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# Comparison of demetalation properties between zinc chlorin and zinc porphyrin derivatives: Effect of macrocyclic structures

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#### 1. Introduction

Metalloporphyrins and metallochlorins (metal complexes of 17,18-dihydroporphyrins) play important roles in biological systems.<sup>1–3</sup> Heme and chlorophyll (Chl) are famous naturally occurring metalloporphyrins and metallochlorins, respectively. Metalloporphyrins mainly function as key cofactors in enzymes and oxygentransporting proteins. In contrast, metallochlorins are major photosynthetic pigments in nature. In some cases, metalloporphyrins are also found in photosynthetic chlorophyllous molecules: Chls *c* are antenna photosynthetic pigments in chromophyte algae and some prokaryotes,<sup>4</sup> and protochlorophyllides are known to be important intermediates in the Chl biosynthetic pathway.<sup>5</sup> The difference of the cyclic tetrapyrrole  $\pi$ -systems in metalloporphyrins and metallochlorins affects their spectral and physicochemical properties.

The stability of metalloporphyrins and metallochlorins has attracted much attention. Removal of central metals from these molecules is one of the major degradation reactions. In addition, demetalation of metallochlorins and metalloporphyrins in photosynthetic systems could be a key reaction in the early process of in vivo chlorophyll degradation.<sup>6–9</sup> From these viewpoints, demetalation properties of metalloporphyrins<sup>10–15</sup> and metallochlorins<sup>16–27</sup> have been extensively investigated in vitro. In the cases of naturally occurring metallochlorins, Mackinney and Joslyn reported demetalation kinetics of natural chlorophyllous pigments, Chls *a* and *b*, in the early 1940s.<sup>16,17</sup> Subsequently, some groups

## ABSTRACT

Metalloporphyrins and metallochlorins are important biological molecules in nature. To examine the effect of macrocyclic structures on removal of central metals from these cyclic tetrapyrrole molecules without peripheral substitution effects, demetalation of zinc methyl pyropheophorbide *a* (zinc chlorin **1**) and zinc methyl protopyropheophorbide *a* (zinc porphyrin **2**) was kinetically analyzed under acidic conditions. Both **1** and **2** exhibited gradual spectral changes from zinc complexes to free-base forms with several isosbectic points in acetone/water (10:1) at the proton concentration of  $6.1 \times 10^{-3}$  M. Demetalation of zinc porphyrin **2** was slower than that of zinc chlorin **1** in the temperature range between 15 and 35 °C. This indicates that the porphyrin macrocycle provides tolerance to removal of the central metal from cyclic tetrapyrrole ligands compared with the chlorin macrocycle.

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investigated demetalation of several Chl derivatives under various conditions.<sup>18–20</sup> Watanabe and co-workers studied physicochemical properties of demetalation of Chls *a*, *b*, and their epimers (Chls *a'* and *b'*, respectively) in detail.<sup>21,22</sup> After the discovery of naturally occurring zinc bacteriochlorophyll (BChl) *a* in the purple photosynthetic bacterium, *Acidiphilium rubrum*,<sup>28–31</sup> demetalation kinetics of this pigment was thoroughly examined by Kobayashi et al.<sup>23,24</sup> Zn BChl *a* was found to show higher resistance to demetalation than BChl *a* possessing a magnesium as the central metal.<sup>23,24</sup> Such a property of Zn BChl *a* could be preferable to *Acidiphilium rubrum*, which grows in acidic conditions. Recently, we reported demetalation properties of light-harvesting BChls, *c*, *d*, and *e* in green sulfur photosynthetic bacteria<sup>25,26</sup> and Chl *d* in a cyanobacterium *Acaryochloris marina*.<sup>27</sup>

Several works indicated that peripheral substituents in cyclic tetrapyrrole rings of these molecules affected demetalation properties of both metalloporphyrins and metallochlorins.<sup>11,13,15–17,21,25–27</sup> However, there is no study, to our best knowledge, on direct comparison of demetalation properties between metalloporphyrin and metallochlorin without substitution effects of the cyclic tetrapyrrole moieties. In the present study, we first report the effect of macrocyclic structures on demetalation kinetics of metallochlorin and metalloporphyrin possessing the same peripheral substituents. The molecular structures of metalloporphyrin and metallochlorin used in the present study are depicted in Figure 1. Zinc methyl pyropheophorbide a (denoted as zinc chlorin 1), whose photofunctional moiety is the chlorin macrocycle, possesses similar peripheral substituents to naturally occurring Chl a. Zinc methyl protopyropheophorbide a (denoted as zinc porphyrin 2) has the same molecular structure as zinc





