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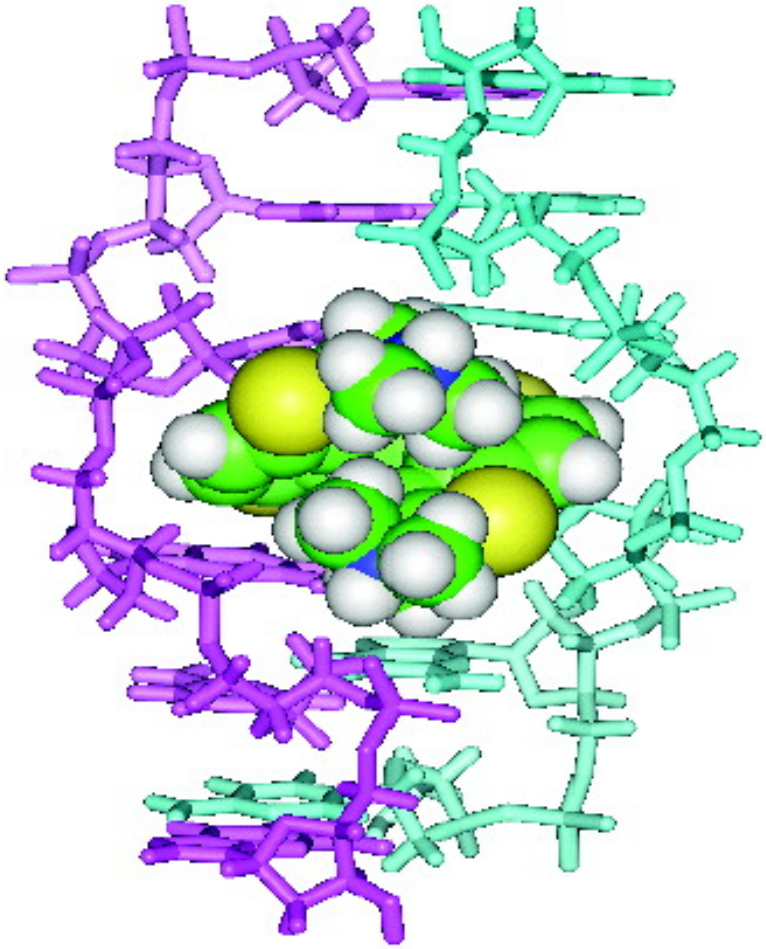

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(*P*)-Helicene Displays Chiral Selection in Binding to Z-DNA

Yan Xu,[†] You Xin Zhang,[‡] Hiroshi Sugiyama,*[,†,§] Tomoko Umano,[⊥] Hideji Osuga,[⊥] and Kazuhiko Tanaka*[,⊥]

Institute of Biomaterials and Bioengineering, Tokyo Medical and Dental University, Chiyoda, Tokyo 101-0062, Japan, Jin Zhou Medical College, China, Department of Chemistry, Graduate School of Science, Kyoto University, Sakyo, Kyoto 606-8501, Japan, and Department of Materials Science and Chemistry, Faculty of Systems Engineering, Wakayama University, Sakaedani 930 Wakayama 640-8510, Japan

Received January 2, 2004; E-mail: hs@kuchem.kyoto-u.ac.jp

Left-handed Z-form DNA is one of the significant characteristic DNA local structures. It has been extensively investigated in relation to transcription, the methylation of cytosine, and the level of DNA supercoiling.[1] Rich and colleagues discovered that double-stranded RNA adenosine deaminase (ADAR1) and the tumor-associated protein, DLM-1, specifically bind to Z-DNA.[2] Recently, the biological relevance of Z-DNA has been further demonstrated by Lui and colleagues. They provided the first evidence that Z-DNA-forming sequences are required for chromatin-dependent activation of the CSF1 promoter.[1b] Recently, there has been increased attention focused on the binding of small molecules to specific DNA structures to inhibit the biological functions in which these particular structures participate.[3]

