Effects of Bridge Ions, DNA Species, and Developing Temperature on Flat-Lying DNA Monolayers

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Recently, we have successfully constructed flat-lying DNA monolayers on a mica surface (*J. Phys. Chem. B* **2006**, *110*, 10792−10798). In this work, the effects of various factors including bridge ions, DNA species, and developing temperature on the configuration of DNA monolayers have been investigated by atomic force microscopy (AFM) in detail. AFM results show that the species of bridge ions and developing temperature play a crucial role during the formation process. For example, the divalent cation Zn^{2+} resulted in many DNA chains stuck side by side in the monolayers due to the strong interactions between it and DNA's bases or the mica surface. Most DNA chain's conglutinations disappeared when the developing temperature was higher than 40 °C. Cd^{2+} and Ca^{2+} produced more compact DNA monolayers with some obvious aggregations, especially for the DNA monolayers constructed by using Ca^{2+} as the bridge ion. Co^{2+} produced well-ordered, flat-lying DNA monolayers similar to that of Mg^{2+}. Furthermore, it was found that the flat-lying DNA monolayers could still form on a mica surface when plasmid DNA pBR 322 and linear DNA pBR 322/Pst I were used as the DNA source. Whereas, it was hard to form DNA monolayers on a (3-aminopropyl)-triethoxysilane−mica surface because the strong interactions between DNA and substrate prevented the lateral movement of DNA molecules. These results suggested that the appropriate interactions between divalent cations and DNA or mica surface were important for the formation of flat-lying DNA monolayers. The obtained information is a necessary supplement to our previous studies on the formation kinetics of such monolayers and may be useful for practical application of the monolayers and further theoretical studies.

Introduction

The monolayers of DNA molecules already attract great interest due to their potential applications as biomaterials in medicine and engineering. Flat-lying, well-ordered DNA monolayers have been successfully constructed on a solid mica surface.[1] The formation of the excellent monolayers on this surface was mainly attributed to ion diffusion and DNA rearrangement. The removal of ion bridged DNA−mica or DNA−DNA surfaces and the lateral movement of DNA resulted in the dispersion of DNA aggregations, rearrangement of DNA molecules, and the formation of well-ordered DNA monolayers. However, the effects of some factors such as bridge ions, DNA species, and developing temperature on the formation of flat-lying DNA monolayers on the mica surface are still unclear.

Divalent cations have been extensively used as bridge ions between DNA's phosphate groups and the mica surface to immobilize negatively charged DNA onto a like-charged mica surface.[2−4] The binding was first ascribed to the inset of divalent cations into the cavities of the mica surface.[2] Further studies in theory suggested that the adsorption of DNA on a mica surface was due to the sharing of the DNA and mica counterions.[3] The correlation between divalent cations on both the DNA and mica surface could generate a net attraction force that pulled DNA onto the surface. Recent studies confirmed that the adsorption was driven by the cooperative effect of divalent metal ion condensation along DNA and their reaction with the surface groups.[5] Furthermore, the ions adsorbed on the DNA surface have been classified into tightly bound ions and diffusively bound ions, which suggests that ion species play crucial roles in the stability and folding kinetics of DNA.[6,7] Raman spectroscopy and atomic force microscopy (AFM) studies also proved that different divalent cations could cause different reactions with the base and phosphate groups of DNA.[8,9] All the studies showed that bridge ions greatly affected DNA immobilization and the morphology of DNA adsorbed on a surface and might further affect the formation of DNA monolayers.

Previous studies of flat-lying DNA monolayers on cationic lipid membranes[10−14] have confirmed that the incubation of the specimens prepared by using relatively large DNA molecules (such as λ-DNA) in a heating solution resulted in the formation of closely packed monolayers, regardless of the existence of DNA molecules in the heating solution. The formation of closely packed monolayers was mainly ascribed to the rapid movement of lipid molecules and some entropic topological constraints from large DNA molecules after the dimension reduction. However, some scattered, free membrane surfaces were also observed because they were not large enough to accommodate single λ-DNA molecules. For short DNA fragments, the formation of closely packed monolayers occurred only after the specimen was incubated in a heated DNA solution. These results suggested that temperature and DNA species might also play

