

Controllable DNA Condensation-Release Induced by Simple Azaheterocyclic-Based Metal Complexes

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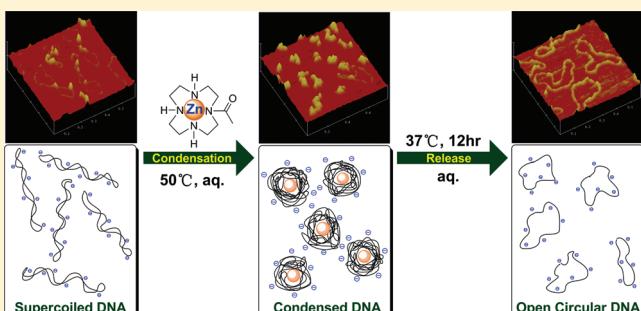
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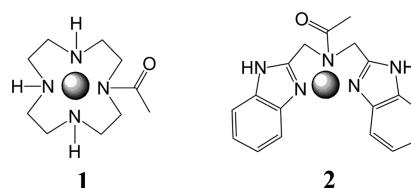
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Supporting Information

ABSTRACT: The condensation of DNA is essential for biological processes such as DNA transcription and replication, and its study receives additional impetus from an interest in gene therapy. Although many efficacious condensing agents have been discovered and investigated, little is known about the conversation of condensation-release under suitable conditions. A novel class of DNA condensing agents based on small azaheterocyclic metal-binding molecules has been discovered and described. Both linear and plasmid DNA can be condensed to nanoparticles by the title compounds with 50 °C incubation, especially in the presence of divalent metal ions. Importantly, this condensation may be released to original forms with little or no damage to the DNA under incubation at physiological temperatures. These changes in DNA morphology over time have been analyzed by gel electrophoresis, circular dichroism (CD), and atomic force microscopy (AFM). The present work might help to develop strategies for the design and synthesis of controllable condensing agents, which may also be applied to control gene expression and delivery.



Scheme 1. Structures of Azaheterocyclic-Based Metal Complexes Studied



= Zn²⁺ or Cu²⁺

may aid in the design and synthesis of controllable condensing agents for DNA nanoparticle formation and their storage as pharmaceutical agents for gene delivery.

EXPERIMENTAL SECTION

All chemicals and reagents were obtained commercially and used without further purification. Spermine and spermidine were purchased from Sigma Chemical Co. Electrophoresis grade agarose and purified plasmid DNA (*pUC18*) were obtained

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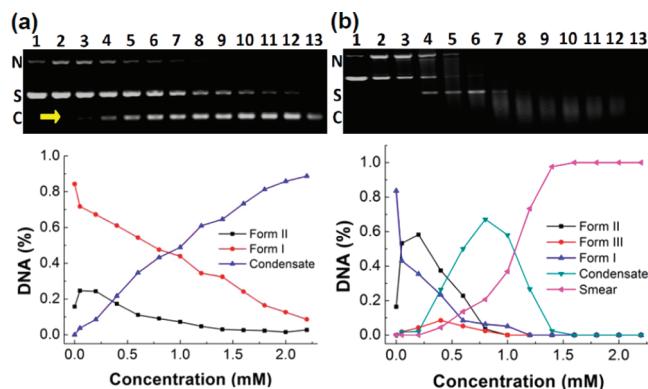


Figure 1. Concentration dependence of pUC18 plasmid DNA condensation by (a) 1-Zn^{2+} and (b) 1-Cu^{2+} complex at $50\text{ }^{\circ}\text{C}$ for 6 h. Lane 1: DNA control; lanes 2–13: 0.05, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8, 2.0, 2.2 mM ligand–metal complex. S: supercoiled DNA, N: nicked DNA, C: DNA condensates.

commercially from Fermentas (China). Linear DNA 1800 bp in length was generated by PCR from *Schizosaccharomyces pombe*. Details of MS (ESI) mass spectral data were recorded on a Finnigan LCQDECA mass spectrometer. ^1H NMR spectra were measured on a Bruker AV400 spectrometer, and chemical shifts in parts per million are reported relative to internal Me_3Si ($\text{DMSO}-d_6$).