Norden and Tjerneld first reported that the Δ enantiomer of tris-(dipyridyl)Fe(II) binds to right-handed B-form DNA.[4] The Barton laboratory developed a series of chiral metal molecules that recognize specific DNA structures including Z-DNA, but the molecular basis of enantioselectivity is not well understood.[5] Although the anticancer agent (+)-daunorubicin and its novel (−)-enantiomer (WP900) display enantioselectivity in binding to DNA, as reported by Qu and colleagues, the synthesis of WP900 is no easy undertaking, requiring some 37 steps.[3c] We report here *a simple helicene* molecule that displays structural selectivity in binding to DNA (Figure 1). We found that the (*P*)-A and (*M*)-A enantiomeric pair can discriminate between B- and Z-DNA and that (*P*)-A selectively binds Z-DNA and effectively converts the B-DNA conformation to Z-DNA.

Synthesis of (*P*)-A and (*M*)-A was initiated with optically active bis(hydroxymethyl)helicenes via the corresponding bis(chloromethyl) derivatives (Supporting Information). Figure 2 shows the circular dichroism (CD) spectra of (*P*)-A, in which a 70% decrease in CD intensity is apparent in binding to Z-DNA, whereas no marked change occurs in binding to B-DNA. In contrast to the stereoselection displayed by (*P*)-A in binding to B- and Z-DNA, (*M*)-A shows no such discrimination, although there is a 20% decrease in CD intensity when it binds B- or Z-DNA. The m^{8}G-containing hexamer d(CGCm^{8}GCG)$_2$ was used to produce Z-DNA at the same salt concentrations as those used for B-DNA.[6,7] The binding constants were measured using a fluorescence titration method in which fixed concentrations of either (*P*)-A or (*M*)-A were titrated against increasing [poly(dGdC)]$_2$ concentrations (Supporting Information).[8] The binding constant of (*P*)-A for Z-DNA is 5-fold greater than that of (*M*)-A, as shown in Table 1. In contrast, the binding constants of (*M*)-A for Z-DNA and B-DNA

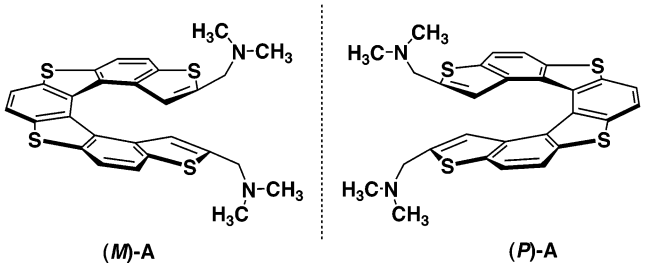

Figure 1. Structures of helicene (*P*)-A and (*M*)-A.

