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Detection of *Listeria monocytogenes* with Short Peptide Fragments from Class IIa Bacteriocins as Recognition Elements

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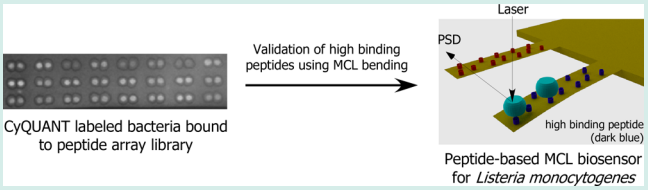
Supporting Information

ABSTRACT: We employed a direct peptide−bacteria binding assay to screen peptide fragments for high and specific binding to *Listeria monocytogenes*. Peptides were screened from a peptide array library synthesized on cellulose membrane. Twenty four peptide fragments (each a 14-mer) were derived from three potent anti-listerial peptides, Leucocin A, Pediocin PA1, and Curvacin A, that belong to class IIa bacteriocins. Fragment Leu10 (GEAFSAGVHRLANG), derived from the C-terminal region of Leucocin A, displayed the highest binding among all of the library fragments toward several pathogenic Gram-positive bacteria, including *L. monocytogenes*, *Enterococcus faecalis*, and *Staphylococcus aureus*. The specific binding of Leu10 to *L. monocytogenes* was further validated using microcantilever (MCL) experiments. Microcantilevers coated with gold were functionalized with peptides by chemical conjugation using a cysteamine linker to yield a peptide density of ~4.8 × 10^{-3} μmol/cm^2 for different peptide fragments. Leu10 (14-mer) functionalized MCL was able to detect *Listeria* with same sensitivity as that of Leucocin A (37-mer) functionalized MCL, validating the use of short peptide fragments in bacterial detection platforms. Fragment Leu10 folded into a helical conformation in solution, like that of native Leucocin A, suggesting that both Leu10 and Leucocin A may employ a similar mechanism for binding target bacteria. The results show that peptide-conjugated microcantilevers can function as highly sensitive platforms for *Listeria* detection and hold potential to be developed as biosensors for pathogenic bacteria.



KEYWORDS: *antimicrobial peptide, class IIa bacteriocins, peptide array, bacterial detection, microcantilever, Listeria monocytogenes*

INTRODUCTION

Rapid, sensitive, and specific detection of pathogenic bacteria using culture-independent methods is critical for monitoring food, the environment, and patients in clinical settings. In general, the conventional culture-based methods for detecting and enumerating bacteria are labor-intensive and time-consuming.[1] On the other hand, rapid methods, like immunoassays and polymerase chain reaction (PCR), are expensive, require state-of-the-art infrastructure, and often require sample pretreatment.[2−4] Pathogenic bacteria like *Listeria monocytogenes* are widely found in nature, such as soil, streamwater, sewage, plants, and food, and have a mortality rate approaching 25%.[5,6] Detecting such pathogens in a timely fashion at low-levels, therefore, remains critical.

Several biosensor platforms have been recently developed that utilize a recognition element, such as an antibody, aptamer, or peptide for sensing, and the binding of these sensing moieties to bacteria is read through transducer in real time. Antibody-based platforms have been popular due to their high affinity and specificity; however, these biomolecules lack stability under harsh conditions and can be expensive.[7,8] In contrast to antibody and oligonucleotide probes, peptides are intrinsically more stable in harsh environments, easier to synthesize, and exhibit broad activities and affinities against wide range of bacterial strains.[9−12] We have explored the viability of using Leucocin A (LeuA), a naturally occurring antimicrobial peptide (AMP) that displays potent activity against *Listeria* strains (minimum inhibitory concentration or MIC ~0.1 nM)[13,14] as a molecular recognition element in biosensor platforms. Using a LeuA-based platform, we selectively detected *L. monocytogenes* from other Gram-positive strains at a concentration of 10^3 cfu/mL.[12] LeuA belongs to class IIa bacteriocins that are characterized by a conserved disulfide bond and a YGNGV sequence near the N-terminus and with an amphiphilic helical C-terminal domain.[14,15] Class IIa bacteriocins possess a narrow activity spectrum, with high potency (typically active in the nanomolar range) against specific Gram-positive strains including *Listeria*, and act by a receptor-mediated mechanism on the target bacterial cells.[16] Such potent activity is presumably achieved by a specific

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binding interaction of the peptides with membrane-localized proteins of the mannose phosphotransferase system (man-PTS) of the target cells.[17,18] The expression levels of the receptor on the target bacterial surface dictate the activities of class IIa bacteriocins from one strain to another.[19]

