Analytical Biochemistry 430 (2012) 39–44

Contents lists available at SciVerse ScienceDirect

Analytical Biochemistry

journal homepage: www.elsevier.com/locate/yabio

Dendron-modified surfaces provide an ideal environment for stem–loop DNA probes

Jonas Boateng [a], Joel Peek [b], Robert Zahorchak [c], Krishnan Chittur [a,*]

[a] Department of Chemical Engineering, University of Alabama in Huntsville, Huntsville, AL 35899, USA
[b] Microarrays, Inc. Huntsville, AL 35806, USA
[c] Hudson Alpha Institute for Biotechnology, Huntsville, AL 35806, USA

ARTICLE INFO

Article history:
Received 31 May 2012
Received in revised form 18 July 2012
Accepted 19 July 2012
Available online 28 July 2012

Keywords:
DNA microarrays
Stem–loop probes
Surface chemistry
Nucleic acid testing

ABSTRACT

Specificity and sensitivity are important factors affecting DNA microarrays. Stem–loop DNA probes (SLPs) can be more specific in their recognition of target sequences than linear DNA probes, but unless they are carefully designed, surface interactions can disrupt the native stem–loop structure. In this study, we show how dendron-modified surfaces with well-defined, uniform spacing of aldehyde chemical functionalities offer an ideal substrate to immobilize SLPs and use them to detect nucleic acid targets. The mesospacing provided by the dendron-modified surfaces produces a solution-like environment that allows the SLPs to detect target nucleic acids at concentrations as low as 1 pM in concentration.

DNA microarray technology has become a fast, reliable, and cost-effective way of monitoring gene expression and for detecting nucleic acids of known sequence. Applications for DNA microarrays range from diagnostics to detection of mutations, and they have played an important role in gene analysis studies, single nucleotide polymorphism (SNP)[1] genotyping, and resequencing.

The advantages of DNA microarrays include the ability to detect numerous targets, functional integration for high-throughput screening, and easy automation [1]. Most traditional microarrays use linear DNA probes that bind to target DNA that has been labeled. However, stem–loop structured DNA probes (SLPs) used in microarrays can be more sensitive and specific than arrays with linear probes [2]. Microarray hybridization is usually based on the interaction of a fluorescently labeled single-stranded target nucleic acid molecule with its complementary molecule, which is sometimes covalently immobilized onto a substrate [3–5].

We have developed a novel two-step hybridization approach [6] using structured nucleic acids that is capable of detecting multiple targets without any need for target modification. The first hybridization event involves the binding of unlabeled targets to the loop region of a surface-immobilized denatured SLP. If the target binds to the probe, the event allows for the binding of a second fluorescently labeled probe (detector) to the complex formed by the target and the SLP (Figs. 1B–D). Conversely, if the target does not bind to the SLP (due to noncomplementarity), then the SLP reforms back into its original stem–loop conformation and the detector fails to bind (i.e., the reformation of the stem "masks" the detector-specific sequence). The technique provides a simple approach for the detection of specific targets in solution without the need for amplification. However, SLP-based microarray platforms can be difficult to implement because of structural changes in the SLP. Some of these changes may involve surface interactions.

In this article, we show how aldehyde-functionalized dendron-modified surfaces (Fig. 2) can improve the specificity of DNA probe microarrays, presumably by providing each DNA probe strand with sufficient space to effectively interact with incoming target DNA [7–9]. A dendron is a well-defined cone-shaped structure that can be functionalized at the apex with an amine or aldehyde group, thereby providing uniform and controlled spacing between immobilized DNA probes—unlike polymer-functionalized substrate surfaces [10–12]. In this study, we show how the use of aldehyde-functionalized dendron-modified surfaces significantly improves both the sensitivity and specificity of our SLP-based microarray platform. The dendron-modified surfaces were compared with glycidoxysilanated (epoxy-functionalized) and non-dendron silylated (aldehyde-functionalized) surfaces.

Materials and methods

Materials

All solvents and chemicals needed for preparing, processing, and using microarrays were purchased from Thermo Fisher

* Corresponding author. Fax: +1 256 824 6839.
E-mail address: chitturk@uah.edu (K. Chittur).

[1] *Abbreviations used:* SNP, single nucleotide polymorphism; SLP, stem–loop DNA probe; SSC, saline–sodium citrate; SDS, sodium dodecyl sulfate; BSA, bovine serum albumin; EtOH, ethanol; NaBH4, sodium borohydride; EDTA, ethylenediaminetetraacetic acid; NSB, NanoSurface Biosciences; rRNA, ribosomal RNA; PBS, phosphate-buffered saline; SSPE, saline–sodium phosphate–EDTA; PMT, photomultiplier tube; RFU, relative fluorescent units; SNR, signal-to-noise ratio.

