fe(III)TPP]$^+$Cl$^-$) by LMCT irradiation and photocatalytic hydrocarbon oxidation.[6] The produced chloride radicals, however, recombine with Fe(II)porphyrin in water, and the ferrous complex cannot be accumulated. We have recently found that an amphiphilic Fe(III)TPP derivative with an intramolecularly coordinated axial imidazole (lipidporphyrinato-iron(III), **1b**)[7] forms a fibrous aggregate with hyaluronic acid, and its central Fe(III) ion coordinates Cl^- in saline solution. This paper describes, for the first time, a clean photoreduction of self-assembled lipidporphyrinato-iron(III) fibers under semi-physiological conditions (pH 7.4, in saline, 25 °C). The obtained lipidporphyrinato-iron(II) (**1a**) aggregate is able to reversibly bind and release dioxygen like hemoglobin. The reduction mechanism was also evaluated using laser flash photolysis experiments.



Upon the rapid injection of methanolic **1b** into phosphate buffered saline (pbs; 1 mM, pH 7.4, [NaCl]: 0.15 M), a homogeneous solution was produced ([**1b**]: 10 μM, [CH_3OH] < 0.3 vol%). The remaining methanol was completely removed by dialysis in pbs for 15 h at 4 °C. Transmission electron microscopy (TEM) of the evaporated **1b** solution showed spherical micelles with a diameter of 10 nm. The UV-vis. absorption spectrum showed that the dominant species of **1b** at pH 7.4 was the five-coordinated Fe(III) high-spin complex with axial chloride.[8,9] We assigned the distinct band at 362 nm (ε: 2.5×10^4 $M^{-1}cm^{-1}$) as a LMCT transition between the central Fe(III) and Cl^-, based on the following results. (i) This band significantly shifted to the lower energy with the decreasing electronegativity of the halides ($I^- < Br^- < Cl^-$), while the Soret or Q-bands, which are π-π^* transitions, were not affected.[6] (ii) The entire absorption spectrum changed with increasing pH and finally showed the five-coordinated Fe(III) complex with axial OH^- at pH 10.[9] This spectral change was reversible and the pKa value was determined to be 7.7. From the absorption pattern, the formation of the six-coordinated Fe(III) low-spin complex with axially bound imidazole and Cl^- was excluded.[10,11]

Upon photoirradiation of this **1b** micellar solution with a 250 W high-pressure Hg arc-lamp (365 nm) under an argon atmosphere, a negligible change in the absorption spectrum was observed. In contrast, the co-existence of a small amount of hyaluronic acid (21 mg L^{-1}, [unit]: 100 μM), which is a scavenger of free radicals in biological systems, led to complete photoreduction of the central ferric ion (Figure 1). After photoirradiation for 45 min, the UV-vis. spectrum of **1b** changed to that of a five-N-coordinated high-spin Fe(II) complex (**1a**) (λ_{max}: 443, 542, 566 nm). The well-defined isosbestic points (431, 462, 531, 584 nm) throughout the measurement revealed that no side reactions occurred; the quantum yield (Φ) was 7.0×10^{-3}.[12] The addition of hyaluronic acid to

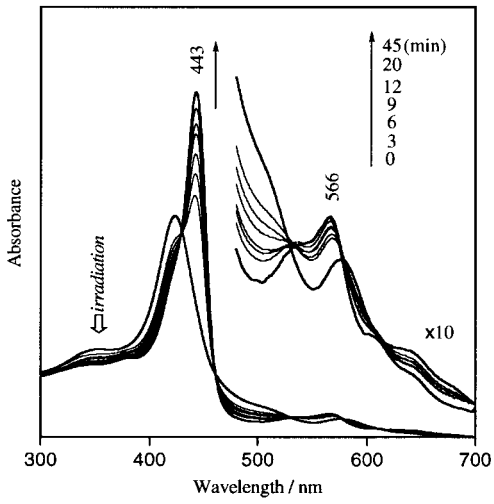


Figure 1. Visible absorption spectral changes in photoreduction of **1b** fibers in phosphate buffer saline (pH 7.4, [NaCl]: 0.15 M) at 25 °C.

