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AFM studies of DNA structures on mica in the presence of alkaline earth metal ions

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Abstract

As counterions of DNA on mica, Mg^{2+}, Ca^{2+}, Sr^{2+} and Ba^{2+} were used for clarifying whether DNA molecules equilibrate or are trapped on mica surface. End to end distance and contour lengths were determined from statistical analysis of AFM data. It was revealed that DNA molecules can equilibrate on mica when Mg^{2+}, Ca^{2+} and Sr^{2+} are counterions. When Ba^{2+} is present, significantly crossovered DNA molecules indicate that it is most difficult for DNA to equilibrate on mica and the trapping degree is different under different preparation conditions. In the presence of ethanol, using AFM we have also observed the dependence of B–A conformational transition on counterion identities. The four alkaline earth metal ions cause the B–A transition in different degrees, in which Sr^{2+} induces the greatest structural transition.

Keywords: DNA; Alkaline metal ion; B–A conformational transition; AFM; Contour length; Persistence length

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1. Introduction

AFM has been successfully used for studying the morphologies of biological molecules for its less required manipulation of sample [1] and subnanometer height resolution [2]. However, one of the characteristics of AFM is the two-dimensional representation of biological molecules. The deposition of DNA molecules from the solution onto the ultra-flat mica surface is a typical process from three-dimension to two-dimension, which is considered to be governed solely by diffusion [3]. Under extreme conditions, DNA molecules can either reequilibrate on the surface to give useful information on the molecular structures or adopt a so-called kinetic trapping mode to preserve their conformation in 3-D solution. The DNA persistence length (P), is a reflection of the correlated relationship between segment directions, i.e. the flexibility of DNA [4]. Quantitative experimental studies of DNA flexibility revealed that the persistence length of a random DNA sequence in moderate salt buffer is approximately 50 nm. In 2-D solution, it is given that:

$$R_{2D}=[4PL(1-2P/L(1-e^{-L/P}))]^{1/2} \qquad (1)$$

where R is the end to end distance and L is the contour length. Because the end to end distance of DNA in trapping form is significantly smaller than that equilibrating on the surface, the comparison between measured R and theoretical calculated

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R in Eq. (1) is useful in distinguishing whether or not the DNA molecules have equilibrated on the mica surface.

Besides the morphologies of DNA on mica, the studies of DNA secondary structures on mica are also interesting and significant. DNA can exist in right-handed A, B or C double helical conformations and left-handed Z conformation [5,6]. DNA conformations are sequence specific [7]. Also, alternative conformations are adopted in response to the environment. In solution, variations in ionic strength, water activity and ligand/protein binding all can strongly modulate DNA structures. The conformational transition from B-form to A-form occurs if either salt of high concentration [8] or dehydrating agents [9], such as ethanol were added to reduce the water activity. Previous studies have indicated that the degree of B–A conformational transition is also dependent on the identity of the counterion. The theoretical model discussed by Shuratovskii [10] suggested that the steric energy of binding to DNA minor groove is correlated with B–A transition. Cs^{+} can form stable complex through favorable direct coordination to minor groove to stabilize the B form. Li^{+} and Mg^{2+}, however, interact with minor groove via water bridges. The position of waters around a DNA structure is correlated with its conformation. Among the other models touching on this viewpoint, the Counterion–water affinity model [11] is put forward to estimate the effect of a particular cation on the DNA conformation transition. It assumed that the high water affinity of counterion is equivalent to further lowering water activity. The stronger the cationic hydration, the fewer water molecules around the DNA chains.

Atomic Force Microscopy (AFM) studies of B–A conformational transition have provided insights into the physical properties of DNA under changed conditions. Different helical rises between B-form and A-form DNA make a shift of contour length of a single DNA molecule with specific base pairs when the environment is changed. The AFM data reflected from the image are then meaningful in assessing the secondary structures of DNA.

It has been known that DNA deposited on freshly cleaved mica from an aqueous solution containing Mg^{2+} or many other divalent cations can yield stable images of DNA [12,13]. Different divalent cations might affect the equilibration and the secondary structures of DNA on mica in a remarkably different way. In this paper, this speculation has encouraged us to investigate the ion species dependence of DNA structure on mica. The trapping effects in the four salt solutions were discussed to estimate the relationship between the behaviors of DNA molecules on mica and ionic conditions. We reported the AFM characterization of DNA molecules in the presence of alkaline earth metal cations, Mg^{2+} Ca^{2+}, Sr^{2+} and Ba^{2+} The measured contour lengths of DNA molecules provided data for analyzing the contribution of different ions to B–A conformational transition in the ethanol water solution.

