Recovery was 98.5–101%. The proposed method has been applied successfully to the determination of ofloxacin in tablets and injections, and the results agree well with those obtained by an official method.

Obelin mutants with altered affinity to calcium and bioluminescence colour

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Since the concentration of free calcium within cell compartments varies noticeably and calcium transients in those compartments run in different manners, it is desirable to have calcium indicators which would have various sensitivities to calcium and which would allow simultaneous measurement of $[Ca^{2+}]$ in different cell compartments. Here we report the properties of some obelin mutants that simultaneously have the altered bioluminescence spectra and calcium affinity. All substitutions were done based on the 3D structures of obelin ligand-dependent conformational states. The mutants display a good bioluminescent activity and the physiological concentration of Mg^{2+} has no effect on their sensitivity to Ca^{2+}. Therefore, they hold much promise for the development of dual-wavelength methods for synchronous monitoring of Ca^{2+} transients in different cell compartments, to reveal how the local changes in $[Ca^{2+}]$ switch the exogenous and endogenous stimuli to the corresponding cell response. Supported by: Grant No. Mκ-1963.2005.4 of the President of Russian Federation; CRDF Grant No. Y4-02-05; the Lavrenteiev Grant for Young Scientists of the SB RAS; and RFBR Grant No. 06-04-08076.

Distribution of luminescence in Ophiuroidea (Echinodermata)

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Although bioluminescence is well known in Echinoderms, it is still poorly understood in terms of phylogenetic and biogeographic distribution. This amazing capability has been mostly studied in the Ophiuroidea over the last 15 years, but on a limited number of species. Recently, a comparative study on brittlestar luminescence was initiated and analyses were conducted after a large sampling effort. The results indicated that, out of 195 species, the total number of known luminous species increased from 33 in 1995 to 64 in 2007. They were mainly collected on hard substrata in deep water, where luminescence appeared more intense. Although brittlestar luminescence was observed in tropical and temperate waters from shallow to abyssal depths, it was least prelevant on tropical coral reefs. Analysis is in progress to try to highlight a possible link between luminescence and ophiuroid phylogeny. New field surveys in a variety of marine regions and habitats will be organized to increase the number of ophiuroid species tested. This is neccesary also in order to understand why so many brittle stars glow in the dark.

Nervous control of luminescence in *Ophionereis schayeri* (Ophiuroidea, Echinodermata)?

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Among echinoderms, luminescence control mechanisms have been mainly studied in the class Ophiuroidea. Initial research performed on *Amphipholis squamata* and *Amphiura filiformis* suggested the preponderance of cholinergic control mechanisms. Within the framework of a comparative study of ophiuroid luminescence, a research programme was developed on *Ophionereis schayeri*, a common southern Australian luminous brittle star. Results show that γ-aminobutyric acid (GABA) is the main neurotransmitter involved in light emission. GABA triggers light emission through the activation of the $GABA_B$ receptor's sub-type located on the membrane of the luminous cells. Nevertheless, acetylcholine induced a weaker luminescence, probably via muscarinic cholinergic receptors. Furthermore, GABA, besides occupying the role of main neurotransmitter, seems to act as a positive neuromodulator of the cholinergic response. The results of this research reinforce the idea that many neurotransmitters and neuromodulators are involved in the control of ophiuroid luminescence.

The function of conserved cystein residues in the bioluminescence of coelenterazine-dependent luciferase from *Metridia longa*; testing with site-directed mutagenesis

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Recently, several secreted luciferases from the copepod *Metridia longa* (MLuc) were cloned and one of them was successfully used as a bioluminescent reporter enzyme in mammalian cells. MLuc is a small Cys-rich protein, which most likely holds intramolecular disulphide bonds. Here we report the results of mutational analysis of conserved 10 Cys residues of MLuc, which were revealed from the protein alignment. All cysteines are found within two non-identical 31 amino acid repeats (five in each). To estimate the role of these Cys residues in bioluminescence, each one was substituted by both Ala and Ser using site-directed mutagenesis. Practically all mutations shift the temperature optimum of MLuc bioluminescence to 0–4°C and result in decreasing bioluminescent activity to a variable degree. However, the replacements of the last Cys residues in the repeats led to almost complete loss of MLuc bioluminescent activity. This suggests the crucial role of these two Cys residues for enzyme catalytic activity. The possible function of cysteines in maintaining structural stability is discussed. Supported by RFBR Grant No. 05-04-48271 and RFBR-Taiwan NSC Grant No. 89502.