Pergamon

Tetrahedron Letters, Vol. 35, No. 8, pp. 1219-1222, 1994
Elsevier Science Ltd
Printed in Great Britain
0040-4039/94 $6.00+0.00

0040-4039(94)E0019-T

Induced Fit of Helical Biphenyl Ligands to the Double-Stranded DNA

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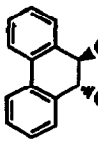
Abstract: Enantiomeric 2,2'-bridged biphenyl derivatives have been used to study an interaction of helical biphenyl group with the double-stranded DNA. The biphenyl group of (9*R*,10*R*)-enantiomer (2a) shows CD spectrum characteristic to predominant equilibrium population in the M-helical conformation either in the absence or presence of double-stranded DNA. The CD signal of (9*S*,10*S*)-enantiomer (2b), which predominantly exist in the P-helical conformation in the solution, changes upon addition of double-stranded DNA. The CD change is indicative of an increase in population of the M-helicity conformer of (9*S*, 10*S*)-enantiomer (2b) upon binding to DNA.

Several examples have been observed that part of the protein become structured only upon binding to DNA.[1] Such induced fit of the protein could be facilitated by complementarity in the surface of the protein and the shape of the local structure of DNA, which is characterized by combination of the several structural components, e.g., the helical twist, tilt, roll, and the propeller twist of base pairs.[2] We report herein an example of induced fit of a small organic molecule with helical biphenyl chromophore to the double-stranded DNA by using enantiomers of 2,2'-bridged biphenyl derivatives.



DHPA (1a, b) : R = OH

enDHP (2a, b) : R = $NH(CH_2)_2NH_2$

Enantiomers of **enDHP** (**2a** and **b**; **a**:9*R*,10*R*; **b**:9*S*,10*S*) are synthesized from *trans*-9,10-dihydro-phenanthrene-9,10-diol to test whether the M- or P-helicity of biphenyl group would sense the chiral structural elements in double helical DNA.[3, 4] Binding constants for the enantiomers **2a** and **2b** with DNA are determined by equilibrium dialysis method.[6] As listed in Table I, binding parameters for both enantiomers to DNA are in the same order of magnitude, although the (9*S*,10*S*)-isomer (**2b**) shows higher equilibrium constant than that of the (9*R*,10*R*)-isomer (**2a**) on interaction with calf thymus DNA and/or poly (dA-dT). Quenching of fluorescence emission of the biphenyl chromophore was observed for both enantiomers upon addition of the double-stranded DNA. This result suggests a stacking interaction between the biphenyl ring and the nucleic acid base pairs.[7] ^{1}H NMR studies of **2a** and **2b** in the presence of poly (dA-dT) reveal that the aromatic protons of the biphenyl groups of both **2a** and **2b** are broadened and shifted upfield more than 0.1 ppm upon binding to poly

Table I. Association Constants (K) and Binding Site Sizes for Binding of **2a** and **2b** to DNA.

	K (x 10^{-5})[a]	binding site size (bp)[a]
2a / CTDNA[b]	0.81	4.4
2b / CTDNA[b]	2.10	4.5
2a / poly(dA-dT)	1.55	4.0
2b / poly(dA-dT)	1.87	2.6

a. Values are obtained from Scatchard plots deduced from equilibrium dialysis performed at pH 7.6 in a buffer containing 5 mM Tris HCl and 10 mM NaCl. *b*. Sonicated calf thymus DNA in average length of 1000 base pairs (bp).

(dA-dT).[8] These observations taken together indicate the stacking interaction mode between biphenyl group and DNA base pairs.

The helical conformations of the biphenyl groups of **2a** and **2b** upon binding to DNA are analyzed by circular dichroism (CD) spectroscopy in the presence or absence of DNA. The CD transitions below 250 nm of enantiomers of 2,2'-bridged biphenyl derivatives (**1** and **2**) are highly sensitive to the helicity of the biphenyl chromophore (Figure 1), which allows us to monitor the equilibrium population of P- and M-helicity conformers in many kinds of solutions.[9] Both **2a** and **2b** exhibit CD spectra characteristic to the predominant population of the diaxial conformer in aqueous solutions (Figure 2A and B), as shown for **1a** and **1b** in Figure 1A.[9a] Specifically, the (9*R*,10*R*)-isomer (**2a**) exists preferentially in a M-helicity conformer with (9*S*,10*S*)- isomer (**2b**) being in a P-helicity conformer in aqueous buffered solution at pH 7.6.

