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A dual DNA biosensor based on two redox couples with a hydrogel sensing platform functionalized with carboxyl groups and gold nanoparticles

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ABSTRACT

Poly(*N*-isopropylacrylamide-*co*-acrylic acid) hydrogels containing —COOH groups and gold nanoparticles were used to propose a new dual-target hybridization assay that involves a dual hybridization event. The target DNA strands were labeled with two different redox probes: ferrocene and anthraquinone derivatives. The targets were simultaneously detected by obtaining two well separated voltammetric peaks. Such good stability of the biosensor can be attributed to the strong covalent bond between Au nanoparticles and the thiolated probe DNA. Also the bond between carboxyl group of the gel and amine-terminated capture-probe DNA is strong in pH ca. 7. The sensor response increased linearly with the logarithm of the target DNA concentration in the range from: 2×10^{-12} to 1×10^{-6} M and 2×10^{-13} to 2×10^{-6} M for target DNA labeled by antraquinone and ferrocene derivative respectively. The determined detection limit was approx. 8×10^{-13} M and 7×10^{-14} M for target DNA labeled with antraquinone and with ferrocene, respectively, which corresponded to the presence of ~2×10^{8} copies of DNA in the solution (0.5 mL). The proposed biosensor can be used for the detection of DNA at the fM level. This achievement is very important for biosensors that use DNA labeled with redox probes, as here we have shown that the sensitivity improved by at least two orders of magnitude compared to the traditional biosensors.

1. Introduction

Specific and selective detection is a major issue in life science. Such diagnostics is mainly performed through the application of genosensors based on the identification of particular DNA base sequences or immunoenzymatic reactions [1,2]. The main factor in the development of DNA biosensors lies in the stable immobilization of a large amount of probe DNA onto a transducer with proper orientation and easy accessibility toward the target DNA. Many DNA hybridization tests performed on various matrixes can be found in the literature: functionalized phenyl layers formed during the electroreduction of the appropriate diazonium salt [3–5] or in the chemisorption process [6,7], conductive polymer and metal nanoparticle composites [8–10] and hydrogel platforms [11–13]. The detection limit so obtained strongly depends on the analytical technique applied as well as on how the biosensors are constructed [14–16]. Recent activity on this subject has been focused on the development of hybridization assays that are reusable and permit simultaneous determination of multiple DNA targets.

Electrochemical devices have shown great promise for genetic testing and are ideally suited for miniaturized DNA diagnostics [17,18]. However, early electrical assays have had the limitation of being inherently single-target protocols. In this paper we present a new solution for simultaneous detection at very low level of two DNA targets. The proposed biosensor involves the application of poly(*N*-isopropylacrylamide-*co*-acrylic acid) hydrogel (pNIPA) functionalized by both: —COOH and gold nanoparticles (Au Nps), as a sensing platform. The detection was based on sensitive, well resolved voltammetric signals corresponding to appropriately tagged targets. The pNIPA platforms can easily be made reusable.

2. Experimental

2.1. Materials and methods

All chemicals were of the highest purity available. KOH, KH_2PO_4, NaCl, KCl, HCl (all from POCH, Poland), anthraquinone-2-carboxylic acid (AQ), ferrocenecarboxylic acid (Fc), *N*-hydroxysuccinimide

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(NHS), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC), *N*-isopropylacrylamide (NIPA), acrylic acid (AA), *N*,*N*′-methylenebisacrylamide (BIS), ammonium persulfate (APS), gold nanoparticles (Au Nps, $\phi = 5$ nm) and *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TEMED) (all from Sigma–Aldrich) were used as received, except for NIPA which was recrystallized twice from a benzene/hexane mixture (90:10 v/v). The following buffers were used: (i) the immobilization buffer, 0.02 M HEPES (2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid) buffer, pH 7.4; (ii) the hybridization buffers: 0.02 M PBS buffer, pH 7.4 with 0.5 M NaCl, 2 mM KCl (for EQCM, voltammetric and spectroscopic measurements) and 0.02 M Tris buffer, pH 7.4 with appropriate additions of NaCl and KCl (for the LA ICPMS measurements).

All oligonucleotide sequences used were purchased from MWG-Operon (Eurofins). The primary thiol modified oligonucleotide sequence was taken from a strain of lactic acid bacteria of the genus *Lactococcus lactis*, and the primary amino-modified oligonucleotide sequence was taken from a strain of *Pseudomonas aeruginosa* Pilin genes. The following oligonucleotide sequences were used:

- thiol probe DNA-1 (5′⇨3′):
  HS-C3-CGCCAACGTTTTCGCCAACG
- amino probe DNA-2 (5′⇨3′):
  H_2N-C6-AGGCGATGCCGAACTGCTCG
- complementary target DNA-1 (5′⇨3′):
  H_2N-C6-CGTTGGCGAAAACGTTGGCG
- complementary target DNA-2 (5′⇨3′):
  H_2N-C6-CGAGCAGTTCGGCATCGCCT
- non-complementary target DNA (5′⇨3′):
  H_2N-C6-AGGCGATGCCGAACTGCTCG
- single G-A mismatch target DNA-1 (5′⇨3′):
  H_2N-C6-CGTTAGCGAAAACGTTGGCG
- single G-A mismatch target DNA-2 (5′⇨3′):
  H_2N-C6-CGAACAGTTCGGCATCGCCT
- double separated G-A mismatch target DNA-1 (5′⇨3′):
- H_2N-C6-CGTTAGCGAAAACATTGGCG
- double separated G-A mismatch target DNA-2 (5′⇨3′):
- H_2N-C6-CGAACAGTTCGGCATCACCT
- single G-C mismatch target DNA-1 (5′⇨3′):
  H_2N-C6-CGTTCGCGAAAACGTTGGCG
- single G-C mismatch target DNA-2 (5′⇨3′):
  H_2N-C6-CGAGCACTTCGGCATCGCCT
- double separated G-C mismatch target DNA-1 (5′⇨3′):
- H_2N-C6-CGTTCGCGAAAACCTTGGCG
- double separated G-C mismatch target DNA-2 (5′⇨3′):
- H_2N-C6-CGAGCACTTCGGCATCCCCT

