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Controlling structural properties of self-assembled oligonucleotide–mercaptohexanol monolayers

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ABSTRACT

The achievement of high hybridization efficiency in DNA microarrays is largely affected by the surface density of immobilized functional DNA probes. We investigated the chemical conditions for the formation of mixed self-assembled monolayers of thiol-modified oligonucleotides and mercaptohexanol deposited onto gold surfaces. The surface density of DNA was studied by means of high-resolution X-ray photoemission spectroscopy. The measurements revealed that the spatial density of DNA strands can be controlled within a wide range by the concentration of $MgCl_2$ in the immobilization buffer. Moreover, improved preparation conditions for high-quality self-assembled hybrid monolayers are presented. Compared to our previous work, a reduction in unbound thiol was achieved by changing from ethanolic to aqueous solvent and lowering the MCH concentration.

1. Introduction

Since the discovery that alkanethiols are capable of forming self-assembled monolayers (SAMs) on gold surfaces [1], many studies have been focussed on the understanding of the properties and mechanisms underlying the structure formation of these organic layers. In particular, the ability to control the properties of a surface by coating it with a SAM has motivated a high research interest in SAMs [2–4]. Nowadays, tethering of thiol-modified DNA probes on gold surfaces has found wide application ranging from DNA microarrays and DNA-based protein sensors up to first attempts in DNA-based nanoelectronics [5–12].

Oligonucleotides end-specifically functionalized with thiol groups tend to interact with the gold surface not exclusively via their anchoring sulfur–gold bond, but to establish also non-specific interactions between their nucleobases and the metal surface [13–21]. To avoid such unwanted adsorption of DNA bases, the functionalized gold surface is typically exposed to mercaptohexanol (MCH) forming a self-assembled monolayer. In addition, deposition of MCH is expected to improve the accessibility of target DNA to the probe DNA during hybridization. Moreover, probe density is a decisive factor for the control of the hybridization efficiency and kinetics [22–27].

Here we investigated to which extend the DNA probe surface density in post-deposited MCH SAMs can be varied by changing the ionic strength of the DNA probe immobilization buffer. In particular, the dependence of the surface density of immobilized probe DNA on the concentration of $MgCl_2$ was studied.

The formation of highly packed alkanethiol SAMs is usually accomplished by assembly from ethanolic solutions [3]. However, since ethanol causes condensation of DNA [28] and is usually used to precipitate DNA [29], the question arises whether the post-deposition of MCH from an ethanolic solution will deteriorate the quality of the mixed layer. This issue was addressed by comparing the properties of hybrid DNA–MCH films prepared by post-deposition of MCH from ethanolic and aqueous solutions.

X-ray photoemission spectroscopy (XPS) can provide qualitative as well as quantitative information on self-assembled alkanethiol monolayers containing DNA [22,30–33]. Here we used this method to characterize our samples. The obtained results are discussed in comparison to our previously reported ones for pure MCH and mixed DNA duplex–MCH SAMs [34].

2. Materials and methods

2.1. Sample preparation

Silicon wafers polished on one side were used as the substrates. The wafers were sonicated for 5 min in acetone and thoroughly rinsed with an ethanolic solution. Then metal was deposited by

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evaporating about 100 nm of gold on top of a 3 nm thick chromium adhesion layer. In our previously reported measurements on self-assembled MCH layers [34], the gold film was cleaned with fuming nitric acid prior to probe immobilization. Here the cleaning step was omitted. Instead, the DNA probe layers were assembled on the as-prepared gold layer surfaces immediately after breaking the vacuum and taking the samples out from the evaporation chamber.

For the immobilization experiments different thiol-modified oligonucleotides have been used: O1-SH (5′-HS-$(CH_2)_6$-PO_4-TTT TTT TTT CAT TTC TTA TCA CAG GCT CAA ACC AGT CC- 3′), GC30-SH (5′-HS-$(CH_2)_6$-PO_4-GCG CCC CGG CGC GGC CCC CCG CCG CCC GCG- 3′) and GC60-SH (5′-HS-$(CH_2)_6$-PO_4-CCC GGG CGC CGG CCG CGG GGC GCG GCG GCG CGC GGG CGG CCC CGG CGG GGG CGG GCC CCC- 3′) (Biozym Scientific GmbH, Germany). The oligonucleotides were purified by the supplier in a two-step procedure. First, HPLC was used to purify the oligonucleotides while their thiol groups were protected by dimethoxytrityl (DMT). Then the thiol group was deprotected and the oligonucleotides purified by PAGE to obtain highest quality oligonucleotides. The oligonucleotides were delivered as lyophilized powders. Directly before use, these powders were dissolved in deionized water and used without additional treatment.

To study the influence of the MCH solvent on the film properties, double-stranded DNA (dsDNA) fragments of 30 and 60 base pairs length (dsGC30 and dsGC60) consisting of only guanine and cytosine were used, respectively. In this experiments double-stranded fragments were used in order to be able to compare the results with previously reported data obtained for samples prepared from ethanolic MCH solutions [34]. The dsDNA was prepared by hybridizing 1 μM GC30-SH and GC60-SH with 1.05 μM of the complementary oligomer (5′-CGC GGG CGG CGG GGG GCC GCG CCG GGG CGC- 3′ and 5′-GGG GGC CCG CCC CCG CCG GGG CCG CCC GCG CGC CGC CGC GCC CCG CGG CCG GCG CCC GGG- 3′) (Biozym Scientific GmbH, Germany), respectively, in aqueous solution with 20 mM $MgCl_2$. The sequence of these oligomers was designed in a way to minimize self-complementary sequence regions. The concentrations of the complementary strands were used in excess to ensure that all single-stranded GC30-SH and GC60-SH were hybridized. Both fragments were hybridized by heating the corresponding solutions up to 95 °C, and then, cooling them down to 4 °C with a cooling rate of 0.1 K/min.

