Structural Basis of Bioluminescent Color Control in Firefly Luciferase

The firefly emits brilliant yellow-green light. The bioluminescence reaction is carried out by the enzyme firefly luciferase, which catalyzes the oxidative reaction involving firefly luciferin, MgATP and molecular oxygen to yield an excited oxyluciferin. The visible light is emitted during the relaxation of the excited oxyluciferin to the corresponding ground state. The catalytic reaction of luciferase is a two-step reaction (Fig. 1(a)). In the first step, luciferin attacks an ATP molecule and luciferase forms an enzyme-bound luciferyl-AMP intermediate. In the second step, a proton is abstracted from the C-4 carbon of the luciferyl-AMP intermediate and molecular oxygen attacks the position, then an electronically excited oxyluciferin molecule, AMP and CO_2 are produced.

One of the most interesting aspects of the emission in firefly luciferase is the bioluminescence color change induced a luciferase mutation. The color change is caused by a single-amino-acid point mutation in firefly luciferase. In the case of Japanese genji-botaru luciferase, yellow-green light and red colored light are emitted by the wild-type enzyme and the S286N mutant, respectively [1]. We determined the crystal structures of genji-botaru luciferase at the various states in the catalytic reaction to elucidate the color control mechanism of luciferase. We synthesized 5'-*O*-[*N*-(dehydroluciferyl)-sulfamoyl]adenosine (DLSA), which is a luciferyl-AMP intermediate analogue, to determine the crystal structure of the intermediate (Fig. 1(b)).

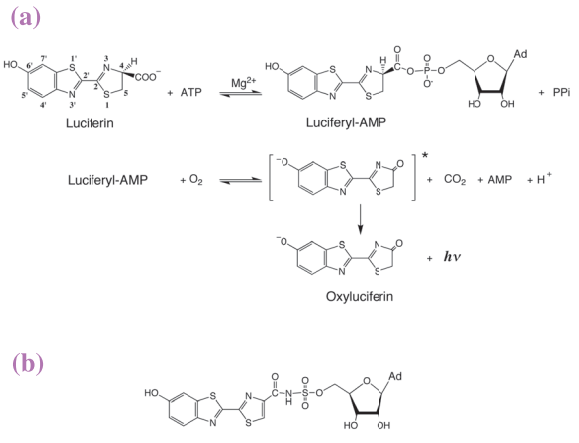

Fig. 1. **(a)** Bioluminescent reaction catalyzed by luciferase. **(b)** Structure of DLSA, luciferyl-AMP intermediate analogue.

The crystal structure of genji-botaru luciferase complexed with DLSA was determined at a resolution of 1.3 Å using beamline **BL45XU**, PX-station (Fig. 2). The overall structure consists of a large amino-terminal domain and a small carboxyl-terminal domain. DLSA was present in the active site pocket formed by these two domains. We also elucidated the structures of genji-botaru luciferase in complex with Mg-ATP and with in complex AMP/oxyluciferin at 2.3 Å and 1.6 Å, respectively. The conformation of DLSA is similar to that of the oxyluciferin and AMP molecules in the AMP/oxyluciferin complex. The overall structure of the AMP/oxyluciferin complex is almost the same as that of the Mg-ATP complex, but significant differences are observed in the DLSA complex. The Ile288 residue in the DLSA complex is much closer to the DLSA molecule than in the other structures. The movement of Ile288 seems to be involved in the switching of the hydrogen bonding network: Ser286 is hydrogen bonded to Glu313 in the AMP/oxyluciferin complex structure; in contrast, the Ser286 residue of the DLSA complex forms hydrogen bonds with Asn231 and Tyr251 through a water molecule in the DLSA complex (Fig. 3(a)). Due to the movement, three atoms of the side chain of Ile288, which is located in the highly hydrophobic active site, form van der Waals contacts with the DLSA molecule (Fig. 3(c)).

Fig. 2. Crystal structure of wild-type genji-botaru luciferase complexed with DLSA (green).

