Selected Paper

Roles of Mono- and Divalent Cations in DNA Compaction Induced by Histone-Mimic Nanoparticles

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The compaction of gene-size T4 DNA induced by the action of silica nanoparticles (10–100 nm diameter), the surfaces of which were modified to various degrees by a 3-aminopropylsilyl groups, was monitored by a fluorescent microscope in an aqueous solution. It was found that DNA compaction was facilitated by mono- and divalent cations such as Na^+, K^+, Mg^{2+}, and Ca^{2+}. This promotion by cations was the result of charge-neutralization of DNA and was more remarkable in compaction by histone-size nanoparticles (10 nm) than in that by larger ones (>15 nm). Divalent metal ions were more effective at promoting compaction due to their greater charge-neutralizing character, but a quaternary diammonium derivative of 1,3-propanediamine deactivated DNA compaction by 10-nm nanoparticles. Although metal ions binding to DNA can reduce electrostatic tension on DNA and provide flexibility to create a DNA loop with a small diameter, such ammonium dication does not make DNA sufficiently flexible.

DNA folding on a histone core (histone octamer) is thought to be the first step in the construction of higher-order DNA molecular assemblies, such as chromatins and subsequently chromosomes in eukaryotes.[1,2] The charge-neutralizing interaction between the phosphate groups of DNA and basic amino acid residues of histone is the first-stage force in the DNA-folding transition. In addition, the tight vs. loose folding of DNA on histone is considered to issue "off" (silence) and "on" (activation) signals to a gene, respectively.[3,4] On the other hand, the folding transition of a giant DNA molecule is known to occur due to the action of various cationic species in vitro, to give a small tightly-compacted DNA particle.[5–14] For example, the DNA compaction induced by spermine (*N*,*N*′-bis(3-aminopropyl)-1,4-diaminobutane) mainly gives doughnut-shaped particles of 100–200 nm ϕ (diameter).[11,12] Since chromatin is constructed by DNA winding around histones of 7 nm ϕ, the bending stress of DNA in chromatin is apparently greater than that in the compacted DNA particle produced by spermine. A loop of DNA in chromatin consists of a small part of the double-stranded DNA with 147 base pairs,[2] but at least a few thousands base pairs are included in the DNA loop of 100 nm ϕ. A short DNA fragment consisting of a few hundred base pairs is known to behave as a rigid wire due to stiffness caused by electrostatic repulsion between negative charges on the surface as well as the duplex structure.[15] Therefore, the spontaneous formation of a small DNA loop on the surface of histone seems to be unlikely without a mechanism that reduces the stiffness of a short DNA duplex. It would be interesting to identify a chemical process that could provide conformational freedom to a DNA chain to ease the formation of a small loop in chromatin.

Abundant metal ions in the cell are known to play an important role in chromatin formation, and the structure of chromatin is maintained by Na^+ and K^+.[16–21] In contrast, such metal ions inhibit the DNA-folding induced by spermine and unfold compacted DNA due to competitive binding to DNA.[11,22,23] An important problem in life science is to solve the mystery of how abundant metal ions prevent competition with the positive charges of histone to work collaboratively in the chromatin formation.

Recently, we reported that polycationic nanoparticles that were prepared from silica beads of 10 to 100 nm ϕ by surface modification with poly(L-lysine) or an aminopropylsilyl coupling agent produced DNA compaction in the presence of Na^+ and K^+.[24–27] It was apparent that the size of the nanoparticles as well as the surface concentration of positive charge was important in DNA compaction, and furthermore metal ions showed greater cooperation with histone-size nanoparticles.

In the present study, we utilized a simple artificial model of DNA compaction in chromatin, in which nanoparticles might play a role of histone proteins, and studied how the presence of abundant in nature inorganic monocations and dications as well as synthetic organic dications could influence the efficiency of DNA compaction on surface-modified nanoparticles of different sizes (10, 15, 40, and 100 nm ϕ).

Experimental

Materials. T4 phage DNA (166 kbp) was purchased from Nippon Gene, Co., Ltd. Silica nanoparticles, IPA-ST (mean diameter: 10 nm), IPA-ST-M (15 nm), IPA-ST-L (40 nm), and IPA-ST-ZL (100 nm),[25] were obtained from Nissan Chemical, Co., Ltd. as 30% (w/w: weight per weight) suspensions in 2-

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propanol. 3-Aminopropyltriethoxysilane was purchased from Sigma-Aldrich, Co., Ltd., and 4′,6-diamino-2-phenylindole (DAPI), sodium chloride, potassium chloride, magnesium chloride, calcium chloride, and a 0.1 M standard solution of HCl were purchased from Wako Pure Chemical Industry, Co., Ltd. *N*,*N*,*N*,*N*′,*N*′,*N*′-Hexamethyl-1,3-propanediammonium dibromide (EtPrEt) and *N*,*N*,*N*,*N*′,*N*′,*N*′-hexamethyl-1,2-ethanediammonium dibromide (EtEtEt) were synthesized following a reported method.[13] Water used in this study was purified by a Milli-Q Labo® (Millipore Corp.).

Surface-Modification of Silica Nanoparticles: A General Method. To a mixture of a 30% suspension of IPA-ST (1.0 mL) and 2-propanol (4.0 mL) was added a solution of 3-aminopropyltriethoxysilane (0.02 mL) in 2-propanol (5.0 mL) at room temperature. The suspension was mixed by sonication for 3 days and subjected to high-speed centrifugal separation (15000 rpm) for 6 h. Precipitates were washed with 2-propanol (10 mL × 3) and then with water (10 mL × 3). The resulting modified NPs (nanoparticles),[27] which were abbreviated as NP_{10} based on the mean diameter of IPA-ST, were stored as 1.0% (w/w) suspensions in water at room temperature until use.