The length of 20 nucleotides is optimal vs. the size of the gel channels. No matter what is the orientation of the strand its length is sufficiently short to enable the easy penetration of the gels.

2.2. Electrochemical measurements

Cyclic voltammetry (CV) and differential pulse voltammetry (DPV) were performed using an Autolab PGSTAT 30 potentiostat. The electrochemical measurements were performed in a special cell with a three-electrode system, consisting of a glassy carbon disk electrode (1.6 mm in diameter, BAS, UK) used as the working electrode, and two platinum rings built into the bottom of the cell used as the reference and auxiliary electrodes. The surface of the working electrode was polished with 1 μm Al_2O_3 powder on a wet pad. After each polishing, to remove alumina completely from the electrode surface, the electrode was rinsed with a direct stream of ultrapure water (Milli-Q, Millipore, conductivity of ~0.056 μS cm^{-1}) and dried.

2.3. EQCM measurements

An electrochemical quartz crystal microbalance (Autolab-model), with a 6 MHz Au/TiO_2 quartz crystal resonator, was used in this study. The resonant frequency of the quartz crystal lattice vibrations in a thin quartz crystal wafer was measured as a function of the mass attached to the crystal interface. For thin rigid films, the interfacial mass change, Δm, is related to the shift in resonance oscillation frequency, Δf, of the EQCM by the Sauerbrey equation [19–21]. The piezoelectrically active (geometrical) surface area of the working Au electrode was 0.353 cm^2 and the real surface area was $A = 0.423$ cm^2 (roughness factor $R = 1.2$). The Au-EQCM electrode was electrochemically pretreated by voltammetric cycling: first, between 0 V and 1.8 V (with a 10 s scan stop at 1.8 V) in 0.5 M NaOH with scan rate 50 mV s^{-1}, and then between −0.3 and 1.5 V (vs Ag/AgCl) in 0.1 M H_2SO_4 solution until a stable voltammogram, typical for a clean gold electrode, was observed [22]. Finally, before the measurements the gold-coated QCM crystals underwent a pretreatment consisting of several potential scans between −0.65 V and 0.95 V at a high scan rate (>1 V/s) in 0.1 M perchloric acid solution.

2.4. ICP MS measurements with laser ablation

Inductively coupled plasma mass spectrometry (ICP MS) makes it possible to measure the intensities of ion streams generated in inductively coupled plasma, separated according to their mass-to-charge ratios in the mass analyzer. High efficiency of ionization in the argon plasma enables very low detection limits to be achieved (on the order of pg L^{-1} for most elements) and high measurement sensitivity to be maintained. Nebulization of the analyte solution is the most common method of introducing samples into the ICPMS system, but direct microsampling by laser ablation (LA) has been gaining recognition in various research areas. Laser microsampling of solids not only reduces the risk of contaminations, but also the entire analytical procedure to be shortened and the demand for costly high purity chemical reagents to be reduced. An inductively coupled plasma mass spectrometer (Perkin Elmer NexION 300) equipped with a laser ablation system (LSX-200$^+$, CETAC, USA) was used. The laser ablation system combines a stable, environmentally sealed 266 nm UV laser (Nd-YAG, solid state) with a high sampling efficiency, a variable 1–20 Hz pulse repetition rate and a maximum energy of up to 6 mJ puls^{-1}. All experiments were performed using Ar as the carrier gas. An open ablation cell was used for this study, with an effective volume of approx. $v = 4.5$ cm^3 [23].

All measurement cycles consisted of three stages of signal registering: (1) black registration – 30 s before the start of laser ablation; (2) registration of signals intensities – 60 s during ablation and (3) subsequent registration of signal intensities – 30 s after the end of laser ablation. For each selected isotope the registered blank values were subtracted from the signals recorded during the ablation of the gel-samples. The averaged values were calculated from the successive replicates of these same samples.

2.5. UV–vis and CD measurements

A J-815 CD spectrometer (Jasco), controlled by the manufacturer's software, was used. A quartz cuvette with special insert for the piece of gel (1 cm long) was used as the optical window. The CD parameters were as follows: scanning speed – 100 nm/min, data pitch – 0.5 nm, bandwidth – 2 nm, accumulation (number of scans) – 10. CD spectra of oligonucleotide solutions were obtained before and after the hybridization process. UV–vis spectra of the solution containing DNA fragments before and after immersion of the piece of gel were obtained in the range of 200–600 nm. A Perkin-Elmer (Lambda-25) spectrometer was used. Measurements were