Summary of this research

Bio-based sensors for various target molecules are being used in a wide range of fields. Among them, protein-based fluorescent biosensors are very useful due to high selectivity and sensitivity. However, when the concentration of protein-based fluorescent biosensors is unknown, it is difficult to detect target molecules as fluorescence intensity changes in a quantitative manner. To solve this disadvantage, protein-based fluorescent biosensors that can detect target molecules as fluorescence ratio changes have been developed on the basis of fluorescence resonance energy transfer (FRET). In this research, I developed fluorescent-labeled single-chain antibody variable fragment (scFv) derivatives which enabled us to detect wide range of antigens in ratiometric manner.

In chapter 2, I synthesized double-labeled scFvs having TAMRA and Rhodamine Green at N- and C-termini, respectively, by using non-natural amino acid mutagenesis. For double-labeled scFvs against BGP (bone gla protein) and bisphenol A, fluorescence intensity ratio changes were observed upon the antigen-binding by the combination with FRET and fluorescence quenching. These result suggested that the double-labeled scFvs will be useful for quantitative detection of various target molecules.

In chapter 3, I explored acceptor and donor fluorophore pairs and optimized flexible linker length between the donor fluorophore and the C-terminus of scFvs to improve fluorescence ratio changes. I found that RhodamineRed significantly improved the fluorescence response for anti-bisphenol A scFv. Moreover, I revealed that the use of BODIPYFL-linked amino acid with a shorter linker and a shorter peptide linker at the C-terminus of anti-cMyc and bisphenol A scFvs improved fluorescence ratio change possibly because of decreased undesirable interaction. These findings will be valuable for construction of various double-labeled scFvs and their improvement.

In chapter 4, I incorporated Dansyl group as an environment-sensitive fluorescent probe into scFvs and examined fluorescence spectral properties of Dansyl-labeled scFvs. Four types of Dansyl-labeled scFvs against BGP, bisphenol A, cMyc, and phosphotyrosine showed fluorescence spectral changes upon the antigen-binding, this demonstrates that the environment-sensitive fluorescent probe can be applied to monitor environmental changes around the antigen-binding site and to detect antigens in a ratiometric manner. Dansyl-labeling will become an alternative strategy to design and synthesis of scFv-based fluorescent ratio probes when double-labeled scFv does not show large ratio change upon antigen-binding.

Throughout this study, I successfully developed new fluorescence ratio probes to detect target molecules. Further improvement of the present strategy will enable us to develop

practical diagnostic reagents and cell imaging tools.

Key word : non-natural amino acid mutagenesis, single-chain antibody fragment, FRET and fluorescence quenching, environmental sensitive probe, fluorescence ratiometric detection