



## Stabilization of 5-aminolevulinic acid by the zinc(II) ion in an aqueous solution

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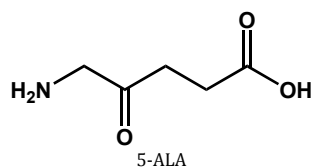
pH

### ABSTRACT

It is known that 5-aminolevulinic acid (5-ALA) is not stable in an aqueous solution because of the auto-condensation reaction between two 5-ALA molecules; however, in this study, 5-ALA was stabilized by the zinc(II) ion in an aqueous solution of neutral pH. This stabilization effect was ascertained by UV-Vis Spectroscopy and <sup>1</sup>H and <sup>13</sup>C NMR Spectroscopy.

### 1. Introduction

5-Aminolevulinic acid (5-ALA) (Scheme 1) is the precursor of all tetrapyrroles in biological systems [1]; in the case of mammals, heme is synthesized from 5-ALA, and, in the case of green plants, chlorophyll is synthesized from 5-ALA. In both biosynthetic processes, two 5-ALA molecules are condensed to give porphobilinogen by an enzyme, 5-aminolevulinic acid dehydratase (EC 4.2.1.24); however, without the enzyme, two 5-ALA molecules are condensed in a different manner to give different products [2–6]. The mechanism of this auto-condensation of 5-ALA was studied by Jaffe and coworkers [2] and Novo and coworkers [3]. They reported that two 5-ALA molecules were condensed to form 2,5-bis(2-carboxyethyl) dihydropyrazine(2,5-dihydropyrazinedipropionic acid, HPDPA) and then HPDPA was oxidized by molecular oxygen to form 2,5-bis(2-carboxyethyl)pyrazine(2,5-pyrazinedipropionic acid, PDPA).



Scheme 1

Recently 5-ALA has been used as a fertilizer for tomatoes, wine grapes, strawberries, and other crops; however, the auto-condensation of 5-ALA reduces the concentration of 5-ALA in the fertilizer solution, which is a problem. Therefore, we are looking for a method to stabilize 5-ALA in an aqueous solution of neutral pH. In the auto-condensation, the first condensation

process is thought to start with a nucleophilic attack by a lone pair of the amino group of 5-ALA to a  $\gamma$ -carbonyl carbon of another 5-ALA molecule. Therefore, if metal ions are added to a 5-ALA solution and bind to the amino groups of 5-ALA molecules, the initial nucleophilic attack will be stopped, and 5-ALA molecules should be stabilized. In this study, we examined the effect of a zinc(II) ion because the zinc(II) ion is colorless and diamagnetic due to its  $d^{10}$  electronic configuration and is suitable for UV-Vis Spectroscopy and NMR Spectroscopy.

### 2. Experimental

#### 2.1. Measurements

Electronic spectra were recorded on a Jasco V-560 spectrometer. <sup>1</sup>H and <sup>13</sup>C NMR spectra (400 MHz) were recorded on a JEOL JNM- $\alpha$ 400 spectrometer in D<sub>2</sub>O using sodium 3-trimethylsilyl-2,2,3,3-tetradeuteriopropionate (TSP) as the internal standard.

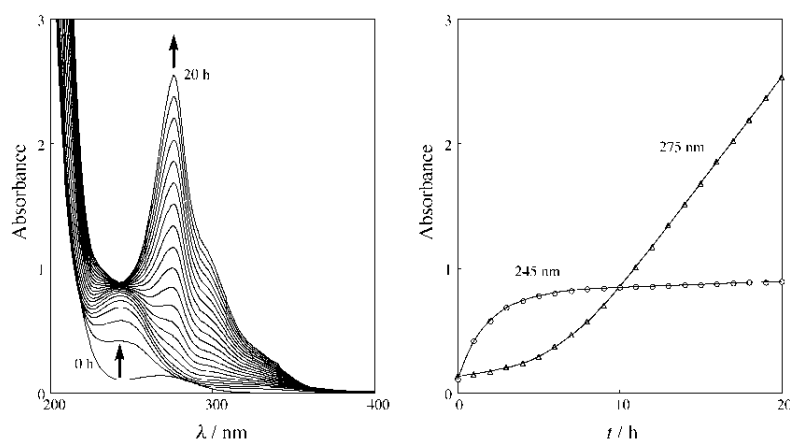
#### 2.2. Materials

Hydrochloric salt of 5-ALA (5-ALA·HCl) was prepared by Cosmo Oil Co., Ltd. using a fermentation method [7]. All other chemicals were commercial products and were used as supplied.

