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Luciferase Enzyme and its Application

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Abstract

Aim: The aim of this review is to know about luciferase enzyme and its application

Background : Luciferase is a generic term for the class of oxidsative enzymes that produce bioluminescence and is distinct from a photoprotein. "Firefly luciferase" as a laboratory reagent often refers to P. pyralis luciferase although recombinant luciferases from several other species of fireflies are also commercially available. Firefly luciferase is a euglobulin protein that catalyses the oxygenation of luciferin using ATP and molecular oxygen to yield oxyluciferin, a highly unstable, singlet-excited compound that emits light upon relaxation to its ground state. Luciferase can act as an ATP sensor protein through biotinylation. Bioluminescence assay systems have become increasingly used in biology and medical research laboratories in addition to (or as alternatives to) fluorescence and chemiluminescence detection strategies. Luciferase enzymes isolated from different animal species have inherent variability in light emission, allowing two or more luciferase enzymes to be used in combination for multiplex analyses, including in vivo imaging, cell viability and single and dual-spectral luciferase reporter assays.

Reason: This review is made to know in detail about the Luciferase enzyme uses and its application in diagnosis

Keywords: *Luciferase enzyme, application, structure, bioluminance imaging, Green fluorescent protein*

Introduction

The term for the class of oxidative enzymes that produce bioluminescence, and is distinct from a photoprotein is. The name is derived from Lucifer, the root of which means 'light-bearer'. One example is the firefly luciferase from the firefly Photinus pyralis[1].

One well-studied luciferase is that of the Photinini firefly Photinus pyralis, which has an optimum pH of 7.8. Firefly bioluminescence color can vary between yellow-green [2]. Firefly luciferase are used to study the role of chaperones in protein folding [3] and of bacterial luciferase to study co-translational folding of polypeptides [4] and of the genes encoding luciferases to monitor transcriptional activities [5,6].

Luciferases from different organisms probably evolved independently, rather than from a common ancestral enzyme. Bacterial luciferase, the first luciferase to be cloned and also the first to be structurally characterized, is a flavin monooxygenase that utilizes flavin mononucleotide (FMN) to activate molecular oxygen, yielding a flavin C4a peroxide [7]. Luciferase is a light-producing enzyme naturally found in insect fireflies and in luminous marine and terrestrial microorganisms. the luciferase and other light-emitting photons a visualizing marker/reporter has drastically expanded the versatility of reporter gene technology. luciferase gene fusion product in expression confers on the host the ability to glow in the dark. The transcriptional and

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translational expression of the attached foreign gene, and enables one to localize it to particular domains, cells, or organelles of almost any organism. This can be done noninvasively, with inexpensive, nonisotopic, easily available substrates, and at extremely high sensitivity under no significant endogenous background.

Two types of luciferase genes cloned from bacteria and firefly are used as sensitive reporter systems in a wide variety of cells such as bacterial, yeast, insect, animal, and plant cells. Bacterial luciferases are flavoenzymes composed of two subunits each encoded by the *lux A* and *lux B* genes, while the firefly luciferase is a single polypeptide specified by the *lux* gene. The two types of luciferase catalyze different reactions. The bacterial luciferase oxidizes decanal (and some homologous long-chain aldehydes) with the energy transfer from $FMNH_2$ and produces blue-green light with an absorption maximum at 490 nm. The firefly enzyme couples the oxidation of luciferin with the energy transfer from ATP and produces yellow–green light with a pH-dependent absorption maximum. The bacterial and firefly luciferase systems present respective advantages and disadvantages associated with inherent differences in substrate profiles and enzyme structures. Recent developments and improvements on the two luciferase systems have rendered the two systems almost equally amenable to a wide variety of applications. To utilize the reporter systems to their full capacity, however, one has to make a judicious choice based on an understanding of their respective characteristics.

This section describes the bacterial luciferase (Lux) in the first part and the firefly luciferase (Luc) in the second part [8,9].

Structure of Luciferase

The fold assumed by the luciferase polypeptide appears to be unique. The N-terminal domain consists of a β barrel and two β sheets flanked by α helices which form a five-layered αβαβα structure. The C-terminal domain, consisting of five β strands and three α helices, is folded into a compact structure that is connected to the N-terminal domain by a disordered loop (connecting residues 435 and 441). There are three other disordered loops not visible in the electron density, one in the C-terminal domain connecting residues 523 and 529, and two in the N-terminal domain (connecting residues 198–204 and residues 355–359). Conti et al. have taken advantage eof the homology of firefly luciferase with other enzymes that catalyze similar reactions [8,9]. In the large N-terminal domain of the molecule, the central regions of the two -sheet subdomains share a similar structure. These regions can be superposed such that 87 pairs of topologically equivalent -carbon atoms, have a separation ≤2.5 Å, giving an overall rms separation of 1.6 Å. The two 'modules' are approximately related by twofold symmetry with a rotational component of 178.4° and a translational component of 2.0 Å[10].