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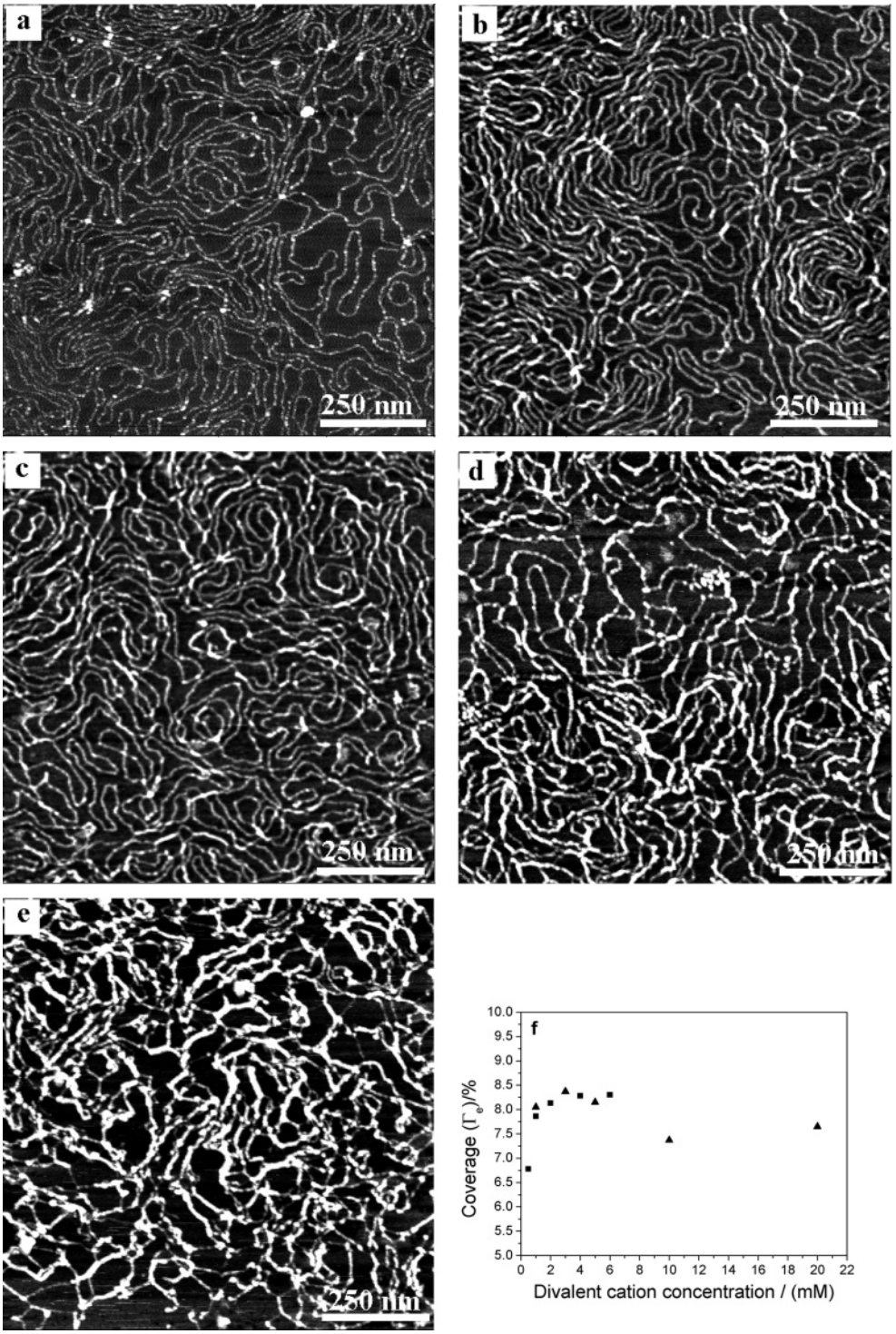

Figure 1. Typical AFM images of DNA films prepared from various Zn^{2+} concentrations: 1 mM (a), 3 mM (b), 5 mM (c), 10 mM (d), and 20 mM (e). The vertical scales are 3 nm. (f) The plot of the estimated surface coverage (Γ_e) of DNA films vs the concentration of Zn^{2+} (▲) and Mg^{2+} (■, ref 1). The DNA concentration used in this study is 7.5 ng/μL and the developing time is 1 h.

an important role in the formation of flat-lying DNA monolayers on a mica surface.

Ex situ AFM has been extensively used to investigate the formation mechanism of molecular monolayers on a solid surface owing to its extraordinary resolution and precision.[15−22] Although there are some disadvantages in ex situ AFM observation as previously mentioned,[19] a lot of information on the formation process of the molecular monolayers could still be revealed by ex situ AFM studies.

In this paper, ex situ AFM was employed to investigate the effects of the above-mentioned factors including bridge ions, DNA species, and developing temperature on the formation of flat-lying DNA monolayers on a mica surface. Although this study is supplementary to our previous work,[1] it is indispensable for practical application of the monolayers and further theoretical studies.

