Atomic Force Microscopy of spermidine-induced DNA condensates on silicon surfaces

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ABSTRACT

In the present work, we show that oxidized silicon may be successfully used to image multivalent cation-induced DNA condensates under the Atomic Force Microscope (AFM). The images thus obtained are good enough, allowing us to distinguish between different condensate forms and to perform nanometer-sized measurements. Qualitative results previously obtained using mica as a substrate are recovered here. We additionally show that the interactions between the cation spermidine (the condensing agent) and the DNA molecules are not significantly disturbed by the silicon surface, since the phase behavior of an ensemble of DNA molecules deposited on the silicon substrate as a function of the cation concentration is very similar to that found in solution.

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1. Introduction

DNA condensation induced by multivalent cations has been studied intensively in the last three decades motivated mainly by the fact that condensed DNA produced in vitro resembles, to some extent, the condensed DNA inside living organisms [1]. Among the main techniques used so far to study the formation and the structure of condensed DNA molecules, we may mention light scattering, circular dichroism, gel electrophoresis, single molecule experiments such as those performed with magnetic and optical tweezers, and microscopy techniques.

Atomic Force Microscopy (AFM) is among the techniques often used to obtain high resolution images of DNA (both in the linear and circular form) [2,3,4] as well as images of DNA–ligand complexes such as DNA–multivalent cations [5] and DNA–intercalating drugs complexes [6]. The direct visualization of the conformational changes undergone by a single DNA molecule in the presence of those ligands is the main advantage of this technique in comparison with others in which the measurement is performed over an ensemble of molecules.

An important aspect of this technique is the need of a smooth surface onto which the DNA molecules can be deposited and afterwards scanned by the microscope tip. The universally used mica surface is thus the one which matches this criterion since it is smooth enough [3]. Moreover, a number of different protocols have been developed in order to attach the DNA molecule onto that surface. This has been accomplished in a number of ways: functionalizing the surface [2], using different divalent cations [4] which can attach the DNA phosphate groups to the substrate, etc.

Apart from mica, silicon has also been used in a few works as a substrate onto which plasmid DNA [3] and λ-DNA [7] have been visualized under the AFM, producing images with good resolution and contrast. In this work, we propose oxidized silicon as an alternative substrate onto which DNA condensates can be visualized, therefore, extending the usage of silicon substrates for DNA imaging under the AFM. Our results show that oxidized silicon surfaces can be used to visualize DNA condensates induced by the trivalent cation spermidine (spd+3) in a straightforward way. Since silicon is much less hydrophilic than mica, the capillary forces are less intense allowing, therefore, DNA imaging at relatively high humidities (60–70%) with good contrast. Additionally, the molecules remain stable on the surface even after repetitive scanning [8].

The typical condensed DNA morphologies such as globular and toroidal are found [5]. The resolution of the images thus obtained is high enough to allow nanometer-sized measurements of DNA condensates height and diameter.

Furthermore, we have investigated the changes in the average sizes of λ-DNA molecules as a function of the amount of the trivalent cation spermidine. The general trend already observed by means of fluorescence microscopy for T4DNA molecules is recovered here [9]: at low enough [spd+3]/[phosphate] ratios, the DNA molecules are unfolded and elongated; on the other hand, at high [spd+3]/[phosphate] ratios, the condensed and compact DNA form is the only one present. At intermediate cation concentrations, straight DNA conformations coexist with more compact ones.

At the highest [spd+3]/[phosphate] ratios, the average sizes of the DNA condensates, as estimated from the images, are shown to be a slight decreasing function of the spd+3 concentration. The DNA molecules appear as small tori/globules with average diameters ranging from 250 nm to 100 nm depending on the cation concentration.

The interaction between the DNA segments and the silicon surface, therefore, does not seem to disturb significantly the electrostatic interactions between this polyelectrolyte and the cations, since the
phase behavior of the DNA–spermidine mixture described here is qualitatively realized in solution [9]. We thus propose oxidized silicon as a substrate for imaging DNA condensates under the scanning force microscope.

