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Fungicidal Effects of Cationic Antimicrobial Peptides and its Application

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In order to investigate of fungicidal effect and its mechanism(s) of the synthetic antimicrobial peptides such as well known cationic antimicrobial peptides, Cecropin A, Magainin 2, Melittin, PMAP-23 and active peptides derived from proteins such as HP (2-20) were used as model peptides. The cationic antimicrobial peptides displays a strong fungicidal activity against various fungi, and the fungicidal activity was inhibited by Ca^{2+} and Mg^{2+} ions. To develop the novel antifungal peptides useful as therapeutic drugs requires strong fungicidal activity against pathogenic fungal cells. To this goal, the several analogues with amino acid substitutions were designed to increase only net hydrophobicity by Trp(W)-substitution at the hydrophilic helix face of cationic antimicrobial peptides without any change at the hydrophobic helix face. These analogues were designed by analysis of the α-helical wheel diagram of cationic antimicrobial peptides. In paticular, the most increased hydrophobic region in cationic antimicrobial peptides induced the enhanced fungicidal activity and antitumor activity and the fungicidal activity was inhibited by salts and the respiratory inhibitor, NaN_3 (1, 2). Thus, this peptide may provide a useful template for design novel antibiotic peptides for the treatment of infectious diseases. The results suggested that the increase of hydrophobicity of the peptides correlated with an antibiotic activity. In order to investigate a relationship of the structure-antimicrobial and hemolytic activities between template peptides and its analogues, several analogues with amino acid substitutions were designed and synthesized. The results showed that the hybrid peptides and its analogues were remarkable increase in antimicrobial activity with less hemolytic activity against human erythrocyte cells than natural model peptides. The analogues were designed by analysis of the α-helical wheel diagram of template peptides. CD analysis showed that all peptides revealed a α-helical conformation in 50% TFE and 30% SDS solution. It was concluded that the hydrophobic region is responsible for the effective antimicrobial activity of the antimicrobial peptides with α-helix structure. The tertiary structures of peptides in DPC micelles, as determined by NMR spectroscopy. The results demonstrate that α-helicity of the peptides is essential for antimicrobial activity.

To study the antimicrobial mechanism of these peptides, fluorescence activated flow cytometry and confocal laser scanning microscopy were performed with the most powerful analogue peptide designed (3-6). The results suggested that the antimicrobial function of these analogues acts by pore formation in the cell membrane. Accordingly, these antimicrobial peptides could be used a good model for the design of

effective antimicrobial agents with a potent antibiotic activity yet without any cytotoxic effects against host organism. Further design of high-value added peptides may require inclusion of unnatural amino acid or small organic compound by using peptidomimetic technology.

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