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 providing a proper spacing between immobilized DNAs showed high single nucleotide polymorphism discrimination efficiency for DNA microarrays, the nanoscale-controlled surface is examined with surface plasmon field-enhanced fluorescence spectroscopy (or SPFS) to elucidate the kinetics of the DNA hybridization. A silica film (d=33 nm) was coated onto a gold surface using the sol-gel technique to suppress fluorescence quenching, followed by self-assembly of a second-generation dendron and covalent immobilization of a catcher DNA strand at the dendron's apex. The association rate for the DNA hybridization on the surface was faster than that reported for a streptavidin-modified surface. In addition, we observed that the capability to capture DNA target strands was maintained even after extended storage at ambient conditions.

Keywords: dendron, self-assembly; DNA hybridization, kinetics, surface plasmon filed-enhanced fluorescence spectroscopy.

1 INTRODUCTION

Immobilization of biomolecules on surfaces is a crucial component for various applications such as microarrays, biosensors, affinity chromatography, ELISA, etc. In particular, since the introduction of DNA microarrays [1,2], the concern in immobilized biomolecules and their behavior has steeply increased. While keeping the inherent activity and the function in bulk aqueous phase, most of the biomolecules seem to frequently lose all or part of them when they are confined within two-dimensional artificial layer [3,4]. In case of DNA microarrays, mainly electrostatic perturbation and steric hindrance experienced by the immobilized DNAs may cause this distortion.

Therefore, to reduce these artifacts, the lateral spacing between the immobilized biomolecules has been controlled [5-10]. Among a variety of ideas, the most commonly employed methodology 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 controlling the density, it could not make sure 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 are likely to associate closely to form aggregates. Therefore, a new methodology is essential to alleviate these problems.

Recently, we have studied self-assembly of a cone-shaped dendron to make the nanoscale-controlled surface providing regular lateral spacing between the immobilized biomolecules.

We demonstrated that performances of DNA microarrays, including SNP (or Single Nucleotide Polymorphism) discrimination efficiency, could be improved with the surface control [11-14]. Also, surface plasmon spectrometry showed that degree of streptavidin binding was enhanced when biotin was immobilized on top of the dendron-modified surface [15].

While kinetic study will deepen our understanding about the effect of the lateral spacing on the DNA hybridization on surface, while the typical DNA microarray is not satisfactory for this purpose. Regardless of lower throughput, this is why the interest on surface plasmon resonance (or SPR) spectroscopy has increased continuously during the last two decades. Many studies have employed SPR because the method allows measurement of the kinetic parameters as well as thermodynamic ones for biomolecular interactions without labeling of the biomolecules [16-21].

However, the relatively low sensitivity has been the foremost limitation for a wider application. In case of a low lateral density of the capture 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 result, an approach utilizing a fluorescence labeled analyte in combination with a surface plasmon spectrometer has been developed to enhance the signal of the interfacial binding event. 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 [22]. In addition, SPFS can monitor reorientation or reorganization of the analytes after the binding to the corresponding receptor, because the

Name	DNA Sequence
Probe DNA	5'-NH_2-$(T)_{15}$ TGT ACA TCA CAA CTA-3'
Complementary Target DNA (MM0)	3'-ACA TGT AGT GTT GAT-Cy5-5'
Single Mismatched Target DNA (MM1)	3'-ACA TGC AGT GTT GAT-Cy5-5'

Table 1: The sequence of probe and target oligonucleotides.

fluorescence intensity is sensitive to the distance between the metal and the chromophore labeled to a biomolecule [23].

In this paper, SPFS was employed to study 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 METHODS

2.1 Preparation of silica sol-gel films

A silica film was prepared on top of the gold surface immediately after the gold deposition. The Au substrate was immersed in 3-MPS (3-mercaptopropylsiloxane, 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 rinsed with water. A thin SiO_x layer was prepared according to the method reported previously [24,25]. The hydrolyzed solution of the sol–gel precursor was delivered onto the 3-MPS-modified gold 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-MPS/SiO_x surface was stored in a desiccator at room temperature for a minimum of 2 days to complete the condensation process as well as to eliminate any residual solvent.

