Talanta 78 (2009) 1102–1106

Contents lists available at ScienceDirect

Talanta

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

Anti-fouling characteristics of surface-confined oligonucleotide strands bioconjugated on streptavidin platforms in the presence of nanomaterials

Mònica Mir[a,b,*], Petra J. Cameron[c], Xinhua Zhong[d], Omar Azzaroni[a,e,*], Marta Álvarez[a], Wolfgang Knoll[a]

[a] Max-Planck-Institut für Polymerforschung, Ackermannweg, 10, 55128 Mainz, Germany
[b] Nanobioengineering Group, Institute for Bioengineering of Catalonia (IBEC), Josep Samitier 1-5, 08028 Barcelona, Spain
[c] Department of Chemistry, University of Bath, Bath BA2 7AY, United Kingdom
[d] Department of Chemistry, East China University of Science and Technology, 200237 Shanghai, China
[e] INIFTA-CONICET-UNLP, C.C. 16 Suc. 4 (1900) La Plata, Argentina

ARTICLE INFO

Article history:
Received 10 October 2008
Received in revised form 8 January 2009
Accepted 14 January 2009
Available online 23 January 2009

Keywords:
Anti-fouling
Non-specific binding
DNA biosensor
Streptavidin SAM
Surface plasmon fluorescence spectroscopy (SPFS)
Surface acoustic wave (SAW)
Quantum dots (QDs)
Surface plasmon resonance (SPR)
CdSe nanoparticles
Thiol-biotin

ABSTRACT

This work describes our studies on the molecular design of interfacial architectures suitable for DNA sensing which could resist non-specific binding of nanomaterials commonly used as labels for amplifying biorecognition events. We observed that the non-specific binding of bio-nanomaterials to surface-confined oligonucleotide strands is highly dependent on the characteristics of the interfacial architecture. Thiolated double stranded oligonucleotide arrays assembled on Au surfaces evidence significant fouling in the presence of nanoparticles (NPs) at the nanomolar level. The non-specific interaction between the oligonucleotide strands and the nanomaterials can be sensitively minimized by introducing streptavidin (SAv) as an underlayer conjugated to the DNA arrays. The role of the SAv layer was attributed to the significant hydrophilic repulsion between the SAv-modified surface and the nanomaterials in close proximity to the interface, thus conferring outstanding anti-fouling characteristics to the interfacial architecture. These results provide a simple and straightforward strategy to overcome the limitations introduced by the non-specific binding of labels to achieve reliable detection of DNA-based biorecognition events.

1. Introduction

The fields of nanobiotechnology and nanomedicine are becoming increasingly important in research and industry. Within this growing research field, the ability to control the adsorption of biomolecules, nanomaterials and bio-inorganic hybrids to solid supports plays a key role in achieving reliable and competitive devices. For example, deposition of proteins, cells or bacteria on the surface of an implant or an *in vivo* biosensor usually leads to failure of these devices [1]. Health problems can be caused due to surface-fouling by microorganisms during the food preparation process. Electrochemical analysis is also affected by the adsorption of biomolecules on the surface, where the unwanted molecules result in the passivation of the electrode surface [2]. The diffusion of reagents in devices containing microfluidic circuits can change drastically if the surface of the channels is affected by fouling [3]. The undesirable non-specific adsorption of different materials on surfaces must be eliminated in effective biosensors as it leads to a reduction in the sensitivity and specificity of the device. For applications such as biosensing, affinity chromatography, biocatalysis or microfluidics it is important not only to build a non-fouling surface, but also to have suitable recognition sites on the non-fouling surface in order to attach specific ligands with desired orientation and coverage, and to ensure a reproducible and reliable response. Thus, the molecular design of a biosensing platform that exhibits: (a) a reproducible and stable surface with resistance to non-specific binding and (b) good control over ligands immobilization is not a trivial task as is of high priority in biosensing community.

Within the great variety of biosensing platforms, of particular relevance is that one concerning to the detection of DNA hybridization. The most common interfacial architecture consists of monolayers of thiolated oligonucleotide probes assembled on gold surfaces forming a brush-like layer [4–6]. However, during last years the use of streptavidin (SAv) as an anchoring layer received increasing attention [7,8]. This is due to the fact that the SAv platform

* Corresponding author at: Institute for Bioengineering of Catalonia (IBEC), Josep Samitier 1-5, 08028 Barcelona, Spain.
E-mail address: mmir@ibec.pcb.ub.es (M. Mir).

0039-9140/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.talanta.2009.01.026

enables an optimized distribution and spacing of the probe strands on the Au electrode, thus facilitating the hybridization process. Moreover, depending on the characteristics of the read out system, it is a common practice to use labeled-biomolecules, enzymes, fluorophores or nanoparticles (NPs) to enhance the detection of the biorecognition process [9].

Fluorescence-based transduction is probably one of the most sensitive strategies to detect DNA or oligonucleotide hybridization. Quantum dots (QDs) are nanomaterials that represent a very interesting type of fluorescent nanoparticles [10]. At present, they constitute the greatest promise as labels in fields like biosensing or biological imaging [10]. This is based on their remarkable photostability, high fluorescence yield, low rates of photobleaching and extinction coefficients comparable to conventional organic fluorophores, which render them outstanding candidates for fluorescent labeling of biomolecules. In spite of the widespread use of QDs combined with DNA in biosensing [11] and design of functional materials [12], little is known about the interaction and non-specific binding interactions between these nanomaterials and oligonucleotide strands at solid-liquid interfaces.