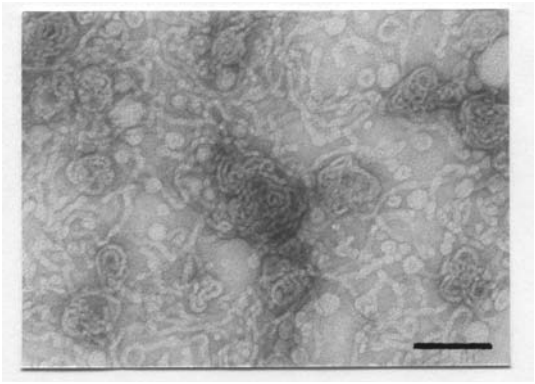


Figure 2. TEM of evaporated aqueous solution of the self-assembled **1b** with hyaluronic acid; short fibers before photoirradiation (bar: 100 nm).

the **1b** solution in the dark induced no absorption change, indicating the polysaccharide neither affects the coordination structure nor reduces the Fe(III) center. Irradiation into this CT band only causes the reduction of the ferric complex in the presence of hyaluronic acid. As expected, the complete reduction was not observed at pH 10. Glucose also showed efficacy for the photoreduction as well, but a large excess amount (0.15 M) was needed for achieving a 100% reduction. Other mucopolysaccharides (chondroitin sulfic acids, *etc.*) were also examined, but they were not as efficient compared to hyaluronic acid.

Interestingly, the addition of hyaluronic acid induced a remarkable morphology change in the **1b** aggregate from the spherical micelles into the unique short fibers (Figure 2). The width of the fiber is estimated to be 5 nm, corresponding to the molecular length of **1b** (4.6 nm). The fibers are presumably made of the polysaccharide chain combined with lipidporphyrins. These co-assembled structures of **1b** and hyaluronic acid may be responsible for the effective photoreduction process. On the other hand, addition of small amount of glucose did not induce any morphology change of the **1b** micelles.

Laser flash photolysis at 355 nm (THG of Nd:YAG) of the deaerated aqueous **1b** solution with hyaluronic acid showed that the photoreduction was finished within the duration of the laser pulse; the transient absorption spectral pattern measured at 50 ns after the pulse was in good agreement with the difference spectrum (five-N-coordinated Fe(II) complex minus Fe(III) complex with Cl^-) (Figure 3). These results suggest that the primary reaction step appears to be the homolytic cleavage of the ferric ion and the axial Cl^-, and that the imidazole arm immediately coordinates to the Fe(II) center within 50 ns. The produced chloride radical probably reacted with the active hydrogen in hyaluronic acid and the reduced ferrous complex was accumulated.[13]

Upon exposure of dioxygen to the aqueous solution of the photoreduced **1a**, the UV-vis. absorption spectrum changed to that of the dioxygenated species (λ_{max}: 425, 548 nm). The O_2 coordination was reversible at 25 °C depending on the O_2-partial pressure and the O_2-binding ability was almost identical to that of the **1a** fibers which were prepared by the previously reported procedure involving the chemical reduction of the Fe(III) center.[7]

In conclusion, photoirradiation into the LMCT band of the self-assembled **1b** fiber with hyaluronic acid leads to a clean

