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学位（博士）論文要旨

(Doctoral thesis abstract)

論文提出者 (Ph.D. candidate)	工学府博士後期課程　生命工学専攻 (major) 令和 3 年度入学(Admission year)　2021 年 10 月 01 日 学籍番号　21831651　氏名　Rawiwan Wongnak (student ID No.)　(Name)
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論 文 題 目 (Title)	Biophysical and immunological analysis of SARS-CoV-2 Omicron BA.5 receptor binding domain (RBD) produced in *E. coli* as a potential vaccine antigen.

論文要旨（2000 字程度）
(Abstract(400 words))
※欧文・和文どちらでもよい。但し、和文の場合は英訳を付すこと。
（in English or in Japanese）

The emergence of highly transmissible SARS-CoV-2 variants like Omicron has posed significant challenges to global COVID-19 vaccination efforts. This dissertation presents two pivotal studies highlighting the potential of low-cost bacterial production of the receptor-binding domain (RBD) of the SARS-CoV-2 spike protein to address this challenge.

The first study details the successful expression of SARS-CoV-2 Omicron BA.5 RBD in *E. coli* T7 SHuffle, an engineered strain that facilitates disulfide bond formation. By expressing the protein at low temperature and employing air oxidation, we achieved a high yield of RBD (3 mg/200 mL culture). The identity of the RBD protein was confirmed by mass spectrometry, matching the theoretical molecular weight. Biophysical characterization showed that the secondary structure content, assessed by circular dichroism (CD), closely matched that of the crystal structure. Thermal denaturation and tryptophan fluorescence confirmed the folded structure and native-like conformation of the RBD. Dynamic light scattering (DLS), static light scattering (SLS), and analytical ultracentrifugation (AUC) confirmed the monomeric form and absence of aggregation. Limited proteolysis indicated biochemical stability similar to RNase A protein. Disulfide bond pairing, determined by LC-MS, matched the native structure, and binding assays showed a strong affinity to hACE2 (K_D = 0.83 nM), comparable to mammalian-expressed RBD. Furthermore, negative control experiments using ovalbumin confirmed the specificity of RBD binding to hACE2.

The second study focuses on the immunogenicity and neutralization capacity of *E. coli*-expressed Omicron BA.5 RBD in a mouse model. Immunization, even without adjuvants, elicited robust antibody responses, with antisera levels reaching 7.3 × 10^5 after the third dose and 1.6 × 10^6 after a booster dose administered 131 days later, indicating a strong memory response. Flow cytometry analysis revealed an increased population of $CD44^+CD62L^+$ T cells, suggesting effective T-cell memory generation. Cross-reactivity assays

showed that mouse antisera against Omicron RBD also reacted with Wuhan RBD, indicating a broad-spectrum response. In vitro assays demonstrated potent neutralization of a pseudovirus and inhibition of the interaction between SARS-CoV-2 RBD and the hACE2 receptor.

These findings underscore the potential of *E. coli*-expressed RBD as a viable and scalable vaccine candidate against SARS-CoV-2 variants. The scalability, cost-effectiveness, and robust immunogenicity of this bacterial expression system highlight its promise for rapid vaccine development to combat emerging variants. This dissertation emphasizes the significant role that bacterial expression systems can play in the ongoing fight against COVID-19.