The smallest isoform of Metridia longa luciferase as a signal partner in hybrid biospecific probe for in vitro assay

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Abstract

Bioluminescent proteins have been shown to be excellent reporter molecules, providing high sensitivity of *in vitro* and *in vivo* assays. Coelenterazine-dependent luciferases and photoproteins are characterized by simple reaction, broad linear measuring range, and low detection limit up to 10-18 mol.
The recently described isoform of *Metridia longa* luciferase, MLuc7, is a high active naturally secreted enzyme that oxidizes coelenterazine yielding bright blue bioluminescence (485 nm). MLuc7 is the smallest (16.5 kDa) among natural luciferases known for today that makes it attractive as a bioluminescent reporter.

In our research, tick-borne encephalitis virus (TBEV), the causative agent of one of the most severe human neuroinfections, was chosen as a target. In order to create a bioluminescent detection probe, MLuc7 was genetically fused with a single-chain variable fragment (scFv) of murine immunoglobulin to TBEV. The variants of constructs involving MLuc7 connected with mini-antibody scFv through flexible GSG-bridge on N- or C-terminus were designed. Bi-functional proteins (44 kDa) with both luminescent activity of luciferase and TBEV-binding ability of scFv were obtained by baculovirus expression and purified from insect cells culture medium by the IMAC.

Fusion proteins were tested as labels in model solid-phase immunoassay of the *virus* envelope *protein E*. After optimization of assay conditions the protein E detection limit was 45 pg. Novel biospecific labels were successfully tested in bioluminescent detection of tick-borne encephalitis virus in native ticks. All the developed variants have demonstrated both high bioluminescent activity and high antigen affinity in *in vitro* assay that implies the suitability of luciferase as a reporter molecule for diagnostic application.
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