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Carbon K-edge X-ray Absorption Spectra of Firefly Luciferin

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1 Introduction

Firefly bioluminescence is the emission owing to the transition from the first excited state to the ground state of oxyluciferin produced by the oxidation reaction of luciferin-adenosine monophosphate intermediate in the enzyme. The luminescence colors are determined from the conjugate acid and base of luciferin and oxyluciferin [1]. However, the relationship between the reaction process and the chemical species is still unclear. The luciferin anion is predominant in pH 7 aqueous solutions, followed by the luciferin dianion in pH 8 aqueous solutions [2]. For understanding the mechanism of bioluminescence reactions, it is important to study the deprotonation of the hydroxy group of the luciferin anion in aqueous solutions. In this study, we focus on C K-edge X-ray absorption spectroscopy (XAS) as a method for investigating the difference in the electronic structure between OH and the O^- groups in luciferin [3].

2 Experiment

The XAS experiments were performed at the soft X-ray beamline BL-7A. C K-edge XAS spectra of luciferin in aqueous solutions were measured using the transmission-type liquid cell, whose details were described previously [4, 5]. The liquid layer in the liquid cell was sandwiched between two Si_3N_4 membranes with a thickness of 100 nm. The thickness of the liquid layer was precisely controlled by adjusting the ambient helium pressure around the liquid cell. The XAS spectra were obtained using Beer-Lambert law, ln (I_0/I), where I is the transmission signal of luciferin solutions and I_0 is that of the bare Si_3N_4 membranes. The different aqueous solutions were exchanged using a syringe pump without changing the position of the liquid cell.

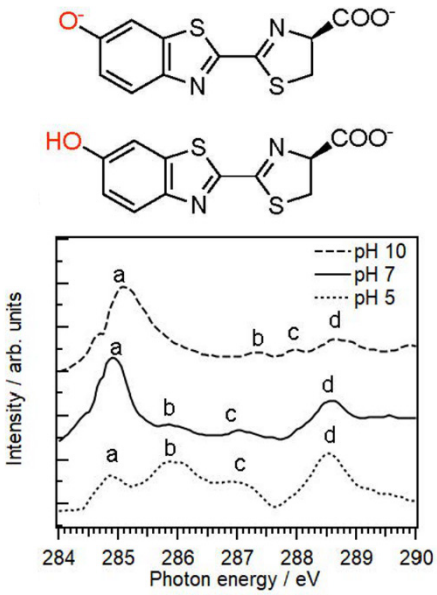

Fig. 1: C K-edge XAS spectra for luciferin in pH 5, 7, and 10 solutions [3]. The inset shows molecular structures for luciferin anion and luciferin dianion.

3 Results and Discussion

Figure 1 shows the C K-edge XAS spectra for luciferin in pH 5, 7, and 10 solutions at 25 °C. Note that the luciferin anion is abundant in both pH 5 and 7 solutions and the luciferin dianion is abundant in pH 10 solution. There were four characteristic peaks (a, b, c, and d) in the spectra. The energetic positions of the largest and second largest peaks (a and d) for pH 10 (285.1 and 288.7 eV) were similar to those for pH 7 (284.9 and 288.6 eV), respectively. On the other hand, the energetic positions of the other two peaks (b and c) for pH 10 show the higher energy shifts from

those for pH 7. Although the peak intensities for pH 5 were different from those for pH 7, the energetic positions for pH 5 were the same as those for pH 7 because the most abundant chemical species is luciferin anion in both pH 5 and 7 solutions.

From the C K-edge inner-shell calculations using time-dependent density functional theory, we found that the deprotonation of the hydroxy group of luciferin is reflected in the energy difference between peak a and peak b in the C K-edge XAS spectra. The energy difference between a and b for pH 7 was 1.0 eV, while that for pH 10 was 2.3 eV because of the red shift of the energy from the 1s orbital of the C atom bonded to the hydroxy group upon its deprotonation.

References

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