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Structural features of bacterial luciferase related to temperature adaptation

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Motivation and Aim: Luminous bacteria are widely distributed in nature, with the majority of species living in the ocean and only a few representatives described as freshwater and terrestrial [1]. Thus, they have adapted to live in various environmental conditions and at different temperatures as well. Based on optimal growth temperature, psychrophilic and mesophilic species of luminous bacteria can be distinguished. The features of bacteria adaptation to spescific temperature range can be seen across all levels of organization, including enzymatic. One of the most studied enzyme of luminous bacteria is luciferase, which is responsible for bioluminescent reaction and widely applied as an element of biosensors in fundamental research, biotechnological applications, ecotoxicology, etc. [2, 3]. The aim of this study is to reveal the structural features of two luciferases from different subfamilies (*Photobacterium leiognathi* and *Vibrio harveyi*) related to temperature dependence of their function.

Methods and Algorithms: The set of 47 amino acid sequences of bacterial luciferase associated with fully or partially sequenced *lux*-operons from NCBI database was obtained mannually. We used the set of sequences to analyze the amino acid substitutions specific to temperature adaptation with GroupSim and multi-Harmony program packages. The temperature dependence of reaction kinetics for *V. harveyi* and *P. leiognathi* luciferases was measured using stopped-flow technique with SX-20 analyzer (Applied Photophysics) in the range 5–45 °C. Additionally, we have run 100-ns molecular dynamics simulations for both enzymes at 5, 10, 27, 45, 60 °C using the GROMACS 2020.4 software package.

Results: Sequence analysis revealed that *P. leiognathi* luciferase is highly homologous to luciferases from psychrophilic strains, while *V. harveyi* luciferase is very similar to the thermostable luciferase from *V. campbellii*. According to experimental results, *V. harveyi* luciferase does have a wider temperature optimum shifted to higher temperatures compared to *P. leiognathi* luciferase. However, the total quantum yeld is lower in the reaction catalyzed by *V. harveyi* luciferase compared to the *P. leiognathi* one. Kinetics analysis indicated that *P. leiognathi* luciferase better stabilizes 4a-hydroperoxyflavin intermediate in the temperature range of 20–35 °C, and is less effective at temperatures of 40–45 °C.

Computational simulation showed that *P. leiognathi* structure is more dynamic, but the shape of the protein does not remarkably change with increasing temperature, which is typical for psychrophilic enzymes. *V. harveyi* luciferase undergoes minor overall fluctuation at studied temperatures indicating its higher rigidity, which is characteristic for thermostable enzymes. We also analyzed the flexibility of sreparate structural elements of the proteins at different temperatures. The fluctuations of a mobile loop

located near the active site becomes higher for both enzymes with increasing temperature. Additionally, a turn in β-sheet of *V. harveyi* luciferase (149–151 a.a.) is more mobile at 45 and 60 °C indicating that stabilizing interactions of this fragment is not the same as those in *P. leiognathi* luciferase. Moreover we have found nine amino acid substitutions which could be specific for observed temperature effects. All of them are located in highly rigid regions of both enzymes except for one found within functional mobile loop mentioned above. These residues could be responsible for compensatory mechanism required to maintain a balance between flexibility and stability of the enzyme.

Conclusion: *V. harveyi* and *P. leiognathi* luciferases are characterized not only by different kinetics of light emission ("fast" and "slow"), but also by structural characteristics which could be responsible for different dependence of the enzymes function on temperature. The obtained results are important for development of enzyme based bioassays that are used for ecological monitoring.

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