Reaction catalysed by luciferase

Firefly luciferase catalyzes a multistep reaction [11]. In the first step, luciferin reacts with Mg2+-ATP to form luciferyl adenylate and pyrophosphate. The luciferyl adenylate is oxidized by molecular oxygen, with the intermediate formation of the cyclic peroxide, a dioxetanone and a molecule of AMP. The is decarboxylated as a result of intramolecular conversions to produce an electronically excited state of oxyluciferin in the enol or keto form. Return to the ground state is accompanied by emission of a quantum of visible light with a wavelength of maximum light intensity of 562–570 nm[12] demonstrated that one oxygen atom of the product CO2 arises from the substrate oxygen. Non-enzymatic oxidation of luciferin yields oxyluciferin without luminescence[13]. The appearance of bioluminescent light varies greatly, depending on the habitat and organism in which it is found. Most bioluminescence, for instance, is expressed in the blue-green part of the visible light spectrum. These colors are more easily visible in the deep ocean. Also, most marine organisms are sensitive only to blue-green colors. They are physically unable to process yellow, red, or violet colors. Most land organisms also exhibit blue-green bioluminescence. However, many glow in the yellow spectrum, including fireflies and the only known land to bioluminesce, native to thetropics of Southeast Asia. Few organisms can glow in more than one color. The so-called railroad worm (actually the larva of a beetle) may be the most familiar. The head of the railroad worm glows red, while its body glows green. Different luciferases cause the bioluminescence to be expressed differently[14].

Application of luciferase enzyme

Luciferase can be produced in the lab through for a number of purposes. Luciferase genes can be synthesized and inserted into organisms or transfected into cells. Mice, silkworms, and potatoes are just a few of the

organisms that have already been engineered to produce the protein[15]. All applications of bioluminescence systems are based on the principle of a chemical reaction; that is, the light intensity as the measurable product depends on the amounts of luciferase, luciferin, and cofactor(s). Using beetle bioluminescence as an example, in the presence of excess luciferin and luciferase, the bioluminescence intensity correlates with the amount of ATP, producing a beetle bioluminescence system that can measure the amount of ATP[16]. This system can be applied to detecting bacteria in food, because bacteria contain ATP as an energy source[17].

Bioluminescent organisms are a target for many areas of research. Luciferase systems are widely used in genetic engineering as reporter genes, each producing a different colour by fluorescence, and for biomedical research using bioluminescence imaging. For example, the firefly luciferase gene was used as early as 1986 for research using transgenic acceptor into a single polypeptide can also allow the detection of ligand-induced conformational switches in monomeric proteins in the millisecond time scale. Many of these approaches are amenable to high throughput screening and the drug discovery process. G protein-coupled receptors (GPCRs) represent a key drug target class. Specific applications of energy transfer techniques to the identification of ligands for this class of protein are highlighted to illustrate general principles.

Bioluminescence imaging

has emerged as a powerful new modality for studies of viral infection and therapy in small animal models. BLI technology captures the light emitted from different luciferase enzymes to detect sites of viral infection and quantify viral replication in the context of a living animal [18].

Green fluorescent protein

The technical revolution resulting from the discovery of relates to a miraculous property of the chromophore that is responsible for its fluorescence. This chromophore is formed spontaneously from a tri-peptide motif in the primary structure of , so that its fluores- cence is "automatically" turned on in every organism where it is expressed. In other words, the maturation of the tri-peptide-based chromophore in only requires oxygen and does not depend on the presence of enzymes or other auxiliary factors. and its related variants thereby provide universal genetic tags that can be used to visualize a virtually unlimited number of spatio-temporal processes in virtually all living systems. This revolution in the biological sciences has been greatly accelerated by a rapid parallel development of quantitative light microscopy, electronics, computational power and molecular modelling of intra- and inter-cellular processes with systems-biology approaches[19].

Bioluminescence and fluorescence resonance energy transfer

A limitation of is the requirement for external illumination to initiate the fluorescence transfer, which can lead to background noise in the results from direct excitation of the acceptor or to photobleaching. To avoid this drawback, Bioluminescence Resonance Energy Transfer has been developed[20,21].

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Conflict of Interest: Nil

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