DOI 10.18699/SBB-2022-102

A low molecular weight protein from Baikal amphipod hemocytes as a potential antimicrobial peptide

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Key words: immune system, antimicrobial peptides, amphipods, Lake Baikal

Motivation and Aim: Protection against foreign bodies, provided by the immune system, is important for all organisms. One of the key immune components are antimicrobial peptides (AMPs), which are expressed by immune cells, hemocytes, into hemolymph [3]. AMPs bind to microbial membranes, causing cell lysis. Over 350 species of endemic crustaceans, amphipods, inhabiting diverse ecological niches of Lake Baikal, could have acquired unique immune components during their evolution. This work is devoted to the study of a potential antimicrobial peptide from the Baikal amphipod *Eulimnogammarus verrucosus* hemocytes.
Methods and Algorithms: The amphipods were caught in the littoral of Lake Baikal near the bay in Listvyanka. Hemolymph was collected from animals, followed by purification of hemocytes [5] and protein isolation [1]. Hemocyte proteome were analyzed using liquid chromatography/tandem mass spectrometry. Data analysis was carried out using SearchGUI v3.3.17 and Peptide Shaker v1.16.44 software. Protein identification was conducted using *E. verrucosus* transcriptome assembly GHHK01 [2]. The search for proteins with antibacterial properties was performed using NCBI Conserved Domain Search web tool. The results of the mass spectrometric analysis are published in Zolotovskaya et al., 2021 [4].
Results: We found a low molecular weight protein, which we designated AMP1. It contains an anti-lipopolysaccharide domain, interacting with the membranes of Gram-negative bacteria. To study the AMP1 properties, we designed the pET20b-AMP1-6His vector. pET20b allows for efficient gene of interest heterologous expression and also contains six histidine sequence for proteins purification by affinity chromatography. The obtained vector was used to transform *Escherichia coli* BL21 pLysS strain. To completely block AMP1 expression, cells were grown in solid LB medium with and without 1 % glucose. On the medium with glucose, the number of bacterial colonies was noticeably fewer than on medium without glucose. Protein expression in BL21 pLysS was induced by adding isopropyl ß-D-1-thiogalactopyranoside (IPTG). Cultivation of bacterial cells in liquid medium was carried out with and without glucose. Cells were grown at the temperature of 37, 28 or 18 °C for 24 hours, for determining the optimal protein expression conditions. During this cultivation we observed low speed of bacterial culture growth. The AMP1 expression was shown in culture without glucose, whereas there was no AMP1 expression in the culture with glucose. The highest amount of protein was formed during 24 hours at the temperature of 28 °C (Fig. 1). Low bacterial growth in liquid medium and small colony size on solid medium indicates pET20b-AMP1-6His toxicity and indirectly demonstrates antibacterial properties of the AMP1 protein.

Conclusion: We analyzed the first hemocyte proteome of an amphipod and found a protein that is a potential antimicrobial peptide.

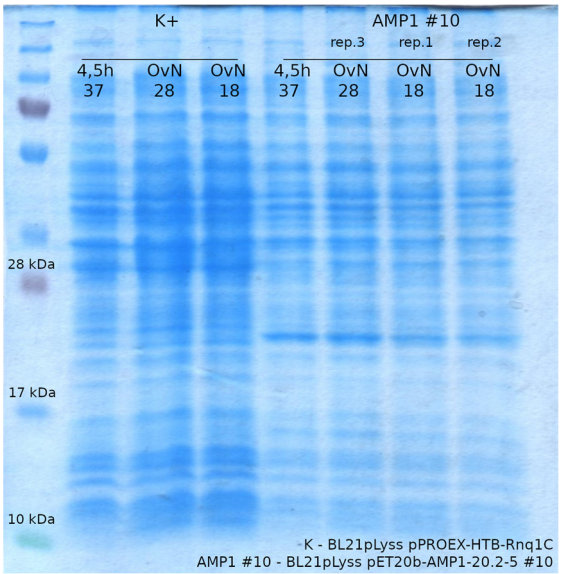

Fig. 1. Protein expression in *E. coli*. K+ – pPROEX-HTb-Rnq1C, used as a positive control; AMP1 #10 – pET20b-AMP1-6His

References

1. Bedulina D.S. et al. Preliminary analysis of hemocyanins in hemolymph plasma of Baikal endemic amphipods. *J Stress Physiol Biochem*. 2016;12(1):74-86.
2. Drozdova P. et al. Comparison between transcriptomic responses to short-term stress exposures of a common Holarctic and endemic Lake Baikal amphipods. *BMC Genomics*. 2019;20(1):712.
3. Rowley A.F. The immune system of Crustaceans. Encyclopedia of immunobiology. Elsevier Academic Press, 2016;1:437-453.
4. Zolotovskaya E. et al. Hemocyte proteome of the Lake Baikal endemic *Eulimnogammarus verrucosus* (Crustacea: Amphipoda) sheds light on the immune-related proteins. *Biological Communications*. 2021;66(4):290-301.
5. Shchapova E. et al. Application of PEG-Covered non-biodegradable polyelectrolyte microcapsules in the crustacean circulatory system on the example of the amphipod *Eulimnogammarus verrucosus*. *Polymers.* 2019;11(8):1246.