Atomic force microscopy (AFM) images were recorded in air with a Nanoscope IIIa microscope operating in tapping mode. The probes were commercially available silicon tips with a spring constant of $42\text{ N}\cdot\text{m}^{-1}$. Freshly cleaved fluoromica ($1\text{ cm} \times 1\text{ cm}$) was covered with $10\text{ }\mu\text{L}$ of samples ($2.5\text{ ng}\cdot\mu\text{L}^{-1}$) mixed with AFM buffer ($10\text{ mM Tris-HCl pH 7.4}$, $1\text{ mM ethylenediaminetetraacetic acid (EDTA)}$, 5 mM MgCl_2). After 5 min, the buffer was rinsed thoroughly with 0.5 mL of distilled water, and the mica was briefly dried under a stream of N_2 (g). The length and height measurements were performed with the Femtoscan online software (version 2.3.94).

Electrophoresis experiments were performed with plasmid DNA (pUC18) and linear DNA (1800 bp). In a typical experiment, DNA ($0.3\text{ }\mu\text{L}$, $0.5\text{ }\mu\text{g}\cdot\mu\text{L}^{-1}$) in Tris-HCl buffer (40 mM) was treated with different small molecules, followed by dilution with the Tris-HCl buffer to a total volume of $10\text{ }\mu\text{L}$. The samples were then incubated at different temperature and time intervals and loaded on a 1% agarose gel containing ethidium bromide ($1.0\text{ }\mu\text{g}\cdot\text{mL}^{-1}$).

Circular dichroism (CD) experiments were performed under a continuous flow of nitrogen using a Jasco-720 spectropolarimeter. A path length cell of 1 cm was used, and all experiments were performed at room temperature. A $0.5\text{ }\mu\text{M}$ solution of azaheterocyclic molecule was titrated into the DNA solutions (at pH 7.4, $10\text{ mM Tris-HCl buffer with }10\text{ mM NaCl}$).

RESULTS AND DISCUSSION

Electrophoresis Results: Compounds 1 and 2 Induce DNA Condensation. The DNA condensation by compound 1 was first revealed by a gel electrophoresis assay. As shown in Figure 1a, as the concentration of 1 increased, in the presence of Zn^{2+} , the amount of supercoiled (Form I) and nicked (Form II) DNA diminished gradually, and a new band (yellow arrow) was observed, which was found to be condensed DNA. At 2.2 mM 1-Zn^{2+} complex both forms of DNA were almost 100% condensed. Unlike previous reports, where condensing products were retarded

in the gel loading well, the DNA condensed by compounds 1 and 2 were more mobile during electrophoresis.^{19,20} This result indicates that negatively charged phosphates in DNA were not completely neutralized during condensation, and nanoparticles were small relative to those created by other condensation agents.

When copper(II) rather than zinc(II) was chelated by compound 1, the concentration dependence on DNA condensation was significantly different. While at low concentrations, the condensed DNA band increased, at concentrations above 0.8 mM a smear of higher mobility DNA was produced. As described in Figure 1b, this smear is due to the cleavage activity of the 1-Cu^{2+} complex, resulting in a range of smaller DNA fragments.

In the control experiments, metal–ligand complexes were more efficacious than free ligands in evoking DNA nanoparticles forming, and no apparent condensates were detected in the presence of metal ions alone (Figure S1 of the Supporting Information, SI). Similarly, experiments with linear 1800 bp DNA produced analogous results to the plasmid DNA experiments (Figure S2 of the SI). Condensation in this manner occurred only with incubation temperatures up to $50\text{ }^{\circ}\text{C}$. No detectable change or single strand cleavage was observed under incubation at $37\text{ }^{\circ}\text{C}$ (Figure S3 of the SI).

