J|A|C|S COMMUNICATIONS

Published on Web 10/07/2009

Meso-Unsubstituted Iron Corrole in Hemoproteins: Remarkable Differences in Effects on Peroxidase Activities between Myoglobin and Horseradish Peroxidase

Takashi Matsuo,*,†,‡ Akihiro Hayashi,† Masato Abe,† Takaaki Matsuda,§,|| Yoshio Hisaeda,§ and Takashi Hayashi*,†

Department of Applied Chemistry, Graduate School of Engineering, Osaka University, Suita 565-0871, Japan, Graduate School of Materials Science, Nara Institute of Science and Technology, Ikoma 630-0192, Japan, and Department of Chemistry and Biochemistry, Graduate School of Engineering, Kyushu University, Fukuoka 819-0395, Japan

Received September 2, 2009; E-mail: tmatsuo@ms.naist.jp; thayashi@chem.eng.osaka-u.ac.jp

Corrole is a porphyrinoid macrocycle which lacks one meso carbon relative to the more common porphyrin framework and may be regarded as an aromatic analogue of corrin, a unit of vitamin B_{12}.[1,2] Although corrole retains the 18π-conjugated system, it has trianionic character because of its contracted framework. Accordingly, it is thought that the ligand shape stabilizes high-valent states of a metal ion bound within the macrocycle core.[1,3] In a past investigation of corrole chemistry, high-valent metallocorroles equipped with electron-withdrawing groups at the three meso positions have been well-characterized because they have accessible synthetic routes and tend to be thermostable.[4] Syntheses of modified corrole compounds (e.g., corrolazine), applications of corroles for light energy conversion, and development of gas sensing by metallocorroles have also been reported.[5] On the other hand, controversies remain regarding electronic configurations and oxidation states of metallocorroles without substituent groups at the meso positions. This is unfortunate because meso-unsubstituted iron corroles are attractive from the standpoint of comparing their chemical properties with those of the naturally occurring prosthetic group, heme.[6,7] Investigation of meso-unsubstituted metallocorroles, particularly iron corroles, is needed to obtain fundamental knowledge in corrole chemistry.

We have recently reported that the incorporation of an iron porphycene, a structural isomer of porphyrin, enhances peroxidase activities of myoglobin (Mb) and horseradish peroxidase (HRP) and that it is possible to evaluate the reactivities of labile high-valent species.[8] These findings indicate that the introduction of an appropriate heme derivative is useful not only for controlling the native functions of hemoproteins but also for gaining an understanding of the physicochemical character of the synthetic cofactor. Consequently, we decided to incorporate iron corrole **1** (the structure shown in Figure 1) into the apo-form of horse heart Mb and HRP to explore the intrinsic characteristics of the meso-unsubstituted iron corrole.[9]

The precursor of **1**, dimethyl ester **2**, was prepared by cyclization of the corresponding [*a*,*c*]-biladiene **3** in the presence of $FeCl_3$ (see Scheme S1).[6a] The ^{1}H NMR spectrum of **2** (Figure S1) indicates spectroscopic features similar to those of the previously reported alkyl corroles.[1,7] The meso protons were observed in an unusually far downfield region (180−200 ppm), which is indicative of Fe(III) combined with a macrocycle π-cation radical.[6] This compound is EPR-silent, suggesting that the spin of the iron with $S = 3/2$ is antiferromagnetically coupled with the macrocycle π-cation radical

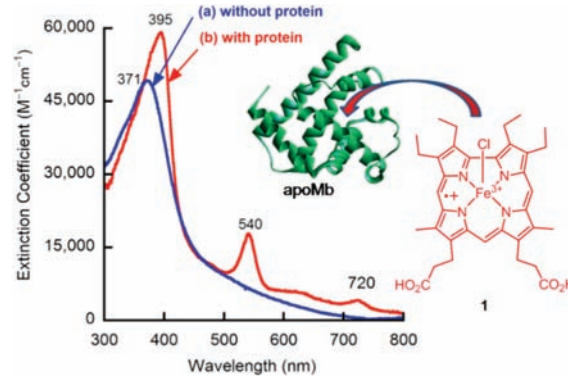

Figure 1. UV−vis spectra of iron corrole **1** in 100 mM KPi pH = 7.0 containing 1% MeOH (blue line, (a)) and in the heme pocket of Mb dissolved in 100 mM KPi pH = 7.0 (red line, (b)) at 25 °C.

($S' = 1/2$) to generate an apparently $S = 1$ electron configuration, which has been reported previously for similar compounds.[7] The hydrolyzed form **1** is proposed to be in equilibrium between the monomer and the μ-oxo dimer under neutral aqueous media.[6b]

After the addition of **1** to apomyoglobin (apoMb) in a KPi buffer solution (pH = 7.0), the band at 371 nm gradually shifts to 395 nm concurrently with the appearance of a strong band at 540 nm and a small band at 720 nm (Figure 1). The change in the higher energy band is indicative of a shift in the equilibrium to the monomer form, followed by incorporation of **1** into the hydrophobic heme pocket of Mb. The appearance of the bands in the visible region indicates the axial coordination of histidine.[10] The prepared Mb was also characterized by ESI-TOF-MS (Figure S2) with a mass number of 17 586. The p$K_{1/2}$ value, the pH corresponding to a 50% loss of a cofactor, of the reconstituted Mb is 4.8 (Figure S3), which is higher by only 0.3 unit than that of the native Mb.[11] The stability of **1**-reconstituted Mb is sufficient for conventional column chromatography, lyophilization, and the experiments for evaluating the oxidation activities described below. Surprisingly, the iron corrole became EPR-active after incorporation into the Mb matrix (Figure S4), suggesting that the ring radical was autoreduced upon imidazole cooridination.[12] Therefore, the oxidation state of the iron corrole in the reconstituted Mb can be assigned as Fe(III) with a neutral ring (*vide infra*).

