Reprogramming the Luciferin-Substrate Activity in NanoLuc luciferase

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Bioluminescence, the process of production and emission of the visible light by living organisms has a great application potential in variety of fields, from basic research, bioimaging to construction of environmentally friendly light sources. Enzymes, generating light by chemical transformation of small organic molecules, so called luciferins, are named luciferases. NanoLuc occupies a sovereign place among luciferases. It is a small but high-performance luciferase with undisclosed blue-light production. Its synthetic luciferin called furimazine has shown cytotoxic effects, poor solubility and expensive preparation that represent significant limitation. Hence, the present research focuses on a structural analysis of NanoLuc complexes with oxidized forms of furimazine and native coelenterazine bonded in the surface allosteric capsule. However, the suppositional active site is located inside the molecule, and, therefore, I made luciferase variants with substituted allosteric site to assess its functionality. Whereas their activities substantially increased in the reaction with coelenterazine, the reaction with furimazine was not significantly affected. The crystallographic analysis of a complex of non-oxidizable luciferin analogue, azacoelenterazine, with the brightest luciferase variant called NanoLuc CTZ has been carried out. The molecular structure confirmed that the catalytic site of this luciferase is located in the centre of ß-barrel. In general, the revealed atomic structures enabled to propose the catalytic mechanism of bioluminescent reaction and subsequent mechanism of homotropic negative allostery of NanoLuc luciferase.

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