Core-Electron Excitation of Firefly Luciferin with Carbon XAS Measurements

Firefly bioluminescence is a phenomenon providing light emission through a chemical reaction of a substrate molecule with adenosine triphosphate catalyzed by enzyme. Many studies have been conducted to understand this luminescence mechanism. We investigated the core-electron excitation of luciferin, the substrate in a firefly bioluminescence, using soft X-ray beamline BL-7A. The C K-edge X-ray absorption spectra of the luciferin anion, which has a C-OH bond, were clearly different from those of luciferin dianion which has a C-O$^-$ bond.

During firefly bioluminescence, an emitter (oxyluciferin) is produced from a substrate (luciferin) and adenosine triphosphate (ATP) by an enzyme [1]. In this reaction, not only the bond cleavage but also deprotonation from these molecules would occur and they are related to color and intensity of firefly bioluminescence. However, the experimental evidence for the luminescence reaction is still insufficient, and thus the details of the reaction remain unclear.

Since absorption measurements using soft X-rays reflect the bonding states of molecules, the soft X-ray time-resolved spectroscopy is expected to elucidate the details of firefly bioluminescence reactions. X-ray absorption spectroscopy (XAS) measurements have been used to detect deprotonation in relatively small, gas-phase isolated molecules in the gas phase or organic molecules. However, it is not obvious that this method can detect bond cleavage and deprotonation in luciferin, intermediates, and oxyluciferin influenced by the solvent and enzyme environment.

In this study, XAS measurements were used to verify whether deprotonation in luciferin can be distinguished [2]. **Fig. 1(a)** shows the molecular structures of the luciferin anion with a hydroxy group and the luciferin dianion, which is formed by deprotonation of this hydroxy group. It is known that the most abundant chemical species of luciferin at pH 7 is the anion and at pH 10 is the dianion [3]. We measured the inner-shell excitation spectrum of luciferin using the solution XAS measurement system shown in **Fig. 1(b)** [4, 5], installed at the soft X-ray beamline BL-7A. Quantum chemical calculations were used for spectral analysis.

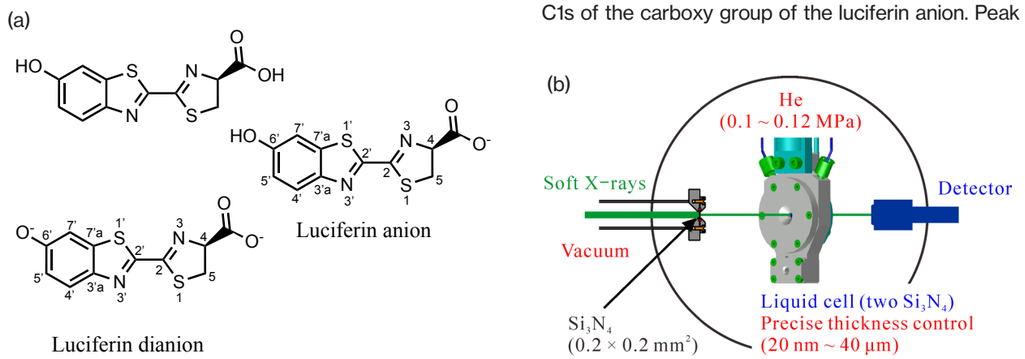

Figure 1: (a) Molecular structures of the luciferin anion and the luciferin dianion. **(b)** The solution XAS measurement system.

Figure 2(a) shows the Carbon (C) K-edge XAS at pH 7 and 10, in which four characteristic peaks appear. The photon energies of the largest peak (285.1 eV) and the second largest one (288.7 eV) at pH 10 are almost the same as those at pH 7 (284.9 and 288.6 eV, respectively). The characteristic difference between the spectra at pH 7 and 10 is the energy difference between peak **a** and peak **b**, which is reduced by approximately 2.3 eV, when changing from pH 10 to 7.

Figure 2(b) shows the theoretical absorption spectra of the luciferin anion and dianion. The bold line corresponds to the excitation from 1s orbital of the C atom (C1s) bonded to the hydroxy group ("6'C" in **Fig. 1(a)**). In the theoretical absorption spectrum of the luciferin anion, the 286-eV peak includes both the excitation from 6'C 1s and that from C1s of the carboxy group. On the other hand, the 285-eV peak in the theoretical absorption spectrum of luciferin dianion includes excitations from 6'C 1s and from 1s orbitals of the C atoms connecting the benzothiazole and thiazoline rings ("2C" and "2'C" in **Fig. 1(a)**). The peak corresponding to the excitation from C1s of the carboxy group appears at 286.3 eV.