AFM Studies: Zinc-Ligand Complexes Result in Larger DNA Condensates than Copper–Ligand Complexes. Atomic force microscopy (AFM) studies were performed in aqueous solution on mica to gain detailed structural information about condensate morphology. An image of uncondensed pUC18 plasmids has been provided for comparison in Figure 2a, which shows a relaxed, open-loop structure with little twisting or fasciculation of the strands, presenting the characteristic plasmid DNA morphology. After the addition of the 1-Zn^{2+} complex (the same conditions as lane 12 in the gel assay), the AFM image (Figure 2b) presents a number of what appear as classical globules²¹ with an average diameter of $88.9 \pm 7.9\text{ nm}$ and height of $0.88 \pm 0.08\text{ nm}$, being much more compact than the untreated plasmid DNA. The nanoparticle size corresponds to nonaggregates derived from one or only a few DNA strands. In contrast, different DNA condensation behaviors in the presence of Cu^{2+} were also observed, as shown in Figure 2c. The average diameter of condensates was much smaller than those condensed by 1-Zn^{2+} complex, presenting an average diameter of $49.3 \pm 4.3\text{ nm}$. This smaller diameter corresponds to DNA fragmentation and various degrees of condensation of these fragments. Histograms show the detailed diameter and height distributions for both complexes in Figure S4 of the SI. Similar AFM results for the condensation induced by 2-Zn^{2+} and 2-Cu^{2+} complexes are shown in Figure S5 of the SI. The AFM data clearly demonstrate the DNA condensation ability of azaheterocyclic-based metal complexes. The driving force of the DNA condensation may involve the electrostatic interactions between the polyamine in 1 and the negatively charged phosphates in DNA and the intercalation of the benzimidazole rigid groups of 2 into the DNA grooves.¹⁴

Temperature Dependence of Ligand-Induced DNA Condensation. Time-course experiments showed that the 1-Zn^{2+} complex-induced DNA nanoparticle condensation is reversible. Figure 3a,b presents the time-dependent decondensation process of linear and plasmid DNA condensates, respectively, with incubations at $25\text{ }^{\circ}\text{C}$ and $37\text{ }^{\circ}\text{C}$. As shown in Figure 3a, more than half of the nanoparticles returned to original linear form when incubated at either $25\text{ }^{\circ}\text{C}$ or $37\text{ }^{\circ}\text{C}$ for 3 h. By 12 h of incubation at $37\text{ }^{\circ}\text{C}$ nearly 100% of the DNA had linearized (Figure 3a). When circular plasmid DNA rather than linear DNA was incubated with 1-Zn^{2+} complex at $37\text{ }^{\circ}\text{C}$ for 12 h, as shown in

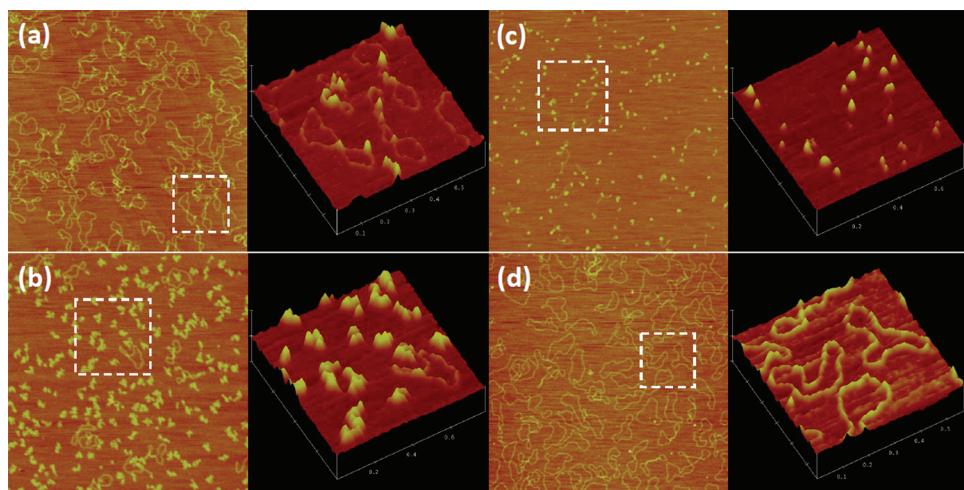


Figure 2. AFM images of pUC18 DNA ($2.5 \text{ ng} \cdot \mu\text{L}^{-1}$) and its condensation induced by metal complexes on mica in tapping mode in air: (a) DNA; (b) DNA + **1**– Zn^{2+} complex; (c) DNA + **1**– Cu^{2+} complex; (d) DNA + **1**– Zn^{2+} complex, 50°C , 6 h, then 37°C , 12 h.

