Dendritic Nucleic Acid Probes for DNA Biosensors

Joseph Wang* and Mian Jiang

Department of Chemistry and Biochemistry
New Mexico State University
Las Cruces, New Mexico 88003

Thor W. Nilsen and Robert C. Getts

Polyprobe Inc., 4170 City Avenue
Philadelphia, Pennsylvania 19131

Received February 24, 1998

DNA hybridization biosensors hold great promise for the rapid diagnosis of genetic and pathogenic diseases.[1−3] Such devices commonly rely on the immobilization of a single-stranded oligonucleotide probe that selectively recognizes its complementary target sequence through hybridization. To date, most activity has focused on the development of different transduction modes, with little attention given to the DNA recognition process. In this paper, we report on the advantages accrued from the use of macromolecular nucleic acid structures such as DNA dendrimers[4−6] or branched DNA[7] as the recognition elements in DNA biosensors. For this purpose we used DNA dendrimers which are highly branched structures possessing multiple single-stranded arms capable of hybridizing with a complementary nucleic acid sequence.[5] The immobilization of these hyperbranched spherical structures onto physical transducers greatly increases the hybridization capacity of the surface and, hence, leads to enhanced sensitivity and extended linearity of DNA biosensors.

DNA dendrimers are assembled via sequential hybridization of designed DNA components, with a controlled exponential growth with each successive generation.[5,8] To our knowledge, no previous attempts have been made to use nucleic acid dendrimers for the creation of DNA biosensors. Branched (comb- and fork-) or dendritic DNA structures, containing multiple hybridization sites, have been used previously for enhancing the sensitivity of solution-phase nucleic acid blot assays.[7,8] The former are restricted by the chemistry of the DNA synthesis to fewer hybridization sites when compared to three-dimensional DNA dendrimers.

In the present work, we employed mass-sensitive piezoelectric transducers,[9] with surface-confined dendrimers, to demonstrate the increased hybridization capacity and detection capability and to monitor directly (without an indicator) the kinetics of hybridization. For this purpose we used adsorption and electropolymeric (polyphenol) entrapment for immobilizing the 4-generation (G4) dendrimer structures, possessing about 30 single-stranded arms specific to the waterborne pathogen *Cryptosporidium parvum*,[5,10,11] onto the quartz-crystal microbalance (QCM). The immobilized dendrimer was physically stable on the crystal, with the numerous probes on the outermost layer accessible to the *Cryptosporidium* DNA target, to yield three-dimensional surface hybridization (Figure 1) and, consequently, a large resonant-frequency response. The amplification properties displayed by solution-phase dendrimers[8] have thus been extrapolated onto the transducer surface.

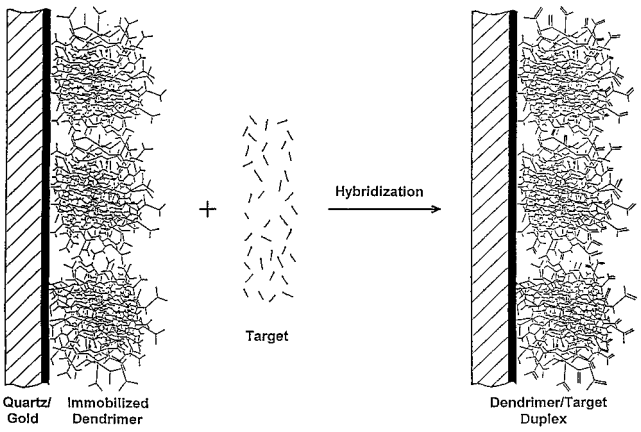

Figure 1. Schematic drawing showing the hybridization detection at the dendrimer/QCM biosensor. For simplicity, the drawing is not to scale and only a monolayer coverage is illustrated. The 38-mer probe is attached to the core dendrimer by complementary oligonucleotide (a(−)) binding on one (a(+)) of the outer arms. The probe sequence for target hybridization is 5′-GGG GAT CGA AGA CGA TCA GAT ACC GTC GTA GTC TTA AC-3′.

