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Single DNA Condensation Induced by Hexammine Cobalt with Molecular Combing

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We investigated the interaction between DNA and hexammine cobalt III [Co(NH₃)₆]³⁺ by a simple molecular combing method and dynamic light scattering. The average extension of λ-DNA-YOYO-1 complex is found to be 20.9 µm, about 30% longer than the contour length of the DNA in TE buffer (10 mmol/L Tris, 1 mmol/L EDTA, pH=8.0), due to bis-intercalation of YOYO-1. A multivalent cation, hexammine cobalt, is used for DNA condensation. We find that the length of DNA-[Co(NH₃)₆]³⁺ complexes decrease from 20.9 µm to 5.9 µm as the concentration of the [Co(NH₃)₆]³⁺ vary from 0 to 3 µmol/L. This observation provides a direct visualization of single DNA condensation induced by hexammine cobalt. The results from the molecular combing studies are supported by dynamic light scattering investigation, where the average hydrodynamic radius of the DNA complex decreases from 203.8 nm to 39.26 nm under the same conditions. It shows that the molecular combing method is feasible for quantitative conformation characterization of single bio-macromolecules.

Key words: Molecular combing, Hexammine cobalt, Condensation, Dynamic light scattering, Fluorescent microscopy

I. INTRODUCTION

The condensation of highly charged DNA molecules into compact structures by condensing agents has been shown to be a fundamental process in various biological processes, including gene therapeutic [1, 2], gene recombination, and strand exchange [3–5], DNA polymerization and ligation [6, 7], and replication [8]. Thus, an understanding of in vitro DNA condensation at the molecular level is of great significance. Traditional research methods for DNA, such as, ultraviolet-visible spectroscopy [9], electrochemistry [10], nuclear magnetic resonance [11], Raman spectroscopy [12], are mainly based on studying a large number of DNA molecules in ensemble. Information about single DNA molecules is buried in thousands of DNA molecules using these methods.

As the structure of DNA is string-like, it is easy to stretch it to promote observation. In previous studies, many techniques have been developed to stretch DNA molecules, including atomic force microscope (AFM) cantilever [13], optical [14] and magnetic tweezers [15] and spin-stretching [16], flowing liquid [17]. These single molecule methods allow us to study the behavior of biological macromolecules under applied tension. With this technique a constant pulling force can be applied on a single DNA molecule while measuring its elongation in real time. For example, Fu et al. have studied the compaction dynamics of single DNA molecule invoked by hexamine cobalt chloride [18]. For the magnetic tweezers experiments, the DNA molecule is unobservable and the end of DNA molecule must be modified by biotin and digoxygenin. Also, a large number of stretched DNA molecules can’t be observed at the same time.

However, Bensimon et al. [19] and Michalet et al. [20] developed a simple and feasible method, molecular combing. In molecular combing, a great quantity of stretched DNA molecules can be directly observed, and the end of DNA chain needn’t be modified. Molecular combing is a natural process, rather like long hair being pulled down a swimmer’s back as she emerges from a pool, and involves two basic steps: binding single DNA molecules by one or both extremities to a surface and using a receding meniscus to extend each molecule in a uniform and parallel manner over the surface [19]. Several combing methods based on interface movement have been proposed: (i) placing and sliding another substrate on a droplet of DNA solution [21], (ii) air-blowing a droplet [22], (iii) spin-stretching [23], (iv) dipping a substrate into a solution and then lifting it up (dynamic molecular combing) [24], and (v) placing a droplet of DNA solution on the hydrophobic surface [25].

Interaction between DNA and monovalent cations seems to be well described by traditional models for polyelectrolyte-counterion interactions, such as Manning-Ocsawa theory and the Poisson-Boltzmann
II. EXPERIMENTS

A. Chemicals and biochemicals

Bacteriophage $\lambda$-phage DNA was purchased from New England Biolabs without further purification. As received from the manufacturer, the concentration of $\lambda$-phage DNA stock solution is 300 ng/$\mu$L. The lambda bacteriophage DNA was diluted in TE buffer (10 mmol/L Tris, 1 mmol/L EDTA, pH=8.0). Poly(methyl methacrylate) (PMMA) was purchased from Japan. Water was deionized and purified by a Millipore system and had a conductivity less than 1$\mu$Ω. Hexammine cobalt III ([Co(NH$_3$)$_6$]$^{3+}$) was purchased as chloride salts and dissolved in bis-Tris (50 mmol/L, pH=6.6) before use. Bis-Tris was purchased from Sigma Company (USA). Fluorescent dye, oxazole yellow dimer (YOYO-1) was purchased from Molecular Probes Company (USA).

