pH Induced DNA Folding at Interface

Hongwei Xia,[†] Yi Hou,[†] To Ngai,[‡] and Guangzhao Zhang*[,†]

Hefei National Laboratory for Physical Sciences at Microscale, Department of Chemical Physics, University of Science and Technology of China, Hefei 230026, China, and Department of Chemistry, The Chinese University of Hong Kong, Shatin, N.T., Hong Kong, China

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DNA chains with cytidine-enriched sequences have been grafted on a gold surface at different pH values monitored by a quartz crystal microbalance with dissipation (QCM-D) in real time. DNA chains grafted under acidic conditions with *i*-motif structures have a lower density due to the larger chain coverage. In contrast, DNA chains grafted under basic conditions with extended conformations are more densely grafted. After the grafted DNA chains completely unfold at pH 8.5, we have investigated the DNA folding with lower and higher grafting densities by use of QCM-D and surface plasmon resonance (SPR). We find that DNA chains with low grafting density can fold into *i*-motif structures, whereas those with higher grafting density only partially fold because crowding provides limited space for their conformational changes.

Introduction

Protein and DNA are the most important biomacromolecules. Their folding with a number of intermediates driven by various molecular interactions relates to their functions in a living cell. Biomacromolecule functions, for instance, DNA/RNA repair and modification or immune recognition/response, always take place in a very crowded environment.[1–3] Namely, the inside of a cell consists of a concentrated solution of proteins, sugars, nucleic acids, and other macromolecules. Such a crowded environment profoundly affects the biological activity of cellular biomacromolecules.[4–6] So far, it is still a challenge to directly detect the behavior of protein or DNA in a living cell. Studies on the folding and interaction of biomacromolecules in crowded or cell-like conditions can be very helpful in shedding light in vivo processes.

DNA with cytidine-enriched sequences can fold into an *i*-motif via intercalated $C-C^+$ base pairing in an acidic environment. On the other hand, it unfolds into a random coil in a basic environment.[7–13] Thus, we can study the folding and unfolding of the DNA by simple alteration of solution pH. Recently, such a conformational change has been utilized to develop DNA-based nanodevices such as a proton-fueled nanomachine,[14–17] a reversibly switched nanocontainer,[18] a colorimetric pH meter,[19] and a pH sensor to detect the pH value inside living cells.[20] Additionally, the DNA conformation can also continuously vary in a pH oscillating system.[21,22]

The DNA chain folding and unfolding kinetics with the *i*-motif structure and G-quadruplex structure were investigated by surface plasmon resonance (SPR).[23,24] However, the DNA structural changes during folding and unfolding are not well understood. The quartz crystal microbalance with dissipation (QCM-D) can provide information about the changes in both mass and structure of macromolecules at liquid−solid interfaces.[25–40] In the present study, we grafted DNA chains onto a gold surface at pH 4.5 or 8.5, leading to DNA chains with different grafting densities. The concentrated or crowded DNA chains at the interface can fully unfold at pH 8.5. We have investigated the DNA folding at pH 4.5 in real time using QCM-D and SPR. Our aim is to have a better understanding of the DNA folding mechanism in concentrated or crowded conditions.

Experiment Section

Oligonucleotides with a sequence of 5′(SH)-TTTTTC-CCTAACCCTAACCCTAACCC-3′ were from Sangon Biological Engineering (Shanghai) and HPLC purified prior to use. They were dissolved in 100 mM phosphate buffered saline (PBS) at pH 4.5 or pH 8.5 with 100 mM NaCl. The final concentration of DNA was 1.0 μM.

Grafting of DNA Chains. DNA chains were immobilized on gold surfaces by immersing gold-coated QCM-D or SPR sensor surface into a solution of the DNA (1.0 μM) in a PBS at pH 4.5 or pH 8.5. Under acidic conditions, DNA chains grow into the folded *i*-motif structure, so that DNA chains have enough room to change conformation. In a basic buffer, DNA chains are extended strands and they are expected to graft more densely on the gold surface. 2-Mercaptoethanol (1.0 μM in PBS) was added for 1 h to remove the physically adsorbed DNA chains. Finally, rinsing with PBS was conducted to ensure only grafted DNA chains remained on the gold surface.

QCM-D Measurements. The QCM-D instrument (Q-Sense D300) and the AT-cut quartz crystal with a fundamental resonant frequency of 5 MHz and crystal constant (C) of 17.7 ng/cm^2 Hz were obtained from Q-sense AB.[41] The quartz crystal was mounted in a fluid cell with one side exposed to the solution. The effects of surface roughness were minimized by using highly polished crystals with a root-mean-square roughness less than 3 nm.[42] The uncertain frequency shift is within ±2 Hz. All the experiments were performed at 25 °C.