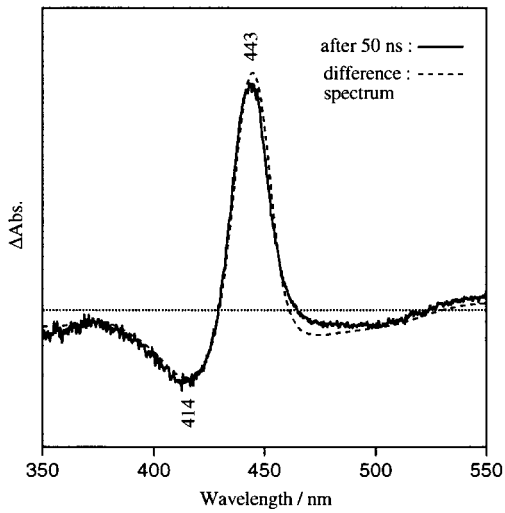


Figure 3. Transient absorption spectrum of aqueous **1b** solution with hyaluronic acid (21 mgL^{-1}) under argon (pH 7.4, [NaCl]: 0.15 M) at 50 ns after the laser pulse. The spectral pattern is in good agreement with the difference spectrum of five-N- coordinated Fe(II) complex minus Fe(III) complex.

reduction of the central ferric ion. Hyaluronic acid strings in the fibers are presumably very effective as a radical scavenger in this reaction.

This work was partially supported by the Core Research for Evolutional Science and Technology, JST, and a Grant-in-Aid for Scientific Research (No. 11650935) from the Ministry of Education, Science, Culture and Sports, Japan.

References and Notes

† CREST investigator, JST
1 I. Hamachi, S. Tanaka, and S. Shinkai, *J. Am. Chem. Soc.*, **115**, 10458 (1993); I. Hamachi, S. Tanaka, S. Tsukiji, S. Shinkai, and S. Oishi, *Inorg. Chem.*, **37**, 4380 (1998).
2 M. Hoshino, K. Ueda, M. Takahashi, M. Yamaji, and Y. Hama, *J. Chem. Soc., Faraday Trans.*, **88**, 405 (1992); H. Seki, M. Hoshino, and S. Kounose, *J. Chem. Soc., Faraday Trans.*, **92**, 2579 (1996); M. Hoshino and T. Baba, *J. Am. Chem. Soc.*, **120**, 6820 (1998).
3 C. Bartocci, F. Scandola, A. Ferri, and V. Carassiti, *J. Am. Chem. Soc.*, **102**, 7067 (1980); C. Bartocci, A. Maldotti, G. Varani, P. Battioni, and V. Carassiti, *Inorg. Chem.*, **30**, 1255 (1991).
4 D. Brault, C. Bizet, P. Morlière, M. Rougee, E. J. Land, R. Santus, and A. J. Swallow, *J. Am. Chem. Soc.*, **102**, 1015 (1980); C. Bizet, P. Morlière, D. Brault, O. Delgado, M. Bazin, and R. Santus, *Photochem. Photobiol.*, **34**, 315 (1981).
5 T. Imamura, T. Jin, T. Suzuki, and M. Fujimoto, *Chem. Lett.*, **1985**, 847.
6 D. N. Hendrickson, M. G. Kinnard, and K. S. Suslick, *J. Am. Chem. Soc.*, **109**, 1243 (1987).
7 T. Komatsu, T. Yanagimoto, Y. Furubayashi, J. Wu, and E. Tsuchida, *Langmuir*, **15**, 4427 (1999).
8 E. B. Fleischer, J. M. Palmer, T. S. Srivastava, and A. Chatterjee, *J. Am. Chem. Soc.*, **93**, 3162 (1971).
9 R.-J. Cheng, L. Latos-Grazynski, and A. L. Balch, *Inorg. Chem.*, **21**, 2412 (1983).
10 F. A. Walker, M.-W. Lo, and M. T. Ree, *J. Am. Chem. Soc.*, **98**, 5552 (1976).
11 C. L. Coyle, P. A. Rafson, and E. H. Abbott, *Inorg. Chem.*, **12**, 2007 (1973).
12 The light intensity was measured by the ferrioxalate acti-nometric procedure. C. G. Hatchard and C. A. Parker, *Proc. R. Soc. London, Ser. A*, **235**, 518 (1956).
13 B. C. Gilbert, D. M. King, and C. B. Thomas, *Carbohydr. Res.*, **125**, 217 (1984).