In what follows, we will present the experimental procedure used to perform our measurements along with some typical images obtained. Additionally, we will discuss some more quantitative aspects based on the estimated average sizes of the DNA condensates.

2. Material and methods

Silicon (Si(111)) wafers of 1×1 cm² in size were treated with a NaOH solution (2 mg/ml) for 15 min and afterwards rinsed extensively with distilled water. The treatment with NaOH has shown to be effective in increasing silicon affinity for water [10] and, therefore, for DNA and to remove some dust as well. The DNA and spermidine solutions were prepared as follows: λ-DNA (Promega Corp.) of about 48.5 Kbp at a concentration equal to 460 μg/ml was dissolved in Tris–HCl buffer 10 mM (pH = 7.0) to a final concentration equal to 4.6 ng/μl. The spermidine (C₇H₁₉N₃ — Sigma Aldrich) solution was initially prepared as a 0.8 M solution and finally dilute to a 1 mM stock solution in the Tris–HCl buffer.

The DNA–spermidine solution was prepared mixing the two solutions described above, keeping the DNA final concentration fixed at 0.02 ng/μl and adding varying amounts of the spermidine solution in order to achieve a given [spd+3](M)/[phosphate](M) ratio. The mixture was allowed to equilibrate for 10–20 min. An aliquot of 20 μl was deposited on the silicon substrate and completely dried out in a flow chamber for 3 h at ambient temperature (~25 °C). The silicon surface was scanned with the AFM (model NTEGRA, NT-MDT-Russia) operating in the conventional semicontact mode at a force of 0.7 nm, a value which is much lower than previously reported values (~60–70%). This experimental procedure has been shown suitable to visualize the condensed DNA in a reproducible and reliable way.

3. Results and discussion

The result of the addition of small amounts of multivalent cations to a highly dilute DNA solution has long been known to induce a drastic conformational change in the DNA molecule, most often producing toroidal, globular and rod-like structures whose volumes are nearly the volume of the DNA molecule itself, indicating the tight packaging of the DNA segments inside the condensates [5,11].

In Figs. 1 and 2, we show typical DNA condensates imaged on silicon surface. The structure seen is clearly round-shaped with a mild central hole (see the height profile). This is the morphology most often presented by cation-induced DNA condensates [5,11] and, to some extent, is independent of the multivalent cation species used.

The measured average height of the DNA condensates is nearly 0.7 nm, a value which is much lower than previously reported values (about 15 nm) [5] and indicates that the DNA segments are all nearly packed in the same plane i.e., forming a more or less flat structure, since this is the reported average height of bare DNA on both silicon and mica surfaces [7,4]. This particular ordering of the DNA segments probably makes the torus central hole not so distinct in the 2D images as in Ref. [5]. The topographical profile of the condensates as the one presented in Fig. 2, for instance, makes it clearer.

On the other hand, the measured average diameter of the tori is about 200 nm, a value 4–5 times higher than the ones reported by Lin et al., at spermidine concentrations close to that used in Ref. [5]. The volume of the toroidal structure remains, however, roughly equal to the volume of one λ-DNA molecule only (~5×10⁴ nm³).

We ascribe the disagreements between the condensate heights and diameters reported here and previous ones to the effect of water surface tension on the DNA segments during the drying sample procedure: the “drying moving front” of the solution drop on the silicon substrate forces the DNA segments to lie side by side on its surface. The same force stretches the DNA molecules at very low spermidine concentrations (see discussion below). This phenomenon is quite similar to that used to align DNA molecules on poly(dimethylsiloxane) surfaces [12], although in our deposition procedure we have not tilted the silicon substrate.

To further investigate a possible influence of the silicon surface on the DNA–spermidine interactions, we have systematically measured the sizes of DNA condensates as a function of different added amounts of the multivalent cation.

We can distinguish between three regimes: (1) at high enough [spd+3](M)/[phosphate](M) ratios (~14:1 or higher), the DNA molecules are predominantly in a toroidal or globular conformation. The tori have a mild central hole which has different sizes, even within the same sample. The globular structures often seen coexisting with the toroidal ones miss this central hole; (2) at low enough [spd+3](M)/[phosphate](M) ratios (~3:10 or lower), the DNA molecules are predominantly unfolded, adopting an elongated conformation. A typical image of straight DNA molecules seen in the regime 2 is shown in Fig. 3. At this regime, the population of tori or globule-like conformations is almost negligible.