When a quartz crystal is excited to oscillate in the thickness shear mode at its fundamental resonant frequency (f_0) by applying a RF voltage across the electrodes near the resonant frequency, the addition of a small layer to the electrodes leads to a decrease in resonant frequency (Δf) that is related to the mass (Δm) of the layer. In vacuum or air, if the layer is rigid, evenly distributed and much thinner than the crystal, the Δf is related to Δm and the overtone number ($n = 1, 3, 5, ...$) by the Sauerbrey equation,

* To whom correspondence should be addressed.
[†] University of Science and Technology of China.
[‡] The Chinese University of Hong Kong.

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$$\Delta m = -\frac{\Delta f\sqrt{\rho_q \mu_q}}{2nf_0^2} \quad (1)$$

where f_0 is the fundamental frequency and ρ_q and μ_q are the specific density and shear modulus of the quartz crystal, respectively.[43]

The dissipation factor is defined by $\Delta D = E_d/2\pi E_s$, with E_d and E_s being the energy dissipated during one oscillation and the energy stored in the oscillating system, respectively. The measurement of ΔD is based on the fact that the voltage over the crystal decays exponentially as a damped sinusoidal when the driving power of a piezoelectric oscillator is switched off. By switching the driving voltage on and off periodically, we can simultaneously obtain a series of changes in the resonant frequency and the dissipation factor. In the present study, Δf and ΔD values from the fundamental were discarded because they were usually noisy due to insufficient energy trapping.[44] All the results obtained were from the measurements of frequency changes in the third overtone ($n = 3$). The results from the fifth and seventh overtones were not presented because they exhibited the same trend as that for the third overtone. The thickness of the layer was fit by theoretical representations based on a Voigt model with Q-tools software from Q-sense AB.[47]

SPR Measurements. SPR experiments were carried out using Biacore X optical biosensors at 25 °C. The gold-coated glass plate was attached to a glass prism with a silicone opto-interface so that they match in refractive index.[45,46] The response is linear to the added mass of the layer with 1000 RU ≈ 1 ng/mm^2, where RU is the response unit. Light from a near-infrared light-emitting diode is focused through the prism onto the sensor surface in a wedge-shaped beam to give a fixed range of incident angles. Light reflected from the sensor is monitored by a linear array of light-sensitive diodes with a resolution corresponding to approximately 0.1°. The phosphate buffer solution was applied to the sensor surface at a flow rate of 5 μL/min.

Results and Discussion

Figure 1 shows the changes in frequency (Δf) and dissipation (ΔD) for the grafting of thiol-terminated single stranded DNA on a gold-coated crystal surface at pH 4.5, where the DNA chains have *i*-motif structure.[14,22] The frequency decreases in the initial stage, indicating the increase of the layer mass and that DNA chains are grafted on a gold surface. Subsequently, Δf levels off in 60 min, indicating that the surface is saturated with DNA chains. On the other hand, ΔD quickly increases in the initial stage and then gradually decreases. It is known that ΔD reflects the changes in the thickness and structure.[44] Typically, a rigid and dense layer has small dissipation while a looser and thicker structure exhibits a larger dissipation. The quick increase of ΔD in the initial stage indicates the increase in the thickness of the DNA layer or the grafting of DNA. The gradual decrease of ΔD indicates that the grafted DNA chains rearrange and pack more densely.

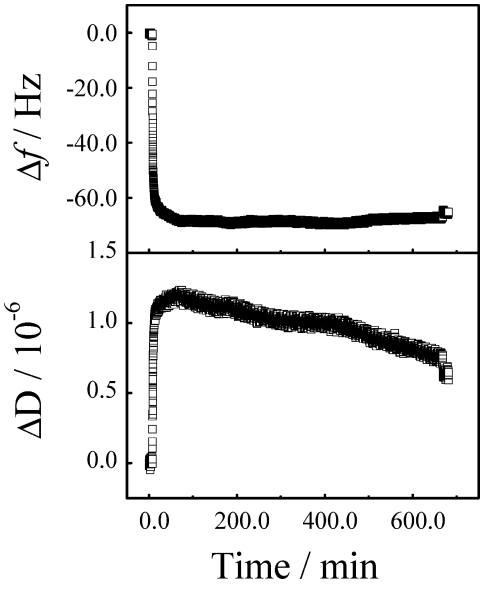


Figure 1. Changes in frequency (Δf) and dissipation (ΔD) for DNA grafting on a gold surface at pH 4.5.

