DOI 10.18699/SBB-2022-184

Molecular modeling of osmolytes' effect on bacterial luciferase

Sukovatyi L.A.[1]*, Lisitsa A.E.[1], Deeva A.A.[1], Kratasyuk V.A.[1, 2], Nemtseva E.V.[1, 2]
[1] *Siberian Federal University, Krasnoyarsk, Russia*
[2] *Institute of Biophysics, SB RAS, Krasnoyarsk, Russia*
** lsukovatyy@sfu-kras.ru*

Key words: bioluminescence, luminous bacteria, bacterial luciferase, osmolyte, molecular dynamics

Motivation and Aim: Intracellular accumulation of organic solutes is one of the strategies used by living organisms to manage the variety of extreme conditions that they encounter in nature. This is also the case for luminous bacteria that inhabit the ocean and can adapt to the variety of temperatures, salinity, concentration of nutrients and toxins. The cells accumulate small molecules to counter the osmotic stress. These low molecular weight protective compounds called osmolytes include polyols, sugars, amino acid derivatives etc. [1]. They are assumed to increase protein stability and prevent protein aggregation. Nevertheless, small organic compounds can cause structural and dynamic changes of proteins and so influence its function, i.e. catalytic activity. For *Photobacterium leiognathi* luciferase it was revealed that integral intensity of the reaction changes in the presence of different cosolvents as follows: sucrose > sorbitol > glucose > glycerol > ethylene glycol that correlates with the relation of hydrodynamic radii of the cosolvent molecules: 5.2 > 3.9 > 3.6 > 3.1 > 2.6 (Å) [2]. Moreover, sucrose increases catalytic constant of excited state intermediate formation up to 5-fold unlike other cosolvents. Atomic-level molecular dynamics (MD) is useful tool to examine effects of osmolytes on *P. leiognathi* structure. Thus, the aim of this study was to reveal the structural and dynamic effects caused by osmolytes on the bacterial luciferase using molecular modelling approach.
Methods and Algorithms: Three-dimension structure of luciferase from *P. leiognathi* was obtained by homology modelling using Swiss-model server. Crystal structure of luciferase from *Vibrio harveyi* (PDB ID: 3FGC) was used as a template. MD simulation of structure of *P. leiognathi* luciferase in water and in water-cosolvent mixtures (40 w/w %) was performed with GROMACS 2020.4 software. Sucrose, sorbitol, glucose, glycerol and ethylene glycol molecules were used as cosolvents. MD of each modeled system was calculated for 100 ns with three independent runs.
Results: To estimate the penetration of cosolvents into active site gorge of *P. leiognathi* luciferase a radial distribution function (RDF) was calculated between centre of mass of αGLU43 and cosolvent molecules. αGLU43 was chosen as reference point because it locates in the deep of luciferase active site. RDF demonstrates that ethylene glycol and glycerol molecules penetrate deeper the active site of luciferase than other cosolvents that may explain the decrease of integral intensity in the presence of these two osmolytes. Minimum distance of RDF for ethylene glycol is 1,5 Å and for glycerol – 1,6 Å, which is close to the value of water (1,45 Å). Sorbitol molecules can be found at 1,7 Å, but this case is extremely rare and the RDF remains close to zero up to the distance of 8 Å relative to the reference point. For glucose and sucrose the RDF function starts to rise from the distance of 4,3 and 5,5 Å respectively, and reaches significant values at >10 Å, i.e. closer to the entrance of the active site gorge.

Calculated gyration radius and solvent accessible surface area of the luciferase in presence of cosolvents indicate that ethylene glycol changes the structure of the protein. These both parameters were found to be significantly higher for this cosolvent. This result is in good agreement with the experimental data demonstrating that only ethylene glycol causes a decrease of the integral intensity of bioluminescent reaction of *P. leiognathi* luciferase.

Analysis of distribution of side chain rotamers of amino acids was carry out to study local conformational transitions in active site of the luciferase. It was found that in presence of sucrose the αGlu175 side chain takes on the only conformation (mt-10, according to the S. Lovell rotamer library), which necessary for binding of luciferase with flavin [3]. However, in aqueous solution and in presence of the other cosolvents it could take additionally on second (known from crystal structure [4]) conformation, which makes impossible binding FMN in the active center. It can be assumed that the presence of sucrose causes local conformation changes in an active site with improved catalytic properties. This fact is in a good agreement with the experimentally observed increase of catalytic constant of the luciferase in presence of sucrose [2].

Conclusion: Thus, the effects of osmolytes on the bacterial bioluminescent reaction were explained by: (i) change in local dynamics of functionally significant regions of luciferase, (ii) global structural alteration of the protein in the presence of ethylene glycol, (iii) different accessibility of the luciferase active center to osmolytes.

Acknowledgements: The study is supported by the Russian Foundation for Basic Research as part of a research project No. 20-34-90118 and the Russian Foundation for Basic Research and the administration of the Krasnoyarsk Territory and Krasnoyarsk Regional Fund of Science within the framework of the scientific project No. 20-44-243002.

References

1. Dandapath I. et al. Bacterial osmolyte system and its physiological roles. In: Cellular Osmolytes. Singapore: Springer, 2017;229-249.
2. Lisitsa A.E. et al. Mechanisms of viscous media effects on elementary steps of bacterial bioluminescent reaction. *Int J Mol Sci*. 2021;22(16):8827-8846.
3. Lovell S.C. et al. The penultimate rotamer library. *Proteins.* 2000;40(3);389-408.
4. Sparks J.M., Thomas O.B. Functional implications of the unstructured loop in the (β/α) 8 barrel structure of the bacterial luciferase α subunit. *Biochemistry.* 2001;40(50);15436-15443.