Peptide-functionalized microcantilever-based systems are an ideal platform for developing miniature sensors, as the presence of a peptide makes bacterial detection highly sensitive, specific, and label-free due to the specific interaction between peptide and bacteria.[20−22] For chemical and biological sensing, one side of a cantilever is coated with a thin film of gold and functionalized with molecular recognition agents such as antibodies or peptides to make it chemically specific. When target molecules or cells bind to the recognition agents, the change in surface energy results in cantilever deflection, which can be detected with different readouts, such as optical beam deflection, capacitance, or piezoresitivity. The sensitivity and reproducibility of a cantilever's bending response depends on the uniformity of the immobilized functional layer as well as the cleanliness of the sensing surface.[23−26] For class IIa bacteriocins, the C-terminal domain determines the target cell specificity.[15] Therefore, we hypothesized that small peptide fragments derived from the C-terminal region of class IIa bacteriocins[27,28] may bind *Listeria* strains with the same specificity as that of the native full-length sequences and that a self-assembled monolayer (SAM) of small peptides on a gold microcantilever may serve as a potential biosensor platform for *Listeria* detection.

Accordingly, in this study, we screened a library of peptide fragments (14-residue) derived from native bacteriocins to identify the specific recognition elements from three class IIa bacteriocins, namely, Leucocin A (LeuA), Pediocin PA1 (PedPA1), and Curvacin A (CurA) (Figure 1). Several fragments from the C-terminal domain displayed high binding toward pathogenic Gram-positive bacteria, including *L. monocytogenes*, *Enterococcus faecalis*, and *Staphylococcus aureus*. Microcantilevers coated with gold and functionalized with a high-affinity peptide fragment, Leu10, showed a much higher deflection signal upon *Listeria* binding compared to that from microcantilevers functionalized with low-affinity fragments or a blank microcantilever (no peptide). In addition, MCL functionalized with a high-affinity peptide fragment showed a similar deflection and response time as that of full-length LeuA upon *Listeria* binding. This study highlights the potential of short peptide-based platforms in the development of biosensors to detect pathogenic bacteria.

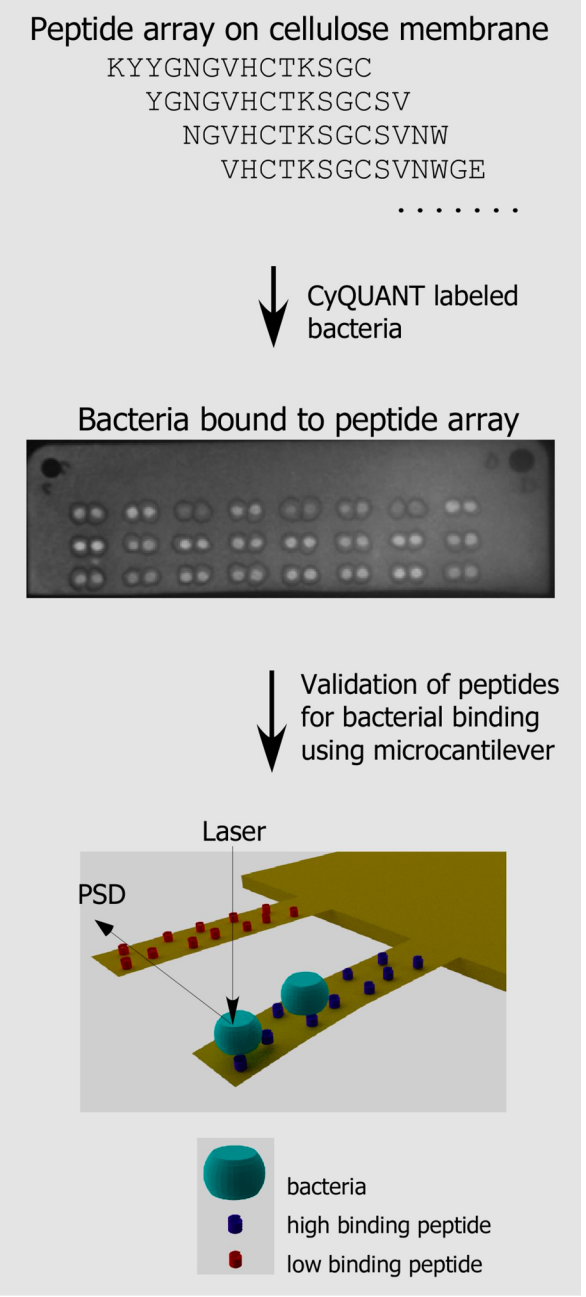

Figure 1. Schematic illustrating the steps used for screening and validating peptides with specific binding toward Gram-positive *L. monocytogenes*. PSD: Position sensitive detector.

