Homogeneous noncompetitive immunological detection of small molecules by ternary luciferase fragment complementation (OS-BLIA)

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Abstract

Homogeneous immunological detection of small molecules with high sensitivity is still a daunting task. Here we attempt sensitive luminescent detection of small peptide based on the Open-sandwich (OS) immunoassay principle, which is combined with sensitive bioluminescent protein-fragment complementation assay (PCA) *in vitro*. First, we tried the detection of antigen-induced approximation of the two antibody variable region fragments VH and VL using the commercially available Nanoluc-based PCA (NanoBiT) by fusing them with N-terminal LgBiT (19 kD) and 11 aa SmBiT fragments. The probes Trx-VH-LgBiT and Trx-VL-SmBiT were successfully expressed and purified using *Escherichia coli* expression system. However, when the two proteins were mixed with substrate furimazine, the signal did not change upon addition of antigen osteocalcin C-terminal peptide (BGP-C7), probably due to improper folding of VH-LgBiT probe. Hence, we decided to synthesize shorter genes corresponding to an N-terminal 18 kD (LnBiT) and an 11 aa C-terminal (LcBiT) fragments of LgBiT, to reduce the size of reporter peptide. Using Trx-LnBiT and Trx-VH-LcBiT proteins successfully prepared as well as Trx-VL-SmBiT, a clear antigen-dependent signal increase up to 70-fold was observed. In addition, through the optimization of SmBiT sequence, the signal was significantly increased while keeping low background signal and limit of detection comparable to those of ELISA, yielding maximal light emission efficiency of 88% compared with the wild-type enzyme. Furthermore, the luminescence of this biosensor was observed with naked eyes as well as with digital camera. Due to its simplicity, this user-friendly OS bioluminescent immunoassay (OS-BLIA) for small antigens might become a foundation of many point-of-care detection devices.

Keywords: protein, fragment complementation assay, immunoassay, Nanoluc

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