Journal of Electroanalytical Chemistry 786 (2017) 58–62

Contents lists available at ScienceDirect

Journal of Electroanalytical Chemistry

journal homepage: www.elsevier.com/locate/jelechem

Improving performance of a rapid electrochemical MRSA assay: Optimisation of assay conditions to achieve enhanced discrimination of clinically important DNA sequences under ambient conditions

D.K. Corrigan [a,b,c], H. Schulze [b], I Ciani [b], G Henihan [b], A.R. Mount [c], T.T. Bachmann [b,*]

[a] Department of Biomedical Engineering, University of Strathclyde, UK
[b] Division of Pathway Medicine, University of Edinburgh, UK
[c] EastChem, School of Chemistry, University of Edinburgh, UK

ARTICLE INFO

Article history:
Received 21 September 2016
Received in revised form 26 December 2016
Accepted 31 December 2016
Available online 3 January 2017

Keywords:
Electrochemistry
DNA
Gold electrodes
Electrochemical impedance spectroscopy
Biosensors
MRSA
Clinical detection
Point of care
Antimicrobial resistance
Screen printed electrodes

ABSTRACT

Electrochemical impedance spectroscopy (EIS) is a highly useful approach for the label free measurement of DNA hybridisation at functionalised electrode surfaces. Since label free detection relies upon a change in the electrochemical signal arising directly from the presence of target oligonucleotide or DNA/RNA sequences it is necessary to improve understanding of the conditions which produce a stable baseline value, promote optimal hybridisation of complementary sequences and can reduce non-specific binding effects. This study investigates both artificial DNA oligonucleotide sequences and clinical samples of MRSA genomic DNA, initially demonstrating that the use of tris(2-carboxyethyl)phosphine (TCEP) during probe layer formation improves both the initial baseline signal reproducibility and also the magnitude of the response upon hybridisation with a complementary target. Having demonstrated enhanced performance from TCEP modified electrodes, the assay is then used to detect clinical samples of MRSA. It is shown that improved performance is achieved both in terms of signal magnitude and discrimination against negative controls. Finally, formamide is added to the EIS measurement buffer and it is demonstrated that EIS measurement is possible in the presence of high formamide concentrations and that non-specific binding is also reduced under such conditions. The importance of these findings lies in the design of future electrochemical assays for nucleic acid biomarkers which are capable of functioning under ambient conditions but still have discriminatory power. A diagnostic device which does not have to operate at elevated temperatures will lead to increased simplicity and substantial battery and time savings which will further the widespread realisation of portable clinical diagnostic devices.

1. Introduction

Chemical and electrochemical sensors have found widespread application in health [1,2] and industrial/environmental monitoring [3–6]. For medical sensing, DNA hybridisation assays typically rely on the base-pair complementarity of two opposing strands of DNA. Upon recognition and hybridisation a signal is provided by e.g. fluorescence-labelled target which reports the binding event. Collection of a threshold signal allows detection of binding and confirmation of target recognition. This approach is common to many diagnostic tools such as microarrays and allows for high throughput screening of genetic sequences [7–9]. The hybridisation conditions necessary to promote specific sequence recognition are relatively well studied and due to the nature of the assays can often involve elevated temperatures and stringency washing. The prospect of transferring DNA hybridisation assays to electrochemical platforms has long been acknowledged [10–12] and studies have been carried out which allow the transfer of microarray sequences to electrode immobilised forms for electrochemical detection [13]. EIS is a powerful tool for monitoring interfacial phenomena and is particularly advantageous when implemented in nucleic acid detection because it permits label free monitoring of DNA hybridisation [14]. A number of studies have been carried out which report the use of EIS for DNA-DNA recognition [15–21], PNA-DNA recognition [22–25] morpholino-DNA recognition [26] and conformational change of the recognition interface [27]. In general, the scheme for detection by EIS involves measurement of the interfacial charge transfer resistance (R_{CT}) at a probe modified electrode in the absence and presence of the target sequence. Depending on the charge of the redox mediator employed, increases and decreases in R_{CT} can be measured as hybridisation takes place. More specifically, the voltage on the working electrode in a three electrode cell is subjected to a series of small magnitude sinusoidal perturbations which decrease in frequency. From the measured response and using an equivalent circuit, various physical

* Corresponding author.
E-mail address: till.bachmann@ed.ac.uk (T.T. Bachmann).

http://dx.doi.org/10.1016/j.jelechem.2016.12.050
1572-6657/

parameters such as the solution resistance (R_S), double layer capacitance (C_{DL}), charge transfer resistance (R_{CT}) and the Warburg Impedance (Z_W) can be extracted. A number of studies have been published which report a series of modifications and improvements to EIS based DNA assays which lead to improved sensitivity and performance [15, 19–21] including the use of dendrimers, nanoparticles and micro/nano electrodes. One significant disadvantage with the majority of these studies is the introduction of additional complexities which make such tests difficult to implement in clinical scenarios where simplicity of engineering and operation are major design concerns. Some recent papers have examined the possibility of making straight forward design alterations to realise the possibility of sensorial application and these involve simple considerations in the form of relative position of the binding sequences [28–30] This paper reports some new relatively simple optimisations to an established EIS assay for the clinically important antibiotic resistant bacteria MRSA. The assay has low limits of detection and high sensitivity (~500 fM) and the optimisations result in improved assay performance through increased reproducibility of electrode starting value, increased reproducibility of detection signal and make the test better able to run at ambient temperatures and therefore more amenable to implementation in routine clinical testing.

