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Adsorption and desorption behaviors of ssDNA molecules on mica surface by surface forces apparatus

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Abstract: An approach for studying the adsorption and desorption behaviors of single-stranded DNA (ssDNA) molecules on the mica surface by the surface forces apparatus (SFA) is reported, which can be used to characterize the precise thickness, configuration and mechanical properties of ssDNA layers on the mica surface at a certain buffer solution. The formation of ssDNA layers is first studied by tuning the ssDNA concentrations, and the experimental results indicate that the ssDNA concentration of 100 ng/μL is ideal for forming a ssDNA monolayer structure on the mica surface, and the hardwall value measured to be 1.04 nm under this circumstance is regarded as the thickness of the ssDNA monolayer confined on mica. The desorption behavior of ssDNA molecules from the mica surface is further studied by observing and comparing different shapes of the force-distance curves under certain conditions. It is found that the desorption of ssDNA molecules from the mica surface occurs as the monovalent salts are added into the gap buffer. It is inferred that the competition effect between monovalent and divalent salts can induce the release of ssDNA from substrate. The results also reveal that 10 mmol/L monovalent salts (Na^+) is sufficient for the desorption of ssDNA from mica. This work provides an applicable method to study the binding mechanism of ssDNA molecules on inorganic substrates.

Key words: single-stranded DNA; mica; adsorption; desorption; competition effect; surface forces apparatus

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Biological molecules play an important role in the fabrication of nanoscale devices due to their adjustable physical and chemical properties on inorganic substrates[1]. It is critical for researchers to manipulate the biological molecules with nanometer resolution in the emerging discipline of the nanotechnology[2]. The DNA molecule, a typical biological molecule, has obtained broad attention for its potential applications, such as bioengineering[3] and nanotechnology[4], which also strongly depends on the size, morphology and shape of the DNA macromolecules. Therefore, it is necessary to study some properties of DNA. As one of the key features, the adsorption of DNA on a liquid-solid interface has been investigated in depth over the past few decades.

To date, various apparatuses have been used for expanding the research on biomolecules' adsorption and desorption, such as the atomic force microscope (AFM)[5-8], scanning polarization force microscopy (SPFM)[9-10], scanning tunneling microscopy (STM)[11-13], and X-ray photoelectron spectroscopy (XPS)[14-15], etc. In this paper, a device named surface forces apparatus (SFA) is adopted to conduct this research. Compared with AFM and other techniques, the SFA stands out from the crowd by virtue of its high sensitivity of 10^{-8} N in force and resolution of 0.1 nm in distance[16]. Most important of all, it can provide accurate mechanical properties, such as the adhesion force between mica and DNA molecules. Besides, it offers the precise thickness of the DNA adsorption layers, which can also be referred to the height of DNA in AFM research.

In this paper, the adsorption properties of single-strand DNA (ssDNA) on the mica surface are investigated with the SFA technique. The interactions between the ssDNA-coated mica and the bare mica are measured, and by comparing the force-distance curves under different ssDNA concentrations, the influence of the ssDNA concentrations on ssDNA adsorption behaviors is evaluated. Besides, the desorption behavior of ssDNA on mica surface is observed when the monovalent salts are added into the gap buffer solution.

1 Experimental

1.1 Materials

In this paper, the mica used as substrate surface is ruby muscovite of Grade 1, which is supplied by the S&J Trading Inc. The muscovite mica is cleaved up to a thickness of 3 to 5 μm with the traditional methods in a laminar flow cabinet. Afterwards, it is cut off by Pt wires on the mica cutting stage. These mica sheets are then covered with a highly reflective silver layer (55 nm in thickness) on the backside with a magnetic sputtering deposition system to engender interfering fringes[17]. Two such

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freshly prepared mica sheets are eventually glued onto two curved silica disks respectively with the Epon 1004, supplied by Edmund Scientific Inc., and mounted into the apparatus in a cross-cylindrical configuration.

The DNA used in this work is ssDNA with 80 bases of adenine, purchased from Sangon Biotech Co., Ltd. (Shanghai), of which the sequences is 5'-[AAAA]$_{20}$-3'. Cobalt chloride ($CoCl_2$, >98%), sodium chloride (NaCl, >99.999%), and HEPES (>99.5%) are obtained from Sigma Aldrich. Two types of buffer solutions are used in this experiment. One is prepared with 1 mmol/L Cobalt chloride solution added to 10 mmol/L HEPES solution, and the other is prepared with 10 mmol/L sodium chloride solution added to 10 mmol/L HEPES, which are titrated to pH 7.5 finally before the experiment. Ultrapure water with a resistivity of 18.25 MΩ · cm is used throughout this work.