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**Figure 1.** Molecular structures of zinc methyl pyropheophorbide *a* (zinc chlorin 1, single bond between 17-C and 18-C atoms) and zinc methyl protopyropheophorbide *a* (zinc porphyrin 2, double bond between 17-C and 18-C atoms).

chlorin **1** except for the bond between 17-C and 18-C atoms, and can be a good model compound of porphyrin-type photosynthetic pigments, protochlorophyllide *a*, which is an important intermediate molecule in the biosynthetic pathway of Chl a.<sup>32,33</sup> It is worth noting that neither zinc chlorin nor zinc porphyrin have been found as natural photosynthetic pigments although Zn BChl *a* was discovered in *Acidiphilium rubrum*.<sup>28–31</sup> Comparison of demetalation kinetics between **1** and **2** enables us to unravel the effect of macrocyclic structures without peripheral substitution effects of the cyclic tetrapyrroles.

## 2. Results and discussion

Zinc chlorin 1 and zinc porphyrin 2 were synthesized from naturally occurring Chl *a* according to previous reports,<sup>33,34</sup> and purified by reverse-phase high-performance liquid chromatography (HPLC) before measurement of demetalation kinetics. Demetalation properties of **1** and **2** were examined under acidic aqueous acetone as described previously.<sup>21–23,25–27</sup> Figure 2 shows spectral changes of zinc chlorin **1** and zinc porphyrin **2** during demetalation processes in acetone/water (10:1, vol/vol) at the proton concentration of  $6.1 \times 10^{-3}$  M at 25 °C. Zinc chlorin **1** exhibited intense Soret and  $Q_v$  bands at 429 and 658 nm, respectively, in this solution. These absorption bands decreased by incubation under the acidic condition, and a new Soret absorption band appeared at 411 nm, which was characteristic of the demetalation form of zinc chlorin 1. Small absorption bands in the range between 500 and 600 nm were gradually changed in acidic aqueous acetone. The isosbestic points could be clearly observed at 420, 453, 553, and 666 nm during the demetalation reaction.

Similar spectral changes occurred during the demetalation process of zinc porphyrin **2** under the acidic condition. The Soret band of zinc porphyrin **2** at 431 nm decreased and a new Soret band of the demetalation form of zinc porphyrin **2** appeared at 415 nm. The isosbestic points could also be observed at 422, 457, 536, 551, 600, and 635 nm during the demetalation reactions of zinc porphyrin **2**.

Demetalation reactions can be quantitatively analyzed by monitoring absorbance changes at the Soret peak positions of **1** and **2**.<sup>21–23,25–27</sup> Time courses of Soret peak absorbance of zinc chlorin **1** and zinc porphyrin **2** incubated in aqueous acetone at the proton concentration of  $6.1 \times 10^{-3}$  M at 25 °C are shown in Figure 3. The logarithm of Soret peak absorbance in the demetalation reactions of both **1** and **2** exhibited linear time dependence. The demetalation reactions of **1** and **2**, therefore, can be regarded as pseudo-first-order reactions.<sup>21–23,25–27</sup> This is consistent with the reaction condition in which the proton concentration



**Figure 2.** Spectral changes of zinc chlorin **1** (A) and zinc porphyrin **2** (B) in acetone/ water (10:1, vol/vol) at the proton concentration of  $6.1 \times 10^{-3}$  M at 25 °C. Compound **1**: spectra from 0 to 15 min at 5-min intervals. Compound **2**: spectra from 0 to 30 min at 5-min intervals. Arrows show the direction of the absorbance changes.



**Figure 3.** Kinetic plots for demetalation of zinc chlorin **1** (open circle) and zinc porphyrin **2** (open square) in acetone/water (10:1, vol/vol) at the proton concentration of  $6.1 \times 10^{-3}$  M at 25 °C. Absorbance changes were monitored at 429 and 430 nm for **1** and **2**, respectively. A<sub>0</sub>, A, and A<sub>∞</sub> are Soret absorbances of **1** and **2** at the onset of measurement, at time *t*, and at the complete demetalation, respectively.

