Review Article

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Structure, Enzymatic Mechanism of Action, Applications, Advantages, Disadvantages and Modifications of Luciferase Enzyme

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Summary

North American Firefly, Photinu spyralis, entertainment countless observers, probably because of human arrival mainland. Between the period of 1950s and 1980s, many young biologists spent their money as a firefly collector, first hired by a Professor William D McElroy at Johns Hopkins University, later became a member of the Sigma Firefly Club. In 1985, when Marlene DeLuca and her colleagues cloned cDNA encoding luciferase, a replacement source of the enzyme becomes available and later in labs around the world, many other organisms began to emit unique yellow-green glow as a result of firefly expression Luciferase is in their cells. Now, luciferase and its genes have become very useful for research purposes and also for a variety of commercial purposes. The structure of firefly and bacterial luciferase will be of great value for the development of applications in many processes. There are many firefly luciferase homologues that can catalyze similar reactions with similar amino acid sequences. Several applications are described in different publications on bioluminescence and chemiluminescence. Firefly flicker is always very interesting to observe it. Firefly luciferase emits light but does not generate heat, which also causes curiosity because it solves the enzyme-catalyzed reaction. Here we mainly review the structure, mechanism, application, advantages and disadvantages of luciferase.

Keyword: Luciferase

Introduction

Luciferase enzyme is one a class of enzyme that catalyze a group of oxidation reaction which then produce the light and also produce bioluminescence that is generally differentiate from the conventional photoprotein; luciferin is a wide-ranging word for the substrate. The name of both luciferin and luciferase are come from the latin word Lucifer which means venus, as like as the morning star. (Lucifer accurately refers light-bringing). The words luciferin and luciferase was first invented by Raphaël Dubois for the substrate and enzyme, respectively. The luciferases enzyme mainly take part in the oxidation which give off light that's why oxygen is needed for this reaction, there are many different type of luciferases enzyme found in the nature which have small or not anything in common, and name as this system is bioluminescent systems [1]. The total luciferases are extensively used in molecular biology, cell biology and biotechnology, microscopic analysis and also in the reporter genes, as well as for fluorescent proteins. In the luciferase reaction, luciferases require the addition of luciferin but do not require an external light source which not likes fluorescent proteins. A diversity of organisms control their own light creation using dissimilar luciferase in the diversity of light producing response [2]. The greater part of calculated luciferases has been established in the animals, as well as fireflies, and a lot of sea animals like as copepods, jellyfish, and the sea pansy. Nevertheless, luciferases have been studied in luminous fungi, example of Jack-O-Lantern mushroom, and also an e.g. in additional kingdoms plus luminous bacteria, and dinoflagellates [3].

In the biochemical process the firefly luciferase is to be investigated as the first enzymes in the molecular biology and biotechnology [4]. Many scientists, researchers and investigators determined the structures of the substrates and products of this enzyme from *P. pyralis* working during the period of 1940s and 1950s [5-9].

Structure

Firefly luciferase structure

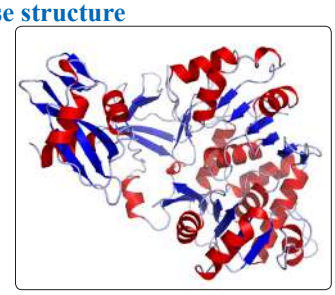

Figure 1: Structure of *Photinus pyralis* firefly luciferase (Adapted from Harvey, E. N. 1920) [3]

A Catalytic domain of the Dinoflagellate Luciferase

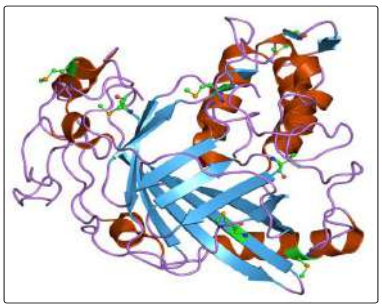

Figure 2: Dino flagellate Lingulodinium polyedrum luciferase domain crystal structure (Adapted from Harvey, E. N. 1920) [3]

Helical bundle domain of the Dinoflagellate Luciferase

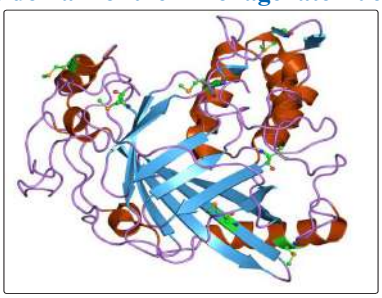

Figure 3: Dinoflagellate Lingulodinium polyedrum luciferase domain crystal structure (Adapted from Harvey, E. N. 1920) [3]

