Ca$^{2+}$-regulated photoproteins of the bioluminescent ctenophores: Cloning, expression, and some properties

Markova SV,1 Burakova LP,1 Golz S,2 Frank LA,1 Vysotski ES1
1. Photobiology Lab, Institute of Biophysics SB RAS, Krasnoyarsk 660036, Russia
2. Pharma Research Molecular Screening Technology, Bayer AG, D-42096 Wuppertal, Germany

The bright blue-green luminescence of the studied ctenophores is reversibly inhibited in sunlight and conditioned by light-sensitive Ca$^{2+}$-regulated photoproteins. We have isolated the cDNAs for ctenophore photoproteins from the expression cDNA libraries of several bioluminescent ctenophores using functional screening method. The open reading frame of each cDNA encodes 206-208 amino acid proteins with calculated molecular weight of 24,500-24,900. The sequence analysis has shown that novel ctenophore photoproteins belong to the EF-hand superfamily of Ca$^{2+}$-binding proteins and have 3 Ca$^{2+}$-binding loops like in the early cloned photoproteins from jellyfishes. However, the novel ctenophore photoproteins display very weak homology with them, sharing maximum identity in 29% with Obelia longissima obelin. The ctenophore photoproteins were overexpressed in E. coli cells and accumulated inside cells in inclusion bodies. The recombinant apo-photoproteins were purified, refolded, and reactivated with coelenterazine into active photoprotein. The recombinant photoproteins reveal light-sensitivity similar to that of native photoproteins. Some biochemical and biophysical properties were studied. The successful expression of the cloned ctenophore photoproteins in mammalian cells suggests that these new photoproteins suit well as bioluminescent reporters for monitoring of gene expression and for measurement of intracellular calcium. This work was supported by Bayer AG (Germany).