Disulfide Cross-linking as a Mechanistic Probe for the B ↔ Z Transition in DNA

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The archetypal B-form structure of duplex DNA can isomerize into a variety of alternate helical structures. Perhaps the most intriguing and controversial amongst these is Z-DNA, so-named for the jagged appearance of its left-handed backbone.[1−3] Although the physiological role of Z-DNA remains a topic of active debate, conclusive evidence indicates that Z-DNA is formed at least transiently in transcriptionally active chromatin as the result of superhelical stress.[4] Short oligonucleotides containing alternating purine-pyrimidine tracts can be induced to form Z-DNA *in vitro* by high salt or organic cosolvents. The B ↔ Z transition requires not only inversion of the helical screw sense and concomitant adjustments in backbone dihedral angles but also requires that each base-pair flip over along its long axis. Various models have been advanced to describe this process, including some that involve strand separation.[1a,5] Here we use disulfide cross-linking to impose severe restrictions on the motions available to DNA during the B ↔ Z transition. These cross-links slow the B ↔ Z transition only slightly and have virtually no effect on the crystallographic structure of Z-DNA.

The rational engineering of disulfide cross-links into nucleic acids[6] has been used extensively to study their structural and functional properties[7] and to gain insight into protein−nucleic acid interactions.[8,9] Whereas this prior work has largely focused on using disulfide cross-linking to control the ground state properties of nucleic acids, here we explore the alternate possibility of using disulfide cross-linking to alter the kinetics of a defined structural transition in DNA. For these studies we chose the self-complementary hexamer sequence, 5′-CGCGCG-3′, which has been extensively studied biochemically and biophysically and for which several X-ray crystal structures have been determined in the Z-form.[1a,10] Molecular modeling suggested that it should be possible to bridge the two symmetry-related guanines in the center of the hexamer using a $-CH_2-CH_2CH_2S-SCH_2CH_2CH_2-$ linker attached on either end to the exocyclic amine of G (Figure 1).

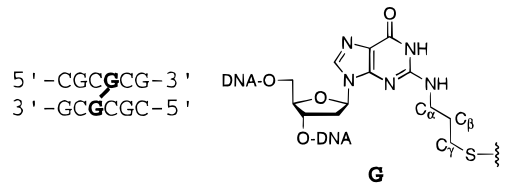

Figure 1. Structure of the cross-linked hexamer used in these studies.

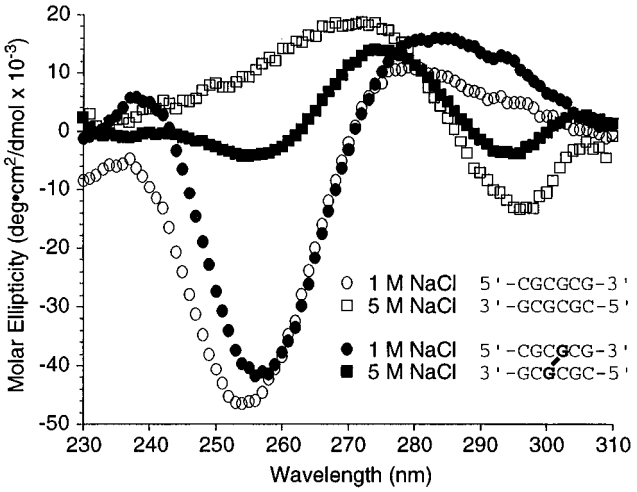

Figure 2. CD spectra of the unmodified and cross-linked hexamers in low (1 M) and high (5.3 M) salt solutions. The oligonucleotides were annealed prior to measurement; all spectra were recorded at 12 °C.

