Template Catalysis: Catalytic Metalation of Porphyrins by Apocytochrome b_{562}

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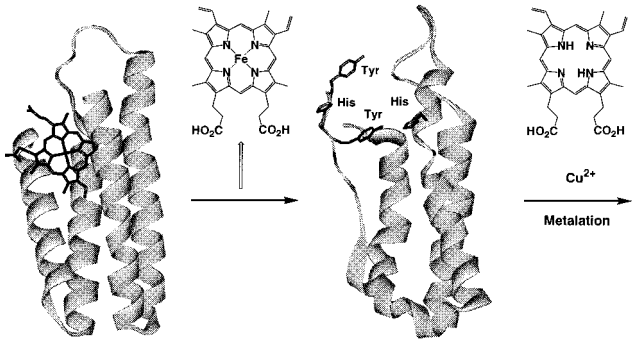
The template effect has attracted great attention in host−guest chemistry.[1,2] In particular, "template catalysis" is a challenging subject, which provides a strategy toward shape-selective transformation of substrates.[3] From this point of view, the conception of catalytic antibody is a promising approach to realize template catalysis.[4] Herein we report a novel example of template catalysis by using the apoprotein of a cofactor-dependent protein. Cofactor-dependent proteins, after removal of the cofactors, are expected to provide certain cavities whose "shapes" are complementary to the cofactors. Therefore, if the cavity has a catalytic activity, one may realize a shape-selective transformation of the cofactor and/or its structural analogues.

We found that the apoprotein of cytochrome b_{562}[5] (apocytochrome b_{562}) is capable of promoting metalation of porphyrins (**1**−**4**),[6] where protoporphyrin IX (**1**), the free-base form of the cofactor (iron protoporphyrin IX), was most preferentially metalated. Cytochrome b_{562} is one of the robust proteins against conformational denaturation because of its four-helix bundled architecture (Scheme 1), and its apoprotein is also thermally stable at room temperature to retain most of the conformational characteristics of the parent holoprotein.[7]

Scheme 1. Schematic Structures of Cytochrome b_{562} (Left) and Apocytochrome b_{562} (Right); Structural Coordinates from Refs 5b and 7b, Respectively



When **1** (1.0 μM) was mixed with $Cu(OAc)_2$ (200 μM) in phosphate buffer (pH 6.3, 10 mM) containing 4% DMSO and 0.05% Triton X-100 at 20 °C in the presence of apocytochrome b_{562} (40 μM),[8] **1** was efficiently metalated to give quantitatively a copper complex (**1**−Cu) within 15 min. In contrast, in the absence of the apoprotein, the reaction took place sluggishly to give **1**−Cu in only 1% yield. The pseudo-first-order rate constant in the presence of apocytochrome b_{562} (1.9×10^{-1} min^{-1}) was 220 times larger than that in the absence of the apoprotein (8.7×10^{-4} min^{-1}). When the concentration of the apoprotein was increased from 10 to 40 μM ($[\mathbf{1}]_0 = 1.0$ μM, $[Cu(OAc)_2]_0 = 200$ μM), the metalation showed a saturation kinetics, indicating that the apoprotein and **1** form a Michaelis complex as a reactive intermediate. Lineweaver−Burk plots gave a Michaelis constant (K_m) of 1.8×10^{-5} M with a rate constant of the metalation (k_{cat}) being 2.5×10^{-1} min^{-1}. From these values, the association constant of the Michaelis complex (K_m^{-1}) and the maximum rate enhancement (k_{cat}/k_{uncat}) by the apoprotein were evaluated to be 5.6×10^4 M^{-1} and 290, respectively (Figure 1).[9]

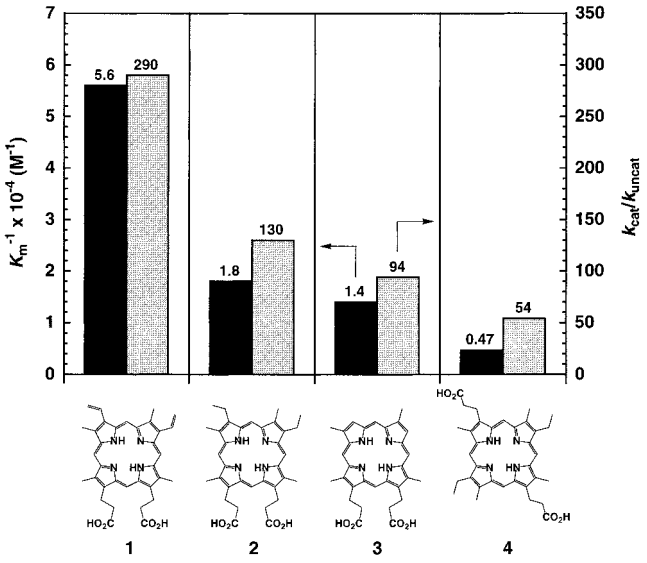


Figure 1. Metalation of porphyrins **1**−**4** (1.0 μM) with $Cu(OAc)_2$ (200 μM) in the presence of apocytochrome b_{562} in phosphate buffer (pH 6.1, 10 mM) containing 4% DMSO and 0.05% Triton X-100 at 20 °C: Association constants of the Michaelis complexes (K_m^{-1}) and acceleration factors (k_{cat}/k_{uncat}).