B. Fluorescence microscope

An inverted fluorescence microscope (FM, Nikon TE-2000E) was equipped with an oil immersion objective (Nikon, 100$\times$, N.A.=1.49) and a ND filter slider (Nikon, 330–385/460–490/510–550 nm). A 100 W high pressure mercury lamp was used as illumination. An intensified charge-coupled device (CCD) camera (512$\times$512 pixels, cascade II512) was utilized to acquire images. The CCD camera was mounted at the FM side port to obtain a wider field of view or at the single-lens reflex port (SLR) to improve the accuracy of measurement through a standard C-mount adapter. NIS-Elements D 3.1 software was used to acquire images and analyze data.

C. Preparation of glass coverslip

In this experiment, a coverslip (50 mm$\times$24 mm) was immersed in washing-up liquid for 12 h, then sonicated in Milli-Q water for 30 min at 45 °C, followed by washing with acetone, ethanol, NaOH, and then rinsed with Milli-Q water. Finally, the rinsed coverslip was put in the drying box.

D. Preparation of PMMA surfaces

The PMMA (570F Japan) in chloroform (10%) was spotted on the center of the cleaned coverslip, which was mounted horizontally on a spin-coating machine, and spread by spin-coating at 6000 r/min for 1 min. After spin-coating, the PMMA film was distributed evenly on the whole surface. The coverslip was baked at 145 °C for about 30 min and then stored at room temperature in a dust-free environment.

E. DNA-multivalent cation complex preparation

DNA was stained with YOYO-1 fluorescent dye at a dye-base pair ratio of 1:10 in TE buffer before use. The complex was incubated for 30 min at room temperature in the dark before using. After incubating, the

equation [26, 27]. These theories ignore the interactions among cations, the discrete nature of DNA, and counterion charges. But DNA in solutions of tri- and higher-valent cations, such as [Co(NH$_3$)$_6$]$^{3+}$ and spermine$^{4+}$, shows a peculiar phase behavior not expected in traditional theories [28, 29]. Much progress has been made over the last decade in DNA condensation induced by multivalent cations and protein. Multivalent cations are generally required in aqueous solutions at room temperature which are known to bind to DNA in the predominantly nonspecific electrostatic manner.

Dynamic light scattering (DLS) also appeared to be a good technique to study the interaction between DNA and counterions. Light scattering techniques are very relevant in the study of colloidal particles, especially in what concerns the size of macromolecules and molecular assemblies. In this work, it is studied with an emphasis on the concentration range for DNA-[Co(NH$_3$)$_6$]$^{3+}$ solution.

The recent developed single molecular methods, such as AFM, optical and magnetic tweezers, have been used to research the DNA condensation induced by multivalent cations. Rocha et al. investigated the spermine induced DNA condensation on oxidized silicon surface by AFM. The results showed that the silicon substrates can provide results as precise as those obtained with the mica ones [31]. Besteman et al. used magnetic tweezers to study the condensation of single DNA molecules induced by [CoCl$_2$H$_6$N$_6$]$^{3+}$ and spermine under tension. They measured the influence of an imposed twist, which showed that condensation was initiated by the formation of a plectonemic supercoil [32]. Broek et al. investigated DNA condensation induced by spermine by optical tweezers [33]. The quantitative force measurements of the stepwise condensation process with high spatial resolution showed that a toroidal structure is likely to be formed in condensing DNA under tension. Clearly, quite a lot of information on the process of DNA condensation has been acquired, but the detail mechanism of DNA condensation has not been completely understood. On the other hand, some professional, specific and expensive facilities are needed in these researches. Here, we investigated DNA condensation induced by [Co(NH$_3$)$_6$]$^{3+}$ using a simple and feasible molecular combing method. With the increase of the concentration of [Co(NH$_3$)$_6$]$^{3+}$, the stretched length of DNA-[Co(NH$_3$)$_6$]$^{3+}$ complex decreased. This condensation phenomenon is confirmed by dynamic light scattering experiment.

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mixture was diluted by bis-Tris, the final concentration of DNA was 6.5 pmol/L. DNA-[Co(NH$_3$)$_6$]$^{3+}$ complex preparation was made by incubating DNA/YOYO-1 (6.5 pmol/L) with [Co(NH$_3$)$_6$]$^{3+}$ at room temperature for about 30 min. The concentration of [Co(NH$_3$)$_6$]$^{3+}$ was 0, 1, and 3 μmol/L, respectively.