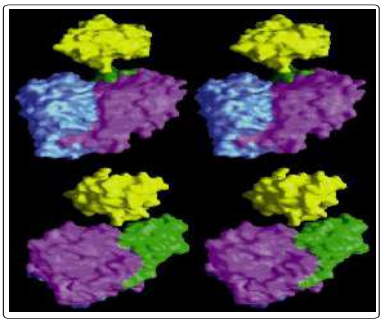

Figure 4: The firefly luciferase structure (3D veiw) has been determined with high resolution (Adapted from Conti, E., N. P, 1996 [1]

Figure 5: Amino acid alignment in the luciferase (Adapted from J.H. Devine, 1993 [10]

Enzymatic mechanism of action

In common, luciferases catalyzed particular type of chemiluminescent reactions. The common reaction format is give below:

Luciferin + Oxygen → Oxyluciferin* → Oxyluciferin + *hv* (1)

Thus,these enzymes generally catalyze the oxidation reactions that oxidize the lucifereins and produceing an exited stated molecule of oxyluciferin (in the reaction 1 given as asterisk indicates an excited state). The light source simply comes from the decomposition of the luciferin from ground state to excited state. When variation in the molecules (like luciferin molecules), or modify in the active site then result is different in the colour of the light emitted. There are various luciferin structures is given below examples that shown in Figure 6 [11].

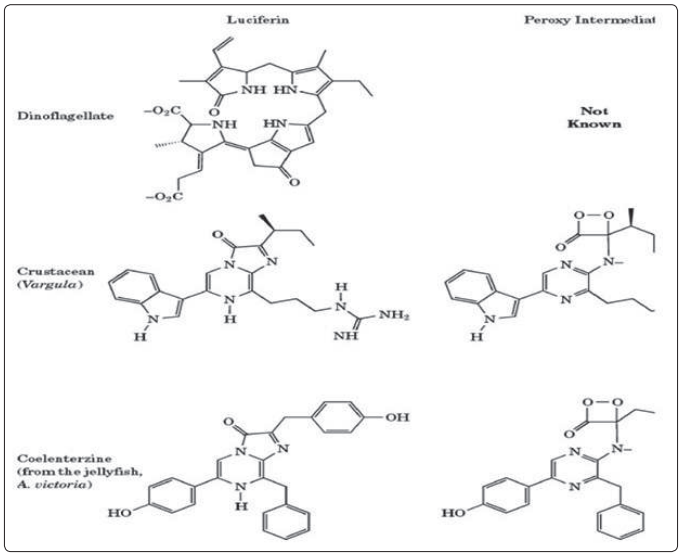

Figure 6: There are many types of luciferin (Adapted from Rees, J.-F., 1998 [11]

Firefly Luciferase

Possibly the largest part usually identified bioluminescent reaction is that of the general firefly. It is an outstanding reaction which gives up of almost 90 percentage (which means around 90 percentage of the reacting molecules will give off a photon of light). Figure7 shown the complete mechanism of action of luciferase in the firefly [12].

In figure 7 reaction the first step is like as to produce an adenylated product which activated by reaction with ATP with firefly luciferin. The C_4 proton is distracted and generate a form of luciferin as carbanionic form which is the form of luciferin that reaction with molecular O_2 to generate the key intermediate (e.g dioxetanone, an activated form) and this intermediate (activated dioxetanone) is not stable for the reason that in the four membered ring contain a high strain so the naturally weak peroxide bond [13]. The decomposition of this activated intermediate is normally supposed to happen by a Chemically Induced Electron Exchange Luminescence (CIEEL) mechanism (given below) and is go together with by loss of CO_2. The last product is the oxyluciferin which excited form, that can be present in both the keto and the enol forms of the excited oxyluciferin, which are accountable for releasing red and yellow-

green light, respectively [14].