 $(6.1 \times 10^{-3} \text{ M})$  was much higher than the concentration of **1** and **2** (< $10^{-5} \text{ M}$ ).<sup>21–23,25–27</sup> In the demetalation processes, the reaction rate constant, *k*, can be obtained by fitting the time courses of Soret absorbance of **1** and **2** to the following kinetic equation:

$$\ln(\mathbf{A} - \mathbf{A}_{\infty})/(\mathbf{A}_0 - \mathbf{A}_{\infty}) = -kt,$$

where  $A_0$ , A, and  $A_\infty$  are Soret absorbances of **1** and **2** at the onset of measurement, at time *t*, and at the complete demetalation, respectively.<sup>21–23,25–27</sup> The demetalation rate constants of zinc chlorin **1** and zinc porphyrin **2** in acetone/water (10:1, vol/vol) at the proton concentration of  $6.1 \times 10^{-3}$  M at 25 °C were estimated to be



**Figure 4.** Arrhenius plots over temperature range of 15–35 °C for the reduction rate constants of zinc chlorin **1** (open circle) and zinc porphyrin **2** (open square) in acetone/water (10:1, vol/vol) at the proton concentration of  $6.1 \times 10^{-3}$  M.

 $2.5 \times 10^{-1}$  and  $1.5 \times 10^{-1}$  min<sup>-1</sup>, respectively (the average of 3–5 independent measurements). The demetalation rate constant of zinc chlorin **1** was 1.7-times larger than that of zinc porphyrin **2**. These indicate that the macrocyclic  $\pi$ -system affects demetalation properties of cyclic tetrapyrrole molecules possessing a central metal. Comparison of demetalation properties between chlorin-type and bacteriochlorin-type pigments by Kobayashi et al.<sup>23</sup> which indicated that Mg BChl *a* and Zn BChl *a* had higher resistance than Mg Chl *a* and Zn Chl *a*, respectively, also suggest the effect of the macrocyclic structures on removal of central metal, although the substituent at the 3-position of BChl *a* was not the same as that of Chl *a*.

Temperature dependence of demetalation rate constants of zinc chlorin 1 and zinc porphyrin 2 at the proton concentration of  $6.1\times 10^{-3}\,M$  was examined between 15 and 35 °C at 10 °C intervals. Demetalation of both 1 and 2 slowed with decrease of the reaction temperature. The logarithm of Soret absorbance of 1 and 2 was linearly changed against reaction time in this temperature range, indicating that these demetalation reactions could be analyzed as pseudo-first-order reactions. Therefore, the demetalation rate constants in this temperature range were estimated by fitting the time course of Soret absorbance of 1 and 2 to the above kinetic equation. Figure 4 depicts Arrhenius plots of the demetalation rate constants of zinc chlorin 1 and zinc porphyrin 2 in acetone/water (10:1, vol/vol) at the proton concentration of  $6.1 \times 10^{-3}$  M in the temperature range between 15 and 35 °C. The activation energies of demetalation of 1 and 2 were estimated to be 95 and 101 kJ mol $^{-1}$ , respectively, from the Arrhenius plots in Figure 4. These values were larger than the reported activation energies of natural Chl a, 47.4 kJ mol<sup>-1</sup>, in acetone/water (3:1, vol/vol) at the proton concentration of  $3.7 \times 10^{-4}$  M.<sup>21</sup> This would be mainly ascribable to the difference of central metals in the macrocycles, since Zn Chl a has higher resistance to demetalation than Chl a possessing a central magnesium.23,24

The difference of demetalation properties between zinc chlorin 1 and zinc porphyrin 2 could be discussed in terms of redox potentials of chlorin and porphyrin macrocycles. We recently reported that resistance of demetalation of natural Chls possessing a formyl group was correlated with their oxidation potentials<sup>27</sup>: the order of resistance of demetalation, Chl b > Chl d > Chl a, was consistent with the order of their oxidation potentials (Chl b (+0.94 V vs SHE) > Chl d (+0.88 V) > Chl a (+0.81 V) in acetonitrile<sup>35</sup>). The electron densities of tetrapyrrole  $\pi$ -systems, which shift redox potentials of cyclic tetrapyrrole molecules, would affect the demetalation properties. In comparison of oxidation potentials between porphyrin and chlorin  $\pi$ -systems, the oxidation potential of porphyrin molecules tended to shift positively compared with chlorin molecules.<sup>36,37</sup> Further physicochemical studies will be necessary to understand thoroughly demetalation properties of cvclic tetrapyrrole molecules.

The products after demetalation reaction under the present condition were analyzed by reverse-phase HPLC. The main product by demetalation of **1** exhibited a visible absorption spectrum of the demetalated form of **1**, methyl pyropheophorbide *a* (the Soret and  $Q_y$  bands were positioned at 408 and 666 nm, respectively, in the HPLC eluent). Few other products were detected after the demetalation reaction of **1**. In the case of zinc porphyrin **2**, the main product after the reaction was assigned to the demetalated form of **2**, methyl protopyropheophorbide *a* (the Soret band was positioned at 415 nm in the HPLC eluent), and other products were hardly observable. These indicate that little side reaction occurred through demetalation of **1** and **2** under the present reaction conditions.