F. Molecular combing

A total of 2 μL of the DNA complex solution was deposited onto the PMMA surface. The complex was incubated at room temperature in the dark before use. After being kept for 10 min, the slide was put on the FM stage and imaged. The tensional force of the moving water meniscus can sweep the DNA off the substrate surface. As the water meniscus moved down, the DNA fibers were aligned.

G. Dynamic light scanning

Light scattering measurements were purchased from Malvern Company. Samples were prepared in a similar way as described for molecular combing study. The DNA complex solution (50 μL) was placed in thin walled UV-Transparent Disposable cuvettes (dimensions: 12.5 mm×12.5 mm×45 mm) and placed in the groove in the instrument. During the measurement, the groove temperature was kept at 25 °C. The light source was a He-Ne gas laser (λ=633 nm) and light scattering detected by the avalanche photodiode mounted on the goniometer arm at 90° to the direction of the incident radiation. Applying the Stokes-Einstein equation to the translation diffusion coefficients provides an intensity-weighted distribution of hydrodynamic sizes [34].

III. RESULTS AND DISCUSSION

A. Effect of YOYO-1 on DNA contour length

DNA molecules can be effectively stretched on hydrophobic surface. We combed λ-DNA stained with YOYO-1 and pre-stained DNA-[Co(NH$_3$)$_6$]$^{3+}$ complex on the PMMA-coated surface respectively, as shown in Fig.1. This phenomenon results from hydrophobic interaction between PMMA surface and DNA.

From Fig.1(a), we find YOYO-1 increases the average length of λ-DNA. When free in solution, a DNA helix behaves as if it was a random elastic coil [35] and the contour length is 16.2 μm. The binding of YOYO-1 lengthens the DNA due to bis-intercalation. During the intercalation of YOYO-1 into λ-DNA, the relative DNA molecule length is increased by 30% [36]. The length distribution of DNA array is presented in Fig.2(a). From Fig.2(a) we find that the stretched length of DNA is about 21 μm. We also observe a rarefied part long DNA with average length of more than 23 μm, this is probably because they were over-stretched. In addition, a rarefied part of short DNA (11 μm) and bright spots are also observed in the experiment.

Consequently, molecular combing can be described as follows. First, DNA extremities bind to PMMA film, and then DNA molecules are stretched by a moving air/water interface [19]. Some base pairs are obliged to bind to PMMA film to resist the stretching force; otherwise, DNA will be taken away from the PMMA film. The interaction between the DNA extremities and PMMA film is also the hydrophobic effect. This hypothesis has been confirmed by some researchers [21, 37]. In the experiment, forces that act on all DNA molecules are described as,

$$ F = EA \left( \frac{l}{l_0} - 1 \right) $$

where $E=1.1\times10^8$ N/m$^2$ is Young’s modulus of DNA molecules, $A=3.8\times10^{-18}$ m$^2$ is their cross-sectional area, $l_0$ is the natural length of DNA, and $l/l_0$ is the relative extension [37]. For λ-DNA, $l_0=16.2$ μm and the measures $l/l_0=1.29$, thus the forces that act on DNA molecules is about 121 pN. In fact, we observed that the lengths of combed DNA molecules are different, which is shown in Fig.2(a). It probably results from the difference of the number of base pairs binding to the PMMA...
film between different DNA and others form coils in solution. The base pairs binding to the PMMA film have no contribution to the combing length of DNA. When DNA molecules are combed, the base pairs binding to the PMMA film will keep coiled, while others will be stretched uniformly. The coil length of base pairs binding to the PMMA film can be ignored compared to that of the uniformly combed part. Therefore, we can observe some short DNA with a stretched length of about 11 μm. This may be because the number of base pairs binding to the PMMA film is large and long DNA molecules are easy to break when treated.

