Monoalkylation and Cross-Linking of DNA by Cyclopropapyrroloindoles Entraps Bent and Straight Forms of A-Tracts

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DNA containing A-tracts has been shown by means of gel electrophoresis and electron microscopy to be bent.[1] While several theories have been proposed for both the nature and the loci of such bending, the origin still remains controversial.[2,3] Crystallographic studies on A-tract-containing sequences have also been inconclusive, and although some studies have shown conformational deviations, the structural and dynamic origins of DNA bending remain unclear.[4,5] X-ray crystallography and high-field NMR studies indicate the presence of an unusually narrow minor groove within A-tract sequences[4,5,6] and show that there is a high degree of propeller twist within the A-tract between the A•T base-pairs.[7]

NMR studies on several sequences reveal distinct junction sites at both the 3′- and 5′-ends of A-tracts.[7] The origin of these junctions is believed to be the transition between the normal Watson−Crick base-pairing of the flanking sequences and the highly propeller-twisted adenines within the A-tracts. Indeed, the 3′-junction of A-tracts is found to be one of the preferred binding sites for the (+)-CC-1065 family of DNA interactive ligands, and based on sequence selectivity studies, it would appear that these molecules can specifically target this unusual junction.[8]

Several workers have proposed, on the basis of spectroscopic techniques (UV and CD), that A-tracts exist as an equilibrium of at least two species.[9,10] Herrera and Chaires[9] found evidence for two species in equilibrium below the melting transition of poly(dA)•poly(dT) but observed only one species for the alternating copolymer poly(dA-dT)•poly(dT-dA). Because they observed isoelliptic points in the CD curves, they concluded that a conformational transition occurs between two helical species. Related studies on the duplex oligomer 5′-GA_4T_4C and homo dA•dT tracts using scanning calorimetry, CD, and UV melting profiles indicated a premelting transition, again suggesting that two different conformations may exist in solution.[3,10] In addition, Wilson and Chaires[11] have also interpreted cooperative binding isotherms for the interaction of propidium and daunomycin, respectively, with poly(dA)•poly(dT) in terms of an allosteric mechanism in which the intercalator preferentially binds to a conformer isomer of the polynucleotide. Several electron microscopy studies on the kDNA form *Crithidia fasciculata* have also provided further evidence for the coexistence of two or more distinct A-tract conformers.[12]

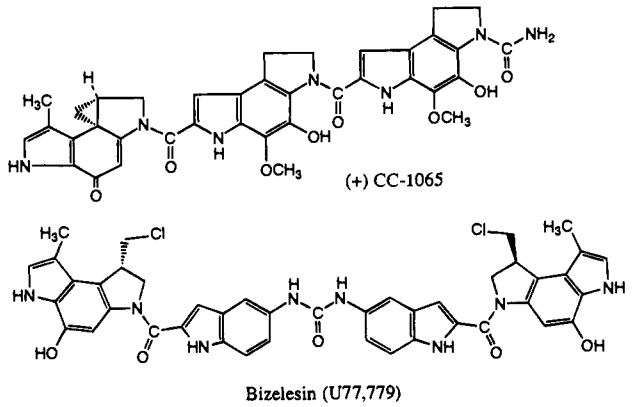

Figure 1. Structures of (+)-CC-1065 and the (+)-CC-1065-based cross-linker bizelesin.

Based on the studies described above, it is possible to envisage a situation where A-tracts exist as an equilibrium of two discrete conformations, one relatively straight and one significantly bent. With this hypothesis in mind, it is also possible to speculate that DNA interactive ligands that react with A-tracts may specifically target and entrap just one of the two conformations.

(+)-CC-1065 (Figure 1) is a potent DNA-reactive antitumor antibiotic produced by *Streptomyces zelensis* that covalently modifies DNA by alkylation of N3 of adenine in the minor groove of DNA.[8,13] (+)-CC-1065 reacts preferentially at two consensus sequences, 5′-PuNTTA* and 5′-AAAAA*.[14] As a structural consequence of DNA alkylation at 5′-AGTTA*, the nonbent helix becomes bent into the minor groove, and high-field NMR and hydroxyl radical footprinting reveal a structure similar to that of an intrinsically bent A-tract. A truncated junction bend model has been proposed to account for this bent structure.[8c] Nondenaturating gel electrophoresis (PAGE) studies have also shown that upon DNA alkylation, (+)-CC-1065 bends DNA into the minor groove by about 14−18°.[8a,15] The intrinsic bending associated with A-tracts is exaggerated by covalent modification with (+)-CC-1065, with the locus of bending within the A-tract moving by about 0.5 base pair to the 3′-side of the central adenine.[8c,15] Bizelesin (Figure 1) is a dimeric analog of (+)-CC-1065 that consists of two alkylating cyclopropapyrroloindole units joined by a rigid linker. Bizelesin interstrand cross-links DNA by alkylating the N3 position of

