DEVELOPMENT OF RAPID IMMUNOASSSAY USING NANOLUC-DERIVED PEPTIDE TAGS

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Introduction

Conventional immunoassays require multiple steps, and are labor and time-consuming. Hence, rapid and handy immunoassays with high sensitivity are desired. Protein-fragment complementation assay (PCA) of an enzyme such as luciferase will be a promising approach to realize such homogeneous immunoassay. Previously, a luciferase from the deep sea shrimp *Oplophorus gracilirostris* (NanoLuc, Nluc) was divided into two fragments, SmBit (11 aa) and LgBit (18 kDa) [Fig. 1 left], and each fused with interacting partners [1]. When expressed in mammalian cells, upon interaction, increased luminescence was observed. However, detection of PCA using purified proteins *in vitro* has been elusive.

The affinity between the two antibody variable region fragments, V_L and V_H, remarkably increases when antigen binds to them (Open-sandwich principle) [2]. In this study, first, LgBit and SmBit were fused with V_H and V_L to detect antigen peptide [Fig. 1, left]. Next, LgBit was divided into MidBit and SmBit2 (11 aa), and SmBit and SmBit2 were each fused with V_L and V_H, respectively, to obtain antigen-dependent luminescent signal upon reconstitution with MidBit [Fig. 1, right].

Methods

cDNA for SmBit, SmBit2 and MidBit were synthesized by Eurofin Genomics (Tokyo, Japan). VL and VH fragments of anti-BGP antibody [3] were each fused with LgBit and SmBit, or fused with SmBit and SmBit2, respectively. They were expressed as a fusion protein with thioredoxin and His6 tag in *E. coli* SHuffle Express T7 LysY, and purified by TALON immobilized metal affinity resin.

Results & Discussion

First, V_H-LgBit and V_L-SmBit were mixed to detect BGP-C7 epitope peptide. However, no change in luminescent intensity was observed when BGP-C7 was added, presumably due to steric hindrance and/or abnormal folding of interacting proteins. Hence, we reasoned that smaller probe fused with interacting proteins could solve this problem. Upon mixing optimal amounts of V_L-SmBit, V_H-SmBit2 and MidBit, the luminescent intensity increased 79-folds when 5 µM of BGP-C7 was added [Fig. 2]. Probably, the complex formation of SmBit and SmBit2 was promoted by the antigen, which resulted in more efficient NanoLuc reconstitution. The detection limit of antigen was 5 nM. Furthermore, by the optimization of the SmBit sequence, the luminescent intensity increased 282-fold, without compromising antigen dependency. It was also detectable by naked eyes.

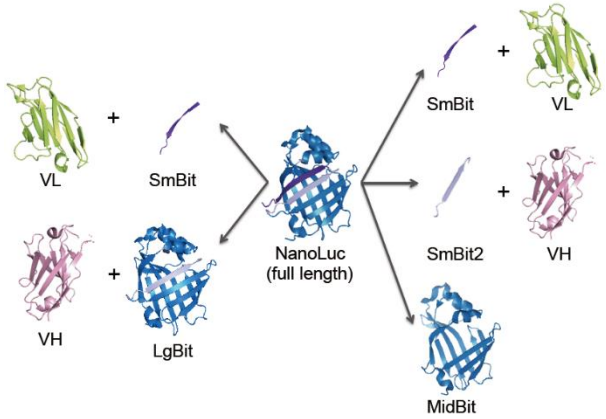

Fig. 1

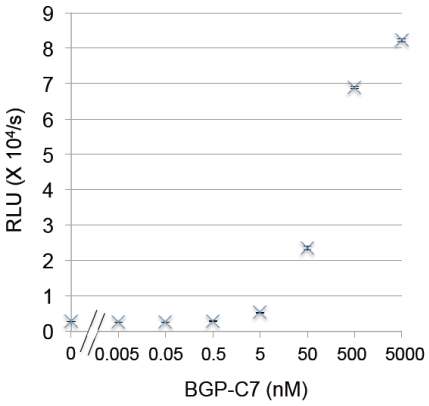

Fig. 2

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