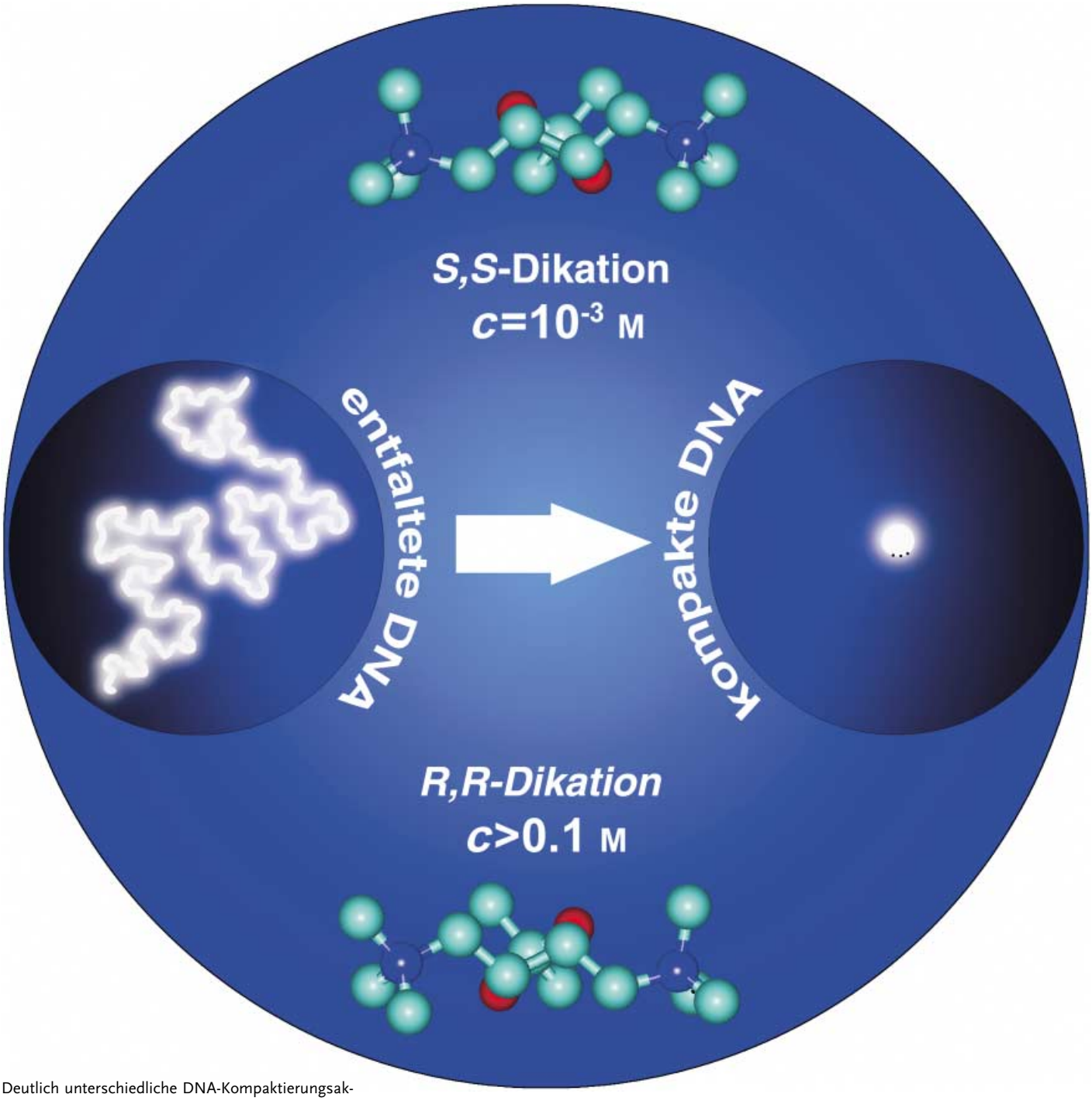
Zuschriften



Deutlich unterschiedliche DNA-Kompaktierungsaktivitäten wurden bei einem Paar enantiomerer Dikationen beobachtet. Als Ursprung der chiralen Diskriminierung wurde die DNA-Faltung identifiziert, woraus sich eine mögliche Methode für die Enantiomerentrennung ableiten lässt. Weitere Informationen entnehmen Sie der Zuschrift von A. A. Zinchenko, S. Murata et al. auf den folgenden Seiten.

