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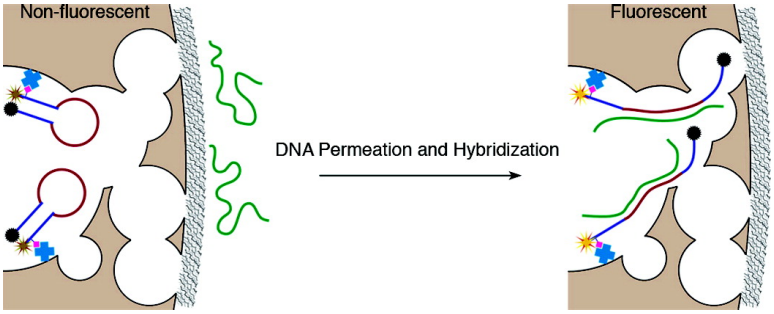

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A Molecular Beacon Approach to Measuring the DNA Permeability of Thin Films

Angus P. R. Johnston and Frank Caruso*

Centre for Nanoscience and Nanotechnology, Department of Chemical and Biomolecular Engineering, The University of Melbourne, Victoria 3010, Australia

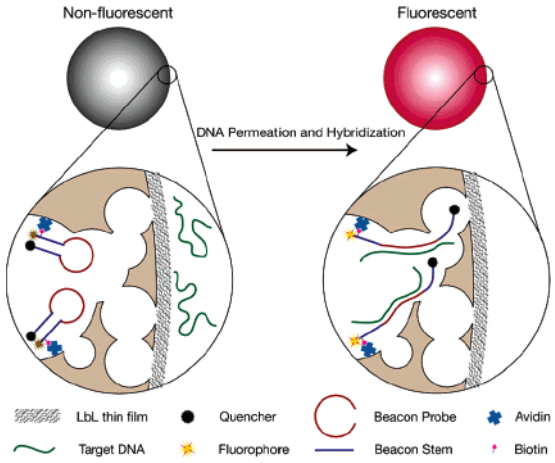
Received April 26, 2005; E-mail: fcaruso@unimelb.edu.au

The controlled release of biomolecules is of great importance for gene therapy[1] and targeted drug delivery.[2] Delivery and in vivo sensing systems must be designed so that specific molecules can be sequestered and/or released. The capability to sense and deliver nucleic acids is controlled largely by the ability to manipulate the permeation of the molecules through a thin film or membrane. Accurately determining the permeability of thin films presents a number of challenges, as low concentrations of the permeating species must be detected; however, labeling alters the permeability of the molecules. Herein, we report a new method for determining DNA permeability of thin films using molecular beacons (MBs). This method is unique because it allows high throughput detection of very low numbers of permeated molecules (sub-attomolar) and does not require labeling of the permeating molecule.

Polyelectrolyte (PE) multilayers, either as planar films or hollow capsules, have significant potential in delivery and sensing, and represent versatile systems for permeability studies.[3] Permeation of high-molecular weight molecules through PE multilayers has been studied previously using dialysis[4] and confocal laser scanning microscopy.[5] However, these methods either require high concentrations of the permeating molecules or labeling. To our knowledge, there are no techniques that can rapidly and accurately determine the permeability of large biomolecules or polymers through thin films.

