doi:10.1246/cl.2010.72
Published on the web December 19, 2009

DNA Compaction on Histone Mimics Prepared from Silica Nanoparticles

Toshio Takenaka,[1] Anatoly A. Zinchenko,[1,2] Kenichi Yoshikawa,[2,3] and Shizuaki Murata*[1]
[1]*Graduate School of Environmental Studies, Nagoya University, B4-1(780), Chikusa-ku, Nagoya 464-8601*
[2]*Spatio-Temporal Order Project, ICORP, JST*
[3]*Department of Physics, Graduate School of Science, Kyoto University, Sakyo-ku, Kyoto 606-8504*

(Received August 18, 2009; CL-090762; E-mail: murata@urban.env.nagoya-u.ac.jp)

Silica nanoparticles, the surface of which was modified by a 3-aminopropylsilyl group, compacted gene-size DNA in aqueous solutions in the presence of Na^+ or K^+. The DNA-compaction efficiency of histone-size (10 nm diameter) nanoparticles is greater than that of large (100 nm) nanoparticles under similar experimental conditions.

The folding of DNA around histones to form a nucleosome is considered to be the first step in the building of higher-order molecular assemblies, such as chromatin, in eukaryotes.[1] In chromatin, the charge neutralization of DNA phosphate groups by basic amino acid residues results in tight or loose DNA folding, which determines the ON/OFF (activation/silencing) signal of a gene.[2] The DNA in chromatin forms a small loop around histone (7 nm diameter) of only 147 base pairs,[1b] but short DNA fragments of a few hundred base pairs are known to behave as a rigid wires.[3] Because in vitro genomic DNA is frequently folded by cationic binders such spermine[3,4] into doughnut-shaped DNA particles with a diameter of 100–200 nm,[3,4b] it is suggested that there is a mechanism providing flexibility to short DNA fragments. Since an appropriate amount of NaCl is known to stabilize the DNA complex with histone,[5] it is likely that Na^+ plays an important role in configuring chromatin and the chromosome.[6] Recently, Zinchenko et al. reported DNA compaction using silica nanoparticles (10–100 nm diameter) coated by poly-L-lysine.[7] Although a similar promoting effect of coexisting Na^+ was found,[7] it is difficult to prepare nanoparticles with the desired poly-L-lysine amount in a surface layer. We describe here the preparation and characterization of histone-mimic nanoparticles (NPs) with a similar charge value (Z) and their application to gene-size DNA compaction.

Silica nanoparticles, Nissan Chemical IPA-ST (mean diameter: 10 nm) and IPA-ST-ZL (100 nm), were treated with 3-aminopropyltriethoxysilane in 2-propanol, and the amount of amino groups on the resulting NPs was analyzed by acid–base titration as summarized in Table 1.

T4 DNA (166 kbp) was compacted by NPs in an aqueous solution in the presence of Na^+ or K^+. Typical fluorescence microscopy images of DNA stained by 4′,6-diamino-2-phenylindole (DAPI) in the coil (A), partly compacted intermediate (B), and globule (C) states are shown in Figure 1. The results of DNA compaction by NP_{10}'s and NP_{100}'s in the presence of Na^+ or K^+ are shown in Figures 2A and 2B, respectively. DNA compaction efficiency, F_g, indicates the percent of DNA in the compact globule state, and the concentration of NPs (C_{NP}) is given as the molar concentration of NP surface NH_2 groups. The smaller NP_{10}'s weakly induce DNA compaction in the absence of the salt, whereas the larger NP_{100}'s under the same conditions compact DNA to a moderate extent ($F_g = 19\%$). The addition of alkali metal ion enhances the efficiency of DNA compaction by both NP_{10}'s and NP_{100}'s, but at higher concentrations the metal ion inhibits DNA compaction. The optimal concentration ($C_{ion} = 200$ mM) was similar to that of the physiological saline solution. The promoting and further inhibitory effects of K^+ were more significant than those of Na^+.

Table 1. Preparation conditions and characteristics of nanoparticles

	NP_{10L}'s	NP_{10}'s	NP_{10M}'s	NP_{10H}'s	histone[a]
Condition (% v/v)[b]	0.02	0.2	2	2[c]	
NH_2/mmol g^{-1} [d]	0.55	1.1	1.8	4.1	
Z/nm^{2} [e]	+1.1	+2.1	+3.4	+7.8	ca. +1.5
Z per particle	340	650	1100	2400	220

	NP_{100L}'s	NP_{100}'s	NP_{100M}'s	NP_{100H}'s
Condition (% v/v)[b]	0.02	0.2	2	2[c]
NH_2/mmol g^{-1} [d]	0.11	0.18	0.30	0.75
Z/nm^{2} [e]	+2.2	+3.5	+5.9	+15
Z per particle	6.8×10^4	1.1×10^5	1.8×10^5	4.7×10^5

[a]Ref 8. [b]Concentration of 3-aminopropyltriethoxysilane. Reactions were carried out at room temperature. [c]Reaction was carried out at 60 °C. [d]Calculated based on the reported density (2.00 g cm^{-3}) of NPs. [e]Assuming that each NH_2 group corresponds to a single charge.

