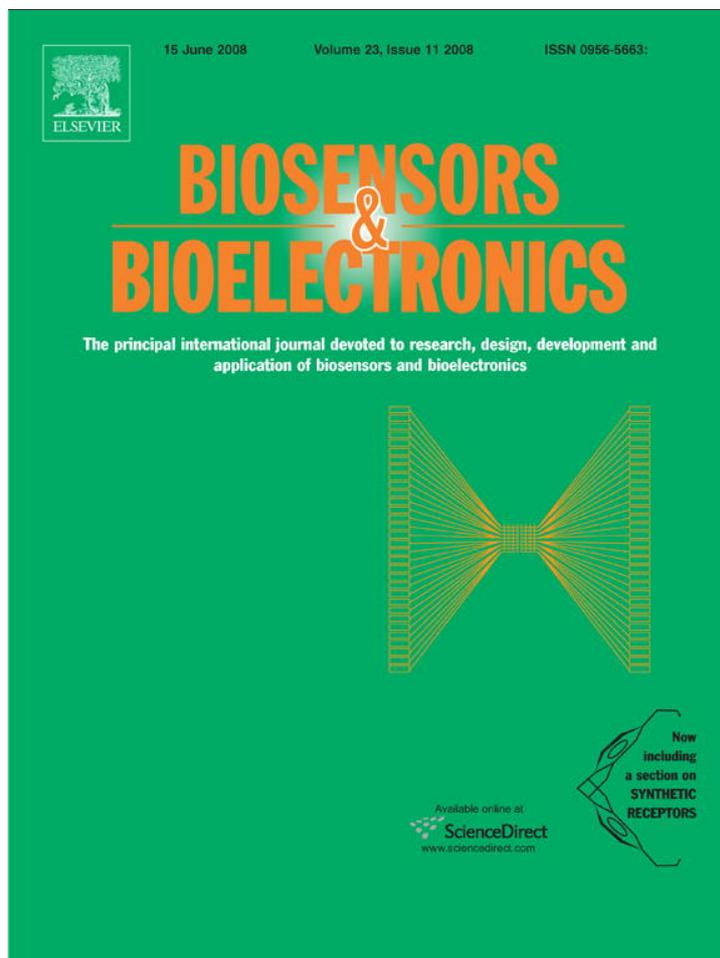


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Biosensors and Bioelectronics 23 (2008) 1721–1727

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Cy5 labeled antimicrobial peptides for enhanced detection of *Escherichia coli* O157:H7

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Received 26 October 2007; received in revised form 26 January 2008; accepted 6 February 2008

Available online 13 February 2008

Abstract

Fluorescently labeled antimicrobial peptides were evaluated as a potential replacement of labeled antibodies in a sandwich assay for the detection of *Escherichia coli* O157:H7. Antimicrobial peptides naturally bind to the lipopolysaccharide component of bacterial cell walls as part of their mode of action. Because of their small size relative to antibodies peptides can bind to cell surfaces with greater density, thereby increasing the optical signal and improving sensitivity. This method combines the specificity of a capture antibody with the increased sensitivity provided by using a labeled peptide as a detection molecule. The antimicrobial peptides cecropin P1, SMAP29, and PGQ were labeled with the fluorescent dye Cy5 via maleimide linker chemistry. Preliminary screening using a whole-cell solution binding assay revealed that Cy5 cecropin P1 enhanced the detection of *E. coli* O157:H7 relative to a Cy5 labeled anti-*E. coli* O157:H7 antibody 10-fold. Detection sensitivity of antibody and peptide were also compared with a prototype immuno-magnetic bead biosensor. Detection using Cy5 cecropin P1 resulted in a 10-fold improvement in sensitivity. Correlation of peptide antimicrobial activity with detection of *E. coli* O157:H7 indicated that activity was not predictive of the sensitivity of the fluorescent assay.

Published by Elsevier B.V.

Keywords: Antimicrobial peptides; Magnetic immuno-capture; *Escherichia coli*; Biosensor; Detection; Pathogens

1. Introduction

Antimicrobial peptides (AMPs) are part of the innate defense system found in all organisms to protect them from microbial infection and are classified primarily by secondary structure (Boman, 1995). They exhibit a relatively broad range of antimicrobial activity toward bacteria, fungi and viruses (Brogden, 2005; Nicolas and Mor, 1995). Although the lysis mechanism is not completely understood, the AMPs used in this study bind to the cell membrane as a precursor to their bactericidal activity. AMPs that target Gram-negative bacteria bind non-specifically to the negatively charged lipopolysaccharide via electrostatic and van der Waals interactions of both pathogenic and non-pathogenic organ-

isms (Piers et al., 1994; Sawyer et al., 1988; Vorland et al., 1999).