RESULTS AND DISCUSSION

Design of Peptide Library Derived from Class IIa Bacteriocins. Previously, we used full-length LeuA (37-mer) and a 24-residue fragment from the C-terminal region of LeuA to show that these peptides bind specifically to Gram-positive strains like *Listeria* and show much less binding toward other Gram-positive bacteria.[29] These peptides do not bind to Gram-negative bacteria such as *Escherichia coli*. The binding between peptide and bacteria was detected using fluorescence microscopy (using labeled bacteria) or impedance spectroscopy (label-free detection).[12] Although fluorescence microscopy allowed detection of about 73 bacteria/100 μm^2, we were able to detect bacteria at a concentration of 1 cell/μL or 10^3 cfu/mL using the impedance method.

Here, we have designed a peptide library with 14-mer overlapping sequences from three class IIa bacteriocins, namely, LeuA, PedPA1, and CurA (Figure 2). Thirteen short fragments (Leu1−Leu13) were derived from the full-length LeuA (37-mer). In addition, six Pediocin fragments (Ped1−Ped6) and five Curvacin fragments (Cur1−Cur5) were derived from the C-terminal regions of PedPA1 (15−38) and CurA (14−35), respectively. For the latter two bacteriocins, fragments were derived from the C-terminal region to limit the number of peptides in the library.

A library of 24 peptides in duplicate was synthesized in an array format on a cellulose membrane derivatized with poly(ethylene glycol)-500 (PEG-500) with free amino groups (Figure 2). Peptides were conjugated to the membrane's amino groups through the C-terminal carboxylate, as we found previously that peptides immobilized from the C-terminus led

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Bacteriocin	Amino acid Sequence
Leucocin A	KY**YGNGV**HCT KSGCSVNWGE AFSAGVHRLA NGGNGFW
Pediocin PA1	KY**YGNGV**TCG KHSCSVDWGK ATTCIINNGA MAWATGGHQG NHKC
Curvacin A	ARS**YGNGV**YCN NKKCWVNRGE ATQSIIGGMI SGWASGLAGM

Fragment #	Fragment (14-mer) Sequence
Leu 1	KY**YGNGV**HCTKSGC
Leu 2	**YGNGV**HCTKSGCSV
Leu 3	**NGV**HCTKSGCSVNW
Leu 4	**V**HCTKSGCSVNWGE
Leu 5	CTKSGCSVNWGEAF
Leu 6	KSGCSVNWGEAFSA
Leu 7	GCSVNWGEAFSAGV
Leu 8	SVNWGEAFSAGVHR
Leu 9	NWGEAFSAGVHRLA
Leu 10	GEAFSAGVHRLANG
Leu 11	AFSAGVHRLANGGN
Leu 12	SAGVHRLANGGNGF
Leu 13	AGVHRLANGGNGFW
Ped 1	SVDWGKATTCIINN
Ped 2	DWGKATTCIINNGA
Ped 3	GKATTCIINNGAXA
Ped 4	ATTCIINNGAXAWA
Ped 5	TCIINNGAXAWATG
Ped 6	IINNGAMAWATGGH
Cur 1	KCWVNRGEATQSII
Cur 2	WVNRGEATQSIIGG
Cur 3	NRGEATQSIIGGXI
Cur 4	GEATQSIIGGXISG
Cur 5	ATQSIIGGXISGWA

Figure 2. Peptide library derived from different class IIa bacteriocins. In all, 24 peptide fragments (14-mers) were derived from Leucocin A (N to C), Pediocin PA1, and Curvacin A by overlapping 12 amino acids and skipping two amino acids. From Pediocin PA1 and Curvacin, peptide fragments were derived from the C-terminal region only (15−38 and 14−35, respectively). The YGNGV motif (bold) and residues in the C-terminal domain forming amphipathic α-helix (red) in the native peptide are highlighted. All peptides are conjugated to cellulose membrane from the C-terminus via a β-alanine (Z) linker.

to a higher number of bound bacteria (81 bacteria/100 μm^2) compared to that with peptide immobilized from the N-terminus (73 bacteria/100 μm^2).[30] Following peptide array synthesis, the membrane was incubated in DMSO (20%) for 10−12 h at 4 °C and then overnight at room temperature to form intramolecular disulfide bonds between the cysteines present in some sequences (Leu1−Leu5).