2. Experimental

Plasmid DNA pBR322 and linear Plasmid DNA pBR322 (pstI) of 4361 bp were purchased from Sino American Biotechnology Co. (Beijing, China). All salts were of analytical pure grade. The stock solution of 500 ng/μl DNA was diluted to a final concentration of 1.5 ng/μl with doubly distilled deionized water. 10 μl 1.5 ng/μl plasmid pBR322 DNA and 10 μl 4 mM Mg^{2+} were mixed and incubated for 30 min at 25 °C. 10 μl of this incubated solution were dropped immediately onto freshly cleaved mica substrate. After air drying for 3 min, the mica was gently rinsed with pure water for 15 s, then dried in air and left sealed. Upon B–A conformational transition, 20 μl 1.5 ng/μl linear DNA, 25 μl 4 mM Mg^{2+} and 5 μl or 12 μl 100% ethanol were mixed and then treated as in water solution, with the exception that the samples were rinsed with ethanol water solution at a particular concentration. The other salts solutions of DNA were treated in the same way as described above.

A Nanoscope IIIa Multimode AFM system (Digital Instruments, Santa Barbara, CA, USA) was performed in Tapping mode. The J scanner with a maximum range of 120 μm was used for all samples. Standard silicon cantilevers were obtained from Digital Instruments. The scanning rate was approximately 1 Hz. All imaging was in air under ambient conditions. Using Top-View

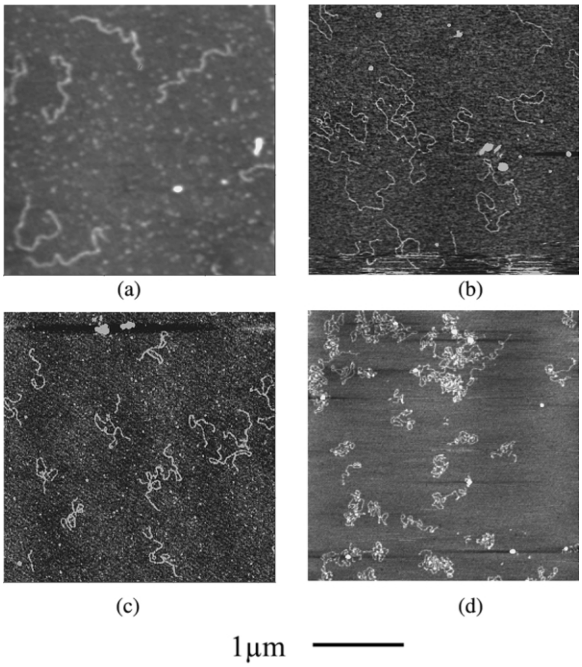

Fig. 1. AFM images of pBR322 (pstI) DNA on mica in the solutions containing 2 mM alkaline earth metal ion. (a) Image of DNA in the presence of Mg^{2+} show the extended DNA molecules, (b) DNA molecules gradually bending in the presence of Ca^{2+} (c) Under the condition of Sr^{2+}, image of more looped DNA molecules, (d) Cross-overed DNA molecules as Ba^{2+} present.

software, lengths were carefully measured by summing the fragment lengths. All average values are reproducible in at least three to five experiments.

3. Results

3.1. The DNA end to end distance

The images of linear DNA molecules on mica in the presence of the four alkaline earth metal ions are displayed in Fig. 1. Ranging from Mg^{2+}, Ca^{2+}, Sr^{2+} to Ba^{2+} DNA molecules tend to be more bending and crossovered. Table 1 summarizes the experimental measured results of the mean DNA end to end distances in the presence of different salt solutions. From Table 1, it can be found that the *R* decreased in the four salt solutions as $Mg^{2+} > Ca^{2+} > Sr^{2+} > Ba^{2+}$ (the number of measured molecules under each condition is approx. 150). In the case of Mg^{2+} solution the mean end to end distance *R* is 805.6 nm. In the solutions of Ca^{2+} and Sr^{2+}, respectively, *R* decreased gradually. The presence of Ba^{2+}, however, displayed a drastically smaller *R* value than that of the other ions.