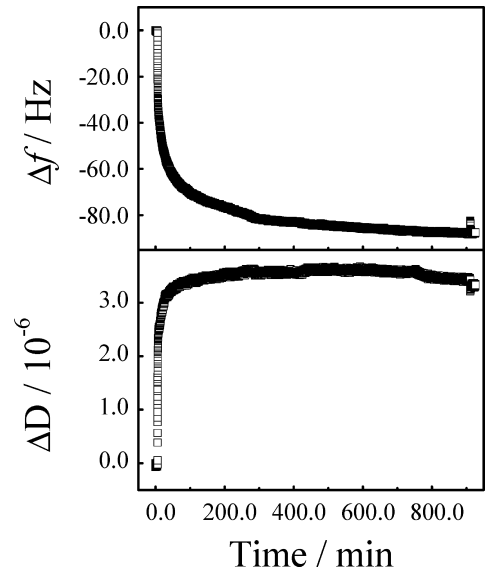


Figure 2. Changes in frequency (Δf) and dissipation (ΔD) for DNA grafting on a gold surface at pH 8.5.

Figure 2 shows the changes in frequency (Δf) and dissipation (ΔD) for the DNA grafting on a gold-coated crystal surface at pH 8.5, where the DNA chains are extended single stranded coils.[22] After a sharp decrease, Δf gradually decreases and finally levels off. Comparing the results in Figures 1 and 2, we know the final frequency shift value (88 Hz) at pH 8.5 is larger than that (68 Hz) at pH 4.5, indicating that more DNA chains grafted at pH 8.5. This is understandable because DNA chains are extended coils with a low chain coverage at pH 8.5. A certain surface can accommodate more such lanky chains. In addition, it takes a much longer time for Δf to reach a constant or the surface to be saturated with DNA chains at pH 8.5. This is because the already grafted chains with a higher density restrict the grafting of more incoming chains, and it is difficult for the subsequent chains to graft on the vacant space between the grafted chains. In contrast, the chains with higher chain coverage at pH 4.5 can quickly saturate the surface, and the space left between the already grafted chains is not enough to further accommodate an incoming chain.

Figure 2 also shows that ΔD quickly increases and levels off, further indicating the grafting of DNA chains. Unlike the gradual decrease of ΔD in the case at pH 4.5 (Figure 1), the level-off of ΔD here indicates that the extended DNA chains are grafted so densely that they do not have space to rearrange or pack.

Figure 3 shows the time evolution of the thickness (t) for grafting a DNA layer on a gold surface. The final thickness values at pH 4.5 and pH 8.5 are about 4.7 and 7.1 nm,

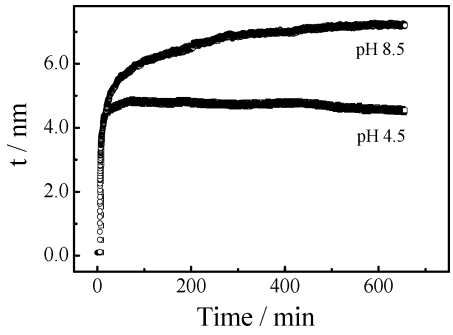


Figure 3. Time evolution of the thickness (t) for DNA grafting on a gold surface at pH 4.5 and 8.5.

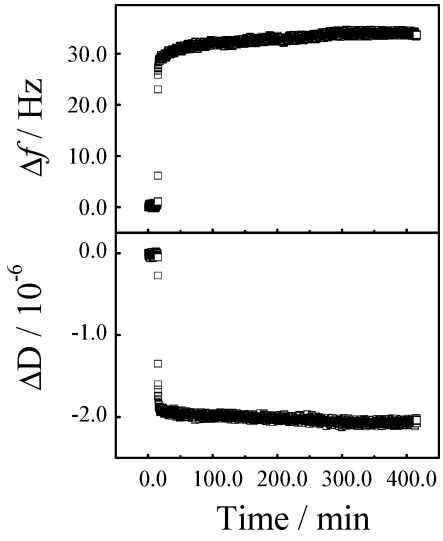


Figure 4. Changes in frequency (Δf) and dissipation (ΔD) for the folding of DNA chains with a low grafting density.