Experimental Section

Chemicals. λ-DNA (48502 bp), plasmid DNA pBR 322 (4361 bp), and linear DNA pBR 322/Pst I (4361) were purchased from Sino-American Biotechnology Corporation

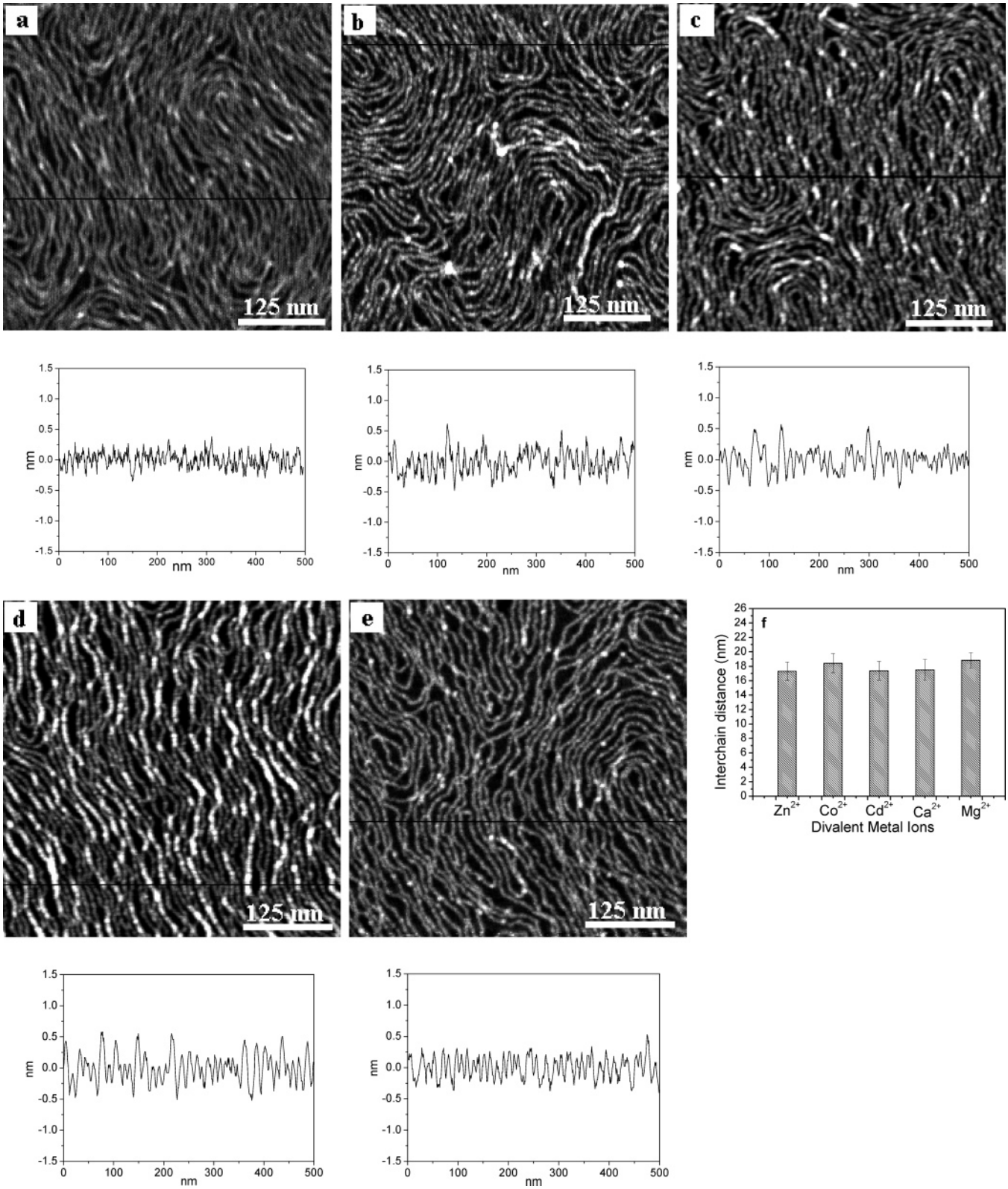

Figure 2. Typical AFM images and cross-section analysis of flat-lying λ-DNA monolayers constructed on a mica surface by using various divalent metal ions as bridge ions: Zn^{2+} (a), Co^{2+} (b), Cd^{2+} (c), Ca^{2+} (d), and Mg^{2+} (e). The vertical scales are 3 nm. (f) A histogram of the interchain distances for the flat-lying λ-DNA monolayers constructed on a mica surface by using the above metal ions as bridge ions. These specimens were developed in ultrapure water at 25 °C for 1 h.

(Beijing, China). Acetate salts ($Mg(OAc)_2$, $Zn(OAc)_2$, $Co(OAc)_2$, $Ca(OAc)_2$, and $Cd(OAc)_2$) were purchased from Sigma. (3-Aminopropyl)triethoxysilane (APTES) was purchased from Aldrich. Other chemicals were obtained from the Beijing Chemical Reagent Factory (Beijing, China). All of the agents were used as received without further purification. Ultrapure water (18.2 MΩ cm) was prepared with a quartz distillatory below its boiling point and then purified with a Milli-Q water purification system (Millipore Co. Ltd.). Muscovite mica ($KAl_2(AlSi_3)O_{10}(OH)_2$,V-1 grade) was purchased from Linhe Street

