Direct Observation of the DNA Multimolecule Condensation with Fluorescence Microscopy

Lian-Sheng Ling,[†] Xiao-Hong Fang,[†] Chen Wang,[†] Li-Jun Wan,[†] Chun-Li Bai,[*†] and Dong-Min Chen[*†,††]
[†]*Institute of Chemistry, Chinese Academy of Sciences, Beijing 100080, P. R. China*
[††]*The Rowland Institute at Harvard, Harvard University, MA 02142, U. S. A.*

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DNA condensation plays a crucial role in DNA strand-exchange, ligation, renaturation and catenation. Thus it has drawn researchers' great attention. In this work, spermine-induced DNA condensation was studied with epifluorescence microscopy. In the presence of $Ru(phen)_2(dppx)^{2+}$ (phen = 1,10-phenanthroline, dppx = 7,8-dimethyl-dipyridophenazine), single λ-DNA with the length of about 10 μm was observed. With the addition of 20 mmol·l^{-1} spermine, there occurred DNA multimolecule condensation, resulting in the formation of a 40 μm rod-like highly compacted and ordered superhelical structure with the diameter of about 7 μm. This provides the direct evidence of DNA multimolecule condensation induced by spermine in solution.

DNA condensation can accelerate a number of biological processes, such as DNA strand-exchange, ligation, renaturation and catenation of closed circular DNA.[1] The study of DNA condensation in vitro is a good model for the study of the process of forming highly compacted and ordered DNA structure in vivo. Many methods, such as light scattering,[2] CD,[3] fluorescence spectrum[4] and atomic force microscopy[5] have been used to study DNA condensation. In the last decades, fluorescence microscopy has been used to study DNA condensation. Most of the previous reports are focused on the coil-globule transition of single DNA induced by different reagents, such as cationic surfactants[6] and cations.[7,8] In this work, we have stained λ-DNA with $Ru(phen)_2(dppx)^{2+}$ (phen = 1,10-phenanthroline, dppx = 7,8-dimethyl-dipyridophenazine),[9] a newly luminescent probe, and further studied DNA condensation induced by spermine with epifluorescence microscopy. The largest condensation structure, a 40 μm rod-like highly compacted, superhelical DNA with the diameter of about 7 μm has been observed for the first time.

$Ru(phen)_2(dppx)^{2+}$ was synthesized,[9] and dissolved in water with a concentration of 1.0×10^{-4} mol·l^{-1}. λ-DNA was obtained from SIGMA Co. and prepared with a concentration of 2.0 μg/mL. Liquid cells and microscope cover glass were bought from Fisher Scientific Co. (Pittsburgh, P.A., U.S.A.). All other chemical reagents were of analytical grade or better. Ultrapure water (18.2 MΩ·cm) was obtained with a Mill-Q filtration system and was used throughout the experiments. The fluorescence image was obtained on Axiovert 200 epifluorescence microscopy (Zeiss, Germany) equipped with Axiocam CCD(Zeiss, Germany).

The cover glass was modified with 3-aminopropyltriethoxysilane(APTES), which was helpful for DNA adsorption. As shown in Figure 1a, λ-DNA also aggregated on the cover glass, which might be induced by $Ru(phen)_2(dppx)^{2+}$. As it is difficult to study DNA condensation under this condition, DNA stretching should be performed prior to the study of DNA condensation. A number of methods such as molecular combing,[10] dynamic molecular combing,[11] electro field,[12] gas flow[13] and spin-stretching[14] have been used to stretch DNA. Here we used the spin-stretching method, as shown in Figure 1b, with the rotation rate of 4300 rpm, λ-DNA was successfully stretched. The majority of the stretched λ-DNA had the length around 10 μm, and a few of them were rather thick rod structure, which was most probably due to multi-chain assembly. The structure of stretched λ-DNA was very stable in the absence of condensing agent. It enabled us to further study DNA condensation.

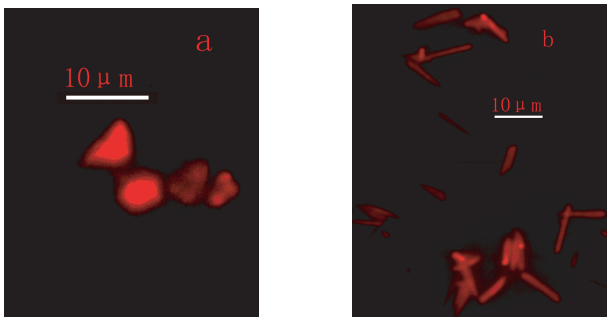

Figure 1. aggregated λ-DNA(a) and spin-stretched λ-DNA(b) stained with $Ru(phen)_2(dppx)^{2+}$. $Ru(phen)_2(dppx)^{2+}$: 1.0×10^{-5} mol·l^{-1}.

There are many reagents which can induce DNA condensation, such as metal cation, peptide, polyamine, surfactant, neutral polydiethanol and organic solvent, et al. As polyamine is a representative reagent to induce DNA condensation, spermine was used in this study.[15] In general, DNA will not be condensed as they have negative charged backbone, which make them electrorepulsive. The addition of spermine revert the charge of the DNA backbone in some regions, forming the electroattractive microenvironment, and inducing DNA condensation. It is reported that spermine did not induce DNA condensation under low concentration. DNA condensation occurs only when the concentration of spermine is higher enough to neutralize the negative charge of DNA backbone to more than 90%. With the addition of 20 mmol·l^{-1} spermine in our experiment, DNA multimolecule condensation was observed. As shown in Figure 2, λ-DNA formed rod-Like superhelical structure with the length of 40 μm and diameter of 7 μm. As a matter of fact, DNA strands having 400 bp to 10^5 bp did fold into highly compacted structure with multivalent cations. As single λ-DNA has the length of about 10 μm and diameter of 2.2 nm in water solution, it can be calculated that the highly compacted DNA structure shown in Figure 2 was composed of more than 1.0×10^9 λ-DNA. When DNA is induced into condensation, its structure become more compact, thus the DNA molecules contained in the superhelical structure might be far more than that calculated number.

Although DNA condensation has been widely studied with atomic force microscopy and many different structures have been

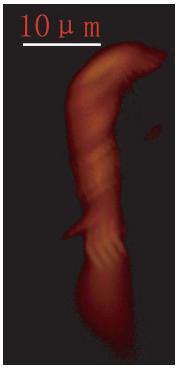

Figure 2. DNA condensation induced by spermine $Ru(phen)_2(dppx)^{2+}$: 1.0×10^{-5} mol·l^{-1}, Spermine: 20 mmol·l^{-1}.

reported, there has been no such a big and ordered structure reported. Our results provide the evidence of DNA multimolecule condensation at surface. Fluorescence microscopy has also been used recently to study DNA condensation with the advantage of carrying out the study in solution. Most of the researches concentrate on the condensation of single DNA molecules, and few reports concentrate on multimolecule DNA condensation. The novel structure of DNA condensation observed in this study is expected to help us obtain a better understanding of the DNA condensation process.

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