How Does DNA Compaction Favor Chiral Selectivity with Cationic Species? Higher Selectivity with Lower Cationic Charge

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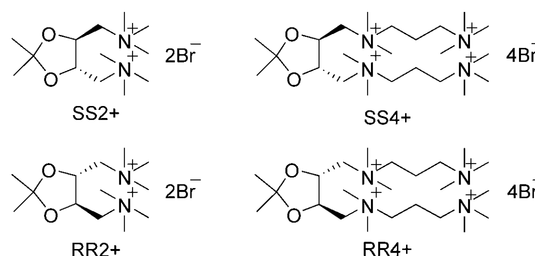
A single-molecule study on giant DNA compaction by enantiomeric dications and tetracations demonstrates that strong chiral discrimination in DNA compaction is manifested only if the positive charge on enantiomeric multications is relatively low. The increase in cationicity of the chiral compaction agent inevitably leads to an increase in nonspecific electrostatic interactions and quenching of chiral discrimination in the DNA-folding phase transition.

Introduction

Despite the original chirality of double-stranded DNA,[1] chiral polymers do not demonstrate significant chiral discrimination in DNA binding or condensation under reversible conditions. For instance, the difference in DNA complexation between polypeptides containing L and D chiral units does not exceed a few percent,[2] neither was any difference in DNA precipitation found between poly-L- and poly-D-lysine.[3] The chiral discrimination of biomacromolecules can be significantly amplified through enhanced DNA asymmetry (rod-like superhelical organization or topologically constrained supercoiled structure).[4,5] On the other hand, our recent investigations demonstrated that small and weakly charged molecules, such as chiral tripeptides[6] or synthetic chiral dications,[7] drastically differ with regard to their DNA-compaction activity, even if there is no difference in their ability to bind to an unfolded DNA chain. Therefore, small weakly charged molecules exhibit strong chiral discrimination in DNA compaction, while there is no difference between chiral polycations in the DNA-folding transition. The details of the origin of chiral discrimination in DNA compaction are still unclear, and when chiral discrimination is manifested depending on charge and compaction potential of cationic species has not yet been explored. To answer this question, we examined giant T4 DNA molecule compaction induced by chiral cationic chemicals with different charges but with the same chiral center.



Scheme 1. Chemical structures of the chiral dications SS2+ and RR2+ and the corresponding chiral tetracations SS4+ and RR4+.

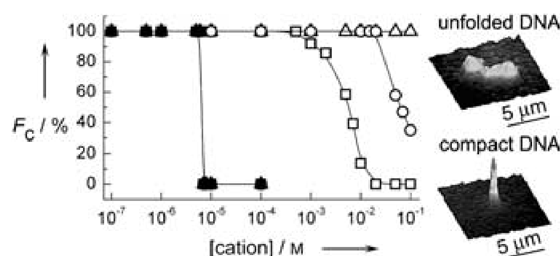


Figure 1. DNA compaction curves with dications SS2+ (□) and RR2+ (△) and their racemic mixture (○), and tetracations SS4+ (■) and RR4+ (▲) and their racemic mixture (●), shown as the dependence of the fraction of DNA in the unfolded (coil) state (F_c) in the ensemble of DNA molecules on the concentration of multications. Right: fluorescence intensity profiles of fluorescent images of unfolded and compact DNA show coil and globule DNA conformations.

Results and Discussion

The compaction of the giant T4 DNA molecule by enantiomeric dications SS2+ and RR2+ (Scheme 1) and their equimolar mixture was studied by conventional fluorescence microscopy (FM). During compaction, the negative charge of DNA is neutralized,[8] and its conformation switches from a coil (unfolded DNA) to a globule (compact DNA), which differ by about 10^4 times in the molecular density of the DNA segments. Typical fluorescence intensity profiles of unfolded and compact T4 DNA molecules observed by FM are shown in Figure 1 (right).