Label-free permeability measurements were achieved by immobilizing a MB[6] inside bimodal mesoporous silica (BMS) particles (pore sizes of 2−3 nm and 10−40 nm)[7] and then encapsulating the MB/BMS particles within a PE multilayer film (Scheme 1). The biotin-functionalized MB was immobilized inside the BMS particles by first modifying the silica surface with avidin. Binding of fluorescently labeled biotin to the avidin/BMS particles showed uniform fluorescence throughout the particle, as observed by fluorescence microscopy (see Supporting Information). This indicated that the avidin was distributed evenly throughout the particle, with no obvious ring fluorescence. This is in accordance with our previous work with BMS particles and other proteins.[8] Attachment of the biotin-functionalized MB to the avidin/BMS particles resulted in minimal fluorescence (data not shown), indicating that the MBs are in their closed state and that immobilization within the BMS did not affect the formation of the stem−loop structure. The MB/BMS particles were encapsulated within a PE multilayer film assembled using the layer-by-layer (LbL) technique,[9] which involves the sequential assembly of oppositely charged PEs onto the particles.[10] The film [polyethyleneimine (PEI) and four poly(styrenesulfonate) (PSS)/poly(allylamine hydrochloride) (PAH) bilayers], which is about 15 nm thick,[11] was deposited on the surface of the particles.[12] To determine the permeability of the film, the encapsulated MB/BMS particles were incubated with varying lengths of permeating molecules (different-length oligonucleotides), and permeation through the PE wall was detected by the increase in fluorescence intensity of the particles (Scheme 1). The fluorescence intensity of individual particles was measured using flow cytometry. The rapid nature of flow cytometry (analysis of >20 000 particles per second) means that a large, statistically significant population of particles can be analyzed quickly.

Scheme 1. Biotin-Functionalized MBs Immobilized inside Avidin-Modified BMS Particles and Encapsulated within a PEI/$(PSS/PAH)_4$ Film



[a] A MB is a single-stranded (ss) DNA molecule that forms a stem−loop structure, with a fluorophore and quencher at opposite ends of the DNA molecule. The stem regions hybridize, bringing the fluorophore and quencher close together, quenching the fluorescence. If a target DNA sequence complementary to the loop is present, the target hybridizes to the loop, opening the MB and resulting in a fluorescent signal.

Four target sequences (15, 25, 35, and 60 bases (b) long) were designed so the middle of each target was complementary to the MB loop (see Supporting Information). Solution studies of the MB (Figure 1) showed that the 15b target had a lower fluorescence signal than the longer targets. This is attributed to the longer targets being more effective at keeping the fluorophore and quencher spaced from each other. A similar but less pronounced trend was observed with the MB immobilized on the BMS particles. The fluorescence signal for the 15b target was significantly higher than that in bulk solution. This is likely due to the decrease in melting temperature (T_m)[13] of the MB stem, caused by immobilization onto the particles, thus making it easier for the target to open the MB. The small decrease in signal from 35b to 60b for the immobilized probe is likely due to slower permeation of the 60b target through the BMS particles. Both of these particles showed homogeneous fluorescence (Figure 2), confirming that the target was able to permeate through the BMS. After 500 h incubation, the fluorescence signal from the 60b and 35b targets were comparable (Figure 3a), indicating complete permeation of the 60b target into the MB/BMS particles.

Assembly of the PEI/$(PSS/PAH)_4$ film on the MB/BMS particles resulted in a marked decrease in the number of MBs opened by the DNA targets (Figure 3b). After 3 h, no 35b or 60b targets were detected inside the "capsules", and a limited signal was detected from the 15b and 25b targets (31% and 4% of beacons opened,

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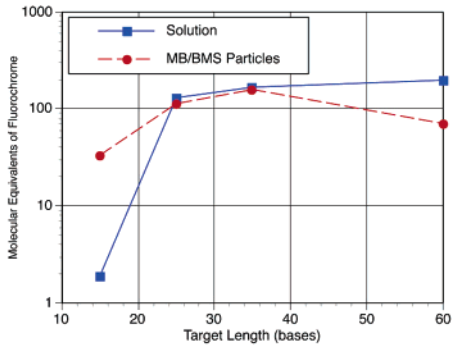

Figure 1. Comparison of fluorescence signal from the MB in solution and the MB immobilized on the BMS particles as a function of DNA target length. Incubation time = 3 h. MB sequence = *TGCTCG***TCCATCT-CATTCAGC***CGAGCA*.

