論文 / 著書情報
Article / Book Information

題目(和文)	タンパク質分子針由来サブユニットワクチンの分子設計
Title(English)	Molecular design of protein needle-based subunit vaccines
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出典(和文)	学位:博士(学術), 学位授与機関:東京工業大学, 報告番号:甲第12157号, 授与年月日:2021年12月31日, 学位の種別:課程博士, 審査員:上野 隆史,上田 宏,金原 数,小畠 英理,丸山 厚
Citation(English)	Degree:Doctor (Academic), Conferring organization: Tokyo Institute of Technology, Report number:甲第12157号, Conferred date:2021/12/31, 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	Life Science and Technology	系 コース	申請学位（専攻分野）： Academic Degree Requested	博士 Doctor of	(Philosophy)
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要旨（英文 800 語程度）
Thesis Summary（approx.800 English Words ）

In this thesis, I employed a protein needle (PN) as a scaffold protein to display various cargo proteins, including sfGFP and non-structural proteins from norovirus and respiratory syncytial virus (RSV); VPg and P protein, respectively. Those constructs were used as a template to investigate the role of linker properties in fusion protein design toward application in vaccine design. In particular, (1) PN was conjugated with sfGFP via linkers of varying length and flexibility, the effects of linkers on the dynamic motions of sfGFPs were investigated by HS-AFM; (2) VPg was genetically fused with the N terminus of PN via various linkers to apply in norovirus vaccine; (3) RSV P was fused to PN via a flexible linker, the construct demonstrate its promising potential as an RSV vaccine candidate. Here I summarize and review each chapter throughout the thesis.

In Chapter 2, a series of sfGFP-X-PNs were established with various linkers in different lengths of flexible $(GGGGS)_n$ linkers and rigid $(PAPAP)_n$ linkers. PN was utilized as a static scaffold protein and sfGFP as a cargo protein. The dynamics of sfGFPs in each construct was observed directly using HS-AFM techniques. By analyzing the width of every hexameric molecule in a duration time of 40 sec, I investigated the dynamic effects of linkers on the width of each construct and the fluctuation of the cargo proteins. The results revealed that both the linker length and the sequence-dependent flexibility contribute to the fluctuation of sfGFP and the intermolecular interaction between the cargo proteins and the scaffold protein. Since PN can penetrate cells, there is potential to use PN for effective drug delivery and vaccine applications with fine-tuning provided by choosing target proteins and adjusting the number of residues and components of the linkers.

In Chapter 3, a non-structural protein derived from human norovirus (HuNV) known as viral

protein genome-linked (VPg) is used as a cargo protein genetically fused with PN. VPg is essential in norovirus genome replication by translation from genomic and subgenomic RNAs and serves as a cap substitute for ribosomal recruitment. Thus, it is expected to be effective in stimulating the immune response. By displaying such non-structural protein on the surface of a repetitive structure such as the trimer-dimer structure of PN, the construct could serve as an engineered subunit vaccine. I conjugated VPg to the N-terminus of PN via different kinds of linkers (VPg-X-PNs) and investigated the efficiency of linker properties and conformational dynamics of constructs using high-speed atomic force microscopy (HS-AFM). The trimer-dimer of PN is retained after conjugation, and the β-sheet structures of these constructs are confirmed. However, the flexibility of VPg may remain, which causes difficulty in identifying its image by HS-AFM. These results indicate that the effect of linker length and flexibility are different on each cargo protein. In addition, PN is a competent scaffold to conjugate with non-structural viral proteins for vaccine development.

In Chapter 4, subsequently from the results of Chapter 3, I developed a new engineered subunit vaccine candidate by exhibiting respiratory syncytial virus (RSV) phosphoprotein (P) on the surface of the PN scaffold via genetic fusion. Since rigid linker was toxic to cells, and $(GGGGS)_n$ with precise lengths are highly flexible and widely used in vaccine application, the flexible linker is preferable. In addition, the results in Chapter 2 demonstrate that the GGGGS linker provides a high fluctuation of sfGFPs in sfGFP-PN constructs. GGGGS was utilized to maintain the distance between P and PN while retaining the dynamics of P protein based on the design of a previous report. The vaccine candidate induced the production of anti-P antibodies (IgA and IgG) in immunized cotton rats. Notably, RSV titers in cotton rats were produced about two folds less than non-immunized or immunized rats with P only by inoculating with P-PN. These results indicate the promising potential of PN in the development of subunit vaccines for RSV.