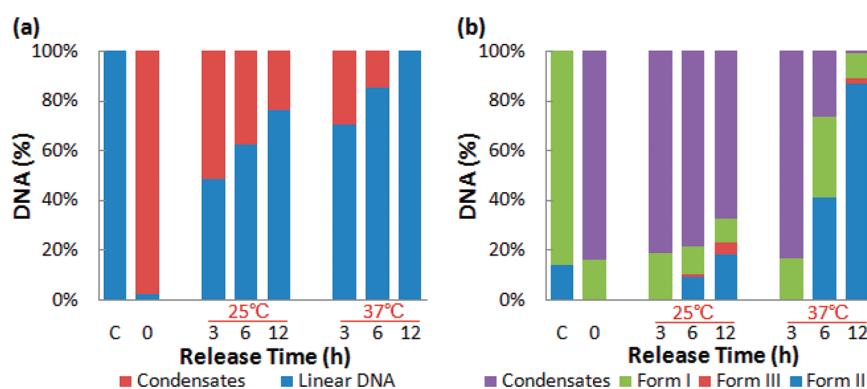


Figure 3. Quantitation of various DNA forms after release from (a) 1800 bp linear DNA and (b) pUC18 plasmid DNA condensates formed with the **1**– Zn^{2+} complex under different temperatures and incubation times.

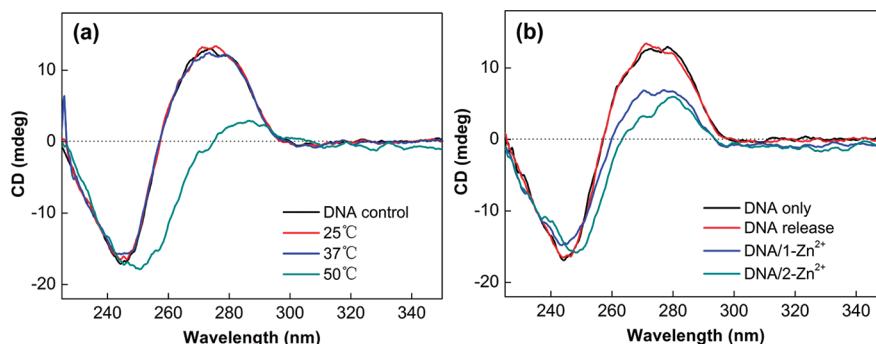


Figure 4. CD spectra of (a) DNA/**1**– Zn^{2+} under different incubation temperatures (black: DNA only; red: 25°C ; blue: 37°C ; green: 50°C) for 12 h and (b) DNA condensation with different complexes under 50°C incubation for 6 h (black: DNA only; green: DNA/**1**– Zn^{2+} complex; blue: DNA/**2**– Zn^{2+} ; red: DNA/**1**– Zn^{2+} complex condensate incubated at 37°C for 12 h).

Figure 3b, the DNA was observed to be 10.0% supercoiled (Form I), 86.6% open circular (Form II), 2.4% linear (Form III), and 0.9% condensed. The corresponding AFM image (Figure 2d) and gel assay (Figure S6 of the SI) clearly depict DNA relaxation from the condensed form. These results demonstrated that DNA condensates can be released with little damage more quickly and thoroughly by aqueous incubation at 37°C . A higher

temperature would be beneficial to compact DNA to nanoparticles, but also favorable for disruption of external hydrogen bonds and interaction of amphoteric charges, retrieving the original DNA form. The temperature effects on DNA form may be complicated and discontinuous.

