Ratcheting Torsional Stress in Duplex DNA

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Received September 3, 1993

DNA inside cells is relentlessly subjected to torsional stress induced by the action of proteins. DNA responds to this stress by adopting a variety of structures that deviate from the canonical B-form duplex.[1] Such noncanonical structures remain poorly understood in many respects, largely because (i) they have been difficult to capture in short DNA molecules devoid of proteins and (ii) methods for comparing the energetic cost of various DNA distortions have not been developed. We have proposed that interstrand alkane disulfide cross-links, introduced site-specifically into DNA, could be used to induce torsional stress in the duplex.[2,3] As a step toward synthesizing DNA containing noncanonical structure, we decided to explore the result of incrementally increasing the torsional stress in a DNA duplex by systematically shortening its interstrand disulfide cross-linked tether. Herein we report the results of this excercise, which has yielded an unforeseen insight into the energetic cost of DNA distortion.

Among the most prevalent kinds of stress to which DNA is subjected *in vivo* is a local underwinding of the helix.[4] In this study we set out to construct a locally underwound oligonucleotide by using a disulfide cross-link to constrain the axial rotation of adjacent base pairs with respect to each other. In the design of this oligonucleotide construct, we reasoned that if the cross-link were slightly shorter than the distance separating its intended attachment points in relaxed DNA, then closure of the cross-link (by disulfide bond formation) might force the DNA to decrease this distance by underwinding. To test this concept, we introduced cross-links bridging the central cytosines (C̲) of a self-complementary decamer, 5′-d(CCAGGC̲CTGG)-3′ (Figure 1A).[5] Computer-aided modeling suggested that a bis(propanethiol)-tethered (C_3-tethered) disulfide cross-link could readily span the distance between attachment points, while a bis(ethanethiol)-tethered (C_2-tethered) disulfide cross-link would fall more than 1.5 Å short.[6]

The C_2- and C_3-thiol-tethered decamers were synthesized by the convertible nucleoside approach[3,7] and oxidized to generate disulfide cross-linked duplexes **C_2X** and **C_3X**, respectively.[8,9] Both decamers were found to form cross-linked duplexes in nearly quantitative yield. Thermal denaturation experiments revealed that both **C_2X** and **C_3X** were stabilized significantly as compared to the corresponding unmodified duplex, with the shorter cross-link being less stable.[10]

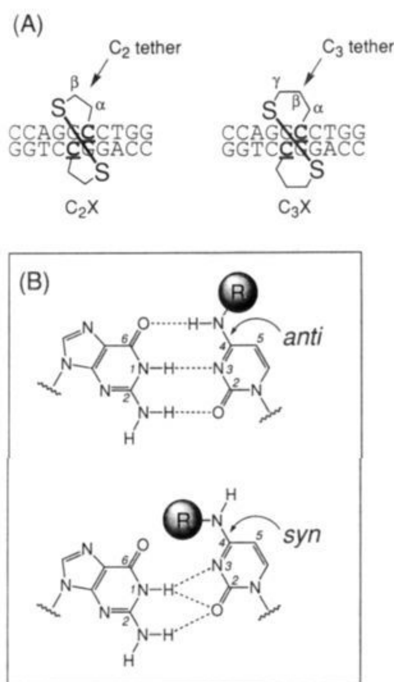

Figure 1. Schematic diagram of the disulfide cross-linked decamers **C_2X** and **C_3X** (A). The tethers are attached to the central C̲ residues as depicted in B; the upper structure represents the G·C̲ base pair with the tether adopting the *anti* rotamer; this has ordinary Watson–Crick hydrogen bonds; the lower structure depicts the *syn* rotamer, which for steric reasons is incapable of Watson–Crick hydrogen bonding, but may form bifurcated hydrogen bonds as shown.

^1H and ^{31}P NMR spectra were used to characterize the structural changes induced by cross-linking. The ^1H-decoupled ^{31}P spectrum of **C_3X** is similar to that of the unmodified control (data not shown), indicating that the C_3 cross-link has little effect on the structure of its host sequence. Due to the symmetry of these molecules, their spectra contain only half as many peaks (9) as phosphates (18). On the other hand, the spectrum of **C_2X** is radically different from **C_3X** or the control. In particular, the spectrum of **C_2X** contains a peak for each phosphodiester in the molecule, indicating that it is asymmetric on the NMR time scale. Furthermore, many of the ^{31}P resonances have shifted significantly as compared to the control, particularly around the site of cross-linking.

The imino proton spectra of **C_2X** and **C_3X** provided further insight into the structural differences between these two molecules (Figure 2). The imino spectra of the unmodified decamer and

(1) For recent reports and reviews on noncanonical DNA, see: Pérez-Martín, J.; Espinosa, M. *Science* **1993**, *260*, 805–807. Travers, A. A. *Curr. Opin. Struct. Biol.* **1991**, *1*, 114–122. Crothers, D. M.; Gartenberg, M. R.; Shrader, T. E. *Methods Enzymol.* **1991**, *208*, 118–146. Travers, A. A. *Annu. Rev. Biochem.* **1989**, *58*, 427–452.