The plot in Figure 1 represents the dependence of the fraction of unfolded DNA in the ensemble of DNA molecules (F_c)

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on the concentration of multications. Chiral dications demonstrate strong chiral discrimination during the compaction of individual DNA molecules. Millimolar concentrations of the SS2+ dication caused DNA to collapse. The transition between the unfolded and compact DNA conformations proceeds via a region of coexistence, with a bimodal distribution between coils and globules indicating an all-or-none mechanism of DNA phase transition. In contrast, compaction of DNA by the RR2+ enantiomer was not achieved even at 0.1 M concentration. Thus, there was a more than 100-fold concentration difference between the DNA compaction activities of enantiomeric dications. An equimolar mixture of chiral dications causes DNA collapse at intermediate concentrations and demonstrates a significant inhibition of SS2+ compaction activity by the RR2+ dication.[7] This chiral discrimination in the DNA molecules' phase transition is even more striking given the fact that, in the unfolded state, the DNA molecule does not discriminate between chiral dications, as shown by the same melting (denaturation) temperature of the DNA double helix in the presence of different dications.

To compare the degree of chiral discrimination in DNA compaction with more highly charged analogues of chiral dications, we synthesized the optically active tetracations SS4+ and RR4+ (Scheme 1) with the same chiral centers as in the respective dications. These tetracations were expected to be much more efficient DNA-compaction agents than dications as a result of their doubled cationic charge.[9] Indeed, fluorescence microscopy observations showed that the compaction activity of tetracations was 3–4 orders of magnitude higher than that of the dications (Figure 1). However, contrary to the profound chiral discrimination observed between SS2+ and RR2+, there was no difference in concentration necessary for DNA collapse caused by SS4+ and RR4+ compaction agents. The DNA-compaction potential of a 1:1 SS4+/RR4+ mixture was also the same. Thus, with an increase in the charge of the chiral compaction agent of only +2 to +4, chiral discrimination completely vanishes. Evidently, in all cases in which the charge on analogues of studied multications is higher than +4, chiral discrimination in DNA phase transition is not expected.

In comparing discrimination between enantiomeric pairs of 2+ and 4+ multivalent cations, it becomes clear that the electrostatic charge on cationic compounds and the degree of chiral discrimination in DNA compaction are tightly interrelated properties, and that an increase in the cationicity of the DNA-condensing molecule inevitably leads to the quenching of enantiomeric discrimination.

Generally, the difference in the extent of chiral discrimination is determined by a balance between nonspecific electrostatic interactions (between DNA phosphates and multications' quaternary ammonium groups) and other weaker interactions (e.g. interaction of the chiral dioxolan ring with the DNA minor groove) that might cause discrimination. Since dications are weakly charged molecules, specific nonelectrostatic interactions that are favored or disfavored by chiral complementarity to DNA's own chiral geometry and powered by the highly cooperative nature of DNA compaction become exceptionally important in the promotion or inhibition of DNA-compaction transition. On the other hand, specific interactions do not contribute significantly when more highly charged tetracations interact with DNA, and, in this case, the compaction potential of tetracations is entirely determined by their electrostatic charge.

It is also worth noting that, in buffer solution, the negative charge of unfolded DNA is partially neutralized (by about 76%) by small counterions, and the addition of multivalent cations induces DNA compaction when about 90% of DNA negative charge is neutralized.[8] Because the value 90% is independent of the nature of the multication, in order to compact DNA, dications must replace almost all the monovalent counterions. Therefore, in the case of tetracations, the DNA might contain a significant portion of monocations. Thus, DNA compacted by dications incorporates more chiral molecules in condensate; this gives additional grounds for chiral discrimination.

To examine the possibility of recovering chiral discrimination between tetracations, we set up experimental conditions to decrease the DNA compaction potential of tetracations by adding the monovalent salt NaCl, based on the simple suggestion that an artificial decrease in the higher compaction potential of SS4+ and RR4+ to the level of dications might lead to a recovery of chiral discrimination. Figure 2 shows the effect of adding 0.1 M NaCl on DNA compaction by chiral tetracations.

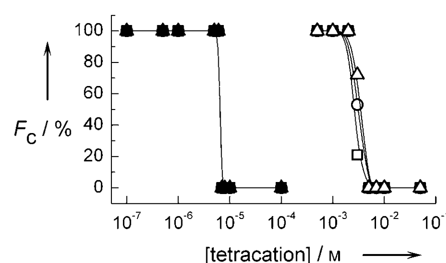

Figure 2. DNA compaction curves with SS4+ (■), RR4+ (▲), and racemic mixture (●) in Tris-HCl buffer solution and in the presence of 0.1 M NaCl (□, △, and ○, respectively) shown as the dependence of the coiled fraction (F_c) in the ensemble of DNA molecules on the concentration of tetracation.