CD Experiments: Ligand-Induced DNA Condensation and Relaxation. Conformational changes of DNA interacting with

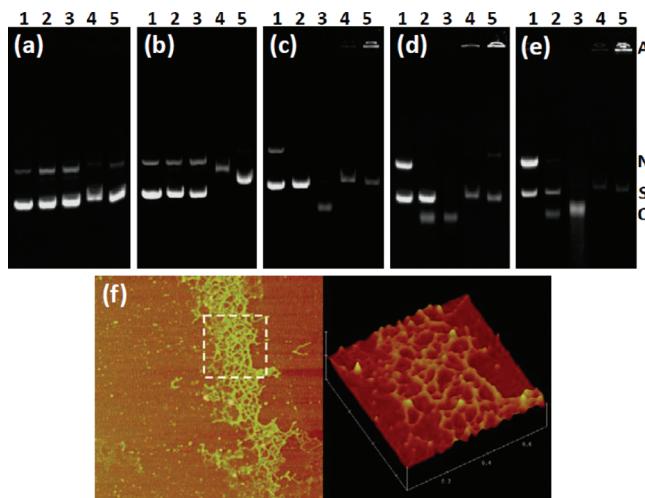


Figure 5. pUC18 plasmid DNA condensation by various polyamines (2.0 mM) for 6 h at (a) 25 °C, (b) 37 °C, (c) 50 °C, (d) 60 °C, (e) 70 °C. Lane 1: DNA control; lane 2: compound 1; lane 3: 1-Zn²⁺; lane 4: spermidine; lane 5: spermine. S: supercoiled DNA, N: nicked DNA, C: DNA condensates, A: DNA aggregates. (f) AFM image of DNA aggregates induced by spermine at 50 °C for 6 h, corresponding to lane 5 in part c.

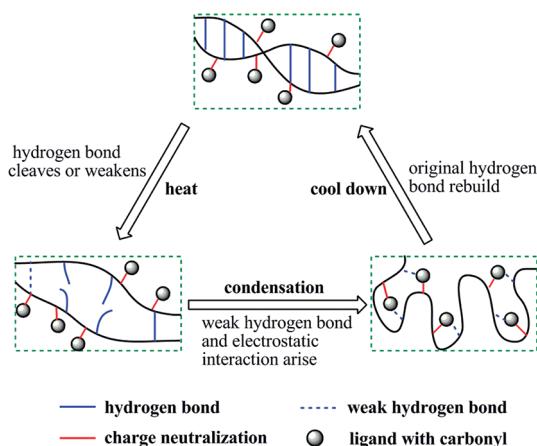


Figure 6. Possible process of temperature-dependent reversible DNA condensation.

azaheterocyclic complexes were further verified by CD experiments. The sample of the DNA/1-Zn²⁺ complex was incubated under 25 °C (red line) and 37 °C (blue line) for 24 h, and no detectable change was observed compared with the conservative spectrum of uncondensed DNA (black line) as shown in Figure 4a. Substantial changes in the CD spectra are, however, observed upon mixing DNA with the 1-Zn²⁺ complex under 50 °C incubation (green line), namely, a more than 70% decrease in the positive band and an overall shift of the bands toward higher wavelengths. The sharp decrease in the positive CD band suggested that the DNA was condensed and there was a secondary structural change.^{22,23} In Figure 4b, similar signal changes in both DNA/1-Zn²⁺ and DNA/2-Zn²⁺ complexes under the same conditions were observed. Importantly, the CD spectrum returned to the characteristic spectrum for uncondensed DNA when DNA/1-Zn²⁺ condensate was incubated at 37 °C for 12 h. These results are consistent with DNA condensation release transformation in the presence of

azaheterocyclic molecules, also shown by gel electrophoresis and AFM assay.

