Base and chain sense in d(CGCGCG)-BP and d(TATATATATATA). Analysis of CD measurements and x-ray crystal data.

Kazimierz Grzeskowiak, Hirofumi Ohishi

1 University of California Los Angeles, Los Angeles, CA 90095-1570, USA
2 Osaka University of Pharmaceutical Sciences, Osaka 569-1094, Japan

ABSTRACT
We use CD spectra and x-ray crystal data to compare structures of d(CGCGCG)-BP, which is d(CGCGCG) modified on 2-amino group of guanine by (+)7R,85-dihydroxy-9S,10R-exoxy-7,8,9,10-tetrahydroBenzo(a)Pyrene (7R-BPDE), with the d(TA)$_6$ dodecamer which with increasing ionic strength of the buffer adopts CD spectrum similar to d(CGCGCG)-BP in 0.1M MgCl$_2$ solution but opposite in sign. We indicates that DNA bases in both oligomers may be in a B-form however as a mirror image of stereo structure of right handed d(CGCGCG)-BP and left handed d(TA)$_6$.

INTRODUCTION
CD measurements were the techniques by which a Z conformation of poly(dG-dC) was first identified, and subtle changes in secondary structures within the B-DNA provided evidences on existence of mixed conformations. X-ray crystal data provide us with practical data per unique DNA base pair. Having two methods at the hand we wish to learn how the base modification of guanine in d(CGCGCG)-BP and intrinsically badly stacked T-A base steps in d(TA)$_6$ are compatible in evoking conformational reconfiguration of CG$_{BP}$ and TAT steps in each oligomer, so CD spectra of both oligomers have a mirror image features. Current crystallographic picture of deoxyguanosine-BP (dG-BP) with (7R-BPDE) is in agreement with the structure previously determined by CD and NMR measurements. CD spectrum of dG-BP and relative spatial orientation of pyrene and guanine chromophores is depicted in Figure 1 - taken from reference 3.

RESULTS
We synthesized d(CGCGCG)-BP adduct with BPDE attached to 2-amino group of guanine according to procedure described for DNA modification. From results of 2D NMR is known that the trans (+)
BPDE adducted DNA duplex has the glycosidic torsion angle 260$^\circ$ and guanine is in anti position as it is in normal B-DNA. Using CD measurements it has been determined earlier that the bands near 250nm in the CD spectra arose from an exciton between the aromatic ring of BP moiety and the guanine base. And the bands at 285nm are derivative mainly from interaction between cytosine and guanine bases.

CD spectra in Figure 2 show that increasing from 0.1M MgCl$_2$ to 3.0M MgCl$_2$ make an ellipticities at 285nm (solid line) decrease and simultaneously an ellipticities at 255nm (broken line) intensify. Spectrum is extremely complex since it shows strong interactions at 255nm between chromophores of guanine and cytosine bases perturbed by pyrene ring. Nevertheless CD spectrum shows a B to Z transition like that observed in d(CG)$_6$.

Figure 1. a, CD and b, UV spectra of deoxyguanosine-BP. CD spectrum is dominated by pyrene chromophore transition at 310-355 nm short axis, L$_4$ and long axis at 260-290 nm, L$_4$. These interact with guanine transitions at 260-270nm.

In 0.1M MgCl$_2$ (Figure 2, solid line) the split Cotton effects centered at 255 nm and 285 nm are indicative of DNA bases being not perpendicular to helix axis as it is in typical B-DNA form, instead the bases are untwisted and more propeller.

CD spectrum of d(CGCGCG)-BP in 3.0 M MgCl$_2$ (broken line) is virtually identical to the CD spectrum of dG-BP adduct (Figure 1), which the structure has the same features as the structure found in crystal of dG-BP. Crystal structure of dG-BP reveals that glycosidic torsion angle equals 73$^\circ$ and the 7R-BPDE enantiomer attached to guanine induces rotation around glycosidic
bond from anti to syn position, a feature observed in Z-DNA. This adds to the evidence that in 3.0 M MgCl₂, the guanine bases of d(CGCGCG)-BP are indeed in syn position similar to those observed in crystal of dG-BP³, and in Z-form of the crystal of d(CGCGCG)³ and found from this work based on CD measurements.

In contrary, CD spectra of d(TA)₆ (Figure 3 white line) shows that at the high MgCl₂ concentration the transition from a B-form to Z-form does not occur. This would be reversing the Cotton effect like that observed in a typical B to Z-form transition of d(CG)₆⁴ or in d(CGCGCG)-BP. Instead there are two bands of negative ellipticities appearing at 285nm and 247nm, and these are opposite in sign to those seen in right-handed B-form of d(CGCGCG)-BP in solution of 0.1M MgCl₂ (Figure 2 solid line).

In the CD spectrum of d(TA)₆ in solution of 3.0 M MgCl₂ and 600μM cobalt hexamine (white line) is virtually identical to the CD spectrum of d(CGCGCG)-BP in 0.1 M MgCl₂ (Figure 2 solid line) but opposite in sign. For detailed description of these spectra see Figure 3 in reference 6.

DISCUSSION
We are aiming to explain what a structure of TA tract in DNA is. The theoretical predictions and results of x-ray crystal structures conclude that in the alternating B-helix, the T-A step is intrinsically badly stacked to avoid steric clash with adenine bases from opposing strand⁷. Such stereochemistry brings D-deoxyribose rings coming very close together that in turn enhance restraining the rotation around glycosidic bond of thymine bases. CD spectra of d(TA)₆ in Figure 3 is not characteristic a B to Z transition. In fact CD of d(TA)₆ in 3M MgCl₂ is similar but opposite in sign to the CD of d(CGCGCG)-BP in 0.1M MgCl₂ that exists as a B-form. This suggests that since the CD of d(TA)₆ is reverse of d(CGCGCG)-BP than d(TA)₆ in 3.0M MgCl₂ must undergo twisting of the ends of helix followed by unstacking and flipping over adenine bases perhaps in preparation for conversion from the right-handed double helix to its left-handed state - a calculated modelling earlier presented in Figure 7 of reference 8.

If CD spectra of d(TA)₆ in 0.1-3.0M MgCl₂ are the mixture of right-handed B-DNA and left-handed state than the Locally Linearized Model (LLM)⁴ could be used to find the intermediate state of d(TA)₆ transition as the linear combination of the spectrum of each component with the weight equal to their concentration.

CONCLUSION
Available CD spectra and x-ray crystal data of dG-BP and d(CGCGCG)-BP compared with d(TA)₆ are very handy in giving us an approximation of possible structure of left-handed B-form of d(TA)₆. These suggest that d(TA)₆ undergoes the transition from right-handed to left-handed B-form when ionic strength of the buffer is increased. It is to be noted that an electron transition vector is known only for a guanine base and that makes interpretation of CD spectra of d(TA)₆ incomplete. Knowing transition vectors of T-A base step and/or x-ray crystal structure of d(TA)₆ would answer to the question - can flexible d(TA)₆ exist as so called W-DNA, left-handed duplex or it possesses another structural feature. The work also is in progress on the crystallization of d(TA)₆ and related DNA lengths.

REFERENCES

*) Corresponding author. Email: kaziu@mbi.ucla.edu