Screening of Peptide Fragments through a Whole Cell Binding Assay. Peptides displaying high binding to pathogenic bacteria, especially *Listeria* strains, were screened by incubating the peptide library with several Gram-positive strains. Bacteria were labeled with the fluorescent CyQUANT dye, by incubating with the dye for 2 h in dark, prior to incubating with the peptide library. CyQUANT was selected for bacteria labeling due to its low intrinsic fluorescence and high fluorescence and quantum yield on nucleic acid binding compared to that of other dyes like DAPI and CFSE.[31,32] The relative binding affinities of peptide fragments were determined through the net fluorescence intensity of the bound bacteria measured with a fluorescence Kodak imager (Figure 3).

The library was incubated with individual bacterial strains, and the binding was recorded followed by regeneration of the peptide array to remove all of the bound bacteria. The binding experiment was repeated once. The membrane was reused for up to 6−8 regenerations. After the binding assay was performed with five different bacterial strains, the binding to peptides was

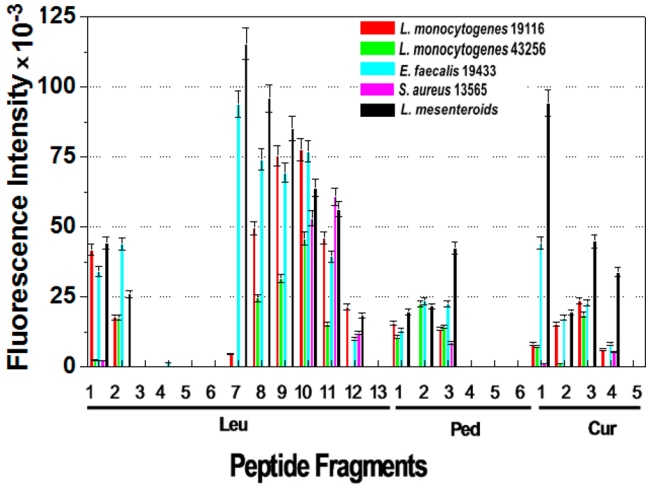

Figure 3. Screening of peptide fragments showing high binding to *Listeria monocytogenes*. A peptide array library on a cellulose membrane was incubated with fluorescently labeled bacterial cells (OD 0.05) for 3 h followed by extensive washing. Fluorescence intensity of bound bacterial cells was measured using a Kodak imager, with excitation and emission at 467 and 535 nm, respectively. The results presented here are mean fluorescence intensity ± SD. Red, *L. monocytogenes* ATCC 19116; green, *L. monocytogenes* ATCC 43256; blue, *E. faecalis*; pink, *S. aureus*; and black, *Leuconostoc mesenteroids* (or UAL 280).

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compared by plotting the fluorescence of the bound bacteria to each peptide fragment in the library, as shown in Figure 3. Several fragments from the C-terminal domain of LeuA (Leu7−Leu11), PedPA1 (Ped1−Ped3), and CurA (Cur1−Cur4) showed good binding to the bacterial strains. LeuA fragments displayed higher binding compared to that of PedPA1 and CurA, as shown by the fluorescence intensities of the bound bacteria. Moreover, Leu10 displayed the highest binding toward all of the pathogenic strains. In order to further validate the differential affinity of the peptide fragments, we selected two peptide fragments, Leu10 (high binding) and Ped3 (relatively lower affinity peptide), for subsequent experiments. Leu13 was selected as a negative control peptide that demonstrated no binding toward any of the tested bacterial strains.

Previously, we used cellulose membrane to screen peptides for mammalian cell line binding.[32,33] Here, we show that a peptide library on a cellulose membrane can also be used to screen peptides with affinity toward bacterial cells. In general, functionalized cellulose membrane is an ideal platform to evaluate a library of peptides (or other synthetic molecules) for whole cell binding or binding with biomolecules like proteins, peptides, and oligonucleotides.[34−36] Kaga and co-worker utilized a membrane-derived peptide array library to identify an octamer peptide that induces caspase-dependent cell death, and this study was performed through direct interaction of membrane-adhered peptide with the cells.[37] Similarly, Hilpert and Hancock used a membrane array library to dig out a highly active antimicrobial peptide from already known Bac2A.[38] In this study, the authors synthesized 100 analogues of Bac2A on the cellulose membrane and identified several antimicrobials that performed better than the parent through monitoring luciferase activity of transformed bacterial cells in an antimicrobial activity assay. However, in these studies, the authors punched out the peptide spots into a 96-well plate to perform antitumor or antimicrobial activity. We have used the intact peptide array library on the membrane to study peptide−cell binding interactions.