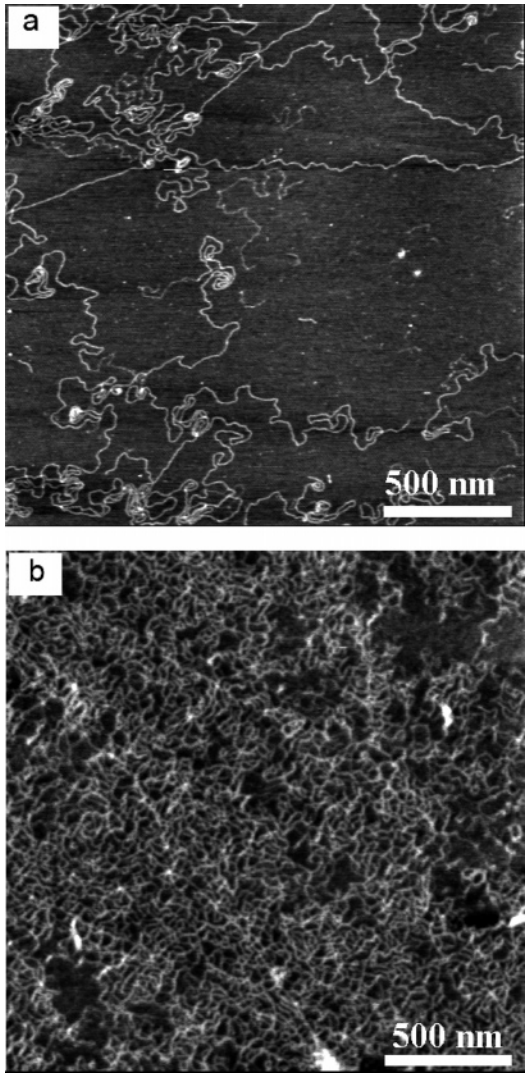

Figure 3. Typical AFM images of flat-lying λ-DNA films constructed on an APTES−mica surface with a silanized time of 5 min (a) and 120 min (b). The vertical scales are 3 nm. The specimens were developed in ultrapure water at 25 °C for 1 h.

Commodity Marketplace (Changchun, China) and was cut into about 1.2 × 1.2 cm^2 square pieces as substrates. Both sides of the mica were freshly cleaved before use.

Solution Preparation. The stock solutions of various DNA were diluted to 50 ng/μL with ultrapure water. The dilute DNA solutions were mixed with 2 mM various acetate salts ($Mg(OAc)_2$, $Zn(OAc)_2$, $Co(OAc)_2$, $Ca(OAc)_2$, and $Cd(OAc)_2$) of equal volume, respectively, as DNA monolayer formation solutions. For DNA films on a silanized mica surface, the dilute DNA solutions of 50 ng/μL were further diluted to 25 ng/μL with ultrapure water and used directly. In a control experiment which estimated the effect of the bridge ion concentration on the formation of DNA monolayers, the DNA solutions were diluted to 15 ng/μL and then were mixed with various concentration $Zn(OAc)_2$ solutions of equal volume, respectively.

The silanized solution was prepared by mixing APTES with a mixture of 50% anhydrous ethanol and 50% ultrapure water. The final concentration of APTES was 0.5% by volume.

Mica Silanization. Freshly cleaved mica sheets were immersed in the silanized solutions for 5 and 120 min, respectively, and subsequently rinsed thoroughly with anhydrous ethanol and ultrapure water in a stepwise manner.

Formation of DNA Monolayers. The construction of DNA monolayers is according to our previous method.[1] Briefly, after incubation at 38 °C for 1 h, an aliquot of 20 μL of the formation solution was dropped onto a freshly cleaved mica surface or silanized mica surface and remained for about 8 min to allow sufficient immobilization of the DNA molecules. Then, the specimen was immersed in a tube with 2 mL of ultrapure water to develop for 1 h. The immersion caused both the removal of ion bridged DNA−DNA or DNA−mica surface and the rearrangement of DNA molecules and generated a flat-lying DNA monolayer. After development, the ultrapure water was sucked up, and then the specimen was taken out and immersed in anhydrous ethanol for 20 s. The treatment with anhydrous ethanol could both stop the development of monolayers immediately to keep the structure of DNA monolayers because of rapid diffusion of water molecules into anhydrous ethanol and enhance stabilization of the DNA films.[1] Finally, the specimen was dried under air. Although the process of removal, treatment, and drying might affect the structure of DNA monolayers, it was possible that it could not greatly disturb the DNA monolayers.

AFM Measurements. All AFM experiments were carried out using a Digital Instruments Nanoscope IIIa (Santa Barbara, CA). Typical AFM images were acquired in tapping mode with silicon (Si) cantilevers (spring constant, 0.6−6.0 N/m) below their resonance frequency (typically, 67−150 kHz) at room temperature under ambient conditions. All AFM images were raw data except flattening. All average values were measured from at least five different AFM images.

