EARTHWORMS AS A SOURCE OF NEW APPROACHES IN BIOMEDICAL RESEARCH

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Traditional medicine has been using earthworms to treat a wide range of pathological conditions for many centuries. Current research focuses primarily on the isolation and characterization of biologically active compounds, including notable examples such as glycoprotein G90, which exhibits thrombolytic and antitumor properties; the enzyme complex lumbrokinase; and the extracellular oxygen transport protein erythrocruorin, regarded as a potential basis for blood substitute development. Model organisms such as *Caenorhabditis elegans*, which are used to study the mechanisms of aging, neurodegenerative diseases, and evaluate the effectiveness of medicines, also play an important role. Bioluminescent systems of annelids are of particular interest because they exhibit a variety of chemical mechanisms of luminescence and unique cofactors that hold promise for applications in biomedicine. The combination of these properties and abilities underscores the importance of worms as a source of enzymes, proteins, and metabolites, as well as model systems for finding solutions to both fundamental and applied biomedical problems.

Keywords: bioluminescence, biomedical research, earthworms, *Henlea*, C-mannosyl tryptophan

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ПОЧВЕННЫЕ ЧЕРВИ КАК ИСТОЧНИК НОВЫХ ПОДХОДОВ В БИОМЕДИЦИНСКИХ ИССЛЕДОВАНИЯХ

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Применение почвенных червей в традиционной медицине имеет многовековую историю и охватывает широкий спектр патологических состояний. Современные исследования сосредоточены в основном на выделении и изучении биологически активных соединений. Среди значимых примеров можно выделить гликопротеин G90 с тромболитическими и противоопухолевыми свойствами, комплекс ферментов люмброкиназу, а также внеклеточный кислородтранспортный белок эритрокруорин, рассматриваемый как основа для разработки заменителей крови. Важную роль играют и модельные организмы, такие как *Caenorhabditis elegans*, используемые для изучения механизмов старения, нейродегенеративных заболеваний и оценки эффективности лекарственных средств. Особый интерес представляют биолюминесцентные системы кольчатых червей, продемонстрировавшие разнообразие химических механизмов свечения и наличие уникальных кофакторов, перспективные для развития практических приложений биомедицины. Совокупность этих направлений подчеркивает значимость червей как источника ферментов, белков, метаболитов и модельных систем, способных решать как фундаментальные, так и прикладные задачи в биомедицине.

Ключевые слова: биолюминесценция, биомедицинские исследования, почвенные черви, *Henlea*, C-маннозил триптофан

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Earthworms have long been used in traditional medicine for the treatment of various diseases. Extracts from various worm species were prescribed for allergic and asthmatic conditions, to lower blood pressure and detoxify the body, and more generally in cases requiring antithrombotic, antipyretic, diuretic, or antispasmodic treatment [1]. The development of biochemical and pharmacological analysis methods allowed to translate these empirical observations into systematic research and to purposefully isolate biologically active compounds of animal origin. For example, in 1992, glycoprotein G90, which has thrombolytic and antitumor properties, was isolated from the homogenate of earthworm tissues. Lumbrokinase, a complex of fibrinolytic enzymes also sourced from earthworms, is used as a dietary supplement supporting cardiovascular system — it can improve blood circulation and dissolve blood clots [1]. In addition to enzyme complexes, there is erythrocruorin, an extracellular molecular complex that is a functional analog of hemoglobin; isolated from annelids, it is a promising base for the development of blood substitutes [2]. Erythrocruorin is considered as a potential next-generation oxygen carrier for the following reasons: it is an extracellular protein with high stability and low susceptibility to oxidative processes, it maintains stability across a wide temperature range, and it can bind and transfer nitrogen monoxide, reducing the risk of vasoconstriction [3]

Currently, worms are considered not only as a source of biologically active compounds and protein complexes but also as model organisms. One of the species fit for this purpose is a soil nematode *Caenorhabditis elegans*. Short life cycle, transparent body, fully sequenced genome, low cultivation requirements, and ability to produce numerous offspring in a short time through self-fertilization make this model a popular choice for studies on aging processes, the pathogenesis of neurodegenerative diseases such as Alzheimer's disease, and the testing of antitumor and antimicrobial drugs [4].