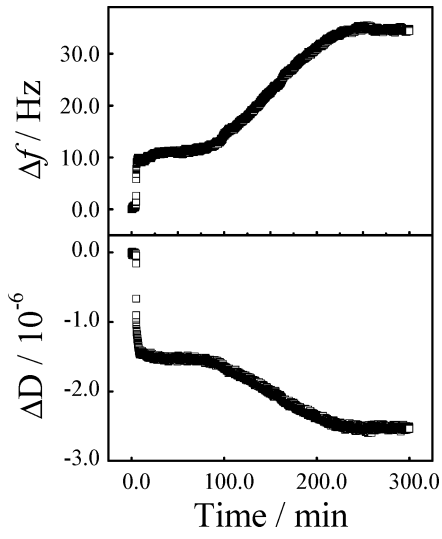


Figure 5. Changes in frequency (Δf) and dissipation (ΔD) for the folding of DNA chains with a high grafting density.

respectively. As discussed above, DNA chains have an extended conformation at pH 8.5 but form the *i*-motif structure at pH 4.5, so a thicker layer is formed at pH 8.5. We also estimated the surface coverage of the grafted DNA chains at pH 4.5 and 8.5 with the data about thickness and density. The graft density values at pH 4.5 and pH 8.5 are 923.1 and 935.2 kg/m^3, respectively. Considering that the molecular mass of DNA is 7920 g/mol, the surface coverage values at pH 4.5 and 8.5

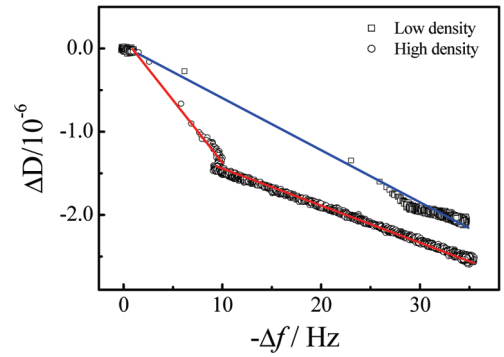


Figure 6. Changes in ΔD plotted versus changes in Δf for the folding of DNA chains at different grafting densities.

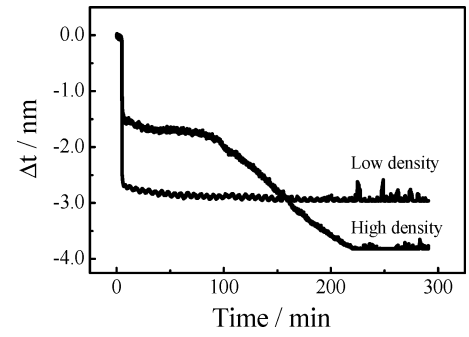


Figure 7. Time evolution of relative thickness (Δt) of DNA chains at different grafting densities during folding.

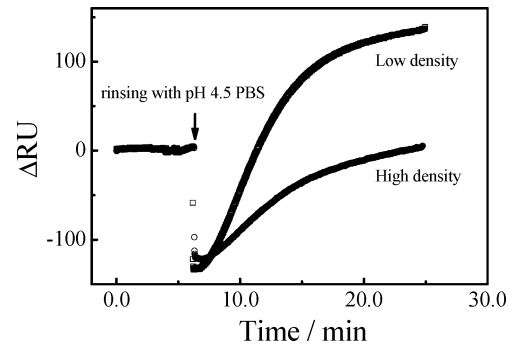
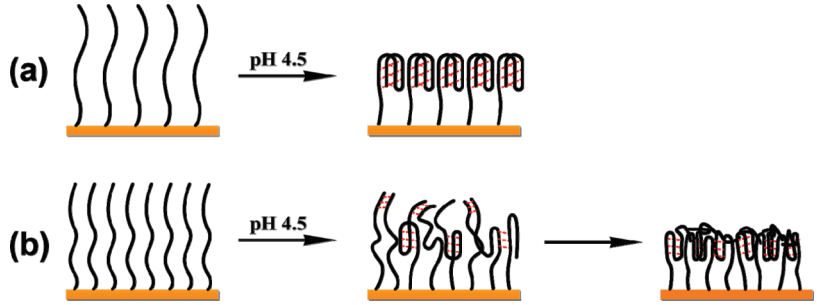


Figure 8. SPR sensorgrams (ΔRU) for the folding of DNA chains at different grafting densities.

estimated are about 3.3 × 10^{13} and 5.1 × 10^{13} chains/cm^2, respectively. These results are close to those reported before.[18] In short, grafting at pH 4.5 and 8.5 leads to a low and higher grafting density, respectively.