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Biological Chemistry

Stereoisomeric Discrimination in DNA Compaction**

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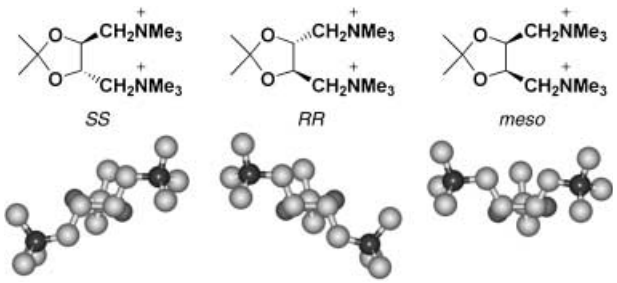
The compaction and decompaction of native DNA are vital to the functions of cells, as these processes cause the necessary conformational transformations of the DNA for the storage, transcription, and replication of genetic information. DNA itself is a chiral molecule characterized by a right-handed helical configuration.[1,2] Therefore, it is expected to exhibit discrimination of chiral compounds. In nature, the behavior of molecular DNA in cells is regulated by a diverse range of chiral molecules, and it is well known that naturally occurring polypeptides have L chirality, which is opposite to the D chirality of native DNA.

The discovery of the principle of biochemical complementarity has prompted numerous investigations into the interaction of DNA with chiral compounds in attempts to account for the origin of natural homochirality and to develop applications aimed at DNA recognition. To date the main classes of chiral compounds that have come under examination have been macromolecules,[3–5] transition-metal complexes,[6–11] and various drugs.[12–14] In most cases, the discrimination of the interaction of DNA with different chiral compounds has been studied exclusively with respect to DNA binding. Although several reports have discussed the effects of chirality on DNA condensation, these physicochemical and biochemical studies were performed under conditions that do not promote single-molecule DNA compaction: with relatively short DNA molecules (< 1 kbp length; bp = base pair) or at high DNA concentrations. Short DNA molecules can not undergo a folding transition (single-molecule condensation), but only undergo transition from a dissolved state into a precipitate. At high concentrations of DNA in solution, multimolecular DNA aggregation occurs instead of single-molecule DNA compaction. Thus, the phenomenon of chiral discrimination in relation to DNA compaction, which is inherent only to long DNA molecules that consist of more than 1 kbp, has not yet been explored, in spite of the fact that all of the genome DNA in living cells is above the size of several hundred thousand base pairs. Recently we showed that enantiomeric trimers of arginine and lysine differ in their DNA-compaction potential.[15] Herein we report dramatic chiral discrimination in DNA compaction by conformationally rigid stereoisomeric dications (Scheme 1). These dications were designed and



Scheme 1. Chemical structures and molecular models of the *SS*, *RR*, and *meso* dications used in this study. (In the molecular models, the counter ions and hydrogen atoms are not shown.)

synthesized[16] as small molecules with minimal geometrical and electrostatic differences, so that we could focus on the intrinsic chirality effect on single-molecule DNA compaction.