Titration of Modified NPs. A stored suspension of NPs was subjected to centrifugal separation, and precipitates were dried under reduced pressure (ca. 100 Pa) at 50 °C for 24 h. The resulting powder (0.500 g) was dispersed in water (10.0 mL), and the suspension was titrated with a standard solution of HCl ($C = 1.00$–100 mM, depending on NPs) in the presence of methyl orange as an indicator. Results of the titration of NPs are shown as a table in Supporting Information.

Measurement of ζ-Potential. A 1.0% (w/w) stored suspension of NPs (0.50 μL) was diluted in water to a total volume of 0.50 mL, and this gave a sample suspension ($C = 1.0 \times 10^{-3}$ wt %: weight percent) for analysis of the ζ-potential. The sample was subjected to a measurement of electrophoretic mobility on a Sysmex Zetasizer®.

Fluorescent Microscope Observation of T4-DNA Molecules: A Typical Example. A sample solution was prepared by sequential mixing of water (0.385 mL), a 1.0 M aqueous solution of NaCl (0.10 mL), a 0.010 mM aqueous solution of DAPI (5.0 μL), a 1.0×10^{-4} wt % suspension of NP_{10} (5.0 μL), and a 0.010 mM solution of T4 DNA (5.0 μL); the resulting concentrations of Na^+ (C_{ion}), NP_{10} (C_{NP}), and DNA (C_{DNA}) in this specimen were 200 mM, 1.0×10^{-6} wt %, and 0.10 μM, respectively. The specimen was subjected to fluorescent microscopic observation on a Nikon TE2000-E with a 100× oil immersed lens, and at least 100 images of the DNA molecules were classified as coil, partial globule, and globule.

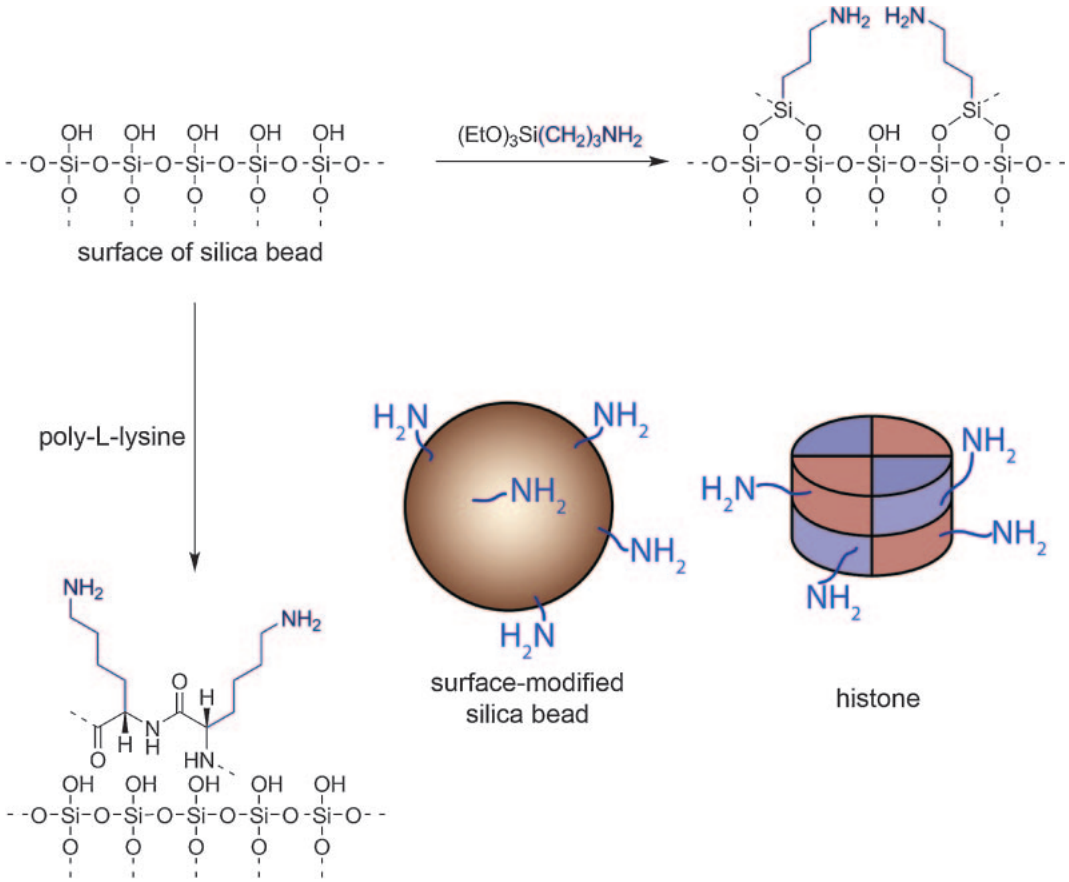
Results and Discussion

Preparation and Characterization of NPs. Surface-modification of silica nanoparticles (IPA-ST, IPA-ST-M, IPA-ST-L, and IPA-ST-ZL) was carried out by treatments with various concentrations of 3-aminopropyltriethoxysilane (0.2%–2% v/v: volume per volume) in 2-propanol at room temperature or 60 °C for 3 days to give NPs with various amounts of NH_2 groups (NP_{10L}–NP_{100H}). During this reaction, silanol (SiO–H) groups on the surface of NPs were converted to 3-aminopropylsilyl ethers ($SiO–SiCH_2CH_2CH_2NH_2$),[27–30] where the terminal amino group mimicked lysine residues in histone, as illustrated in Scheme 1. The concentrations of the surface NH_2 group were analyzed by acid–base titration using a standard solution of HCl, and the results are given in Table 1 together with the characteristics of NPs. When surface treatments of NPs of different size were carried out under the same conditions, the concentration of NH_2 was greater in larger-size NPs. A series of NPs of different sizes, NP_{10M}, NP_{15}, NP_{40}, and NP_{100}, with similar amine concentrations (4.6–5.9 μmol m^{-2}) were obtained.

