Mutant firefly luciferase with enhanced oxidative luminescent activity improved the response of protein-protein interaction assay FlimPIA

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Abstract

Previously we developed a protein-protein interaction (PPI) assay named firefly luminescent intermediate-based protein interaction assay (FlimPIA) 1, which utilizes the characteristic two half reactions catalyzed by firefly luciferase (Fluc). In the first step LH2 and ATP are converted to luciferyl-adenylate (LH2-AMP), while LH2-AMP is converted to oxyluciferin in the second step. We designed two mutant Flucs named the Donor and the Acceptor, where the Donor lacks oxidative luminescent activity and the Acceptor shows very week adenylation activity. When these mutants are each tethered with interacting proteins, transfer efficiency of LH2-AMP from the Donor to the Acceptor is increased by the interaction, resulting in the higher luminescent intensity. Recently, we improved the signal/background (S/B) ratio of the assay by suppressing remaining adenylation activity of the Acceptor 2. However, it resulted in lower luminescent signal.
It is hypothesized that the C-terminal domain of Fluc rotates depending on the half-reaction to be catalyzed 3, 4. Here, we aimed at enhancing the oxidative luminescent activity of the Acceptor by mutating L526, which is supposed to exist on the surface of Fluc in the second conformation. When FKBP12 and FRB were used as interacting proteins, the mutant Acceptor with L526P or L526S mutation significantly enhanced the luminescent intensity and S/B ratio of FlimPIA. More specifically, while the overall luminescent intensity using LH2 and ATP as substrates decreased, the oxidative luminescent activity was accelerated. These results suggest suppression of the adenyaltion activity due to L526 mutation near the active site in adenylation conformation, while the oxidation conformation becomes more stable.

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