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Review

The emerging use of bioluminescence in medical research

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ABSTRACT

Bioluminescence is the light produced by a living organism and is commonly emitted by sea life with Ca^{2+}-regulated photoproteins being the most responsible for bioluminescence emission. Marine coelenterates provide important functions involved in essential purposes such as defense, feeding, and breeding. In this review, the main characteristics of marine photoproteins including aequorin, clytin, obelin, berovin, pholasin and symplectin from different marine organisms will be discussed. We will focused on the recent use of recombinant photoproteins in different biomedical research fields including the measurement of Ca^{2+} in different intracellular compartments of animal cells, as labels in the design and development of binding assays. This review will also outline how bioluminescent photoproteins have been used in a plethora of analytical methods including ultra-sensitive assays and in vivo imaging of cellular processes. Due to their unique properties including elective intracellular distribution, wide dynamic range, high signal-to-noise ratio and low Ca^{2+}-buffering effect, recombinant photoproteins represent a promising future analytical tool in several in vitro and in vivo experiments.

1. Introduction

Bioluminescence is the primary source of light deep under water and is a natural chemical reaction where visible light is emitted. The famous English chemist, Boyle, was the first to discover the importance of oxygen for bacterial bioluminescence in 1667 [1]. In 1969, a German chemist, Brand, conducted the first study on the light production process [2]. Novel research on the bioluminescence phenomenon began in 1885 when Dubois studied the luminescent beetle *Pyrophorus* sp. and later, Harvey discovered the luciferin-luciferase system in marine crustacea *Cipridina sp.* in l917 [1] (Fig. 1). Subsequently, Shimomura and his colleagues identified an unusual bioluminescent protein in jelly fish *Aequorea victoria* and named it "Aequorin" in 1961 [3]. Collectively these light -emitting proteins in light-emitting organisms, are named "photoproteins" [4,5].

Aequorin is the most common bioluminescent photoproteins emitted by marine coelenterates and is composed of a single polypeptide chain with high sequence homology. Although, original research utilizing bioluminescent properties began with terrestrial organisms (fire-flies) [6], studies have now expanded into using marine organisms such as bacteria [7,8], copepods [9], and cnidarians [10]. Along with a high variety of luminescent organisms, there is also approximately 30 naturally occurring bioluminescence systems [11]. Currently, chemical pathways for the emission of light have been divided into two types of living organisms: 1. The classical luciferin–luciferase system where luciferin acts as the substrate and the light emitting molecule which is oxidized by the luciferase enzyme, in the presence of oxygen and/or cofactors [1,12]. 2. Luciferins such as aequorin generates light in conjunction with the pre-charged enzymatic- oxygen complex [13]. Aequorin can emit light in the absence of both calcium and oxygen. This phenomenon does not explicitly follow the classic concept of the luciferin-luciferase system [5]. Moreover, another significant difference of aequorin with the luciferase enzyme is the proportion of emitted light with the total amount of protein required. In contrast, in a luciferin-luciferase reaction, the total emitted light is proportional to the amount of luciferin available [5,13]. The subsequent binding with cofactors, such as Ca^{2+} or Mg^{2+} gives the organism a mechanism to precisely regulate the level of light emission.

The substrates for bioluminescent reactions (commonly known as luciferins meaning light bearers) [11], vary substantially in chemical structure across different organisms. The substrates include FMNH2 in bacteria, tetrapyrrole in dinoflagellates, coelenterazine in cnidarians, N-isovaleryl-3-amino propanal in annelids, enol formate in molluscs, (benzo) thiazole in insects and imidazopyrazine nucleus in crustacea

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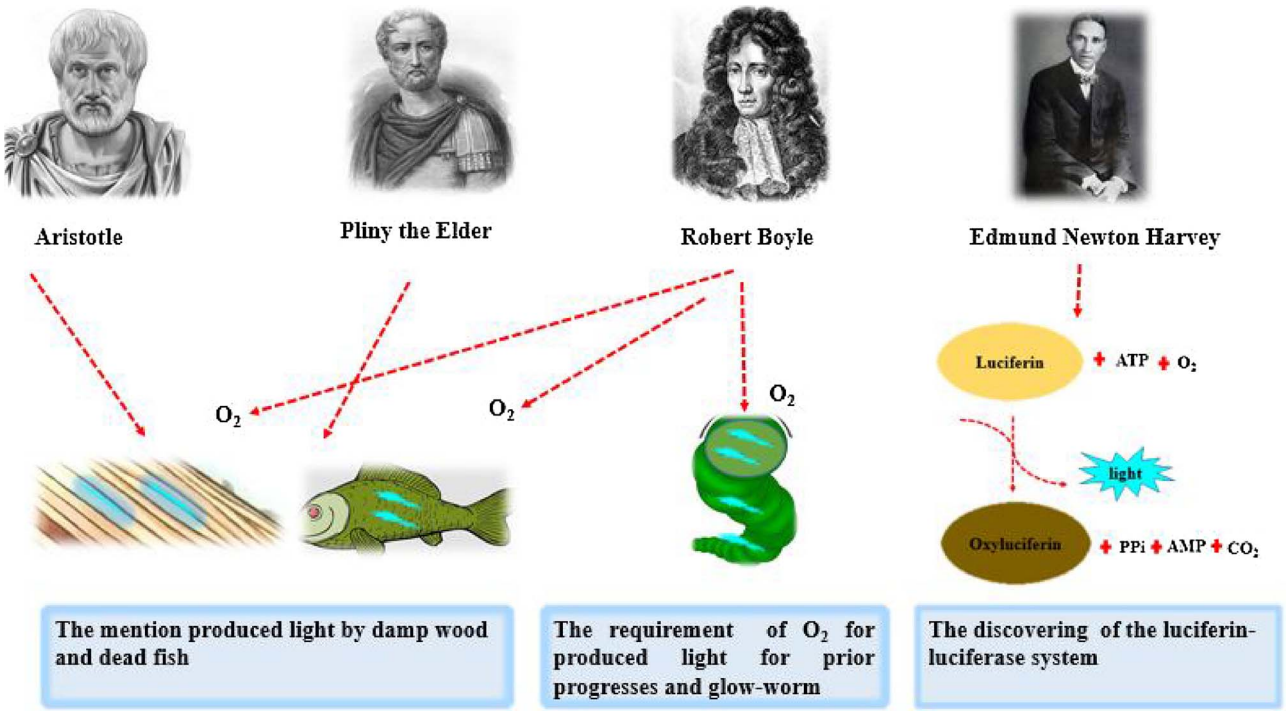