At last, (3) at intermediate [spd+3](M)/[phosphate](M) ratios (between the regimes 1 and 2), DNA molecules coexist in both compact and unfolded states in the same sample. Furthermore, we can also observe loosely folded structures, within which is possible to distinguish between the different segments comprising the chain. In addition, molecules partially folded or partially unfolded are also found in this regime. The image shown in Fig. 4 illustrates the coexistence between different DNA conformations in the same sample.
As discussed earlier, the stretching force responsible for the elongated DNA conformations may be produced during the drying process, as the solution drop shrinks on the silicon surface. Note that similar straight λ-DNA conformations were also observed in Ref. [7] under similar conditions. Here we hypothesize that, if the DNA has a few weak attractive contacts with the silicon substrate, this force may be high enough to stretch the DNA molecule. Of course, more experiments would have to be performed to decide whether this is the real scenario. We strongly feel, however, that the silicon surface does not play a big role in aligning the DNA molecules by means of strong DNA–Si interactions. As we state, the opposite happens, actually.

Anyway, it seems clear that our results are consistent with previous experimental data under similar solution conditions [9]. Based on these results, we conclude that oxidized silicon surfaces can be conveniently used as an alternative substrate to image DNA condensates under ambient conditions, to follow the phase behavior of DNA–multivalent cations mixtures and to perform nanometer-sized measurements on the condensates.

To summarize the general trend of the overall sizes of the DNA molecules found in the three regimes mentioned, in Fig. 5 we show the behavior of the average sizes of the DNA molecules as a function of the [spd⁻³](M)/[phosphate](M) ratio. On the one hand, at the lowest [spd⁻³](M)/[phosphate](M) ratios used in our experiments (corresponding to regime 2), the DNA molecules appear elongated on the silicon surface. The DNA end-to-end distance $R_E$ (nm) seems to be the best statistical parameter which characterizes the overall sizes of the straight DNA molecules at this regime. On the other hand, at the highest [spd⁻³](M)/[phosphate](M) (corresponding to regime 1) the DNA molecules appear as compact globules or tori, with an average diameter $D$ (nm) which decreases slightly with the increasing spermidine concentration. In between these two regimes, there is a broad coexistence zone (corresponding to regime 3). For clarity, these three regimes are indicated in Fig. 5.

The general trend sketched here regarding the sizes of DNA molecules in a solution containing the multivalent cation spermidine is also in agreement with precipitation experiments which suggest the same qualitative behavior: the continuous decrease of the overall ensemble average sizes of the DNA molecules in solution with the increasing [spd⁻³](M)/[phosphate](M) ratio [13].

At last, we should mention at this point, that the DNA “reentrant condensation” observed when an excess of spermidine is added to the DNA solution [13] was not observed in our experiments. This
may be explained either by the use of rather lower spd\textsuperscript{+3} concentrations than that used in Ref. [13] or by noting that we have used the nonacid form of spermidine instead of the spermidine trihydrochloride (C\textsubscript{7}H\textsubscript{19}N\textsubscript{3}·3HCl). The absence of the anion Cl\textsuperscript{−} in solution would disfavor the ion-pairing assumed to occur by Todd and Rau at high spermidine concentrations [14] and thus, the spd\textsuperscript{+3} cations would not be taken off the DNA condensed phase, preventing the DNA redissolution. Clearly, more work is needed to clarify this issue.

4. Conclusion

In this paper, we have shown that oxidized silicon can be used as a substrate onto which spermidine-induced DNA condensates may be deposited and imaged under the atomic force microscope. We thus propose silicon as an alternative substrate for DNA imaging and possibly for imaging other biomolecules as well, with the advantage to permit imaging at relatively high humidities (60–70\%) with good resolution, which is difficult to perform with mica substrates. In addition, the quantitative results obtained for the average height and diameter of the condensates show that the silicon substrates can provide results as precise as those obtained with the mica ones.

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