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Short communication

A capacitive DNA sensor-based test for simple and sensitive analysis of antibiotic resistance in field setting

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ABSTRACT

To meet urgent needs for solving serious worldwide drug-resistance problems, a sensitive label-free capacitive sensor developed in our group was investigated as a tool to be applied in the field of antibiotic resistance genotyping, for instance the detection of ampicillin resistance gene (ampR). Proof-of-concept data demonstrated its detection sensitivity of pico-molar without any signal amplification step and a dynamic range of at least three orders of magnitude. The detection limits of less than 1 pM for the single-stranded ampR oligonucleotide and 4 pM for the double-stranded target can reliably be achieved after only 2.5 min sample reaction. Reusability of the probe-functionalized disposable electrode was investigated by comparing different regeneration solutions; mix of 25 mM NaOH/30% formamide was employed to regenerate the electrode for at least six cycles without significant loss of sensing ability. Assay is performed automatically and result is retrieved in 20 min. The developed sensitive genotyping tool is expected to provide simple, fast and affordable screening for monitoring spread of antibiotic resistances, which is suitable for testing in field setting.

1. Introduction

Antibiotic resistance is a major global health threat today, largely due to the misuse of antibiotic drugs. It leads to less effective or failed treatment by current drugs, longer-period hospitalization and resulting rapid expansion of drug-resistant infections, which dramatically give impact on morbidity, mortality and increased healthcare costs (Baku, 2011). Infections from methicillin-resistant *Staphylococcus aureus*, multidrug-resistant tuberculosis and other common respiratory pathogens occur with increasing frequency worldwide. Antibiotic resistance tests range from the conventional resistant microbial growth phenotyping, e.g. disc diffusion and antimicrobial gradient tests, to genotyping, e.g. real-time PCR (Jorgensen and Ferraro, 2009). Due to the constraints of cost and centralized facilities, the problem is particularly serious in developing countries (Laxminarayan and Heymann, 2012). Therefore, an affordable and functional assay is needed to acquire antidrug information in point-of-care or field setting and to provide a possibility for doctors to treat patients with the proper drugs at an early stage of the infections.

To address this urgent need, an automatic label-free capacitive sensing system, CapSenze sensor involving a disposable gold electrode, was employed to develop a simple, fast, low-cost and sensitive genotyping test for detection of antibiotic resistance-encoding genes presented in pathogens. This sensor has been successfully applied for immunoassay by our group, which offers important advantages, i.e. high sensitivity, simple, reusable and portable (Mattiasson et al., 2010; Loyprasert et al., 2010). The developed capacitive immunosensor enables the fast and real-time detection of HIV-1 p24 antigen down to 5 fM in the plasma-spiked sample (Teeparuksapun et al., 2010). Here, this capacitive sensing platform was applied in the field of nucleic acid analysis and demonstrated detection of the ampicillin-resistance gene (ampR) within 20 min after sample injection. Our approach, a disposable gold working electrode, which is first functionalized with capture DNA oligonucleotide probe, mounted in a flow chamber to carry out target–probe complementary sequence hybridization. This surface reaction triggers a capacitance drop due to the change of the dielectric property, which can be measured in real-time by the capacitive DNA sensor.

2. Materials and methods

2.1. Reagents

Tyramine (99%, $HOC_6H_4CH_2CH_2NH_2$) was obtained from Sigma (Steinheim, Germany). Water used in all experiments was

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ultrapure water (Milli-Q, Millipore purification system, MA, USA). Phosphate buffer (PB, 10 mM) was prepared by dissolving 2.8 mM NaH_2PO_4 and 7.2 mM Na_2HPO_4 in water and adjusting to pH 7.2. PB buffer was degassed before use. Phosphate-buffered saline (PBS) contained 150 mM NaCl and 25 mM Na_2HPO_4/NaH_2PO_4 in water with a pH of 7.2. TNE buffer was prepared by dissolving 10 mM Tris, 10 mM NaCl and 1 mM EDTA in water and adjusting pH to 8 with concentrated HCl. DreamTaq DNA polymerase (5 U/μl), 10 × DreamTaq buffer, dNTP mix (2 mM each) and water (nuclease-free) were obtained from Thermo Scientific (Germany).

Oligonucleotides: poly-guanidine 25 (polyG25-amine) with 5′-end amino group label, poly-cytosine 25 (polyC25) and poly-adenine 25 (polyA25) were synthesized from Eurofins MWG operon (Ebersberg, Germany).

2.2. Escherichia coli BL21 (DE3) culture and DNA sample preparation

E. coli BL21 (*E. coli* BL21), which was transformed with the ampR-selective pET-22b(+) vector, was kindly provided by Gashaw Mamo (Department of Biotechnology, Lund University, Sweden). Bacteria were grown aerobically in 50 ml Luria–Bertani (LB) nutrient broth (Difco, Lennox), supplemented with 50 μl ampicillin, at 37 °C with shaking (180 rpm). Cells from the overnight culture were collected by centrifugation (3000*g* at 4 °C, 10 min). Pellets were washed once with TNE buffer, suspended and recollected by the same centrifugation. Afterward, cell pellets were resuspended in TNE buffer containing 1% Triton X-100. Freshly prepared lysozyme (0.5 mg/ml) was added to the cell suspension and incubated at 37 °C for 30 min. The enzyme was denatured at 80 °C for 15 min. Supernatant containing bacterial DNA material was collected by centrifugation (3000*g* at 4 °C, 30 min). Samples containing 30 ng/μl DNA and serial dilutions thereof were used as PCR templates.

