Modified DNA immobilized on bioreactive self-assembled monolayer on gold for dynamic force microscopy imaging in aqueous buffer solution

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Recent advances in DNA specimen preparation techniques for dynamic scanning force microscopy based on terminal anchoring of the DNA segments on bioreactive self-assembled monolayers on ultraflat gold surfaces or on the gold surfaces themselves are presented DNAs of well-defined lengths and sequences carrying amino- or thiol-modified nucleotides at each end have been prepared. DNA imaging in the ac mode has been reproducibly achieved in buffer solution. Since the DNA segments are anchored at their ends only, their backbones are lying free on the self-assembled monolayer and are accessible to DNA–protein interaction. These procedures are suitable to follow in real time biological processes involving DNA. © *1996 American Vacuum Society.*

I. INTRODUCTION

Scanning force microscopy (SFM)[1] potentially allows direct imaging of individual macromolecules under ''physiological'' conditions, i.e., undenatured, in aqueous buffers, and also in the presence of appropriate ligands or effectors.[2–5] During the past few years many groups have demonstrated the general applicability of SFM to a wide range of biological systems. Nucleic acids have played a particularly important role, mostly for methodological reasons. Images of DNA have been obtained in air,[6–9] and a few examples of successful imaging of DNA in aqueous buffers have also been reported.[10–15]

Nevertheless, the number of imaged dynamic events involving biological objects in real time is still quite small, such as the measurement of the viscoelastic properties of platelets[16] and the extrusion of pox virus particles.[17] Dynamic studies on the morphology of single, native macromolecules, individually adsorbed onto a surface, are still fewer, such as the degradation of DNA with DNase I,[14] the assembly of RNA polymerase-DNA complexes,[15] and the disassembly of clathrin cages to triskelia.[18]

The development of the ac mode in liquids made it possible to reduce the disruptive influence of the lateral tip forces, which results in less deformation of soft samples.[14,19–22] The interaction of the tip with the sample should ideally be strictly localized and involve very low forces. If the interaction of the sample with the tip is stronger than with the substrate, the sample is wiped off during scanning.

In this article we focus on the preparation of DNA segments for dynamic force microscopy (DFM) in aqueous buffer solutions. We show that it is possible to image DNA strands anchored at their ends only. This was achieved in either of two ways: by introducing thiol groups at phosphates of the 5′ ends and anchoring them to bare ultraflat gold surfaces[13,23–25] or by introducing primary amino group via a spacer arm to thymine residues and covalently binding them to ω-functionalized N-hydroxysuccinimide (NHS) dialkyldisulfide self-assembled monolayer (SAM)[26] on gold (see the present data). These procedures result in freely accessible DNA backbones for studying DNA–protein interactions.

II. EXPERIMENT

Amino-modified thymidine was purchased from Glen Research (Sterling, VA). Two well-characterized double-stranded DNAs were used as templates: a standard plasmid (pBluescript KS-) and the plasmid of pro SIΔ,[27] which includes a 668 bp segment of wild type pro-sucrase isomaltase cDNA in pBluescript KS-. All chemicals and solvents were commercial grades of highest purity. Ultraflat template-stripped gold (TSG) was prepared as described previously.[23–25] Synthesis of dithio*bis*(succinimidylundecanoate) (DSU) was carried out via the oxidation of the intermediate Bunte salt of 11-bromoundecanoic acid.[26]

The DNA segments were amplified by the polymerase chain reaction (PCR) using amino-modified dT oligonucleotides. The resulting lengths were specified by the location of the oligonucleotides in the plasmid sequence (806 bp, 1561 bp, 2249 bp). The PCR was performed at 65 °C according to Ref. 28. The annealing temperature of the oligonucleotides was ~10 °C below the calculated value. All DNA purification steps were done at a *p*H above *p*H 9.0. Finally, the DNA was dissolved in 10 mM borate buffer (*p*H 9.0).

