Development of Antibodies That Show Antigen-dependent Fluorescence Enhancement

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Abstract
Protein-based fluorescent biosensor is an undoubtedly powerful tool for detection and visualization of target biomolecules. Recently, it was reported that single-chain variable fragment (scFv) antibody-based fluorescent biosensor, namely Quenchbody, showed a fluorescence intensity change in an antigen concentration-dependent manner. Because structurally conserved protein (i.e., antibody) was employed as the scaffold of biosensor, measurements of various antigens were achieved by preparing fluorescent amino acid-incorporated scFv. However, time-consuming gene manipulation and usage of less productive cell-free translation system is required for genetic incorporation of fluorescent amino acid into scFv. Thus, alternative approach for preparation of antibody-based fluorescent biosensor is highly demanded.

We here report a novel design and synthesis of the antibody-based fluorescent biosensor. Previous study proved that fluorescent amino acid at the N-terminal side of scFv was quenched by controlled tryptophans, but the binding of its antigen triggered a structural rearrangement of scFv followed by a cancellation of the fluorescence quenching. Accordingly, it is considered that N-terminal-selective fluorescent labeling will be useful for preparation of antibody-based biosensor. As a proof of concept, immunoglobulin (IgG) monoclonal antibody-based fluorescent biosensor was semi-synthesized by reductive alkylation of N-terminal α-amino groups of IgG with dye-aldehyde in a weak acidic buffer. The N-terminal-selective conjugation of fluorescent dye was evaluated by tandem mass spectrometric analysis of peptide fragments. Also, antigen-dependent fluorescence intensity change of the labeled IgG was confirmed by fluorescence spectral measurement. Fluorescent detection of several peptides and biomolecules was successfully achieved by using the IgG-based biosensor. Because a variety of different IgG are commercially available, wide applications of the IgG-based biosensor will be developed.

Results
1) N-terminal α-amino group-selective fluorescent-labeling

2) Synthesis and characterization of TAMRA-labeled anti-FLAG IgG

3) Optimization of fluorescent dye and alkyl linker length

4) MS/MS analysis of the trypsinized IgG (anti-c-Myc 9E10 monoclonal)

5) TAMRA-labeled IgG antibodies might expand various applications