Hydration Layer Scanning Tunneling Microscopy of "G-wire" DNA

Tamieka Armstrong\textsuperscript{a}, Jeffrey Root\textsuperscript{b}, and James Vesenka\textsuperscript{a}

\textsuperscript{a}University of New England
11 Hills Beach Road
Biddeford, ME  04005
Email: jvesenka@une.edu

\textsuperscript{b}California State University Fresno
2345 E. San Ramon, MS# MH37
Department of Physics,
Fresno, CA  93740-0037

Abstract. Hydration Layer Scanning Tunneling Microscopy (HLSTM) of quadruplex ("G-wire") DNA on mica was carried out under controlled humidity conditions. The G-wires showed remarkable similarity with atomic force microscope images of the same DNA in air, i.e. increased lateral width due to tip broadening but with diameters similar to those measured by x-ray techniques. The G-wire height above the mica substrate and width appeared to decrease slightly with increasing humidity. Though much of the lateral broadening is likely a result of residual buffer salts and the lower resolving ability of HLSTM, the dependence of the DNA height and width on humidity suggests a simple explanation in terms of the hydration layer. An estimate of the increased thickness of the hydration layer of up to 0.6 nm was observed.

INTRODUCTION

G-DNA is a polymorphic family of four-stranded structures containing guanine tetrads motifs (G-quartets) \cite{1,2}. Guanine rich oligonucleotides that are self-complimentary, as found in many telomeric (chromosome ends) repeat sequences, form G-DNA in the presence of monovalent and/or divalent metal cations. The atomic force microscope (AFM) and hydration layer scanning tunneling microscope (HLSTM) are high resolution, near-field, three dimensional imaging devices that were used to explore the structure of linear G-4 polymers at different humidities. These “G-wires” \cite{3} are speculated to form by the self-assembly of the telomeric oligonucleotide sequence d(GGGGTTGGGG) also called d"Tet1.5" monomers (Fig. 1). HLSTM images \cite{5} have suggested that the G-wires are semiconductors \cite{4}. The mechanism of conductivity may be the result of base stacking of G-quartets and caged monovalent cations (Fig. 1). G-wires are well known for their stability when adsorbed to the surface of mica \cite{3,6}. The potential for conduction, stability, uniformity and long lengths make G-wire DNA interesting candidates for molecular wiring \cite{7,8,9}. HLSTM imaging is a sensitive function of both the relative humidity and ionic concentration of the hydration layer (Fig. 2). This study describes a model explaining features of HLSTM images and estimates the thickness of the hydration layer.
The stability of the G-wire DNA is better understood through examination of the hypothesized growth mechanism. The Tet1.5 monomer can form a dimer pair with either a “closed”, “looped”, or “staggered” conformations as shown in Fig. 1a. In either of the closed or looped conformations no more growth of the G-wires can occur. In the staggered conformation another dimer can attach to the G-wire ladder creating a succession of “sticky ends”, enabling multimers to assemble. The process is driven thermodynamically [9]. Monomeric cation species such as potassium or sodium are thought to help stabilize the G-wires down the base-stacked core of the structure as seen in Fig. 1b [3,7]. The thymine groups may act as flexible links that can compress or stretch in solution or after adsorption onto a substrate.

**MATERIALS AND METHODS**

Quadruplex G-wire DNA was prepared according to the procedure outlined by Marsh et al. [3]. Melting of the G4T2G4 monomers (Tet1.5) was maintained with a PCR Thermocycler (Thermo Hybaid, U.K.), i.e. the growth cocktail and Tet1.5 were is raised to 95°C for ten minutes to promote the melting of fortuitous G-4 structures, i.e. to ensure a monomeric concentration of G-wires. Samples of concentrated G-wires (monomer concentration 1.0 mM) were diluted and incubated for five minutes either directly on freshly cleaved muscovite mica, or on parafilm for ten minutes followed by direct adsorption onto mica at room temperature in a buffer consisting of 10 mM Tris (pH 7.6) and 1 mM MgCl2. The samples were then rinsed with 1 ml de-ionized water to remove excess buffer salts. Freshly prepared G-wire DNA was imaged with a PicoSPM (Molecular Imaging, Tempe AZ) low current STM under controlled humidity with a Digital Instruments (Santa Barbara CA) Nanoscope E controller. G-wires were imaged at RH ≈ 75-85%, bias voltages of -5 to -10 V, and tunneling current of ≈ 1-3.0 picoamperes. A Nanoscope IIIa controller and Multimode AFM operated in Tapping Mode was used to image freshly prepared and dessicated samples.
RESULTS AND DISCUSSION