2.2 Preparing the dendron-modified substrates

The sol-gel coated substrate was reacted in a chamber with a few drops of N-(Triethoxysilylpropyl)-o-poly ethyleneoxideurethane (TPU) for 12 h at 120 ℃ in order to introduce hydroxyl groups which can react with the dendron molecules. After cooling to room temperature, the substrate was rinsed with copious amounts of ethanol. For the dendron-modification, the dendron (1.0 mM), 1,3-dicyclohexylcarbodiimide (DCC) (9.3 mM), and 4-dimethylaminopyridine (DMAP) (0.90 mM) were dissolved in the mixture of methylene chloride (18 mL) and DMF(2.0 mL) solution. After allowing the plate in the reaction solution for 12 h at room temperature, the plate was rinsed with methanol, water, and methanol in a sequential manner each for 3 min. Finally the washed plate was dried in a stream of nitrogen gas (Figure 1)

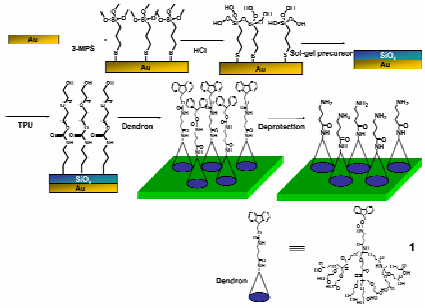

Figure 1: The scheme illustrating steps for the dendron modification on a sol-gel silica film.

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2.3 Immobilization of a probe DNA on the NHS-modified substrate

A spotting buffer solution (25 mM $NaHCO_3$, 5.0 mM $MgCl_2$, pH 8.5) dissolving an amine-tethered probe DNA (1.0 μM) was placed onto the NHS-modified substrate by di(N-succinimidyl) carbonate linker (Table 1). After 12 h, the substrate was rinsed with deionized (Milli-Q) water.

3 RESULTS AND DISCUSSION

3.1 Surface modification

Because of the compatibility with the silylation process and its ability to generate an appropriate separation ($d \approx 35$ nm), we employed a sol-gel silica layer. In particular, such an inorganic layer is suitable for the immobilization of the dendron if a proper silane interlayer is in place.

Applying a TPU coating on the sol-gel film introduced reactive hydroxyl functional groups, which formed ester bonds with the carboxylic acid groups at the termini of the dendron. The combination of the DSC linker and the amine tethered oligonucleotides resulted in an effective immobilization of probe DNA, while the hybridization with the target DNA was followed with SPFS.

3.2 DNA hybridization to the dendron-modified surface

The employed silane reagent, TPU, that has ethylene glycol units (n = 4 – 6) has been suitable for generating an interlayer suppressing nonspecific binding of DNA. Upon immobilization of the dendron, the median lateral spacing between the amine groups would be 3.2 nm [12], and because the spacing between the binding pockets of a streptavidin layer is comparable to that, it would be worthwhile to compare the non-natural nano-controlled architecture to the one based on the protein layer.

Upon the addition of the fully complementary target DNA, the fluorescence intensity increased rapidly and reached a constant value. The decay of the fluorescence was slow if the sample was rinsed with a buffer solution (Figure 2(a)). A contrasting behavior was observed in the case of injecting a mismatched target DNA on the same surface. The rate of the increase upon the injection was smaller, and the rate of the decrease upon the washing was larger (Figure 2(b)).

Kinetic information could be collected by fitting the SPFS data with a simple one-to-one interaction model. According to the Langmuir approach, the association process should follow

$$I_{fl}(t)=I_{max}(1-\exp(-(k_{on}c_0+k_{off})t)) \quad (1)$$

and the dissociation process should be described by

$$I_{fl}(t)=I_{max}\exp(-k_{off}t) \quad (2)$$

In order to enhance the reliability of the analysis, the data were collected at various concentrations. For the complementary case, the concentrations varied from 2.0 nM to 50 nM, and higher concentrations (10 nM to 200 nM) were employed for the mismatched case. The individual k_{on} from each concentration was calculated, and the values from the five fittings were averaged. The corresponding k_{off} was obtained in the same way. The resulting kinetic values are given in Table 2. As expected, the k_{off} rate constant for the double stranded DNA with one base mismatch was larger than that for the perfectly matched double stranded DNA, and k_{on} rate constant was in the reverse order. The affinity constant K_A is given by