In contrast, when **1** is incorporated into apoHRP, a band at 540 nm does not appear in the UV−vis spectrum (see Figure S5). Upon addition of sodium dithionite to the HRP solution, a new band at 540 nm is observed and the spectrum becomes similar to that of **1**-reconstituted Mb (Figure S5). It is known that an axial anionic ligand (for example $C_6H_5^-$) provides a large ligand field splitting

† Osaka University.
‡ Nara Institute of Science and Technology.
§ Kyushu University.
|| Current address: Kurume National College of Technology, Kurume, Fukuoka 830-8555, Japan.

10.1021/ja907428e CCC: $40.75

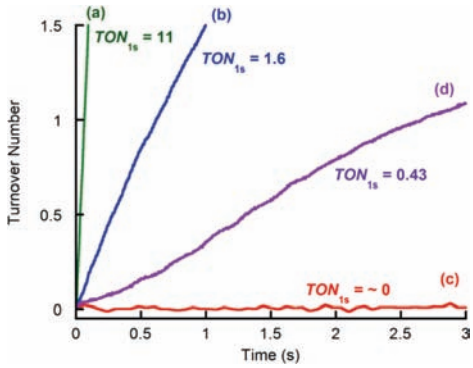

Figure 2. Catalytic activities for guaiacol oxidation mediated by Mbs or HRPs; (a) HRP with the native heme; (b) HRP with **1**; (c) Mb with the native heme; (d) Mb with **1**; $[\text{guaiacol}]_0 = 0.25$ mM, [protein] = 2 μM, $[H_2O_2]_0 = 5$ mM; 50 mM NaPi buffer pH = 7.0 at 20 °C; TON_{1s} represents the turnover number of the catalytic reaction at 1 s.

of the corrole iron. This affords an Fe(IV) corrole with a neutral ligand,[6c] which is chemically equivalent to the Fe(III) corrole π-cation radical. Because the proximal imidazole in HRP can provide anionic imidazolate-like coordination,[13] the corrole iron in HRP would be expected to attain the +4 metal oxidation state. When dithionite is added to **1**-reconstituted HRP, the corrole iron is expected to be reduced to a +3 metal oxidation state. The similarity of the UV−vis spectra between the reduced HRP (Figure S5b) and the above-mentioned Mb (Figure 1b) supports the assignment for the oxidation state of **1** in Mb described above.[14]

To address the spin state of **1** in Mb, the total magnetic susceptibility of **1**-reconstituted Mb was investigated by the Evans method.[15] When *tert*-butanol is employed as a standard sample, the peak of the butyl protons in the ^{1}H NMR spectrum is shifted as a result of the paramagnetism of **1** in the interior of Mb (Figure S6). From the variance of the downfield shift, the effective magnetic moment, μ_{eff}, of **1** in Mb is calculated to be 3.79 μ_B, suggesting that the total spin of the Mb is $S = 3/2$. Therefore, as a result of the dithionite reduction, the electron configuration of **1** in Mb is assigned as Fe(III) with the intermediate spin state.

The apparent difference in the electron configurations of the iron corrole in these two proteins provides us with a good opportunity to compare iron corrole driven peroxidase activities in these proteins. When the guaiacol (2-methoxyphenol) oxidations mediated by these proteins were examined, the increase in the absorbance around 470 nm was observed in the presence of HRPs and **1**-reconstituted Mb, indicative of the catalytic activity toward the oxidation (Figure S7). It was found that the order of the catalytic activity (based on the turnover numbers measured at 1 s) is as follows: HRP with heme > HRP with **1** > Mb with **1** ≫ Mb with heme (Figure 2). Interestingly, the incorporation of **1** into apoMb distinctly enhances the peroxidase activity, whereas the incorporation of **1** into apoHRP produces peroxidase activity which is inferior to the peroxidase activity of native HRP for the same reaction. The origin of the improved peroxidase activities in **1**-reconstituted Mb is attributable to the extension of the lifetimes of oxidized heme intermediates such as compounds I and II in **1**.[16] The trianionic nature of the corrole ligand can help to suppress any uncoupling processes, such as catalase activity, and to shift reactivity toward the oxidation of the organic compounds. The decreased catalytic activity of **1**-reconstituted HRP is a result of the iron attaining the +4 oxidation state which causes poor binding and/or activation of H_2O_2 on the iron.

In summary, we have successfully reconstituted Mb and HRP with an iron corrole without substituent groups at the meso positions of the framework. To the best of our knowledge, this is the first example of introduction of a formal ferryl cofactor into an apohemoprotein. The reconstituted proteins have remarkable differences in electronic configurations and peroxidase activities, which are influenced by the environments of the heme pockets in these proteins. Efforts to precisely characterize the high-valent chemical species of the iron corrole in the protein matrix are in progress.

Acknowledgment. The authors thank Dr. Motohiro Nakano (Osaka University) for the suggestion of the Evans NMR measurements. This work was financially supported by MEXT Japan (to T.M. and T.H.) and Global COE program at Osaka University (to T.M.).

Supporting Information Available: The synthesis of **1**, UV−vis spectra of the HRP with **1**, the NMR spectra, the EPR spectrum, the preparation of the proteins with **1** and the evaluation of peroxidase activity are described. This material is available free of charge via the Internet at http://pubs.acs.org.

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JA907428E