B. Effect of hexamine cobalt on DNA condensation

As we know, DNA configurations are stabilized in large measure by multivalent cations and positively charged proteins, whose likely roles are both to neutralize the large negative charge of the DNA phosphates, thereby overcoming the coulomb force that opposes close packing, and to produce the conformation changes that lead to the compact form. During this experiment, we directly observed the pre-stained DNA condensation induced by [Co(NH₃)₆]³⁺ (Fig.1 (b) and (c)). Lengths of pre-stained DNA-[Co(NH₃)₆]³⁺ complex straightened by combing are listed in Fig.2 (b) and (c). From Fig.2(b) we can see the most of DNA-[Co(NH₃)₆]³⁺ complex whose length abruptly decreased (Fig.1(b)), and the average stretched length is 14.7 μm, as the concentration of [Co(NH₃)₆]³⁺ is 1 μmol/L. With further changes of the concentration of [Co(NH₃)₆]³⁺ (3 μmol/L), the length of complexes decrease (Fig.1(c)), and the average length is 5.9 μm (Fig.2(c)). As the above-mentioned discussion, the average stretched length of the pre-stained DNA-[Co(NH₃)₆]³⁺ complex decrease with the increase of the concentration of [Co(NH₃)₆]³⁺ as shown in Fig.3.

When adding [Co(NH₃)₆]³⁺, these cations bind to nitrogen base sites and the backbone PO₄⁻ groups and most of the DNA charge were neutralized. It overcome the strong electrostatic repulsion which led DNA to condense into compact structure. Under this circumstance, the force acting on DNA molecular can be divided into three forces, namely coagulative power, stretching force, and hydrophobic effect. The coagulative power and hydrophobic effect resists the stretching force, so the contour length is shorter than the DNA molecular without the addition of [Co(NH₃)₆]³⁺. These results indicate that molecular combing technique can be used to obtain the quantitative conformation characterization of DNA-[Co(NH₃)₆]³⁺ complex on PMMA surface.

C. Dynamic light scattering

Dynamic light scattering experiments measure the relative diffusion constant of particles in solution via the time autocorrelation of the fluctuations in the intensity of the scattered light. From the diffusion constant, an average hydrodynamic particle radius may be derived. DNA was mixed in distilled bis-Tris buffer with different amounts of [Co(NH₃)₆]³⁺ so that the concentration ratio in solution varied between 0 and 3 μmol/L. We started by performing DLS measurements on the DNA-YOYO-1 and pre-stained DNA-[Co(NH₃)₆]³⁺ complex independently. The DNA used in these experiments

FIG. 2 Statistical data of the length of combed DNA-YOYO-1-hexammine cobalt complex with different [Co(NH₃)₆]³⁺ concentration, [DNA]=6.5 pmol/L, with different concentration of [Co(NH₃)₆]³⁺. (a) 0, (b) 1 μmol/L, (c) 3 μmol/L.

FIG. 3 Extension of individual DNA-YOYO-1 molecule with different concentration of [Co(NH₃)₆]³⁺.
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IV. CONCLUSION

We study the interaction between DNA and [Co(NH$_3$)$_6$]$_3^+$ by a simple molecular combing method, in which individual DNA molecules are combed on surfaces coated with PMMA. The visualization of DNA can be realized when they are stained with YOYO-1 fluorescent dye. The intercalation of YOYO-1 increases lengths of DNA about 30%, in agreement with the previous study [36]. When [Co(NH$_3$)$_6$]$_3^+$ and DNA coexisted in the solution, [Co(NH$_3$)$_6$]$_3^+$ made DNA more condensed and compacted with smaller volume. The stretched length of complex decrease from 20.9 µm to 14.5 and 5.9 µm, when [Co(NH$_3$)$_6$]$_3^+$ of 1 and 3 µmol/L is added, respectively. The visual condensation of DNA is confirmed by the results of dynamic light scattering. Under the same condition in the combing study, the average hydrodynamic radius of DNA-[Co(NH$_3$)$_6$]$_3^+$ complex decrease from 203 nm to 141 and 39.26 nm in DLS measurements. The zeta experiment data show that there is no effect on the interaction between DNA and [Co(NH$_3$)$_6$]$_3^+$ with the present of YOYO-1. So the condensation of DNA is only due to the presence of [Co(NH$_3$)$_6$]$_3^+$. In conclusion, molecular combing provides a simple and feasible method for quantitative conformation characterization of single bio-macromolecules. It can be used to investigate the interaction between protein or ligand and DNA.

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FIG. 4 (a) The DNA-YOYO-1 size distribution by intensity. (b) Hydrodynamic radius of the DNA-[Co(NH$_3$)$_6$]$_3^+$ complex vs. the concentration of [Co(NH$_3$)$_6$]$_3^+$.

FIG. 5 Zeta potential of DNA and the complex of DNA and YOYO-1, respectively.
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