In previous studies on the treatment of DNA by various nanomaterials, the amount of surface charge has been discussed in terms of the ζ-potential (zeta-potential).[24,28,30,31] To compare the DNA-folding effect of the NPs in this study with those previously reported the ζ-potentials of NPs in Table 1 were measured in 1.0×10^{-3} wt % aqueous solution at room temperature. For example, the ζ-potential values of NP_{10M}, NP_{15}, NP_{40}, and NP_{100} were observed in the range of +18.8–+37 mV, although their surface charge densities (Z/nm^2) were similar (+2.8–+3.5). Since we could not find any relations between the ζ-potential and their actual charge amounts obtained by titration or the size of the NPs, we do not discuss the surface charge of NPs based on the ζ-potential.

DNA Compaction Carried Out by NPs in the Presence of Mono- and Divalent Metal Ions. When a solution of T4-DNA (166 kbp; 56 μm contour length) stained by DAPI is treated with NPs in the presence of Na^+ or K^+, three typical kinds of DNA fluorescent images are observed depending on the progress of the DNA-folding transition.[27] In the stage at which NPs do not significantly affect the structure of DNA, the DNA molecule is observed as a coil. Since the DNA strand spreads to maintain a low molecular density due to electrostatic repulsion between the phosphate negative charges on the DNA surface, the DNA image in the coil state looks like a "hazy cloud," which is transformed freely by Brownian motion. When the intramolecular charge repulsion in DNA is weakened by increasing cationic NPs, atomic attraction in the DNA molecule partly overcomes the repulsion to create a high-density nucleus that is the partial globule state.[12,32] The DNA in the partial globule stage looks like a coil image, and includes one to several bright particles. Finally, sufficient charge neutralization completes DNA folding to give the globule form, which is observed as a single rapidly-moving particle. Dynamic images of DNA in the coil, partial globule, and globule stages at a 0.1 s interval are shown in Figure 1.

In a previous paper, we have reported that DNA compaction by both small (10 nm ϕ) and large (100 nm) NPs was facilitated optimally by the presence of sodium or potassium ion at $C_{ion} = 200$ mM, and the promoting effects of K^+ for both NPs were greater than those of Na^+.[27] To gain insight into the relation between the surface charge and the size of NPs with regard to DNA-folding efficiency, DNA compaction induced by various concentrations of NP_{10M}, NP_{15}, NP_{40}, and NP_{100} ($C_{NP} = 0.01$ nM–0.1 mM, based on molar amounts of surface charge per volume) with similar charge densities (+2.8–+3.5/nm^2) in the presence of the optimal concentration of K^+ was studied in a 10 μM (based on molar amounts of base pairs) solution of DNA. Distributions of DNA in the 3 typical stages



Scheme 1. Models of histone and surface-modified silica nanoparticles. The poly(L-lysine) modification is shown as a monolayer model.

Table 1. Specifications and Surface Charge Values of NPs

	NP_{10L}	NP_{10}	NP_{10M}	NP_{10H}	NP_{15}	NP_{40}	NP_{100L}	NP_{100}	NP_{100M}	NP_{100H}
Mean radius/nm[a)]	5.0	←	←	←	7.5	20	50	←	←	←
Mean surface area $\times 10^{16}/m^2$	3.1	←	←	←	7.7	50	310	←	←	←
Concentration of NH_2/mmol g^{-1} [b)]	0.55	1.1	1.8	4.1	1.0	0.41	0.11	0.18	0.30	0.75
Concentration of NH_2/μmol m^{-2}	1.8	3.5	5.7	13	4.6	5.5	3.6	5.9	9.8	25
Surface charge density/nm^2 [c)]	+1.1	+2.1	+3.4	+7.8	+2.8	+3.3	+2.2	+3.5	+5.9	+15
Net charge per a particle (Z)	340	650	1100	2400	2200	17000	68000	110000	180000	470000
ζ-Potential/mV	−19.3	+22.0	+34.9	+41.1	+18.8	+23.3	+21.0	+36.7	+37.8	+41.5

a) Reported values from the manufacturer. b) Calculated using the reported density (2.00 g cm^{-3}) of the series of IPA-ST. c) Calculated assuming that one NH_2 group corresponds to one + charge.

are shown in Figure 2. NP_{10M} is more effective (Figure 2A) than the others at the same surface-charge concentrations. At $C_{NP} = 1.8 \times 10^{-4}$ μM, about 30% of DNA was converted to the partial globule or globule state by the action of NP_{10M}, and almost all DNA was in the compacted state at $C_{NP} = 1.8 \times 10^{-2}$ μM. With the other NPs (Figures 2B–2D), although their sizes varied greatly from 15 to 100 nm ϕ, more than 60% of DNA remained in the coil state at $C_{NP} = 1.0 \times 10^{-2}$ μM, and a concentration of at least 10 μM was necessary to complete DNA compaction. Similar to the results seen for NP_{10} and NP_{100},[27] K^+ is generally more effective than Na^+ in DNA compaction induced by all types of NPs.