A: $MgCl_2$ concentration. In order to investigate the dependence of the probe density on the $MgCl_2$ concentration of the immobilization buffer, the gold substrates were immersed for 1.5 h in solutions consisting of 1 μM O1-SH and 0, 2, 20, and 120 mM $MgCl_2$. Thereafter, the samples were treated with 1 mM aqueous MCH solution (Aldrich) for 1 h.

B: Two different preparation methods for DNA–MCH SAMs. The influence of the solvent for MCH on the film properties was studied by preparing samples where the thiol-modified dsDNA fragments dsGC30 and dsGC60 were immobilized on the gold surfaces, and the free gold surface was subsequently blocked with an aqueous MCH solution. To this end, the gold substrates were immersed in 1 μM dsDNA fragment solution for 1.5 h, and then, treated with 1 mM aqueous MCH solution for 1 h. The obtained results are compared to previously reported measurements where the blocking was accomplished with an 117 mM ethanolic MCH solution [34].

After each immobilization step the samples were carefully rinsed with deionized water and blown dry with nitrogen. The samples were stored in argon atmosphere.

2.2. Electron spectroscopy and data analysis

The X-ray photoemission spectroscopy measurements were performed at the Berliner Elektronenspeicherring für Synchrotronstrahlung (BESSY) using radiation from the Russian-German beamline. This bending magnet dipole beamline provides a moderate photon flux continuously distributed over the wide range between 30 and 1500 eV photon energy. These beam properties have proven to be well suited for studies on sensitive organic and biological molecules, such as self-assembled MCH monolayers [34] and proteins [35–38]. Particularly, X-ray radiation induced damage effects are rather minor granting sufficiently long time to record unaffected spectra from the same spot on the sample. The experimental station was equipped with a SPECS PHOIBOS 150 electron energy analyzer (AE) for high-resolution photoemission (PE) experiments. All measurements were performed at room temperature and in the kinetic energy range of roughly 100–400 eV with an energy resolution better than 120 meV. A reference Au $4f_{7/2}$PE signal of the underlying gold substrate was recorded immediately after measurement of every sample spectrum. Each time the sample spectrum and the corresponding reference signal were acquired at the same photon energy, without change of the monochromator settings. The well-known position of the Au $4f_{7/2}$peak at 84.0 eV below the Fermi level in metallic samples was used for careful alignment of the spectra on the binding energy (BE) scale. Least-squares fit analysis of the acquired XPS spectra was done using a series of Lorentzian line shapes convoluted with a Gaussian accounting for the finite experimental resolution. In the case of MCH, the line shapes and binding energies were specified by using the parameters from our previous work [34]. For the analysis of the of the DNA spectra, we used data derived from recent measurements on genomic DNA [39] which are in full agreement with other published data on DNA [31,33,40–42]. Upon fitting of a particular core-level spectrum, the peak width was set identical for all subspectra. Inelastically scattered electrons were modeled in terms of an integral Shirley background [43].

3. Results and discussion

3.1. $MgCl_2$ concentration

DNA molecules are negatively charged polyelectrolytes. Repulsive forces between their phosphate groups caused by inter- and intrastrand Coulomb interactions are reduced by association with cations. Depending on the concentration and type of cations, conformational changes of the DNA strands take place [44–47]. These occur, because cations mask the negative charges on the DNA backbone, and thus, decrease the radius of gyration R_g of a coiled single-stranded DNA. This allows to achieve a higher probe density which is proportional to $1/R_g^2$ [48,49]. It is known that molar concentrations of $Na^+$and K^+ lead to an increase of the probe density as well as of the immobilization and hybridization rate [22,25]. At comparable concentrations, the impact of divalent cations such as Ca^{2+} and Mg^{2+} is even larger. Investigations carried out at Mg^{2+} concentration of 5 mM [50,51], 20 mM [52,53] and 100 mM [30] revealed that even millimolar concentrations have a considerable effect on the preparation of highly dense DNA SAMs. Petrovykh et al. compared the influence of $CaCl_2$, $MgCl_2$, KH_2PO_4, K_2HPO_4, KCl and NaCl on the immobilization efficiency of 5'-HS-$(dT)_{25}$-3' oligonucleotides by XPS and Fourier transform infrared spectroscopy [30]. At 100 mM concentration, the application of Mg^{2+} showed the highest impact on the DNA surface coverage among all investigated ions [30]. However, systematic data on the dependence of the surface coverage on the Mg^{2+} concentration of the immobilization buffer is not available so far.

To investigate this important issue in more detail, we prepared layers from aqueous solutions of 1 μM ssDNA (O1-SH) with $MgCl_2$ concentrations in the immobilization buffer of 0, 2, 20 and 120 mM. Immobilization of the thiol-modified DNA probe molecules on the gold surface was followed by subsequent MCH deposition. In partic-