(2) (a) Ferentz, A. E.; Verdine, G. L. *J. Am. Chem. Soc.* **1991**, *113*, 4000–4002. (b) Ferentz, A. E.; Keating, T. A.; Verdine, G. L. *J. Am. Chem. Soc.* **1993**, *115*, 9006–9014.

(3) For a review, see: Ferentz, A. E.; Verdine, G. L. In *Nucleic Acids and Molecular Biology*; Eckstein, F., Lilley, D. M. J., Eds.; Springer-Verlag: New York, 1994; Vol. 8, in press.

(4) See, for example: Liu, L. F.; Wang, J. C. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 7024–7027.

(5) In the unmodified form, this sequence has been structurally characterized by X-ray crystallography. Heinemann, U.; Alings, C. *J. Mol. Biol.* **1989**, *210*, 369–381. Heinemann, U.; Alings, C.; Bansal, M. *EMBO J.* **1992**, *11*, 1931–1939.

(6) This value represents a lower limit, since in this model the dihedral angle of a disulfide bond formed between the sulfur atoms would be ~175° (length 3.75 Å). To achieve a more optimal dihedral angle of ~100°, the distance separating the two thiols would have to be lengthened further. The optimal S–S bond distance in alkane disulfides is ~2.02 Å: Jiao, D.; Barfield, M.; Combariza, J. E.; Hruby, V. J. *J. Am. Chem. Soc.* **1992**, *114*, 3639–3643 and references contained therein.

(7) MacMillan, A. M.; Verdine, G. L. *Tetrahedron* **1991**, *47*, 2603–2616. MacMillan, A. M.; Verdine, G. L. *J. Org. Chem.* **1990**, *55*, 5931–5933.

(8) Further details are available in the supplementary material or can be obtained directly from the authors by FAX, (617) 495-8755.

(9) Following our initial report,[2a] two alternative methods of introducing disulfide cross-links into DNA have appeared. The nucleoside analogs used in the latter methods are incapable of Watson–Crick pairing and hence were deemed unsuitable for the present purposes. Milton, J.; Connolly, B.; Nikiforov, T.; Cosstick, R. *J. Chem. Soc., Chem. Commun.* **1993**, 779–780. Glick, G. D. *J. Org. Chem.* **1991**, *56*, 6746–6747.

(10) Melting temperatures (T_m) of **C_3X**, **C_2X**, and the unmodified decamer were 71.2, 65.1, and 55.1 °C, respectively, while a control decamer containing 2-(methylthio)ethyl (MTE) tethers had a T_m of 41.3 °C. The MTE-tethered duplex allows one to estimate the thermodynamic penalty of simply attaching the tethers to DNA. The duplex stabilization attributable to cross-linking is proportional to the difference in T_m of the MTE-tethered and cross-linked duplex: 29.9 °C, C_3 cross-link; 23.8 °C, C_2 cross-link. Melting temperatures (T_m) were collected on a Perkin-Elmer Lambda 3B spectrophotometer equipped with a thermoelectrically controlled cell holder and interfaced to an IBM-XT personal computer using ASYST (version 1.53) data collection software. Samples with initial OD_{260} ~0.5 AU were prepared in 1 M NaCl, 1 mM in EDTA, and 10 mM in sodium phosphate buffer at pH 7.

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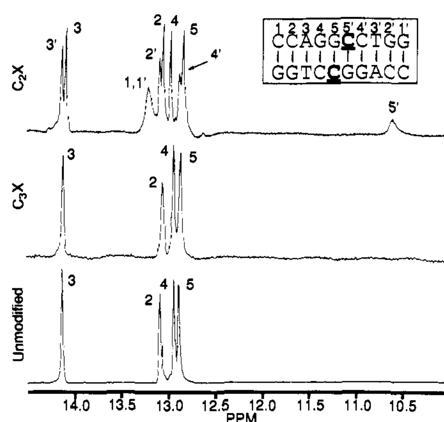

Figure 2. Imino region of the 500-MHz ^{1}H spectra of C_2**X** (top), C_3**X** (middle), and the unmodified decamer (bottom). Spectra were taken in 90:10 H_2O/D_2O containing 100 mM NaCl, 10 mM sodium phosphate (pH 7.5), and 0.2 mM EDTA. Spectra were acquired with a 1–3–3–1[18] pulse sequence to suppress the H_2O signal. NMR spectra of C_3**X** and the unmodified decamer were taken at 17 °C, while the spectrum of C_2**X** was taken at 12 °C. This difference accounts for the appearance of the 1 and 1′ signals in only the spectrum of C_2**X**.[11]

C_3**X** are very similar, displaying only four peaks due to their inherent symmetry.[11] In sharp contrast, the imino spectrum of C_2**X** displays twice the number of peaks, confirming the asymmetry of the molecule. Particularly striking is the pronounced broadening and upfield shift of one of the central guanine imino protons (peak 5′); such behavior indicates that this residue is not involved in ordinary Watson–Crick base-pairing.[12] The imino proton of the symmetry-related G (peak 5) has a chemical shift and line width indicative of Watson–Crick base-pairing.

