Abstract of Doctoral Dissertation

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Amphibian derived Cathelicidin -DM and -BG: recombinant overexpression in *Escherichia coli*, and comparison of their structures and antimicrobial activities
(両生類由来カテリシジン-DM および-BG：大腸菌における組換え過剰発現、およびそれらの構造と抗菌活性の比較)

Amphibians are a widely studied source of antimicrobial peptides (AMPs). Cathelicidin -DM and -BG were discovered in frogs that live in Asia, *Duttaphrynus melanostictus* and *Bufo gargarizans*, respectively. Their amino acid sequences are homologous, but differences in predicted secondary structures and activity have been reported, although in non-identical conditions. Therefore, a direct experimental comparison was deemed important. In this study, we constructed recombinant overexpression systems for both cathelicidins and conducted an experimental comparison. In the general recombination overexpression system using *E. coli* as the host and thioredoxin as fusion partner protein, both cathelicidins were highly insoluble and difficult to purify. Conversely, in the system utilizing calmodulin as the fusion partner protein, there was an enhancement in solubility and cleavage efficiency, enabling the attainment of sufficient quantities of recombinant cathelicidins for a range of experiments.

Cathelicidin-DM was predicted to have a structure with a predominance of β-sheets using the structure prediction tools. However, CD measurement results confirmed that both cathelicidins form helical structures in micelles that mimic the membrane environment. In addition, it was confirmed that both cathelicidins have clear activity against Gram-positive and Gram-negative bacteria, and that the mechanism is mainly due to membrane disruption. From these results, it was confirmed that both peptides are highly similar, as their sequence homology indicates.