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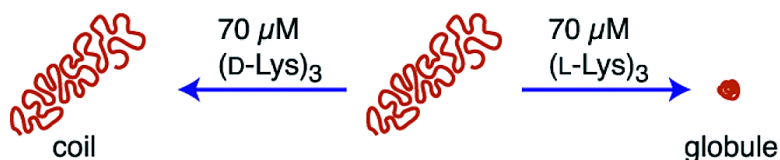
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Nonspecificity Induces Chiral Specificity in the Folding Transition of Giant DNA

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On the 50th anniversary of the discovery of DNA, numerous genius findings have led to the complete analyses of the whole human genome. During that period, changes in the higher-order structure of DNA, as well as in the genetic sequences, have been known to play important roles in living organisms, and many challenging problems still have to be solved. DNA exists in the cell nucleus as a compacted and hierarchic structure of chromatin, in which a right-handed double helical DNA chain winds counterclockwise 1 and 3/4 turns around histone proteins to produce a nucleosome. Because DNA constructs such a higher-order chiral molecular assembly, it is natural to expect that some chemicals interactive with DNA can exhibit different binding modes depending on the chiralities. However, the occurrence of change on the higher-order structure of DNA induced by chiral compounds is still unclear due to the uncertainties inherent in classical assays.[1] In relation to this, it is known that polypeptides afford only a slight difference in their binding ability with DNA,[2] and, for example, the difference in the complex-forming efficiencies of poly-L-lysine and poly-D-lysine was approximately 3% at maximum.[2c] So far, chiral interactions on the large-scale structural transition of giant DNA molecules have not been significantly examined, despite the recent discovery and development of the all-or-none type switching in the higher-order structure of DNA.[3] Very recently, we found that slight structural differences of polyamines were amplified into large differences during DNA folding transitions.[4] In this paper, we would like to report that the DNA folding transition can be significantly differentiated by asymmetric structures of triamines related to the histone protein.

A 0.02 μM (in base pair) solution of T4 phage DNA (166 kbp with 57 μm contour length) was treated with a 0–150 μM solution of a tripeptide (tri-L-lysine, tri-D-lysine, tri-L-arginine, or tri-D-arginine) in a pH 7.6 buffer solution. Figure 1c shows the histograms for the distributions of the long axis length (l) of individual T4 DNA molecules in the presence of (L-Lys)$_3$ and (D-Lys)$_3$ at various concentrations. At the lower tripeptide concentrations, the distribution is wide, and almost all of the DNA molecules exist in the elongated coil state (l = 1–7 μm).[5] When the concentration is increased, DNA collapses into the compacted globule state (l < 1 μm), and, finally, T4 DNA exhibiting a narrow unimodal distribution is recognized. On the basis of the successive observations of the conformation and also the Brownian motion of individual DNA molecules by fluorescence microscopy, we can easily distinguish between the coil and globule states. Typical fluorescent microscope images are illustrated in Figure 1a and b, respectively.

Figure 1d and e shows the proportion of the compact state depending on the concentrations of the tripeptides. It is to be noted
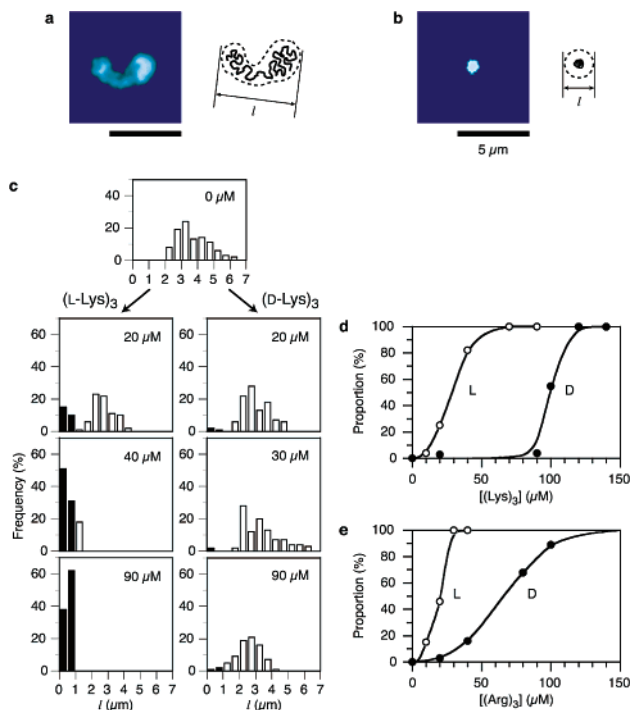

Figure 1. Fluorescence microscope images of T4 DNA: an elongated coil state (a) in the absence of a condensing agent and a globule state (b) in a 70 μM (L-Lys)$_3$ solution. Histograms of the distribution of the long-axis lengths (l) of DNA at various concentrations of (L-Lys)$_3$ and (D-Lys)$_3$ are given in (c). The populations of molecules in the coil and globule states are indicated by the different shading in the histogram. The ratios of the folded DNAs are given in (d) and (e), where the open and closed circles denote the L- and D-enantiomers, respectively.

that (L-Lys)$_3$ has a higher potentiality to induce the folding transition than does (D-Lys)$_3$. The DNA compaction begins and finishes in the presence of 20 and 70 μM (L-Lys)$_3$, respectively, whereas (D-Lys)$_3$ did not compact DNA even at the 90 μM concentration. As for the arginine trimers, which are more effective because of their large pK_a values,[6] the L-isomer again exhibits a higher ability to cause the folding transition. These results clearly indicate that the L- and D-series of the tripeptide interacted with DNA in different manners. The most drastic difference is observed in the 70 μM solutions of trilysine and 30 μM solutions of triarginine. In the L-series solutions of these concentrations, all DNA molecules existed in the globule state, while no folding occurred in the presence of the same amounts of the D-isomers.