Results and Discussion

The divalent metal ions, Cd^{2+}, Zn^{2+}, Co^{2+}, and Ca^{2+}, could bind both to the phosphate groups of DNA and to bases, while the Mg^{2+} ion could only bind to the phosphate groups of DNA.[8,23,24] Their binding affinities for the bases were in decreasing order ($Cd^{2+} > Zn^{2+} > Co^{2+} > Ca^{2+}$) and for the phosphate groups were in increasing order ($Cd^{2+} < Zn^{2+} < Co^{2+} < Ca^{2+}$).[24,25] It was also found that Zn^{2+} and Co^{2+} could bind DNA to mica tightly as bridge ions, while Ca^{2+}, Mg^{2+}, and Cd^{2+} could not bind DNA to the mica surface tightly or could not bind DNA at all.[2,5] The large discrepancies in interactions between these metal ions and DNA or mica resulted in the above-mentioned ions being chosen as typical divalent ions to estimate the effect of bridge ions on the formation of flat-lying DNA monolayers.

The concentration of bridge ions might influence the amount and morphology of DNA bound to the mica surface, since the binding of DNA to the mica surface was accomplished by their conglutinations between DNA and mica surface. The effect of bridge ion concentration varying from 1 to 20 mM on the DNA monolayers was first investigated. The 1 mM concentration was chosen as the lower limit because under this concentration DNA was maximally bound to the mica surface when the DNA concentration was 0.5 ng/μL.[2] A DNA concentration of 7.5 ng/μL was used in this experiment. Although this concentration could not produce the most compact DNA monolayers, the change in the amount and morphology of DNA bound to the mica surface as the concentration of bridge ions was increased could be observed clearly. Figure 1 is a series of AFM images of DNA films prepared in various Zn^{2+} concentrations and the plot of estimated surface coverage vs Zn^{2+} concentration. In the case of low concentration (lower than 10 mM), DNA molecules could disperse on the mica surface randomly with similar morphology as shown in parts a, b, and c of Figure 1. The amount of DNA molecules bound to the mica surface was estimated from the surface coverage[1] and almost was the same