1.2 DNA-coated surface preparation

Three methods of surface preparation are generally utilized to investigate the adsorption properties of DNA on mica. The first method employs a biological tool called the Langmuir-Blodgett technique[18]. In the second method, the mica is aminated (AP-mica) to a positive surface to induce electrostatic binding of DNA to mica[19-20]. The last method uses the mica with a buffer solution containing divalent ions, such as Co^{2+}, Mg^{2+}, Ni^{2+}, Zn^{2+} and Ba^{2+}[21-26], which ascribes the adsorption of DNA onto mica to the "salt bridge" effect between the negatively charged mica surface and the negatively charged phosphate groups of the DNA backbone, and thus is used in this paper.

The ssDNA is first diluted to a stock solution of 750 ng/μL in the buffer solution: 10 mmol/L Tris-HCl, 1 mmol/L EDTA, pH 7.5, which is further diluted in 1 mmol/L Cobalt chloride and 10 mmol/L HEPES buffer solution at pH 7.5 to a final concentration of 100, 150, and 200 ng/μL. The freshly cleaved mica is first rinsed sufficiently by the aforementioned buffer solution, i.e., 1 mmol/L Cobalt chloride, 10 mmol/L HEPES, pH 7.5. Then, a 20 μL droplet of the ssDNA solution (100, 150, or 200 ng/μL) is placed onto the treated mica surface. After 10 min incubation, the surface is flushed completely with plenty of buffer solution to remove unbound ssDNA molecules, followed by a drop of this buffer solution left on the surface. This prepared surface is kept in a culture dish prior to use.

1.3 Surface forces apparatus

The SFA 2000 system from the SURFORCE, LLC at Santa Barbara, is utilized throughout this study. The distance between the two mica surfaces can be estimated by analyzing the optical interference fringes, namely, the multiple beam interferometry and fringes of equal chromatic order (FECO), which can be controlled over a range of 5 mm with a resolution of 0.1 nm by a four-stage mechanism[16]. The interaction between the two surfaces is measured by the defection of the cantilever spring with known stiffness, as shown in Fig. 1. The value of D_H in Fig. 1 is the abbreviation of the hardwall, which represents the thickness of the adsorption layer on the mica surface.

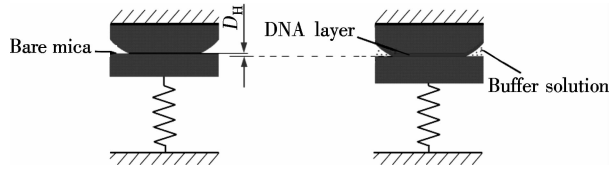


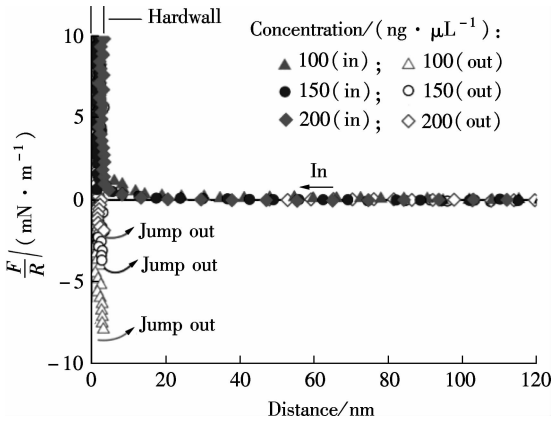
Fig. 1 Schematic diagram of the SFA for force measurements

2 Results and Discussion

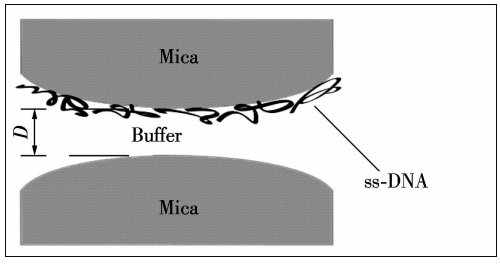
2.1 The effect of ssDNA concentration on ssDNA adsorption

