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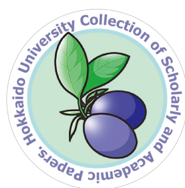

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Abstract of Doctoral Dissertation

Degree requested Doctor of Soft Matter Science Applicant's name Yan Shaonan

Title of Doctoral Dissertation

Recombinant Production of Human α-Defensin 5 via Calmodulin Fusion and Investigation of the Mechanism Enhancing Its Expression Level

（カルモジュリン融合によるヒト α-Defensin 5 の組換え生産と発現レベル向上機構の解明）

Defensins, which are cationic, cysteine-rich, and amphipathic peptides, stabilized by intramolecular disulfide bonds, constitute a major class of antimicrobial peptides (AMPs) found in mammalians, insects, and plants, and play an essential role in the host immune system against microbial invasion.

Human α-defensin 5 (HD5), a cysteine-rich antimicrobial peptide critical for intestinal innate immunity, has been extensively studied for its structural and functional properties. Both the oxidized form (HD5oxi) and reduced form (HD5red) exist *in vivo* and exhibit distinct antimicrobial activity spectra. However, the structure–function relationship of HD5 remains unclear.

To enable detailed analysis of HD5 using techniques such as nuclear magnetic resonance (NMR) spectroscopy, efficient production of stable isotope-labeled peptides is essential. While *Escherichia coli* (*E. coli*)-based recombinant expression provides practical advantages, small peptides like HD5 are prone to degradation and misfolding. Fusion protein strategies, including the use of calmodulin (CaM) as a partner, have shown efficiency in recombinantly expressing AMPs. Although considered suitable for α-helical AMPs production by its binding ability, CaM has also succeeded fusing with β-sheet peptides containing disulfide bonds. The interaction between CaM and peptides may inhibit the disulfide bond formation, underscoring the need to balance protection and folding. Thus, HD5oxi production via CaM fusion provides a valuable model to study these competitive processes.

In this study, we developed an efficient method to overexpress recombinant HD5 in *E. coli* BL21 (DE3) strain by using CaM as a fusion partner. Fusion expression suppressed the degradation of HD5 and reduced its toxicity to host cells. Following purification of the fusion protein and enzymatic cleavage to release the HD5 region, we successfully obtained sufficient amounts of active recombinant HD5oxi retaining desirable antimicrobial activity and native structure as well as HD5red for various applications. We further investigated the protective effect of CaM fusion and the mechanism of disulfide bond formation using circular dichroism (CD) and NMR spectroscopy,

structural prediction, and molecular dynamics simulations. Our results suggested that the appropriate interaction strength between CaM and the HD5 region in the fusion state is a key factor for stable production.

In conclusion, we demonstrated that HD5 fused with CaM can be efficiently recombinantly expressed in a soluble form using *E. coli* as a host. This method was applicable to both HD5oxi and HD5red, and stable isotope-labeled samples for NMR studies were efficiently prepared. Furthermore, we showed that disulfide bond formation, which is necessary for the conversion of reduced HD5red to oxidized HD5oxi, is not inhibited even in the CaM fusion state. Insights into the interactions and structures between CaM and the target peptides may provide important clues for extending the CaM fusion expression system to the production of other AMPs and peptide classes.