Secondary Structure of Select Peptides in Solution. To understand the structural basis of Leu10's higher and selective binding to *Listeria* strains, we used circular dichroism (CD) spectroscopy to study the solution conformation of selected peptides. CD spectra of Leu10 were compared to those of Ped3 and Leu13 in 40% TFE (Figure 4). The CD spectra of fragment Leu10 showed typical negative minima at 207 nm ($\theta = -5.0 \times 10^3$) and 222 nm and a positive band at 195 nm, suggesting a helical conformation. On the other hand, the spectra of Ped3 exhibited a negative minimum at 208 nm, suggesting a partial helical conformation or transformation from a β-sheet to a helical shape in the presence of TFE. Finally, Leu13 displayed a negative band at ∼195 nm, which is characteristic of a random coil. The CD spectrum of Leu10 resembled that of native LeuA, which is a helical peptide, as observed by CD and NMR spectroscopy.[39,40] The CD spectrum of native LeuA shows a negative band at 206 nm ($\theta = -10.5 \times 10^3$) and a negative shoulder near 220 nm ($\theta = -6.0 \times 10^3$).[28] Usually, it has been seen that helical conformations of a peptide play an important role in its interaction with a membrane receptor or antimicrobial activity.[41,42] The presence of a helical conformation in Leu10 and not in Ped3 and Leu13 confirms our conjecture that helical conformation may play an important role in the differential affinity of these peptides toward Gram-positive bacteria.

Peptide fragment	Sequence (14-mer)
Leu 10	H_2N-GEAFSAGVHRLANG-$CONH_2$
Leu 13	H_2N-AGVHRLANGGNGFW-$CONH_2$
Ped 3	H_2N-GKATTCIINNGAXA-$CONH_2$

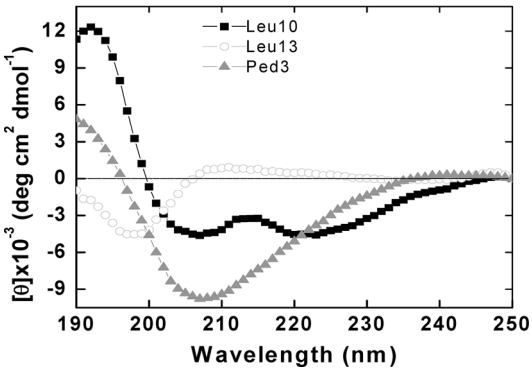

Figure 4. CD spectra of peptide fragments (Leu10, Leu13, and Ped3) in 40% TFE/water. The concentration of peptides was 0.3 mg/mL (∼200 μM).

Peptide Conjugation on Gold Surface of the Microcantilevers. Different chemistries have been used for the immobilization of recognition elements, such as peptides, antibodies, or carbohydrates, on a gold surface.[11,43,44] We previously used direct conjugation of thiolated peptides to a gold surface using gold−thiol chemistry.[29] Here, we used a thiolated linker, cysteamine hydrochloride, which provides a consistent peptide density during immobilization of different peptides on the gold surface. First, a gold chip was incubated overnight with cysteamine to make a monolayer on the gold surface. The free amino groups of cysteamine were then reacted with activated peptide by incubation with the peptide solution for 6 h. Peptide density using this method was obtained for two different peptides, Leu10 and Leu13.

In order to calculate the relative density of peptides on the microcantilever chip, peptides with N-terminal Fmoc (Fmoc-Leu10 and Fmoc-Leu13) or without Fmoc (Leu10 and Leu13) were immobilized independently onto a gold-layered silicon wafer chip. The Fmoc group was removed using piperidine, and the amount of peptide was estimated by measuring the Fmoc absorbance (extinction coefficient 7800 cm^{-1} M^{-1} dm^3) of the resulting solution at 300 nm (Table 1). The results show that both peptides formed a monolayer in the range of $\sim 4.8 \times 10^{-3}$ μmol/cm^2 or 2.9×10^{15} molecules/cm^2. A similar trend was seen by Jung and Campbell[45] for the surface coverage of a series of alkylthiols (CH_3CH_2SH−$CH_3(CH_2)_{17}SH$) from an ethanol solution onto gold using surface plasmon resonance (SPR) spectroscopy. The authors found that 80% surface

Table 1. Peptide Density on Gold Surface Measured Using the Absorbance of an Fmoc Group

peptide	absorbance (300 nm)[a]	peptide density
Fmoc-Leu10	0.021 ± 0.002	$\sim 4.8 \times 10^{-3}$ μmol/cm^2
Leu10	0.001 ± 0.001	
Fmoc-Leu13	0.021 ± 0.003	$\sim 4.8 \times 10^{-3}$ μmol/cm^2
Leu13	0.001 ± 0.001	

[a]Fmoc was removed from the peptides using 20% piperidine. The absorbace of the removed Fmoc group in solution was measured at 300 nm to calculate peptide density.