Much of the literature has focused on peptides for potential use as therapeutic agents or their interaction with LPS and artificial membranes to elucidate mechanisms of antimicrobial activity. Recently AMPs have been immobilized on solid substrates for capturing and detecting microorganisms. Cecropin P1 immobilization on maleic anhydride microplates using amine residues was reported for the capture of pathogenic and non-pathogenic strains of *Escherichia coli* (Gregory and Mello, 2005). AMPs have also been immobilized via an engineered cysteine to control peptide orientation for the investigation of binding specificity of various microorganisms (Mello and Soares, in press). Kulagina et al. (2005, 2006) immobilized AMPs onto glass slides using biotin-avidin chemistry for detection of *E. coli* O157:H7 and *Salmonella typhimurium*. In these studies fluorescently labeled cells were detected directly or in a sandwich assay using a fluorescently labeled antibody. Detection limits *E. coli* and *Salmonella* were 5×10^4 to 5×10^5 and

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1×10^5 to 5×10^6 CFU ml⁻¹, respectively. To date, the use of antimicrobial peptides as a labeled molecule for detection of microorganisms has not been reported.

A number of immunologically based techniques are employed for the detection of *E. coli* O157:H7, one of numerous pathogenic microorganisms that continue to cause food-borne illness. While the majority of outbreaks associated with *E. coli* O157:H7 have involved ground beef (Naugle et al., 2005), outbreaks have also occurred in unpasteurized apple juice and orange juice (Cody et al., 1999), unpasteurized milk, alfalfa sprouts (Breuer et al., 2001), lettuce, and water (Friedman et al., 1999). Immuno-magnetic separation (IMS) is a common method used to capture cells with antibody conjugated paramagnetic beads and subsequently detected with a second labeled antibody. IMS has been used in food and environmental samples to capture *E. coli* O157:H7 (Liu et al., 2003; Parham et al., 2003; Shelton and Karns, 2001), *S. typhimurium* (Yu and Bruno, 1996) and *Bacillus sterootherophilus* (Blake and Weimer, 1997). Although detection of single organisms has been reported with IMS, a pre-enrichment step of up to 18 h was required (Geng et al., 2006; Padhye and Doyle, 1991; Tsai et al., 2000). To avoid widespread illness rapid detection methods require an enhancement of sensitivity without the need for enrichment.

Antibodies are used in IMS because of their relative specificity and ability to minimize false positive results. Liu et al. (2003) demonstrated the ability to avoid cross-reactivity in a biosensor format distinguishing *E. coli* O157:H7 from *S. typhimurium*, *Listeria monocytogenes* and *Campylobacter jejuni*. However, the elimination or reduction of cross-reactivity is not always possible and is dependent on the target organism and use of monoclonal or polyclonal antibodies. While possessing specificity, antibodies lack the sensitivity for direct measurement of low cell concentrations. The detection limit by fluorescence is generally 10^3 to 10^4 bacterial cells ml⁻¹ in food matrices (DeMarco and Lim, 2001; Demarco and Lim, 2002; Geng et al., 2006).

To address antibody limitations, AMPs were investigated as an alternative detection molecule. The class of linear, cationic peptides that form amphipathic α -helical structures upon cell binding are the focus of this study. Cecropin P1, SMAP29 and PGQ were used as labeled detection molecules for sensing *E. coli* O157:H7. Cecropin P1 is found in nematodes from the stomach of pigs (Pillai et al., 2005) with antimicrobial activity against predominantly Gram-negative bacteria. Binding studies with phospholipid vesicles has identified the N-terminus as the binding region (Gazit et al., 1995). SMAP29 is from the cathelicidin family of peptides found in sheep (Bagella et al., 1995). It is highly active against Gram-positive and Gram-negative bacteria as well as fungi. Structure function studies have identified N- and C-terminal binding domains and that the N-terminal binding region is also responsible for antimicrobial activity (Tack et al., 2002). PGQ was isolated from frog skin but is the least active of the three investigated (Moore et al., 1991) and is not well characterized.