0003-2697/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.ab.2012.07.021

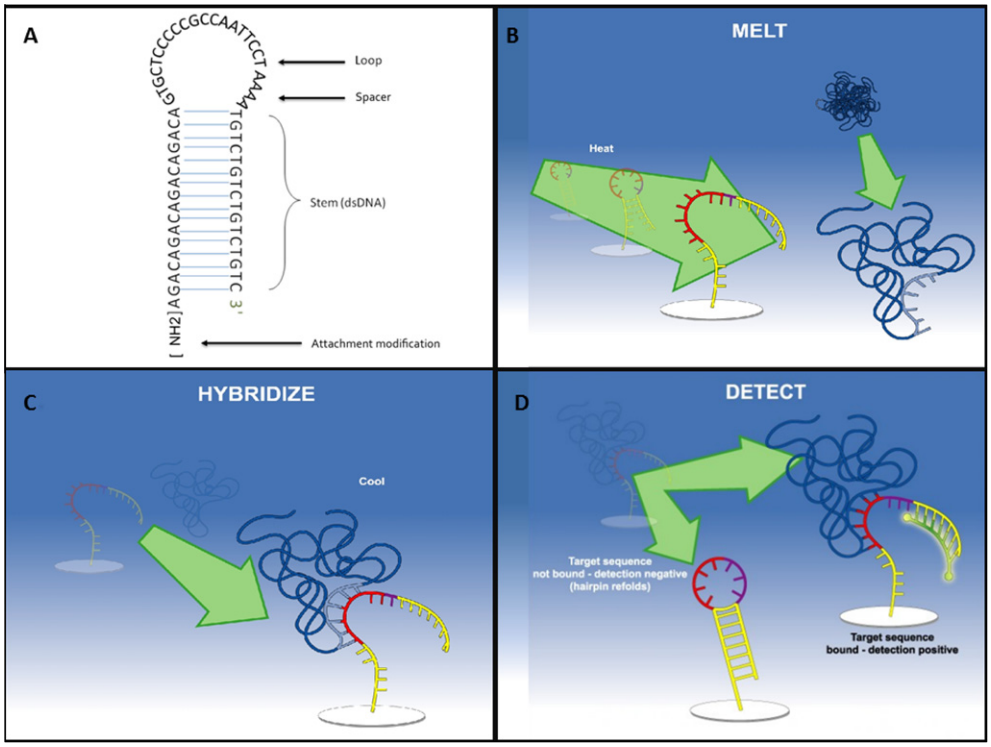

Fig.1. Schematic depicting the microarray platform used in this study. (A) Stem–loop probe showing a loop region (complementary to a target), two complementary stem sequences, a spacer, and a chemical functionality for surface attachment. (B) Denaturation of the SLP using heat in the presence of a sample that may have target nucleic acids leads to an open hairpin orientation for the SLP. (C) Hybridization of the open hairpin with its target DNA. (D) The hybridized probe maintains its open hairpin orientation when subjected to renaturation conditions, thereby allowing the detector to bind with the free stem tail region (unattached stem region). All unhybridized probes return to the closed hairpin orientation on renaturation, preventing the detector from binding to the stem tail region.

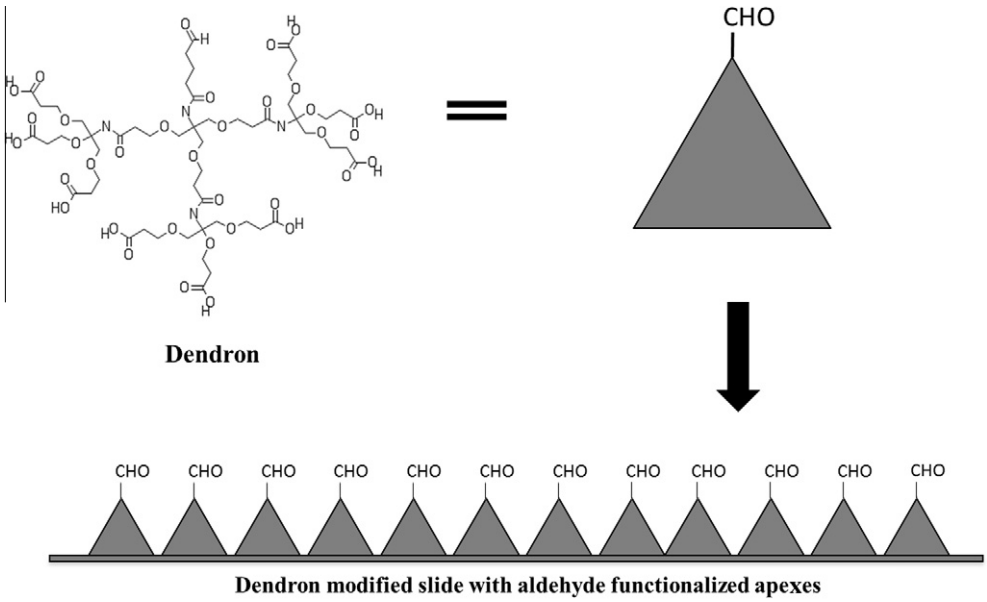

Fig.2. Illustration of the dendron molecule functionalized with an aldehyde group and its incorporation onto the surface of a glass slide.

Scientific (Pittsburgh, PA, USA) as reagent grade. These included saline–sodium citrate (SSC), sodium dodecyl sulfate (SDS), bovine serum albumin (BSA), ethanol (EtOH), sodium borohydride ($NaBH_4$), NaCl, NaH_2PO_4, ethylenediaminetetraacetic acid (EDTA), Na_2HPO_4, HCl, and NaOH. Glycidoxysilanated glass slides with epoxide functionalization (2.5 × 7.5 cm) were purchased from Corning (Lowell, MA, USA), SuperAldehyde silylated glass slides (2.5 × 7.5 cm) were purchased from ArrayIt (Sunnyvale, CA, USA), and aldehyde-functionalized dendron-modified NanoSurface Biosciences (NSB) glass substrates were purchased from NanoSurface Biosciences POSTECH (Pohang, South Korea). Oligonucleotides were purchased from Integrated DNA Technologies (Coralville, IA, USA). Ultrapure water (18 MΩ/cm) from a Millipore purification system was used where needed.