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coverage (4.8×10^{14} molecules/cm^2) was achieved in the first 40 s and that the remaining 20% adsorption occurred at a much slower rate. Since we incubated the gold with thiols (cysteamine hydrochloride) overnight followed by incubation with peptide solution for 6 h, the gold surface seems to be maximally covered, reaching 2.9×10^{15} molecules/cm^2.

Detection of Bacteria by Deflection of Peptide Coated Microcantilevers. Peptide fragments identified from library screening were evaluated using peptide-functionalized microcantilevers (MCL). A deflection in MCL upon bacterial binding was used for the detection of *L. monocytogenes* due to the specific interaction between peptide and bacteria. MCL deflection, which corresponds to the absorption of cells (bacteria) or molecules, can be accurately measured by a position-sensitive detector (PSD) and is described by Stoney's formula for electroplated metal films.[46] For MCL systems, where the secondary coatings are quite thin compared to the thickness of the cantilever, a no-slip boundary condition at the substrate−film interface can be assumed and the stress relaxation due to substrate deformation is negligible, so the deflection of the free end of a microcantilever, δ, can be related to differential surface stress, Δs, by

$$\Delta s = \left[\frac{Et^2\delta}{3L^2(1-\nu)}\right] \quad (1)$$

where E is Young's modulus for the substrate (Si, 155.8 GPa), t is the thickness for the cantilever (1 μm), L is the length of the cantilever (500 μm), and ν is Poisson's ratio (Si, 0.28). This formula (eq 1) was used to determine the surface stress differential upon bacteria binding. In addition, in our system, an optical beam deflection-based response readout was used. There are several procedures to readout the response of a microcantilever such as optical beam deflection, capacitance, piezoresitivity, piezoelectricity, and interferometery. Optical beam deflection-based response readout delivers the highest sensitivity and is used in most microcantilever-based biochemical applications.[47]

The three peptide fragments, Leu10, Ped3, and Leu13, were separately immobilized onto MCLs. The binding behavior of these fragments was compared with that of full-length LeuA. MCL coated with cysteamine alone (linker) was used as a blank reference. The sample was flowed through the MCL system at a constant flow rate of 5 mL/h. First, buffer (PBS, 50 mM, pH 7.4) was allowed to flow through the system to establish a stable baseline, followed by introduction of the sample solution containing *L. monocytogenes* at a concentration of 10^5 cfu/mL. As shown in Figure 5, we observed that the response or signal builds over time after the introduction of the bacterial sample and that the signal saturates in about 20 min when the peptide−cantilever surface reaches maximal bacterial binding. The deflection on the Leu10-functionalized MCL was measured at around 100 nm, which refers to a surface stress of 29 mN/m. In comparison, the LeuA-functionalized sensor showed a response of about 120 nm, indicating similar binding processes between LeuA and *L. monocytogenes* as well as Leu10 and *L. monocytogenes*. On the other hand, the response of the Ped3-functionalized sensor to *L. monocytogenes* was significantly reduced. The deflection observed for Ped3 was about 40 nm, whereas Leu13 showed no significant response to *L. monocytogenes*. Furthermore, the response time for Leu10 MCL is comparable to that of the LeuA MCL, suggesting that

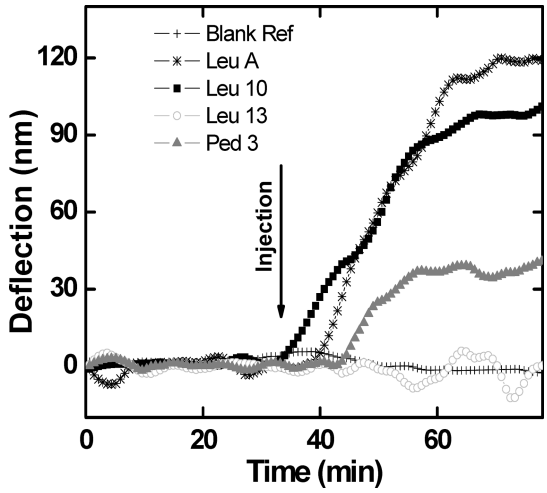

Figure 5. Deflection of microcantilevers functionalized with LeuA-derived short peptides upon *L. monocytogenes* ATCC 19116 binding. *L. monocytogenes* was injected at a concentration of 10^5 cfu/mL (in PBS) at a flow rate of 5 mL/h. LeuA is a 37-residue full-length antimicrobial peptide, whereas Leu10, Leu13, and Ped3 are 14-residue peptide fragments.

Leu10 is an optimum peptide candidate for *L. monocytogenes* detection.