Such a gain in the hybridization signal, compared to that of the common use of oligonucleotide probes, is illustrated in Figure 2. The dendrimer/QCM biosensor responds rapidly to changes in the target concentration over the 5−30 (A) and 25−200 (B) μg/mL range. The fast attainment of steady-state signals (ca. 90 s) indicates that the adsorptive surface immobilization does not compromise the favorable hybridization kinetics. The response increases linearly with the target concentration up to 100 μg/mL, with a leveling off thereafter. Statistical treatment yields a sensitivity of 1.04 Hz·mL/μg and a detection limit around 1 μg/mL. Similar results were obtained upon attaching the dendrimers during the electropolymeric growth of polyphenol (not shown). Significantly lower sensitivity (0.11 Hz·mL/μg) and a higher detection limit (8 μg/mL) have been estimated from the response of the conventional oligonucleotide/QCM biosensor. The latter also displays deviation from linearity above 25 μg/mL and a similar response time. The higher sensitivity and wider linear range of the dendrimer-based biosensor are attributed to its substantially higher hybridization capacity. No response was

(1) Xu, X. H.; Bard, A. J. *J. Am. Chem. Soc.* **1995**, *117*, 2627−2631.
(2) Mikkelsen, S. R. *Electroanalysis* **1996**, *8*, 15−19.
(3) Peterlinz, K.; Georgiadis, R.; Herne, T.; Tarlov, M. *J. Am. Chem. Soc.* **1997**, 119, 3401−3402.
(4) Hudson, R. H. E.; Damha, M. J. *J. Am. Chem. Soc.* **1993**, *115*, 2119−2124.
(5) Nilsen, T. W.; Grayzel, J.; Prensky, W. *J. Theor. Biol.* **1997**, *187*, 273−284.
(6) Service, R. F. *Science* **1995**, *267*, 458−459.
(7) Horn, T.; Urdea, M. S. *Nucl. Acids Res.* **1989**, *17*, 6959−6967.
(8) (a) Nilsen, T.; Vogelbacker, H. H.; Getts, R. C.; Tian, N.; Grayzel, J. *Clin. Chem.* **1996**, *42*, S202. (b) Vogelbacker, H. H.; Getts, R. C.; Tian, N.; Labaczewski, R.; Nielsen, T. *Proc. Am. Chem. Soc. Div. Polym. Mater.* **1997**, *76*, 458−460.
(9) (a) Okahata, Y.; Matsunobo, Y.; Ijiro, K.; Murakami, A.; Makino, K. *J. Am. Chem. Soc.* **1992**, *114*, 8299−8300. (b) Caruso, F.; Rodda, E.; Furlong, D. N.; Niikura, K.; Okahata, Y. *Anal. Chem.* **1997**, *69*, 2043−2049. (c) Su, H.; Chong, S.; Thompson, M. *Langmuir* **1996**, *12*, 2247−2255.

(10) The DNA dendrimers used in this work were prepared in a "four-layer" (G4) assembly process, using seven oligonucleotides strands, in accordance with refs 5 and 8b. The term "layer" (generation) refers to each sequential growth of the dendrimer structure in the pairwise hybridization process. (Up to 12-generation dendrimers, possessing as many as 2 million arms at their outer surface, can thus be assembled.) Trioxsalen was used to cross-link the dendritic structure at each generation of assembly. A 68-base long oligonucleotide, containing 38-mer nucleotides complementary to the *Cryptosporidium* DNA target and 30-mer nucleotides complementary to the c(+) arm of the G4 dendrimer, was hybridized and covalently cross-linked with 4,5′,8-trimethylpsoralen (trioxsalen) to the outer surface of the **G4** dendrimer. Additional details of the dendrimer synthesis are included in the Supporting Information.
(11) Wang, J.; Rivas, G.; Parrado, C.; Cai, X.; Flair, M. *Talanta* **1997**, *44*, 2003−2010.