According to the Manning's condensation theory,[33–35] divalent cations can neutralize DNA negative charges more effectively than monocations, but do not induce the DNA-folding transition. Therefore, they are thought to promote DNA compaction by NPs at a lower concentration than monocations, and in fact, Mg^{2+} and Ca^{2+} promoted DNA compaction by NP_{10} and NP_{100} at 1/10 the concentration ($C_{ion} = 20$ mM) of Na^+ and K^+. The results are shown in Figures 3A and 3B, where the DNA-compacting efficiency is indicated as F_g, which is a percentage of DNA in the globule state. At $C_{ion} = 20$ mM, Ca^{2+} promoted DNA compaction by NP_{10} more effectively than Mg^{2+}, but there was no significant difference between the two ions with NP_{100}. The trend in the difference of the DNA-compacting activity between Mg^{2+} and Ca^{2+} was similar to that observed between Na^+ and K^+, as illustrated in Figures 3C and 3D.[27]

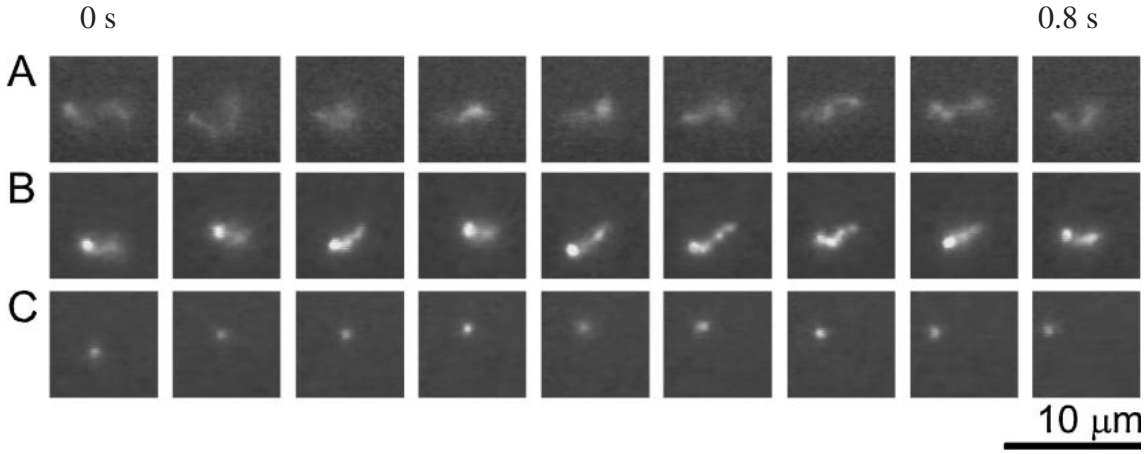


Figure 1. Dynamic fluorescent microscopic images of typical DNA-folding stages. Lanes A, B, and C show dynamic images of DNA in the coil, partial globule, and globule states, respectively, and the interval between photographs is 0.1 s.

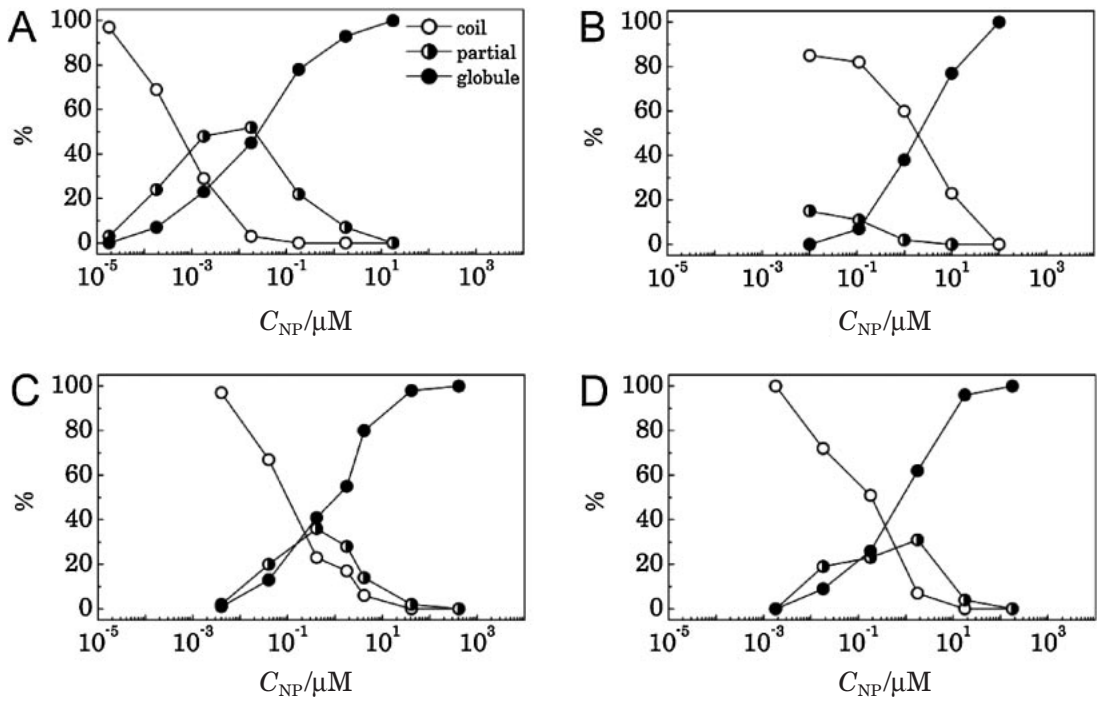


Figure 2. Distribution of DNA conformational states in DNA compaction with various concentrations of NP_{10M} (A), NP_{15} (B), NP_{40} (C), and NP_{100} (D) in the presence of K^+. The concentrations of DNA and K^+ are $C_{DNA} = 0.10\,\mu M$ and $C_{ion} = 200\,mM$.

