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Single DNA molecules as probes for interrogating silica surfaces after various chemical treatments

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ABSTRACT

We examined the adsorption of single YOYO-1-labeled λ-DNA molecules at glass surfaces after treatment with various chemical cleaning methods by using total internal reflection fluorescence microscopy (TIRFM). The characteristics of these surfaces were further assessed using contact angle (CA) measurements and atomic force microscopy (AFM). By recording the real-time dynamic motion of DNA molecules at the liquid/solid interface, subtle differences in adsorption affinities were revealed. The results indicate that the driving force for adsorption of DNA molecules on glass surfaces is mainly hydrophobic interaction. We also found that surface topography plays a role in the adsorption dynamics.

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1. Introduction

Single-molecule detection (SMD) techniques have gained plenty of attention in the field of life science [1] because it can reveal detailed kinetics of physical processes and chemical reactions that are often hidden in ensemble measurements [2]. Total internal reflection fluorescence microscopy (TIRFM) has played an important role in this field [3,4] because excitation by the evanescent field suppresses fluorescence background from the bulk solution and allows one to observe single-molecule fluorescence at solid/liquid interfaces with high signal-to-noise ratios. The observation and manipulation of single biomolecules allow their dynamic behaviors to be recorded to provide insights into molecular genetics [5–7], biochip assembly [8–10], biosensor design [11,12], biophysics [13–21] and basic separation theories of liquid chromatography and capillary electrophoresis [22–29].

Glass is the most widely used substrate for immobilization of biomolecules in optical microscopy, DNA arrays, and biosensor assays [30–32]. A biomolecular receptor can be specifically immobilized on the glass-based substrate to facilitate the measurement of the interaction between the receptor and its ligand. The properties of such surfaces are particularly important since they affect the density, uniformity, and reproducibility of sensing films through the adsorption of analyte molecules onto the active area of the biosensor. Typically the surface must be cleaned to remove contamination, to create surface attachment sites, and to control surface roughness. In the literature, a wide variety of cleaning methods have been described [33–37], including gas plasmas and combinations of

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acids, bases, and organic solvents that are allowed to react at different temperatures and for different times. Most of these procedures are thought to be “ideal” as the authors believe that the cleaning method does not change the topology of the surfaces, that contaminations are completely removed and that the surfaces are activated to expose a high density of hydroxyl groups to the solution. Contact angle (CA) measurements, atomic force microscopy (AFM), X-ray photoelectron spectroscopy (XPS), imaging ellipsometry (IE) or Fourier transform infrared spectroscopy (FT-IR) [38,39] are generally used to study the effects of surface treatment.

Single-molecule spectroscopy is uniquely suited to probe spatial and temporal heterogeneity of a system. In the present study, the interaction between single λ-DNA molecules labeled with YOYO-1 in aqueous solution and cover-glass surfaces treated with five different chemical cleaning methods was studied using objective-type TIRFM. The adsorption and motion of individual DNA molecules were analyzed as a function of buffer pH and surface characteristics. Such interactions can be used to model biomolecule adsorption onto the sensor surface. We also identified the driving force for adsorption of individual DNA molecules. These five surfaces were also characterized using CA and AFM and the results were compared with TIRFM measurements.

2. Experimental

2.1. *Reagents*

Concentrated sulfuric acid (98%), hydrogen peroxide, ethanol, sodium hydroxide, potassium dichromate, acetic acid, sodium acetate, and sodium chloride or higher glacial acetic acid, sodium acetate, and sodium chloride were obtained from Sinopharm Chemical Reagent, China. Sodium dodecyl sulfate (SDS) was purchased from Sigma (St. Louis, MO, USA). All solutions were prepared with ultrapure water (18-MΩ).

Buffer solutions (pH 3.97–7.47) were prepared from 1.0 M solutions of acetic acid, sodium acetate, and sodium chloride or higher glacial acetic acid, sodium acetate, and sodium chloride dissolved in ultrapure water. Unless specified, the final mass balance of acetate was 25 mM.