Two effects are possibly involved in the changes in *R*. The first one is that the persistence lengths of DNA molecules are dependent on ion types in three-dimensional solution [14]. Another one is the trapping effect of the interaction between sample and mica surface. Control experiments were carried out to clarify the extent to which trapping effects preserve smaller end-to-end distance in the 2-D situation. Sample preparation is the same as the process described in Section 2 with the exception that the sample is rinsed with pure ethanol after being dried for 3 min (I) or deposited for 1 min and 6 min, respectively, and then rinsed with water (II). Experimental results revealed that these control preparation methods do not significantly modify measured *R* when Mg^{2+}, Ca^{2+} or Sr^{2+} is counterion. The observed difference in apparent end-to-end distances should result from the effects of ion types on the flexibility of DNA molecules. In the case of Ba^{2+}, however, longer deposition time or rinsing without water all causes DNA molecules to be relatively stretched.

Table 1
Change in measured contour length and measured *R* by different metal ions

Ion	Measured end to end distance *R* (nm)	Measured contour length in 10% ethanol solution (nm)	Measured contour length in 20% ethanol solution (nm)
Mg^{2+}	805.6	1428.1	1362.4
Ca^{2+}	684.1	1342.2	1271.2
Sr^{2+}	502.5	1306.4	1207.4
Ba^{2+}	210.4	1320.6	1289.3

Even under these conditions, the *R* of DNA molecules in the 3-D solution of Ba^{2+} is still significantly smaller than that in the solution containing Mg^{2+}. Thus, the structures on mica should result from the combination of these two effects and sample preparation methods play an important role in the Ba^{2+}-mediated interaction between DNA and mica.

3.2. Contour length of DNA molecules

To evaluate the influence of counterions of different types on the DNA conformations, we performed the same manipulation procedure to obtain the AFM images of DNA in the four salt solutions. Plasmid pBR322 DNA was chosen due to the fact that its circular shape facilitates identifying definite contour lengths and avoids artifacts caused by chains broken or contamination. The images of DNA molecules at 10% and 20% ethanol water solution, respectively, were shown in Fig. 2. The DNA molecules in Fig. 2a–c are well separated, allowing for unambiguous measurement of the contour length of each molecule. The values of contour length for the presence of different cations are shown in Table 1. When Mg^{2+} was present, the mean contour length was approximately 1428.1 nm, corresponding to a mixed composition of B-DNA and A-DNA at the low ethanol concentration. In the solution containing Ca^{2+} the measured length was 1342.2 nm. It can be seen that in the presence of Sr^{2+}, the contour length shortens further to 1306.4 nm, an indication of increased B–A conformational transition. In contrast to the solutions of Mg^{2+}, $Ca^{2+}$and Sr^{2+}, DNA molecules appeared to have intramolecular interaction with a lot of cross-overs in the Ba^{2+} solution. The chains are difficult to be traced. At a larger statistic range (500 molecules), the roughly measured length is 1320.6 nm, which is more than 1306.4 nm in the solution of Sr^{2+}. This experimental result exhibited less change in DNA conformation upon addition of Ba^{2+} than that of Sr^{2+}. At higher concentration of ethanol, the contour lengths decrease further in the same order as 10% ethanol is present as shown in Fig. 2. We investigated the morphologies of DNA molecules on mica only at the concentration of ethanol less than 20% for the purpose of observing the B–A transition. Even higher ethanol concentration likely induce DNA condensation in the presence of divalent ions, which is worth systematically studying in future. The interaction tendency between two DNA molecules toward condensation in the bottom image of Fig. 2a might indicate the agreement with the previous studies of the condensation intermediate of linear DNA in the ethanol solution containing Mg^{2+} [15].

4. Discussion

4.1. The deposition of DNA on mica

Excluded volume effects result from coulombic repulsion. The repulsive coulombic interactions between the phosphate groups of the chains prevent the DNA segments from overlapping and thus alter the chain dimensions [16]. To the DNA molecules with contour length larger than ~20 persistence length, it is necessary to take into account the excluded volume effect. It has been uncovered that the DNA solution containing Mg^{2+} can equilibrate on mica [17]. As presented, the non-negligible excluded volume effects for the DNA length of 4362 bp in our experiment introduce a larger value of *R* in the presence of Mg^{2+} because end-to-end distance should be 525.9 nm when *L* is the measured contour length of all B-form DNA and $P = 50$ nm. Taking the measured *R* in the case of Mg^{2+} and measured contour length into the Eq. (1), we determined that the persistence length is 141.7 nm, which is in the range of *P* for long fragments observed previously [17].