DNA compaction by *SS*, *RR*, and *meso* stereoisomeric dications was monitored by fluorescence microscopy (FM) as the conformational change of giant T4 DNA molecules from an elongated coil with an average long-axis size (L) of the fluorescent image of about 3.5 μm into a compact globule (L = 0.6–0.7 μm; Figure 1a). The process of DNA compaction is accompanied by a dramatic decrease in the long-axis size and effective molecular volume of the DNA. The three-dimensional plots in Figure 1b show the change in the distribution of L with respect to the concentration of the dications. The *SS* isomer showed the highest potential to induce DNA collapse. Upon the addition of the *SS* dication to the solution of T4 DNA, the T4 DNA underwent compaction from coil into globule through a coexistence region with bimodal size distribution. In contrast, the *RR* dication did not promote DNA compaction at concentrations up to 0.1M, and only a slight decrease in the DNA coil size was observed with increasing concentration of the dication. Thus, the concentration of the *RR* dication required to induce DNA compaction is at least 100 times higher than that of the *SS* dication. The achiral *meso* compound showed moderate compaction activity, which was slightly lower than that of the *SS* compound. Figure 1c summarizes the results in terms of the compaction activities of the dications. The fraction of the DNA molecules in the sample solution in the coil state is plotted against the dication concentration. The dication with the "favorable" configuration (*SS*) is about three times as

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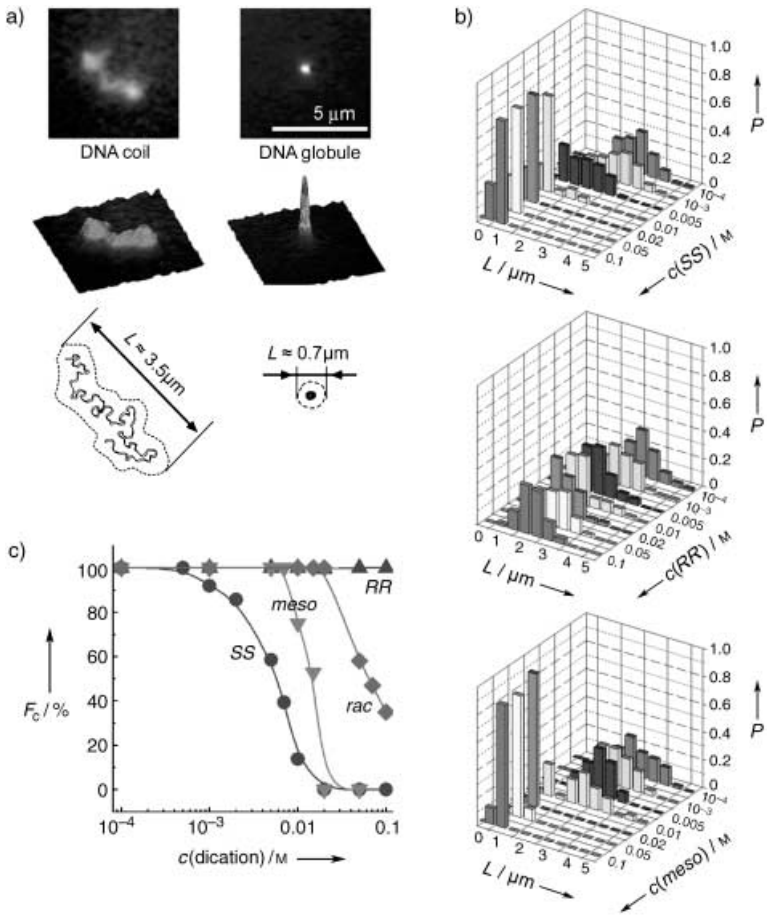

Figure 1. T4 DNA folding transition promoted by *SS*, *RR*, and *meso* dications. a) Fluorescent images of a T4 DNA molecule in unfolded (coil) and collapsed (globule) states, light-intensity distributions in images of a coil and a globule, schematic illustrations of a coil and a globule, and definition of the characteristic parameters of the long-axis length (*L*). b) Dependence of the distribution of the T4 DNA long-axis size (*L*) on the concentration (*c*) of the *SS*, *RR*, and *meso* dications (*P* is relative population). Sample solutions contained Tris-HCl buffer (10 mM, pH 7.8), 2-sulfanylethanol (v/v 4%), T4 DNA (0.2 µM), and the fluorescent dye DAPI (4′,6-diamidino-2-phenylindole dihydrochloride; 0.2 µM). All starting solutions of T4 DNA used in this study were prepared in this way. c) Compaction curves for the *SS* (●), *RR* (▲), racemic (*SS*:*RR* = 1:1; ◆), and *meso* (▼) dications. The fraction (F_c) of the DNA molecules in the sample that are in the coil state is plotted against the concentration of the dication.

