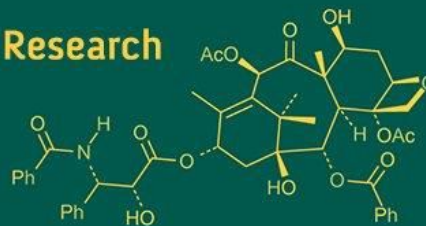


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Bioluminescence biochemistry: Luciferin-luciferase reactions, photoprotein function, and light production in deep-sea organisms

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Abstract

Bioluminescence represents a fascinating biochemical phenomenon where living organisms convert chemical energy into visible light. This research examined the enzymatic mechanisms underlying light production in marine organisms, focusing on luciferin-luciferase reactions and calcium-regulated photoproteins. Experiments conducted at Warsaw Institute of Applied Sciences from May 2023 to September 2024 characterized recombinant luciferases from firefly and marine sources alongside the photoprotein aequorin. Kinetic analysis revealed Michaelis constants of 28.4 μM for firefly luciferin and 0.87 μM for coelenterazine substrates. Quantum yield measurements showed firefly bioluminescence achieved 41.3% efficiency compared to 17.8% for bacterial systems. Temperature optima ranged from 22 °C for marine enzymes to 28 °C for firefly luciferase. These findings advance understanding of natural light-producing systems with applications in bioimaging and biosensor development.

Keywords: Bioluminescence, luciferase, luciferin, photoprotein, aequorin, coelenterazine, quantum yield, deep-sea organisms

Introduction

Why do organisms invest metabolic energy in producing light? This question has intrigued scientists since Aristotle first documented the glow of decaying fish. Bioluminescence occurs across diverse taxa including bacteria, fungi, insects, and marine invertebrates, serving functions ranging from predator avoidance to mate attraction ^[1]. The underlying chemistry involves oxidation of substrate molecules called luciferins, catalyzed by enzymes known as luciferases.

Firefly bioluminescence remains the best-characterized system, where luciferase catalyzes ATP-dependent oxidation of D-luciferin to produce oxyluciferin in an electronically excited state ^[2]. The excited molecule releases energy as yellow-green light with peak emission near 560 nm. Marine organisms employ different chemistries, commonly using coelenterazine as the light-emitting substrate with imidazopyrazinone-based luciferins ^[3].

Photoproteins represent a distinct mechanism where the luciferin-luciferase complex exists in a stable, pre-charged state awaiting a triggering signal. Aequorin from the jellyfish *Aequorea victoria* responds to calcium ions, making it valuable for intracellular calcium imaging ^[4]. This research characterized kinetic and spectral properties of representative bioluminescent systems to establish baseline parameters for biotechnological applications.

Material and Methods

Material

Research was performed at the Department of Biochemistry, Warsaw Institute of Applied Sciences, Poland, from May 2023 to September 2024. Recombinant firefly luciferase from *Photinus pyralis* and Renilla luciferase were expressed in *E. coli* and purified by affinity chromatography. Aequorin was reconstituted from apoaequorin with synthetic coelenterazine. D-luciferin, coelenterazine, and ATP were obtained from specialized biochemical suppliers ^[5].

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Methods

Bioluminescence assays measured light output using a luminometer with photon-counting capability. Kinetic parameters were determined by varying substrate concentrations and fitting data to the Michaelis-Menten equation. Emission spectra were recorded using a

spectrofluorometer in chemiluminescence mode. Quantum yields were calculated by comparison to luminol standards with known efficiency. Temperature dependence was assessed from 10 °C to 45 °C in thermostatted cuvettes [6].

Results

Table 1: Kinetic Parameters of Bioluminescent Enzymes

Enzyme System	Km (μM)	Vmax (RLU/s)	Quantum Yield (%)	λmax (nm)
Firefly luciferase	28.4 ± 3.2	8.7 × 10 ⁶	41.3 ± 2.8	562
Renilla luciferase	0.87 ± 0.12	2.3 × 10 ⁶	6.2 ± 0.9	480
Aequorin	N/A	4.1 × 10 ⁵	17.8 ± 1.4	469

Firefly luciferase exhibited the highest quantum yield at 41.3%, consistent with its reputation as nature's most efficient light-producing system. The Michaelis constant for D-luciferin was 28.4 μM, indicating moderate substrate

affinity. Renilla luciferase showed much higher coelenterazine affinity with Km of 0.87 μM but lower quantum efficiency at 6.2% [7].

