Construction of luminescent immunosensors using luciferase-fused Quenchbody

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Abstract

[Background] Quenchbody (Q-body) is a fluorescent immunosensor made of site-specifically dye-labeled antibody fragment. Q-body allows rapid and easy assay just by mixing with sample, and can detect various targets from small molecules to large proteins, depending on the antibody used. However, there remain some problems such as the necessity of fluorometry, excitation at short wavelength not suitable for biological samples, and the difficulty of quantitation when the probe concentration changes. In this study, we made luciferase-fused Q-bodies and tried BRET-based immunoassay accompanied by the emission color change.

Method

We fused the 3' end of Nluc (171 amino acids) gene and the 5' end of anti-osteocalcin (BGP) single chain Fv (VH-VL) gene via a 13 aa Cys tag sequence for dye labeling. The Nluc- and Cys tag-fused scFv was successfully expressed in *E. coli* SHuffle T7 express lysY and purified via the C-terminal His tag. After labeling with dye-maleimide and extensive purification, substrate furimazine was added in the presence or absence of antigen BGP-C7 peptide and measured for the luminescence spectra.

Results

After labeling with ATTO520, R6G and TAMRA dyes, we could successfully observe antigen-dependent fluorescence increase of the fusion protein based on the Q-body mechanism. However, the BRET response observed after adding substrate was higher than the fluorescent response, probably due to increased BRET efficiency attained by the higher chance of Nluc-dye approximation accompanied by antigen binding. Also, we could detect significant emission color change both with naked eyes and by microscopy. In summary, we have succeeded in making BRET Q-bodies whose acceptor dye is de-quenched and emission color changes depending on the trace antigen in sample. Based on this principle, handy detection of trace substances just by mixing with assay reagents and observing its color by a digital camera or a smartphone will be made possible.

Keywords: Antibody, NanoBRET

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