Biosensors and Bioelectronics 25 (2009) 778–783

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

Selective immobilization of oligonucleotide-modified gold nanoparticles by electrodeposition on screen-printed electrodes

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ARTICLE INFO

Article history:
Received 29 June 2009
Received in revised form 17 August 2009
Accepted 19 August 2009
Available online 26 August 2009

Keywords:
Electrodeposition
Gold nanoparticles
DNA biochip
Multimodular
Electrochemical biosensor

ABSTRACT

Here, we describe a proof of concept procedure for the selective immobilization of oligonucleotides functionalized gold nanoparticle probes (affinity modules) on arrayed screen-printed gold electrodes. Current microarrays are using many different ways to address their DNA probes onto the transducer area. For that reason, we have mixed the electrodeposition of metals, which is a very well known process, in addition with the DNA-gold nanoparticles formation, which is an area of great interest in biosensing applications in the field of genomics, clinical and warfare applications. Combining these fields, we have developed a novel method for the immobilization of gold nanoparticles conjugated with oligonucleotides (affinity modules) onto screenprinting gold electrodes through electrodeposition at a current positive potential of 800 mV vs. Ag/AgCl. The modules were selectively immobilized onto the electrode surface being, afterwards, ready for a successful hybridization. The gold colloids take the advantage of being a carrier that allows the immobilization of any kind of bioreceptor in the same conditions and the capability of quality control analysis before the electrodeposition procedure. With this system, we avoided non-specific interactions between the transduction layer and the bioreceptor and in the case of DNA oligonucleotides allowed us the immobilization of multiple sequences in a multimodular device for a further industrial process of cheaper biochip fabrication.

1. Introduction

In the last three decades, the advances in biosensor and biochip technologies have triggered the capability of analyzing nucleic acids and proteins for novel biological and medical research applications. The DNA sequencing or expression analysis of mRNA has involved the development of miniaturized DNA arrays or so called DNA biochips that are revolutionizing the field of such as different disciplines as microfabrication, organic and analytical chemistry, molecular biology and genetics (Cosnier and Mailley, 2008; Wang, 2000). DNA biochips are small DNA biosensor devices based on micro-nanotechnologies fabrication, which consists in a biological recognition element (bioreceptor) which is retained in direct spatial contact with a transduction element (Thevenot et al., 2001). DNA biochips use nucleic acids as bioreceptors and the base pair complementarities as a recognition mechanism. Another important component of these devices is the transduction element that can be based on many different properties of the transduction layer. From optical techniques, such as fluorescence (Christensen and Herron, 2009; Vercoutere and Akeson, 2002), surface plasmon resonance (Homola, 2003; Lechuga, 2005) or colorimetric detection (Elghanian et al., 1997; Storhoff et al., 1998) to those based on electrochemical measurements like amperometry or potentiometry (Cosnier and Mailley, 2008; Drumond et al., 2003; Kerman et al., 2009; Zhang et al., 2009; Zhu et al., 2009) or nanomechanics (Alvarez et al., 2004).

Immobilization of bioreceptors on the sensor surface has strong consequences on the quality of analyses since it influences not only the efficiency of DNA attachment, but also the degree of non-specific binding and the accessibility to its targets. Moreover, the bioreceptor layer directly affects to the reproducibility, selectivity and the resolution of the device. The immobilization process should allow the correct orientation and accessibility to the DNA target for a correct hybridization process. In the last years many different strategies have been developed in order to improve the formation of self assembly monolayers (SAMs) onto the sensors surface (Boozer et al., 2004; Cosnier and Mailley, 2008; Peterson et al., 2001; Pividori et al., 2000; Wang et al., 2004). All these strate-

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doi:10.1016/j.bios.2009.08.028

gies depend on the final application and the kind of supporting element to be used (Beier and Hoheisel, 1999). Silicon and gold are two of the most popular platforms as they take advantage of well established chemistries and microfabrication techniques. But new immobilization schemes and advanced sensing materials are highly desired to improve the analytical capabilities of biosensing devices (Wang, 1999).

Here, we report an improved approach of previously reported methods (Campas and Katakis, 2006; Stonehuerner et al., 1992), which consists in the electrodeposition of DNA-capped gold nanoparticles, referred as affinity modules in this work. This is a proof of concept which opens new ways for the electro-selective address of DNA probes on arrayed electrodes. We have electrodeposited two different in sequence affinity modules with no complementary sequences on a four gold screen-printed electrode. The electrodeposition of each affinity module led to the selectivity immobilization of each sequence. Every batch of affinity modules was independently checked after production and before electrodeposition. This is an essential step for a further implementation of this technique in a future industrial development. Furthermore, the DNA biosensor so formed was utilized to detect a PCR product, with a 10-time higher signal relative to that previously reported (Campas and Katakis, 2006). The final hybridization reaction clearly shows the correct assembly of the process and the ability to improve to a large scale fabrication low cost DNA arrays.