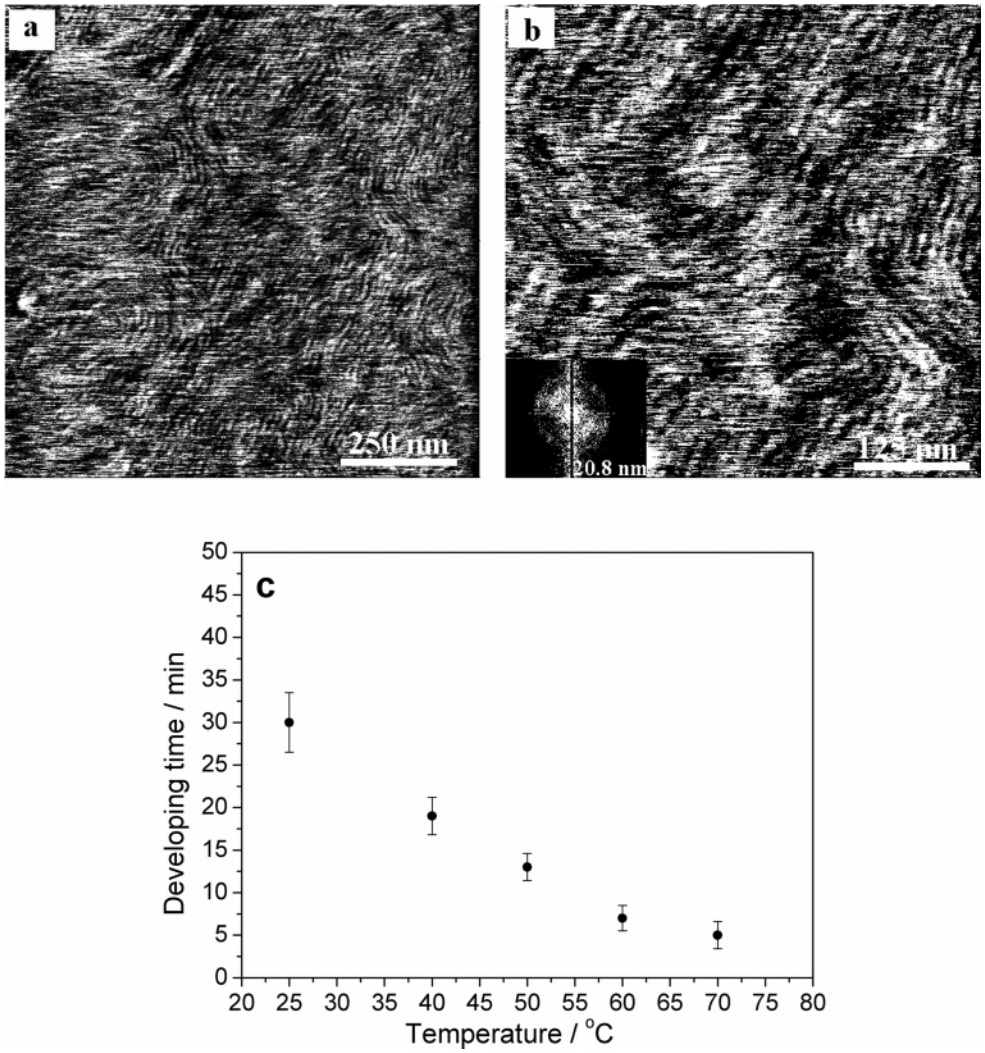

Figure 4. (a) Typical AFM phase images of flat-lying λ-DNA monolayers constructed on a mica surface by the development of specimen in ultrapure water at 40 °C for 1 h. (b) An enlarged image of (a) and the inset shows its 2D Fourier transforms, suggesting the interchain distance is 20.8 nm in the case that the coefficient of tip broadening, 5.9, has been considered. (c) A plot of approximately minimal time of the monolayer formation as a function of developing temperature. Zn^{2+} was used as the bridge ion in the experiments.

for different specimens (as indicated in Figure 1f). These results suggested that the Zn^{2+} concentration in this range did not greatly disturb the amount and morphology of DNA molecules adsorbed on the mica surface, which was similar to that of Mg^{2+} (Figure 1f). After that, some obvious aggregations between DNA chains appeared (parts d and e of Figure 1). These aggregations might be ascribed to the short developing time in which the overlapping DNA chains did not sufficiently disperse. The amount of DNA molecules adsorbed on the mica surface in such a situation was hard to estimate because of the aggregation of DNA molecules. Other bridge ions had a similar behavior (data not shown here).

It was noticeable that the concentration of bridge ion in the lower than 10 mM range did not greatly disturb the amount and morphology of DNA molecules adsorbed on the mica surface. This might be ascribed to the formation mechanism of the DNA monolayers. The formation of the DNA monolayers was mainly due to ion diffusion and DNA rearrangement. The removal of ion bridged DNA−mica or DNA−DNA surfaces and the lateral movement of DNA molecules resulted in the dispersion of DNA aggregations, rearrangement of DNA molecules, and the formation of DNA monolayers. In the formation process of DNA monolayers, most of the divalent cations were removed, which resulted in a weak effect on bridge ion concentration. Hence, 1 mM was chosen as the bridge ion concentration in the following experiments.

The effect of ion species on the formation of flat-lying DNA monolayers on a mica surface is shown in Figure 2. Parts a, b, c, d, and e of Figure 2 are AFM results of flat-lying λ-DNA monolayers constructed on a mica surface by using Zn^{2+}, Co^{2+}, Cd^{2+}, Ca^{2+}, and Mg^{2+} as the bridge ions, respectively. The images are typical of those obtained from at least five separate specimens. Each ion produced a compact DNA film with similar structure: a rule of thumb. Cross-section analysis showed that the main height of the fibers in the DNA films was about 0.5 nm for all of the images. The height was approximately the reported chain height of a single double-stranded DNA by tapping mode AFM in air,[26,27] which suggested that most of the DNA molecules arranged themselves on the mica surface into a separated one. The interchain distances estimated by their two-dimensional (2D) Fourier transforms were approximately 17.3, 18.4, 17.4, 17.5, and 18.8 nm, respectively (Figure 2f). The coefficient of tip broadening has been considered in estimation of the interchain distances. The large interchain distances might result from the removal of the bridge ions. The removal of the bridge ions caused the effect that the direct repulsive interactions between adjacent molecules could not been screened effectively, which resulted in the large interchain