In order to study the adsorption behaviors of ssDNA on the mica surface at different concentrations, the ssDNA is diluted in the buffer solution to various concentrations. The force is measured as a function of distance between two mica surfaces as shown in Fig. 2(a). A sight repulsion force appears at the distance of about 20 nm as the two mica surfaces are brought together, and such repulsion force increases as the distance decreases. When the two surfaces come into contact, the distance between these two mica surfaces tends to be constant, which is defined as the hardwall, as shown in Fig. 2(a). In this paper, the value of the hardwall keeps constant when the applied load increases beyond 10 mN/m. It can be easily seen since the hardwall is actually a reflection of the thickness of the adsorption ssDNA layer confined between the mica surfaces. Considering that the diameter of a double-stranded DNA molecule is approximately 2.2 nm[27], the diameter of the ssDNA molecule can be taken as around 1.1 nm. When the concentration of ssDNA is 100 ng/μL, the average value of the hardwall is approximately 1.04 nm, which is approximately equal to the diameter of the ssDNA molecule (1.1 nm). As the concentration of ssDNA increases to 200 ng/μL, the average value of the hardwall changes to approximately 2.03 nm, which is analogously consistent with the thickness of a bilayer of ssDNA on the mica surface. Therefore, one can speculate that the configuration of the adsorption layer of ssDNA on the mica surface may be a monolayer structure for 100 ng/μL and a bilayer structure for 200 ng/μL. It is noted that these structures (monolayer and bilayer) are stable for ssDNA adsorption on mica, because the measurements can be repeated and these adsorption layers can even exist when the applied loads exceed 10 mN/m. For the ssDNA concentrations between 100 and 200 ng/μL, such as 150 ng/μL, the hardwall is approximately 1.50

nm, which is a median between 1.04 and 2.03 nm. Hence, the structure of the ssDNA layer in this case may be a complex formation falling in between the monolayer and the bilayer.



(a)



(b)

Fig. 2 The mechanical properties at different ssDNA concentrations. (a) Force-distance curves between ssDNA layer and bare mica across the buffer solution (1 mmol/L $CoCl_2$, 10 mmol/L HEPES, pH 7.5); (b) Schematic diagram of ssDNA adsorption on mica surface

The adhesion force F/R is measured at the interface between the adsorbed ssDNA layer and the opposing bare mica (see Fig. 2 (b)) when the two surfaces are separated, as shown in Tab. 1. The results show that the adhesion force decreases roughly as the ssDNA concentration increases, which is, for example, −7.64 mN/m at the ssDNA concentration of 100 ng/μL and −1.93 mN/m at the concentration of 200 ng/μL, respectively. This indicates that the characteristics of the ssDNA adsorption layer are quite different from 100 to 200 ng/μL.

Tab. 1 Adhesion forces under different ssDNA concentrations

Concentrations of ssDNA/(ng · μL^{-1})	$\frac{F}{R}$/(mN · m^{-1})
100	−7.64 ± 0.10
150	−3.52 ± 0.08
200	−1.93 ± 0.05

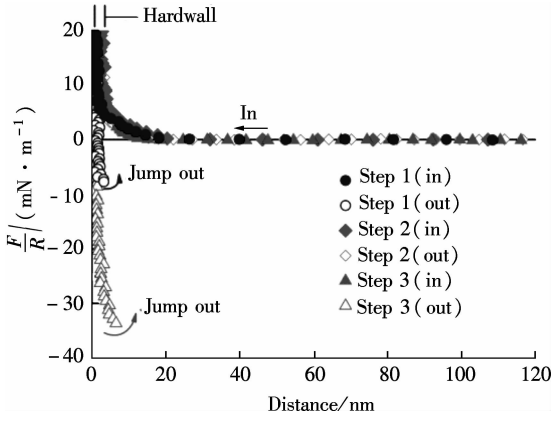
The mechanical properties between the ssDNA layer and bare mica at different ssDNA concentrations are represented in Fig. 2. In the analysis of hardwall, it is discovered that ssDNA molecules are doubtlessly immobilized on mica in all three concentrations by deposition from a solution containing divalent Co^{2+}. It is well known that the mica surface can attract DNA molecules through the correlation of the shared counterions in the presence of divalent salts[28]. That is, the divalent salts act as bridges between DNA molecules and the mica surface, which leads to the adsorption of DNA on mica. In this study, this view is confirmed as well. Besides, due to the influence of the ssDNA layers on mica, the adhesion force is sharply declined, compared to the situation in which only buffer solutions exist between two surfaces without any ssDNA molecules. Furthermore, it is observed that the values of hardwall are quite different at different ssDNA concentrations, which means that the adsorption structures of ssDNA are varied at different ssDNA concentrations.