S0002-7863(98)00619-2 CCC: $15.00
Published on Web 07/30/1998

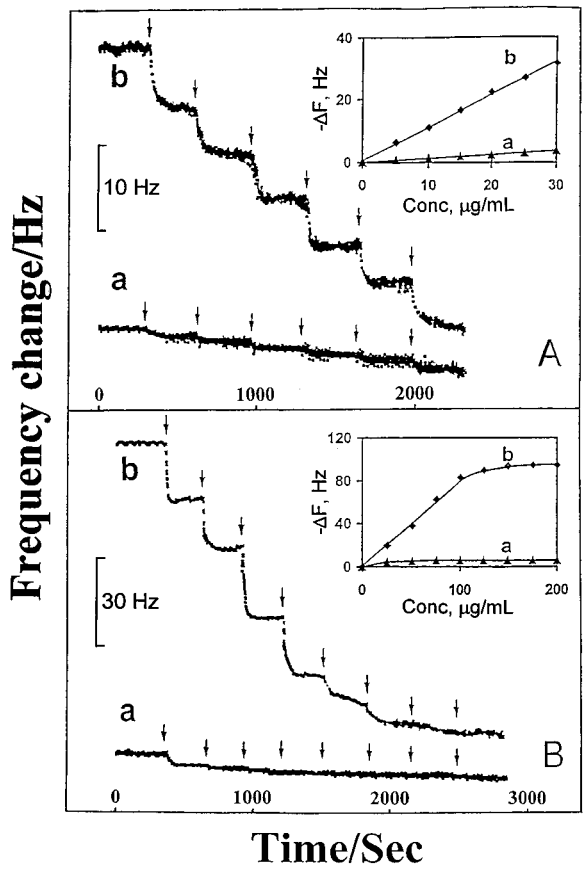

Figure 2. Frequency-time response of conventional (a) and dendritic (b) QCM DNA biosensors to multiple additions of 5 (A) and 25 (B) μg/mL of the 38-mer DNA target. Target sequence, 5′-GTT AAG ACT ACG ACG GTA TCT GAT CGT CTT CGA TCC CC-3′. Hybridization medium was a stirred Tris-HCl buffer (pH 7.0, 1.0 M) containing 0.1 M NaCl (25 °C). Adsorptive immobilization of the dendrimers was accomplished by casting 10 μL of the dendrimer solution (820 μg/mL in 50 mM Tris-HCl/1 mM EDTA, pH 8.0) onto the gold surface on the quartz wafer and allowing the solvent to evaporate for 2 h at 40 °C. The conventional biosensor was prepared by self-assembly of a 38-mer thiolated probe (sequence as in Figure 1; product of IDT Co., Coralville, IA) from its 1000 μg/mL solution (50 mM NaCl/5 mM phosphate buffer, pH 7.0), followed by 48 h incubation under argon atmosphere. The AT-cut gold-coated quartz crystals (with 41-mm^2 gold area on each side and a 5-MHz fundamental frequency) were received from ICM Co. (Oklahoma City, OK). Frequency changes were monitored with a model PM-740 Maxtek monitor (Torrance, CA) in connection with a Universal Sensors (Metaire, LA) model 1120 piezocell operated in the batch mode.

observed for similar target additions using the uncoated crystal (not shown). The high sensitivity is coupled with good selectivity and elimination of nonspecific adsorption effects. A large excess of various noncomplementary oligomers had a negligible effect upon the response of the DNA target (see the Supporting Information). The absence of nonspecific effects is attributed to the dense and hydrophilic dendrimer surface layer.

The 10-fold sensitivity gain is lower compared to that which is expected for the numerous oligonucleotide arms, indicating that not all of the dendrimer binding sites contribute to the biosensor response. Apparently, the surface confinement of the dendrimer probes leads to a steric hindrance and lower accessibility compared to that of blot protocols. Further improvements in the sensitivity are expected upon using dendrimers containing more single-stranded arms and in connection with higher resonant- frequency QCM.

The dendrimer surface coverage (0.278 pmol/cm^2), estimated from the 126-Hz frequency change (in air) following the adsorptive immobilization, corresponds to an 18-layer packing, on the basis of the 120 nm diameter and 8×10^6 dalton of the G4 dendrimers. Profilometric measurements (with a stylus force of 5 mg, scan length of 1000 μm ,and scan rate of 0.5 μm/s) yielded an average film thickness of 1.08 μm, which corresponds to a 9-layer dendrimer packing. Such discrepancy between the mass and film-thickness measurements is attributed to the entanglement/overlap of neighboring dendrimer molecules. Possible flattening upon adsorption can also not be ruled out. Such adsorptive immobilization is also reproducible, as was indicated from the 18% relative standard deviation of the response to the 10 μg/mL target sequence at 6 different crystals. In view of the relatively thick and nonrigid dendrimer layer (and related effects of interfacial liquid properties and film viscoelasticity), the Sauerbrey equation cannot be simply applied, and hence, quantitation should rely on calibration experiments. Hybridization reversibility was not investigated since the thermal melting may also effect the QCM resonant frequency.[9b]

While the advantages of the dendrimer surface probes have been presented in connection with piezoelectric biosensors, other (e.g., optical, electrochemical) transduction modes should benefit from their unique properties. Such use of surface-confined branched DNA structures opens up new opportunities for DNA hybridization biosensors and for DNA diagnostics in general.

Acknowledgment. This work was supported by the NM Water Resources Research Institute.

Supporting Information Available: Experimental details of dendrimer synthesis and additional data (5 pages print/PDF). See any current masthead page for ordering information and Web access instructions.

JA980619P