In addition, we determined the crystal structure of the red-light-emitting S286N mutant luciferase complexed with DLSA at 1.45 Å using beamline **BL44B2**, and compared its structure with those of three types of wild-type crystal. The structure of the S286N mutant complexed with DLSA is similar to the structure of the wild-type enzyme complexed with AMP/oxyluciferin, but not to the structure of wild-type enzyme complexed with DLSA. Note that, the conformational change observed in the wild type was not seen in the S286N mutant. The Ile288 residue of the S286N mutant complexed with DLSA is not close to the DLSA molecule. This might be due to the side chain atoms of Asn286 hydrogen bonding to Glu313 and a water molecule, which seems to prevent Ile288 from shifting (Fig. 3(b)). As a result, only one atom of the side chain of Ile288 forms van der Waals contact in the S286N mutant complexed with DLSA (Fig. 3(c)). Therefore, the structure of the S286N-DLSA complex creates less of a hydrophobic microenvironment than that of the wild-type enzyme-DLSA complex.

The conformation of the DLSA molecule in the wild-type complex is almost same that in the S286N complex. Thus, the difference in both structures is only in the conformational change in luciferase, which controls the hydrophobic microenvironment for the DLSA molecule (Fig. 3(a,b)). We consider that the structure of the DLSA complex indicates the structure just before the light is emitted. Therefore, the difference in the structure of DLSA complex reflects the difference in the energies of the excited state of oxyluciferin. The wild-type luciferase tightly binds the excited state of oxyluciferin in the highly rigid and nonpolar microenvironment created by the hydrophobic side chain of Ile288, minimizing energy loss before emitting yellow-green light. In contrast, the S286N luciferase forms a less rigid microenvironment, which allows some energy loss from the excited state of oxyluciferin due to thermal relaxation. Therefore, the S286N mutant emits the red light low in energy, which is compared with yellow-green light.

To confirm our hypothesis that the degree of the hydrophobicity is related to bioluminescence color, we prepared I288V and I288A mutants and examined the emission colors. As expected, the I288V and I288A mutants emitted orange and red colored light, respectively. Therefore, the amino acid residue at position 288 directly influences the emitted light color and the molecular rigidity of the excited state of oxyluciferin, which is controlled by the transient movement of Ile288, and determines the color control of bioluminescence during the emission reaction.

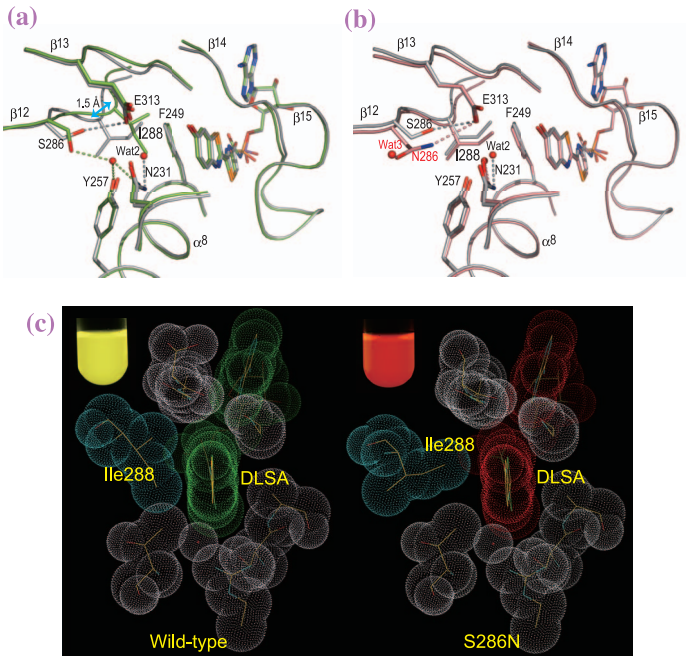

Fig. 3. Comparison of active site in luciferase structures. **(a)** Superposition of wild-type DLSA (green) and wild-type AMP/oxyluciferin (white) complexes. **(b)** Superposition of S286N DLSA (pink) and wild-type AMP/oxyluciferin (white) complexes. **(c)** Comparison of van der Waals interactions in structures of wild-type (left) and S286N (right) luciferases complexed with DLSA. The van der Waals radii of Ile288 (blue), DLSA in wild-type (green) and DLSA in S286N (red).

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