POLYSACCHARIDE TEMPLATED SILVER NANOWIRE FOR ULTRASENSITIVE ELECTRICAL DETECTION OF NUCLEIC ACIDS ON NANOOGAPPED BIOSENSOR

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ABSTRACT

An ultrasensitive DNA electrical detection method has been developed. In this study, peptide nucleic acid (PNA) probes were immobilized in the gaps of interdigitated microelectrodes and hybridized with their complementary target DNA. Then, pectin molecules were associated to captured DNA strand via zirconium-phosphate/zirconium-carbonate chemistries; and oxidated by periodate. The resultant aldehyde groups reduce ammoniacal silver ion to produce silver nanoparticles, which bridged the gap of the interdigitated microelectrodes. The resistance of silver nanoparticles correlated directly with the amount of the hybridized DNA. Near 1 femto molar DNA sample can be detected; this method is also applicable to RNA detection.

KEYWORDS: DNA, pectin, electrical detection

INTRODUCTION

Electrical based biosensors have drawn great attention recently because of their inherent advantages such as low cost, high sensitivity, portability with fast detection process. Some reports [1-3] about electrical detection of DNA on nanogapped biosensors have already appeared by describing the use of metallic wires formed on DNA templates. In these reports, metal ions either react with base on DNA molecules, or adsorbed on the anionic phosphates of the DNA backbone, and then an enhancement step was carried out. However, these approaches for DNA detection either suffered from low sensitivity, high background noise or required labeling biomolecules with gold nanoparticles [4], which prevented them from being widely used.

To overcome these drawbacks, we developed a novel approach on nanogapped biosensor which can achieve ultrahigh sensitivity with low noise. In this approach, after target DNA molecules hybridized with immobilized capture PNA probes localized in the gaps, polysaccharide pectin molecules were introduced to target DNA strand via zirconium ions by zirconium-phosphate-carboxylate chemistry (5, 6). In the ensuing step, vicinal diols on pectin were oxidized by periodate to produce a great number of aldehyde groups on pectin backbone (7), which is an established technique for aldehyde production on polysaccharide. Then, by employing Tollen’s reduction, metal silver was deposited on pectin template through localized chemical reduction; which is the basis for ultrahigh sensitivity and high specificity detection.
EXPERIMENTAL

Nanogapped chips used in this experiment were fabricated by using a lift-off method. There are 10 x 10 interdigitated electrodes on each chip, the gap distance between each pair of interdigitated electrodes is 500 nm. The electrodes consist of 10 nm titanium layer and 15 nm gold layer above it. Surface modification of the biosensor and functionization was realized according to a reported method (8).

Hybridization was performed at room temperature for 60 min in TE buffer (10 mM Tris-HCl, 1.0 mM EDTA and 0.1 M NaCl). Pectin was attached to the DNA strand via zirconium-phosphate/carboxyl chemistry, and the chips were then incubated in a freshly prepared solution of 25mM of NaIO₄ in 0.2M sodium acetate buffer (pH 3.98) for 2 hours in the dark at 4°C. In the ensuing step the newly formed aldehyde groups reduced ammoniacal silver nitrate (Ag(NH₃)₂NO₃) solution (pH 9.3) for 60 minutes to silver in the dark at room temperature. A continuous silver nanowire was formed and thus the electrodes were bridged, dramatically reducing their electrical resistance. The scheme is shown in Figure 1.

RESULTS AND DISCUSSION

Results (Figure 2) shows that by using this approach, near 1 femo Molar of the DNA sample can be detected on nanogapped biosensor with very low background noise, which is more than one hundred times higher than previously reported [4]. The calibration curve is also in good linear relationship with regression coefficient 0.99.

Single base mismatch (SBM) detection has also been achieved using the biosensor array with a SBM selectivity factor of 50:1, much higher than conventional optical microarray and most other previously reported methods (9, 10). This approach readily allows discrimination between the perfectly matched and mismatched DNAs.

CONCLUSIONS

Ultrasensitive detection of DNA on a nanogapped biosensor, enhanced by the pectin templated formation of silver nanoparticle was demonstrated in this paper.
For the first time, pectin, a polysaccharide molecule, was used for deriving aldehyde groups for DNA electrical detection. This method showed very high sensitivity and high specificity together with much lower noise. This approach enables the integrations of nanogapped biosensors for multiplexed detection in wide applications.

**Figure 2.** Calibration curve of complementary DNA and Single Base Mismatch

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**REFERENCES**