The amino-reactive monolayer was prepared by immersing the TSG platelet in a 1 mM solution of DSU in 1,4-dioxane for 30 min at room temperature. After extensive rinsing with 1,4-dioxane, the NHS-terminated monolayer

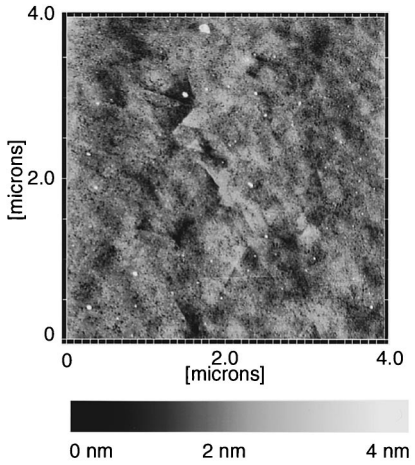

FIG. 1. DFM image of the DSU SAM on Au (111) taken in air in the noncontact mode.

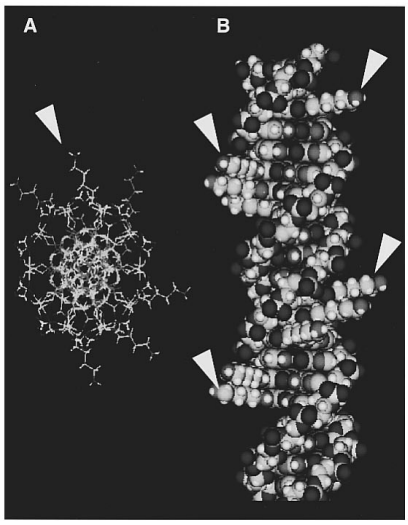

FIG. 2. (A) Molecular structure and (B) van der Waals model of the end of the DNA fragment carrying nine amino-modified thymidines at each end. (A) End-on top view and (B) side view in the B conformation.

was dried in air and immediately used for the immobilization step.

The amino-modified DNAs were immobilized by transferring a droplet of 2 μl (1 ng/μl) of DNA in 10 mM borate buffer *p*H 9.0 onto the NHS-terminated monolayer, and left for 2–3 h at room temperature at 100% relative humidity. After the incubation the sample was rinsed with 10 ml of buffer and always kept wet.

Thiol-modified DNA strands were prepared according to Ref. 13 and directly chemisorbed onto the bare gold surface (see Figs. 5 and 6).

The SFM imaging was done on a homebuilt SFM and on a Nanoscope III multimode SFM (Digital Instruments, Inc., Santa Barbara, CA) using silicon cantilevers with integrated silicon tips.[29] The spring constants ranged from 20 to 80 N/m for ac mode and from 0.02 to 0.66 N/m for repulsive dc imaging. The cantilevers were silanized prior to measurement with 1% trimethylchlorosilane (FLUKA, Buchs, Switzerland) in dry toluene. For ac measurements in aqueous environment we either used the lock-in technique or a rectifier (rms-to-dc converter) for amplitude detection. The cantilever was dynamically driven close to its mechanical resonance frequency f_0. All data presented in this work are unfiltered.

III. RESULTS AND DISCUSSION

In the present article we report the immobilization of amino-modified DNA segments on a bioreactive SAM of DSU chemisorbed onto flat gold surfaces, and the anchoring of thiol-modified DNA segments directly to the bare gold surface imaged with SFM in buffer solutions. We have recently described[23–25] a procedure to prepare very flat ''template-stripped'' (TSG) polycrystalline Au (111) surfaces with a mean roughness of 2–5 Å over areas as large as 100 μm^2. A typical TSG surface covered with a monolayer of DSU is shown in Fig. 1, taken in air at ambient conditions. The atomically flat terraces with diameters of 50–500 nm are completely annealed, and differ only in 3–5 atomic steps in *z* height. The triangular facets stem from the (111) lattice. The topography of the SAM reproduces the structure of the underlying gold terraces. Characteristics structural features of the SAM are small depressions, which are single-atom-deep pits resulting from an etching process during the adsorption of the disulfide.[30] Images of DSU-covered TSG surfaces taken with dc contact mode were similar.

We chose the terminal anchoring of the DNA strands because it is unlikely that the physisorption of the DNA backbone onto the surface allows it to be fully accessible to interactions with proteins. Onto these DSU SAMs we have chemisorbed amino-modified DNA strands and compared them with thiol-modified DNA strands directly chemisorbed on the bare TSG surface. The chemisorption of the amino-modified DNA strands did not include any drying step and yielded a more homogenous coverage of DNA on the substrate than the thiol-modified DNA directly anchored to the bare TSG surface.