The addition of NaCl results in a dramatic shift in the concentrations of tetracations required to collapse DNA from about 10^{-5} M to 10^{-2} M. Under these conditions, the potentials of SS4+ and RR4+ to fold DNA become similar to those of SS2+ and RR2+ (Figure 1). However, even under conditions in which tetracations show a low compaction potential, all of the DNA compaction curves are almost coincident; this indicates that a recovery of discrimination to the degree observed for chiral dications does not occur. We only noted a slight difference in DNA compaction with SS4+, RR4+, and their racemic mixture, which qualitatively resembles the order of DNA-compaction activity of the corresponding dications. For instance, at 3×10^{-3} M of tetracations, the fraction of DNA compacted into the globule state by SS4+ was about 80%, by a 1:1 mixture—about 50%, and by RR4+—about 30%. However, this difference is fairly small compared to the discrimination

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between chiral dications. The fact that chiral discrimination between tetracations is not manifested at high salt concentrations indicates that discrimination between enantiomers does not depend on their absolute DNA compaction activity. On the contrary, the balance between electrostatic and weaker specific interactions with the DNA helix inherent to a certain structure and cationicity of enantiomers determines the degree of chiral discrimination in DNA-compaction transitions.

To compare the morphologies of DNA molecules compacted by chiral di- and tetracations, we performed an electron microscopy study of DNA condensates. Typical electron microscopy images of DNA collapsed by dication SS2+ and tetracation SS4+ are shown in Figure 3.

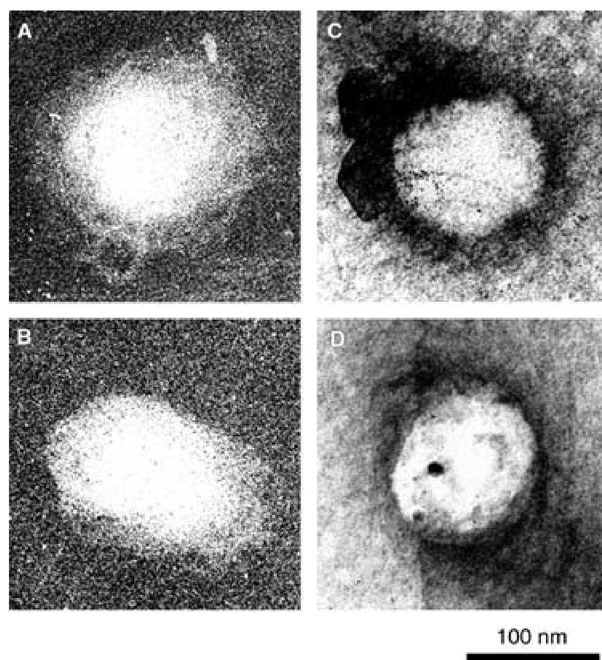


Figure 3. Transmission electron microscopic images of T4 DNA (1×10^{-6} M) collapsed by 5×10^{-5} M SS4+ (A, B) or 5×10^{-2} M SS2+ (C, D) in Tris-HCl buffer solution. A and B are negatives.

In both cases, DNA was collapsed into spherical particles. However, definite differences were found in their morphologies. DNA molecules that had been collapsed by dications appeared as tightly packed particles with an outer diameter smaller than 120 nm. In contrast, DNA that had been collapsed by tetracations was larger (around 150 nm), and it was easy to see that DNA packing in such condensates was rather loose. A tighter packing of DNA by dications implies a highly ordered assembling of dications on DNA in the condensate; this can be forced by stricter conditions for chiral complementation between DNA and the dications during compaction. In a more loosely packed DNA structure formed by tetracations, such requirements regarding the fit of the chiral interaction are much less strict.

