doi 10.18699/bgrs2024-3.1-48

Thermal stability of the rhodopsin of Antarctic UV resistant bacteria

Okhrimenko I.S.*, Lyubaykina N.A., Zagryadskaya Yu. A.

Research Center for Molecular Mechanisms of Ageing and Age-related Diseases of Moscow Institute of Physics and Technology (State University), Dolgoprudny, Russia

* *okhrimenko.is@mipt.ru*

Key words: rhodopsins; ion pumps; ion channels; X-ray crystallography; photocycle

Motivation and Aim: The most abundant rhodopsin family is the family of proteorhodopsins (PRs)[1]. As many microbial rhodopsins PRs are often used by host organisms to survive in extreme environment. PRs genes were firstly discovered in the marine uncultured proteobacteria and characterised *in vitro*[2]. Later the PRs genes were found in plankton *Archaea*, where they probably appeared due to the lateral gene transfer[3]. The reported PRs have proton pump[2,4,5] or sensory activity[6], they are spread all over the Earth and have the major impact to the solar energy capturing in the world ocean[7]. The PRs genes were found even in Antarctic Sea ice samples[8] (moderate UV areas) and in *Modern Stromatolites* living a High-Altitude Andean Lake in Argentinean Puna in the extreme UV irradiation areas[9]. According to the data of modelling rhodopsin named HbR1 may bind carotenoids. Its gene together with the HbR2 gene were found trough bioinformatics research[10] in the genome of the Antarctic UV resistant bacteria *Hymenobacter sp.* PAMC 26554. This bacterium was isolated from an Antarctic lichen *Usnea sp.*[11] at Barton Peninsula, King George Island, Antarctica (62°13'S, 58°47'W).

It is the open question, if mentioned rhodopsins itselves have special properties originated from Antarctic environment, and what properties are important for the global ecology. We hope that our functional and structural studies will contribute to the answer on these question.

Conclusion: The gene optimized for the best yields of HbR1 was expressed in *E. coli* in presence of all-trans retinal, the purified protein had absorption maximum at 516 nm. HbR1 has not show any light-induced ion transporting activity and had remarkably long photocycle. The length of photocycle is approximately the same at the grate range of H^+ concentrations (pH4.2-9.5) and in the low or hight ion strength of the solution (0 or 1M NaCl). This could show the absence of the connection of proton acceptor group (which should be close to the retinal Shiff base, as analogue of Asp 85 in bacteriorhodopsin of *H. salinarum*) with the environment. HbR1 is stable protein: by means of TSA it's melting temperature was determined as ~80℃, and it increases in case of lowering pH and growing of ion strength. So stability of the protein increases at low pH and high salt conditions.

Funding: The research was performed with the support of Russian Scientific Foundation, project # 23-14-00160.

References

1. Bamann C., Bamberg E., Wachtveitl J., Glaubitz C. Proteorhodopsin. *Biochim Biophys Acta*. 2014;1837(5):614-625
2. Béjà O., Aravind L., Koonin E.V. et al. Bacterial rhodopsin: evidence for a new type of phototrophy in the sea. *Science*. 2000;289(5486):1902-1906

3. Frigaard N.U., Martinez A., Mincer T.J., DeLong E.F. Proteorhodopsin lateral gene transfer between marine planktonic Bacteria and Archaea. *Nature*. 2006;439(7078):847-850
4. Petrovskaya L.E., Balashov S.P., Lukashev E.P. et al. ESR – A retinal protein with unusual properties from Exiguobacterium sibiricum. *Biochemistry (Mosc).* 2015;80(6):688-700
5. Yoshizawa S., Kawanabe A., Ito H., Kandori H., Kogure K. Diversity and functional analysis of proteorhodopsin in marine Flavobacteria. *Environ Microbiol*. 201214(5):1240-1248
6. Hartz A.J., Sherr B.F., Sherr E.B. Photoresponse in the heterotrophic marine dinoflagellate Oxyrrhis marina. *J Eukaryot Microbiol*. 2011;58:171-177
7. Gómez-Consarnau L. et al. Microbial rhodopsins are major contributors to the solar energy captured in the sea. *Sci Adv*. 2019;5(8):eaaw8855
8. Koh E.Y. et al. Proteorhodopsin-bearing bacteria in Antarctic sea ice. *Appl Environ Microbiol*. 2010;76(17):5918-5925
9. Albarracín V.H. et al. Functional green-tuned proteorhodopsin from modern stromatolites. *PLoS One*. 2016;11(5):e0154962
10. Ushakov A., Grudinin S., Okhrimenko I. et al. Knowledge-based prediction model for characterization of microbial rhodopsins for optogenetics. *FEBS J*. 2016;283:127-128
11. Bubach D. et al. Elemental composition of Usnea sp lichen from Potter Peninsula, 25 de Mayo (King George) Island, Antarctica. *Environ Pollut*. 2016;210:238-245