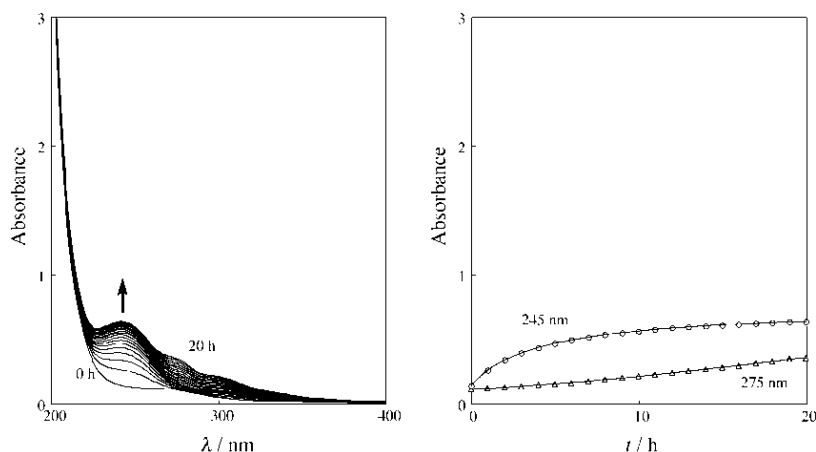
### 3. Results and discussion

#### 3.1. Auto-condensation reaction of 5-ALA monitored by electronic spectra

Before examining the stabilization effect of zinc(II) ion, the auto-condensation reaction of 5-ALA was monitored by the



**Figure 1.** Electronic spectral change for an aqueous 5-ALA solution (left) and time dependencies of the absorbance for the solution at 245 nm ( $\circ$ ) and 275 nm ( $\Delta$ ) (right). Conditions: 5-ALA-HCl (20  $\mu$ mol) and LiOH (40  $\mu$ mol) in 5 mL of water under aerobic conditions at room temperature (pH=8.0). Measurements were conducted for 20 hours at one-hour intervals.



**Figure 2.** Electronic spectral change for an  $N_2$ -exchanged aqueous 5-ALA solution (left) and time dependencies of the absorbance for the solution at 245 nm ( $\circ$ ) and 275 nm ( $\Delta$ ) (right). Conditions: 5-ALA-HCl (20  $\mu$ mol), LiOH (40  $\mu$ mol) in 5 mL of water under  $N_2$  condition at room temperature. Measured for 20 hours at one hour intervals.

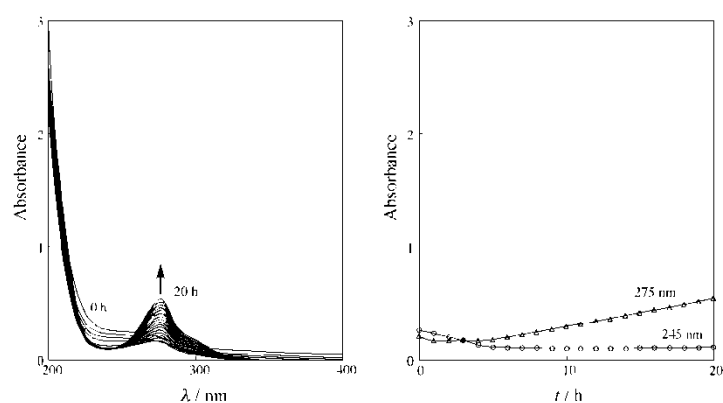
electronic spectra as follows. An aqueous solution of 5-ALA (pH $\approx$ 8) was prepared by mixing 5-ALA-HCl and lithium hydroxide (LiOH) in a 1:2 molar ratio, and the absorption spectra of the resulting solution were measured for 20 hours at one-hour intervals (Figure 1). At first, a new absorption band appeared at around 245 nm, and then another band appeared at around 275 nm together with two shoulder bands at around 300 nm and 330 nm. The result is consistent with the results of Novo and coworkers [3]; the appearance of the first band at around 245 nm corresponds to the formation of HPDPA, and the second band at around 275 nm corresponds to the formation of PDPA. An experiment was also carried out under nitrogen (Figure 2), and as anticipated, the first band appeared at around 245 nm as well as in the aerobic experiment; however, the intensity of the second band at around 275 nm was significantly smaller than that in the aerobic experiment.

