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DNA–DNA interaction on dendron-functionalized sol–gel silica films followed with surface plasmon fluorescence spectroscopy

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Abstract

Since we observed that dendron-assembled surface provided high single nucleotide polymorphism discrimination efficiency for DNA microarrays, and that the binding yield for streptavidin increased when biotin was immobilized on top of it, the nanoscale-controlled surface is examined for surface plasmon field-enhanced fluorescence spectroscopy (or SPFS). Firstly, a silica film was coated onto a gold substrate using the sol–gel technique, followed by the covalent immobilization of a layer of second-generation dendrons with a DNA catcher strand at their apex. The thickness of the inorganic interlayer ($d = 33$ nm) was effectively suppressing fluorescence quenching. Thus, the kinetics and affinity characteristics of DNA hybridization could be investigated very sensitively by SPFS. The kinetic rate constants found for DNA hybridization on the dendron-modified surface were larger than those reported for a streptavidin-modified surface by one order of magnitude, except for dissociation rate constant for a single mismatched case. In addition, we observed that the DNA on the cone-shaped linker maintained its capability to capture DNA target strands even after extended storage at ambient conditions.

Keywords: Dendron; Self-assembly; DNA hybridization; Kinetics; SPFS; Sol–gel

1. Introduction

Immobilization of biomolecules on surfaces is an essential component for various applications such as microarrays, biosensors, affinity chromatography, ELISA, etc. Especially, since the introduction of DNA microarrays [1,2], the interest in immobilized biomolecules and their behavior has steeply increased. Most biomolecules seem to lose all or a part of their inherent activity and function in the bulk aqueous phase when confined to a two-dimensional interfacial layer [3,4]. In case of DNA microarrays, mainly electrostatic perturbation and steric hindrance experienced by the immobilized DNAs may cause this distortion.

Therefore, in order to minimize these artifacts, the lateral spacing between the immobilized biomolecules has been controlled [5–10]. Among the various ideas, the most widely employed approach is based on a mixed self-assembled monolayer (SAM) in which two kinds of surfactants are mixed in the desired ratio. While the mixed SAMs providing a reduced density of an active functional group showed better performance for the DNA microarray than the surface-active materials without the density control, it could not ensure regular spacing between the surface-immobilized DNAs, because statistics governed the lateral distribution. In addition, the situation is even worse, because molecules of the same type tend to associate closely to form aggregates. Therefore, a new methodology is required to alleviate these problems.

Dendrimers are highly branched polymers with uniform size and molecular weight as well as well-defined structure. They consist of a central multifunctional core, repeating units attached around the core and terminal or end groups. Typically, they can be categorized into two groups. The first one has circular or elliptic shape of which repeating units are stretched from the core in a rather isotropic manner, whereas the sec-

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ond type, frequently called dendron, has conic shape of which repeating units are directionally stretched from the core. Because dendrimers manifest a highly ordered structure and give thin films with a various functional group on the top surface of a substrate, it is possible to create unique materials of which surface characteristic is controlled at the molecular level. Successful adsorption of the dendrimers on gold, mica, graphite, and silica substrates was demonstrated and the usefulness for chemical sensor [11], chromatographic packing material [12], multiple redox reaction center [13], interface for metal colloid insertion [14], and resist for scanning probe lithography [15] was shown. In particular, Whitesell showed that degree of alpha helix formation was increased when oligopeptides were immobilized on the dendron-assembled surface [16]. Recently, we demonstrated that performances of DNA microarrays, including SNP (or single nucleotide polymorphism) discrimination efficiency, could be enhanced with the surface control [17–20]. Also, surface plasmon spectrometry showed that degree of streptavidin binding increased when biotin was immobilized on top of the dendron-functionalized surface [21].

While kinetic studies will deepen our understanding of the impact of the lateral spacing on the DNA hybridization reaction on a surface, the standard DNA microarray is not adequate for this purpose. This is why, in spite of the lower throughput, the interest in surface plasmon resonance (or SPR) spectroscopy has increased continuously during the last two decades. Many studies have employed SPR because the method allows for measurements of the kinetic as well as thermodynamic parameters for biomolecular interactions without labeling of the biomolecules [22–27].