Fig. 1. The history of bioluminescence from the observations of Aristotle to the discovering of the luciferin-luciferase system by Harvey. The glowing of damp wood and dead fish was mentioned by both Aristotle and Pliny the Elder. Later, Boyle showed the involving of oxygen in the process including wood and in glow-worms. Finally, the acting of light-emitting substances known as luciferins on by enzymes called luciferases was discovered by Harvey.

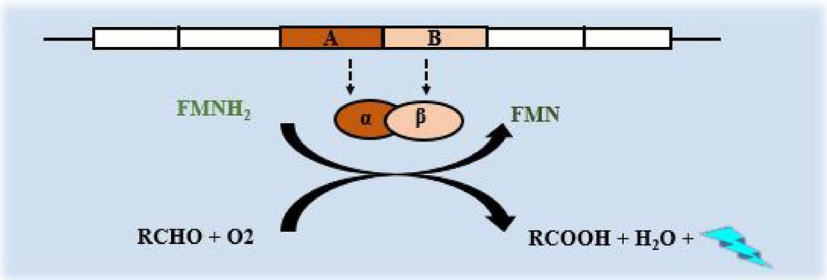

Fig. 2. The schematic view of bacterial bioluminescence that light-emitting reaction is occurred using substrates reduced flavin by heterodimeric luciferase (luciferase composing two homologous α- and β-subunits encoded by the luxA and luxB genes, respectively), long chain aldehyde, and oxygen and produces oxidized flavin, carboxylic acid, and water that associated with emission of blue-green light around 485–490 nm and quantum yield approximately 10–16% reported from well-studied two genera, *Vibrio* and *Photobacterium*.

[11]. In bioluminescent bacteria, a flavin-dependent monooxygenase catalyzes a light-emitting reaction by using substrates including reduced flavin, long chain aldehyde, and oxygen and produces oxidized flavin, carboxylic acid, and water as products with concomitant emission of bluegreen light around 485–490 nm (Fig. 2). The production of light is either continuously or rapidly transient with the latter requiring the triggering proprerties such as a rapid pH change within an organelle present in the organism, the presence of oxygen, and the binding to calcium ions [14].

Photoproteins are known as bioluminescent proteins that emit a rapid flash of light upon binding to calcium ions [15]. Photoproteins however, do not turn over enzyme as the emitted light is derived from the charged photoprotein. Moreover, photoproteins can operate as the sole organic molecular species in bioluminescent reaction systems as they do not require oxygen or other cofactors [11].

Over the past several decades, marine bioluminescence has attracted growing interest among different scientific fields including comparative biology [16], biochemistry [17], physiology [18], neuroscience [19], population dynamics [20], and naval applications [21]. Up to now, the literature contains more than 350,000 references utilizing applications for bioluminescence represented in 553 phylogenetic concepts and distributed within 13 broader taxonomic categories [22]. Bioluminescence, the phenomenon of emitting light by animals, is a major factor in ecological interactions and an important ecological trait in organisms found on at ground level and deep within the sea [22]. The evolution of bioluminescence over time from bacteria to fish powerfully influences behavioral and ecosystem dynamics [23]. Marine organisms use bioluminescence for important physiological functions including mating, the recycling of nutrients, concealment and defense [24]. Approximately, 76% of marine organisms in the deep sea including crustaceans, cnidarians, cephalopods, echinoderms, annelids, dinoflagellates and bacteria possess bioluminescence capabilities [22]. Interestingly, there is a large spectrum of bioluminescence observed in organisms that live in deeper water such as planktonic compared to low levels in benthic or shallow species.

During the past 20 years, there has been a shift of research interest from chemistry and biochemistry into genetic biotechnology [5]. High detectability and the rapid speed of bioluminescence spectroscopy have assisted in making advances in molecular biology. These properties allow for the monitoring of biological processes longitudally in clinical, diagnostic and drug discovery applications [25]. Because of these unique properties, bioluminescence can be used as ultrasensitive and selective bioanalytical tools [25]. Indeed, ultrasensitive binding assays and cell-based assays can be made through bioluminescent proteins [26]. Bioluminescence has also been used as a critical analytical tool in the environmental sciences [27].

While marine organisms are a potentially important source of marine photoproteins, the majority of studies use bioluminescent