### 3.2. Stabilization of 5-ALA by zinc(II) ion

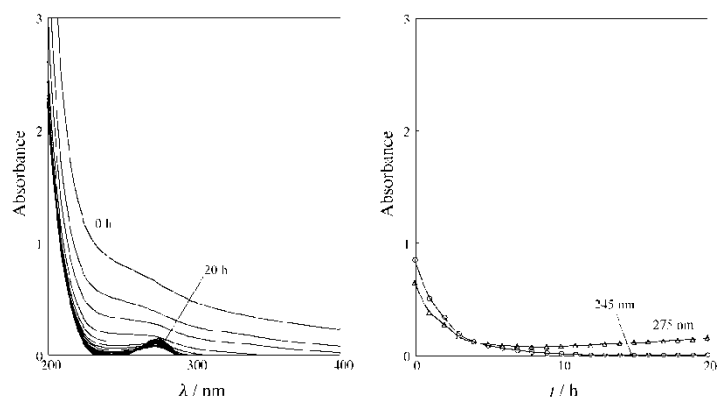
The effect of zinc(II) ion on the auto-condensation of 5-ALA was examined by the method described in Section 3.1. In the presence of  $ZnCl_2$  in the molar ratio of 5-ALA: $ZnCl_2$  (2:1), the rate of PDPA formation decrease to about 15% (Figure 3) [slope of absorbance at 275 nm:  $2.5 \times 10^{-2} \text{ min}^{-1}$  in Figure 3 and  $1.7 \times 10^{-1} \text{ min}^{-1}$  in Figure 1]. It is noteworthy that the subtle precipitation of zinc hydroxide caused an increase in the absorbance in a wide wave-length range just after preparing

the solution, but this disappeared within several hours. When  $ZnCl_2$  was added to a 5-ALA solution in a 1: 1 molar ratio, the rate of PDPA formation became less than 5% (Figure 4) [slope of absorbance at 275 nm:  $7.1 \times 10^{-3} \text{ min}^{-1}$  in Figure 4]. The formation of HPDPA and PDPA was significantly inhibited by the zinc(II) ion, and we can conclude that the zinc(II) ion stabilizes 5-ALA in solution.

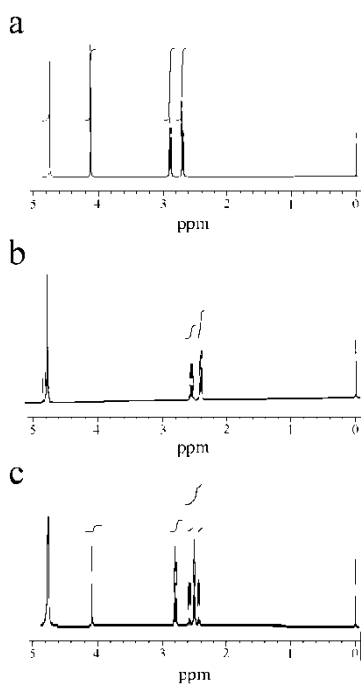
The stabilization effect of the zinc(II) ion was also ascertained by  $^1H$  and  $^{13}C$  NMR (Figure 5 and 6). Without the zinc(II) ions, the signals of 5-ALA [ $^1H$  NMR:  $\delta = 2.71$  (t, 2H); 2.90 (t, 2H); 4.13 (s, 2H) ppm;  $^{13}C$  NMR:  $\delta = 30.4$ ; 37.3; 50.0; 179.8; 207.0 ppm] disappeared, and the signals of HPDPA [ $^1H$  NMR:  $\delta = 2.40$  (s, 2H); 2.42 (t, 2H); 2.57 (t, 2H) ppm;  $^{13}C$  NMR:  $\delta = 36.2$ ; 36.2; 36.3; 175.8; 184.5 ppm] appeared; however, with zinc(II) ions (5-ALA: $ZnCl_2$ , 1:1), the signals of 5-ALA remained, and a small amount of HPDPA was observed [ $^1H$  NMR:  $\delta = 2.51$  (t, 2H); 2.81 (t, 2H); 4.09 (s,  $\sim$ 1.1H) ppm;  $^{13}C$  NMR:  $\delta = 33.5$ ; 36.08 (HPDPA); 38.8; 50.2; 183.9; 208.9 ppm]. In the  $^1H$  NMR in the presence of zinc(II) ions (Figure 5c), the integrated intensity of the  $\delta$ -methylene protons was smaller than expected; this may be explained by the D-H exchange through enolization of 5-ALA in  $D_2O$  [2]. In the presence of zinc(II) ions, the  $^1H$  NMR signals shifted downfield as compared with the solution without zinc(II) ions at pH 7 ( $\delta = 2.45$ ; 2.75; 4.05 ppm) [2]; this may suggest the coordination of 5-ALA to the zinc(II) ion to form a zinc(II) complex.