The thiol-tethered self-complementary hexamer 5′-CGC**G**CG-3′ (**G** = N^2-(3-thiopropyl)deoxyguanosine was synthesized by the convertible nucleoside method as previously described.[8] Oxidation to the disulfide cross-linked hexamer was carried out under conditions that favor a B-form duplex.[11] To compare the global helical structure of the unmodified and disulfide cross-linked hexamers, we measured their circular dichroism (CD) spectra under conditions that favor either the B- or Z-form (Figure 2). At 1 M NaCl, the unmodified and disulfide cross-linked hexamers exhibited similar CD spectra, which reveal features characteristic of B-form DNA. Increasing the NaCl concentration to saturation (5.3 M) results in a marked change in the CD spectra for both the unmodified and cross-linked hexamers. However, whereas the CD spectrum of the native hexamer at high salt was prototypical for Z-DNA,[2] that of the cross-linked hexamer still has a slight negative inflection in the 255 nm range, suggesting it retains a small residual population of B-form duplex structure under these conditions. Thus it appears that cross-linking causes a slight shift in the position of the B ↔ Z equilibrium in favor of B-form.

To determine the effect of disulfide cross-linking on the rate of the B ↔ Z isomerization, we performed a series of salt-jump experiments, in which oligonucleotides were instantaneously exchanged from one buffer to another at a different

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(11) The reduced DNA was mixed with 0.5 equiv of 5,5′-dithiobis(2-nitrobenzoic acid) to facilitate disulfide bond formation, and the cross-linked oligonucleotides were separated from unreacted DNA by anion exchange chromatography (see ref 9b).

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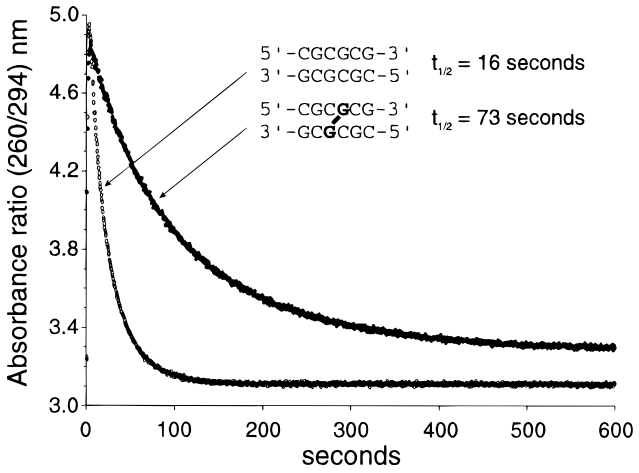

Figure 3. Representative salt-jump experiment, in which an oligonucleotide stock was rapidly diluted from 1 to 4 M NaCl. The experiment shown was carried out at 5.8 °C.

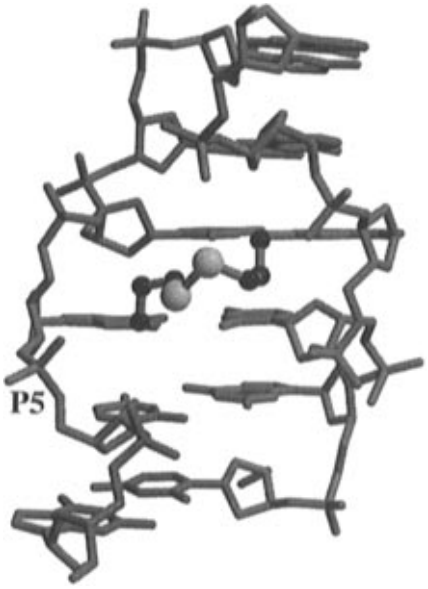

Figure 4. View looking into the minor groove of the final structure. The carbon atoms of the tether are shown as black spheres, and the sulfur atoms are shown as slightly larger light gray spheres.

salt concentration, and the rate of duplex isomerization was measured by ultraviolet (UV) spectroscopy.[12,13] A representative measurement shown in Figure 3 reveals that both the unmodified and cross-linked oligonucleotides isomerize from the B to Z form with simple exponential behavior but do so with markedly different kinetics. Specifically, the half-life of the B ↔ Z interconversion reaction was increased roughly 4-fold by disulfide cross-linking (16 vs 73 s); a similar increase was observed for the Z ↔ B transition (37 vs 152 s; data not shown).[14] Thus, disulfide cross-linking clearly alters the conformational dynamics of the B ↔ Z transition but not so profoundly as to lock the duplex in either form.