$$K_A = k_{on}/k_{off} \quad (3)$$

3.3 Comparison of the DNA hybridization on the dendron-modified surface with that on a streptavidin-modified surface

The kinetic characteristics of the DNA hybridization on the dendron-modified surface is compared with that of the streptavidin-modified surface reported previously [22]. The association rate constant (k_{on}) of the DNA hybridization for the complementary and the mismatch target DNA on the dendron-modified surface (43 x 10^4 $M^{-1}s^{-1}$ and 6.8 x 10^4 $M^{-1}s^{-1}$, respectively) is larger than that for the streptavidin-modified surface (3.7 x 10^4 $M^{-1}s^{-1}$ and 0.89 x 10^4 $M^{-1}s^{-1}$, respectively) (Table 2). It is not straightforward to understand why the dendron-modified surface gives the association rate constant of one order of magnitude larger value. Difference in the lateral spacing realized by the two approaches could be one of the reasons. High resolution electron microscopy showed that the median spacing generated with the second-generation dendron was 3.2 nm. Meanwhile, there are two categories of the spacing in the streptavidin surface, the one dictated by the distance between the two binding pockets (d = 2.5 nm), and the other one determined by the packing degree of streptavidin on the surface. While the former is the inherent value of the protein, the latter is dependent on the way of the sample preparation, including the architecture of the base layer immobilizing the protein. Because 1.5 biotin-tethered DNA probes bind on average to each streptavidin [26] under typical conditions, the spacing between the probes could be either 2.5 nm or a larger one governed by the distance to the nearest streptavidin.

Another difference worth of consideration is the electrostatic influence. While the backbone of the dendron is neutral in terms of electrostatics, streptavidin (pI = 5.5) is negatively charged at the hybridization condition (pH = 7.4). The partial negative charge of the streptavidin-modified surface could slow down the association rate of the incoming target DNA.

The dissociation rate constant (k_{off}) of the complementary target DNA on the dendron-modified surface (12 x 10^{-5} s^{-1}) turned out to be larger than that of the streptavidin surface (0.7 x 10^{-5} s^{-1}), and both dissociation

	k_{on} [in 10^4 $M^{-1}s^{-1}$]	k_{off} [in 10^{-5} s^{-1}]	K_A [in 10^8 M^{-1}]
Complementary Target DNA	43 (3.7)*	12 (0.7)	35 (53)
Single Mismatched Target DNA	6.8 (0.89)	32 (37)	2.2 (0.24)

* Numbers in parenthesis: previously reported data for the streptavidin surface [22].

Table 2: Rate constants for the DNA hybridization on the dendron-modified surface.

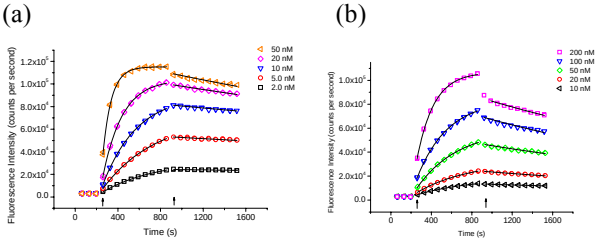

Figure 2: SPFS measurement of DNA hybridization on a dendron-modified surface. (a) Hybridization of complementary target DNA (MM0) (b) hybridization of single base mismatched target DNA (MM1).

constants for the mismatched case were larger than those for the matched case, and were very similar to each other (32×10^{-5} s^{-1} and 37×10^{-5} s^{-1}, respectively).

In order to examine temporal stability, the probe DNA-tethered dendron-modified surface was stored at the ambient conditions for days. In particular, the surface was exposed to air in the absence of the buffer solution. The fluorescence intensity profile observed when the target was injected after 7 days was essentially same as the one obtained with the fresh sample (1.3×10^4 cps at 800 seconds). The similar stability was observed when the probe DNA-tethered streptavidin-modified surface was stored in a buffer solution, while the signal intensity diminished significantly when the surface was exposed to air in the absence of the buffer (3.9×10^5 cps to 3.6×10^4 cps). The result implies that the conically shaped molecule on surface is relatively rigid and its structure is less sensitive towards the environment, presumably because the three-dimensional structure is dictated mainly by the covalent bonds.

4 CONCLUSION

Sol-gel silica films were effectively introduced as interlayers between a gold substrate and a second generation dendron (**1**) monolayer, with probe DNA strands attached to the apex of the dendrons. This architecture provided fluorescence signals sufficient for surface plasmon field-enhance fluorescence spectroscopic analysis of hybridization reactions. Monitoring the kinetics of DNA association and dissociation yielded rate constants that were larger than those found for a catcher matrix based on biotin-streptavidin coupling scheme by one order of magnitude, except for k_{off} of the mismatched case. The capturing activity for hybridization reactions of the probe DNA on the dendron apex was maintained for long times at ambient conditions. The result further suggests that the conically shaped molecules are relatively rigid and do not induce nonspecific binding of the probes. In addition, the control of the lateral spacing realized by the conical shape of the dendrons of various generations makes the approach attractive for the immobilization of functional molecules in bio-assays.