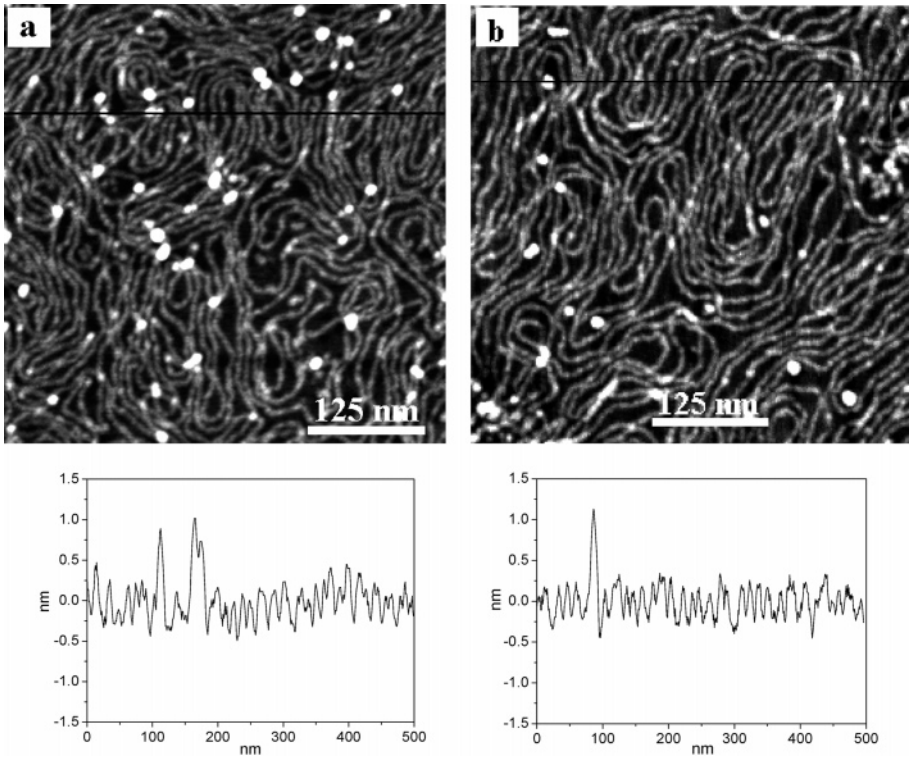

Figure 5. Typical AFM images and cross-section analysis of flat-lying DNA monolayers constructed on a mica surface by using plasmid DNA pBR 322 (a) and linear DNA pBR 322/Pst I (b) as the DNA sources. The vertical scales are 3 nm. The specimens were developed in ultrapure water at 25 °C for 1 h. Mg^{2+} was used as the bridge ion in the experiments.

distances. The approximately uniform interchain distance indicated that the DNA molecules in the film were densely packed and well organized. All of these results confirmed that the flat-lying, well-ordered λ-DNA monolayers could be constructed on a solid mica surface when the above-mentioned divalent cations were used as bridge ions.

However, there are several slight discrepancies among the DNA monolayers constructed by using the above-mentioned divalent cations as bridge ions. The conglutinations of DNA chains frequently appeared in the DNA monolayers constructed by using Zn^{2+} as the bridge ion (Figure 2a). Cross-section analysis showed that the main height of the conglutinations was about 0.5 nm. Ca^{2+} and Cd^{2+} produced more compact DNA monolayers with some obvious aggregations, especially for the DNA monolayers constructed by using Ca^{2+} as the bridge ion (parts c and d of Figure 2). The height of the aggregations was approximately 0.8−1.0 nm and so they might possibly be overlapped DNA bundles with several DNA chains.[28−30] Co^{2+} produced well-ordered, flat-lying DNA monolayers similar to that of Mg^{2+}. The differences may mainly result from the different interactions between the bridge ions and DNA or mica, since the formation of DNA monolayers is a result of equilibration of all these interactions.[1] For Zn^{2+}, the strong interaction between it and the bases of DNA caused segmental DNA changes in structure including partial disordering of the B-form backbone and reduction in base stacking and pairing.[8,24,31] Such segmental denaturation might enhance the conglutinations of DNA chains by hybridizing with complementary bases of other denatured chains in the formation solution, especially in solution of high DNA concentration. When DNA molecules adsorbed on the mica surface, most of the conglutinations might be kept because DNA molecules were immobilized on the mica surface tightly enough to prevent them from dispersing.[2,5] The main height of the conglutinations, 0.5 nm, also suggested that the conglutinations came mainly from hybridization of complementary base pairs, since it approached the chain height of a single double-stranded DNA. It was noticeable that the conglutinations could be frequently observed only in high DNA concentration. Cd^{2+} could also interact as strongly with DNA molecules as that of Zn^{2+}, but it did not immobilize DNA on the mica surface tightly.[2] When the specimen was immersed in ultrapure water to develop, some conglutinations of DNA chains could gradually disperse as the Cd^{2+} was removed because of the weak interaction between it and the mica surface. Under the weak interaction, the DNA molecules can easily move and be stretched so as to decrease the electrostatic repulsion between DNA chains and interfacial Gibbs free energy to a minimum. Thus, most of the conglutinations were not kept in the DNA monolayers. However, the strong interactions between it and the bases of DNA produced more compact monolayers. Although Ca^{2+} could not alter DNA structure as extensively as Zn^{2+} and Cd^{2+}, [25,32] it strongly interacted with the phosphate groups of DNA.[2] The unique affinity between it and the phosphate groups of DNA might result in the extensive contacts between DNA chains. The contacts would form overlapping DNA bundles with several DNA chains in DNA solution, especially in high concentration DNA solution. During the formation of DNA monolayers in ultrapure water, the contacts gradually dispersed as the Ca^{2+} removed, which induced the formation of more compact DNA monolayers. Some contacts that failed to disperse into separated DNA molecules formed aggregations in DNA monolayers. The heights of the aggregations were approximately 0.8−1.0 nm, suggesting that they were overlapped DNA bundles. Co^{2+} could not extensively interact with DNA molecules but could immobilize DNA to the mica surface tightly. Hence, it produced well-organized DNA monolayers similar to that of Mg^{2+}. It was concluded that other factors

might also be involved and a more reasonable theoretical model was needed to further discuss this issue.