We have used peptide-conjugated microcantilevers with optical beam deflection for detecting the specific interaction between the sensor and bacteria with high sensitivity. Such a nanomechanical platform offers advantages over other biosensor methods such as surface plasmon resonance (SPR), quartz crystal microbalance (QCM), and electrochemical methods, like impedance, admittance, or capacitance. SPR real-time detection is based on a change in the refractive index when an analyte interacts with the recognition surface. It is highly sensitive, but SPR detection may require pretreatment of the sample to first isolate bacteria, e.g., using antibodies.[48] Bouguelia and co-workers used SPR to detect low levels of *Salmonella* and other Gram-negative pathogens.[49] However, their culture−capture−measure (CCM) approach utilizes monoclonal antibodies immobilized on an SPR surface, and the detection takes place in several hours (>6 h). QCM, which is a mass-based method, has not yet been optimized for high sensitivity.[50] Similarly, electrochemical methods like impedance spectroscopy that provide a fast readout are still in the early stages of development.[51,9,12]

CONCLUSIONS

Peptides function as specific recognition elements for the detection of a variety of biomolecules and cells. Using a synthetic peptide array library on a cellulose membrane, we screened short peptide fragments for high and specific binding to *L. monocytogenes*. Peptide fragments were derived from three potent anti-listerial peptides that belong to class IIa bacteriocins. Several fragments from the C-terminal region showed high-to-moderate binding to five Gram-positive bacteria. Fragment Leu10 (GEAFSAGVHRLANG), which displayed the highest relative binding to bacteria compared to that of other fragments, was chosen for validation using a microcantilever (MCL)-based technique. MCLs were functionalized with fragments Leu10, Ped3, Leu13 or with full-length LeuA. The Leu10-functionalized MCL was able to detect *Listeria* with the same sensitivity as that of the LeuA-

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functionalized MCL. It is interesting to note that the binding response of a short fragment was similar to that of the full-length peptide. However, the antibacterial activity of this class of peptides is dramatically reduced even with a small change in the sequence, and short sequences are generally inactive.[28,52] In addition, class IIa bacteriocins have a well-folded C-terminal amphiphathic helix that is considered to be important for its target bacteria binding and antimicrobial activity.[15] Leu10, derived from the C-terminal region of LeuA, folds into a helical conformation (Figure 4), suggesting that Leu10 may possess a similar binding mechanism as that of full-length bacteriocins to the target bacteria. The study highlights the use of peptide array membranes to screen short fragments from antimicrobial peptides with specificity toward pathogenic bacteria and demonstrates that such peptides can be used as recognition elements in biosensor development.

EXPERIMENTAL PROCEDURES

Materials. All reagents and solvents were of analytical grade and used as received with no further purification. Five bacterial strains, pathogenic (level II) and nonpathogenic, namely, *L. monocytogenes* (ATCC 19116 and ATCC 43256), *L. mesenteroids* (or UAL 280), *S. aureus* (ATCC 13565), and *E. faecalis* (ATCC 19433), were used in this study. All strains were obtained from the cell bank of CanBiocin, Inc. (Edmonton, AB, Canada) and were subcultured in their respective appropriate media, like APT or LB broth.

Peptide Array Synthesis. A peptide array library comprising 24 short peptide sequences (each 14 amino acids long) was synthesized in duplicate on a cellulose membrane using SPOT synthesis (Figure 2).[34] Short peptide sequences were derived from the sequence of full-length class IIa bacteriocins Leucocin A, Pediocin A, and Curvacin A. Briefly, from the full-length Leucocin A, 13 peptides of 14 amino acids in length, skipping two amino acids, were derived, and six and five peptides were derived from the central amphipathic α-helical region of Pediocin A and Curvacin A, respectively. The peptide array was synthesized on a PEG-500-derivatized cellulose membrane with a free amino terminal group using a semiautomatic robot AutoSpot ASP222 (Intavis AG, Germany), as described in the Supporting Information.[32,33]

Peptide Array-Cell Binding Assay. Bacteria were labeled with CyQUANT dye before performing the cell binding assay with the peptide array membrane. The peptide array membrane was incubated with the labeled bacteria, and, after washing, the net fluorescence intensity of each peptide spot due to bound bacteria was quantified using a Kodak imager, as described in the Supporting Information.