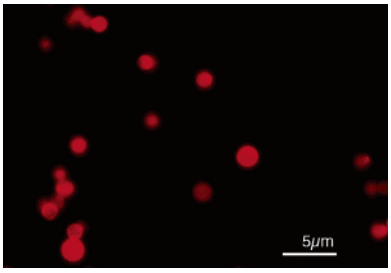

Figure 2. Fluorescence microscopy image of MB/BMS particles after exposure to a 60b DNA target for 3 h.

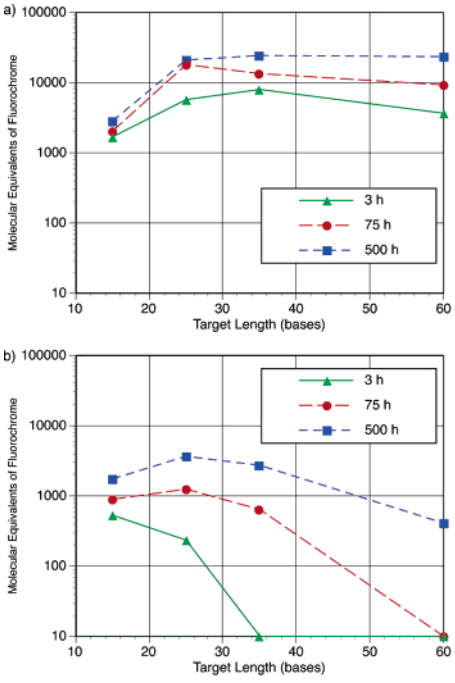

Figure 3. Flow cytometry data of MB/BMS particles exposed to different-length DNA targets. (a) MB/BMS particles with no film, (b) MB/BMS particles encapsulated within a PEI(PSS/PAH)$_4$ film. The limit of detection for the flow cytometer is ~100 dye molecules. 600 000 mol equiv of fluorochrome corresponds to 1 attomole of MB.

respectively). After 75 h, there was still no 60b target detected inside the capsules, but 5% of the MBs were opened by the 35b target. After 500 h, all four targets were detected inside the capsules. This shows that DNA can slowly permeate through the PEI/(PSS/PAH)$_4$ multilayers and, as would be expected, the shorter DNA sequences can permeate through the layers faster. The permeation of large molecules through the film is probably facilitated by conformational changes in the permeating molecule and by rearrangement of the PE molecules within the film.

The permeability of the films is related to the size of the permeating DNA. ssDNA is highly flexible (persistence length = 3 nm) and for the size used in this study will adopt a random coil conformation. Using the Kratky−Porod equation[14] (see Supporting Information) to calculate the radius of gyration (R_g) of the DNA targets, over a period of 3 h DNA molecules larger than 4.7 nm in diameter did not permeate the PEI/(PSS/PAH)$_4$ film, and over 75 h DNA molecules larger than 6 nm in diameter were excluded. Other work has shown that even when the pore size is smaller than the R_g of the molecule, DNA permeation still occurs.[15] This makes the direct comparison of permeation of large flexible PEs to smaller rigid molecules difficult.

We have demonstrated that MBs can be used to probe the permeability of thin films. This technique provides a convenient and rapid approach to measure the permeability of DNA sequences through polymer films without labeling the permeating molecule and is applicable to a wide variety of film types and assembly methods. The size of the DNA permeable to the film can be easily determined by employing a number of different-length DNA targets. This information can be used in the synthesis of smart films with controlled permeability. We are currently using this method to determine the permeability of a variety of LbL films, including DNA[16] and nanoblended porous films.[17] While this technique is limited to molecules for which MBs can be constructed, it is broadly applicable to studying the permeability of DNA and its analogues. For example, DNA conjugates (e.g., DNA−nanoparticles) and nucleotide analogues (such as peptide nucleic acids (PNA) and intercalating nucleic acids (INA)) can also be investigated. This technique is likely to find applications in determining the permeability of LbL systems such as hollow PE capsules, where control over the trafficking of specific sized molecules through the film is required, for example in gene delivery and gene interference using PNA and INA.[18]

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Supporting Information Available: Experimental details and DNA size calculations. This material is available free of charge via the Internet at http://pubs.acs.org.

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