

ANTIMICROBIAL PEPTIDES FOR USE IN BIOSENSING APPLICATIONS

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Current pathogen detection systems lack the stability, sensitivity, and time-independent functionality required for real-time biosensing in the field. Antibodies exhibit specificity for pathogenic bacteria but lack the sensitivity to detect reduced pathogen levels and the stability needed for detection in harsh environments. PCR-based techniques are extremely accurate and sensitive but lengthy sample preparation and the need for secondary analysis equipment is causing major hurdles. To overcome these inefficiencies, naturally occurring antimicrobial peptides (AMPs) are being investigated due to their intrinsic stability in harsh environments, low molecular weight (3000-5000Da), and binding affinity toward bacteria. AMPs possess a broad range of activity and affinity toward microorganisms, including gram-negative and gram-positive bacteria, but often lack selectivity for pathogenic bacteria.^{1,2} Our research focuses on tailoring AMPs not for antimicrobial activity but for selective binding to pathogenic bacteria for the development of new molecular recognition elements for use in pathogen detection platforms.

Six full-length peptides (pleurocidin, cecropin P1, PGQ, cecropin A, ceratotoxin A, and SMAP-29) were chemically synthesized with the addition of a c-terminal cysteine for site-directed immobilization onto a maleimide reactive plate. A whole cell binding assay was developed to utilize horseradish peroxidase (HRP)-conjugated antibodies specific to the target cells with subsequent color development for the quantitative determination of a peptides' ability to bind specific bacteria (Fig 1). The full-length peptides were analyzed for their discriminatory binding behavior to the gram-negative food pathogen *E. coli* O157:H7 relative to gram-positive *S. aureus* 27217 and a non-pathogenic gram-negative *E. coli* 45827. All of the full-length peptides exhibited preferential binding of the gram-negative food pathogen *E. coli* O157:H7 versus gram-positive *S. aureus* 27217. This initial discrimination verification was necessary before proceeding to binding studies to discriminate between two gram-negative species, which possess similar cell wall composition. Even more difficult is discrimination between two gram-negative bacterial strains (i.e. *E. coli* O157:H7 vs. *E. coli* 45827) since the cell wall components are even more similar. Three of the six peptides (cecropin P1, PGQ, and ceratotoxin A) exhibited selective capture of pathogenic *E. coli* O157:H7 versus non-pathogenic *E. coli* 45827 gram-negative bacteria (Fig 2). The ability to discriminate between pathogenic and non-pathogenic bacteria is essential for peptide-based molecular recognition elements in a pathogen detection platform since there is an abundance of non-pathogenic bacteria in a soldier's environment.

Full-length PGQ, cecropin p1, pleurocidin, and SMAP-29 were truncated (9-15 amino acids) to elucidate portions of the native sequence essential for binding to target cells. Development of short peptides with equivalent or enhanced affinity and selectivity for *E. coli* O157:H7, compared to full-length peptides, will lead to ease of commercial production. The fragments were rationally designed to encompass the entire native peptide sequence and overlap at amino

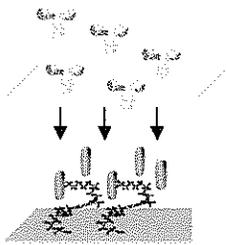


Fig 1. Schematic of whole cell binding assay

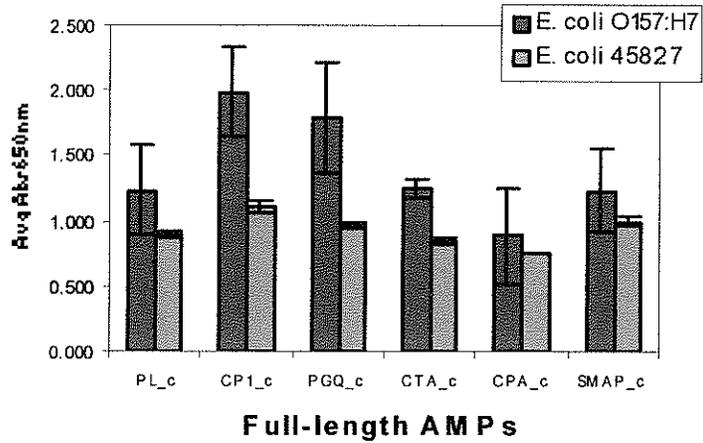


Fig 2. Full-length AMP preferential capture of *E. coli* O157:H7 vs. non-pathogenic *E. coli* 45827

acid residues known to be essential in activity analyses. Peptide fragments were synthesized and analyzed to determine the discriminatory binding behavior of previously identified *E. coli* O157:H7 binding domains. PL_1_c exhibited selective binding of *E. coli* O157:H7 versus *E. coli* 45827 while PGQ_2_c and CP1_4_c discriminatory binding properties remain inconclusive. Unexpectedly, several peptide fragments, most notable PL_4_c and SMAP_5_c, exhibited selective binding of *E. coli* 45827 vs. *E. coli* O157:H7. This is in contrast to the behavior of the corresponding full-length peptides identifying potential non-pathogenic bacterial binding domains. Non-pathogenic selective peptide fragments within a peptide-based array would allow for monitoring of false positive signals during real-time pathogen detection.

Continued investigation of discriminatory binding behavior of the peptides and their corresponding fragments versus different non-pathogenic gram-negative bacteria is needed to verify selective behavior. The ability to impart selectivity of antimicrobial peptides is an important initial step toward developing selective peptides for use in applications such as food safety, drug therapeutics, and water monitoring. In addition, with the current emphasis on homeland defense and the Army capability requirements for detection of CB warfare agents, the durability of these peptides may allow for the development of biological threat detection systems for incorporation as soldier-uniform embedded sensors.

- (1) Soares, J. W.; Mello, C. M. In *SPIE International Symposium: Photonics East 2003*; Bennedsen, B. S., Chen, Y.-R., Meyer, G. E., Senecal, A. G., Tu, S.-I., Eds.; Monitoring Food Safety, Agriculture, and Plant Health; SPIE-The International Society for Optical Engineering (USA): Providence, RI, 2003; pp 20-27
- (2) Maloy, W. L.; Kari, U. P. *Biopolymers* **1995**, *37*, 105-122.