2.3. Design of PCR primers and probes

Upper/lower primer pair, capture probe, target and helper oligonucleotides (oligo) were designed by using open IDT SciTools programs (Integrated DNA Technologies, USA) according to the ampR-coding gene sequences available from NCBI database. The NCBI BLAST function was employed to evaluate the sequence specificity of all selected primers and probes by aligning with all bacterial gene sequences available from NCBI.

PCR/real-time PCR upper primer (5′-CTTCATTCAGCTCCGGTTCC), lower primer (5′-CAGTGCTGCCATAACCATGA), target oligo (5′-GGGATCATGTAACTCGCCTTGA), ampR capture probe (5′-amine TCAAGGCGAGTTACATGATCCC), helper oligo (the same sequence as upper primer) and partial *E. coli* BL21 16S rRNA gene sequence (5′-GAGTAAAGTTAATACCTTTGCTCATTGACG) were synthesized from Integrated DNA Technologies (Leuven, Belgium).

2.4. Conventional PCR and real-time PCR

PCR reaction components in 50 μl volume contained 5 μl *E. coli* BL21 DNA template, 0.25 μM primers, 200 μM each of dNTPs and 1.25 U DreamTaq polymerase in 1 × DreamTaq buffer. PCR reactions were performed in a DNA Thermal Cycler (PTC-200, MJ Research, MN, USA) according to the designed program: predenaturation (95 °C, 10 min); followed by 35 cycles of denaturation (95 °C, 45 s), primers annealing (53 °C, 40 s) and elongation (72 °C, 40 s); final extension (72 °C, 10 min). Resulting PCR amplicons were analyzed by 1.5% agarose gel electrophoresis.

Real-time PCR was performed on a LightCycler™ 1.2 instrument (Roche Diagnostics GmbH, Mannheim, Germany) by using LightCycler® DNA Master SYBR Green I kit. The reaction components in 20 μl volume contained 0.2 μM primers and 5 μl *E. coli* BL21 DNA template. The protocol was according to the designed program: predenaturation (95 °C, 5 min); followed by 40 cycles of denaturation (95 °C, 10 s), primers annealing (53 °C, 5 s) and elongation (72 °C, 12 s). Quantification of the target ampR sequence in serially diluted DNA templates was analyzed by the LightCycler software version 3.5 (Roche Diagnostics GmbH).

2.5. Disposable electrode surface polymerization

Thin film electrode with the diameter of 3 mm was coated with a 200 nm thick layer of gold and prepared according to the previous work (Teeparuksapun et al., 2010). Gold electrode surface was cleaned with acetone for 10 min, ethanol for 5 min and rinsed with Milli-Q H_2O. Afterwards, electrode was dried with N_2 gas and plasma cleaned for 20 min.

A cleaned gold electrode surface was subjected to tyramine (0.02 M in PB buffer) electro-polymerization. Polymerization was performed by cyclic voltammetry with the potential range of 0–1.5 V (vs. Ag/AgCl) and the scan rate 50 mV/s for 15 scans. The electrode was washed thoroughly with PB buffer to remove physically adsorbed tyramine and finally dried with N_2 gas.

2.6. Electrode functionalization of DNA capture probes

Electrode surface was immersed in crosslinker glutaraldehyde solution (5% in PB buffer) for 30 min. After washing and drying, the modified gold surface was derivatized by adding 25 μl amino group-labeled capture probes (10 μM of polyG25-amine or ampR-amine in PBS buffer) and incubated overnight in a humidity chamber at room temperature (RT). After immobilization, the electrode was first immersed in ethanolamine solution (100 mM in PB buffer) for 20 min to passivate unreacted aldehyde groups; and followed by soaking in 1-dodecanethiol (10 mM in ethanol) to block any pinholes. Functionalized electrode was washed under streaming water to remove any unbound chemicals and then dried for later analysis.

2.7. PCR amplicon target preparation and capacitive sensor measurement with current pulse

Before sample injection, helper oligo (20 nM working concentration) was added to the serial-diluted PCR amplicon targets in PB buffer. The mixture (300 μl volume) was incubated in 95 °C water bath for 10 min and then immediately cooled on ice to recover to RT. Afterwards, sample was directly flow injected to the reaction chamber equipped with probe-functionalized electrode for sensor assay.

CapSenze capacitive sensing system is built by four main components, i.e. DNA probe-functionalized gold electrode, current source, capacitance measuring card and automated flow injection system (Fig. 1). The flow injection system consists of a flow cell (chamber) for mounting electrode and sample reaction, degasser unit, pump and multiport valves for automatic sample/buffer injection. The injection flow rate was 100 μl/min. All assay steps are controlled by PC-based CapSenze software command. Binding of injected target to the immobilized DNA probe leads to a capacitance reduction due to pushing-away of the electric double layer at the electrode/solution interface. This dielectric change is sensed in real-time by the measuring card under constant current pulse (+10 μA), recorded by the software. Details of the instrumentation and sensing principle were described previously (Erlandsson et al., 2014).

Sample was introduced to the flow cell with sensing electrode during a 2.5 min pump injection with the flow rate 100 μl/min. This triggers target–probe interaction when sample flowing through the electrode. PB buffer was constantly injected to wash