Characterization of a Novel Coelenterazine-Dependent Luciferase and Photoprotein

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ABSTRACT

Bioluminescence imaging (BLI) is a crucial technique in biomedical research, allowing for the non-invasive visualization of cells and biochemical events within living organisms through light emission. This technique relies on the utilization of bioluminescent enzymes, specifically luciferases and photoproteins, which are responsible for the light-emitting reactions. Despite the vast number of bioluminescent species, only a small fraction of the corresponding luciferase and photoproteins have been thoroughly characterized. Therefore, the primary objective of this project is to isolate and characterize novel luciferases and photoproteins from deep-sea organisms that have not been extensively studied. The findings from this research demonstrate a positive dependency of light emission when coelenterazine(CTZ) or Ca2+ is added to luciferase or photoproteins, which encourages further work on modifying the characteristics of these proteins to enhance their performance for various biomedical applications.

INTRODUCTION

Bioluminescence is the emission of visible light by an organism due to a biochemical reaction (Haddock et al., 2010): luciferin, which is considered to be unique to specific species, is oxidized by an enzyme known as luciferase, resulting in light emission.

These enzymes can be categorized under a group known as coelenterazine-dependent luciferases.

There are two distinct types of luciferase. One type is luciferase itself which facilitates the oxidation of coelenterazine(CTZ) through a typical enzyme-substrate interaction, resulting in blue light emission. Another type is Ca-regulated photoproteins(Php), which rely on Ca^{2+} for their bioluminescent activity (Markova & Vysotski., 2015).

With these characteristics, luciferases and photoproteins are powerful analytical tools in applications such as reporters, bioimaging probes, etc. (Krasitskaya et al., 2020). This work aims to characterize novel luciferase and photoprotein for further implication in various applications.

METHODOLOGY

Expression and Purification from E. coli
The photoprotein and luciferase coding sequences were inserted into pET-30b(+) vectors for expression in E. coli. An N-terminal His-tagged protein was produced by inducing E. coli strain cells with 1 mM IPTG. After harvesting by centrifugation, the cells were resuspended in Protein Extraction Reagent lysis buffer. The recombinant proteins (RP) were initially purified. RPs were eluted using a stepwise gradient of imidazole (20 mM, 50 mM, 100 mM).

Light-emission Data Acquisition Tools

- A tube luminometer, SiriusBerthold(RLU/s).
- Light acquisition duration of 1-2min. at 25°C.

Figure 1.
Luminometer Sirius II

Statistical Analysis

- Either means ± standard deviation (SD) or means ± standard error of the mean (SEM).
- Data integration, statistical analysis, and function fit analysis: Excel & SciDAVis software.

RESULTS

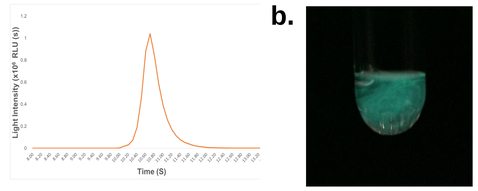

Fig. 2 Novel Photoprotein
(a) Light intensity of the photoprotein in presence of Ca^{2+} ions.
(b) Light emission of the photoprotein.

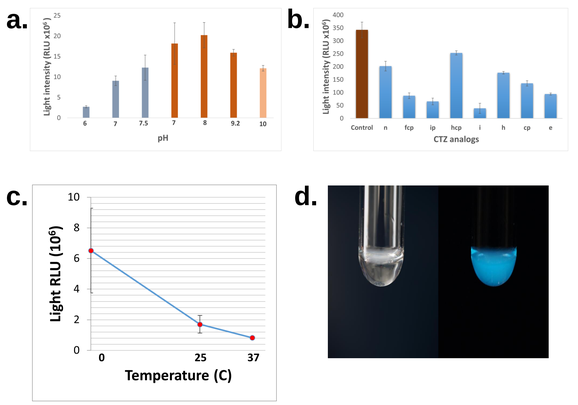

Fig. 3 Novel Luciferase
(a) Llight intensity and pH of the buffers: phosphate buffer (gray), Tris-HCl (dark orange), and CAPSO (orange).
(b) Luciferase light emission with various CTZ analogs and Native CTZ (as control).
(c) Luciferase light intensity and the sample temperature.
(d) Luciferase light emission.

CONCLUSIONS & RECOMMENDATIONS

For the first time, novel CTZ-dependent luciferase and photoprotein have been characterized. When CTZ was added, novel luciferase eluted intensive light, demonstrating an explicit reliance on this substrate.

Concentration and light emission are positively correlated.

- Best buffer & pH is Tris-HCl Buffer pH=8.
- Native CTZ is best, and hcp analog shows promising potential with coral luciferase.
- Thermostability is not satisfying. Stabilizing protein at body temperature is needed.

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