However, the relatively low sensitivity has been the major limitation for a wider application. In case of a low lateral density of the capture (or the receptor) molecules or for analytes of low molecular mass, only a minute change of the refractive index occurs, eventually resulting in a SPR angular shift too small to be detected. As a consequence, an approach utilizing a fluorescence labeled analyte in conjunction with a surface plasmon spectrometer as a means to enhance the signal of the interfacial binding event has been developed. In SPFS, a novel recently introduced optical detection scheme, advantageous aspects of both surface plasmon excitation and fluorescence detection are combined, while avoiding fluorescence quenching from the metal [28]. In addition, SPFS is capable of monitoring reorientation or reorganization of the analytes after the binding to the corresponding receptor, because the fluorescence intensity is sensitive to the distance between the metal and the chromophore label conjugated to a biomolecule [29].

In this paper, SPFS was employed to investigate the kinetic rate constants, k_{on} and k_{off}, as well as the affinity constant, K_A, between the incoming fluorescently labeled target DNA in solution and the probe DNA on the dendron-modified surface. In addition, temporal stability of the probe DNA immobilized surface was measured at ambient conditions.

2. Materials and methods

2.1. Materials

All materials employed were of research grade, and were used as received from commercial sources without any additional purification with the exception of ethanol, which was dried through distillation, and 3-mercaptopropylsiloxane (3-MPS, Lancaster Synthesis GmbH), which was distilled prior to use. Tetramethoxysilane (TMOS) and hydrochloric acid (HCl) were purchased from Fluka GmbH. *N*-(Triethoxysilylpropyl)-*o*-polyethyleneoxideurethane (TPU) were purchased from Gelest, Inc. All chemicals and solvents for synthesis of the dendrons were of reagent grade from Sigma-Aldrich and Mallinckrodt Laboratory Chemicals. While all other chemicals for the surface reaction were of reagent grade from Sigma-Aldrich, washing solvents for the substrates were of HPLC grade from Mallinckrodt Laboratory Chemicals. Oligonucleotides were purchased from MWG-Biotech. Deionized water (18 MΩ cm) was obtained from a Milli-Q purification system (Millipore). Hybridization buffer solution was prepared using phosphate-buffered saline (PBS) tablets (Sigma-Aldrich) with a Na^+ concentration of 0.137 M. The employed dendron with an F-moc (or fluorenylmethoxycarbonyl) protection group was prepared according to the procedure published previously [21]. In this method, we coupled *N*-[tris(carboxyethoxymethyl)methyl]-4-(9-fluorenylmethoxycarbonyl amino)butyramide with 3 equivalents of tris[(2-(*tert*-butoxycarbonyl)ethoxy)methyl]methylamine in the presence of 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDC) and 1-hydroxybenzotriazole (HOBT), and hydrolyzed the resulting second generation dendron with formic acid to make the target dendron (**1**).

2.2. Thermal evaporation of gold onto the glass substrate

Gold (99.9999%, Balzers) of 50 nm thickness was deposited onto clean optical Schott glass slides (LaSFN9 type) by thermal evaporation (deposition rate = 0.1 nm/s, $p < 10^{-6}$ mbar) in an evaporation apparatus (Edwards). In order to improve the adhesion of the gold film to the glass substrate a chromium film of approximately 2 nm thickness was evaporated.

2.3. Synthesis of silica sol–gel films

An ultrathin silica film was prepared on top of the gold surface immediately after the gold deposition. The Au substrate was immersed in 3-MPS (20 mM) in dry ethanol for 2 h. The surface was then rinsed with copious amounts of ethanol and deionized (Milli-Q) water. Hydrolysis of the 3-MPS layer was followed by dipping in aqueous HCl (0.10 M) solution for 1 h. The substrate was removed from the HCl solution, and rinsed with water. A thin SiO_x layer was prepared according to the method reported previously [30,31]. The hydrolyzed solution of the sol–gel precursor was delivered onto the 3-MPS-modified Au surface, and the substrate was spun at ∼3400 rpm for 1 min, resulting in a film with thickness of ca. 33 nm. The Au/3-