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DNA Nanotube Ultrastructural Analysis Using Scanning Tunneling

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ABSTRACT

This article describes the possible uses of atomic force microscopy and scanning tunnelling microscopy for characterising DNA nanotubes at the nanoscale. Using M13mp18 DNA as a scaffold and the Cadnano programme, the nanotubes were created. Following ligase treatment, DNA nanotubes were created using the origami approach. The morphology of DNA nanotubes was validated by transmission electron microscopy. In contrast to a scanning tunnelling microscope, an atomic force microscope was employed to characterise the topography of DNA nanotubes. The results of scanning tunnelling microscopy in the constant-current mode showed a high-resolution topography of DNA nanotubes; however, atomic force microscopy in the contact mode explored additional information about the self-assembly in DNA strands in nanotubes (or constant height). Our results indicated that the two for the purpose of creating two- and three-dimensional micrographs of DNA nanotubes, microscopes may be the best tool.

Keywords: Atomic force microscope; DNA nanotube; Scanning tunneling microscope

INTRODUCTION

Simplified processes for DNA nanotube production and characterisation are required for their potential uses in nanotechnology and medicine, notably as intracellular delivery systems. Recently, these designs have been considered as potential peptide delivery systems for accelerating neural stem cell differentiation into neurons [1]. DNA double-stranded helix is used to create the filamentous structures known as DNA nanotubes. DNA nanotubes are characterised by a high aspect ratio, a long and narrow central channel, and DNA-derived sidewalls. Numerous techniques have been developed to create DNA nanotubes since this is a fascinating area of study in DNA nanobiotechnology [2]. Recently, we described a streamlined procedure for creating DNA nanotubes with the least amount of staples possible, assisted by an origami technique and a ligation treatment of sticky-ended DNA nanostructures. The microscopic characterisation of DNA nanotubes can only be done using a few techniques. The transmission electron microscope (TEM) is the most well-known microscope, yet even in topographic mode, the TEM was unable to show further details of these DNA nanostructures [3].

As a result, scanning probe microscopes, especially the atomic force microscope (AFM), have been often used for this. The AFM requires specialised cantilever probes for the characterisation of DNA nanotubes, which are costly and require caution in use [4]. But another scanning probe microscope, the scanning tunnelling microscope (STM), provides additional information about DNA nanostructures in topographic mode. Additionally, the STM may investigate the electrical properties of the nanostructures in part thanks to the electron tunnelling interaction between the sample and surface molecules [5]. Additionally, the price of tips for In comparison to the AFM probes, the STM is smaller. Here, we evaluated how well the AFM and STM characterised the ultrastructure of DNA nanotubes. Additionally, important elements that significantly impact the accuracy of the structural data for DNA nanotubes are introduced.

MATERIALS AND METHODS

Chemicals and Instruments

The C1000 thermal cycler made by Bio-Rad, California, USA was used to set the heat conditions for the self-assembly in the origami reaction. The Philips EM028 TEM, located in Aachen, Germany, was used for transmission electron microscopy. JPK-AFM (JPK Instruments AG, Berlin, Germany) produced the micrographs. Mica was prepared by Tehran We bought phage genome and T4 DNA ligase from New England Biolabs Massachusetts, USA. Sigma-Aldrich Chemie GmbH produced and desalted the desired single-stranded oligonucleotides Munich, Germany [6]. Bio-Rad provided the Quantum Prep Freeze 'N Squeeze DNA gel-extraction spin columns. We bought SYBR Gold nucleic acid gel dye from Molecular Probes.