Apocytochrome b_{562} also promoted the metalation of other free-base porphyrins such as mesoporphyrin IX (**2**), deuteroporphyrin IX (**3**), and mesoporphyrin II (**4**), where the Michaelis−Menten kinetics was again operative.[9] Of interest to note here is that the K_m^{-1} and k_{cat}/k_{uncat} values were both dependent on the structure of the substrate (Figure 1): When **2** was the substrate in place of **1** ($CH{=}CH_2 \rightarrow CH_2CH_3$), the K_m^{-1} and k_{cat}/k_{uncat} values were both decreased significantly from 5.6×10^4 to 1.8×10^4 M^{-1} and from 290 to 130 ($k_{cat} = 2.8 \times 10^{-1}$ min^{-1}), respectively. Larger drops in K_m^{-1} and k_{cat}/k_{uncat} resulted when the vinyl groups of **1** were replaced by hydrogen atoms (**3**: $K_m^{-1} = 1.4 \times 10^4$ M^{-1}; $k_{cat}/k_{uncat} = 94$). Thus, only a subtle change of the peripheral substituents on the substrate significantly affected the metalation

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(9) See Supporting Information.

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process. On the other hand, the topology of the substituents also had a great influence on the metalation: The metalation of **4** having two propionate residues at diagonal positions showed definitely smaller K_m^{-1} (0.47×10^4 M^{-1}) and k_{cat}/k_{uncat} (54) values than those for **2**, a topological isomer of **4**.[10]

Upon titration with apocytochrome b_{562} at 20 °C in bis-Tris buffer (pH 6.1, 10 mM) containing 4% DMSO, **2** showed a 29.5 nm red shift at its Soret absorption band, where the spectral change profile indicated the formation of a 1:1 complex between **2** and the apoprotein.[11] Unlike apocytochrome b_{562}, cytochrome b_{562}, whose heme pocket is occupied by the native guest, did not promote the metalation of **2**. Accordingly, titration of **2** with cytochrome b_{562} resulted in no spectral change associated with the complexation. Thus, the metalation with apocytochrome b_{562} is most likely to occur at the heme pocket of the protein. In contrast with apocytochrome b_{562}, apomyoglobin, a conformationally labile apoprotein,[12] did not accelerate the metalation under similar conditions. Binding of Cu^{2+} to the apoprotein[13] is also essential for the metalation, since saturation kinetics with respect to [$Cu(OAc)_2$] was observed.[9,14] Titration of apocytochrome b_{562} with Cu^{2+} resulted in quenching of the fluorescence from the tyrosine residues,[9,15] which are located within the heme pocket near His 102 and His 63 (Scheme 1),[7] suggesting that Cu^{2+} is bound to either or both of their imidazole groups.

The metalation with apocytochrome b_{562} was found to be significantly suppressed at pH values lower than 6.0, where the protonation of imidazole can occur. Considering also the geometry of the heme pocket (Scheme 1), the transition state of the metalation possibly involves a cooperation of two imidazole groups, where one imidazole accommodates a Cu^{2+} ion in proximity to the porphyrin core, while the other assists deprotonation of the core NH. When the substrate is protoporphyrin IX (**1**), such a transition state is considered to be best fit to the heme pocket, since the pocket has originally been constructed for a protoporphyrin IX complex, coordinated with His 102. Thus, a parallel correlation between K_m^{-1} and k_{cat}/k_{uncat} (Figure 1) may indicate that the metalation is a "template"-assisted reaction.[16]

With a catalytic amount of apocytochrome b_{562} (0.5 μM) under similar conditions,[17] the metalation of **2** (10−30 μM) with $Cu(OAc)_2$ (200 μM) also proceeded to 100% conversion. The reaction obeyed a Michaelis−Menten kinetics, from which K_m and k_{cat}/k_{uncat} were evaluated to be 1.1×10^{-4} M and 680 ($k_{cat} = 3.8 \times 10^{-1}$ min^{-1}), respectively. This observation indicates that the apoprotein turned over at a rate 22.8 h^{-1} without significant product inhibition. Accordingly, the association constant of the apoprotein with **2**−Cu, as determined by a spectroscopic titration ($K_a = 6.5 \times 10^6$ M^{-1}), was almost comparable to that with free-base porphyrin **2** (9.2×10^6 M^{-1}).

