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Accuracy of determination of mass of DNA films on gold electrodes

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ABSTRACT

Mass and organization of layers of unmodified DNA strands of various lengths deposited from solutions of its various concentrations on the surface of a gold electrode were examined using EQCM, UV–vis and AFM. The accuracy of mass determination is vital for the electrochemical examination of interactions between DNA and ligands, especially drug molecules. For such studies, the parallel orientation of the DNA fragments/strands vs. electrode surface may be advantageous. We have found that the Sauerbrey equation can give satisfactory results for the mass of accumulated oligonucleotides if the deposition process is done under appropriate conditions. Apparently, the viscoelastic effect did not lead to substantial errors.

1. Introduction

Films of single-stranded or double-stranded DNA immobilized on the electrode surface play a very important role in biotechnological and biomedical applications including DNA microarrays [1,2], biosensors [3–5] and the studies of interactions with drugs [6,7]. A DNA film can be attached to the gold substrate chemically or physically. The first method usually requires an additional step: the surface modification with an intermediate layer suitably functionalized. To form a chemical bond between the intermediate layer and the DNA strand, the immobilized oligonucleotide must also be modified with a suitable functional group, for example, $-NH_2$ and $-SH$ [8,9]. The formation of a DNA film on the gold surface due to the chemical bonding results in a homogenous distribution and mainly perpendicular orientation vs. the electrode surface. Homogenous orientation of DNA strands in the sensing layer plays the crucial role in the construction of the DNA biosensor. In contrast, from the biophysical and biomedical point of view, the perpendicular orientation of the strands is not preferable because the access to DNA strands for the drug molecules may be limited. The drugs are relatively big molecules, so they require enough free space to interact efficiently with the DNA components [10]. Additionally, the theoretical modeling of DNA–drug interactions assumes that the DNA strands are accessible over the entire length [11–13]. For such studies, the parallel (vs. electrode surface) orientation of the strands in the film may help.

The simplest method to immobilize a DNA film with parallel orientation of the strands to the gold surface is its adsorption at a constant potential [14,15]. However, this procedure leads to the formation of multiple direct contacts of the phosphate residues of the DNA strands with the gold surface. In such cases, the accurate measurement of the DNA surface coverage, a crucial parameter for quantitative description of the interactions, is challenging [16,17]. Absolute quantification of DNA strands in the film is possible only by application of radiolabeling method, but the conditions of such experiments are very hazardous to the researcher and the environment [18]. The characterization of radioactive label-free DNA films is possible by applying such techniques as surface plasmon resonance or elipsometry [17,19,20]. However, the presence of non-specific adsorption substantially complicates the data interpretation. The required information can also be obtained from X-ray photoelectron spectroscopy and quartz crystal microbalance [21–23]. We think it is very useful to have the complete knowledge on the quantity and state of the DNA deposit on the substrate surface.

The literature reports on the determination of the adsorbed amount of DNA on the electrode surface do not provide evidence that the amount deposited is equal to the amount detected [24]. The DNA coverage of the gold electrode can be easily determined from the number of $Ru(NH_3)_6^{3+}$ ions electrostatically bound with DNA according to the procedure described by Steel et al. [25]. The ruthenium(III) complex interacts with the phosphate groups in the

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DNA chain. The situation is very simple in the case of perpendicular or any non-parallel orientation of DNA vs. the electrode surface. In the case of a DNA film formed by adsorption of DNA chains through the sugar-phosphate backbone, the Steel procedure cannot be used, because many phosphate groups are not available for the interactions with ruthenium(III) cations. Therefore, another procedure that allows the quantitative and qualitative descriptions of the layer of the immobilized DNA on a solid substrate is much needed.

While the construction of the DNA electrochemical biosensors is well advanced and widely reported in the literature, the problem of reliable, controlled deposition of an exact amount of DNA strands oriented parallel in the layer still needs perfection. We report here on the conditions that assure accurate determination of mass of DNA films on the gold electrode surface. The electrochemical quartz crystal microbalance (EQCM), electrochemical impedance spectroscopy (EIS), atomic force microscopy (AFM) and UV–vis spectroscopy were applied for physicochemical quantitative and qualitative characterization of the DNA films. In particular, our aim was to find the conditions of quantitative characterization of DNA layers by using the Sauerbrey equation. We applied UV–vis spectroscopy for independent determination of the mass of DNA attached to the electrode surface. In the examinations, synthetic oligonucleotides of different length were employed. The application of the nucleotides of different length allowed us to get the information on how the number of nucleotides in the strands and their conformation in the solution affect the quality of the formed DNA layer and in consequences the electrooxidation of guanine in the chain. The accumulation of DNA on the gold electrode surface was done by adsorption at a constant potential; this should lead to a monolayer-thick DNA film.