The measured *R* (Table 1) varies as a function of the type of counterion. The present cations are in the same Group 2. Their ionic radius might determine the degree of their mediating DNA and mica. Mica surface is arranged as hexagonal recessed cavities with a spacing of 0.5 nm. Freshly cleaved mica has a negative surface charge. When DNA solution containing divalent cations is injected onto the mica surface, only those cations with enough small radius can fit into the mica cavities and substantially neutralize the negative charge of the mica surface. In this case, the binding of DNA to mica is eased and the effect, in turn, precludes

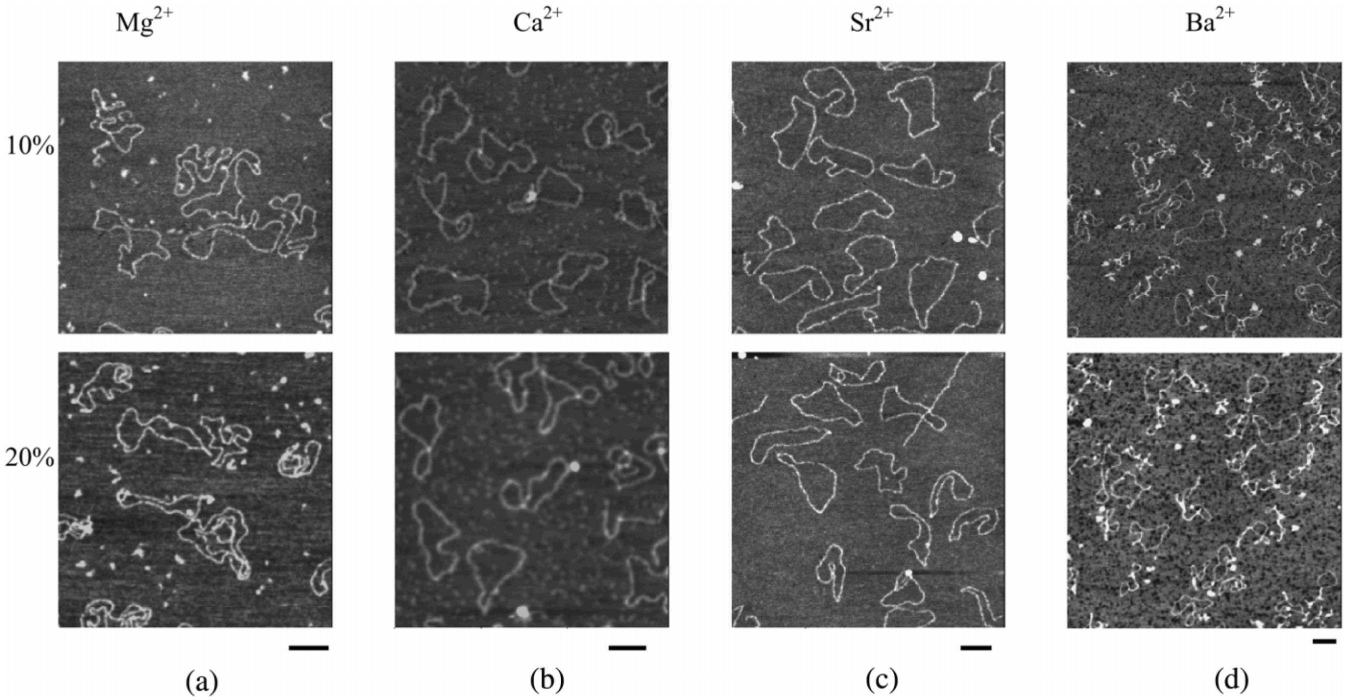

Fig. 2. AFM imaging DNA molecules in the presence of 2 mM alkaline earth metal ions. Top images: plasmid DNA pBR322 in the solution of 10% ethanol. Bottom images: plasmid DNA pBR322 in the solution of 20% ethanol. Every scale bar represents 200 nm used for the two images corresponding to the same ions.