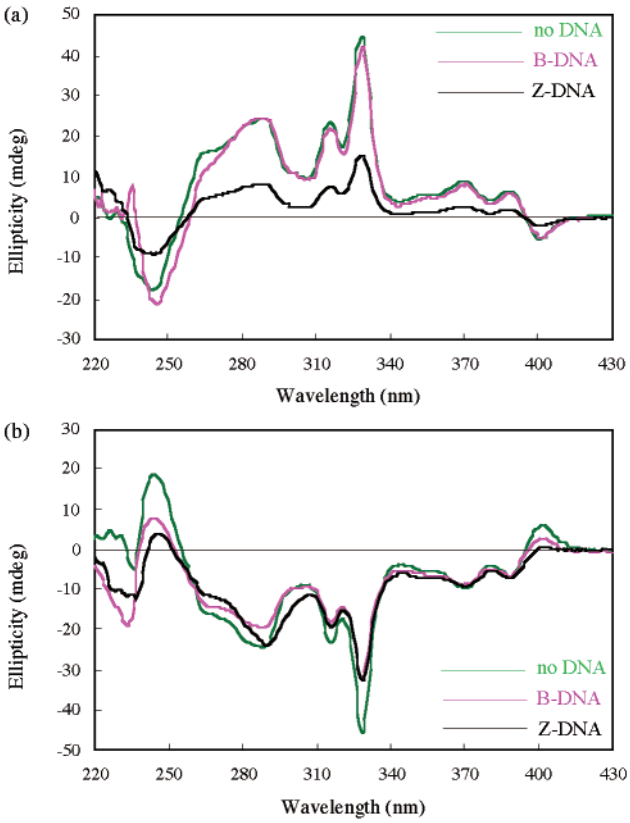

Figure 2. CD spectra of (*P*)-A (a) and (*M*)-A (b) (12.5 μM, 25 °C) with or without B-DNA d(CGCGCG)$_2$ or Z-DNA d(CGCm^{8}GCG)$_2$ (10 μM strand concentration) in 5 mM Na-cacodylate buffer, pH 7.0.[6,7]

are similar. These results quantitatively confirm that only the (*P*)-A enantiomer selectively binds to Z-DNA.

Dialysis experiments were designed to determine the structural selectivity of the helicene enantiomers. A mixture of helicene enantiomers was prepared by mixing equimolar amounts of (*P*)-A and (*M*)-A (Figure 3a). The enantiomeric mixture was dialyzed

[†] Tokyo Medical and Dental University.
[‡] Jin Zhou Medical College.
[§] Kyoto University.
[⊥] Wakayama University.

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Table 1. Binding Constants for the Interactions of (*P*)-A and (*M*)-A with B-DNA and Z-DNA[a]

DNA	(*P*)-A	(*M*)-A
B-DNA	$(1.4 \pm 0.3) \times 10^4 M^{-1}$	$(2.8 \pm 0.3) \times 10^4 M^{-1}$
Z-DNA	$(8.0 \pm 0.5) \times 10^4 M^{-1}$	$(2.4 \pm 0.2) \times 10^4 M^{-1}$

[a] Experiments were carried as described in the Supporting Information.