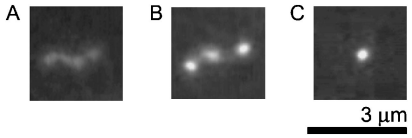

Figure 1. Typical images of DNA in the coil (A), partial globule (B), and globule (C) conformational states.

In the presence of K^+ ($C_{ion} = 200$ mM), the lowest concentration (C_{NP}) of NP_{10}'s to give the globule state ($F_g = 4\%$) was 0.011 μM, and 13% of DNA existed in the intermediate state.[9] DNA compaction was almost complete ($F_g = 92\%$) at $C_{NP} = 17.7$ μM under similar conditions. Large NP_{100}'s were less active and compacted DNA up to $F_g = 89\%$ under the same conditions. These results are shown in Figures 2C and 2D. When the efficiency of DNA compaction was compared between NP_{10}'s and NP_{100L}'s ($Z = +2.2$/nm^2) and between NP_{10M}'s and NP_{100}'s ($Z = +3.4$/nm^2), the following relations were found: NP_{10}'s > NP_{100L}'s and NP_{10M}'s > NP_{100}'s. These results show that histone-size NP_{10}'s have higher DNA affinity[5] than large NPs, and the results were opposite to those seen in DNA compaction induced by the NPs with poly-L-lysine.[7a]

When we compared the C_{NP} values at $F_g = 50\%$, the DNA-compacting ability of NP_{10L}'s was about 1/1000 that of NP_{10}'s

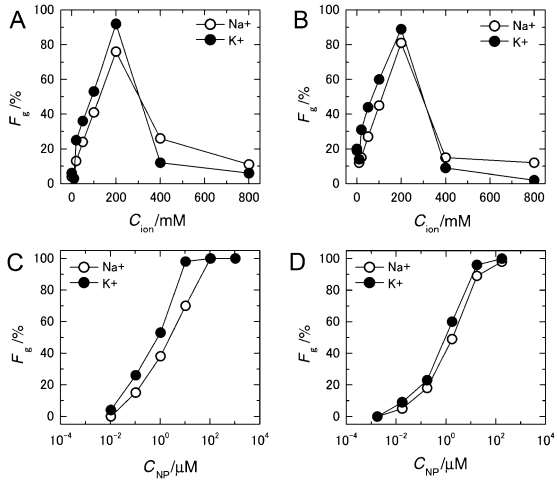

Figure 2. Compaction of 0.1 μM DNA by NP_{10}'s and NP_{100}'s in the presence of Na^+ or K^+: A and B, Dependence of the DNA folding efficiency F_g by 17.7 μM of NP_{10}'s (A) and NP_{100}'s (B) on monocation concentration C_{ion}; C and D, Dependence of the DNA folding efficiency F_g in solution of 200 mM of Na^+ or K^+ on concentration of NP_{10} (C) and NP_{100} (D) surface amino groups C_{NP}.

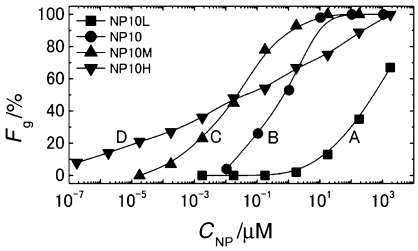

Figure 3. Compaction efficiency F_g of 0.1 μM DNA by 10-nm NPs of different surface charge density in solution of 200 mM K^+: A, NP_{10L}'s; B, NP_{10}'s; C, NP_{10M}'s; D, NP_{10H}'s.

(Figure 3), although the surface concentration of amino groups is only 1/2. NP_{10M}'s with a 1.5-fold higher charge induced DNA compaction with 80-fold higher efficiency than NP_{10}'s. NP_{10H}'s, which had ca. four times more charge than NP_{10}'s, compacted DNA even at 0.1 pM ($F_g = 8\%$), but at least $C_{NP} = 1$ mM was necessary to complete DNA compaction. Therefore, DNA compaction is optimally achieved when NPs have a moderate charge density as shown in curves B and C of Figure 3. As a result of low cationicity, NP_{10L}'s with a lower charge are less active, while there might be repulsion between NP_{10H}'s loading DNA chain. However, the mostly histone-like[8] NP_{10L}'s ($C_{NP} = 1.77$ μM) convert a significant amount of DNA (31%) to the intermediate state.