The results regarding the DNA-folding transition by the action of NP_{10M}, NP_{15}, NP_{40}, and NP_{100} in the presence of Ca^{2+} ($C_{ion} = 20\,mM$) are shown in Figure 4. Although the promoting effect of Ca^{2+} in DNA compaction induced by NPs was somewhat less than that of K^+, the smaller NP_{10M} again tends to be more active than the others. For example, at $C_{NP} = 1.8 \times 10^{-3}\,\mu M$, NP_{10M} gave 19% partial globule together with 2% globule images in the presence of Ca^{2+} ($C_{ion} = 20\,mM$), while larger NPs did not give compacted DNA images even at higher concentrations. More than 96% DNA was in the coil form at $C_{NP} < 1.8 \times 10^{-2}\,mM$ with NP_{15}, NP_{40}, and NP_{100}.

Competition is observed between NPs and the metal ion for DNA charge neutralization. The inhibition of DNA compaction by the metal ion is apparent at lower ion and higher DNA concentrations, and, in fact, the ratio of compacted DNA by NP_{100} ($C_{NP} = 18\,\mu M$) is reduced from 76% (without ion) to 50% in the presence of Na^+ ($C_{ion} = 20\,mM$). At the optimal concentration of the metal ion (200 mM for Na^+ and K^+; 20 mM for Mg^{2+} and Ca^{2+}), both the metal ion and NPs work collaboratively in DNA compaction. However, Na^+ and K^+ have suppressive effects at higher concentrations ($C_{ion} > 300\,mM$) on DNA compaction by NPs.[27]

Effect of Quaternary Ammonium Dication on DNA Compaction. Despite the fact that mono- and divalent metal ions cannot trigger the DNA compaction in an aqueous solution,[11] we reported that the 1,3-propanediammonium derivative, EtPrEt $[(C_2H_5)_3NCH_2CH_2CH_2N(C_2H_5)_3]^{2+}$, could effectively induce DNA compaction.[13] For example, EtPrEt

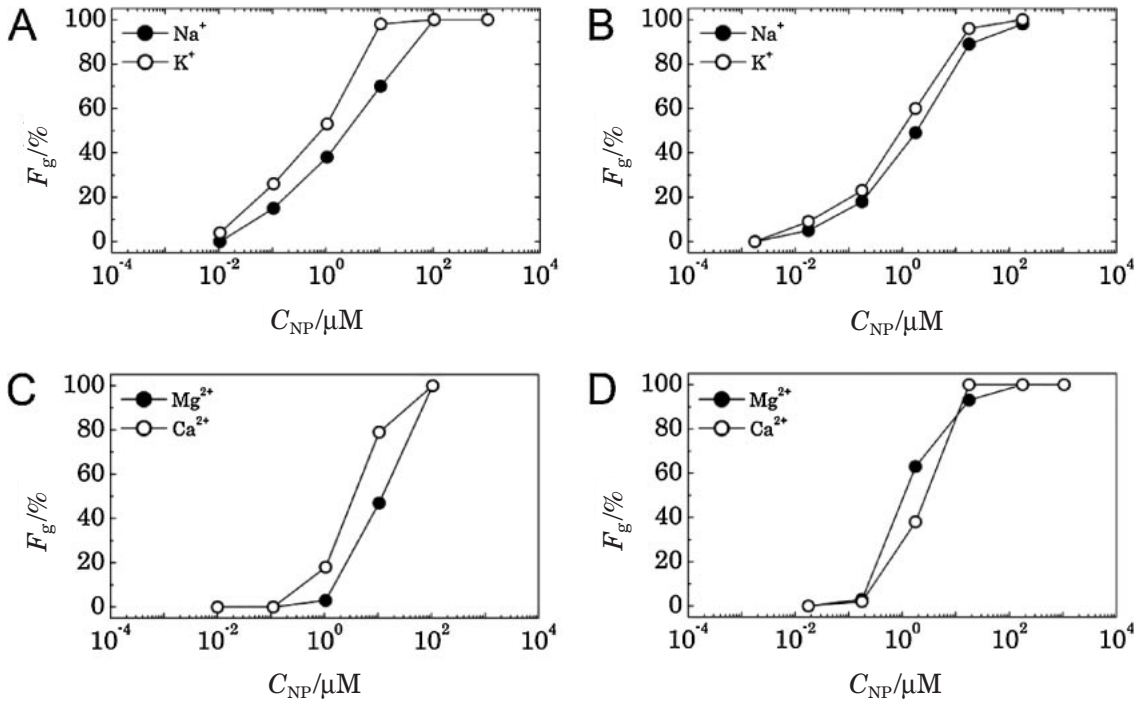


Figure 3. DNA-compacting efficiencies (F_g) of NP_{10} (A and C) and NP_{100} (B and D) in the presence of Na^+ and K^+ (A and B), and Mg^{2+} and Ca^{2+} (C and D). The concentrations of DNA and metal ions are $C_{DNA} = 0.10\,\mu M$ and $C_{ion} = 200\,mM$ (A and B) or 20 mM (C and D). The results in A and B are reproduced from Ref. 27.