2. Material and methods

2.1. Materials

All chemicals were of the highest quality available. NaOH (p.a., POCh, Poland), KH_2PO_4 (p.a., POCh, Poland), K_2HPO_4 (p.a., POCh, Poland) were used as provided by the manufacturer. The synthetic oligonucleotides of different length and containing the guanine–cytosine pairs at the level 50% were purchased from MWG-Biotech, Germany. The following sequences of the bases were used:

- telomere fragment: 5′ TTAGGG 3′
- probe-1 DNA (20 nucleotides): 5′ GCAA TGGG TTAC TGAT GGGT 3′
- probe-2 DNA (40 nucleotides): 5′ GCAA TGGG TTAC TGAT GGGT GCAA TGGG TTAC TGAT GGGT 3′
- probe-3 DNA (60 nucleotides): 5′ GCAA TGGG TTAC TGAT GGGT GCAA TGGG TTAC TGAT GGGT GCAA TGGG TTAC TGAT GGGT 3′
- complementary 1 target DNA: 5′ ACCC ATCA GTAA CCCA TTGC 3′
- complementary 2 target DNA: 5′ ACCC ATCA GTAA CCCA TTGC ACCC ATCA GTAA CCCA TTGC 3′
- complementary 3 target DNA: 5′ ACCC ATCA GTAA CCCA TTGC ACCC ATCA GTAA CCCA TTGC ACCC ATCA GTAA CCCA TTGC 3′

To get double-stranded DNA fragments, the following hybridization procedure was applied. The concentration of the batch DNA solutions was 20 μM. To make sure that the solutions contain only single-stranded DNA, the vials with the probe and complementary target DNA were heated for 15 min at the melting temperature determined by the producer. After this time, the solutions were rapidly cooled in ice, diluted with distilled water and mixed in equimolar ratio. Such prepared mixtures were left for 24 h at room temperature for complete hybridization. For electrochemical and spectroscopic measurements, the concentration of dsDNA solutions was 0.2 μM (except for CD experiments; 2 μM).

We also used natural calf thymus double-stranded DNA (ctDNA). This reagent was purchased from Fluka; it was sufficiently pure and virtually free of protein. The criterion for the acceptable DNA purity is met when the values of the DNA absorbance ratios: $A_{\lambda = 260\ nm}/A_{\lambda = 280\ nm}$ are in the range 1.7–2.0 and $A_{\lambda = 260\ nm}/A_{\lambda = 250\ nm}$ in the range 1.4–1.7 [26]. The ctDNA samples chosen by us gave an absorbance ratio in the middle of the indicated ranges. ctDNA solution of 1 mg DNA per 1 ml of water was prepared at least 24 h before experiments to get full renaturation. The concentration of ctDNA was determined from the value of the absorbance at $\lambda = 260$ nm and at $\varepsilon = 13{,}200\ M^{-1}\ cm^{-1}$ [27]. All solutions were prepared with Milli-Q water.

2.2. Immobilization of DNA strands

The accumulation of non-modified synthetic single- and double-stranded DNA fragments and natural double-stranded DNA (ctDNA) on the gold electrode surface was done by adsorption at a constant potential from a pure-water solution. A freshly cleaned Au-EQCM electrode was immersed in an appropriate DNA solution (for synthetic DNA: 0.1–2 μM, for ctDNA: 1–6 μM) for 30 min. Since the DNA molecules are negatively charged at pH 7 [28], the immobilization of DNA on Au-EQCM surface was performed by holding the potential of the electrode at a constant positive value of +0.08 V for 30 min. After that time, the frequency change stabilized. In the case of ssDNA solutions, to be sure that ssDNA strands are not bonded together, before the immobilization step the solutions containing ssDNA were heated for 10 min at melting temperature. After this time, the solutions were rapidly cooled to room temperature. Such solutions were ready for immobilization step.

2.3. Electrochemical measurements

Electrochemical impedance spectroscopy (EIS) and electrochemical quartz crystal microbalance (EQCM) measurements were performed using an Autolab, model PGSTAT 12 potentiostat equipped with an ECD amplifier module (RC time settings: 0.0 s for scan rates >10 mV/s and 0.1 s for scan rates <10 mV/s) and the electrochemical analysis system based on the GPES and FRA software package (Eco Chemie B.V., Utrecht, the Netherlands). For each voltammetric measurement, the three-electrode system consisting of a 6 MHz Au/TiO_2AT-cut quartz crystal resonator (Au-EQCM; Eco Chemie B.V., Utrecht, the Netherlands) used as the working electrode. The thickness of the adhesion layer (TiO_2) was 100 Å and the thickness of Au coating was 1000 Å. The piezoelectrically active (geometrical) surface area of the working Au electrode was 0.361 cm^2 and the real surface area $A = 0.532\ cm^2$ (roughness factor $R = 1.5$). The real surface area of the Au-EQCM electrode was determined from the Pb underpotential-deposition voltammetric peak (0.01 M lead(II) perchlorate in 0.1 M perchloric acid). The generally accepted value for the charge of a Pb monolayer on Au is $Q_{Pb} = 302\ \mu C/cm^2$ (manufacturer data). A saturated Ag/AgCl electrode was used as the reference electrode and a gold wire was employed as the auxiliary electrode. The Au-EQCM electrode was electrochemically pretreated/cleaned by voltammetric cycling: first, between 0 and 1.8 V (with a 10-s scan stop at 1.8 V) in 0.5 M NaOH and with a scan rate of 50 mV/s, 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 [29]. Finally, before the deposition of DNA film, the gold-coated QCM crystals underwent a pretreatment consisting of several potential scans between −0.65 and 0.95 V at a high scan rate of 2 V/s in 0.1 M perchloric acid solution. This cycling was continued until a stable cyclic voltammogram consistent with that of a polycrystalline gold