To pinpoint the nature of the structural distortion within C_2**X**, we examined its two-dimensional ^{1}H–^{1}H NOESY[13] spectrum. Whereas the normally paired C residue (C-5, Figure 2) of C_2**X** showed a strong NOE from the α-CH_2 of the tether to its own 5H, this diagnostic NOE was absent in the abnormally paired C residue (C-5′) of C_2**X**. In addition, the tether of C-5′ exhibited NOEs to the imino proton of the neighboring G·C base pair. These NOEs are consistent with the α-CH_2 of the tether at C-5′ adopting a *syn* orientation with respect to N3 (refer to Figure 1B). In this position a severe steric clash occurs between the α-CH_2 of the tether and the carbonyl of the complementary G. This forces the guanine to slide into the minor groove, where it may adopt a weakened wobble pair with the C-5′. NOEs from the 8H proton of the displaced G to neighboring bases indicate that it is at least partially stacked in the helix, but that the base has shifted away from the sugar on its 5′ side. The C-5′ appears to be stacked in the helix as well, with typical NOEs from its 5H and 6H protons to the neighboring base and sugar protons, although the chemical shifts of its 5H and 6H protons suggest that this base experiences an unusual local environment in the helix.[14]

This report demonstrates the utility of alkane disulfide cross-links for the investigation of torsional stress in duplex DNA. The presence of the cross-link itself is benign to the duplex structure, as demonstrated by the NMR spectra of C_3**X**. From this relaxed structure, torsional stress was increased simply by ratcheting the tether length from three to two methylene units. However, instead of unwinding by ~30–40° according to our design, the DNA relieved this torsional stress by disrupting a Watson–Crick base pair. We thus conclude that the energetic cost of disrupting a G·C base pair is smaller than the cost of unwinding the helix to ~0° at a GpC step. Consequently, proteins that severely unwind DNA must do so only at considerable thermodynamic expense.[15] On the other hand, proteins that cause local disruption of Watson–Crick pairing, such as DNA methyltransferases,[16] would seem to require a relatively smaller expenditure of energy.[17,19]

Supplementary Material Available: Selected regions of the NOESY spectra of the cross-linked decamers, their ^{1}H-decoupled ^{31}P spectra, and a representative ^{31}P–^{1}H hetero-TOCSY spectrum of the control (7 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

(11) The imino protons of the terminal base pairs (1 and 1′ in Figure 2) are seen only in the spectrum of C_2**X**, because this was acquired at a lower temperature than the spectra of C_3**X** and the control to slow the exchange of the G-5′ imino proton.

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(13) States, D. J.; Haberkorn, R. A.; Ruben, D. J. *J. Magn. Reson.* **1982**, *48*, 286–292.

(14) An alternative but perhaps less likely structure is one in which C-5′ and the complementary G are completely unpaired, sliding past each other in the helix. Experiments are underway to determine the structure of C_2**X** at high resolution.

(15) Winkler, F. K.; Banner, D. W.; Oefner, C.; Tsernoglou, D.; Brown, R. S.; Heathman, S. P.; Bryan, R. K.; Martin, P. D.; Petratos, K.; Wilson, K. S. *EMBO J.* **1993**, *12*, 1781–1795.

(16) Erlanson, D. A.; Chen, L.; Verdine, G. L. *J. Am. Chem. Soc.*, preceding paper in this issue.

(17) The objective of synthesizing locally underwound DNA could presumably be realized by utilizing tethers attached to endocyclic positions in DNA, such as the 5 position of pyrimidines. Derivatizations suitable for this purpose have recently been reported. Goodwin, J. T.; Glick, G. D. *Tetrahedron Lett.* **1993**, *34*, 5549–5552.

(18) Hore, P. J. *J. Magn. Reson.* **1983**, *55*, 283–300.

(19) This research was supported by grants from the NIH (GM 44853-03) and NSF (PYI program). G.L.V. is a Sloan Fellow, a Lilly Grantee, a Dreyfus Teacher-Scholar, and a 1994 ACS Cope Scholar. S.A.W. was supported by a predoctoral fellowship from the National Science Foundation. We thank A. Ferentz and A. M. MacMillan for assistance with synthesis and NMR experiments, V. Grantcharova for help with DNA preparation and purification, Larry McLaughlin for use of the T_m instrument, and G. Kellogg and S. Huang for help with the ^{31}P–^{1}H hetero-TOCSY experiment. The AM-500 was funded by grants from the NIH (1-S10-RR04870-01) and NSF (CHE88-14019).