

Nematode Cecropin P4 Pro-piece Inhibits Microbicidal Activity of Antimicrobial Peptides

S. Ueno^{1,2}, K. Kusaka¹, H. Zhang¹, M. Minaba¹, and Y. Kato^{1*}

¹National Institute of Agrobiological Sciences, Tsukuba, 305-8634, Japan; ²University of Tsukuba, Tsukuba, 305-8572, Japan
E-mail: kato@affrc.go.jp

Introduction

Antimicrobial peptides are immune effectors produced in various multicellular organisms. These peptides are selectively toxic against microbes, but not against higher eukaryotes. The nematode cecropin is an α -helical-type antimicrobial peptide isolated from the body fluid of the pig intestinal parasitic nematode, *Ascaris suum* [1-3]. Until date, four members of nematode cecropin (P1-P4) have been identified in *A. suum*. In addition, clear homologues have been found in other Ascarididae nematodes (*Ascaris lambricoides*, and *Toxocara canis*) by EST database searches [4]. The nematode cecropin precursor consists of a secretory signal region at the N-terminal, a mature peptide, and a C-terminal acidic pro-region (Fig. 1). The C-terminal pro-region is conserved in all known nematode cecropins, suggesting that the pro-region may be important for the functioning of nematode cecropins. However, the physiological role of the acidic pro-region is still unclear.

In this study, we chemically synthesized the C-terminal pro-piece of cecropin P4 (P4P) to examine whether it affected the activity of mature cecropin P4. In addition, its effect on various other antimicrobial peptides was also investigated.

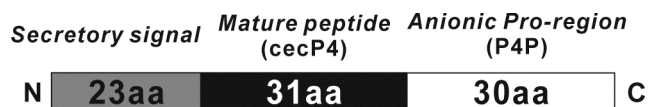


Fig. 1. Organization of nematode cecropin P4.

Results

We assessed the influence of mature cecropin P4 on bactericidal activity with or without P4P at various molar ratios. P4P inhibited the bactericidal activity at an equimolar (1:1) concentration or higher, both against *Escherichia coli* (Fig. 2A) and *Staphylococcus aureus* (Fig. 2B). This inhibition was observed to be dose-dependent. P4P without mature cecropin P4, is not bactericidal below a concentration of 300 μ g/ml.

Cecropin A is an insect antimicrobial peptide isolated from *Cecropia* moth. Cecropin A consists of two α -helices connected via a hinge-region. Although such α -helix-rich structure is not completely identical to that of nematode cecropins, both the structures are clearly similar to each other. We tested the effect of P4P on the bactericidal activity of cecropin A (Fig. 3). P4P also inhibited the activity of cecropin A.

ASABF- α is another antimicrobial peptide isolated from *A. suum*. ASABF- α , which is structurally different from nematode cecropin, since it contains a single α -helix and a pair of antiparallel β -sheets, stabilized by four

intramolecular disulfide bridges, called CS $\alpha\beta$ motif. ASABF- α is especially effective against Gram-positive bacteria. It also shows some bactericidal effect against Gram-negative bacteria and yeasts. Nisin is a bacterial antimicrobial peptide (bacteriocin) produced in the Gram-positive bacterium, *Streptococcus lactis*. Nisin contains post-translationally modified 34 amino acid residues and 5 disulfide bridges. Gram-positive bacteria are sensitive to nisin. Polymyxin B is a peptidic antibiotic isolated from Gram-positive bacterium, *Bacillus polymyxa*. Polymyxin B circularly polymerized with 10 amino acids is active against Gram-negative bacteria. Although ASABF- α , nisin, and polymyxin B are membrane-attacking antimicrobial molecules, similar to nematode cecropins, these molecules are structurally distinct from nematode cecropins. P4P inhibits not only the bactericidal activity of cecropin P4 but also that of cecropin A, suggesting that the inhibition by P4P is not highly specific for the naturally coupled antimicrobial peptide, cecropin P4. To test whether P4P also inhibited the activity of antimicrobial agents that are structurally unrelated to cecropin P4, the effect of P4P against ASABF- α , nisin, and polymyxin B were explored (Fig. 4A-C). Although the activity of ASABF- α and nisin was not affected, P4P inhibited the activity of polymyxin B, similar to that of cecropin P4 and cecropin A.

Discussion

In this study, we demonstrated that P4P inhibited the bactericidal activity of mature cecropin P4. Mature nematode cecropin P1 attacks mitochondria resulting in the uncoupling of respiration, since the mitochondrial membrane is similar to that of bacteria [5]. Such mitochondrial toxicity is also found in insect cecropins. In insect cecropin, a small pro-region at the N terminus suppresses membrane-attacking activity of mature peptide, suggesting that the N-terminal pro-region inhibits unexpected activity of mature peptide, such as the mitochondrial toxicity in the cytoplasm. Thus, P4P should have a similar physiological role, and the suppression of mitochondrial toxicity may be essential for in vivo production of cecropin-type antimicrobial peptides. The suppression of mature peptide activity by acidic pro-region has also been found in mammalian α -defensin, further fortifying our conclusion. Some other antimicrobial peptides, such as mollusk defensins and tunicate styelins, also contain a C-terminal acidic pro-region, suggesting that such precursor organization may be a typical structure for regulating the activity of antimicrobial peptides.