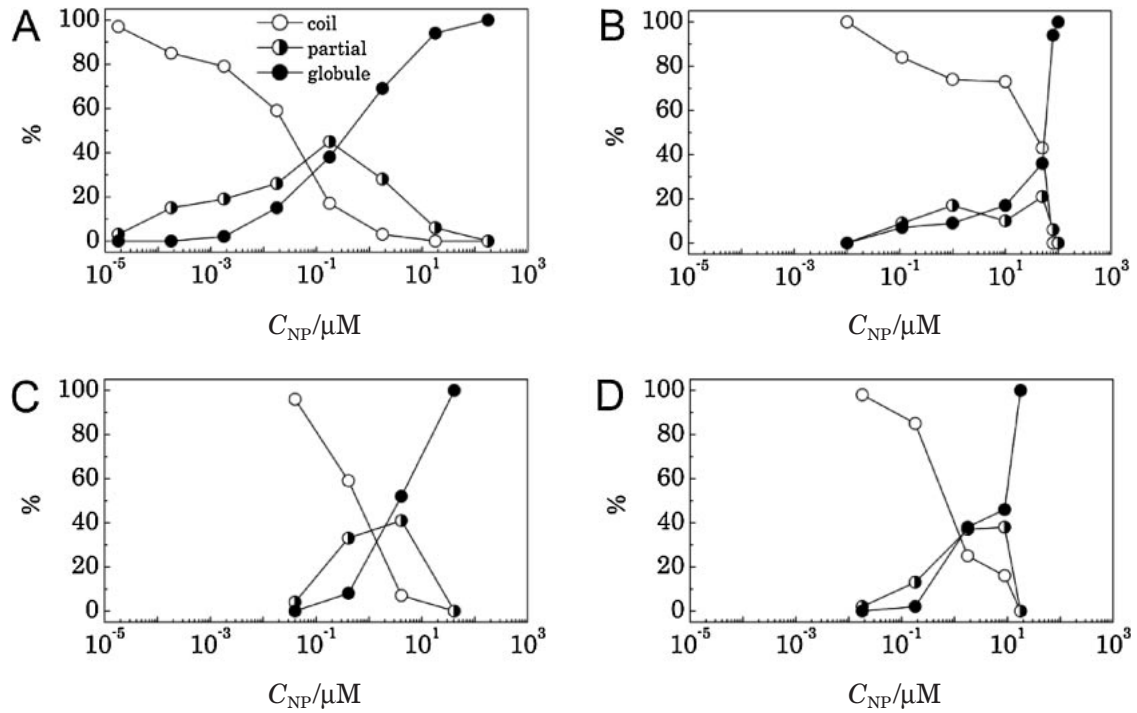


Figure 4. Distribution of DNA conformational states in DNA compaction with various concentrations of NP_{10M} (A), NP_{15} (B), NP_{40} (C), and NP_{100} (D) in the presence of Ca^{2+}. The concentrations of DNA and metal ion are $C_{DNA} = 0.10\,\mu M$ and $C_{ion} = 20\,mM$.

induces DNA compaction even at low concentrations (<1.0 mM) to a significant extent ($F_g = 56\%$) without any promoting agents.[13] What role does EtPrEt play in DNA compaction by NPs: competition, independent action, or collaboration? The results of DNA compaction by NP_{10} and NP_{100} ($C_{NP} = 1.8\,\mu M$) at various concentrations of EtPrEt ($C_{ion} = 0.050$–$1.0\,mM$) are shown in Figures 5A and 5C. The combination of EtPrEt with NP_{10} reduced their DNA-compact-

