Polymorphic G:G mismatches act as hotspots for inducing right-handed Z DNA by DNA intercalation

Roshan Satange[1,2] and Ming-Hon Hou[1,2,3,*]

[1]Institute of Genomics and Bioinformatics, National Chung Hsing University, Taichung, 402, Taiwan.
[2]Ph.D. Program in Medical Biotechnology, National Chung Hsing University, Taichung, 402, Taiwan.
[3]Department of Life Science, National Chung Hsing University, Taichung, 402, Taiwan.
*Correspondence to: mhho@nchu.edu.tw

Abstract

DNA mismatches are highly polymorphic and dynamic, albeit poorly characterized structurally. We utilized the antitumour antibiotic $Co^{II}(Chro)_2$ (Chro=chromomycin A3) to stabilize a G:G mismatched DNA duplex, allowing X-ray crystallography-based monitoring of mismatch polymorphism. For the first time, the unusual geometries of several G:G mismatches can be simultaneously observed in the crystal structure. The G:G mismatch sites can also act as a hotspot for the formation of alternative DNA structures with a GC/GA-5' intercalation site for binding by the GC-selective intercalator actinomycin D (ActiD). Direct intercalation of two ActiD molecules to G:G mismatch sites causes DNA rearrangements, resulting in backbone distortion to form right-handed Z-DNA structure with a single-step sharp kink. Our study provides insights on intercalators-mismatch DNA interactions and a rationale for mismatch interrogation and detection via DNA intercalation.

Keywords – DNA mismatch, DNA intercalation, DNA deformation, Z-DNA, X-ray crystallography

Introduction

DNA mismatches are potentially mutagenic and are associated with a number of diseases including cancers [1]. The presence of mismatches can exert a significant impact on local base step geometry and accordingly alters the structural topology of DNA. Guanine-guanine (G:G) mismatches are known to be flexible and can affect DNA significantly [2]. G:G mismatches are reported in various structures viz. G-quadruplexes, purine-purine DNA, triplexes, and RNA stem bulge structures associated with various disease state [2]. On the other hand, small molecules that recognize mismatched DNA can induce various degrees of structural deformations, with many having pharmaceutical and/or diagnostic potential [3].

To understand polymorphism and structural consequences that exist in the G:G mismatches, we utilize a self-complementary $d(TTGGCGAA)_2$ DNA containing two G:G mismatches. We use $Co^{II}(Chro)_2$ as a mild stabilizer to stabilize and enable crystallization of this mismatch containing duplex. Our structural analysis shows different arrangements of G:G mismatches can be tolerated in a single duplex that acts as 'hotspot' for binding of a GC-selective DNA intercalator, ActiD. Interactions between ActiD and mismatched duplex resulted in the formation of a right-handed 'zigzag' (Z) DNA-type of backbone structure through a sharp kinking of the DNA helix that is more marked than that induced by sequence-specific DNA-binding drugs. These structures altogether display extraordinary features that can be exploited as unique mismatch DNA recognition characteristics revealed through DNA intercalation.

Experiments

The major technique involved in the current study was X-ray crystallography. Crystals were grown by the sitting drop vapour diffusion method at 4 °C. X-ray diffraction data from single crystals were collected at synchrotron radiation facilities. Data integration and reduction were processed using the HKL-2000. Phases were solved by SAD and MR methods. DNA helical parameters were analysed using Web-3DNA and the CURVES+ [4]. Binding affinity and stoichiometry of ActiD were determined by SPR and fluorescence measurements to support structural results.

Results

Structure of the $d(TTGGCGAA)_2$ DNA duplex with G:G mismatches stabilized by $Co^{II}(Chro)_2$

The oligonucleotide self assembles into an antiparallel duplex comprising three continuous independent duplexes in one asymmetric unit with the help of $Co^{II}(Chro)_2$ **(Fig. 1).** These three duplexes are held together by van der Waals contacts between the stacking bases to form a pseudo-continuous right-handed plectonemic supercoil duplex with an r.m.s.d. of 0.6 Å.

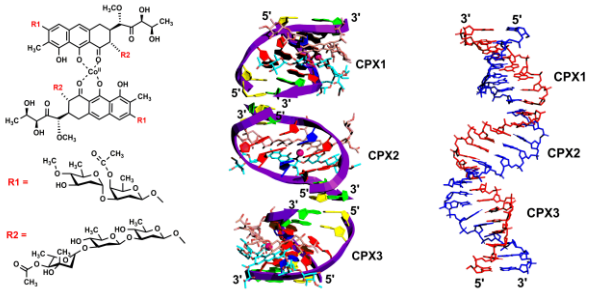

Fig.1: Overall structure of $Co^{II}(Chro)_2$-DNA containing three independent duplexes.

Asymmetric G:G mismatch base-pairing and its effects on DNA duplex geometry

Three complexes contain six G:G mismatches one on each side of a duplex flanked with central G-C pairs. Out of which the G6:G11 mismatched base pairs adopt *anti-syn* conformations in all three complexes. The G3:G14 mismatched pairs are found to be more flexible and adopt more diverse conformations including a *syn-syn* form in CPX1, a water-stabilized *anti-syn* type in CPX2, and an unusual *syn-syn* like base pair type in CPX3 **(Fig. 2)**.