Fig. 3 is an example of a G-wire network that was created by depositing a sample containing a concentrated 24 hour-old G-wire solution onto freshly cleaved mica. The sample is rinsed and immediately imaged in TappingMode™ revealing an oriented network of G-wire strands over the surface of mica in Fig. 3a. The orientation affect is due to G-wire alignment with potassium vacancy sites on the surface of freshly cleaved mica [9]. The density of the G-wire DNA appears to depend upon local variations of the mica surface. For example, imaging a region of the mica surface a millimeter away may provide a distribution of G-wires similar to those seen in Fig. 3c in which the G-wires are clearly separated. In Fig. 3b the same sample from Fig. 3a had been imaged after drying for 24 hours. Note that the G-wire DNA appears much narrower (all three images have the same scale bar) because of the dessication of the hydration layer over the surface. The hydration layer is absolutely essential for imaging of the molecules via HLSTM as seen in Fig. 3c [5].

FIGURE 3. G-wires freshly adsorbed onto mica imaged via tapping mode (Fig. 3a) and the same sample imaged by the same tip 24 hours later after drying in an oven at 37°C (Fig. 3b). After drying in an oven the G-wires appear much narrower and the buffer salts appear to distribute themselves in between the DNA strands. It is the freshly made, hydrated form of the G-wires that is essential for HLSTM imaging (Fig. 3c), recorded at 1pA tunneling current, -7V bias and 80% relative humidity in a sealed imaging chamber. Successful HLSTM images of G-wires occur most frequently on freshly prepared samples at high G-wire densities, but NOT G-wire “networks” as seen in Figs. 3a and b. Vertical height range is 10 nm from black to white.
**FIGURE 4.** Freshly made G-wires imaged by HLSTM at 3pA tunneling current, -7V bias and four different relative humidities in a sealed chamber with different Pt-Ir tips. N.B. the apparent decrease in width as the humidity increases. Vertical height range is 5 nm from black to white.

**FIGURE 5.** Height and width information plotted as a function of relative humidity. The trend lines decrease in both with increasing humidity, but the exact relationship with relative humidity is not possible to establish because of the large error bars and limited range of the humidity measurements. Unlike the decrease in width the slight reduction in height, about 0.6 nm, as the humidity increases, is within the measurement error and cannot be established as being significant.

Fig. 4 is a panel representing the humidity dependence of G-wire contrast. Most notable is the apparent reduction in width as the humidity of the imaging chamber was increased, even over a very small range. Fig. 5 reflects the quantitative measurements of the images in Fig. 4, clearly indicating decreasing trend lines. The error bars are so large in the average width measurements, and the relative humidity range so small, it is not possible to glean the exact type of relationship between the two, though it appears the reduction in width is significant. Height information from the same samples indicate a decrease of 0.6 nm as the humidity increased, but the measurement error bars are so large that we are unable to determine if this decrease is significant. In our hands the relative humidity range at which images can be intermittently collected is between 65% and 90%, and only stably between 75% and 85%. Crashing tips is a common casualty as the humidity changes, and all these measurements involved different tips. Consequently the trend lines could represent fortuitous tip broadening as HLSTM can involve a slightly different contrast mechanism compared to regular
STM, i.e. through ballistic tunneling [5]. In ballistic tunneling many apical atoms contribute to the tunneling current, reducing the resolution of the image.

The cartoon in Fig. 6 speculates about a possible contrast mechanism that would explain the data in Fig. 5, namely a reduction in height and width of the G-wire DNA as the humidity in the chamber rises. With increased humidity comes greater adsorption of water into the hygroscopic hydration layer on the surface of the sample. If the DNA is firmly anchored onto the surface of the mica, the rising hydration layer would slowly submerge the DNA. The decrease in the height of the DNA is thus reflective of the increase in the thickness of the hydration layer, about 0.6 nm over the range indicated. This thickness is substantially larger than thicknesses measured for water on mica by scanning polarization microscopy [10]. However those results are for pure water without any ions in solution.

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