論文 / 著書情報
Article / Book Information

題目(和文)	バイオプロセスで生産される組換えタンパク質検出のための蛍光バイオセンサーQ-bodyの構築
Title(English)	Construction of fluorescent biosensor Q-body for detecting recombinant proteins produced in bioprocess
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出典(和文)	学位:博士(工学), 学位授与機関:東京工業大学, 報告番号:甲第12125号, 授与年月日:2021年9月24日, 学位の種別:課程博士, 審査員:上田 宏,田中 寛,中村 浩之,三重 正和,吉村 奈津江,北口 哲也
Citation(English)	Degree:Doctor (Engineering), Conferring organization: Tokyo Institute of Technology, Report number:甲第12125号, Conferred date:2021/9/24, Degree Type:Course doctor, Examiner:,,,,,
学位種別(和文)	博士論文
Category(English)	Doctoral Thesis
種別(和文)	論文要旨
Type(English)	Summary

（博士課程）
Doctoral Program

論 文 要 旨

THESIS SUMMARY

系・コース： Department of, Graduate major in	生命理工学 系 ﾗｲﾌｴﾝｼﾞﾆｱﾘﾝｸﾞ ｺｰｽ	申請学位（専攻分野）： Academic Degree Requested	博士 （工学） Doctor of (Engineering)
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要旨（英文 800 語程度）
Thesis Summary（approx.800 English Words ）

This thesis is entitled "Construction of fluorescent biosensor Q-body for detecting recombinant proteins produced in bioprocess", and consists of 5 chapters.

In Chapter 1 “Introduction”, the current situation that many useful substances are produced in the form of recombinant proteins by bioprocess is introduced, and some examples are described. As people become more and more aware that the monitoring and control of bioprocess is necessary to obtain high-quality products, several detection sensors have been developed. However, the existing sensors only target environmental variables, such as temperature and dissolved oxygen. For analyzing the products, usually time-consuming purification and concentration steps are required. Therefore, this research is aiming to develop a sensor that can directly detect the recombinant protein product in culture medium. Such sensor is believed to help speed up the mutation screening and improve the monitoring efficiency of large-scale production process. On the other hand, most of the recombinant proteins have poly-histidine (His6) tag, which is used to obtain high-purity target protein in a mild condition by immobilized metal affinity chromatography (IMAC). In the rapidly changing bioprocess, especially when microorganism is used as expression host, a sensor that can rapidly detect His6-tagged recombinant protein will be very attractive. In this thesis, a fluorescent immunosensor, Quenchbody (Q-body), which is site-specifically labeled with fluorescent dye at the N-terminus of an antibody fragment was used to achieve the goal.

Chapter 2 is entitled “Evaluation of single- and double-labeled Q-body’s performance”. Here, by fluorescent dye labeling the sequence at the N-terminus of 3D5 single-chain antibody (scFv) and its Fab fragment, Q-bodies that can recognize C-terminal His6 tag were constructed. Generally, the fluorescent dye is quenched by tryptophan (Trp) residues inside the antibody, or by another dye, due to photoinduced electron transfer (PeT) effect. When an antigen binds to a Q-body, the quenching effect becomes weakened, resulting in a fluorescence increase. However, the TAMRA-labeled scFv-type Q-body did not exhibit expected characteristic. On the other hand, after labeling TAMRA at the N-termini of H and L chains of Fab 3D5 antibody, significant fluorescence quenching was observed in Fab-type Q-body. However, the fluorescence response of Fab Q-body to its antigen His6 peptide was about 2-fold at most.

Chapter 3 is entitled “Effect of aromatic amino acid mutation in the VH-CDR1 region of Q-body”. In this chapter, to improve the antigen response of the Q-body constructed in chapter 2, Trp near the antigen binding site and the aromatic amino acid tyrosine (Tyr) that has similar structure with Trp were focused. Two consecutive Tyr residues in VH-CDR1 region were mutated into Trp, and the YW-, WY- and WW-Fab variants were made into Q bodies. The association and dissociation rates determined by biolayer interferometry (BLI) method and the fluorescence measurement results showed that in YW- and WW-mutated Q-bodies, not only the quenching effect was enhanced, but also the antigen binding activity and the detection sensitivity were increased. Interestingly, it was found that although the antigen binding activity of WW-mutated Fab was strong, the antigen binding might be hindered by increased interaction between Fab antibody and TAMRA after dye-labeling, so that YW-mutated Q-body’s detection sensitivity was higher. Moreover, the experimental

results of the antigen binding change caused by mutation were consistent with the energy calculation results of the structure-based computer-aided simulation method.
In Chapter 4 "Application of Q-body in bioprocess monitoring", the two kinds of Fab variants that were developed in chapter 3 were labeled with TAMRA or another fluorescent dye ATTO520, respectively. These four types of Q-bodies were used to quantitatively determine the concentration of C-terminal His6-tagged anti-SARS-CoV-2 nanobody (VHH) that was secreted by Gram-positive *Brevibacillus choshinensis* into the culture medium. After optimizing the expression system, medium and the measurement method, the *Brevibacillus* were pre-cultured in a rich-nutrient medium, and then were transferred to a minimal medium. The culture medium was diluted to 50% during Q-body assay. The results showed that the VHH-His secreted into the culture medium could be accurately quantified by Q-body in the concentration range of 1.5 - 4 μM.
Chapter 5 "Conclusion" summarizes the results of each chapter and describes the future prospects. In short, the anti-His tag Q-body was successfully developed. Its capability of quantitative determination and ease of use are expected to contribute to the monitoring of His-tagged recombinant products in both academic research and industrial bioprocess. In addition, the experience in improving Q-body's performance through aromatic amino acid mutation could provide useful ideas for designing other effective Q-bodies in the future.