Variants of these peptides were synthesized containing a C-terminal cysteine for selective attachment of the fluorescent dye Cy5. Labeled peptides were evaluated as a

substitute for secondary antibodies in a whole-cell solution binding assay and a prototype magnetic bead immuno-capture sensing system. We hypothesized that enhanced sensitivity would be achieved using peptides for detection, taking advantage of the selectivity of binding by the capture antibodies and the high density binding of peptides to the cell surface. In the whole-cell binding assay, Cy5 labeled cecropin P1 (Cy5 CP1) was shown to enhance detection of *E. coli* O157:H7 10-fold relative to a Cy5 labeled anti-*E. coli* O157:H7 antibody. Use of Cy5 CP1 in an immuno-magnetic bead based biosensor resulted in detection of 10^3 CFU ml⁻¹, a 10-fold improvement in sensitivity relative to a secondary antibody.

2. Materials and methods

2.1. Bacteria, growth conditions and reagents

Phosphate-buffered saline (PBS) consisted of 137 mM NaCl, 2.7 mM KCl, 4.4 mM Na₂HPO₄ and 1.4 mM KH₂PO₄. PBST was PBS supplemented with 0.05% Tween 20. *E. coli* O157:H7 (ATCC 43888) was grown to mid-log in Luria broth (LB) at 37 °C to OD₆₀₀ = 1 (approximately 10^8 CFU ml⁻¹) and washed 2× in an equal volume of PBST before being resuspended in PBST. Cells were serially diluted 10-fold in PBST for detection sensitivity experiments.

2.2. Peptides and labeling

Antimicrobial peptides containing a C-terminal cysteine were chemically synthesized by SynPep Corp. (Dublin, CA, USA).

Cecropin P1	SWLSKTAKKLENSAKKRISSEGIAIAIQGGPRC
SMAP-29	RGLRRLGRKIAHGKVKYGPVLRIRIAGC
PGQ	GVLSNVIGYLLKLGALNAVLKQC

Peptides solubilized in phosphate-buffered saline (PBS), pH 7.4, at 1 mg ml⁻¹ were quantitated by BCA Protein Assay Kit (Pierce Biotechnology, Rockford, IL, USA). A 3 M excess of Tris(2-carboxyethyl)phosphine (Sigma Chemical Co., St. Louis, MO, USA) was added to reduce the peptide. Peptide was fluorescently labeled at 90 nmol/vial Cy5 dye from Cy5 mono-reactive maleimide kit Amersham Biosciences (Piscataway, NJ, USA). Cy5 labeled peptides CP1, SMAP, and PGQ were purified by RP-HPLC using a C₄ column, 250 mm × 4.6 mm, 5 μm pore size (YMC, Inc., Wilmington, NC, USA). HPLC fractions were lyophilized, resuspended in PBST and analyzed by SDS-PAGE. Fractions with the labeled peptide were pooled and quantitated by reverse phase HPLC using unlabeled peptide as a standard curve. Quantitation was confirmed with the Modified Lowry Protein Assay Kit (Pierce Biotechnology).

2.3. Antibody and labeling

Affinity purified polyclonal antibody to *E. coli* O157:H7 was obtained from KPL Inc. (Gaithersburg, MD, USA). One mg of antibody was reacted with a Cy5 mono-reactive maleimide kit

(Amersham Biosciences) and purified according to the manufacturer's instructions.

2.4. Whole-cell solution binding assay

Cells were grown and prepared in PBST as described above. A 100 μl aliquot of serially diluted cells was added to 900 μl of PBST containing Cy5 peptide (at a final concentration of 0.5–10 $\mu\text{g ml}^{-1}$) or Cy5 anti-O157 antibody (1:1000 final dilution) in PBST and mixed on a Dynal rotary mixer (Dynal Biotech, Browndeer, WI, USA) at approximately 20 rpm (setting 20–25) for 30 min at ambient temperature. Cells were harvested at 10,000 $\times g$ for 3 min, the supernatant removed with a pipet, and the pellet washed 3 \times with 1 ml PBST and spun as above. Cells were resuspended in 200 μl PBST and transferred to a black microplate (Nalge Nunc International, Rochester, NY, USA). A 900 μl aliquot of peptide solution was added to 100 μl buffer without cells and assayed as the zero cell negative control. The microplate was imaged using the Storm 860 (Amersham Biosciences) using red fluorescence at 1000 V PMT, 200 μm . The image was quantitated by TotalLab Version 2003.03 software (Nonlinear Dynamics, Newcastle upon Tyne, UK).

