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Article / Book Information

題目(和文)	頑健な免疫センサー酵素の開発及び人工細胞バイオセンサーへの応用
Title(English)	Creation of a robust immunosensor enzyme and its application to protocell array-based digital immunodetection systems
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種別(和文)	論文要旨
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（博士課程）
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論 文 要 旨

THESIS SUMMARY

系・コース： Department of, Graduate major in	生命理工 系 コース	申請学位（専攻分野）： Academic Degree Requested	博士 Doctor of （ 工学 ）
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要旨（英文800語程度）
Thesis Summary（approx.800 English Words ）

The thesis named "**Creation of a robust immunosensor enzyme and its application to protocell array-based digital immunodetection systems**" is written in English and composed of five chapters.

In **Chapter 1 "General introduction",** the immunoassay using antigen-antibody binding for detection as a highly specific and sensitive analysis method is introduced. Immunoassay plays a significant role in environmental and diagnostic settings as well as biological studies, such as enzyme-linked immunosorbent assay (ELISA). However, the widely used ELISA systems need extensive washing step(s) to remove background signal, which hampers its performance. To address this problem, development of enzyme-based homogeneous immunosensors that detect small molecules in one step were attempted. Other backgrounds including the enzyme used (β-glucuronidase, GUS) and protocells are introduced.

In **Chapter 2 "Development of a novel immunosensor based on mutant β-glucuronidase"**, engineering of β-glucuronidase (GUS) as a self-assembling tetramer that needs four subunits to be active to a biosensor that detects protein-protein interaction is described. By using a set of reported interface mutations (M516K, Y517E) that prohibits the GUS dimers to form tetramer and leave inactive dimers (GUSm), the affinity between variable regions of an antibody was measured, which increases in the presence of antigen (open sandwich principle). A system using a pair of variable regions of an antibody as detector tethered via a flexible linker to GUSm as reporter was constructed. By the antigen induced increase in affinity between the variable regions, GUSm dimers were dimerized and gave antigen-dependent enzyme signal that allowed the detection of antigens (5-iodo-)4-hydroxy-3-nitrophenyl acetyl (NP/NIP) and osteocalcin C-terminal peptide BGP-C7 quantitatively. Thus, the developed immunodetection system was simple, easy to use and fast. However, problems of low stability of GUSm fusion proteins and existence of background signal remained to be solved.

In **Chapter 3 "Optimization of thermostabilized β-glucuronidase mutant"** attempts to solve the above-mentioned two problems are described, by using a thermostabilized *E. coli* GUS. To this end, previously reported thermostabilized GUS_{IV5} was used as a backbone to screen optimized interface mutants that gave lower activity at dimer state and higher activity at tetramer state. By measuring the activity of His_6-tagged GUS_{IV5} variants with different interface mutations before and after dimerization by anti-His_6 antibody, GUS_{IV5}_KW (M516K, F517W) mutant was screened out to show the lowest activity before dimerization and the highest activity after dimerization, as high as the wild-type GUS. The system using GUS_{IV5}_KW as the reporter was able to detect NP with a higher sensitivity than the system using GUSm. To further demonstrate its utility in immunodetection, another fusion protein of a single domain antibody (V_HH) that recognizes caffeine to GUS_{IV5}_KW was constructed. By antigen-dependent dimerization, it succeeded in the quantitative detection of caffeine. Hence, by thermostabilization and optimization of reporter enzyme, propagating background signal was significantly decreased and a stable and highly regulated enzyme switch was created. However, due to its homogenous nature, the immunosensor might be affected by the presence of contaminated GUS in sample, such as human blood. To solve this problem, further development of the protocell array was attempted.

In **Chapter 4 "Creation of a protocell-base biosensor"**, the engineering of an artificial cell-based protocell biosensor system that gives digitalized binding signal using GUS_{IV5}_KW is described. To this

end, a transmembrane domain derived from epidermal growth factor receptor with a tag sequence sat its N-terminus was tethered to the GUS_{IV5}_KW and expressed inside vesicles to make tag-displaying fusion proteins to detect tag-specific antibodies. The fusion proteins were synthesized by an in vitro transcription and translation system in lipid-based protocells made by inverted emulsion method. The protocells could detect tag-specific antibodies that dimerized the tags displayed on the surface of protocells and activated GUS_{IV5}_KW inside to give visible fluorescent signal. To demonstrate its ability to give digitalized signal for antibody binding, flow cytometry (FCM) was used to count positive protocells responding to antibody in gradient concentrations. The counts of fluorescent protocells increased corresponding to the concentration of antibody, indicating the quantitative detection ability of this biosensor. To explore the ability of protocell biosensor for antigen detection, SpyTag/SpyCatcher system was successfully used to connect variable region of the anti-caffeine V_{HH} outside of protocell and GUS_{IV5}_KW reporter inside to give digitalized signal responding to caffeine. A digitalized signal corresponding to external caffeine concentration was obtained, which gave the potential to detect multiple antigens quantitatively.

In **Chapter 5 "Summary and perspectives",** this study was summarized and perspectives are discussed. In this thesis, a stable and optimized enzyme switch to detect protein interactions such as those of antibody fragments in homogeneous solution and on the protocell was created. Although digital immunodetection might need further system optimization, the developed system will form a basis of future developments of wash-free digital immunoassay systems.