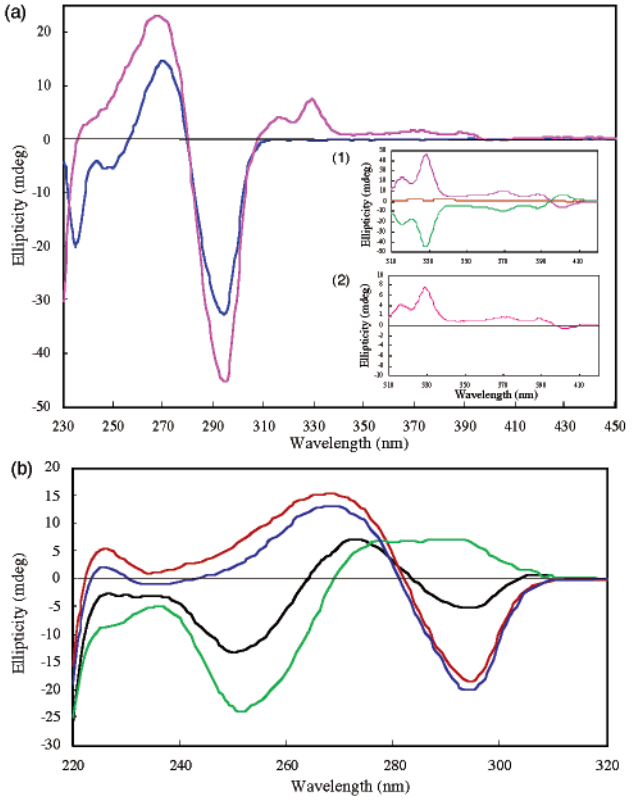

Figure 3. (a) Pink curve, CD spectrum of the residue after dialysis of the helicene mixture against Z-DNA; blue curve, CD spectrum of $[poly(dGdC)]_2$ solution (3 M NaCl) before dialysis. Inset 1: pink curve, (*P*)-A; green curve, (*M*)-A; red curve, a 1:1 molar ratio mixture of (*P*)-A and (*M*)-A. Inset 2: pink curve, a shorter wavelength scale for the residue after dialysis. (b) CD spectra of $[poly(dGdC)]_2$ at 100 μM (bp) in buffered aqueous solution containing 2.25 M NaCl. Black curve, DNA alone, showing a spectrum characteristic of a mixture of B- and Z-DNA. Red curve, DNA with (*P*)-A added to a final concentration of 2 μM; the resultant spectrum is characteristic of Z-DNA. Blue curve, DNA in 5 M NaCl. Green curve, DNA in 0.1 M NaCl showing a spectrum characteristic of B-DNA.

against Z-DNA $[poly(dGdC)]_2$ in 3 M NaCl. CD was used to monitor the residue for enrichment with the enantiomer with stronger affinity for the DNA conformation contained within the dialysis tube. The residue after dialysis showed a stronger Cotton effect around 330 nm and was enriched in (*P*)-A, confirming the preferential binding of (*P*)-A to Z-DNA compared with (*M*)-A (Figure 3a). It is important to note that the negative peak around 295 nm in the CD spectrum that is characteristic of Z-DNA increased after dialysis relative to that before dialysis. It is assumed that (*P*)-A selectivity is sufficiently strong to drive the allosteric conversion of DNA to the preferred Z-DNA conformation. To verify this, we designed an experiment to qualitatively demonstrate the allosteric binding of (*P*)-helicene to Z-DNA. A solution of $[poly(dGdC)]_2$ containing 2.25 M NaCl was prepared, in which the polymer existed as a mixture of B- and Z-DNA (1:1) (Figure 3b). When (*P*)-A was added to the solution, the CD spectrum changed to one characteristic of Z-DNA with an increase in the Cotton effect around 295 nm, as shown in Figure 3b. These results suggest that (*P*)-A not only binds selectively to Z-DNA over B-DNA but also drives DNA to adopt a left-handed helical Z-DNA form. Moreover, we found that this structural selectivity was completely abolished by substitution of the amino group of (*P*)-A and (*M*)-A with a hydroxy group, suggesting that the protonated amino group in (*P*)-A and (*M*)-A plays a key role in the interaction of the helicenes with DNA (Supporting Information). The detailed molecular basis of the molecular recognition of Z-DNA by (*P*)-A is currently under investigation by NMR analysis.

Chiral metal complexes failed to convert B-DNA to Z-DNA, which is assumed to be a consequence of their weak structural selectivity for Z-DNA.[5c] To the best of our knowledge, this is the first report demonstrating that (*P*)-helicene is an enantioselective ligand capable of binding Z-DNA and converting B- to Z-DNA.[9] The biological function of Z-DNA remains poorly defined, but it is an area of active research.[10] Kim and colleagues recently discovered that the ability to bind Z-DNA is essential to the activity of the E3L protein of *Vaccinia* virus.[11] Z-DNA has also been demonstrated in the transcriptional regulatory regions of the *c-myc* gene in cancer.[10] The enantioselectivity of the helicenes offers a new route for the rational design of inhibitors of biological functions that may depend on Z-DNA.

Supporting Information Available: Information on the synthesis of (*P*)-A and (*M*)-A and the binding study (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(6) We have already demonstrated that the incorporation of a methyl group at the guanine C8 position (m^8G) dramatically stabilizes the Z conformation. Samples were prepared by the addition of 1.3 μL (10 μM strand concentration) of DNA solution into 0.5 mL of 12.5 μM helicene (*P*)-A or (*M*)-A solution in same buffer. The m^8G-containing hexamer $d(CGCm^8GCG)_2$ (ODN 1) and unmodified hexamer $d(CGCGCG)_2$ (ODN 2) were used. ODN 2 retains a B conformation in 100 mM NaCl, and ODN 1 adopts a Z conformation in the same solution.

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