Nucleic Acid Organizations Visualized by Scanning Force Microscopy

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Both DNA and RNA domains and microdomains—to a certain degree comparable to self-organizational mesophase areas—have been visualized as graphite surface adlayers by scanning force microscopy in contact force mode. More polar substrate surfaces, sharper tip geometries and tapping mode procedures proved less favourable, due to sample distortions and prevention of organization within the adlayer by dominant adhesive forces in the interface. © 1997 by John Wiley & Sons, Ltd.

INTRODUCTION

Organizational phenomena, especially in artificial liquid crystals, have been widely studied also by scanning force and scanning tunnelling microscopy (SFM/STM). The field of biopolymeric species, however, with the exception of some early approaches and quite recent new findings, has mainly been devoted to studies of molecular individuals rather than molecular ensembles. Only preorientated two-dimensional arrangements as in Langmuir-Blodgett layers and, of course, membranes have attracted attention.

Thus a lot of artificial and native objects—e.g. single-, double- and triple-stranded nucleic acids as well as helical superstructures and plasmids, proteins, bacteriophages, complex viruses and membranes—have been visualized, deposited on a variety of quite different surfaces such as graphite, (modified) mica, silicon, MoS₂ and various other minerals. The quality of the images appears as a complex function of sample, sample preparation, substrate, scanning probe and scanning mode.

The obvious neglect of mesophase patterns in previous nucleic acid near-field investigations seems surprising, because nucleic acids (Plate 1) in vitro are mainly present and active in condensed forms, as in cell nuclei, genetic materials, ribosomes and other biological objects. The formation of amphotropic liquid-crystalline phases in a concentrated solution of these semi-rigid macromolecules (Fig. 1) has received considerable attention. The supramolecular biosogenic nucleic acid organizations are of fundamental interest with respect to biopolymer packing, replication, information processing and general regulations in vivo.

In first attempts to visualize these supramolecular organizations, we have carried out corresponding measurements with the help of SFM and STM, polarized light microscopy, differential scanning calorimetry, x-ray diffraction and Langmuir-Blodgett techniques. In the special case here, it was our aim to elucidate prerequisites and conditions for the study of nucleic acid adlayer organizations with respect to sample, sample preparation, tip, support and mode variations. As model systems we have chosen natural, highly polymerized polydispersed chicken and plasmid DNA for DNA and RNA duplexes and simplexes for RNA organization.

MATERIALS AND METHODS

Polynucleotides

The SFM samples and the corresponding Tₘ characterizations have been obtained as follows: plasmid DNA (pUC-19, 2686 base pairs) according to Ref. 40, 35 μg ml⁻¹, 10 mM Tris HCl, 1 mM EDTA in water (pH 7.4); high-molecular-weight polydispersed chicken-erythrocyte DNA (REANAL), 35 μg ml⁻¹, 1 mM NaCl in water (pH 7.0); (U)ₙ, (A)ₙ duplex (polyuridylc-polyadenylic acid), 0.1 mM solutions in water, PBS (0.15 mM NaCl and 0.01 mM Na-phosphate, pH 7.0), hybridized according to Ref. 40 from polyuridylc acid (SERVA, K-salt, S₂₀w = 5.7) and polyadenylic acid.
Figure 1. Mesophase textures (left to right): high-molecular-weight polydispersed chicken B-DNA (170–180 mg ml$^{-1}$, magnification 250×) in unspecific and thread-like domain appearances after 6 days of slow evaporation; (U)$_n$ - (A)$_m$ (100 mg ml$^{-1}$, magnification 320×) in thread-like and unspecific domain appearances after 2 days of evaporation—results that might even be valid for the somewhat different relationships within an adlayer.

(SERA, K-salts, $S_{20w} = 8.8$); (dA)$_n$ (polydeoxyadenylic acid) (Boehringer, K-salt), 0.1 mm solution in water, 1 mm NaCl (pH 7.0).

Polarizing microscopy probes have been prepared according to SFM samples, except for polynucleotide concentrations: chicken DNA $= 180$ mg ml$^{-1}$; (U)$_n$ - (A)$_m$ = 100 mg ml$^{-1}$.

Polarizing microscopy

The liquid-crystalline textures of DNA and RNA probes (Fig. 1) have been detected by means of a polarizing microscope (Leitz LaborLux 12S) equipped with a Hitachi video colour camera KP-551. In all cases samples were placed on a slide under a partially sealed coverslip, so that slow evaporation took place. Samples were observed through crossed polarizers and photographed with magnification 320×.

For sample preparation, 20 µl of a diluted solution of each nucleic acid complex in water were slowly poured across the substrate surface of freshly cleaved HOPG or oxygen-sputtered silicon(111) at room temperature.

Scanning force microscopy (SFM)

The SFM images were collected at room temperature in air using the commercially available Nanoscope III with three differently shaped tips (I, II, III) fixed on silicon nitride and silicon cantilevers. Tip I versions (silicon nitride) have a rather obtuse pyramidal form with spring constant $= 0.06$ N m$^{-1}$ and tip radius $= 4–40$ nm. Tip II (silicon) is a more acute pyramidal form with tip radius $= 27$ nm and spring constant $= 0.1$ N m$^{-1}$. Tip III (ion-beam-sharpened silicon) has a 27 nm tip radius (Digital Instruments, Santa Barbara, CA). For a detailed survey of the three tip versions, see Fig. 2. The substrates used were highly oriented pyrolytic graphite (HOPG) and oxygen-sputtered silicon(111).