Current biotechnology progress allowed to exploit another unique biological phenomenon characteristic of annelids — bioluminescence, the emission of light by living organisms — which is used in biomedical research. Most of the two dozen luminous annelids live in tropical regions, but some are found in Siberia, where their glow can be observed at night with the naked eye. Practical significance of studying bioluminescent systems lies in the creation of new analytical tools and the improvement of molecular imaging methods. Bioluminescent bioimaging offers several advantages over fluorescent methods: it does not require an external excitation source, thereby preventing autofluorescence, produces a low background signal, provides high sensitivity and allows detection of emission at the level of individual cells [5]. Currently, only a few insect and marine luciferins are used, including D-luciferin, coelenterazine, and its synthetic analog furimazine [6]. However, the expansion of the set of luciferin-luciferase pairs for multicolor imaging, which allows simultaneous monitoring of various molecular processes, is an actively discussed subject [7].

For a long time, it was believed that there is only *Diplocardia longa* peroxide-dependent mechanism (λ_{max} = 490 nm) that is responsible for the bioluminescence of all earthworms (Fig. 1A) [8]. With *D. longa*, the luminescence is the result of oxidation of a low-molecular-weight luciferin substrate, N-isovaleryl-3-aminopropanal, in the presence of the luciferase enzyme. However, the discovery of three new species in Siberia — *Fridericia heliota*, *Henlea petushkovi*, and *Henlea rodionovae* — has shown that annelids have at least two other distinct luminescence systems [9]. *F. heliota's* luciferin (λ_{max} = 478 nm) is a tetrapeptide oxidized by oxygen in the presence of ATP, Mg^{2+}, and luciferase (Fig. 1B). In *Henlea sp*. (λ_{max} = 464 nm), luciferin has tryptophan residue and is oxidized by oxygen in the presence of Ca^{2+} and a luciferase [8] (Fig. 1B). The additional luminescence enhancer in this system is a cofactor F0 (ActH), a structural analog of riboflavin, which receives energy from excited oxyluciferin and re-emits it, the bioluminescence is amplified 33-fold and shifts from 410 to 464 nm, using the Förster Resonance Energy Transfer (FRET) mechanism [10, 11]. Due to the high stability of luciferin and the presence of an enhancer cofactor, it is the *Henlea sp*. bioluminescent system that is of particular interest as a base for the development of new platforms for bioluminescent bioimaging and multicolor monitoring of intracellular events.