The DNA chains with low and higher grafting density were immersed in a PBS with pH 8.5 so that they were able to completely unfold. Subsequently, a PBS with pH 4.5 was quickly introduced to replace the PBS with pH 8.5. Thus, we investigated the folding of DNA chains with different grafting densities at pH 4.5. Figure 4 shows Δf quickly increases when pH is changed from 8.5 to 4.5. This is indicative of the collapse or folding of DNA chains since the layer thickness decreases as Δf increases. On the other hand, it is known that cytidine groups are protonated at pH 4.5 forming intrachain and interchain hydrogen bonds due to C−C$^+$ pairing.[11–13] The interactions lead some of the water molecules coupled on DNA molecules to leave them, so the layer mass decreases. Namely, the dehydration of DNA also gives rise to the increase of Δf. Later, Δf gradually increases, indicating the completion of folding. On the other hand, the quick decrease in ΔD also

SCHEME 1: Folding of DNA Chains with a Low (a) and High (b) Grafting Density



indicates the decrease of the thickness of DNA layer, further revealing the collapse or folding of DNA chains. The final level-off of ΔD indicates that the folding is over.

Figure 5 shows the changes in Δf and ΔD for the folding of DNA chains with higher grafting density. It can be seen that Δf increases and ΔD decreases quickly in the initial stage because the pH-induced folding leads to the decrease of thickness and the dehydration of DNA chains. Then, Δf increases and ΔD decreases gradually. As stated above, because of the high grafting density, the DNA chains restrict each other to fold into the *i*-motif structure. The DNA chains have to gradually fold as they rearrange and shape themselves. After a long time, Δf and ΔD level off, indicating that the folding is finished. However, due to the limited space, the DNA chains are expected to partially fold.

The folding of DNA chains can be viewed in terms of ΔD vs Δf relation. Figure 6 shows that the folding of DNA chains with a low grafting density roughly involves one-stage. This is because the space around a DNA chain is enough for its full folding. However, an obvious two-stage folding is observed for DNA chains with higher grafting density. As discussed above, the DNA chains are expected to partially fold in the first stage, where the chains are not completely restricted. In the second stage, the DNA chains have to rearrange themselves to have more space for further folding.

Figure 7 shows the time evolution of the relative thickness (Δt) of DNA layer during grafting, where the thickness of the unfolded DNA chains at pH 8.5 was used as the reference. The decrease in Δt indicates that DNA chains change from extended state to collapse state. At low grafting density, Δt quickly decreases and levels off, suggesting a one-stage folding. At high grafting density, a two-stage folding can be observed. Δt first sharply decreases, implying that DNA chains fold and entangle each other. Subsequently, Δt gradually decreases and levels off, indicating that DNA chains further fold in a limited space. In addition, at the folded state, DNA chains with high grafting density have a larger Δt value than those with lower grafting density. This is because the former are much thicker than the latter in the extended state, as shown in Figure 3.

Figure 8 shows SPR sensorgrams for the folding of DNA chains with low and high grafting density. As the pH is varied from 8.5 to 4.5, it is not surprising that the replacing buffer leads to a quick decrease of ΔRU because protonation of cytidine groups at pH 4.5 leads to the dehydration of DNA chains and thus the thickness of DNA layer decreases. This is consistent with the QCM-D results shown in Figures 4 and 5. Subsequently, ΔRU increases and tends to level off, indicating that the folded DNA chains cause the refraction index of the layer to increase. Clearly, DNA chains with a low grafting density exhibit a change in ΔRU larger than that for DNA chains with higher grafting density. This is because the former can completely fold, which leads to a larger increase of refraction index. In contrast, the folding of the latter is not complete, and the change in the refraction index is limited, resulting in a smaller change of ΔRU. Clearly, the SPR results are consistent with QCM-D results. However, QCM-D can also provide the information about the structural changes of the folding DNA chains.

Scheme 1 illustrates the folding of DNA chains with a low and high grafting density. The folding involves only one-stage at low grafting density (a); that is, DNA chains form an *i*-motif structure. At higher grafting density, the folding involves two stages (b). Namely, DNA chains first fold and entangle each other. Then, they further fold in a limited space and form a more dense structure.

Conclusions

In conclusion, the grafting of DNA chains on gold surface at different pH values can be monitored by a quartz crystal microbalance with dissipation (QCM-D) in real time. In an acidic environment, DNA chains which have *i*-motif structures with a larger chain coverage are grafted with a low density. In a basic environment, DNA chains that are extended coils with a low chain coverage can be densely grafted. The folding of DNA chains with different grafting densities has been investigated by use of QCM-D and surface plasmon resonance (SPR). The crowding profoundly influences the DNA folding. DNA chains with low grafting density can readily fold into *i*-motif structures and display a one-stage folding. DNA chains with high grafting density exhibit a two-stage folding and can only partially fold due to the steric restriction.

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