These differences suggested that the appropriate interactions between bridge ions and DNA or mica surface were important for the refined structure of flat-lying DNA monolayers. The differences in the refined structure of flat-lying DNA monolayers are a result of all these interactions between bridge ions and DNA or mica. If the interactions are enough prevent the movement of DNA molecules, then the DNA monolayers will not form on the mica surface. It was confirmed by the fact that the flat-lying DNA monolayers could not form on an APTES−mica surface in our experiment because the strong interactions between DNA and substrate prevented the movement of the DNA (Figure 3).[33] After the mica surface was silanized completely, the DNA molecules obviously crossed each other and formed DNA networks (Figure 3b).

Figure 4 shows the result of a specimen developed in heated ultrapure water, and Zn^{2+} was used as the bridge ion here. When the specimen was developed in ultrapure water at 40 °C, the conglutinations of DNA chains observed in Figure 2a almost disappeared as shown in Figure 4a. The flat-lying DNA monolayers became better than the ones observed in Figure 2a as indicated by the insert in Figure 4b. The uniformity of the average radii of the ring in the vertical axis and the horizontal axis suggests that the monolayers are well ordered. The formation of such excellent monolayers may be ascribed to the two following factors. One factor is that the energy of DNA lateral movement increased as the developing temperature increased. Thus, some DNA segments that could not rearrange at room temperature now could further rearrange into an ordered structure and finally form more ordered monolayers. The other factor is that more cations can be removed from the DNA surface and mica surface, which might enhance the rearrangement of DNA molecules. It was noticeable that the interchain distances were increased from 17.4 to 20.8 nm as the development proceeded in heated ultrapure water. This phenomenon suggested that the loss of DNA molecules occurred during the development in heated ultrapure water. Furthermore, the developing time for the formation of well-ordered monolayers in heated ultrapure water was also shortened due to the rapid rearrangement of DNA molecules and ion diffusion, as shown in Figure 4c.

Figure 5 shows the effect of DNA species on the formation of DNA monolayers. In this experiment, the plasmid DNA pBR 322 and linear DNA pBR 322/Pst I were investigated due to their large property differences from λ-DNA. It is well-known that the pBR 322 is supercoiled and has some degree of writhing under normal conditions. The linear DNA pBR 322/Pst I is so far shorter than λ-DNA. The huge length of λ-DNA can facilitate the formation of DNA monolayers by some entropic topological constrains after its dimensionality reduces from three to two dimensions.[11] Despite their large differences in properties, the flat-lying monolayers could both form on the solid mica surface when either DNA were used as the DNA source as observed in Figure 5. The DNA monolayers are similar to that of λ-DNA except for larger interchain distances and several bright dots dispersed in the monolayers. Their interchain distances were estimated approximately to be 20.7 and 20.2 nm by their 2D Fourier transforms, respectively. Cross-section analysis showed that the height of the bright dots was approximately 1.0−2.0 nm. The bright dots were also found in previous studies of the Col E1 plasmid and pZT plasmid DNA monolayers on cationic lipid membranes[11] and have been ascribed to the instable free ends that cause the tip to jump up while scanning across them. In our experiments, the bright dots might be DNA aggregations caused by the treatment of anhydrous ethanol, since the scanning was carried out in air.[34] The interactions between a large number of linear molecular ends and strong supercoiled press of plasmid DNA molecules in densely packed monolayers might also produce such bright dots.

Conclusions

In summary, the flat-lying, well-organized DNA monolayers can be constructed on a mica surface by developing the specimen in ultrapure water, regardless of DNA or bridge ion species in the range of our studies. However, there are several slight discrepancies among the DNA monolayers constructed by varying DNA and bridge ion species. AFM results showed that when Zn^{2+} was used as the bridge ion, there are some conglutinations of DNA chains in the monolayers. Cd^{2+} and Ca^{2+} produced more compact DNA monolayers with some obvious aggregations. For Co^{2+}, the DNA monolayers are similar to that formed by Mg^{2+}. The differences are mainly due to the different interactions between bridge ions and DNA or mica surface. It is observed that the developing temperature increased to higher than 40 °C is beneficial to remove the conglutinations of DNA chains and form a better monolayer. These findings confirm that appropriate interactions between divalent cations and DNA or mica surface are very important for the formation of well-organized DNA monolayers. When plasmid DNA pBR 322 and linear DNA pBR 322/Pst I were used as the DNA source, the flat-lying DNA monolayers could still form on the mica surface but there are some bright dots dispersed on the monolayers. This information may be useful for the practical application of the monolayers and further theoretical studies.

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