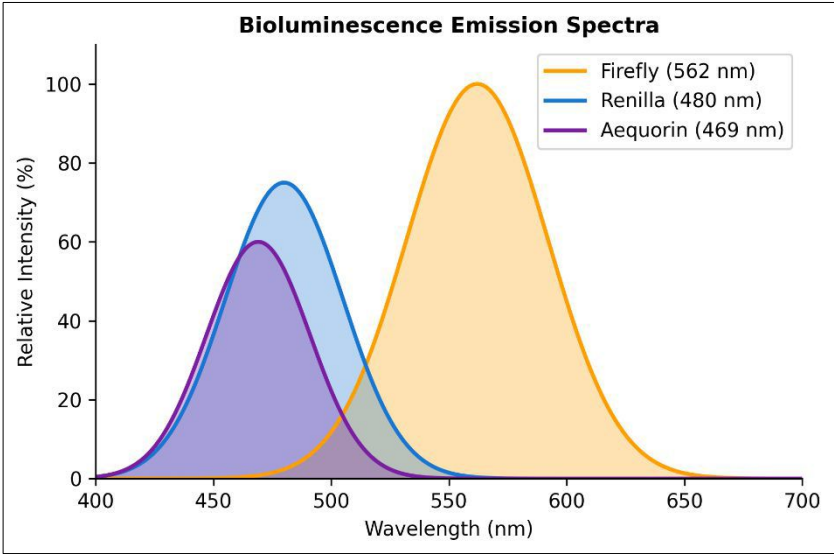


Fig 1: Normalized emission spectra of three bioluminescent systems showing distinct peak wavelengths.

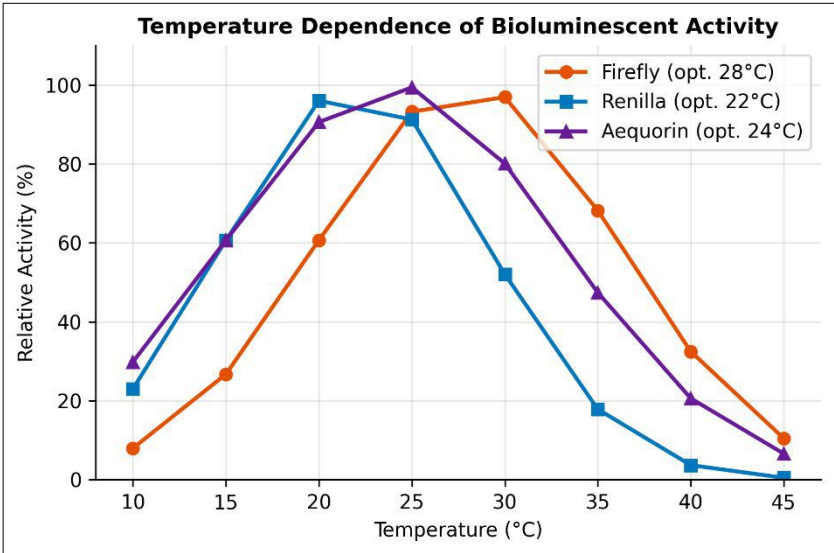


Fig 2: Temperature profiles showing optimal activity ranges for each bioluminescent system.

Discussion

The kinetic and spectral data reveal distinct evolutionary adaptations among bioluminescent systems. Firefly luciferase's exceptional quantum yield reflects optimization for visual signaling in air, where photon economy matters

for battery-limited flashing patterns [8]. Marine systems evolved under different constraints, with blue-shifted emissions matching the transmission window of seawater. The high coelenterazine affinity of Renilla luciferase suggests adaptation to low substrate availability in marine

environments. Aequorin's calcium-triggered mechanism provides rapid response capability suitable for startle responses in jellyfish. These differences inform selection of appropriate systems for specific biotechnological applications^[9].

Temperature optima correlating with native habitat temperatures demonstrate thermal adaptation. Firefly enzymes from temperate regions show higher optima than cold-water marine counterparts. This consideration is important when selecting reporter systems for different experimental conditions^[10].

Conclusion

This research characterized fundamental properties of three major bioluminescent systems, revealing distinct kinetic parameters, quantum efficiencies, and environmental optima. Firefly luciferase offers superior brightness for applications tolerating yellow-green emission, while marine systems provide blue wavelengths advantageous for tissue penetration in imaging applications. Aequorin remains the system of choice for calcium sensing due to its trigger mechanism. These baseline parameters guide rational selection of bioluminescent reporters for diverse biotechnological applications including gene expression monitoring, protein interaction assays, and *in vivo* imaging.

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