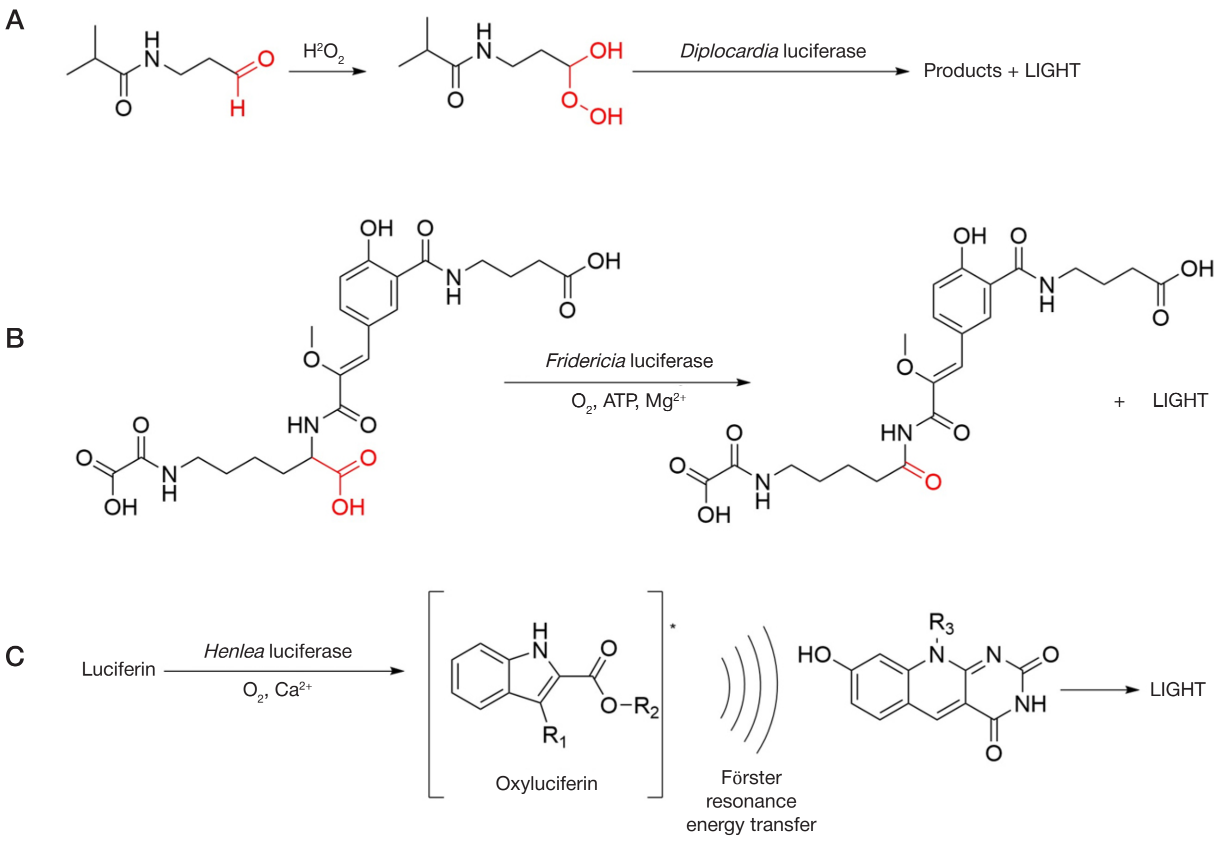

Fig. 1. Bioluminescent reactions of *D. longa* (**A**), *F. heliota* (**B**), *Henlea sp.* (**C**)

Additionally, analysis of *Henlea sp*. metabolites revealed a significant amount of α-C-Mannosyltryptophan (ManTrp), a new compound to bioluminescent systems. The absorption and fluorescence spectra of ManTrp coincided with those of *Henlea's* luciferin, therefore ManTrp was considered as its probable metabolic precursor. In nature mannosyltryptophan occurs either as a free ManTrp monomer or as part of a protein's polypeptide chain, resulting from an unusual C-glycosylation at the C2 position of the indole ring. The mechanism of C-glycosylation in a polypeptide chain was discovered in 1994 using human RNase. Monomeric ManTrp was found in blood and urine of humans and other mammals, including mice and rats, as well as some sea sponges [12]. The concentration of ManTrp in blood plasma increases in a number of pathologic conditions, including myelofibrosis, type 2 diabetes mellitus, chronic kidney disease, ovarian cancer, and platelet growth dysregulation. Thus, ManTrp can be considered as a promising biomarker for the diagnosis of oncological diseases.

One of possible options of practical application of the *Henlea sp.* luciferin-luciferase system is using it in a bioluminescent test system for the diagnosis of ovarian cancer based on specific recognition of ManTrp. The change in the BRET index associated with ManTrp binding can be exploited as a highly sensitive diagnostic signal with ActH/F0 cofactor acting as luminescence enhancer. Similar strategies found practical implementation in "luciferase-nanoparticle" systems in BRET-activated photodynamic therapy [13]. Moreover, stable hybrid constructs combining gold-based nanoparticles, the luciferase enzyme (including *Phrixotrix hirtus*) and a photosensitizer were created. These bioconjugates exhibit a stable bioluminescent signal and retain their functional activity in cellular conditions [14]. The results demonstrate that luciferase systems can be integrated into nanoplatforms and enable both visualization and activation of therapeutic processes [15]. This opens up the possibility of creating molecular theranostics systems that use ManTrp as a diagnostic marker and an activating trigger for the bioluminescent system in future.

Thus, sensors based on the *Henlea* sp. bioluminescent system and ManTrp specific recognition can serve as a basis of new platforms for early diagnosis and monitoring of ovarian cancer and other metabolic disorders.

CONCLUSION

The use of earthworms in medicine has evolved beyond traditional practices: once material for folk remedies, they are currently screened for biologically active compounds using modern scientific methods. Today, earthworms and nematodes are considered as sources of enzymes and protein complexes, as well as model systems for finding solutions to both fundamental and applied biomedical problems.

Bioluminescent systems of annelids are of particular interest: they exhibit a variety of chemical mechanisms of luminescence and have unique cofactors. Studying them not only deepens the understanding of the evolution and biochemistry of luminescence, but also opens the way to the creation of new molecular imaging tools [16]. In particular, the combination of luciferin-luciferase systems with energy transfer mechanisms (e.g., FRET) makes them potentially applicable in photodynamic therapy (PDT), especially in cases when it is necessary to generate light directly inside body cells. The study of bioluminescent worm systems remains a vital and promising field in modern biology, integrating evolutionary and biochemical perspectives with innovative applications in diagnostics, therapy, targeted drug delivery, and bioluminescent imaging.

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