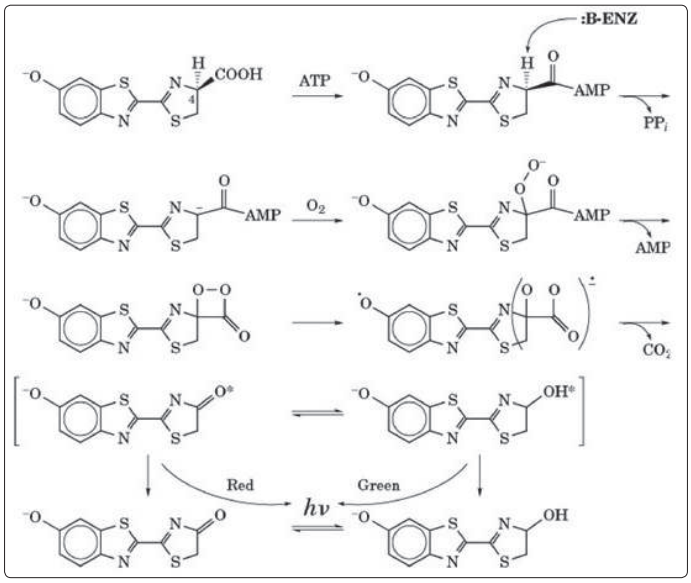

Figure 7: In the firefly the bioluminescence mechanism at a glance (Adapted from Close, D. M., 2009 [12]

During the CIEEL mechanism, an excited state is generated and this hypothesis stats that benzathiazole part perform as an electron rich donor (which is simply oxidizable) molecule which provide an electron to the peroxide part that assist the breakdown of the OO peroxide bond, giving an oxygen atom along with a complete negative charge and one neutral charge. The electron is return to donating part after loss of the CO_2 and yielding an excited state oxyluciferin [15].

Not only the firefly species but also a number of organism like as sea pansy, rnillareform is, sea crustacean cyprdinahilgedorfii and Jellyfish have luciferins of obviously dissimilar structure sto make use of a dioxetanone intermediate [16].

Bacterial Luciferase

Bacterial luciferase is one of the best bioluminescence techniques and there are many species of bacteria that are able to luminescent but the luciferases obtained from a variety of organisms are homologous. Bacterial bioluminescent does not have a luciferin molecule which is completely unlike to other bioluminescent systems and it is exclusive to the bioluminescence reaction. This system is mainly responsible for the conversion of $FMNH_2$ to FMN (oxidized flavin) and the subsequent carboxylic acid [17]. This technique require molecular oxygen which similar to other bioluminescent reactions.

The peroxide species leading to the light production is a linear relatively than a cyclic peroxide and it the most important dissimilarity between this reaction and most of the bioluminescent reactions [17]. Below is the overall chemical reaction:

$$FMNH_2 + O_2 + R\text{-}CHO \rightarrow FMN + H_2O + R\text{-}COOH + h\nu \quad (2)$$

Here, RCHO is the long chain aldehyde, RCOOH is the carboxylic acid and FMN is the oxidized flavin.

In above reaction the reactants have to combine to the enzyme in the specific sequence; first $FMNH_2$, then O_2 and then aldehyde. In figure 8 describe the complete design/scheme of the bacterial bioluminescence reaction [17]. The creation or generation of a 4a-peroxyflavin from $FMNH_2$ and $O_2$and it is a first step in the given reaction. After that the 4a-peroxyflavin and aldehyde reaction will happen and give a tetrahedral intermediate. Cleavage of peroxide bond by breakdown of the tetrahedral intermediate together with the development of an excited state of 4a hydroxyflavin and a RCOOH (carboxylic acid). The hydroxyflavin release light since it rest in the ground state and then go through dehydration to give up oxidized flavin (FMN) and water (H_2O).

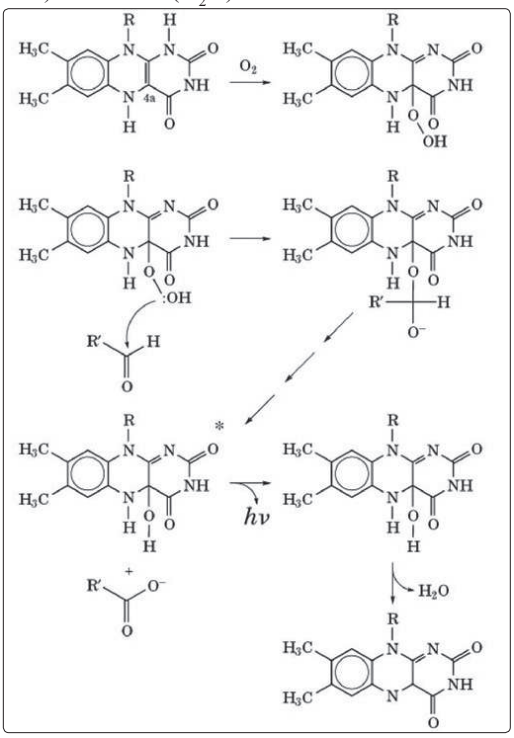

Figure 8: Bioluminescence mechanism in the bacteria (Adapted from Hastings, J. W., 1963 [17]