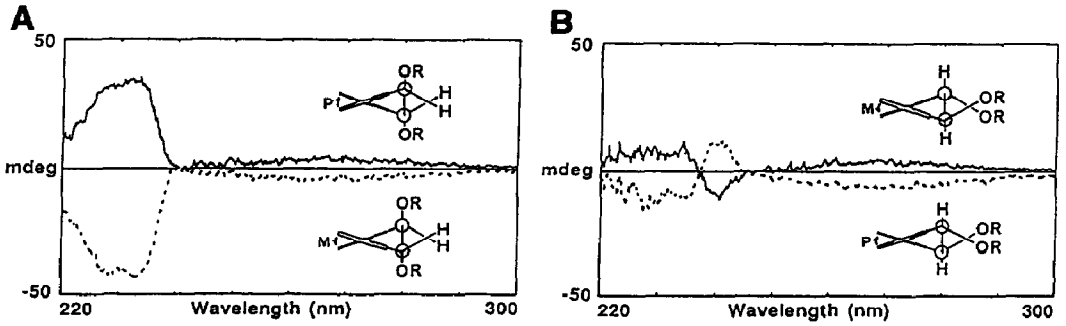


Figure 1. CD spectra of (9*R*,10*R*)- and (9*S*,10*S*)-**DHPA** (**1a** and **1b**). Panel **A** shows CD spectra of (9*S*,10*S*)-**DHPA** (**1b**) (——) predomonantly exists in a P-helical conformation and (9*R*,10*R*)-**DHPA** (**1a**) (-----) in a M-helical conformation in methanol. Panel **B** shows CD spectra of **1b** (——) predominantly exists in a M-helical conformation and **1a** (-----) in a P-helical conformation in THF. Each spectrum is taken at **DHPA** concentration of 10 μM. Representative changes in helicity of the biphenyl group are shown in each pannel.

The conformational change of **2a** and **2b** upon binding to DNA is judged by using their differential CD spectra which are obtained by subtracting the CD signal of DNA from that of the complex. In case of **2a**, the negative CD transition below 240 nm in the presence of DNA is slightly decreased, but is almost identical to that of free **2a** (Figure 2A). However, the

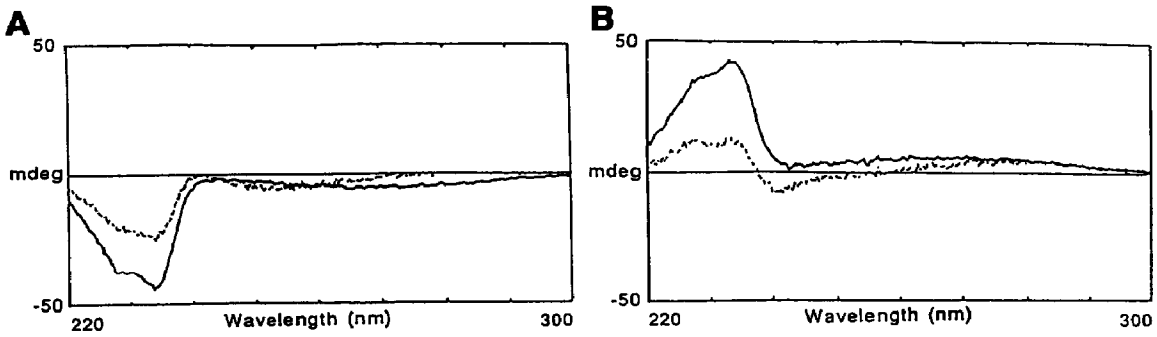


Figure 2. CD spectra of (9*R*,10*R*)-**enDHP (2a)** (panel A) and (9*S*,10*S*)-**enDHP (2b)** (panel B) free (——) in a buffered solution (5 mM Tris HCl, 10 mM NaCl, pH 7.6) and in the presence of 200 μM calf thymus DNA (-----). Each spectrum is taken at **enDHP** concentration of 10 μM.

positive signal at 230 nm observed for the free **2b** decreases substantially and a negative signal at 240 nm is observed upon binding to DNA (Figure 2B). The decrease of positive signal at 230 nm and an appearance of the negative signal at 240 nm are characteristic of (9*S*,10*S*) - enantiomers of 2,2'-bridged biphenyl derivatives preferentially existing in the M-helicity conformer,[9] as shown for **1b** in Figure 1B. Since the CD change below 240 nm indicates an alternation of the helicity of the biphenyl ring, the deference spectrum of **2b** (Figure 2B) indicates that *population of the M-helicity conformer of* **2b** *increases upon binding to the double-helical DNA*. That the difference between CD signals around 270 nm of free and bound ligands is small indicates little effect of DNA conformational changes on the differential CD spectra. Thus the CD signal changes below 240 nm would mainly result from the changes in helicity of the biphenyl ligands. No such a change in CD spectrum is observed on addition of a single stranded polynucleotide poly G to each enantiomer (**2a** and **2b**), thus indicating that the duplex structure is required for inducing the change in helicity of the biphenyl group. Moreover, lack of the CD change upon addition of double-stranded polyribonucleic acid, poly G-poly C, precludes a possibility that the right-handedness of the duplex is a sole determinant for the observed change in helicity of the biphenyl group.

Since the base-pairs are propeller-twisted to uniform direction (negatively propeller-twisted) in the right-handed B-DNA,[2, 10] one possible interpretation of these results is that the P-helicity of (*9S,10S*)-**enDHP (2b)** changes to M-helicity upon binding to DNA to fit the helicity of base pair propeller twist, while the M-helicity of (*9R,10R*)-**enDHP (2a)** remains the same upon binding to DNA.[11] Our results open a possibility that a molecule with axial chirality can be utilized as an element for designing sequence-specific DNA recognition molecules.[12]

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(*Received in Japan* 13 *March* 1993; *accepted* 21 *June* 1993)