The molecular structure (A) and the van der Waals model (B) of the terminal sequence of the amino-modified DNA strands (40 mer) are shown in Fig. 2. Amino-modified dTs replaced unmodified thymidine residues during the oligonucleotide synthesis. For our experiments we introduced nine modified dTs of a 20 mer oligonucleotide, in order to have enough reactive amino groups which jut out in all directions. Some of them are marked with an arrow in Fig. 2. The distance of the primary amine to the base is determined by a spacer arm with a total length of 10 atoms. The amino groups bind to the NHS-activated SAM covalently in aqueous buffers (borate buffer *p*H 9.0). Immobilization times of 2–3 h resulted in a coverage of 5–10 strands per μm^2. Longer incubation times (e.g., overnight) led to a totally covered surface, which made it impossible to detect individual molecules. DNA strands which had not been amino modified did not chemisorb or physisorb to the amino-reactive mono-

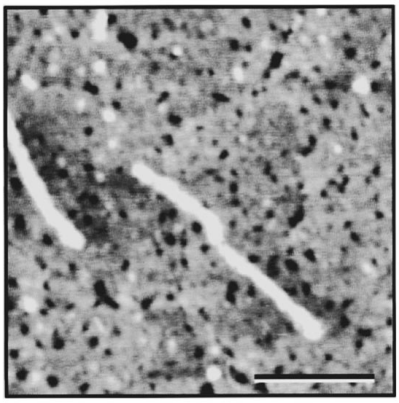

FIG. 3. DFM image of the amino-modified 806 bp DNA segment (length ≈ 250 nm), bound to a DSU SAM on Au(111), taken in air; bar=100 nm.

layer (i.e., after rinsing they were not to be detected by SPM) (data not shown).

We found that PCR and purification of the amino-modified DNA strands had to be performed under slightly basic conditions (*p*H 9.0). At lower *p*H values the DNAs tend to aggregate to larger networks, probably because the positively charged amino groups interact with the negatively charged DNA backbone. These aggregates did not disaggregate even in buffers of high ionic strength (>0.5 M) (data not shown).

A typical amino-modified DNA fragment chemisorbed onto the DSU SAM is shown in Fig. 3. Imaging was carried out in air in the noncontact mode with our homebuilt DFM. The cantilever was driven by a bimorph with an amplitude of 1–5 nm in contrast to the commercially available tapping mode, where the cantilever is usually oscillating with an amplitude of 50–100 nm. The measured contour length of the strand correspond to those of DNA in a B conformation (806 bp; ~250 nm). The holes in the background resulted from an etching process during the assembly of the monolayer as mentioned above.

The same DNAs were also investigated by DFM in aqueous buffer (Fig. 4); however, this was successfully carried out only if silanized hydrophobic cantilevers were used. Operation in the ac mode under liquids reduced the disruptive

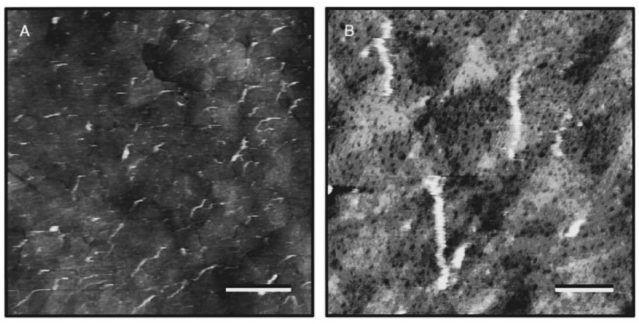

FIG. 4. DFM images of the amino-modified 806 bp DNA segments (length ≈ 250 nm), bound to a DSU SAM on Au(111), in 10 mM borate buffer (*p*H 9.0): (A) measured with the homebuilt DFM, bar=500 nm; (B) measured with Nanoscope III, bar=200 nm.