Applications

By genetic engineering Luciferase enzyme can be produced in the laboratory for different purposes. Firstly, synthesized luciferase genes and then it will be inserted into the organisms or transfected into cells. There are a few numbers of the organisms like mice, silkworms and potatoes which is used to produce the protein [18].

When luciferase enzyme works on the suitable luciferin substrate then light is emitted during the reaction takes place. Light sensitive apparatus like luminometer or modified optical microscope can detect the photons that are emitted from the luciferase reaction. And it is for the observation of biological system or processes.

Only very small (for example, the concentration is 0.02pg) amount can be precisely calculated by using of standard scintillation technique [19], because there is nominal auto fluorescence and thus practically background free fluorescence, Luciferase bioluminescence is not need the light excitation [20].

Luciferase is mainly used as a reporter to evaluate the transcriptional movement in cells that are transfected with a targeted genetic material that contain the luciferase gene under a control of promoter

of interest [21]. It is also used to determine another enzyme activity in the assay. There are many enzyme but like caspase activity, CYP450 activity and others [21].

In the kinase activity assays or in cell viability assays, also these enzymes are able to identify the cellular intensity of ATP and this ATP is the energy giving step, so this step is very important for cell [21,22]. The enzyme acts as an ATP sensor protein by biotinylation, which then luciferase will immobilize on the cell-surface by binding to a streptavidin-biotin complex which permit luciferase to identify the efflux of ATP from the cell and will successfully show the real time release of ATP during bioluminescence that is very important [23]. Luciferase can as well be prepared most susceptible for ATP revealing by raising the luminescence concentration by altering definite amino acid molecules in the sequence of the protein [24].

A very swift and susceptible assay has been confirmed which mainly base on the conventional dual luciferase reporter method that can be used as a very original technique to differentiate the smallest amount of promoter region of a gene. It is very convenience of this method for studying both constitutive (telomerase) and inducible (NF-kappaB-dependent) promoters [25].

It is a powerful technique in the whole animal imaging (both in-vivo and in-vitro imaging) for analyzing the cell population in live animals like mice [26]. There are many cells (e.g T cells, bone marrow stem cells, many cancer cell etc) can be modified to state the luciferase permitting their noninvasive image within the living animal cells by means of the susceptible charge-couple device camera that has been used to learn the tumorigenesis and reply of the tumours to healing in the animals model [27,28]. The strength of the signal can be calculated by in vivo imaging method that may depend on a variety of factors, such as d-luciferin corporation through the peritoneum, blood circulation, permeability of the cell membrane, co-factors availability, intracellular pH and clearness of overlying tissue, as well as to the amount of luciferase [29].

As luciferase enzyme is heat susceptible protein, which is applicable for studying the protein denaturation for checking the defensive capability of the heat shock proteins, so many many opportunities for using the enzyme [30].

Advantages and Disadvantages

Scientist generally detects the ATP for the application of bioluminescence and it is very oldest applications which are commonly used for the swift quantification of cell feasibility in the laboratory. As compared to conventional tetrazolium-based assays for the assay of mammalian cells analysis performed, the bioluminescence technique is about five minutes and is over 100-fold more sensitive. Similar assays for bacterial feasibility are susceptible to about 100 cells which depend on the type of cell. In the bioluminescence assays, ATP detection procedure may also be used to calculate the enzymes that consume ATP which is very important, the majority remarkably kinases which gives a practically widespread assay for kinase activity, in spite of either the phosphate acceptor is a protein, lipid or polysaccharide or not [21,31].

Luciferin may also be included into assay drawing in aapproach comparable to fluorogenic assays. The luciferin becomes occupied to the luminescent reaction until the modifier is removed during some biochemical process by joining a modifying group. For example, the luciferin derivative, Asp-Glu-Val-Asp-6′-aminoluciferin (DEVD-Aminoluciferin), cannot support luminescence until the tetrapeptide sequence is cleaved by the caspase-3 protease.