Peptide Synthesis and Purification. The three selected peptide fragments (Leu10, Leu13, and Ped3) were synthesized by an automated synthesizer (Tribute, Protein Technology Inc., USA) utilizing solid-phase methods, as described in the supplementary data.[29]

Circular Dichroism. The circular dichroism (CD) spectra of the peptides were recorded on an Olis CD spectropolarimeter (GA, USA) in 40% TFE (40% TFE in water, v/v). The spectropolarimeter was calibrated routinely with 10-camphor sulfonic acid. The samples were scanned at room temperature (~25 °C) with the help of a capped quartz cuvette with a 0.2 cm path length at a wavelength range of 250−185 nm. The final concentration for CD measurements was 0.3 mg/mL (~200 μM) for each peptide. The baseline of blank (40% TFE) was subtracted from the peptide sample reading. An average of 4−6 scans was taken for each sample with a scan speed of 20 nm/min and a data interval of 1 nm.

Peptide Immobilization on Microcantilever System (Gold Surface). Gold-coated silicon microcantilever (MCL) arrays, 500 × 90 × 1 μm^3 (Micromotive Octosensis microcantilever arrays), were obtained from IBM Research Laboratory (Rüschlikon, Zurich). The gold-coated MCL array was rinsed with aliquots of ethanol followed by Piranha cleaning for 3 min and finally with DI water and ethanol rinse before functionalization. The backside of the MCL was passivated by immersing the array into a bis-(triethoxysilylpropyl) poly(ethylene oxide) ethanol solution for 20 min followed by rinsing and drying in air. After passivation, the microcantilever array was kept in 0.01 M cysteamine hydrochloride in a phosphate buffer solution (8× PBS) at pH 8.1 for 6 h at room temperature for the formation of a self-assembled monolayer (SAM) of cysteamine linker. After rinsing with ethanol and PBS, MCL arrays were immersed into a 1× PBS solution at pH 7.4 containing class IIa bacteriocin fragment peptides (Leu10, Leu13, or Ped3; 1.0 mg/mL). EDC (0.2 M) and NHS (0.05 M) were also added into the PBS solution to activate the carboxyl group (−COOH) of the peptides. The formation of amide bond between the cysteamine and the peptides led to immobilization of the peptides. The cantilever array was functionalized using a dip-in method.[53] By dipping the cantilever beam into the peptide solutions, the MCL array was selectively functionalized. The unmodified cantilevers served as the reference in bacterial detection. Prior to the deflection test, the MCL array was removed from the solution and rinsed with copious amounts of PBS to remove any physically adsorbed materials. The array was then dried with nitrogen and setup in the flow cell of the detection system.

Peptide Density on a Gold Surface. Peptide density on a gold surface was determined using two Fmoc-peptides, Fmoc-Leu10 and Fmoc-Leu13 (Figures S4 and S5). As described above, Fmoc peptides were conjugated to the gold surface (0.45 cm^2). Leu10 and Leu13 (without Fmoc) were also immobilized independently on the gold surface to serve as negative controls. Briefly, four gold surface chips were incubated with 0.01 M (75:25) of cysteamine hydrochloride (7.5 mM) and 3-mercapto-1-propanol (2.5 mM) in absolute alcohol overnight at room temperature. Thereafter, the gold surface was rinsed with ethanol and deionized water. After functionalization with cysteamine, chips were incubated with and without Fmoc peptides (~1 mg/mL) in PBS (pH 7.4) containing EDC (0.2 M) and NHS (0.05 M) for 6 h at room temperature. Chips were washed with excess PBS to remove adsorbed peptide and dried with a nitrogen flow. After the immobilization process, the four chips were incubated separately with 20% piperidine in DMF (0.5 mL for 30 min × 2). The chips were removed, and the absorbance of the solution was measured at 300 nm to calculate the concentration of immobilized peptides. The extinction coefficient of Fmoc at 300 nm (7800 cm^{-1} M^{-1} dm^3) was used to calculate peptide densities.

Microcantilever Deflection System and Detection. The detection of *L. monocytogenes* by the peptide-functionalized MCL array is based on the concept of changes in intrinsic stress leading to cantilever bending. All of the experiments were performed in 1× PBS (pH 7.4) solution with a constant flow rate of 5 mL/h. A syringe pump was used to introduce the mobile phase (PBS) and the bacterial solution into the flow cell where the MCL array was located. Due to the constant flow,

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the microcantilever response from fluidic dynamics was also recorded. This was considered as background noise for the study of peptide-assisted bacteria detection using the MCL system. This noise was minimized by taking the differential signal between the functionalized beam and the reference beam. After a steady baseline was achieved, the bacterial sample solution was injected into the experimental system at the same flow rate of 5 mL/h. The response of each microcantilever bending was recorded as a function of time as well as the total volume of PBS and bacterial solution flowed through the system. Deflection of the cantilever array was measured by a position-sensitive detector (PSD) assembled with the home-made system.

■ ASSOCIATED CONTENT

Supporting Information

Additional experimental details and MALDI-TOF characterization of peptides. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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