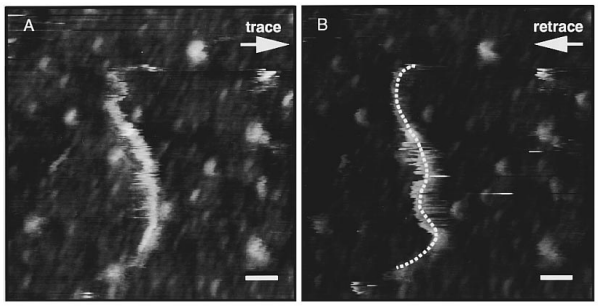

FIG. 5. SFM images of a thiol-modified 2249 bp DNA segment (length ≈ 750 nm), on bare TSG in propanol in dc mode. Simultaneously captured data set of the same segment, but with different scanning directions: (A) left- to right-hand side, and (B) right- to left-hand side; bars=100 nm (scan speed 1 Hz) (from Ref. 13).

lateral forces, which results in less deformation of the soft DNA strands. Image 4(A) shows the DNAs visualized by the homebuilt ac instrument, and image 4(B) shows the same DNAs imaged with the Nanoscope III in the tapping mode, both in buffer solution. The DFM measurement, with amplitudes of only 1–5 nm, exhibited fewer details of the underlying SAM assuming conditions close to the noncontact regime in liquids.

In previous experiments we have anchored thiol-modified DNA directly to the bare gold surface via thiolate bonding.[13] SFM imaging in the contact mode showed a lateral movement of the terminally anchored DNA due to the lateral forces exerted from the scanning tip. An image of these DNA strands (2249 bp) taken in propanol is shown in Figs. 5(A) and 5(B). These two images were obtained simultaneously, either with the scan direction from (A) left to right-hand side or (B) from right-to left-hand side. A white dotted line shows the contour length of the fragment in Fig. 5(B). The DNA fragment followed the movement of the cantilever with a frequency of 1 Hz, indicating that the backbone was lying free on the substrate. Such an approach makes the DNA accessible for DNA–protein interactions, but this has to be paid for with a significantly reduced sharpness of the DNA strand. This problem could be overcome by running DFM in the ac mode in liquids[14,19–22] which potentially allows following slow molecular events in real time.

We have shown in preliminary experiments in dc mode that the digestion of such a DNA (1561 bp) on TSG by a restriction enzyme (e.g., EcoRI) could be observed (see Fig. 6); however, in comparison to *ex situ* experiments in solution the restriction enzymes had to be used at much higher concentrations (e.g., 100 U/μg), and exhibited much slower kinetics. At higher scan rates (4 μm/s) no DNA cleavage was observed, probably because the enzymes were swept away from the scanning tip. No change was detected in control experiments without enzyme (data not shown). We conclude that the changes are caused by the action of the restriction enzyme on the DNA fragments. The particular final appearance of the fragments after digestion [Fig. 6(b)] may be due to coiling up of the DNAs around their anchoring points or to the lateral ''wiping off'' effect of the DNA molecules attached at only one end during contact measurements.

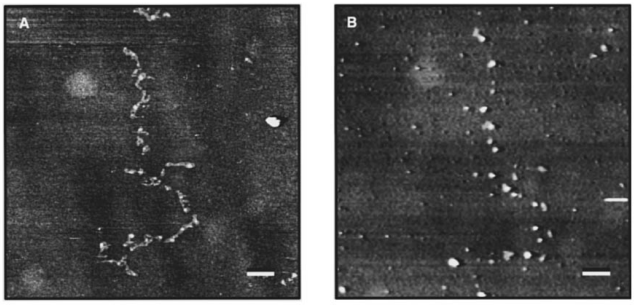

FIG. 6. Digestion of DNA (1561 bp) terminally anchored via thiol groups onto bare TSG: (A) before restriction; (B) 30 min after EcoRI injection; bars=1 μm.

IV. CONCLUSIONS

We have described immobilization procedures of modified DNA strands on amino-reactive SAMs on ultraflat gold surfaces. This procedure is an alternative to the physisorption of DNA onto mica surfaces or chemisorption of thiolated DNA on bare gold. Our approach is based on anchoring DNAs at their ends only, thus providing a highly accessible DNA backbone. Measurement with the DFM in liquids reduced the lateral forces exerted by the scanning tip compared to SFM in dc mode which often resulted in a deformation of the biomolecules; however, dc mode SFM allowed the *in situ* observation of DNA digestion by a restriction enzyme under native conditions. We showed that the SPM could be used to investigate biomolecules in aqueous buffer solutions with molecular resolution and to follow processes involving changes in the morphology of DNA. This will make it possible to eventually characterize the dynamics of such processes.

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