2.5. Immuno-capture biosensor assays

Cells were grown and prepared in PBST as described above. 20 μl anti-*E. coli* O157 paramagnetic Dyna-beads (Dynal Biotech) were added to 1 ml of 10³ to 10⁶ CFU ml⁻¹ cells and incubated 30 min by rotary mixing. A zero cell sample of beads and buffer was run as the negative control. Beads were collected and washed 3 \times with 1 ml PBST. Cy5 CP1 (5 μg) or Cy5 anti-O157 antibody (1:1000 dilution) in 1 ml PBST were added to cells captured on the magnetic beads. After 30 min of rotary mixing, beads were collected and washed in PBST to remove unbound peptide. Beads were resuspended in 500 μl PBST for analysis on a magnetic focusing fiber optic biosensor.

2.6. Sensor platform

A second generation prototype magnetic focusing fiber optic fluorometer (Pierson Scientific Associates, Andover, MA, USA) was used as the sensor platform. It consisted of a 5 milliwatt, 635 nm diode laser excitation source (Lasermate Corporation, Walnut, CA, USA), a compact photomultiplier detector (Hamamatsu Corporation, Hamamatsu City, Japan) and an optical system. Optical filters were purchased from Omega Optical, Inc. The custom built optical system consisted of two optical fibers, an excitation fiber and emission fiber, for the transmission of light at a wavelength of 650 nm. The fibers were interfaced with a sample chamber that held a standard disposable semi micro-cuvette. The excitation fiber delivered laser excitation light to the sample chamber and the emission fiber carried the emitted fluorescence light to the photomultiplier. The custom built sample chamber consisted of a metal probe that housed the fibers and a magnetic holder that securely positioned the probe at the cuvette wall. The holder also generated the magnetic field required to

pull the paramagnetic microspheres out of solution to a spot in front of the excitation and emission fibers where the fluorescence was measured.

2.7. Antimicrobial activity assay

Bactericidal kinetics were determined using a modified version of the assay described by Lehrer et al. (1983); 10⁵ CFU ml⁻¹ *E. coli* O157:H7 in PBST was incubated with 5 μg Cy5 labeled peptide for 30 min with rotary mixing to reflect the solution binding assay conditions. Aliquots were serially diluted 10-fold in high ionic strength buffer (PBST–1 M NaCl), plated on LB medium and incubated overnight at 37 °C. Peptide free controls were also run to determine control cell concentrations.

2.8. Statistical analysis

Statistical analyses were carried out using SigmaPlot 10 (Systat Software, Inc., Point Richmond, CA, USA). An unpaired *t*-test was used to test the mean differences and generate *P*-values. Each labeled molecule was analyzed by calculating the *P*-value of signal from each cell concentration and comparing to the zero cell control. The lowest cell concentration with *P* < 0.1 was considered to be the detection limit. *P*-values were also used for comparison of signal of labeled molecules for each cell concentration; *P*-values less than 0.1 were deemed to be a statistically significant difference.

3. Results and discussion

3.1. Peptide labeling

Peptides synthesized with a C-terminal cysteine were labeled with the fluorescent dye Cy5 via maleimide linker chemistry. The Cy5 mono-reactive maleimide kit was designed for one mg of antibody and modifications were necessary for peptide labeling. One milligram of peptide failed to label with high efficiency. Peptide quantity was reduced to 90 nmol per reaction, comparable to the moles of labeling sites found on the antibody. HPLC purification was necessary due to the similar size of the peptides relative to that of free dye. The degree of peptide labeling with Cy5 dye was peptide dependent; molar ratios of dye:peptide were 1.44, 0.53 and 0.4 for SMAP, CP1, and PGQ, respectively. A theoretical maximum molar ratio of one dye per peptide molecule is possible due to labeling the single cysteine residue engineered on each peptide. Co-purified free Cy5 dye is suspected for the Cy5 SMAP degree of labeling result, due to insufficient separation by HPLC. However, any free dye present with Cy5 SMAP would not have contributed to the fluorescent signal in these binding studies, since unconjugated dye alone does not bind to cells or magnetic beads.