REFERENCES

[1] S.P.A. Fodor, R.P. Rava, X.C. Huang, A.C. Pease, C.P. Holmes and C.L. Adams, Nature 364, 555, 1993.

[2] R.K. Saiki, P.S. Walsh, C.H. Levenson and H.A. Erlich, Proc. Natl. Acad. Sci. U.S.A. 86, 6230, 1989.

[3] E. Southern, K. Mir and M. Shchepinov, Nature Genet. 21, 5, 1999.

[4] P. Angenendt, J. Glökler, D. Murphy, H. Lehrach and D.J. Cahill, Anal. Biochem. 309, 253, 2002.

[5] K.L. Prime and G.M. Whitesides, Science 252, 1164, 1991.

[6] K.L. Prime and G.M. Whitesides, J. Am. Chem. Soc. 115, 10714, 1993.

[7] B.E. Rabinow, Y.S. Ding, C. Qin, M.L. McHalasky, J.H. Schneider, K.A. Ashline, T.L. Shelbourn and R.M. Albrecht, J. Biomater. Sci. Polym. Ed. 6, 91, 1994.

[8] R.G. Chapman, E. Ostuni, M.N. Liang, G. Meluleni, E. Kim, L. Yan, G. Pier, H. S. Warren and G.M. Whitesides, Langmuir 17, 1225, 2001.

[9] R.E. Holmlin, X. Chen, R. Chapman, S. Takayama and G.M. Whitesides, Langmuir 17, 2841, 2001.

[10] Y. Luk, M. Kato and M. Mrksich, Langmuir 16, 9604, 2000.

[11] B.J. Hong, J.Y. Shim, S.J. Oh and J.W. Park, Langmuir 19, 2357, 2003.

[12] B.J. Hong, S.J. Oh, T.W. Youn, S.H. Kwon and J.W. Park, Langmuir 21, 4257, 2005.

[13] B.J. Hong, V. Sunkara and J.W. Park, Nucleic Acid Res. 33, e106, 2005.

[14] S.J. Oh, J.M. Ju, B.C. Kim, E.S. Ko, B.J. Hong, J.G. Park, J.W. Park and K.Y. Choi, Nucleic Acids Res. 33, e90, 2005.

[15] Y.-S. Choi, C.W. Yoon, H.D. Lee, M., Park and J.W. Park, Chem. Commun. 11, 1316, 2004.

[16] M. Brigham-Burke, J.R. Edwards and D.J. O'Shannessy, Anal. Biochem. 205, 125, 1992.

[17] B.C. Cunningham and J.A. Wells, J. Mol. Biol. 234, 554, 1993.

[18] K. Kukanskis, J. Elkind, J. Melendez, T. Murphy, G. Miller and H. Garner, Anal. Biochem. 274, 7, 1999.

[19] S. Löfås, B. Johnsson, K. Tegendal and I. Rönnberg, Colloids Surf. B: Biointerf. 1, 83, 1993.

[20] H. Miyachi, K. Yano, K. Ikebukuro, M. Kono, S. Hoshina and I. Karube, Anal. Chim. Acta 407, 1, 2000.

[21] H. Morgan and D.M. Taylor, Biosens. Bioelectron. 7, 405, 1992.

[22] T. Liebermann, W. Knoll, P and Sluka, R. Herrmann, Colloids Surf. A 169, 337, 2000.

[23] T. Neumann, M. Johannson, D. Kambhampati and W. Knoll, Adv. Funct. Mater. 12, 575, 2002.

[24] D.K. Kambhampati, T.A.M. Jakob, J.W. Robertson, M. Cai, J.E. Pemberton and W. Knoll, Langmuir 17, 1169, 2001.

[25] D.H. Kim, K.H.A. Lau, J.W.F. Robertson, O.J. Lee, U. Jeong, J.I. Lee, C.J. Hawker, T.P. Russell, J.K. Kim and W. Knoll, Adv. Mater. 17, 2442, 2005.

[26] X. Su, Y. Wu, R. Robelek and W. Knoll, Langmuir 21, 348, 2004.