the occurrence of kinetic trapping. *R* in the case of Mg^{2+} was considered here to be a criterion to estimate the effects of the other cations on the deposition of DNA on mica. In the solutions of Ca^{2+} and Sr^{2+}, with the increasing of cations radius compared with that of Mg^{2+}, *R* becomes slightly smaller, apparently indicating somewhat trapping tendency. If it is true, longer deposition time should be favorable for further equilibration of DNA molecules and the trapping effect should also be enhanced when the sample is rinsed with water immediately. Nevertheless, the obtained results confirm that DNA can equilibrate on mica in a short moment even when Ca^{2+} and Sr^{2+} have larger radius than that of Mg^{2+} A good explanation can be specified in the case of Ba^{2+}. Because the radius of Ba^{2+} is too large to fit into the hexagonal hole, the negative surface charge is still high enough to interact with DNA quickly. The steric hindrance of Ba^{2+} may limit the degree of binding, so some portion of unbinding fragments can equilibrate farther with longer incubated time. If the deposition time is limited and the sample is directly rinsed with deionized water, the initial Ba^{2+} loosely binding on the cavities is readily exchanged by H^+ in the rinsing process with water. The consequent H^+-exchange surface brings increased negative surface charge to further enhance DNA–mica interaction and facilitate trapping occurrence [17].

4.2. The B–A conformational transition

As known, addition of ethanol to the water solution decreases the water activity and causes a B–A conformational transition. The B–A transition is enhanced with the increase of the concentration of ethanol [15]. In our work, we found that the degree of B–A form transition is strongly correlated with the identities of alkaline earth cation at the same concentration of ethanol. As the mechanism studies described by Shuratovskii, we assumed the size of counterions determine the pattern of binding to DNA and thus contribute differently to the conformation DNA adopted. With the size increase of counterions, Sr^{2+} changes B-DNA into A-DNA in the greatest degree in comparison with the other ions. Although Ba^{2+} has larger size than Sr^{2+}, the significantly trapped DNA chains were hindered to adopt conformational transition [15].

The hydration abilities of counterions change in the order $Mg^{2+} > Ca^{2+} > Sr^{2+} > Ba^{2+}$. When viewed from the counterion–water affinity model, Mg^{2+} should most readily induce B–A form transition. The present results instead are in accord with the theoretical model investigation that Mg^{2+} can stabilize B-form through water bridge. Therefore, due to the different binding mode, Mg^{2+} is not comparable using the counterion water affinity model. The remaining three counterions also incompletely agrees with the counterion–water affinity model in which Sr^{2+} should cause less B–A transition than Ca^{2+} This indicated that the counterion–water affinity model cannot explain well the experimental results. At least, the hydration affinity of counterion should not be the governing effect on the B–A form transition in the solution of the Group 2 metal cations.

There are possibly potential doubts on our data interpretation needed to be clarified. Questions might arise from that interaction of DNA with mica surface, the drying process or the measuring method can also become a source of the conformational changes in DNA. Although mica can facilitate the B–A form transition, the binding of DNA to mica is fast enough to trap the conformation of DNA before mica induces B–A transition, especially in the case of long DNA, where numerous binding sites of DNA fragments constrain the B–A transition [18]. Thus, mica does not cause a significant artifactual change of contour length. In the drying process, it was reported that DNA molecules shorten 20–32 nm more in air than in buffer [19]. It is clear that compared with the observed shortened values the distortion of the DNA molecules during rinsing step of sample preparation do not strikingly change the secondary structures of DNA bound to mica. Meanwhile, the broadening effect of scanning tip leads to apparent lengthening of the DNA fragment. As pointed out by Rivetti et al. [20], the effect is constant and relatively negligible for long DNA fragment. It should be pointed out that all the potential factors of B–A transition above have no negative effect on the comparison of contour lengths among the investigated salt solutions.

5. Conclusions

AFM data indicated that both the topological structures and secondary structures of DNA molecules on mica are depended on the type of alkaline earth metal ions. The trapping effects on mica in the presence of the four metal ions demonstrated that DNA could equilibrate on mica in the solutions containing Mg^{2+} based on the measured end-to-end distance. Apparent small end-to-end distances in Ca^{2+} and Sr^{2+} solutions should be due to small persistence lengths inherent in three-dimensional solution. DNA molecules are more readily trapped in the 2-D Ba^{2+} solution and the interaction of Ba^{2+}-mediating is affected by sample preparation. We have shown that the conformation of DNA exhibits significant and specific conformational changes in response to various divalent counterions. The different changes in contour length of DNA in the four salt solutions under the same concentration condition of ethanol illustrate that the magnitude of inducing B–A conformation transition on mica varies in the following order: $Sr^{2+} > Ba^{2+} > Ca^{2+} > Mg^{2+}$.

Acknowledgments

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