effective at promoting compaction (in terms of half-transition concentrations) as the achiral (*meso*) compound. The dication with the "unfavorable" configuration (*RR*) has a dramatically lower compaction activity than the achiral analogue.

DNA compaction is driven by negative-charge neutralization in DNA.[17] Therefore, charge value and charge distribution in the compaction agent are responsible for the potential compaction activity.[18–20] Based on data from computer modeling the *SS*, *RR*, and *meso* dications have similar intercharge (N^+-N^+) distances of approximately 6 Å, and the charge distributions on the nitrogen atoms are the same for all three compounds. It was also found that the values for the degree of dissociation of all the dications are the same at about 90%. Thus, all the dication dibromide salts were expected to possess the same electrostatic binding affinity for DNA, and we aimed to prove this experimentally. To compare binding abilities, we investigated the stability of the DNA double helix against thermal denaturation in the presence of the dications.[21] As expected, the addition of a cationic compound to DNA in solution resulted in a significant increase in the temperature of DNA melting. However, when equal amounts of different dications were added to the sample solution of DNA, identical melting profiles were recorded. The melting temperature of DNA is known to be very sensitive to the nature of interacting cations,[22] and the coincidence of melting profiles is strong evidence that the DNA-binding potentials of the *SS*, *RR*, and *meso* dications are the same; that is, the DNA exhibits no chiral discrimination in binding these small divalent molecules.

Apparently there is no direct correlation of the type observed for achiral compaction agents between the ability of chiral polycations to bind with DNA and their ability to collapse DNA. Additional structural conditions are required to extend the efficiency of binding to DNA to compaction activity. It has been established that the compaction of DNA proceeds in an all-or-none fashion. This means that in the case of giant T4 DNA, hundreds of thousands of chiral molecules must be incorporated into the DNA complex to induce the transition from the coil to the globule state. Thus, a small stereochemical difference in the dication should be enhanced significantly because of the highly cooperative nature inherent to the folding transition of giant DNA. The importance of the condensed state of DNA in chiral discrimination was suggested by Minsky and co-workers,[23,24] who showed that enhanced DNA asymmetry (rodlike superhelical organization or topologically constrained supercoiled structure) was required for the manifestation of chiral effects in the interaction of DNA with chiral peptides. However, no previous study has described the stereoisomeric discrimination that occurs during the generation of the compact DNA state. Our results clearly demonstrate that the formation of the DNA compact state provokes strong chiral discrimination.

It is known that macromolecules that contain units of random chirality possess a lower ability to condense DNA than optically pure polycations.[3] Enantiomeric "cross-inhibition" in mixtures of enantiomers is another well-known phenomenon, which hinders template-directed DNA replication severely.[25,26] To gain insight into the combined effect of the *SS*, *RR*, and *meso* dications on DNA compaction, we investigated the conformational behavior of DNA in the presence of binary equimolar mixtures of the dications. First, T4 DNA was added to an equimolar mixture of the *SS* and *RR* enantiomers. The compaction activities of the pure *SS* and *RR* components and those of a mixture of the two compounds are