2.2 Desorption of the ssDNA layer from mica surface

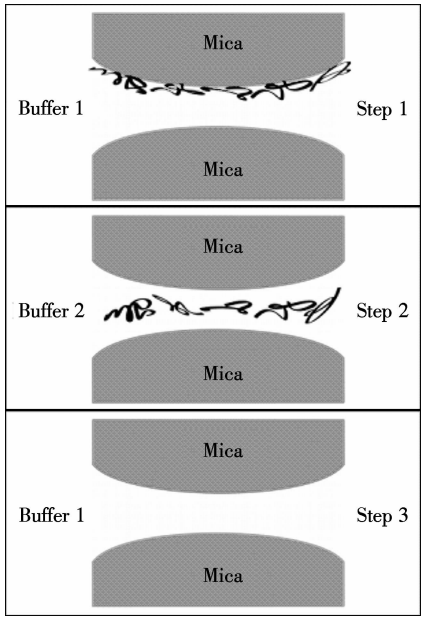
As is well known, there is a competition effect between monovalent and divalent salts, which can lead to the release of DNA molecules from the mica surface[7, 28-29]. In this study, the desorption behavior of the ssDNA layer on the mica surface is investigated by adding monovalent salts into the gap buffer. The ssDNA molecules are first adsorbed on the mica in $CoCl_2$ solution (Buffer 1: 1 mmol/L $CoCl_2$, 10 mmol/L HEPES, pH 7.5), then the gap buffer solution is replaced entirely with the buffer solution containing monovalent cations (Buffer 2: 10 mmol/L NaCl, 10 mmol/L HEPES, pH 7.5). As shown in Fig. 3, the interactions between the two surfaces have almost the same features during the approaching process under these three different conditions. When the surfaces are brought towards each other, no net force is observed until a separation distance of about 20 nm is reached, below which a repulsion force appears, leading to a constant separation finally. According to Fig. 3(a), there are distinctions in the hardwall values. In step 1, the hardwall is 1.21 nm that approaches the thickness of the monolayer of ssDNA as mentioned above. However, the hardwall in step 2 increases to 2.38 nm, which is far beyond the thickness of the ssDNA monolayer, and the hardwall finally decreases to 0.62 nm in step 3, which is smaller than the thickness of the monolayer of ssDNA. The hardwall value obtained in step 2 can be attributed to the impact of the residual ssDNA molecules suspended in the gap and the hydration force due to Na^+, whose apparent effect is to prevent the surfaces from being brought together further in a certain concentration approximately 10 mmol/L[30]. Furthermore, by the analysis of the hardwall value measured in step 3, it is believed that the ssDNA monolayer has been eliminated under this condition, and the small hardwall value is induced merely by the residual ssDNA molecules on mica.

Furthermore, the adhesion forces of these situations are also measured for investigating the desorption behavior of the ssDNA. Comparing the retraction process of these three steps, there are significant differences as well. In

step 1, there is an adhesion force of -7.82 mN/m, which disappears as the buffer solution is replaced with 10 mmol/L NaCl in step 2. Then in step 3, the adhesion force appears again at an even much greater magnitude. The adhesion force in step 3 (-33.75 mN/m) is at least 3 times larger than that in step 1 (-7.82 mN/m). The strong adhesion force measured in step 3 might be only the value measured between two bare mica surfaces across the buffer solution of 1 mmol/L $CoCl_2$, 10 mmol/L HEPES, pH 7.5, which indicates that the ssDNA molecules have been released from the mica surface during the action of the monovalent salts Na^+.



(a)



(b)

Fig. 3 The mechanical properties in different steps. (a) Force-distance curves between ssDNA layer and bare mica across different buffer solutions; (b) Schematic diagram showing the transition of the structure of ssDNA layer on mica surface

In order to further verify this hypothesis, another experiment is developed to investigate the mechanical properties between two bare mica surfaces across the buffer solution: 1 mmol/L $CoCl_2$, 10 mmol/L HEPES, pH 7.5. As shown in Fig. 4, it demonstrates that there is an adequate comparison between such two conditions by comparing these two different force curves. At very large separation (beyond 20 nm), there is no obvious interaction force between two surfaces. When the distance decreases to less than 20 nm, a slight repulsion force appears. Then, the two surfaces mildly jump from 6.6 nm into contact owing to the van der Waals interaction. We discover that the force-distance curves are almost the same at these two conditions. The only differences lie in the hardwall and the peak value of adhesion forces, which can be ascribed to the effect of residual fragments of ssDNA in the gap. We suspect that it is because of the presence of the residual ssDNA molecules that leads to the increase of hardwall from 0 to 0.62 nm. Moreover, the residual ssDNA molecules whittle down the adhesive attraction between the two mica surfaces, which is manifested by the decrease in the peak value of the adhesion force.

