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ScienceDirect
Biosensors and Bioelectronics 22 (2007) 1532–1537
BIOSENSORS & BIOELECTRONICS
www.elsevier.com/locate/bios

Short communication

Sensitivity enhancement of DNA microarray on nano-scale controlled surface by using a streptavidin–fluorophore conjugate

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Received 16 March 2006; received in revised form 18 May 2006; accepted 24 May 2006
Available online 30 June 2006

Abstract

High throughput analysis of DNA in low concentration and small volume is an important issue and a continuing challenge in the field of DNA microarray and sensor. Recently, we have demonstrated that the DNA microarray on nano-scale controlled surface provides ample space for hybridization resulting in the best discrimination efficiency for SNP analysis. Here, we report the utility of the nano-scale controlled surface in conjunction with a multiply tagged protein. Application of streptavidin–fluorophore conjugates in combination with the highly controlled surface that suppresses non-specific binding of DNA allows highly sensitive detection of DNA while maintaining superior SNP discrimination efficiency comparable to our earlier results. The sensitivity of DNA microarray on the mesospaced surface is two orders of magnitude higher than that of the generic surface when a streptavidin–fluorophore conjugate was employed, and the detection limit on the former surface was found to be 50 fM of 15-mer target DNA. Various streptavidin–fluorophore conjugates including streptavidin–Cy3, streptavidin–Cy5, streptavidin–Alexa Flour 555 and streptavidin–phycoerythrin were examined.

Keywords: DNA microarray; Dendron; Streptavidin–fluorophore conjugate; Detection limit

1. Introduction

DNA microarray is a revolutionary tool for high throughput, multiplexed analyses of large number of genes (Fodor et al., 1991). It is important to develop highly sensitive detection methods for the microarray-based analysis as sometimes only minute amounts of genetic material is available. Typically, the signal output is enhanced through one of amplification methods, which are classified into two classes, i.e., target amplification and signal amplification (Schweitzer and Kingsmore, 2001). Importance and shortcomings of target amplification in gene expression analyses were recently reviewed (Wang, 2005). Despite the wide applicability, the target amplification by PCR has drawbacks like contamination of the material through amplicon carry-over, limited ability for multiplexing, variations in amplification efficiency, etc. Amplification of mRNA from a sample of small copies also suffers from distortion of pristine RNA ratio and increased noise ratio (Nygaard et al., 2005). In order to get around the above complications, efforts have been made to amplify the signal for detection of nucleic acids (Collins et al., 1997; Capaldi et al., 2000; Hall et al., 2000; Taton et al., 2000; Nallur et al., 2001; Saghatelian et al., 2003; Zhou and Zhou, 2004; Ho et al., 2005; Crut et al., 2005; Simon et al., 2005), and the techniques for low abundant DNA sensing have been also developed (Bao et al., 2002; Epstein et al., 2002; Zhao et al., 2003; Baca et al., 2004; Hahm and Lieber, 2004; Wang et al., 2005; Flechsig et al., 2005; Lee et al., 2005; Bowden et al., 2005). However, most of these methods deal with sensing nucleic acids in a sequential manner, hence, the methods applicable for high throughput analysis are to be developed. Recently, an approach utilizing PAMAM dendrimer-activated glass slide has been investigated in order to enhance the signal intensity (Le Berre et al., 2003). It is clear that the signal intensity becomes larger at high concentration of target DNA as the number density of the probes increases, but suppressing non-specific binding is also important to push down the detection limit (Oh et al., 2002). Among various functionalities, the oligoethyleneglycol monolayer was found to effectively lower the non-specific binding of biomolecules on various surfaces (Clare et al., 2005). We explored the advantage of the ethyleneglycol layer in con-

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doi:10.1016/j.bios.2006.05.027

junction with cone-shaped dendron that provides mesospacing among neighboring biomolecules on surface and lessens non-specific adsorption of biomolecules on the surface (Hong et al., 2005a,b; Oh et al., 2005; Choi et al., 2004).

Fluorescent conjugates of streptavidin are often used as amplifiers to detect biomolecules in DNA hybridization technique (Liu et al., 2005; Lucarelli et al., 2006; Lawrence et al., 1990; Fan et al., 1990) and as secondary detection reagents in histochemical applications, flow cytometry, blot analysis and immunoassays (Zhiqiang et al., 2005; Guido et al., 2004). Because biomolecules can be easily labeled with biotin, the biotin–streptavidin interaction has been established as a representative system for amplifying the signal after the hybridization.

Herein, we present application of a streptavidin–fluorophore conjugate to improve detection limit of DNA microarray, while securing extraordinary SNP discrimination efficiency by employing the dendron-modified glass substrate.