Condensation and Aggregation Induced by Different Polyamines. Earlier studies by Thomas et al.¹⁸ indicated the effects of chemical structure, ionic strength, and temperature on DNA nanoparticle formation induced by analogues of spermine, but no remarkable temperature sensitivity was presented. To compare the interactions with two kinds of polyamines, temperature dependence assays were performed including spermine and spermidine as controls, and the results are shown in Figure 5. At 50 °C, DNA nanoparticles began to form in the presence of 1-Zn²⁺, and the result still held until 70 °C, which were consistent with the conclusion above. However, spermine or spermidine can induce DNA retarding at room temperature. Up to 50 °C, most DNA molecules stayed in gel loading well, suggesting the formation of bulky DNA aggregates rather than single-molecule-scale condensation. The AFM image, as shown in Figure 5f, clearly presented the shape of aggregates induced by spermine under higher temperature. No matter whether equivalent Zn²⁺ exists or not, the results are the same.

Structurally, linear and cyclic polyamines with the same positive charges possess a different sphere of influence. The stretched structure and dispersed charges of linear polyamide may provide more opportunities for cross-linking of DNA. However, the existence of carbonyl moiety in molecules may be the most intrinsic reason for the diversity, which could form external hydrogen bonds between carbonyl and bases.

A Possible Process: Competitive Formation of External Hydrogen Bonds. When DNA are incubated at higher temperatures for a long time, the original hydrogen bonds between A-T or G-C could become less stable and even break. The carbonyl of condensing agents competitively interacts with the H atom in DNA bases, and then the new weak hydrogen bonds form. In spite of the higher temperature, the distance between carbonyl moieties and bases is close enough because of the electrostatic interactions, easily resulting in the form of external hydrogen bonds. Due to the existence of numerous charge neutralization and external hydrogen bonds at various orientations, DNA molecules are liable to be compacted to smaller particles. However, with the decrease in temperature, the broken hydrogen bonds in DNA are retrieved according to the principle of complementary base pairing. It seems to be an annealing process. The external hydrogen bonds disappear, and the interaction of charge neutralization remains, which could not maintain the compact conformation of condensed DNA, resulting in the release of condensates. This probable mechanism is depicted in Figure 6.

To further prove the speculative process mentioned above, the effect of ionic strength on condensation was evaluated by gel electrophoresis in Tris buffer containing Na⁺ with different concentrations. Admittedly, the melting temperature of DNA (T_m) ascends gradually with the increase in the ionic strength of the medium under the same conditions. If DNA are incubated in solution with higher salt concentrations, the double helix structure will become more stable due to the higher T_m value, and the external hydrogen bonds will hardly form. Therefore, none or less of the condensing phenomenon would happen. The results are shown in Table S1 of the SI. An abrupt decrease in the content of DNA condensates was observed when the concentration of Na⁺ was more than 1 mM. In the buffer containing 20 mM sodium chloride, hardly any condensate was detected. These results indicated that the appearance of condensation may result from external hydrogen bonds forming soon after double helix

untwisting. T_m values under different ionic strength were defined on the basis of eq 1 and listed as references (see the SI).

CONCLUSIONS

In this study, we have demonstrated that (i) two azaheterocyclic-based small molecules have the ability to condense DNA to single-molecule-scale globules in the aqueous solution under 50 °C incubation, (ii) the degree of condensation increased remarkably in the presence of metal ions, and (iii) condensed DNA nanoparticles can be released to their original DNA forms with little or no damage by incubation at 37 °C for some time. One possible mechanism for condensation induction involves original hydrogen-bond breaking and external ones building under higher temperature, forming multiple interactions from different orientations. Further investigations into the mechanism behind this phenomenon are still ongoing; the present results should be of value for the further understanding of DNA condensation and release, as well as offer the promising method to design better condensing molecules. Furthermore, we anticipate that these controllable DNA condensing agents may also be applied to control gene expression and delivery and thus have the potential for novel gene vector applications.

ASSOCIATED CONTENT

S Supporting Information. Detailed synthetic routes and data of all compounds; histograms of diameter and height distributions for both complexes; gel electrophoresis for control experiments, including ligands, metal ions only, different temperatures, and substrates; relative assay of ligand **2** for condensation research; and ionic strength dependent assay. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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