3.2. Dose response

The solution binding assay with *E. coli* O157:H7 cells revealed a linear dose response curve between 0.5 and

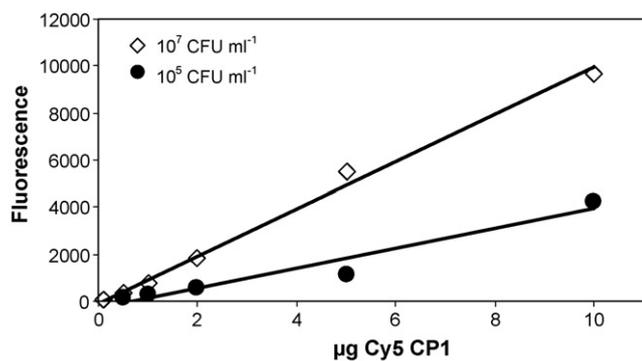


Fig. 1. Dose response of Cy5 CP1 vs. *E. coli* O157:H7 in PBST in solution binding assay. Saturation does not occur up to 10 µg peptide at either 10⁷ or 10⁵ CFU ml⁻¹ cell concentration.

10 µg ml⁻¹ with purified Cy5 CP1 (Fig. 1). This was an effort to determine the concentration of labeled peptide required to saturate the cells to generate maximum signal; however, saturation was not achieved. Cell densities of 10⁵ and 10⁷ CFU ml⁻¹ both resulted in a greater fluorescent signal as the quantity of peptide increased to 10 µg ml⁻¹. Based on the antimicrobial activity of CP1, a concentration of 5 µg ml⁻¹ was chosen. 5 µg ml⁻¹ was also used for SMAP and PGQ.

3.3. Detection

There are many methods for detection of food pathogens that employ labeled antibodies as detection molecules. Antibodies possess target specificity, but also have the limitations of insufficient detection sensitivity and instability. Using the same antibody for both capture and detection may result in competition for the same epitopes, causing steric hindrance and reduced sensitivity. Due to these factors, antimicrobial peptides were evaluated as alternative detection molecules in an immuno-capture assay to improve sensitivity. Peptides alone are not suitable for detection of microorganisms, due to their mode of binding non-selectively to the negatively charged LPS. Specificity for a particular organism would be addressed during target capture, using the selectivity of the capture antibody.

3.3.1. Peptide screening by solution binding assay

To determine the detection sensitivity of *E. coli* O157:H7, 5 µg ml⁻¹ labeled peptides were tested in solution against 10⁷ to 10⁴ CFU ml⁻¹ cells and compared to a 1:1000 dilution of Cy5-anti O157 antibody. Improved detection of *E. coli* O157:H7 using a labeled AMP in place of an antibody was demonstrated (Fig. 2). A *t*-test was run on each labeled detection molecule to determine the minimum cell concentration which is statistically different than the zero cell control. The confidence limit of *P* = 0.1 was utilized to determine the detection limit. Of the three peptides evaluated, Cy5 CP1 was the most sensitive. The detection limit for antibody was 10⁵ CFU ml⁻¹ (*P* = 0.01), and for Cy5 CP1 was 10⁴ CFU ml⁻¹ (*P* = 0.032). It was therefore concluded that a detection sensitivity of 10⁴ CFU ml⁻¹ was achieved using Cy5 CP1, a 10-fold improvement over Cy5 antibody detection of 10⁵ CFU ml⁻¹ (*P* < 0.05).

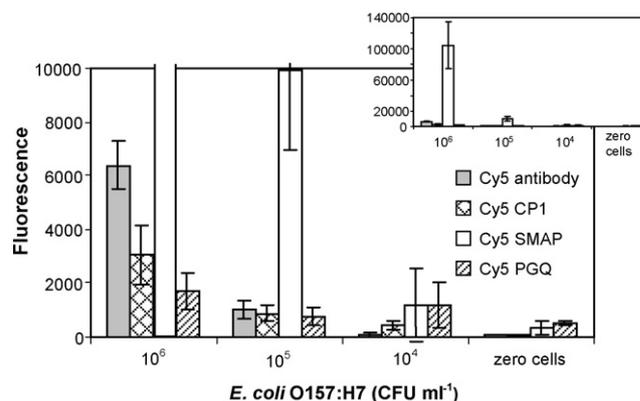


Fig. 2. Detection of *E. coli* O157:H7 in solution binding assay. Cy5 anti-O157 antibody (1:1000) and Cy5 labeled peptides (5 µg) were tested. Zero cells used as the negative control to exhibit background. Y-axis scale reduced to visualize detection limits. Full scale graph in the insert is to show elevated signal from Cy5 SMAP. Although Cy5 SMAP displayed elevated signal at increased cell concentration, Cy5 CP1 exhibits the greatest sensitivity based on net signal (*P* < 0.05).

