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Author: Mojtaba Mortazavi Saman Hosseinkhani

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Surface charge modification increases firefly luciferase rigidity without alteration in bioluminescence spectra

Mojtaba Mortazavi[a], Saman Hosseinkhani[b]

[a]*Department of Biotechnology, Institute of Science and High Technology and Environmental Science, Graduate University of Advanced Technology, Kerman, Iran.*
[b]*Department of Biochemistry, Faculty of Biological Sciences, Tarbiat Modares University, Tehran, Iran*
[a]*To whom correspondence should be addressed:*
Phone: 034-33776611
Fax: 03433776617. E-mail: m.mortazavi@kgut.ac.ir

Highlight

- Bioluminescence emission spectra of luciferase is preserved with substitution of Arg in surface loops.
- Temperature property of mutant luciferases were improved.
- Engineering the enzyme based on surface loops modification approach further enhanced its activity.

1. Introduction

Lampyris turkestanicus (Iranian firefly) luciferase is an example of bioluminescent enzymes, which can converts the chemical energy into light [1]. Firefly luciferase (EC 1.13.12.7) catalyzes the oxygenation of luciferin using Mg^{2+}-ATP to yield excited state of oxyluciferin that emits light [2-4]. The specific advantages of the luciferase reaction as high sensitivity and low-cost [5, 6], have led to an important insight into the use of this approach in the biological systems [7-9]. However, like any other technology tool, a series of problems hindered the use of this technology [3, 10, 11]. According to the diverse ideas and routine techniques in the protein engineering, several approaches as site directed mutagenesis [12-20] have been conducted to find the variants of luciferase that overcome these problems.

One issue to be considered in the protein engineering is pay special attention to the loops. Previous studies have shown that loops have the highest mobility in protein crystallization [21]. Comprehensive studies of hyperthermophilic proteins have shown that modifying the loop through loop shortening or anchoring, have increased the thermostability of proteins [22, 23]. Loop anchoring can be achieved through many ways as ion pairing, hydrogen bonding or hydrophobic interactions [24]. On the other hand, the sequence and structure of mesophilic and thermophilic proteins are compared to find the thermal stability mechanisms of proteins [25]. This comparison shows that thermostable proteins stabilize their solvent-exposed surfaces with higher frequency of Arg [26]. This amazing feature of thermophilic proteins, is a reason for providing the thermal stability with increasing in the ion interactions, salt bridges and ions pairing [27, 28]. In this context, *Strub* and coworkers achieved interesting results with mutation