2. Materials and methods

2.1. Materials

All chemicals and solvents for synthesis of the dendron were of reagent grade from Sigma–Aldrich and Mallinckrodt Laboratory Chemicals. The silane coupling reagent (3-glycidoxypropyl)methyldiethoxysilane (GPDES) was purchased from Gelest Inc. and all other chemicals for surface reaction were of reagent grade from Sigma–Aldrich. Reaction solvent for the silylation is anhydrous one in a Sure/Seal bottle from Aldrich. All washing solvents for the substrates are of HPLC grade from Mallinckrodt Laboratory Chemicals. Glass slides (2.5 cm × 7.5 cm) were purchased from Corning Co. Silanated slides which are one of typical generic amine-modified slides, were purchased from TeleChem International Inc. and Cel Associates Inc. All of the oligonucleotides were purchased from Metabion, streptavidin–Cy3 conjugate from Sigma–Aldrich, streptavidin–Alexa Fluor 555 and streptavidin–*R*-phycoerythrin from Invitrogen Detection Technologies, and streptavidin–Cy5 from eBioscience. Ultrapure water (18 MΩ/cm) was obtained from a Milli-Q purification system (Millipore).

2.2. Instruments

Oligonucleotides were spotted by using a MicroSys5100 microarrayer (Cartesian Technologies Inc.) and a Piezorray (Perkin-Elmer, LAS). Hybridization was performed with a HS4800 Hybridization Station (Tecan). Fluorescence signal of the array was measured with a ScanArray Lite (GSI Lumonics) and an ArrayWoRx (Applied Precision Inc.), and the image was analyzed with an Imagene 4.0 software (Biodiscovery).

2.3. Preparation of dendron-modified slide

The dendron-modified slides were prepared according to the published procedure (Hong et al., 2005a).

2.4. Linker modification and arraying probe oligonucleotides on dendron-modified surface and silanated one: DSC linker modification

The dendron-modified slides and silanated slides were immersed in a solution of *N*,*N*′-disuccinimidyl carbonate (DSC, 25 mM) dissolved in acetonitrile, with a catalytic amount of diisopropylethylamine (DIPEA) under nitrogen for 4 h, then the slides were placed in DMF with stirring for 20 min, washed with methanol. After drying under vacuum for 30 min, the oligonucleotides (20 μM) dissolved in a spotting buffer (25 mM $NaHCO_3$, 5 mM $MgCl_2$, pH 8.5) with 10% (v/v) DMSO, were arrayed in a 4 × 4 format with a microarrayer and were incubated at 80% relative humidity overnight. Pre-hybridization washings were performed by placing the slides in a stirring hybridization buffer solution [2× SSPE, SDS (7 mM), pH 7.4] at 37 °C for 20 min, washed with deionized water and dried under a stream of nitrogen and stored at 4 °C.

2.5. Hybridization

The arrayed slides were hybridized with 100 μl of a target oligonucleotide dissolved in a hybridization buffer [2× SSPE, SDS (7 mM), pH 7.4] at 37 °C for 1 h. Washed with the hybridization buffer solution at 37 °C for 1 min and 1× PBS buffer at 25 °C for 10 s in a Tecan hybridization station.

2.6. Incubation with a streptavidin–fluorophore conjugate

After hybridization with the biotinylated target, the slides were incubated with a solution of streptavidin–fluorophore (Cy3, Cy5, Alexa Fluor 555, or *R*-phycoerythrin) conjugate in 1× PBS buffer (1:100 of 1 mg/ml solution) at 37 °C for 30 min and washed with 1× PBS buffer at 25 °C for 1 min in a Tecan hybridization station.

3. Results and discussion

3.1. Sensitivity of dendron-modified slide and silanated one with a Cy3 tagged complementary oligonucleotide

To investigate the sensitivity of DNA microarrays on the dendron-modified surface and silanated one, a probe oligonucleotide (P1) of 20 μM was spotted on both slides, and the arrays were hybridized with a target oligonucleotide (T1) in various concentrations (1.0 nM, 100, 10, 1.0 pM and 100 fM). On the dendron-modified slide, fluorescence signal could be detected at a target concentration as low as 1.0 pM whereas on the silanated slide the lowest target concentration detectable was 5.0 pM. This result indicates that the detection sensitivity of the dendron-modified slide is five-fold higher than that of the silanated one. In order to optimize the sensitivity of the silanated slide, different linker modifications were tried, one of which was a PDITC linker, as described in our previous report (Hong et al., 2005b) and the other was a NHS linker, which gives similar functionality at the surface like the DSC linker (procedures for the surface activation is given in supple-