Spectral difference between obelin and aequorin is determined by the residue in position 88.

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Bioluminescence of Ca\textsuperscript{2+}-regulated photoproteins arises from chemical breakdown of "coelenterazine", an imidazolopyrazine derivative substituted by a hydroperoxy at the C2-position and tightly but non-covalently bound within the protein. Ca\textsuperscript{2+} binding initiates decarboxylation resulting in the excited state of the product, coelenteramide. The well-studied representatives are aequorin and obelin. The aequorin bioluminescence maximum is at 465 nm, whereas that of obelin is at longer wavelength, $\lambda_{\text{max}}=485$ nm. Unreacted photoproteins are hardly fluorescent but Ca\textsuperscript{2+}-discharged aequorin has a strong fluorescence ($\lambda_{\text{max}}=465$ nm) coinciding with the bioluminescence spectrum, Ca\textsuperscript{2+}-discharged obelin having green fluorescence with $\lambda_{\text{max}}=510$ nm. According to spatial structures there is only one remarkable difference between the two photoproteins in the nature of residues making up the substrate-binding site. In obelin Phe is found at position 88, whereas in aequorin the corresponded 82-position is occupied with Tyr, i.e. hydrogen-bonded with the oxygen atom of the 6-(p-hydroxy)-phenyl group of coelenterazine. To elucidate the influence of the residue in this position on spectral properties, two mutants were constructed: F88Y-obelin and the corresponding Y82F-aequorin. Both mutants show no change in specific activity versus the WT photoproteins. They mainly differ in light emission spectra. The obelin mutant shifts both bioluminescence ($\lambda_{\text{max}}=455$ nm) and fluorescence ($\lambda_{\text{max}}=488$ nm) to the blue, while the aequorin mutant emits green bioluminescence ($\lambda_{\text{max}}=501$ nm) and fluorescence ($\lambda_{\text{max}}=505$ nm). These results clearly indicate that the residue in this position controls the excited electronic energy level of coelenteramide. Work was supported grant 02-04-49419 of RFBR and Physical and Chemical Biology Program of RAS.