Comparison of demetalation properties between zinc chlorin **1** and zinc porphyrin **2** might be in line with the early processes of the degradation of biological cyclic tetrapyrrole molecules. In the degradation pathway of Chl *a*, demetalation occurs prior to oxidative cleavage of the *meso*-position of the chlorin macrocycle by pheophorbide *a* oxygenase.<sup>6–9,38,39</sup> Therefore, smooth removal of central magnesium from the chlorin macrocycle would be important in the Chl degradation, although the in vivo demetalation mechanism of natural Chls or chlorophyllides has not been thoroughly unraveled.<sup>40–42</sup> It might be worth noting that one of the famous metalloporphyrins, heme, breaks down into biliverdin by heme oxygenase, followed by release of central iron.<sup>43,44</sup>

To summarize, the present kinetic analyses directly revealed the difference of physicochemical properties on the removal of central metal from the tetrapyrrole macrocycles between porphyrin and chlorin  $\pi$ -systems without effects of their peripheral substituents on the cyclic tetrapyrrole rings. The porphyrin-type macrocycle was more tolerant to demetalation than the chlorin-type macrocycle. These results should be useful in chemistry and biochemistry of cyclic tetrapyrrole molecules.

# 3. Experimental

## 3.1. Apparatus

Visible absorption spectra were measured with a Shimadzu UV-2450 spectrophotometer, where the reaction temperature was regulated with a Shimadzu thermo-electric temperature-controlled cell holder TCC-240A. Mass spectra were measured with a Shimadzu KOMPACT MALDI 4 spectrometer. HPLC was performed with a Shimadzu LC-20AT pump and an SPD-M20A or SPD-20AV detector.

# 3.2. Measurements of demetalation kinetics

Measurements of demetalation kinetics of **1** and **2** were performed in the similar procedure to previous reports.<sup>21–23,25–27</sup>

A 3 mL acetone solution of **1** and **2** (Soret absorbance = 1.0) was mixed with 0.28 mL of distilled water. In kinetic analysis of demetalation processes,  $20 \,\mu$ L of aqueous 1 M HCl solution was added to the pigment solution, and absorbance at the Soret peak position of pigments was measured under the control of reaction temperatures between 15 and 35 °C.

#### 3.3. Materials

Zinc methyl pyropheophorbide *a* (1) and zinc methyl protopyropheophorbide *a* (2) were synthesized from naturally occurring Chl *a* according to previous reports.<sup>33,34</sup> General synthetic procedures of these compounds were as follows. Methyl pyropheophorbide *a* was synthesized through three steps from Chl *a*, which was extracted from *Spirulina geitleri*. A methanol solution saturated with Zn(CH<sub>3</sub>COO)<sub>2</sub>·2H<sub>2</sub>O was added to a dichloromethane solution of methyl pyropheophorbide *a*, and the solution was stirred at room temperature for ca. 2 h to afford zinc methyl pyropheophorbide *a* (1).<sup>33,34</sup> Zinc methyl pyropheophorbide *a* (1) was reacted with 2,3dichloro-5,6-dicyano-*p*-benzoquinone (1.1 equiv) in distilled acetone for ca. 5 min to give zinc methyl protopyropheophorbide *a* (2).<sup>33</sup> Zinc complexes 1 and 2 were purified by reverse-phase HPLC.

# 3.4. Spectral data

### 3.4.1. Zinc methyl pyropheophorbide a (1)

UV-vis (acetone)  $\lambda_{max}$  = 655 (relative intensity, 0.68), 608 (0.12), 567 (0.07), 524 (0.04), 426 (1.00), 405 nm (0.61). MS (MALDI): found: *m*/*z* 611.2, calcd for C<sub>34</sub>H<sub>35</sub>N<sub>4</sub>O<sub>3</sub>Zn (MH<sup>+</sup>), 611.2. See also Ref. 33.

# 3.4.2. Zinc methyl protopyropheophorbide *a* (2)

UV-vis (acetone)  $\lambda_{max} = 613$  (relative intensity, 0.13), 563 (0.05), 428 nm (1.00). MS (MALDI): found: m/z 609.6, calcd for  $C_{34}H_{33}N_4O_3Zn$  (MH<sup>+</sup>), 609.2. See also Ref. 33.

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