2-6-11. Bioluminescence

Overview

Bioluminescence (BL) is chemiluminescence (CL) used by luminous organisms such as fireflies. Most of the BL substrates react as luciferin under the enzymatic action of luciferases to show luminescence. A BL reaction generally consists of multiple processes, in which an excited product is produced by decomposition of a peroxide intermediate as chemiexcitation and emits light. The reaction can proceed in vivo and is widely used for luminescence analysis including bioimaging. Its use in life science field has developed, and the development and improvement of luciferins and luciferases are still progressing. Fundamental reaction mechanism research has also progressed at the same time.

Current Status and Frontiers

Chemiluminescence (CL) used by luminous organisms such as fireflies is called bioluminescence (BL). In many cases, BL is based on the luciferin-luciferase (L-L) reaction, in which a luciferin (substrate) undergoes a luminescent reaction under the action of a luciferase (enzyme). The reaction proceeds in multiple steps involving oxygenation and the step of peroxide-intermediate decomposition producing a product (oxyluciferin) in the excited state as chemiexcitation. Luciferases catalyze the reactions and regulate the luminescent property of the excited oxyluciferins. In the BL research, the elucidation of reaction mechanisms and the analytical applications of the luminescent materials of fireflies and marine organisms are progressing. Because BL is characterized by visible light production at high quantum yields, it is widely used in bioimaging in life science field. Similar to CL, BL provides a low-background luminescence analytical method. In recent years, the development of near-infrared (NIR) BL systems has been advanced for deep in vivo imaging. Research in Japan has contributed to the field from basic to applied studies, and several studies are introduced as follows.

1. Fundamental chemistry

Studies on the reaction mechanisms of the substrates for fireflies, jellyfish, and sea fireflies (Fig. 1) have progressed. Luminous beetles including fireflies use common luciferin (FL). Both the jellyfish substrate, coelenterazine (CTZ) and sea firefly (*Cypridina*) luciferin have the imidazopyrazinone (IP) core structure. The reaction of FL consists of adenylation, oxygenation, chemiexcitation, and light emission, and that of IP consists of oxygenation, chemiexcitation, and light emission. The oxygenations proceed via electron transfer to O_2, and chemiexcitations commonly arise from dioxetanone

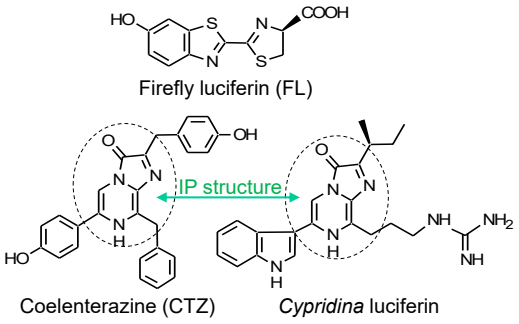

Figure 1. Representative BL substrates.

intermediates (Fig. 2). The highly efficient chemiexcitation will be caused by the CTIL mechanism proposed by a theoretical study (see Yamaguchi et al., in "Chemiluminescence"). Since the BL reactions proceed in enzymes, it is important to know the luciferase structure. For a firefly case, the complex structure of a luciferase and an adenylated FL-model has been elucidated (Nakatsu, Kato, et al.), to give the basis to understand the reaction. Luminous beetles show various emission colors from green to red. Differences in emission wavelengths reflect differences in the state of oxyluciferin in the excited singlet state in the luciferase active site. The keto-phenolate anion of oxyluciferin will be the emitting structure for various emission colors depending on changes in environmental polarity (Hirano et al.), while it has not yet been elucidated.

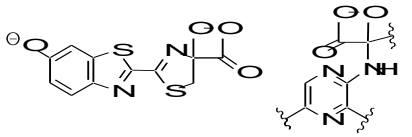

Figure 2. Dioxetanones.

2. Improvement of luciferins and luciferases

Luminescence characteristics suitable for the purpose of application were realized by both structural modification of luciferins and the preparation of luciferase variants. A NIR FL analog, AL (λ_{BL} 670 nm), for instance, has been developed for imaging deep in mammals (Maki, Niwa et al., Fig. 3). A luciferase mutant, Akaluc with a high BL efficiency with AL was also created (Iwano, Miyawaki et al.), and the use of AL/Akaluc became a world standard for long-wavelength BL imaging.

In life sciences, small enzymes may be advantageous for various BL analysis methods. A U.S. company has developed a 19 kDa enzyme for CTZ from a shrimp luciferase, that is smaller than firefly luciferase (61 kDa), and are used worldwide. A 13 kDa enzyme for CTZ has also been developed in Japan (Ohmuro et al.), and its application will expand.

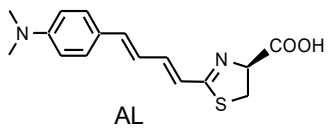

Figure 3. A NIR FL analog.

References

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4. S. Iwano, S. Maki, A. Miyawaki, et al., *Science*, **359**, 935–939 (2018).
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Future Perspectives and Directions

Issues to be resolved and realized within the next 10 years: Flexible design of luciferins and luciferases by AI prediction of BL reactivity and luminescence characteristics, Development of photofunctions to expand analytical utilization, Experimental establishment of reaction mechanisms including emission-wavelength modulation mechanisms and chemiexcitation mechanism.

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