While rapid protein degradation is advantageous for genetic reporters to increase response dynamics, high enzyme stability is preferred when luciferase is a component of the assay reagent. Thus, for assays based on the ATP detection or luciferin, a modified version of luciferase has been designed for enhanced physical robustness. This stabilised luciferase, called Ultra-GlorLuciferase, is also resistant to chemical inhibitors such as those found in pharmaceutical compound libraries2.

Bioluminescence has already verified method to be adjustable for designing quick, sensitive and very simple assays for biochemical and cell based assays. Widely popularised for use in genetic reporters, bioluminescence has become regularly useful to a wide range of assay methodologies. These assays are generally recognised for their quantitative precision, low inherent backgrounds, and low sample interference [31].

Modifications

Modification is necessary to improve the activity of the enzyme. There are many technique to modify the efficacy and also the activity of the enzyme. Here present some basic technique such as (1) Mutations of Luciferase- Enhanced Activity and Thermostability, (2) Interaction with Hydrophobin-1-Increased thermostability and catalytic efficiency, (3) Coupling with other enzyme (Halophilic β-Lactamase)- Improved expression in bacteria, higher solubility and stability of luciferase, (4) Using Ionic Liquid Mediator- Improved Thermostability [32-35].

Here discuss about four above Modification of this enzyme.

1. Mutations of Luciferase- Enhanced Activity and Thermostability [32]

- First, mutation of the enzyme luciferase on amino acid then produce mutant product of these enzyme like Mutant E which is very thermostability of the enzyme and also produce Mutant product LGR which is very catalytic activity (Figure 9).
- When the combination of the two mutant product of Mutant E and Mutant LGR, produce another mutant (YY5) product which is composed of 8 amino acid and it vary from the first form (wild-type-WT) of luciferase thatrevealed both superior thermostability and brilliant luminescence at a very low concentration of luciferin. As a result, the final mutant product of YY5 possibly will be functional for reporter gene applications (Figure 9) [32].

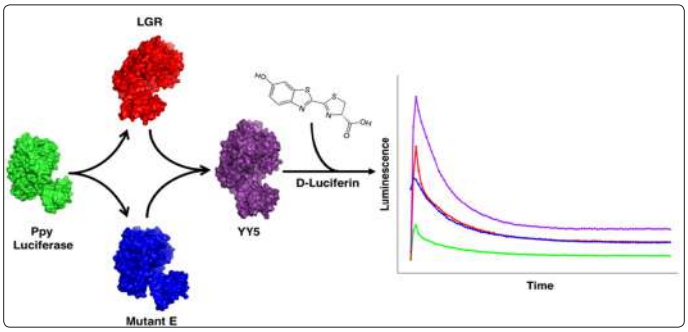

Figure 9: Modification through Mutation of Luciferase (Adapted from Tania Pozzo, et al. 2018 [32]

2. Interaction with Hydrophobin-1-Increased thermostability and catalytic efficiency [33]

- Hydrophobin 1, (HFB1) is a small protein (it is secretory protein)and the molecular weight is 7.5 kDa.
- When the HFB1 interacts with luciferase enzyme (with the hydrophobic site of the enzyme)
- Byadding the HFB1 directly to the luciferase enzyme solution then improve the catalytic effectiveness of luciferase and consequently develop the usefulness of the enzyme.
- Which leads to increase the thermostability and catalytic efficiency of the lucuferase enzyme

3. Coupling with other enzyme (Halophilic β-Lactamase)-Improved expression in bacteria, higher solubility and stability of luciferase [34]

- A classic halophilic enzyme for example AmpCβ-lactamase (HaBLA-which is very soluble either in native or in heat denatured structures), from a bacteria of Chromohalobacter which is assemble like aggregation-prone protein .
- This fusion technique is the highly powerful technique.
- Finally this halophilicβ lactamase enzyme gives to higher solubility and stability of the firefly luciferase as a lactamase and luciferase fusion protein product.

4. Using Ionic Liquid Mediator- Improved Thermostability [35]

- By adding 1, 1, 3, 3-tetramethylguanidine (TMG) and acetate to the enzymatic solution then luciferase shown anenhanced activity and increased thermal stability.
- On the other hand, the enzyme has revealed decreased and unchanged activity when add TMG and trichloroacetate and also add TMG and triflouroacetate.

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