Comparison of the spectra in **Figs. 2(a)** and **2(b)** allows for the assignment of the experimental peaks as follows. Peak **b** at pH 7 in the experimental spectrum is attributed to the excitations from 6'C 1s and C1s of the carboxy group of the luciferin anion. Peak

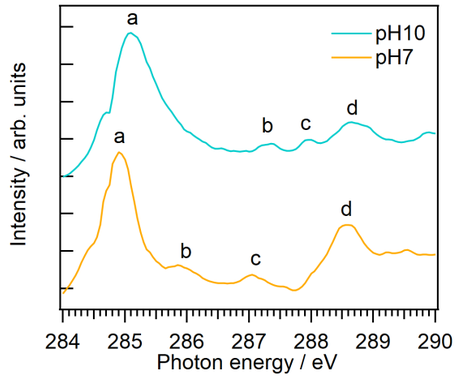

Figure 2: (a) XAS experimental spectra of luciferin at pH 7 and 10. This figure is partially modified from Figure 2 in [2].

a at pH 10 in the experimental spectrum is attributed to excitations from 6'C 1s and from 1s orbital of 2C and 2'C of the luciferin dianion, while peak **b** corresponds to excitation from C1s of its carboxy group.

Our results demonstrate that the effect of deprotonation of the hydroxy group in luciferin is reflected in the energy difference between peaks **a** and **b**, which arises from the difference in excitation energy from 1s orbital of C atom bonded to the hydroxy group in the luciferin anion and dianion. The difference is 2.3 eV, indicating that C K-edge XAS spectral measurements can be used to investigate the change from C-OH bonds (luciferin anion) to C-O$^-$ bonds (luciferin dianion). The C K-edge spectrum associated with the carbon atom which is part of the benzothiazole ring may be affected by electron delocalization described by resonance. If the C–O single bond is cleaved directly, a more significant change in the C K-edge spectrum can be expected.

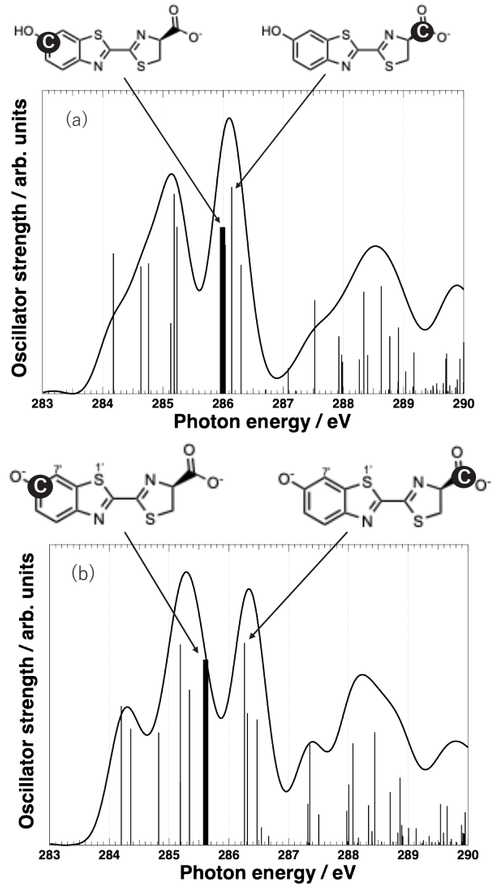

Figure 2: (b) Theoretical core-electron excitation spectra for luciferin anion (upper panel) and luciferin dianion (lower panel). Bold line: the excitation from 1s orbital of C atom bonded to the hydroxy group. This figure is partially modified from Fig. 3 in [2].

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BEAMLINE

BL-7A

Y. Kudo[1], F. Kumaki[2], M. Nagasaka[3], J. Adachi[2], Y. Noguchi[4], N. Koga[5], H. Itabashi[1] and M. Hiyama[1] ([1]Gunma Univ., [2]KEK-IMSS-PF, [3]IMS, [4]Shizuoka Univ., [5]Nagoya Univ.)