2. Materials and methods

Colloidal gold sols were prepared as described previously (Frens, 1973). Briefly, a solution of aqueous sodium citrate was added to a boiling, rapidly stirred solution of gold (III) chloride ($HAuCl_4$) and the solution was refluxed for 30 min. The final concentrations (w/v) were 0.01% $HAuCl_4$ and 0.03% sodium citrate. The particle medium size was estimated by transmission electronic microscopy (TEM) as approximately 18 nm.

Table 1
Set of oligonucleotides used in this work.

Name	Base sequence 5′–3′	Modifications
A	GGC GTA ATA GCG AAG AGG C	5′-Digoxigenin (A_{dig})
Ap	GCC TCT TCG CTA TTA CGC C	5′-SH (Ap_{SH})
B	CCA CAC AAC ATA CGA GCC G	5′-SH (B_{SH})
Bp	CGG CTC GTA TGT TGT GTG G	5′-Digoxigenin (Bp_{DIG})
XABc	ACC CAA CTT AAT CGC CTT GCG GCG TAA TAG CGA AGA GGC CGG CTC GTA TGT TGT GTG G	
X	ACC CAA CTT AAT CGC CTT GC	5′-Digoxigenin
B	CCA CAC AAC ATA CGA GCC G	

The screen-printed electrochemical sensor with four working electrodes (AC81) was purchased from BVT Technologies (Czech Republic). The sensor was formed on a corundum ceramic base. On to this surface, four working electrodes were applied with diameter of 1.00 ± 0.05 mm (~ 0.785 mm^2). Electrodes were made of pure gold. At the end of the sensor, there was a contact connected with the active part by the silver conducting path which was covered by a dielectric protection layer.

The oligonucleotides used in the affinity modules with the C(6) thiol modified in 5′ were purchased from Cruachem (Poland) and those with the 5′ digoxigenin or none modification were obtained from TIB MOLBIOL (Germany). The sequences are listed in Table 1.

Affinity modules were obtained applying the technology developed by Mirkin et al. (Mirkin et al., 1996; Storhoff et al., 1998). In our particular approach, the final colloidal gold concentration was 30 nM and the final oligonucleotide concentration was 3.7 μM. Thus, 7.25 μL of a 500 μM solution of thiol-modified oligonu-

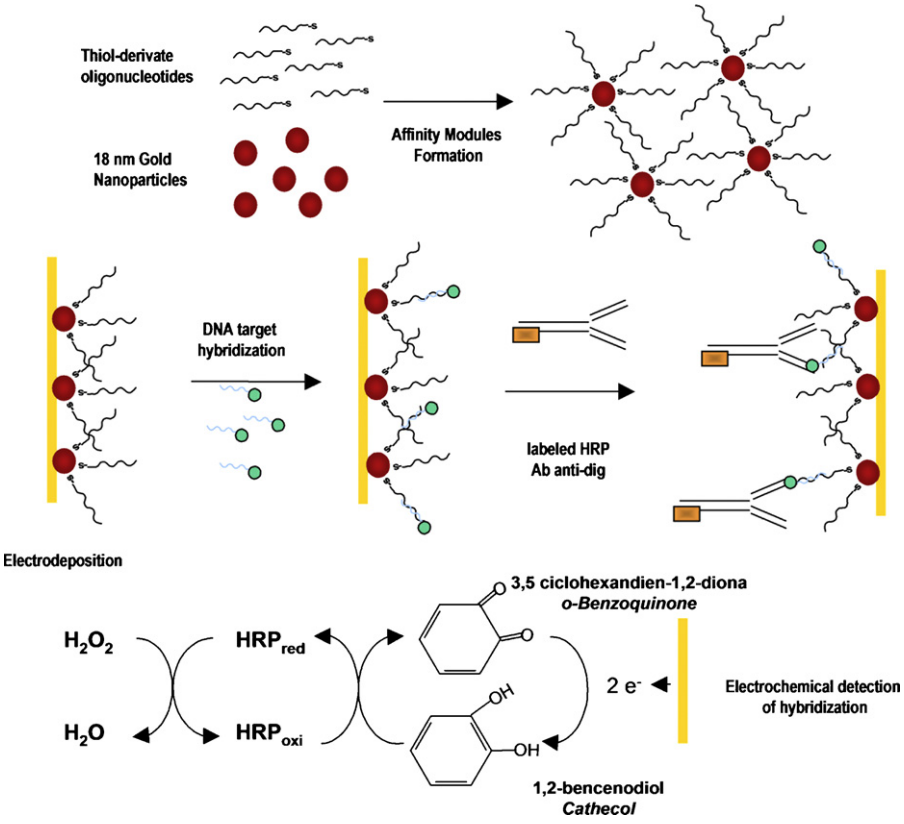

Fig. 1. Reaction scheme of the development of the novel DNA biosensor device proposed.