Neither Cy5 SMAP nor Cy5 PGQ improved sensitivity, detecting 10⁵ and 10⁶ CFU ml⁻¹, respectively. While it was not determined if Cy5 CP1 could detect fewer cells, it appears that 10⁴ CFU ml⁻¹ may be approaching the sensitivity limit. Note that while Cy5 SMAP signal at 10⁶ CFU ml⁻¹ is 40× greater than Cy5 CP1, it is not as sensitive (detection limit of 10⁵ CFU ml⁻¹, *P* < 0.005). Free, unconjugated Cy5 dye did not exhibit cell binding (data not shown).

Bactericidal activity appears to be an important factor influencing detection sensitivity. This was tested under conditions that simulated the whole-cell solution-binding assay to determine if cell lysis was occurring during the 30 min of peptide-cell incubation. Although Cy5 SMAP had a much greater signal at a cell concentration of 10⁶ CFU ml⁻¹, it was not as sensitive as Cy5 CP1. Following a dramatic reduction at 10⁵ CFU ml⁻¹, Cy5 SMAP signal was completely lost at 10⁴ CFU ml⁻¹. Bactericidal activity for Cy5 SMAP yields, a 5-log reduction within

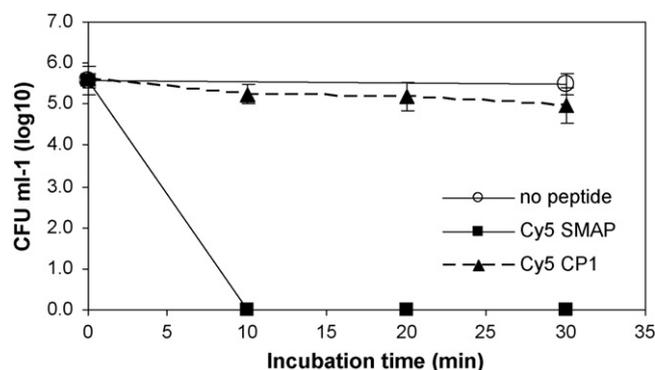


Fig. 3. Antimicrobial activity of Cy5 labeled peptides against *E. coli* O157:H7. Bactericidal activity of Cy5 labeled CP1 and SMAP. 10⁵ CFU ml⁻¹ organisms were incubated with 5 µg peptide for 30 min in PBST, 137 mM NaCl. Cells were then diluted in PBST, 1 M NaCl and plated to determine viable cell counts. Cells with no peptide added were the negative control. Cy5 PGQ bactericidal activity was not determined.

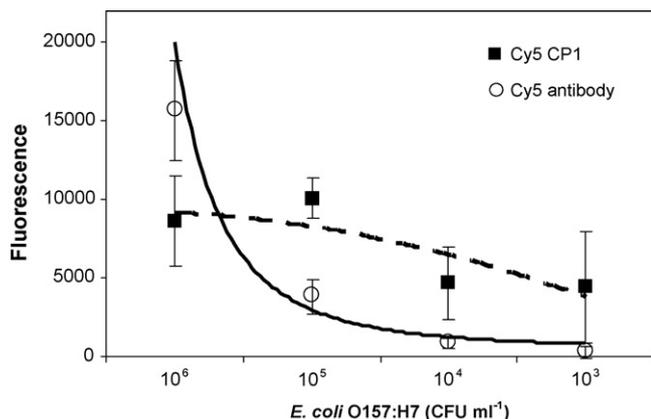


Fig. 4. Detection sensitivity of *E. coli* O157:H7 using magnetic immuno-capture assay. Cy5 anti-O157 antibody at a 1:1000 dilution and 5 μg Cy5 CP1 were tested. Zero cells used as the negative control; background was subtracted out to yield net signal. Cy5 CP1 increased sensitivity compared to Cy5 antibody ($P < 0.01$).