To gain insight into the microscopic structure change of DNA during the folding transition, we have performed AFM measurements on the DNA molecules weakly absorbed on a mica surface. The AFM images on compact DNA molecules with (L-Lys)$_3$ and (D-Lys)$_3$ are displayed in Figure 2b and c, respectively, together

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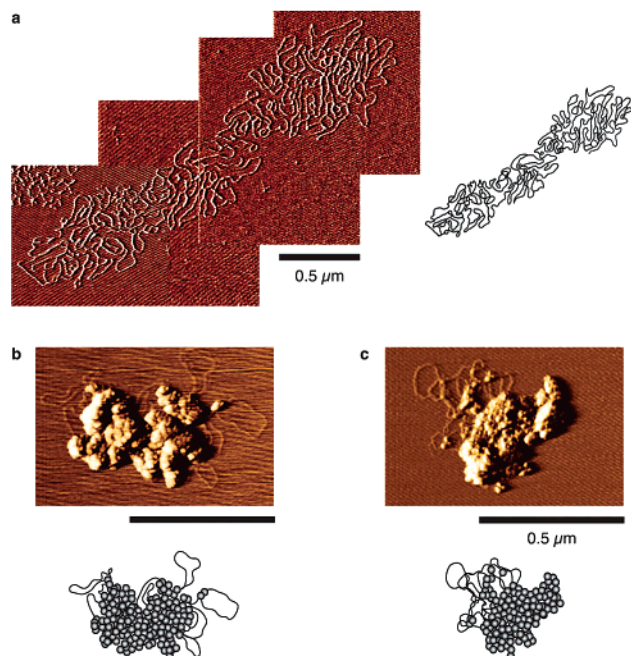
Figure 2. AFM images of the coil (a) and globule states (b and c) in the presence of 70 μM (L-Lys)$_3$ and 120 μM (D-Lys)$_3$, respectively.

with the image of the elongated coil state (a). It is noted that the condensed DNA is composed of many small beads with a diameter of ca. 20 nm. The structure is different from geometric toroidal and rodlike nanostructures which are generally reflecting the stiffness of the double-stranded DNA chain by the interaction with polyamines and multivalent metal cations.[7] The characteristic shape with many small beads as in Figure 2b and c is somewhat similar to the morphology of chromatin composed of small beads, that is, a nucleosome.[8]

Thus, we have successfully observed marked differences in the giant DNA folding transition based on the chirality interaction with the much lower molecular weight (1/10^6) tripeptide. It is generally accepted that a highly specific molecular recognition can be achieved by a biopolymer based on the complementary interaction through a binding site, that is, the key-and-lock philosophy. Indeed, enzymes can recognize differences in the substrates and bind specifically to the binding site.[9] Contrary to such current beliefs in the specific molecular recognition, the chirality specific DNA folding transition was carried out by the L-series tripeptide without help of the binding sites. Seemingly, this specific chiral differentiation is classified into the same category as the chromatographic optical resolution on the chiral polymers[10] and the DNA−ruthenium complexes,[1,11] but is conceptually different from those examples. The chromatographic separation is performed by a successive accumulation of small differences generated by a 1:1 molecular interaction between the substrate and the stationary phase. On the contrary, the folding transition of giant DNA molecules is induced as the cumulative effect of a large number of nonspecific interactions with small chemical species.

In the present study, we have demonstrated that the "nonspecific" weak interaction of small molecules causes a "specific" effect on the compaction of the giant DNA. Such a result could be

rationalized by the following consideration. During the past decade, it has been established that a single giant DNA molecule undergoes an on/off transition[3,12] (see, e.g., Figure 1c). This implies that the binding equilibrium of cationic species to DNA remains essentially constant before the compaction or folding transition, in accordance with past observations,[2] and that the effective binding constant markedly increases with the folding transition of the on/off nature. Even if the interaction energy difference on chiral species is minute, a large free energy difference would be generated by the on/off folding transition of the giant DNA as a result of the accumulative effect with a large number of the small molecules.

Here, it becomes obvious that natural L-series amino acids have higher potentialities than D-enantiomers to induce the folding transition on DNA, and this result affords considerable discussions in the studies of stereochemical complementarity[13] and molecular evolution to understand the origin of biochemical homochirality.

Supporting Information Available: HPLC purification of tripeptides, experimental procedures of fluorescence microscopy, and AFM (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(5) The apparent size in the fluorescence image is larger than the actual size of the DNA chain due to the blurring effect of approximately 0.3 μm. (See: Yamasaki, Y.; Yoshikawa, K. *J. Am. Chem. Soc.* **1997**, *119*, 10573−10578). The appearance of very small fraction of compact state as in Figure 1c is attributable to the artifactual effect of imperfect mixing.

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JA036745X