For sample preparation, 20 µl of a diluted solution of each nucleic acid complex in water were slowly poured across the substrate surface of freshly cleaved HOPG or oxygen-sputtered silicon(111) at room temperature.
allowing for evaporation and approaching the adlayer formation conditions of Fig. 1.

The nucleic acid images were obtained in constant force mode (Figs 3 and 4 and Plate 2) at 15–20 nN net repulsive force, collecting the scans from left to right with a 512 × 512 pixel information density at scan rates of 30.5 Hz (Plate 2, top right), 10.2 Hz (Plate 2, top left), 3.1 Hz (Fig. 3 and Plate 2, lower part), 1.7 Hz (Fig. 4) and, additionally in tapping mode, 2.0 Hz (Fig. 5). All images are presented as row data except for flattening.

Molecular modelling

Both B-DNA and A-RNA (Plate 1) models have been generated on Silicon Graphics workstations by HAMOG- and SYBYL programs according to Brookhaven Protein Data Bank.

RESULTS AND DISCUSSIONS

As a continuation of our initial investigations of self-organizational phenomena in nucleic acids (Plate 1 and Fig. 1) concerning both volume and adlayer investigations,12 we compare here different surfaces, tip geometries and force mode variations with respect to their usefulness in imaging nucleic acid duplex adlayer organizations. As indicated by general experimental evidence,1–4 but also following on from hydrogen bond organizations of model compounds,41 the complex interactions between the graphite surface and the nucleic acid adlayer appear to be rather weak. As nucleic acids on graphite surfaces are not strongly adsorbed, they are able to undergo molecular association, orientation and organization processing during the evaporation procedure. On the other hand, following a general tendency for single-macromolecule imaging on mica and comparable substrates,9,13,16–19,24,28,42–44 the more polar modified silicon surface (SiO; undefined ratio of x/y) of an oxygen-sputtered silicon wafer builds up stronger interactions with the nucleic acid domains. Under these conditions, which are especially meaningful for chemical sensing,45 organization of the macromolecules is adversely affected by solid support/macromolecule interactions in the interface. The results compare with the above-cited numerous literature data.

Orientationally, Fig. 3 shows plasmid DNA on
HOPG. Contrary to previous investigations, which concentrated mainly on improvements in the resolution of single-molecule details, we obtained—by our sample preparation and imaging procedures, pyramidal tip I versions and the convenient contact force mode—more expressed association domains as well as individual molecules. The calculated length of the circular DNA compared well with experimental data (indication in

Figure 4. \((U)_n \cdot (A)_n\) imaging—tip II, contact force mode (upper part, left to right): rudimentary domains on different areas of oxygen-sputtered Si(111) surface; (lower part, left to right): more expressed domains on graphite in 2D representation; zoomed strand-array with fine structures, indicative of biomaterial impairments by tip abrasion in 3D representation.

Figure 5. \((dA)_n\) in single- and/or double-strand appearances—graphite substrate, tip III, tapping mode (left to right): 3D representation of overall and zoom views.
Fig. 3, lower part).13,14,24

An alternative approach with respect to support and tip variations is shown in Fig. 4. Using the more polar oxygen-modified silicon as a substrate and, moreover, the sharper tip II version (Fig. 4, upper part), we preferably obtained coiled single molecules, 'frozen' in accidental interaction adherent to the surface. As the association processes will be dominated by the strong adhesion forces to the substrates, only weak orientation within the nucleic acid adlayer is found.

So, notwithstanding certain drawbacks with the well-known mimicking of helical structures by graphite surface steps and clefts,46 we returned for further experiments to HOPG as the preferred substrate. Maintaining the sharper tip II in contact mode led to a \((U)_n \cdot (A)_n\) domain visualization as given in the lower part of Fig. 4, with a zoomed cut exhibiting fine-structure details probably caused by abrasion rather than by imaging nucleic acid duplex details (Plate 1).

Within continued trials to improve detail resolutions and also to compare with literature data,23 the tapping mode44 has been used further (Fig. 5); unfortunately, however, as is the case also with our contact force measurements, without equipment facilities for providing a water milieu within liquid cells. The organizational behaviour of polyoxybenzenedic acid, regardless of whether single- or double-strand versions appear,29,47 is nicely visualized. Again, however, the surface of the DNA individuals seems to be impaired by the action of the extremely sharp tip III version.

Surprisingly, the best results with respect to the overall imaging of organizational phenomena in the nucleic acid adlayers are obtained with a more primitive commercial pyramidal tip I version on a graphite surface in the convenient contact force mode (Plate 2). The imaging of both high-molecular-weight polydispersed DNA and high-molecular-weight \((U)_n \cdot (A)_n\) duplexes—the molecular prerequisites and the texture presentations in the volume phase are given in Plate 1 and Fig. 1—nicely reveals impressive arrangements with domains and microdomains. Although in this case there also remains the open problem as to the imaging of real duplex width, the radial resolution quality compares to the tapping mode variant, avoiding, however, the disadvantages of macromolecule impairments and macromolecule disruptions by the sharper tip II and tip III versions.

CONCLUSIONS

Initial results in the near-field microscopy of nucleic acid organizations12 have been extended to the elucidation of substrate, cantilever and operation mode dependencies. While overall views of nucleic acid adlayer association domains may successfully be imaged even under convenient standard conditions (pyramidal tips, graphite, contact force mode), ways to better resolution of single-molecule design with facilities to switch into dynamic processes will require further improvements in equipment and preferably investigations within the native milieu in liquid cells. Nevertheless, by the imaging of nucleic acid (mesophase) domains and microdomains in nucleic acid adlayers, this paper might contribute to the overall picture of experimental approaches to organizational phenomena in the nucleoprotein system.

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