compared in Figure 2a. It is evident that the compaction activity of such a mixture is significantly lower than the expected value based on the assumption that all the *SS* compound in solution "works" for DNA collapse. In other words, whereas the pure *SS* compound induces DNA collapse at a concentration of 1 mM (Figure 1c), in the equimolar mixture with the *RR* enantiomer the concentration of the *SS* enantiomer required to induce a switch in the DNA conformation is as high as 25 mM. Thus, not only is the *RR* compound itself unable to collapse DNA, but it significantly interferes with the DNA collapse induced by the *SS* compound. The chirality conflict observed between the *RR* and *SS* compounds is in good agreement with the previously suggested antagonistic effect in systems containing enantiomers.[3,25,26] DNA compaction begins with the formation of dense nuclei on the DNA chain, which grow until the complete DNA chain collapses.[27,28] The formation of the nuclei requires the neutralization of charge on the DNA over a relatively long region (on the order of several hundred base pairs) and, as has been shown, a structural correspondence between the chirality of the DNA and that of the dication. When the mixture of the *RR* and *SS* enantiomers is added to the DNA, the phosphate groups on the DNA become randomly occupied by isomers of opposite chirality. Therefore, partial occupation of the DNA chain with the *RR* dication prevents nucleation to a certain extent, and thus prevents DNA compaction.

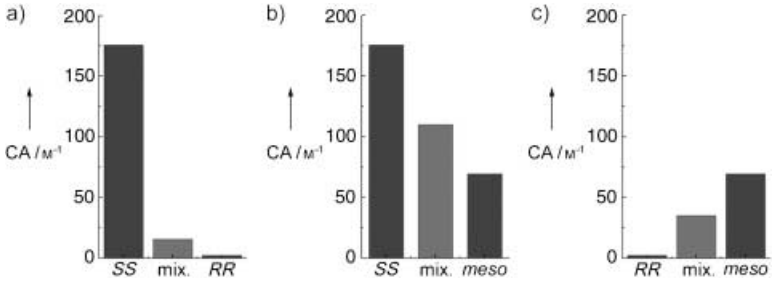

Figure 2. Compaction activities of the pure *SS*, *RR*, and *meso* dications, and of equimolar binary mixtures of the dications. Compaction activity (CA) was calculated from the corresponding compaction curves as $1/C_{50\%}$, where $C_{50\%}$ is the dication concentration at 50% conversion of the DNA from the coil into the globule state. a) Compaction activities of the *SS* and *RR* dications, and of an equimolar mixture of these two compounds. b) Compaction activities of the *SS* and *meso* dications, and an equimolar mixture of these two compounds. c) Compaction activities of *RR* and *meso* dications, and an equimolar mixture of these two compounds.

In contrast to the *SS*/*RR* mixture, the activity of the *SS*/*meso* mixture is an average of the individual activities of the *SS* and *meso* compounds, as both components participate in the compaction of DNA coherently (Figure 2b). A similar synergetic effect was found for the *RR*/*meso* mixture (Figure 2c). In mixtures of chiral and achiral dications, both components are involved in the DNA compaction process; otherwise, the non-active dication would prevent DNA collapse by its own ionic strength through electrostatic competition with the active dication for DNA binding, which would inevitably result in the prevention of DNA compaction. However, we observed no loss of overall compaction activity in these mixtures. Thus, the achiral *meso* dication is able to contribute to the DNA compaction in accordance with the chirality of either the *SS* or *RR* enantiomer and in this way decrease the complementary limitation of DNA compaction.

The finding of the strong antagonism between the *SS* and *RR* dications in DNA compaction raises a new interesting question regarding the possibility of the reverse process: the decompaction (unfolding) of DNA complexed with the *SS* dication by addition of the *RR* dication. To evaluate this possibility we performed the following experiments. First, T4 DNA was treated with the *SS* compound at a concentration of 7 mM. Coils were observed in solution and compact globules were observed on a microscope glass slide simultaneously. The T4 DNA bimodal size distribution at this concentration is represented by the front distribution shown in Figure 3a. After the addition of the *RR* compound (20 mM), an increase in the proportion of DNA in the coil state was observed (middle distribution in Figure 3a). After 1 day, the distribution had shifted even further toward DNA unfolding (see Figure 3a), thus indicating that a certain period of time is required for the equilibrium to be attained in this process. FM analysis of the solution of