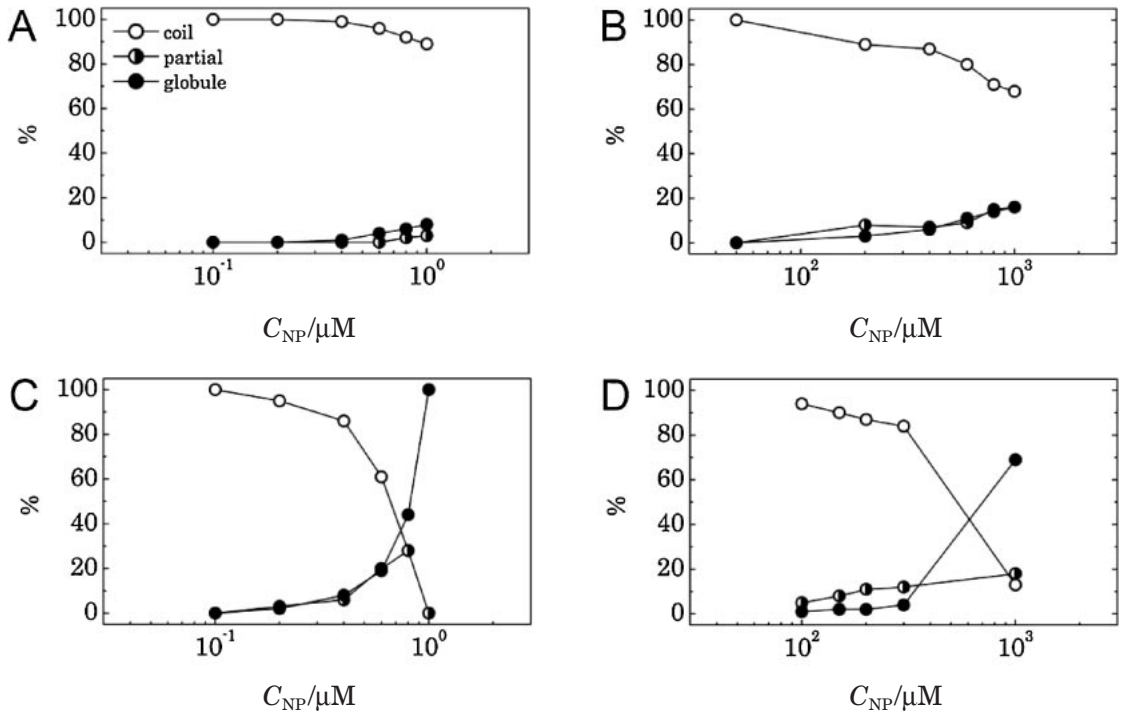
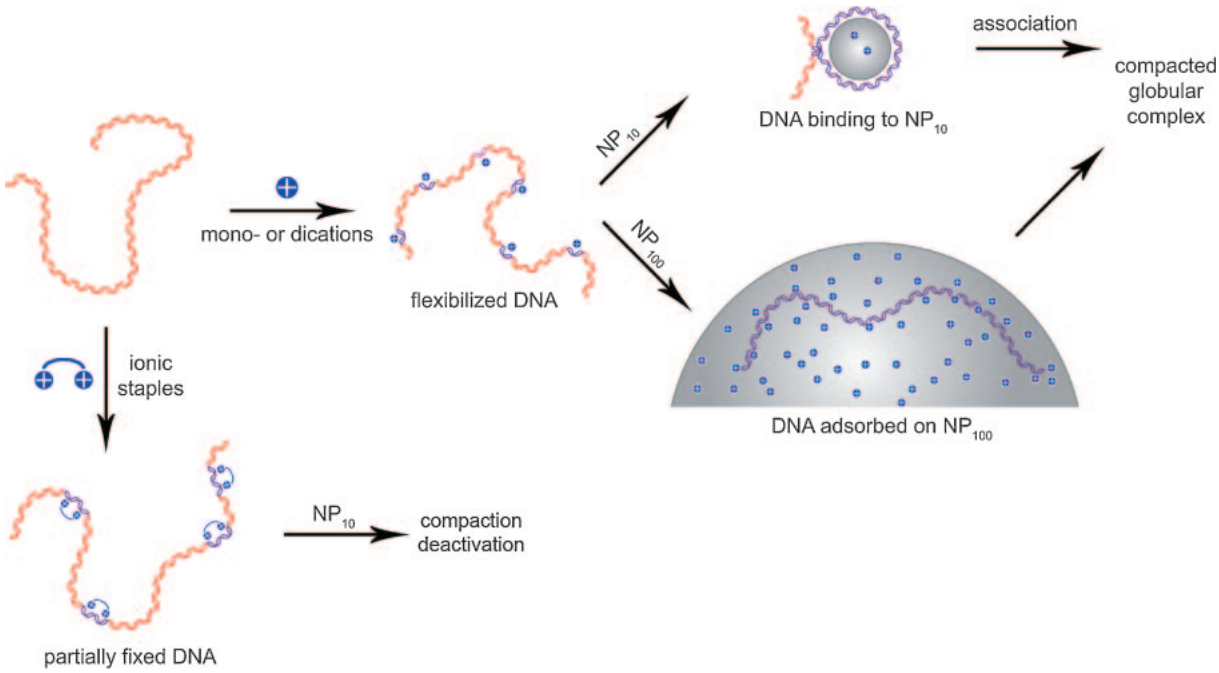


Figure 5. Distribution of DNA conformational states in DNA compaction with various concentrations of NP_{10} and NP_{100} in the presence of EtPrEt and EtEtEt. A and B correspond to DNA compaction by NP_{10} in solutions of EtPrEt (A) and EtEtEt (B), and C and D correspond to DNA compaction by NP_{100} in solutions of EtPrEt (C) and EtEtEt (D). The concentrations of DNA and NPs are $C_{DNA} = 0.10\,\mu M$ and $C_{NP} = 1.8\,\mu M$.

ing activities: for example, DNA compaction proceeded in the presence of a mixture of EtPrEt ($C_{ion} = 1.0\,mM$) and NP_{10} ($C_{NP} = 1.8\,\mu M$) to give the globule form at only 1/7 ($F_g = 8\%$) the efficiency of that with EtPrEt. At the same time, EtPrEt inhibited the DNA-compacting action of NP_{10}. All DNA molecules were in the coil form in the presence of a trace amount ($C_{ion} = 0.10\,mM$) of EtPrEt, although NP_{10} could produce DNA self-compaction without the assistance of a metal ion to give partially compacted DNA in 4% efficiency. EtPrEt also disturbed the action of NP_{100} ($C_{NP} = 1.8\,\mu M$) at a lower concentration ($C_{ion} < 0.40\,mM$), and the ratio of unfolded DNA (86%) was greater than that observed with NP_{100} alone (70%). On the other hand, EtPrEt promoted DNA compaction by larger NPs at a higher concentration ($C_{ion} = 0.80\,mM$): for example, NP_{100} ($C_{NP} = 1.8\,\mu M$) gave 44% globule together with 28% partial globule DNA. This DNA-compacting efficiency reached a maximum value in the presence of the optimal concentrations of K^+ and Ca^{2+}.

An analogous divalent ion with a shorter distance between two cationic centers, EtEtEt ($[(C_2H_5)_3NCH_2CH_2N(C_2H_5)_3]^{2+}$), is known to be ineffective for DNA compaction.[13] Within the concentration range of $50\,mM < C_{ion} < 1\,M$, EtEtEt did not appear to promote DNA compaction induced by NP_{10} ($C_{NP} = 1.8\,\mu M$). A weak promoting action (at high concentration) and inhibitory action (at low concentration) were observed when EtEtEt was used for DNA compaction by NP_{100} ($C_{NP} = 1.8\,\mu M$). These results are shown in Figures 5B and 5D.