2.2. *DNA sample preparation*

λ-DNA (48 502 bp) was obtained from Promega (Madison, WI, USA). All DNA samples were prepared in 10 mM Gly–Gly (Sigma) buffer, pH 8.2. DNA samples were labeled with YOYO-1 (Molecular Probes, Eugene, OR, USA) at a ratio of one dye molecule per five base pairs. DNA stock solutions were prepared at a concentration of 200 pM. For single-molecule imaging, these solutions were further diluted to 10 pM with appropriate buffer solutions prior to the start of the experiment.

2.3. *Cleaning of cover glass*

The glass substrates used in this study were standard microscope cover glass purchased from Erie Scientific (Portsmouth, NH, USA). The chemical composition of the cover glass obtained from the manufacturer is: 72.15% silicon dioxide, 14.25% sodium oxide, 6.25% calcium oxide, 4.1% magnesium oxide, 1.12% aluminum oxide, 1.15% potassium oxide, 0.3% sulfur trioxide, 0.3% iron oxide, and 0.05% titanium oxide. The cover glasses were manufactured as drawn (versus float) glass. Therefore, both faces of the cover glass possess identical chemical composition and surface characteristics. The detailed procedures of the five cleaning methods (ethanol, SDS, Piranha, $K_2Cr_2O_7 + H_2SO_4$ and NaOH) are listed in Table 1.

Table 1 – Chemical cleaning methods employed in this study

Method	Steps
1	Ultrasonic bath for 30 min in DI H_2O, 95% ethanol, dry
2	1 h in 0.077 mol/L SDS, rinse in H_2O, ultrasonic bath for 30 min in DI H_2O, 95% ethanol, dry
3	1 h in piranha, rinse in H_2O, ultrasonic bath for 30 min in DI H_2O, 95% ethanol, dry
4	1 h in $K_2Cr_2O_7 + H_2SO_4$, rinse in H_2O, ultrasonic bath for 30 min in DI H_2O, 95% ethanol, dry
5	1 h in 0.1 M NaOH, rinse in H_2O, ultrasonic bath for 30 min in DI H_2O, 95% ethanol, dry

2.4. *Single-molecule detection (SMD) measurements*

Each 5 μL of sample solution was sandwiched between a microscope cover glass and another microscope cover glass. A 488-nm argon ion laser (ILT5500, Aiao Laser Machinery, Shanghai) was used as the excitation source. SMD was performed using a Nikon ECLIPSE TE2000-U inverted microscope in TIRF mode. The microscope objective was a Nikon 60× PlanApo/TIRFM (oil 1.45 NA). The objective was coupled to the microscope cover glass with immersion oil (type A, $n = 1.515$, Nikon, Japan). In TIRFM, the laser beam was focused and coupled into the microscope using a single-mode optical fiber. Total internal reflection was achieved by translating the laser position adjustment knob, which in turn moved the position of the incoming laser beam to the edge of the objective until total internal reflection was observed at the glass/solution interface. Images of the irradiated region through the objective were recorded on a CoolSNAP HQ2 CCD camera (Roper scientific Inc.). The full size of the CCD was used (1392 × 1040) without binning (6.45 μm × 6.45 μm). Images were acquired by RS Image software (Roper scientific Inc.). The CCD exposure time was 100 ms throughout. All frames were analyzed off-line using Image J (1.37v, NIH).

In order to ensure reliability of the experimental results, cover glasses are treated with five different chemical cleaning methods at the same time, and the time of each SMD measurement were kept same. Firstly, cover glasses were placed into different beakers and treated with different solutions simultaneously. Prior to SMD measurements, the cover glasses were dried. Secondly, experimental reproducibility was examined using two different approaches. One approach is that cover glass treated by the first cleaning method was tested at the pH range of 8.2–3.97, and then cover glass treated by the second cleaning method was tested at the pH range of 8.2–3.97, etc. The other approach is that cover glasses treated by five different methods were first tested at pH 8.2, and then tested

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