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Fabrication of DNA Nanotube

Staple sequences for folding and creating DNA nanotubes were chosen using the Cadnano software in honeycomb design. The staples were created based on their complementarities with the specific places of the scaffold sequence for moulding sticky-ended nanostructures, which was the M13mp18 phage genome employed as the scaffolded DNA [7]. The DNA scaffold (M13mp18 single-stranded DNA) was mixed with 100 nM of each staple oligonucleotide in 1 Tris base, acetic acid, and EDTA buffer (40 mM Tris-acetic acid buffer, pH 8.0, and 12.5 mM magnesium acetate). This was done to prepare the origami reaction. The mixtures were then kept at 95 °C for 5 minutes, and then annealed from 95 [8]. The origami products were handled by ligase in order to create DNA nanotubes. It was made the ligation-reaction mixture. Containing 1 L of T4 DNA ligase enzyme, 2 L of 50% polyethylene glycol, 10 L of self-assembled DNA nanotubes, and 2 L of 10 T4 DNA ligase reaction buffer. The ligation mixture was then incubated for 2 hours at 16°C. DNA nanotube size and morphology were determined using the TEM. In order to achieve this, DNA nanotubes that had been gel-extracted using Quantum Prep Freeze 'N Squeeze DNA gel-extraction spin columns were immobilised by 300-mesh Cu syringe spraying on Agar Scientific holey carbon film in Stansted, Essex, the United Kingdom [9].

Atomic Force Microscopy of DNA Nanotubes

Five microliters of the DNA nanotubes that had been isolated from the gel were immobilised for 4 hours at room temperature (25 °C) in order to be dried. Using a JPK Nano Wizard control, the samples were imaged in contact mode with JPK-AFM at 150 Hz IGain, 0.0048 PGain, and 1.0 V set point. The ACTA-10 probe model cantilever (silicon, N-type, 0.01-0.025 /cm) was used. The JPK Nano analyzer programme was used to graphically process rough data [10].

Characterization of DNA Nanotubes

The DNA nanotubes from the gel extraction process were diluted 103-fold in a buffer containing Tris, acetic acid, and EDTA-Mg2+ (pH 8.0). Then, after drying for 3 hours at room temperature, 5 L of the diluted sample was immobilised on the highly ordered pyrolytic graphite (HOPG). With an STM in topographic mode, the samples were scanned using a platinum-iridium tip, 0.1 Na current set point, and 0.2 V sample bias. Line adjust, plain adjust, and average filters of the NAMA-STM Nanoanalyzer software Nanotechnology System Corporation, Tehran, Iran were used to first process rough data [11]. The colouring procedure was then evaluated at various degrees on the produced micrographs.

RESULTS

A picture of DNA nanotubes taken using a TEM. The nanotubes were shaped like filaments. The nanotubes' morphologies proved their efficient production, and their lengths were in the micron range. The produced DNA nanotubes were characterised using the AFM. The created DNA nanotubes' atomic force microscopy clearly showed their tube-like nanostructures. Although the DNA nanotubes' self-assemblies and ligations were effectively confirmed by the presence of these filamentous nanostructures, the ultrastructures of the nanotubes are magnified in the inset. DNA nanotubes using STM micrograph in three dimensions with zoom-in ultrastructures [12]. The two-dimensional micrograph of the nanotubes is seen in the micrograph. The sticky ends of the primary nanotemplates could be successfully connected together amid the elongated DNA nanotubes in this micrograph, which also clearly showed highly organised nanotemplates.

DISCUSSION

Designing DNA nanostructures, which under the correct circumstances self-assemble into discrete nanotools with innovative uses, is one of the many fascinating promises of DNA nanotechnology. The fabrication procedures of the nanotubes are crucial based on their components and designs, and they are also promising nanomaterials for use in applications beyond the nanoscale. The ability to efficiently self-assemble in the liquid phase and modify the side chains to add functionality to the surface of DNA nanotubes is a benefit. This makes it possible to modify their physical and chemical properties for certain medical or other technological uses. Characterization of these novel nanomaterials is difficult, though, because they are delicate and easily damaged by conventional microscopy methods. Here, we sought to present the thorough examination of DNA-nanotube ultrastructures with standard dimensions using the AFM and STM's capabilities. DNA nanotubes' ultrastructural properties provide crucial information regarding the proper shape. Topographic information is provided by nanostructures with more accurate measurements than by electron microscopes. Here, we examine the possibilities of AFM and STM for the characterisation of self-assembled DNA nanotubes, among the plethora of characterization techniques for researching materials with lower dimensions. As, the TEM gave some information on DNA nanotubes in their cross-sectional and longitudinal dimensions, but it couldn't tell us anything specifically about their topologies. As a result, the AFM technique was used to access this information. However, in order for the AFM to characterise objects, it was crucial to take into account a number of parameters, including sample preparation, the right tips, and the ideal compression power. Silicon cantilevers with low spring constants were employed in soft tapping to achieve the desired height profile.

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