

Antimicrobial Mechanism of an Antimicrobial Peptide, Pseudin-2, Derived from the Skin of the Paradoxical Frog in Various Membrane Lipid Compositions

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Introduction

Pseudin-2 was isolated from an extract of the skin of the paradoxical frog *pseudis paradoxa* (Pseudidae) [1]. Pseudin-2, a naturally occurring 24 residues antimicrobial peptide (GLNALKKVVFQGIHEAIKLNHVQ), had broad-spectral antimicrobial activity and relatively low cytotoxicity. This peptide was believed to exert its antimicrobial activity *via* formation of pores in the target cell membrane [2-7].

In this study, the mechanical action of pseudin-2 on microorganisms and artificial model membranes were investigated. Circular dichroism (CD) studies showed that Pseudin-2 has an more amphipathic α -helical structure in negative liposome than in zwitterionic liposome. The peptide had no structural change in the increased salt concentration which affected in antimicrobial activity. Therefore, it was found that salt did not affect peptide conformation but did influence membrane potential energy.

Results and Discussion

Pseudin-2 is an antimicrobial peptide isolated from the skin of the *Pseudis paradoxa* (Pseudidae). In the previous study, pseudin-2 is a naturally occurring 24 residues (GLNALKKVVFQGIHEAIKLNHVQ-NH₂) antimicrobial peptide and it has broad-spectrum antimicrobial activity and low cytotoxicity activity (Table 1). This peptide is believed to exert its antimicrobial activity *via* formation of pores in the target cell membrane. In this study, we investigated the relationship between structure and mechanical action of pseudin-2 in microorganism and liposomes. In the studies of CD, Pseudin-2 has α -helical structure in zwitterionic liposome than negative charged liposome. A study of the interaction with various liposomes revealed that it causes perturbation of different liposomes and that the action of peptide is modulated to some extent by membrane lipid composition. In addition, pseudin-2 could form self-associated oligomeric structure in zwitterionic liposome and the release of fluorescent markers caused by pseudin-2 is size-dependent on liposome (Fig. 1). We suggest that pseudin-2 forms pores *via* peptide oligomerization using the toroidal pore-forming mechanism in zwitterionic liposomes, whereas the barrel-stave model occurs in negatively charged liposomes.

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References

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	MIC (μ M)			
	Pseudin-2		Melittin	
	Buffer I	Buffer II	Buffer I	Buffer II
Bacteria cell G(+)				
<i>L. monocytogenes</i>	1	8	2	2
<i>S. aureus</i>	1	2	2	1
<i>B. subtilis</i>	1	2	2	2
<i>S. epidermidis</i>	2	8	2	16
Bacteria cell G(-)				
<i>E. coli</i>	2	4	2	4
<i>P. aeruginosa</i>	2	8	2	8
<i>S. typhimurium</i>	1	1	1	1
Yeast				
<i>C. albicans</i>	8	16	8	16
<i>T. beigellii</i>	8	16	4	16
Fungi				
<i>A. fumigatus</i>	32	64	32	64
<i>F. oxysporum</i>	64	>64	32	64
<i>A. flavus</i>	32	64	32	64

Table 1. Antimicrobial activity of pseudin-2

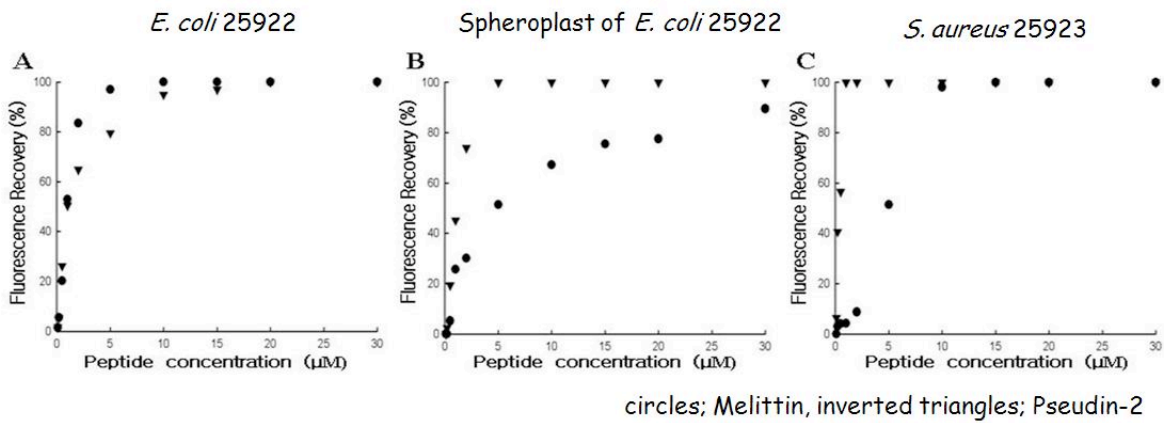


Figure 2. Depolarization of the membrane potential of bacteria