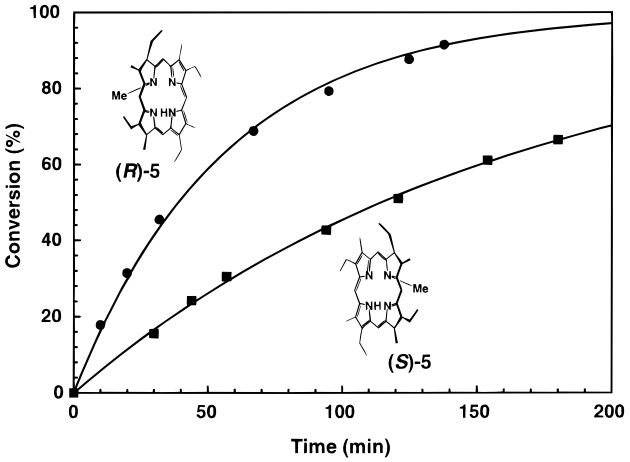

Figure 2. Metalation of (*R*)-**5** (●) and (*S*)-**5** (■) (1.7 μM) with $Zn(OAc)_2$ (200 μM) in the presence of apocytochrome b_{562} (34 μM) in phosphate buffer (pH 6.8, 10 mM) containing 4% DMSO at 20 °C.

Protein catalysis has a potential for enantioselective transformation.[18] We have reported a highly selective chiral recognition of apocytochrome b_{562} in reconstitution with chiral metalloporphyrins.[10] We found that an attempted metalation of chiral *N*-methyletiporphyrin I[19] (**5**, 1.7 μM) with $Zn(OAc)_2$ (200 μM) in phosphate buffer[20] at 20 °C proceeded enantioselectively in the presence of apocytochrome b_{562} (34 μM),[21] where (*R*)-**5** ($k^R_{obsd} = 1.8 \times 10^{-2}$ min^{-1}) was metalated three times faster than (*S*)-**5** ($k^S_{obsd} = 0.61 \times 10^{-2}$ min^{-1}) (Figure 2), indicating a possibility of kinetic resolution of **5**. Thus, racemic **5** (3.4 μM) was incubated with $Zn(OAc)_2$ (100 μM) at 5 °C in the presence of the apoprotein (6.8 μM), where the [*R*]/[*S*] ratio of unreacted **5** was decreased with time to furnish 33:67 in 66% conversion (19 h).[19]

In the present communication, we demonstrated a new catalysis of the apoprotein of cytochrome b_{562} in metalation of porphyrins at the heme pocket, where protoporphyrin IX (**1**), the free-base form of the native guest, is most preferentially metalated (template effect). The observed rate constant of the metalation, for example, of **2** ($k_{cat} = 3.8 \times 10^{-1}$ min^{-1}), is comparable to those with a catalytic antibody (1.4×10^{-1} min^{-1}) and a ribozyme (9.2×10^{-1} min^{-1}).[6] Utilization of apocytochrome b_{562} for the transformation of other organic substrates is a subject worthy of further investigation, considering its chiral recognition ability and "amphiphilic" binding activity to hydrophobic guests and metal ions.

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Supporting Information Available: Lineweaver−Burk plots and kinetic parameters of matalation of **1**−**4**, plots of rate constant of metalation of **2** versus initial concentration of $Cu(OAc)_2$, and results of fluorescence titration of apocytochrome b_{562} with Cu^{2+} (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(13) Apocytochrome b_{562}−Cu^{2+} adducts were detected by MALDI-TOF-MS spectroscopy.

(14) $[Cu(OAc)_2]_0 = 5.0-50$ μM at [apocytochrome $b_{562}]_0 = 5.0$ μM in phosphate buffer (pH 6.3, 10 mM) containing 4% DMSO at 20 °C.

(15) Titration of apocytochrome b_{562} (23 μM) with $CuCl_2$ in phosphate buffer (pH 6.3, 10 mM) at 5 °C showed quenching of the fluorescence from the tyrosine residues ($\lambda_{ext} = 276$ nm, $\lambda_{em} = 304$ nm), which, however, was saturated at $[CuCl_2]_0$/[apocytochrome $b_{562}]_0$ around 1.9 (see Supporting Information). For Cu^{2+}-induced fluorescence quenching of proteins, see: Luk, C. K. *Biopolymers* **1971**, *10*, 1229.

(16) A preliminary experiment under similar conditions (phosphate buffer [pH 6.3, 10 mM] containing 4% DMSO at 20 °C) showed that apocytochrome b_{562} can also promote metalation of **2** with other metal ions such as Co^{2+} and Zn^{2+}.

(17) At 20 °C in phosphate buffer (pH 6.3, 10 mM) containing 4% DMSO and 0.25% Triton X-100.

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(20) In phosphate buffer (pH 6.8, 10 mM) containing 4% DMSO.

(21) Under the conditions employed, (*R*)- and (*S*)-**5** were completely bound to apocytochrome b_{562}.