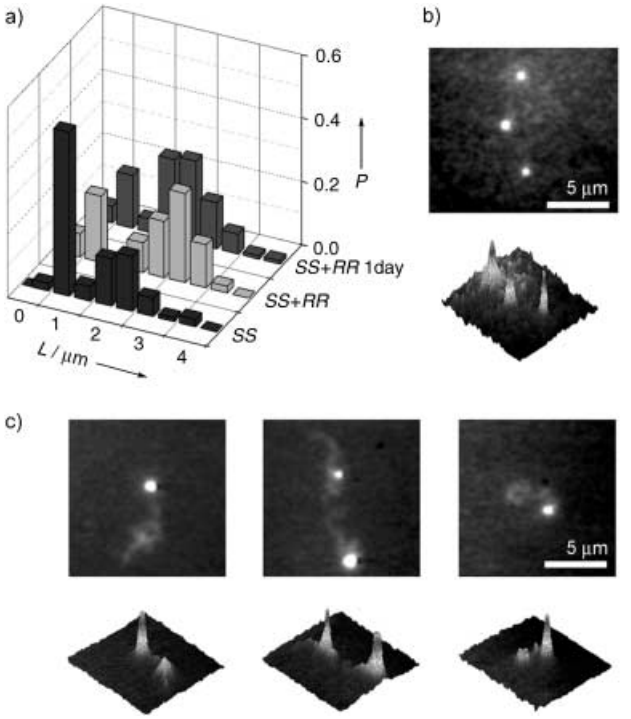

Figure 3. Unfolding by the *RR* enantiomer of T4 DNA collapsed by the *SS* enantiomer, monitored by FM. a) Long-axis size (*L*) distributions (*P* is relative population) of T4 DNA after the addition of the *SS* enantiomer (7 mM; front) to the solution of DNA, after the addition of the *RR* enantiomer (20 mM; middle) to the resulting mixture, and in the resulting mixture after 1 day (back). The starting solution of T4 DNA was the same as described in the legend of Figure 1. b) Fluorescent images of T4 DNA globules on a glass surface in the presence of the *SS* enantiomer (7 mM). c) Partially unfolded structures of T4 DNA observed on a microscopic glass slide after the successive addition of the *SS* enantiomer (7 mM) and the *RR* enantiomer (20 M).

DNA in the presence of the *SS* enantiomer in the coexistence region showed only globules on the glass surface (Figure 3b); after the addition of the *RR* enantiomer the number of globules on the glass surface decreased, and many DNA molecules containing both folded and unfolded parts appeared (Figure 3c). The appearance of these structures on the glass surface upon the addition of the *RR* compound is also attributed to the phenomenon of unfolding. This phenomenon appears to be a direct result of the antagonistic effect that the *SS* and *RR* enantiomers have on one another in DNA compaction. Since DNA compaction is a reversible process, the exchange of dications at suitable sites in the exposed regions of the DNA chains in a compact globule results in the partial populating of DNA by the *RR* dication, which initiates unfolding. The importance of this finding is that a noncomplementary enantiomer can be used not only to prevent the formation of the DNA compact state by the complementary enantiomer, but also to promote the reverse process, that is, the decompaction of the compact DNA complex formed with the complementary dication.

In summary, a dramatic difference and conflict was observed between two synthetic enantiomeric dications in their effect on the compaction of giant T4 DNA. As no difference was found in the DNA-binding activity of the two enantiomers, the results obtained regarding chiral discrimination in DNA compaction indicate that the selectivity for an enantiomer with complementary chirality has its origin in the DNA-folding process itself. It was also demonstrated that an achiral dication can remove the structural chirality restrictions during DNA compaction by enantiomers. In nature, where DNA folding and unfolding are fundamental processes, chiral discrimination in the DNA compaction may be one of the selection mechanisms toward biochemical homochirality.

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Keywords: chiral discrimination · chirality · DNA compaction · DNA recognition · fluorescence microscopy

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