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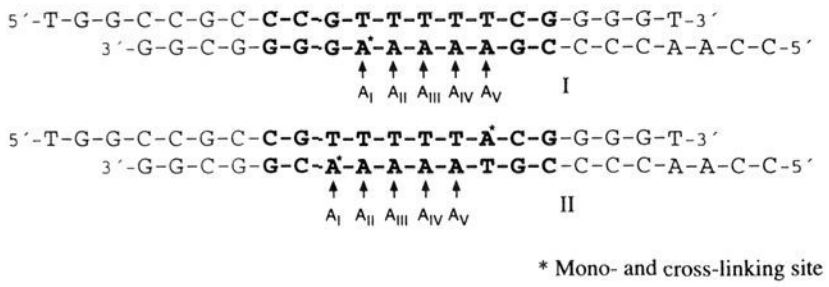

Figure 2. DNA sequences used in the PAGE and high-field NMR studies (NMR sequences shown in bold), indicating the location and covalent modification (marked with an asterisk). The A-tract numbering scheme is used in Figure 3.

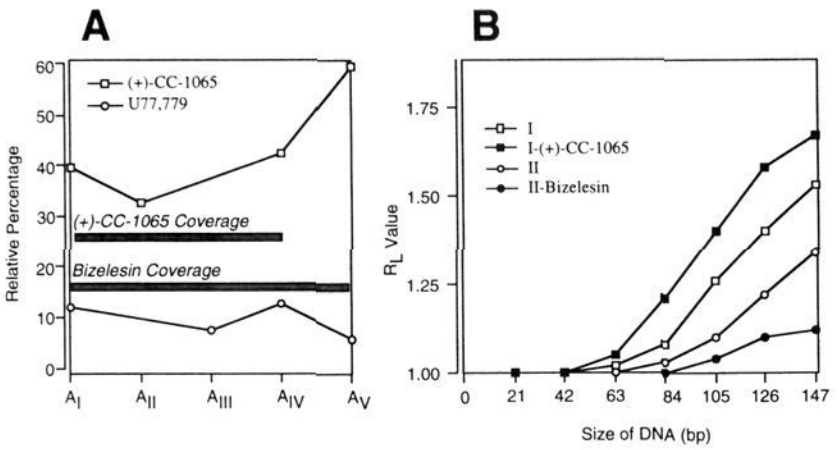

Figure 3. (A) Relative intensities of the normalized cross-strand NOESY peak volumes between the AH2 and the H1′ of the 3′-neighboring residue on the complementary strand for the I + (+)-CC-1065 and II + bizelesin adducts. Results are expressed as a percentage of the normalized (see text) corresponding peak volumes of the unmodified duplexes. (B) R_L values of the ligation products of 21-bp oligomers of A-tract-containing DNA duplexes and their respective (+)-CC-1065 and bizelesin adducts.

two adenines separated by four base pairs.[16] Sequence selectivity studies have shown that bizelesin has a cross-linking preference for 5′-TAATTA* and the A-tract 5′-TAAAAA*, although several other sequences are also targeted, with much lower efficiency.[17,18]