10 min. The 30 min peptide-cell incubation during detection of *E. coli* O157:H7 provides ample time for cell lysis to occur. Cy5 CP1 has at least 10-fold more sensitivity than Cy5 SMAP but showed little activity in 30 min, causing only a 1/2–1 log reduction (Fig. 3). It is possible that the lysed cell fragments containing bound Cy5 SMAP were washed away during the assay. During the whole-cell solution assay soft blue cell pellets were observed with Cy5 SMAP using 10^4 CFU ml⁻¹ or less cells. Under light microscopy the pellets appeared to be cell debris, further suggesting that lysis had taken place. Bactericidal activity of Cy5 PGQ was not tested because its activity was greater than the $5 \mu\text{g ml}^{-1}$ used in the solution or immuno-capture assays.

3.3.2. Immuno-detection

Detection sensitivity of *E. coli* O157:H7 by Cy5 CP1 peptide and anti-O157 antibody was also determined using a prototype immuno-capture biosensor. Various concentrations of cells (10^6 to 10^3 CFU ml⁻¹) were captured on anti-O157 antibody conjugated magnetic beads and detected with Cy5 labeled antibody (1:1000 dilution) or Cy5 CP1 ($5 \mu\text{g ml}^{-1}$) (Fig. 4). As noted above a *t*-test was run on the both the antibody and Cy5 CP1 data to determine the detection limit, using $P = 0.1$ as the threshold for a statistically significant difference. For antibody the limit was 10^5 CFU ml⁻¹ ($P = 0.086$), and Cy5 CP1 the limit was 10^4 CFU ml⁻¹ ($P = 0.056$). It was therefore concluded that Cy5 CP1 detection was 10^4 CFU ml⁻¹, 10-fold more sensitive than the Cy5 antibody detection sensitivity of 10^5 CFU ml⁻¹ ($P < 0.01$). Cy5 CP1 also has a net positive signal at 10^3 CFU ml⁻¹ cells, suggesting further enhancement in detection sensitivity. However, further investigation is needed due to the uncertainty raised by the large standard deviation. Cy5 SMAP and PGQ detection in the immuno-capture assay did not improve sensitivity relative to the antibody (data not shown).

The large amount of variability for Cy5 CP1 makes it difficult to definitively determine a detection limit. The cause of inconsistency is not known, but does not appear to be instrument related since the antibody data is relatively consistent. Unlike labeled

antibody, Cy5 CP1 does not respond proportionally to cell concentration. There is little statistical difference in signal between cell concentrations throughout the range tested. The zero cells control also exhibits variability, possibly caused by incomplete removal of unbound label when washing the magnetic beads. Changes in peptide solution may also affect interaction with the beads. Further efforts are needed to investigate the cause of the variability as well as finding a way to improve assay to assay consistency.

In contrast to Cy5 antibody, there was significant non-specific binding of Cy5 CP1 to the magnetic beads. For 10^6 CFU ml⁻¹ cells non-specific binding resulted in a signal to noise ratio of about 2. This ratio fell with decreasing cell concentration, significantly reducing net signal and possibly overall sensitivity.

To investigate how peptide quantity affects non-specific binding, 10^6 CFU ml⁻¹ cells were assayed using 2, 5, and 10 $\mu\text{g ml}^{-1}$ Cy5 CP1. High background signal was observed in the zero cell negative controls for each concentration (Fig. 5). In fact, background binding increased with greater peptide quantity, although the signal to noise ratio remained a relatively constant value of 2. Inclusion of non-ionic detergent did not effectively reduce the signal. Free Cy5 dye did not exhibit binding to the magnetic beads; background is therefore thought to be due to non-specific peptide binding (data not shown). Background reduction would improve signal to noise and possibly sensitivity. A number of blocking agents are being investigated to reduce non-specific binding, including BSA, non-fat dry milk, fetal bovine serum, and casein.