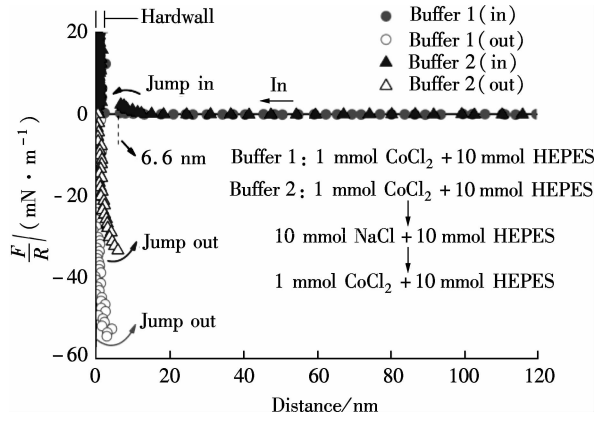


Fig. 4 Force-distance curves between two surfaces across the certain buffer solution

The desorption of ssDNA molecules from the mica surface is observed by adding the monovalent salts into the buffer solutions, which is attributed to the competition effect between the monovalent and divalent salts in the buffer. As the divalent salts in the buffer are replaced by the monovalent salts, the density of divalent salts on mica is markedly decreased, which conclusively lowers the binding strength of ssDNA[28]. Furthermore, the monovalent salts in the buffer can neutralize the negative charges on both the mica and ssDNA molecules, so that it can screen the binding sites of the divalent cations binding to ssDNA[29], and subsequently weakens the attachment of ssDNA molecules on mica. Under such conditions, the ssDNA molecules are easily squeezed out as the two surfaces approach each other under the applied force. Our results reveal that the required concentration of monovalent salts (Na^+) to release the deposition of ssDNA is about 10 mmol/L, which is much lower than that mentioned in other experiments[7, 29].

3 Conclusion

In this paper, the SFA technique is utilized to study the

adsorption and desorption behaviors of ssDNA molecules on mica surface. The thickness of the ssDNA layer on mica at different ssDNA concentrations can be precisely measured. The results show that the ssDNA concentration of 100 ng/μL is the most appropriate concentration for the formation of ssDNA monolayer on mica surface, and the thickness of the ssDNA layer at 100 ng/μL, measured to be 1.04 nm, is believed to be the exact thickness of a monolayer of ssDNA confined on mica. Besides, the desorption of ssDNA from the mica occurs when the monovalent salts are added. It is the competition effect between monovalent salts and divalent salts that induces the release of ssDNA from mica. Our results demonstrate that 10 mmol/L Na^+ can cause the desorption of the ssDNA layer from mica surface. Generally, we believe that the SFA can become a promising technique for researchers to unveil the mechanism of ssDNA action on mica and other solid substrates. It is not unreasonable to expect that the studying of ssDNA with the SFA can make a considerable contribution to the understanding of ssDNA properties.

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利用表面力仪研究单链 DNA 在云母表面的吸附与解吸附特性

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摘要:利用表面力仪(SFA)研究了单链 DNA 分子在云母表面的吸附与解吸附特性,获得了在特定缓冲液环境中,云母表面吸附的单链 DNA 分子层的精确厚度、几何结构及力学特性. 首先,通过改变单链 DNA 的浓度,研究了不同浓度条件下云母表面吸附的单链 DNA 分子层的几何结构. 此外,通过比较不同缓冲液中测得的力-距离曲线,研究了单链 DNA 分子的解吸附行为. 结果表明:当单链 DNA 浓度为 100 ng/μL 时,其在云母表面吸附形成单分子层,该单分子层的厚度约为 1.04 nm;而当 10 mmol/L 的单价阳离子(Na^+)加入到缓冲液中时,原本吸附在云母表面的单链 DNA 分子发生了解吸附现象,进一步分析表明,单价阳离子与高价阳离子之间的竞争效应是引起单链 DNA 分子解吸附的根本原因. 本研究为进一步研究单链 DNA 分子在无机基底表面的吸附机理提供了实用方法.

关键词:单链 DNA; 云母; 吸附; 解吸附; 竞争效应; 表面力仪

中图分类号:O561.4