Two different A-tract sequences (I and II, Figure 2) and their (+)-CC-1065 and bizelesin-modified duplex adducts have been examined by high-field NMR and PAGE. For NMR studies, 10-mer sequences were examined, while for PAGE, 21-mer sequences were used to determine the relative bending magnitude. From the ^{1}H-NMR data, the volumes of the connectivities between the AH2 and the H1′ of the deoxyribose in the 3′-neighboring residue on the complementary strand were determined for each NOESY spectrum of duplexes I and II and their respective adducts.[19] To allow for a comparison between individual samples (which varied in concentration), these volumes were then used as a percentage of the average volume of the cytosine H5 to H6 connectivities within that spectrum. To compare the effects of adduct formation on the intensities of the cross-strand connectives, the relative volumes were compared (as a percentage) between the unmodified duplexes and their respective adducts (Figure 3A). In both cases, the relative volumes of the cross-strand connectivities decreased significantly upon adduct formation, due to the increased proton density within the minor groove as a result of the bound ligand's protons. However, the residual percentages showed marked differences for the NOE intensities between the bizelesin and (+)-CC-1065 adducts (Figure 3A), which indicates that the distances between the AH2 and the H1′ of the deoxyribose in the 3′-neighboring cross-strand residue had significantly increased in the bizelesin cross-linked adduct relative to the (+)-CC-1065 monoalkylated product.

The results from the PAGE study (Figure 3B), in which 21-mers were ligated using T4 ligase, clearly show that upon monoadduct formation with (+)-CC-1065, the R_L values and corresponding bending magnitude observed within the unmodified 5′-AAAAA* duplex are significantly increased.[20] This observation is (at least partially) supported by the increased cross-strand connectivities in the (+)-CC-1065 adduct relative to the bizelesin adduct. These relative intensities indicate that there is a significantly reduced distance between the AH2 and the cross-strand H1′ in the (+)-CC-1065 adduct, suggesting that the base pairs are still propeller twisted. In contrast, the equivalent PAGE data for the bizelesin adduct of the intrinsically bent 5′-TTTTTA* sequence show that the cross-linked duplex is now insignificantly bent. From the NMR data, it is impossible to precisely determine the degree of base-pair distortion within the bizelesin adduct.[21]

Evidence for junctions at both the 3′- and 5′-ends of the A-tract can also be seen in the NMR spectra for both unmodified duplexes (I and II) and the (+)-CC-1065 adduct. These junctions are believed to be the result of the transition between the highly propeller-twisted A-tract and the surrounding B-form DNA and can be visualized by an upfield shift in the cytosine H5 signal at the 3′-end of the tract and upfield shifts in the H1′ of the base adjacent to the 5′-end of the adenine tract. In contrast, there is no evidence from the NMR data for any junctions within the bizelesin cross-linked adduct, again providing support for the loss of propeller twist within the A-tract.

In conclusion, we propose that (+)-CC-1065 and bizelesin each entrap one of the two interconverting A-tract conformers. (+)-CC-1065 freezes out the bent conformer, while bizelesin entraps the straight conformer. While solution studies[9,10] have previously detected more than one species in A-tract oligomers, to the best of our knowledge this is the first time that the two equilibrating conformers have been trapped and examined independently, although intercalation by propidium and ethidium has been proposed to occur preferentially to one conformer isomer.[11]

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Supplementary Material Available: 2-D NOESY spectra and autoradiographs for duplexes I and II and their respective adducts (5 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, can be ordered from the ACS, and can be downloaded from the Internet; see any current masthead page for ordering information and Internet access instructions.

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(19) Two-dimensional NOESY NMR data sets in H_2O and D_2O buffered solution were recorded on a 500 MHz NMR spectrometer. Proton chemical shifts were referenced relative to the water signal. Phase-sensitive two-dimensional NOESY spectra were obtained for all four samples with a mixing time of 150 ms to reduce the influence of spin diffusion. The spectra were acquired with 16 scans at each of 1024 t_1 values, spectral width of 10.002 ppm, and a relaxation delay of 10 s between scans. During data processing, a 90°-shifted squared sine bell function was used in both $w1$ and $w2$ dimensions. The FID in $w1$ was zero-filled to $2K$ prior to Fourier transformation to give a $2K \times 2K$ spectrum.

(20) Purified [γ-^{32}P]APT-labeled duplexes were reacted with either (+)-CC-1065 or bizelesin.[8] The drug-modified duplexes and unmodified duplexes were then self-ligated in 20 μL of ligation buffer with T4 ligase to produce multimers, which were electrophoresed on polyacrylamide gel, and the bands were located by autoradiography.

(21) A comparison of the relative cross-strand connectivities obtained in a similar study with a sequence-related cross-linked bizelesin adduct, which is known to have little or no propeller twist within the central cross-linked A·T base pairs, indicates that the A-tract bizelesin adduct contains normal B-form AH2 to cross-strand H1′ distances, suggesting that the bases contain little or no propeller twist.