3.4. Optimization

Although Cy5 CP1 improved sensitivity when compared to a labeled antibody, the assay method has not been optimized. Two parameters for optimization are possible; increasing sensitivity and reducing assay time. First, improved sensitivity may be achieved by greater labeling efficiency or increasing the quantity of peptide. At 50% efficiency, Cy5 CP1 labeling could be improved to deliver more label to the cell surface. Increasing peptide concentration may further enhance sensitivity, since $5 \mu\text{g ml}^{-1}$ Cy5 CP1 did not reach cell saturation. However, because the peptide quantity to saturate $< 10^5$ CFU ml⁻¹ has

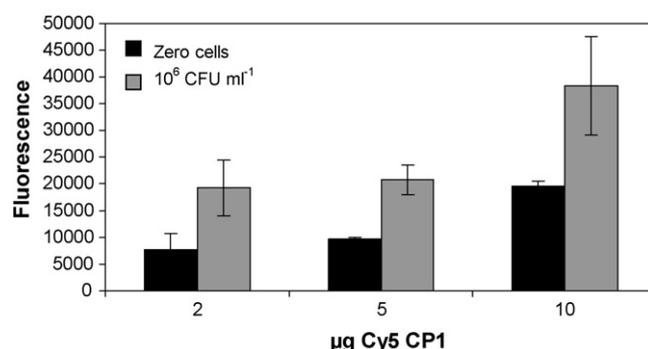


Fig. 5. Signal generated in magnetic immuno-capture determined by amount of Cy5 CP1. While increased peptide causes increased fluorescence, the percent of non-specific binding is high and has a relatively constant signal to noise ratio of approximately 2.

not been determined, it is not known whether increased peptide concentration will improve sensitivity. In addition, it was noted earlier that antimicrobial activity causing rapid cell lysis appears to adversely impact sensitivity. Potential activity from using greater than $5 \mu\text{g ml}^{-1}$ Cy5 CP1 would need to be determined. Identification of peptides with high binding affinity and reduced activity would also be worthwhile candidates to investigate.

Decreasing overall assay time would be desirable and may be possible by reduction of the incubation period of labeled AMP with captured cells. Rapid binding of Cy5 CP1 and Cy5 SMAP to *E. coli* O157:H7 in as little as 5 min has been observed. Signal intensity was not significantly different than the signal resulting from the standard 30-min binding (data not shown). Bactericidal activity in as little as 2 min has been reported for natural cecropin P1 against K12 strain *E. coli* D21 (Boman et al., 1993) and SMAP-29 against *Pseudomonas aeruginosa* (Travis et al., 2000). Since binding of these peptides is a precursor to cell lysis, rapid activity implies that binding is also occurring very quickly. A thorough investigation of how incubation time correlates with detection sensitivity has not yet been conducted.

3.5. Other applications

Immuno-detection is a commonly used method for pathogen detection from a variety of sources. As a detection molecule, the fluorescently labeled peptide Cy5 CP1 has been shown to enhance sensitivity relative to a labeled antibody in buffer. Detection with Cy5 CP1 in food matrices is currently ongoing. Preliminary results indicate that at least a 10-fold greater sensitivity compared to antibody is obtained in apple juice (data not shown). While the impact of food samples on immuno-detection has been investigated with antibodies, it is unknown what effect it will have using peptides for detection. Complex samples such as ground beef will likely present additional challenges, although the effect may be minimized because the majority of interfering substances would be washed from the beads when peptide is added. AMPs may also have application in other biosensor formats where antibodies are currently used for detection. Cy5 labeled antibodies have been used in a fiber optic immuno-sensor to detect *E. coli* O157:H7 (DeMarco and Lim, 2001; Demarco and Lim, 2002), *L. monocytogenes* (Geng et al., 2004), and *S. typhimurium* (Zhou et al., 1997). In addition to fluorescence, *E. coli* O157:H7 and *S. typhimurium* detection with electrochemiluminescence has been demonstrated (Yu and Bruno, 1996). While the data presented here demonstrates improved detection, only a single organism has been examined. Binding of fluorescent AMPs to non-pathogenic *E. coli*, *Staphylococcus aureus*, and *Bacillus* spp. spores has been demonstrated (data not shown), suggesting that peptides may also be useful for detection of other organisms.

4. Conclusions

Enhanced detection of *E. coli* O157:H7 has been demonstrated using a fluorescently labeled antimicrobial peptide in place of an antibody. Evaluation of labeled AMPs is underway in food samples. Assay optimization including improvement

of signal to noise and reduction of assay time may further increase sensitivity. The combination the antibody specificity and increased signal of provided by AMP detection molecules offers the opportunity of improved pathogen detection.

Acknowledgements

The authors would like to thank Jason Soares, Romy Kirby and Laurel Doherty (US Army Natick Soldier Research Engineering and Development Center), Daniel Lim and Marianne Kramer (Center for Biological Defense, University of South Florida) for their valuable ideas and discussions.

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