2. Materials and methods

2.1. Reagents

DNA oligonucleotides were purchased from Metabion (Martinsried, Germany). PNA oligonucleotides were ordered via Cambridge Research Biochemicals (Cleveland, UK) from Panagene (Daejeon, South Korea) (Table 1). PCR kit and DNeasy blood and tissue kit were purchased from Qiagen (Crawley, UK). Potassium ferricyanide, potassium ferrocyanide, sodium saline citrate (SSC), monosodium phosphate, disodium phosphate, dimethyl sulfoxide (DMSO) and tris(2-carboxyethyl)phosphine (TCEP) were purchased from Sigma Aldrich (Poole, UK).

2.2. DNA extraction from S. aureus

MRSA and MSSA bacteria were sub-cultured onto Columbia blood agar and incubated overnight at 37 °C in a 5% CO_2 atmosphere. Cells were inoculated into saline and the optical density measured using a Densicheck (bioMerieux). This gave values in McFarland units, proportional to the cellular concentration of bacteria in the suspension. A bacterial cell suspension of approximately 10^8 cells/mL was produced in this way and ten-fold dilutions ranging down to 10^2 cells/mL prepared from this suspension. Real time PCR was performed to characterise the DNA yields from the dilution series. The bacterial cells were pelleted by centrifuging 1 mL of the suspension at 5000 ×*g* for 10 min. The supernatant was discarded and the bacterial pellet resuspended in 200 μL of enzymatic lysis buffer (2 × TE buffer, 1.2% Triton X, 50 μg/mL Lysostaphin), before incubating for 30 min at 37 °C. 200 μL of bacterial lysate was added to 20 μL Proteinase K and DNA extracted using the bioMerieux NucliSens easyMAG automated platform. Guanidine thiocyanate was the active chaotropic agent in the lysis buffer, acting as a protein denaturant in the purification and extraction of nucleic acids from cellular material. The purified nucleic acid solution was then removed from the vessel without dislodging the magnetic silica pellet - DNA was eluted in 100 μL of water.

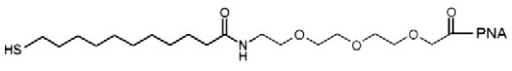
2.3. Electrochemical impedance spectroscopy (EIS)

Gold disk electrodes (2 mm diameter) were purchased from IJ Cambria Scientific (Carms, UK). Each solid gold working electrode was thoroughly pre-cleaned by mechanical polishing with 0.05 μm alumina powder (IJ Cambria Scientific (Carms, UK)) for 1 min, rinsing with water and immersing in an ultrasonic water bath for 1 min (to eliminate any residual alumina) and finally cleaning for 10 min in piranha solution (6 mL concentrated H_2SO_4 + 2 mL 30% (v/v) H_2O_2 solution). Then the electrodes were thoroughly washed with water and dried under a stream of nitrogen. After cleaning, the gold disk electrodes were incubated with a solution of 1.5 μM thiol-modified PNA solution + 30 μM mercaptohexanol in 50% (v/v) DMSO for 16 h at 30 °C. Electrodes were rinsed in 50% (v/v) DMSO and incubated in 1 mM mercaptohexanol in 50% (v/v) DMSO for 1 h at 30 °C. Then the electrodes were washed with 50% (v/v) DMSO and the EIS measurement buffer (0.1 mM $K_3[Fe(CN)_6]$ + 0.1 mM $K_4[Fe(CN)_6]$ + 10 mM phosphate buffer) for 2 h and 1 h respectively. Long buffering and equilibration times were employed because previous work found such rinsing times to be superior for ensuring a stable baseline behaviour when measurements were recorded. For electrodes where the effect of tris (2-carboxyethyl)phosphine (TCEP) was under investigation, 5 mM TCEP was added to the probe and blocking solutions.

EIS measurements in batch end point assays were performed using a three electrode system with an Ag/AgCl/3M KCL reference electrode and a platinum wire counter electrode (both from Metrohm (Runcorn, UK)) connected to an Autolab potentiostat running FRA software (Metrohm, Runcorn, UK). EIS measurements were performed at a DC potential of 0.24 V with an amplitude of 10 mV rms using a frequency range between 100,000 Hz–0.1 Hz (15 frequencies) in 0.1 mM $K_3[Fe(CN)_6]$ + 0.1 mM $K_4[Fe(CN)_6]$ + 10 mM phosphate buffer. The DNA sample was prepared by mixing 45 μL of sample with 5 μL of 20xSSC and then heating at 95 °C for 5 min, storing on ice for 2 min and heating at 30 °C for 5 min. The electrode was incubated with the sample for 2 h at 55 °C with shaking (650 rpm). Following incubation with sample, electrodes were washed with 2xSSC, 0.2xSSC and EIS measurement buffer for 10 min in each. EIS measurements were performed pre and post hybridisation.

The online assay was performed by recording continuous EIS measurements with gold screen printed electrodes (SPEs) (DRP-C223BT) with an integrated Ag pseudo reference electrode and a gold counter electrode (Dropsens, Oviedo, Spain). A single well from a Schott Nexterion 16-well self-adhesive superstructure (Stafford, UK) was cut out and fitted around the electrode in which 50 μL of EIS measurement buffer was present. The well was sealed with an adhesive lid from the Schott Nexterion 16-well self-adhesive superstructure kit (Stafford, UK). 45 μL of sample was mixed with 5 μL of 10× EIS measurement buffer and pretreated by heating at 95 °C for 5 min, storing on ice for 2 min and heating at 30 °C for 5 min. Once the sample was prepared the EIS

Table 1
Sequences and structures of oligonucleotides used during the study.

	Oligo name		5′ modif.	Sequence 5′-3′
1	PNA 48_02	PNA	Thiol-C11-AEEEA-PNA 	ACTAGGTGTTGGTGAAGATATAC
2	DNA 48_c	DNA	–	GTATATCTTCACCAACACCTA GT
3	DNA_nc	DNA	–	ACCTTTGCTCATTGA