To describe in detail the effect of disulfide cross-linking on the structure of Z-DNA, we crystallized the cross-linked hexamer in the Z-form and determined its structure by X-ray diffraction to a final resolution of 2.25 Å.[15] The solution structures of several disulfide cross-linked oligonucleotides have been determined by NMR spectroscopy;[7c,9b] however, to our knowledge this represents the first report of an X-ray structure determination of disulfide cross-linked DNA. The structure, shown in Figure 4, is remarkably similar to that of unmodified Z-DNA,[1a] with an overall root-mean-squared-deviation (RMSD) between the cross-linked and unmodified hexamers of only 0.60 Å. This degree of variation is comparable to that found for the unmodified hexamer crystallized under slightly different conditions. Interestingly, whereas the phosphate P5 is found in the minor Z_{II} conformation in most X-ray structures of the Z-form hexamer,[10b] this phosphate adopts the major Z_I conformation in the disulfide cross-linked hexamer. Nonetheless, P5 does exhibit a higher degree of overall disorder than the rest of the molecule; excluding it lowers the RMSD for the disulfide cross-linked versus unmodified hexamer to 0.47 Å. Electron density corresponding to the sulfur atoms in the tether was obvious from the earliest stages of refinement, but the C_β and C_γ carbons did not refine well and seem to be partially disordered; indeed, the average B factor of the tether is more than double that of the duplex as a whole (14.4 Å^2 vs 6.0 Å^2). The disulfide dihedral angle is −87°, a value characteristic of unstrained disulfides.[16] Overall, the tether fits snugly into the minor groove of the hexamer.

Here we have shown that disulfide cross-linking retards the rate of the B ↔ Z transition in DNA but has little or no effect on the structure of the Z-form duplex. CD, NMR, and molecular modeling experiments further suggest that cross-linking has little effect on the structure of the B-form hexamer (data not shown). At this stage, it is not possible to state conclusively whether the 4-fold decrease in rate of the B ↔ Z transition results from ground-state stabilization,[17] transition state destabilization, or both. Nevertheless, it is clear from NMR melting experiments on these and other oligonucleotides that an unstrained interstrand disulfide cross-link stiffens the duplex and greatly retards strand separation, yet despite this, the cross-link has only a modest effect on the rate of the B ↔ Z transition.[14] These experiments further suggest strongly that strand separation is not required for the B ↔ Z transition to occur. The disulfide cross-link imposes a steric limitation on the relative motions of the two linked base-pairs during the B ↔ Z transition, such that both cannot undergo domino-like movements. It thus seems likely that the dinucleotide repeat of Z-DNA is established in the transition state leading to its formation.

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Supporting Information Available: Details of the crystallization and structure determination and a table of coordinates with the PDB format for the disulfide cross-linked hexamer (7 pages). See any current masthead page for ordering and Internet access instructions.

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(13) By monitoring the UV absorbance ratio at 260 to 294 nm, the B to Z or Z to B transition can be followed. The data can be fitted to a simple exponential equation to obtain the relaxation time τ, where $\tau^{-1} = k_{(B\ to\ Z)} + k_{(Z\ to\ B)}$ and $t_{1/2} = \ln(2)\cdot\tau$.

(14) A 4-fold difference in rate corresponds to an activation free energy difference of about 0.8 kcal·mol^{-1} at room temperature, which is modest in comparison to the 35−50 kcal·mol^{-1} required for the overall B ↔ Z transition (see ref 12a).

(15) Full experimental details are given in the Supporting Information.

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(17) Thermal denaturation experiments followed by UV spectroscopy support this hypothesis. While the unmodified hexamer melts at 47 or 40 °C (in 1 and 4 M NaCl, respectively), the cross-linked hexamer does not show a clear cooperative melting transition in either 1 or 4 M NaCl, suggesting that the tether stabilizes the duplex state. This has previously been seen with other cross-linked oligonucleotides (see ref 7c).