To interpret the results regarding the efficiency of DNA compaction by NPs, the following 3 factors should be considered: (i) surface charge density of NPs, (ii) the size of NPs, and (iii) reduction of DNA stiffness. Small NPs generally has a lower charge than larger NPs; even if the surface charge densities are the same, the net charge would be largely lost when small NPs interacts with DNA. For example, if NP_{10L}'s is surrounded by double-stranded DNA that consists of a chromatin-like 150 bp fragment ($Z = -300$), the original net charge (+340) would be reduced to only +40. Therefore, DNA complexes with small NPs can easily associate with each other to give a nucleus.[7a] The stiffness of double-stranded DNA generally prevents it from forming a loop around small NP. A DNA loop with a diameter of ca. 100 nm created by the self-organization of sufficiently neutralized DNA[10] is considered to be a stable DNA morphology.[3,4b] The bending stress is obviously greater for a DNA molecule wrapped around 10-nm NPs. Nevertheless, small NPs are more active in DNA compaction in the presence of Na^+ or K^+ than large NPs. It has been reported that coexisting metal ions reduce the elasticity of DNA.[11] At low concentrations of Na^+ or K^+ ($C_{ion} < 50$ mM), the DNA-compacting ability of NP_{10}'s was weak, probably due to insufficient DNA flexibility. At higher concentrations ($C_{ion} > 300$ mM), although DNA is sufficiently flexible, the negative charges on DNA are significantly screened by monocations. Because K^+ interacts with DNA more efficiently than Na^+,[12] K^+ has greater promoting (in low salt) and inhibitory (in high salt) effects on DNA compaction by NPs.

This work is partially supported by a Grant-in-Aid for Scientific Research in Priority Area (No. 20034025) from MEXT, Japan.

References and Notes

1 a) R. D. Kornberg, *Science* **1974**, *184*, 868. b) K. Luger, A. W. Mäder, R. K. Richmond, D. F. Sargent, T. J. Richmond, *Nature* **1997**, *389*, 251.
2 a) B. Dorigo, T. Schalch, A. Kulangara, S. Duda, R. R. Schroeder, T. J. Richmond, *Science* **2004**, *306*, 1571. b) N. J. Francis, R. E. Kingston, C. L. Woodcock, *Science* **2004**, *306*, 1574.
3 K. Yoshikawa, Y. Yoshikawa, in *Pharmaceutical Perspectives of Nucleic Acid-Based Therapeutics*, ed. by R. I. Mahato, S. W. Kim, Taylor & Francis, London, **2002**, pp. 137–163.
4 a) L. C. Gosule, J. A. Schellman, *Nature* **1976**, *259*, 333; V. A. Bloomfield, *Biopolymers* **1991**, *31*, 1471. b) A. A. Zinchenko, V. G. Sergeyev, S. Murata, K. Yoshikawa, *J. Am. Chem. Soc.* **2003**, *125*, 4414. c) A. A. Zinchenko, V. G. Sergeyev, K. Yamabe, S. Murata, K. Yoshikawa, *ChemBioChem* **2004**, *5*, 360.
5 a) E. C. Uberbacher, V. Ramakrishnan, D. E. Olins, G. J. Bunick, *Biochemistry* **1983**, *22*, 4916. b) T. D. Yager, C. T. McMurray, K. E. Holde, *Biochemistry* **1989**, *28*, 2271. c) D. W. Brown, L. J. Libertini, E. W. Small, *Biochemistry* **1991**, *30*, 5293.
6 a) J. Bednar, R. A. Horowitz, J. Dubochet, C. L. Woodcock, *J. Cell Biol.* **1995**, *131*, 1365. b) L. M. Carruthers, J. Bednar, C. L. Woodcock, J. C. Hansen, *Biochemistry* **1998**, *37*, 14776. c) R. Strick, P. L. Strissel, K. Gavrilov, R. Levi-Setti, *J. Cell Biol.* **2001**, *155*, 899.
7 a) A. A. Zinchenko, K. Yoshikawa, D. Baigl, *Phys. Rev. Lett.* **2005**, *95*, 228101. b) A. A. Zinchenko, F. Luckel, K. Yoshikawa, *Biophys. J.* **2007**, *92*, 1318. c) A. A. Zinchenko, T. Sakaue, S. Araki, K. Yoshikawa, D. Baigl, *J. Phys. Chem. B* **2007**, *111*, 3019.
8 H. Schiessel, *J. Phys.: Condens. Matter* **2003**, *15*, R699.
9 Supporting Information is available electronically on the CSJ-Journal Website, http://www.csj.jp/journals/chem-lett/index.html.
10 a) G. S. Manning, *Q. Rev. Biophys.* **1978**, *11*, 179. b) G. S. Manning, *Biopolymers* **1980**, *19*, 37. c) G. S. Manning, *Cell Biophys.* **1985**, *7*, 57.
11 a) S. B. Smith, L. Finzi, C. Bustamante, *Science* **1992**, *258*, 1122. b) P. Cluzel, A. Lebrun, C. Heller, R. Lavery, J.-L. Viovy, D. Chatenay, F. Caron, *Science* **1996**, *271*, 792. c) S. B. Smith, Y. J. Cui, C. Bustamante, *Science* **1996**, *271*, 795. d) R. Podgornik, P. L. Hansen, V. A. Parsegian, *J. Chem. Phys.* **2000**, *113*, 9343. e) J. R. Wenner, M. C. Williams, I. Rouzina, V. A. Bloomfield, *Biophys. J.* **2002**, *82*, 3160.
12 N. Korolev, L. Nordenskiöld, *Biomacromolecules* **2000**, *1*, 648.

