DEVELOPMENT OF A NOVEL HOMOGENEOUS IMMUNOASSAY USING MUTANT BETA-GLUCURONIDASE

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Beta-glucuronidase (GUS) catalyzes breakdown of complex carbohydrates, whose activity can be detected quantitatively and sensitively by using fluorogenic and chromogenic substrates. GUS is a tetramer composed of four identical subunits, and assembly of all these subunits is necessary to attain its activity. Based on a previous study, a set of interface mutations (M516K, Y517E) is known to effectively inhibit the assembly and makes it inactive [1]. Usually, the affinity between the two variable region domains (VH and VL) of an antibody recognizing a small molecule is relatively low. However, in the presence of antigen, this affinity becomes higher so that they bind each other more tightly [2]. This gives the idea that a fusion protein system comprising VH and VL of an antibody as the detector each tethered to a mutant GUS subunit (GUSm) as the reporter can be used as a biosensor for small molecules. In this study, we aimed at detecting 4-hydroxy-3-nitrophenylacetyl (NP) and bone Gla protein (BGP) as targets of this novel immunosensor (Fig. 1).

Expression vectors for NP and BGP detection systems were constructed. Fusion proteins for NP detection system, as well as those for BGP detection system were expressed and purified. Performance of these two systems was tested using fluorogenic and chromogenic substrates by time course and antigen dose-dependency measurements. For both systems, signal was observed immediately and increased gradually, after mixing the substrate with the sample. When the two antigens, NP and 3-iodo NP (NIP), were used for antigen dose-dependency test on NP system, higher antigen concentration gave a higher fluorescent signal, while NIP was more sensitively detected (Fig. 2a). In BGP detection system, higher BGP-C7 concentrations gave higher colorimetric and fluorescent signals (Fig. 2b).

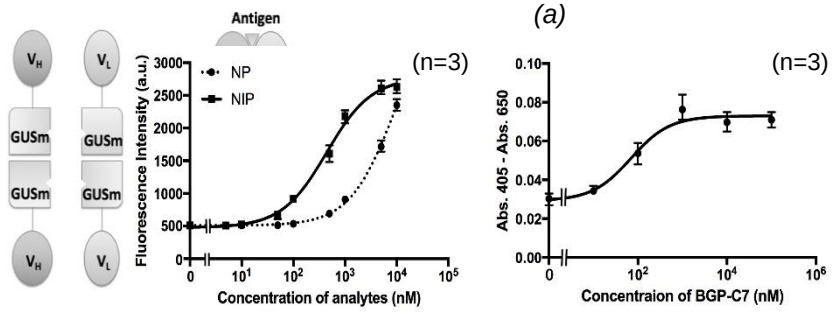

Fig. 2. (a) Antigen dose-dependency measurements of NP detection system. (b) Antigen dose-dependency measurement of BGP detection system

Fig. 1. Tangible scheme of this system

Compared with traditional methods, the proposed homogeneous immunoassay performs well with higher responses in the detection of small molecules without additional steps including separation. The instantaneous response after simple mixing of the components and an analyte gives this system convenient and timesaving features as an efficient high-throughput analysis method. This system will be effective for the analyses of various small molecules in the environmental and in clinical settings.

References

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