Development of new antifungal formulations based on enzymatic site-specific bioconjugation

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論 文 名　：Development of new antifungal formulations based on enzymatic site-specific bioconjugation
（酵素による部位特異的修飾に基づく新規抗真菌製剤の開発）

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論　文　内　容　の　要　旨

Fungal infections are serious diseases that require strict treatment because they can have serious consequences if not treated. Several variables can contribute to higher mortality rates in infected patients and the rapid spread of infection to others. Resistance can arise from controlled variables, such as increased use of antifungal drugs, or uncontrolled reasons, such as global climate change. Biotechnology and bioengineering from the viewpoint of chemistry will propose another approach to reducing either the dose or adverse effects of antifungal drugs. It is also important to consider the level of toxicity of the antifungal formulation being developed. In addition, compliance with drug regulations will facilitate future drug use.

In this thesis, I explored the potential of enzymatic bioconjugation toward new antifungal formulations. Specifically, I focused on microbial transglutaminase (MTG) to achieve site-specific modification of antifungal peptides and proteins. As an antifungal protein, a small chitin-binding domain (LysM) from *Pteris ryukyuensis* chitinase A was used. MTG-catalyzed site-specific introduction of lipid-modified substrates was first conducted to validate the effects of lipid moiety on the antifungal activity of LysM-lipid conjugates (Chapter 2). Then, I attempted to introduce responsiveness to external stimuli by engineering a peptide linker connecting LysM and lipid in the conjugate for effective treatment with a commercial liposomal antifungal drug, AmBisome (Chapter 3). Finally, I focused on the use of epsilon-poly-L-lysine (ε-PL) as an antifungal polypeptide. The consideration of selecting chemical or enzymatic modifications of ε-PL to maintain its antifungal activity was evaluated (Chapter 4).

In Chapter 2, I prepared a series of LysM-lipid conjugates (LysM-lipids) with different lengths (LysM-C12, -C14 and -C16) and different numbers of alkyl chains [LysM-$(C12)_2$, -$(C14)_2$ and -$(C16)_2$] and tested their antifungal activities against *Trichoderma viride* for the first time. The results showed that the cross-linking efficiency was independent of the type of lipid-peptide substrate except for LysM-$(C16)_2$. Since the antifungal activity of the LysM-lipids was influenced by both the length and the number of alkyl groups. For the development of new antifungal formulations, LysM-C14 and -C16 would be good candidates for further use.

In Chapter 3, I combined a liposomal antifungal formulation (AmBisome) with LysM-lipids. I designed LysM-Q with a thrombin digestive (TD) linker to add responsiveness to external environments. The results showed that the formulation of LysM-lipids with AmBisome has the ability to maintain stability at 25°C (for

chitin-binding ability) and at 4°C (for long-term storage). In addition, the insertion of a TD linker at the C-terminus of LysM improved the antifungal activity of AmBisome with LysM-lipids against *Candida albicans*, a common fungal pathogen that exhibits antibiotic resistance. These results demonstrate that the implementation of external stimuli-responsiveness may be a viable strategy for the development of effective treatment techniques for fungal diseases.

In Chapter 4 I have shown that the use of MTG to conjugate small molecules has led to a better understanding of the potential of ε-PL as an antifungal drug. The results indicate that the random chemical labeling caused a negative effect on the antifungal activity of ε-PL. By contrast, MTG-mediated N-terminal specific conjugation with a fluorescence substrate did not show a negative impact on its antifungal activity. Consequently, the visual localization of ε-PL by fluorescent microscopic analysis showed more uniform distribution in *T. viride* hyphae than that of chemically labeled counterparts. These results offer new strategies to develop antifungal cationic biopolymers while minimizing potential adverse effects.

In conclusion, the results obtained in this study indicate the potential of enzymatic conjugation in the creation of new antifungal biomolecules for future developments of antifungal formulations to humans. I hope this thesis will provide a clearer picture of the potential for using biological entities to advance the development of treatments for diseases caused by fungal infections.