It is worth noting that differences in DNA compaction between iso-charged molecules based on differences in the chemical structure other than chirality support the same principle: a higher charge on the compaction agent results in poorer discrimination. For example, there is a more than 1000-fold difference in DNA-compaction ability between homologous linear diamines that having different lengths of methylene spacers between charges,[10] whereas there is only a several-fold difference between homological tetramines.[11]

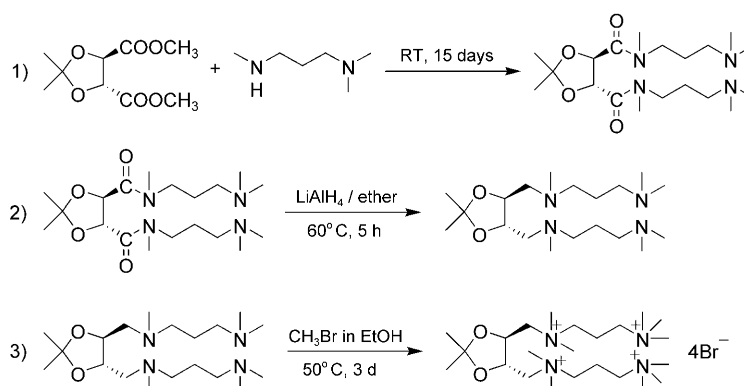
In summary, the best chiral discrimination in the compaction of right-handed DNA macromolecules was achieved when the cationicity of the multivalent cation was relatively low. The balance between electrostatic and weak nonelectrostatic forces in the interaction between DNA and chiral multications is responsible for the manifestation of chiral discrimination in DNA compaction.

Experimental Section

Materials: Bacteriophage T4 DNA (166000 base pairs) was purchased from Nippon Gene Co., Ltd. (Japan), the fluorescent dye 4,6′-diamidino-2-phenylindole (DAPI) dihydrochloride and 2-mercaptoethanol (ME) were obtained from Wako Pure Chemical Industries, Ltd. (Japan) and were used for the fluorescence microscopy observations. Dimethyl (4*R*,5*R*)-(+)-2,2-dimethyl-1,3-dioxolan-4,5-dicarboxilate, dimethyl (4*S*,5*S*)-(−)-2,2-dimethyl-1,3-dioxolan-4,5-dicarboxilate, *N*,*N*-dimethyl-*N*′-methyl-1,3-diaminopropane, and methyl bromide for the synthesis of tetracations were purchased from Tokyo Kasei Kogyo Co., Ltd. (Japan). A solution of $LiAlH_4$ in diethyl ether (1 M) was purchased from Aldrich. Organic solvents were purchased from Nacalai Tescue Inc. (Japan).

Chiral dications were synthesized as descried previously.[7] Chiral tetracations were synthesized according to Scheme 2.

Diamidodiamines (step 1) were prepared in methanol with a fivefold excess of diamine to diester, according to an earlier report.[12] Reduction and quaternization (second and third steps) were performed in the same manner as in refs. [13] and [7], respectively. Elemental analysis calcd (%) for $C_{23}H_{54}N_4O_2Br_4$: C 37.42, H 7.37, N 7.59; found for RR4+: C 37.43, H 8.01, N 6.92; elemental analysis calcd (%) for $C_{23}H_{54}N_4O_2Br_4{\cdot}3\,H_2O$: C 34.86, H 7.63, N 7.07; found for SS4+: C 34.47, H 7.78, N 6.75.



Scheme 2. Synthesis of chiral tetracations.

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Methods

Fluorescent microscopy: The samples were illuminated with 365 nm UV light (high-pressure Hg lamp). Fluorescence images of DNA molecules were observed by using a Zeiss Axiovert® 135 TV microscope equipped with a 100× oil-immersed lens and recorded on S-VHS videotape through a Hamamatsu SIT TV camera. Sample solutions contained Tris-HCl buffer (10 mM, pH 7.8), ME (4%, *v*/*v*), DAPI (0.2 μM), and T4 DNA (0.2 μM in phosphates) were mixed in the order listed. In the experiments with high concentrations of NaCl, NaCl was added to the solution after the buffer. Solutions were equilibrated for 30 min before observations. All observations were carried out at room temperature.

Electron microscopy: Samples were mounted on carbon-coated copper grids (mesh 300), stained with 1% uranyl acetate, and observed on a JEOL 1200 EX (100 kV) transmission electron microscope at the Graduate School of Medicine, Nagoya University. Sample solutions contained Tris-HCl buffer (10 mM, pH 7.8) and T4 DNA (1 μM).

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