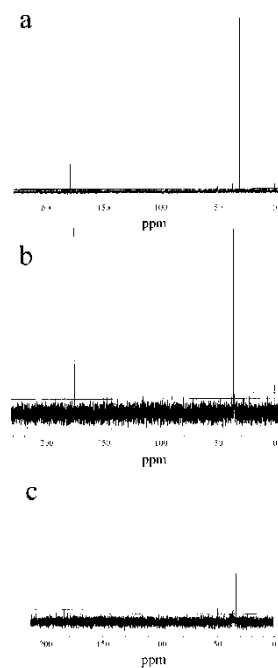
**Figure 3.** Electronic spectral change for an aqueous 5-ALA solution in the presence of zinc(II) ion (left) and time dependencies of the absorbance for the solution at 245 nm (○) and 275 nm (Δ) (right). Conditions: 5-ALA-HCl (20 μmol), ZnCl<sub>2</sub> (10 μmol), and LiOH (40 μmol) in 5 mL of water under aerobic conditions at room temperature (pH = 7.2). Measurements were conducted for 20 hours at one-hour intervals.



**Figure 4.** Electronic spectral change for an aqueous 5-ALA solution in the presence of zinc(II) ion (left) and time dependencies of the absorbance for the solution at 245 nm (○) and 275 nm (Δ) (right). Conditions: 5-ALA-HCl (20 μmol), ZnCl<sub>2</sub> (20 μmol), and LiOH (40 μmol) in 5 mL of water under aerobic conditions at room temperature (pH = 6.7). Measurements were conducted for 20 hours at one-hour intervals.



**Figure 5.** <sup>1</sup>H NMR spectra of 5-ALA in D<sub>2</sub>O: (a) 5-ALA-HCl, (b) neutralized with LiOH, and (c) neutralized with LiOH in the presence of ZnCl<sub>2</sub> (pH = 7).



**Figure 6.** <sup>13</sup>C NMR spectra of 5-ALA in D<sub>2</sub>O: (a) 5-ALA-HCl, (b) neutralized with LiOH, and (c) neutralized with LiOH in the presence of ZnCl<sub>2</sub> (pH = 7).

### 3.3. Stabilization mechanism of 5-ALA by zinc(II) ion

As reported in the introduction, the auto-condensation of 5-ALA starts with a nucleophilic attack by a lone pair of the amino group of 5-ALA to a  $\gamma$ -carbonyl carbon of another 5-ALA molecule. Judging from the downfield shift in  $^1\text{H}$  NMR, 5-ALA seems to coordinate to the zinc(II) ion to form a zinc(II) complex. Since the lone pair is used for the coordination bond in the complex, the initial nucleophilic attack cannot occur, and 5-ALA molecules are stabilized. Although the zinc(II) complex has not been isolated, attempts to do so is underway.

### 4. Conclusion

A method of stabilizing 5-ALA at neutral pH was being required in order to use 5-ALA for some application purposes. It is known that 5-ALA is not stable in an aqueous solution at neutral pH. In this study, the zinc(II) ion was found to stabilize 5-ALA in an aqueous solution of neutral pH by forming a zinc(II) complex.

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