P4P is negatively charged at neutral pH and is directly cis-fused to positively charged mature cecropin P4, suggesting that these regions may be electrostatically

coupled with each other, i.e., the inhibitory effect of P4P on the activity of mature cecropin P4 may be due to direct binding of these regions. P4P inhibited not only the activity of naturally coupling antimicrobial peptide, cecropin P4, but also that of a structurally related peptide, insect cecropin A. In contrast, the activities of structurally unrelated antimicrobial peptides, ASABF- α and nisin, were not affected by P4P. The binding of P4P could be restricted in cecropin-type structure. Polymyxin B exhibits strong affinity to negatively charged macromolecules such as lipopolysaccharides. Although the competitive binding of P4P to polymyxin B is a possible mechanism of inhibition, the nature of P4P inhibition remains to be elucidated.

Nematode cecropins, mainly cecropin P1, are widely investigated for their application in clinical use and for the generation of pathogen-resistant gene-modified plants. The inhibitory effect of P4P can extend the technological applications of nematode cecropins.

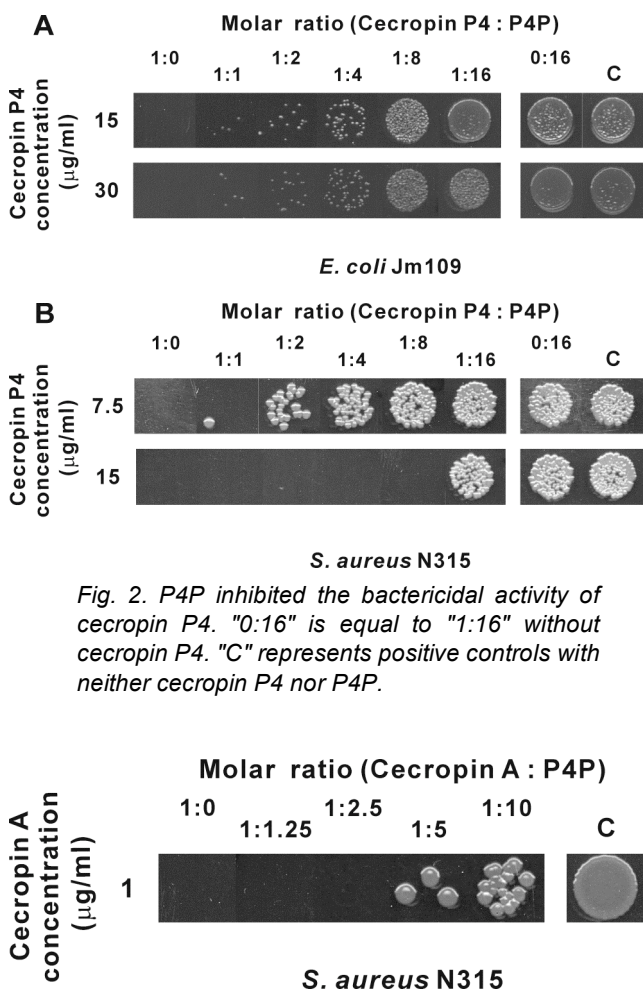


Fig. 2. P4P inhibited the bactericidal activity of cecropin P4. "0:16" is equal to "1:16" without cecropin P4. "C" represents positive controls with neither cecropin P4 nor P4P.

Fig. 3. P4P also inhibited the bactericidal activity of cecropin P4. C; no peptide.

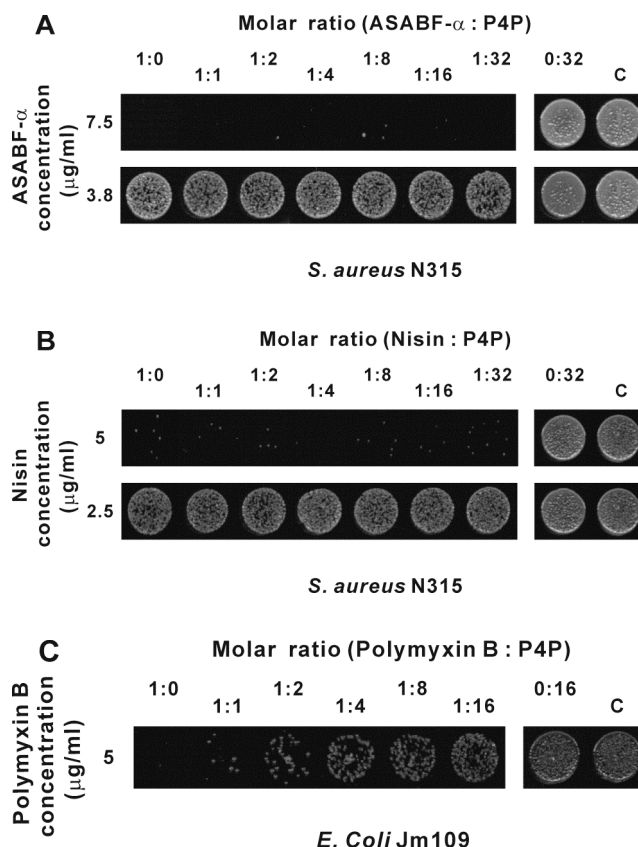


Fig. 4. Effect of P4P on bactericidal activity of peptidestructurally unrelated to cecropins. "0:32" and "0:16" are equal to "1:32" and "1:16" without antimicrobial agents, respectively. "C" represents positive controls with neither antimicrobial agents nor P4P.

Acknowledgments

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