Study on the luciferase reaction systems of beetle bioluminescence

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Luminous beetles, which use an identical luciferin, show a variety of bioluminescence reactions, for example differences on colors. Among them, only the bioluminescence reaction of firefly luciferase has been utilized to some industrial applications because the reaction was controlled artificially *in vitro*, so that instruments can easily measure the light output.

This study has intended to control a variety of bioluminescence reactions, which were catalyzed by luminous beetle luciferases origined from *Phrixothrix hirtus*, *Ragophthalmidae Ohbi*, and *Photinus pyralis*, for applying to industrial applications by use of co-factors. As the results, each reaction showed a variety of luminescence kinetics depended on different luciferases. The addition of pyrophosphate into every reactions was effective to control luminescence kinetics where both rapid decays and initial flash of light outputs were decreased. These results suggested pyrophosphate might be effective to control every luminescence reactions with a variety of luminous beetle luciferases for putting these bioluminescence reactions to practical use. On the other hands, although DTT has been thought as an essential component for control firefly bioluminescence reaction, some reducing reagents increased light activity only at 10% concentration compared with DTT. This result suggested that there might be a new control mechanism by reducing reagents for firefly bioluminescence reaction. Moreover, by optimization of pyrophosphate addition, practical luminescence reaction has been established for both the luciferase assay and the ATP assay methods, where a half-life of luminescence reaction extended until several hours. By this study, some co-factors were found that effected significantly to control every bioluminescence reactions catalyzed by a variety of luminous beetle luciferases. In additional optimization using by the co-factor, they might be easily make luminous beetle bioluminescence reactions put into industrial applications in the future.