In this work we studied with particular emphasis the non-specific adsorption of QDs on surface-confined oligonucleotide strands formed by assembly of thiolated strands and by bioconjugation of biotinylated strands on SAv platforms. Our studies show notable differences between both platforms, indicating that the commonly used thiolated DNA assemblies are prone to non-specific binding of QDs. In contrast, the SAv-based did not evidence any non-specific adsorption of the nanomaterials. These results were also extended to the use of biomolecules, like streptavidin, as labels obtaining similar differences between both platforms.

2. Experimental

2.1. Materials

The oligonucleotide sequences; 18-mer thiol labeled capture probe (SH-C_6-5'-TTTTGTACATCACAACTA-3ĭ), 18-mer biotinylated capture probe (biotin-5'-TTTTGTACATCACAACTA-3ĭ) and 15-mer target (5'-TAGTTGTGATGTACA -3ĭ) used in this work were purchased from MWG Biotech AG. All stock oligonucleotide solutions were 100 μM prepared with milliQ water and stored at -20 °C. Streptavidin, mercaptoundecanol, 2-mercaptoethanol, phosphate buffered saline, polyethylene glycol sorbitan monolaurate (tween 20), trioctylphosphine (TOP), oleylamine, oleic acid, 1-octadecene (ODE), CdO, Se powder and 3-mercaptopropionic acid (MPA) were purchased from Sigma. Biotin-terminated thiol was obtained from Roche Diagnostics.

2.2. Synthesis of MPA-Capped CdSe nanoparticles

Oil-soluble CdSe nanoparticles were prepared according to a literature method [13]. Typically, 5.0 mL of oleylamine and 0.15 mL of Se stock solution (2.1 M in TOP) were loaded in a 50 mL three neck round-bottom flask, and the mixture was heated to 300 °C in a flow of argon. 1.0 mL of Cd stock solution (0.3 M, obtained by dissolving CdO in 6-fold of oleic acid and ODE at elevated temperature) was injected quickly into the reaction flask. The temperature was then set at 280 °C for the subsequent growth and annealing of nanocrystals. After completion of particle growth, the reaction mixture was allowed to cool to ~60 °C, and 10 mL of methanol was added. The obtained CdSe nanocrystals were precipitated by adding methanol into the toluene solution and further isolated and purified by repeated centrifugation and decantation. MPA-Capped water-soluble QDs were obtained by a ligand replacement reaction [14]. Due to the carboxylic group in the MPA ligand, the obtained MPA-capped CdSe QDs are negative charged in aqueous solutions. The mean size of the CdSe QDs used for the following experiment is ~4.5 ± 0.3 nm with emission wavelength λ = 620 nm.

2.3. Biomolecules immobilization

Both interfacial architectures involving thiolated oligonucleotides (DNA-SH) and biotinylated oligonucleotides bioconjugated on SAv monolayers (DNA-SAv) were assembled onto gold surfaces. The DNA-SH architecture was prepared by incubating the gold films in a 1 μM solution of thiolated capture probe in 1 M KH_2PO_4 for 2 h, the slide then was placed in a 1 mM solution of mercaptoethanol in milliQ water for 1 h to backfill any empty spaces between the capture probe strands. It is worth mentioning that the backfilling also improves the orientation of the oligonucleotide strands leading to an improvement of the hybridization process.

The DNA-SAv architectures were constructed by chemisorbing a mixed self-assembled monolayer of 12-mercaptododecanoic-(-8-biotinoylamido-3,6-dioxaoctyl)amide and 11-mercapto-1-undecanol in ethanol in a 1:9 ratio [15]. Then, the biotinylated surface was incubated in a 1 μM streptavidin solution in 10 mM PBS, 100 mM NaCl at pH 7.4, leading to a compact monolayer evenly distributed on the Au surface [16,17]. Considering that SAv has unique properties as an adapter for the binding of a second layer of biotinylated molecules and the extremely high and very specific interaction with biotin ($K = 10^{15}$ L mol^{-1}), the resulting protein layer acts as stable platform for supramolecularly anchoring the biotinylated capture probes. Both surface architectures were hybridized with a 1 μM ss-DNA target solution and afterwards 1 μM solution of streptavidin or QDs respectively were left to interact with both platforms in order to investigate the effect of non-specific binding.

In both platforms, the unbounded molecules on the surface sensor were rinsed away after each immobilization step with the buffer used in the immobilization step.

2.4. Surface acoustic wave (SAW) measurements

The non-specific adsorption of SAv on both platforms was measured by acoustic wave sensor spectroscopy (SAW) (S-sens® k5, Nanofilm Surface Analysis). The sensor chip array consists of five gold sensors with a sensing area of 6.3 mm^2 each. The chips were cleaned before use by plasma treatment for 5 min at 300 W under argon atmosphere. All incubations were programmed and injection was done automatically at a flow rate of 20 μL min^{-1}. After each experiment an injection of 5% glycerol solution was required for calibration purposes [18].

2.5. Surface plasmon fluorescence spectroscopy (SPFS)

The binding of QDs on both interfacial architectures was monitored by surface plasmon fluorescence spectroscopy (SPFS) [19–21]. Laser light at λ = 594.6 nm was used to excite surface plasmons in the gold film (coupled in the Kretschmann configuration). The QDs located near the gold–dielectric interface can be excited by the surface plasmon that propagates along this interface. Photons emitted from the QDs were monitored with a photomultiplier. To avoid collection of scattered and transmitted laser light, a λ = 611 nm narrow band pass filter was placed in front of the photomultiplier. The sensor chip was a ~50 nm evaporated gold film on BK7 glass with ~2 nm of chromium being evaporated just prior to the gold deposition to improve adhesion between the gold and glass. All incubations were done at a flow rate of 20 μL min^{-1}.