Important Factors in DNA Compaction by NPs in Solutions of Electrolytes. The influence of three major factors, i.e., surface charge density of NPs, size of NPs, and DNA stiffness, has to be considered when discussing DNA compaction by NPs. It was reported earlier that DNA compaction by any size of NPs was optimal at an intermediate salt concentrations.[27] The results of this study show that, regardless of charge densities of NPs, DNA-compacting activities of small (10 nm) NPs in the presence of the optimal amount of a metal ion would be the highest in comparison to NPs of larger sizes (Figure 2). The exceptionally efficient DNA compaction by NPs of 10 nm ϕ can be explained based on the differences in residual charge on NPs bound to DNA and the bending stress of the DNA molecule adsorbed on the surface of NPs. Smaller NPs generally have fewer surface charges per particle than larger ones, even if the surface charge densities of NPs of different sizes are the same (Table 1). Therefore, the net surface charge of NPs would be largely lost when small NPs bind to DNA. For example, if NP_{10L} ($Z = +340$) is surrounded by double-stranded DNA that consists of 150 base-pairs ($Z = -300$), which is the same as a unit of chromatin, the net particle charge would decrease to +40. The amount of charges on NPs of 100 nm ϕ increases 100-fold to that of 10 nm ϕ under conditions of the same surface cationic charge density, and if surface concentration of DNA bound to NP is constant, the residual positive charge on large NPs would be largely increased. Therefore, due to the greater reduction in charge repulsion between 10 nm NP, DNA binding to the NPs of 10 nm ϕ occurs readily to create a high-density complex of DNA and NPs.[24] Since the stiffness of double-stranded DNA prevents it from forming a loop around small NPs, both the size of NPs and the stiffness of DNA are interrelated in DNA compaction. The bending stress of a DNA molecule wrapped



Scheme 2. Proposed scenario for DNA compaction by NP_{10} and NP_{100} in the presence of a point charge or ionic staple.

around 10 nm NPs is obviously greater than that of a stable DNA loop of 100–150 nm ϕ induced by binding of multivalent cations such as spermine.[11,12] Therefore, our results suggest that the metal ion which binds to the DNA molecule as a point charge provides flexibility to DNA to ease the formation of a small loop with large stress around 10 nm NPs. The fact that Na^+ and K^+ increase the elasticity of DNA[36–40] supports this speculation.

The low DNA compaction efficiency at a lower ion concentration[27] is probably caused by insufficient flexibility of DNA in low salt due to strong electrostatic repulsion between phosphate groups of DNA. At the optimal concentration of the metal ion, due to a gradual increase in DNA flexibility together with an insignificant increase of competition with NPs, DNA compaction proceeds most cooperatively. However, despite a high flexibility of DNA, a further increase in the concentration of the metal ion over 0.2 M increases electrostatic screening of the DNA molecule and NPs, which causes decrease of the DNA compaction efficiency.[27]

Dications, Mg^{2+} and Ca^{2+}, showed the same qualitative effect on DNA compaction, but at lower concentrations than monocations, in good agreement with the suggestion that providing flexibility to DNA via binding with the dications allows DNA to gain necessary flexibility for efficient DNA compaction at lower dication concentrations.

In contrast to point charges, such as inorganic mono- and dications, a diammonium ion such as EtPrEt, in which two cationic centers are separated by trimethylene spacer, binds to adjacent phosphate groups of DNA like a "staple" to impose local structural rigidity on the DNA cite.[13] Such interaction between DNA and an organic dication limits conformational freedom of double-stranded DNA and increases binding stress, which promotes the conformational transformation from B-type DNA to A-type.[13] Due to the additional stress on DNA, EtPrEt cannot efficiently facilitate DNA compaction by NPs in the way monocations do. Moreover, diammonium dications compete with NPs for DNA binding, and the resulting DNA compaction inhibition is more significant when weakly binding 10 nm ϕ NP are used. However, EtPrEt promotes DNA compaction by large NP_{100} at a higher concentration of dication, because DNA-folding on NPs of 100 nm does not require DNA to be highly flexible for efficient complexation with NPs.

Another diammonium ion, EtEtEt, with an intercharge distance (ethylene) different from that between adjacent DNA phosphates cannot bind to DNA in the same effective manner as EtPrEt; nevertheless, it can also induce the B-type to A-type transition of DNA at high concentrations.[13] The similar effect on EtEtEt in DNA compaction by NP_{10} and NP_{100} can be explained similarly to EtPrEt, although the effective concentrations of EtEtEt are much higher than that of EtPrEt.

In conclusion, the mechanism of DNA compaction by histone-size (10 nm ϕ) and larger (100 nm ϕ) NPs in the presence of biologically essential metal ions and an "ionic staple" can be speculated and is illustrated in Scheme 2.[41] Binding of mono- or dications to DNA provides DNA flexibility to various extent[36–40] and facilitates DNA folding with a larger curvature on even small NPs, which was observed as an increase of compaction efficiency of NPs in high salt solutions. The absorption of DNA on NP_{10} promotes the association between NPs and DNA, to give a compacted assembly due to the DNA and NP charge neutralization. On the other hand, because DNA is not made sufficiently flexible by binding with an organic dication ("ionic staple"), small NPs do not induce DNA compaction in the presence of EtPrEt.

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Supporting Information

Ten tables summarizing distributions of the DNA conformation in the coil, partial globule, and globule states upon treatments with NPs under various conditions and a table showing results on the titration of NPs. This material is available free of charge on the web at http://www.csj.jp/journals/bcsj/.

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