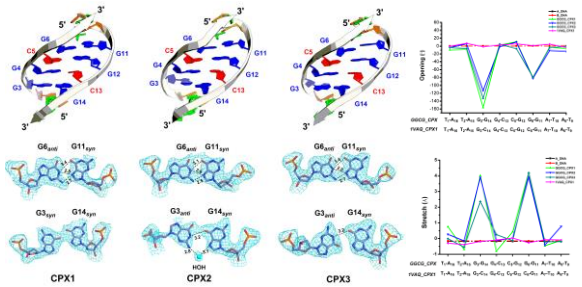

Fig.2: Geometry of G:G mismatches in $Co^{II}(Chro)_2$-DNA complex. DNA parameters showing significant differences at the G:G mismatch sites.

Binding of ActiD forces a DNA rearrangement and right handed Z DNA formation of the G:G mismatched DNA

ActiD **(Fig. 3A)** was found cooperatively stabilizing the G:G mismatched DNA with significant binding affinity. The crystal structure of ActiD-DNA complex shows many unexpected features **(Fig. 3B)**. The phenoxazone rings of ActiD (pink) and ActiD* (cyan) intercalate into the G3:A7*/C5:G6* and G6:C5*/A7:G3* base pairs, respectively, through the minor groove side. The helix axis of the top half is bent with an angle of c.a. 61◦ away from that of the bottom half. The structural rearrangement causes the guanine flipping out that generates a G:A mismatch instead of G:G. Upon comparing the opposite twist characteristics and zigzag-like backbone shape of the DNA duplex with those of the left-handed Z DNA structure [5], we propose that the present complex structure can be called as right-handed Z-DNA **(Fig. 3C)**.

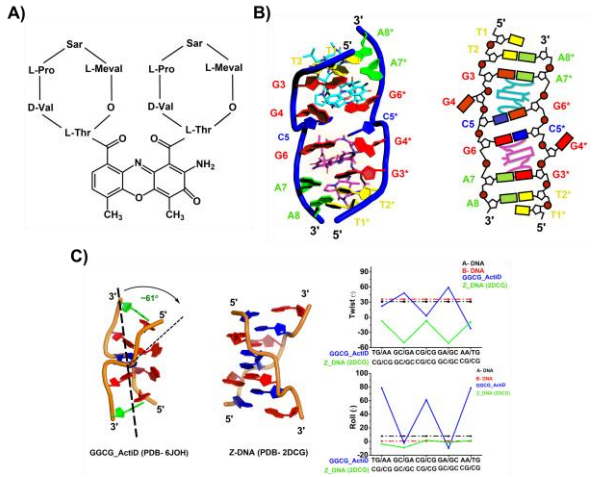

Fig.3: (A) Chemical structure of ActiD. (B) Crystal structure of ActiD-DNA complex. (C) Right-handed Z-DNA formation and comparison.

Structural details of the ActiD binding sites

The two phenoxazone rings are intercalated individually into the (G3pC5)-(G6*pA7*) step and the (G3*pC5*)-(G6pA7) step, respectively. The high G:C preference of ActiD binding can even extrude nucleotides located between a 5'-G and a 3'-C. There are extensive stacking interactions between the phenoxazone ring and the guanine bases from both sides of the ring. There are also strong intermolecular hydrogen bonds between G3-Thr7 and G6-Thr1 of the two ActiD moieties. Additionally, water-cluster, triplet-stranded base pair formation, and Na+-mediated hydrogen bonding also affect the stabilization of ActiD and the right-handed Z DNA complex.

Discussion

Out of the eight known mismatches, the G:G mismatch has been reported to be profoundly destabilizing of DNA duplexes [2]. In this study, we have demonstrated for the first time the existence of G:G mismatch polymorphism through X-ray crystallography. The mismatch adopts a variety of conformations and may act as a hotspot for intercalator binding. ActiD has been used in clinical practice for a long time; however, it is highly cytotoxic with many different side effects [6]. Our results suggest that a well-studied non-metallo intercalator is equally capable of recognizing mismatches. Based on our structural analysis and comparison, we contemplated that the binding of ActiD will follow two general rules: (i) G:G mismatch will break to cause overall structural displacements and (ii) adjacent guanines to the mismatches will invariably flip out in order to form a new GpC step **(Fig. 4)**. The structural understanding from the current study will guide the development of future generations of more selective intercalating agents, such as ActiD derivatives, as chemical tools for the interrogation and detection of mismatch related diseases.

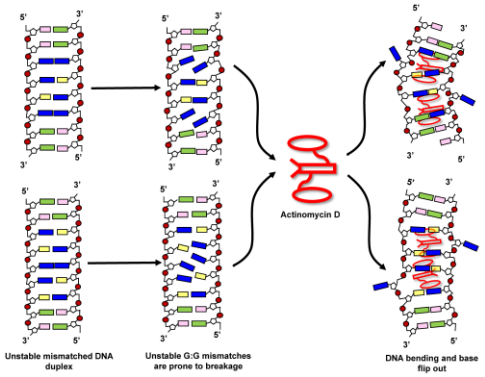

Fig.4: Schematic representation of ActiD-induced conformational changes in 6J0H (top) (current) and 4HIV (bottom) structures.

Acknowledgments

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