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Structural arrangement of DNA constrained by a cross-linker

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Self-complementary cross-linked oligonucleotides with a disulfide linkage were designed and synthesized. Double helix and hairpin structures were controlled using the different diastereochemistry of phosphoramidate where the cross-linker was introduced. The structures of the strained cross-linked DNAs were estimated by gel mobility, circular dichroism spectra, and melting profiles.

Dynamic structural changes of DNA have attracted attention because they are considered to be mechanical movements and can have applications to the construction of molecular devices.[1–2] Structural transitions between the B- and Z-form,[3–4] hairpin–duplex transitions,[5–6] quadruplex–duplex transitions[7–9] and topological changes[10–11] have been utilized as mechanical switches, and these transitions have been observed with fluorescence-based methods and in AFM images. Specific DNA strands and cations are indispensable for these transitions—the thermally unfavourable DNA conformations are created by the addition of these third reagents. In addition, unfavourable conformations (such as strained DNA structures) can be created by connecting the two DNA strands using a covalent linkage which induces distortion of the duplex structures.[12–15] In previous work, we connected two double helix DNAs with disulfide and bismaleimide linkers and thus controlled the structural arrangement of the duplex using cross-links.[16–20] Further extension of these structural arrangements could be developed for the creation of molecular devices and novel DNA structures, utilizing this DNA cross-linking method.

In this report, we control hairpin and double helix DNA structures by constraining the DNA backbone with the modification of phosphorus atoms and cross-linking the two DNA strands (Fig. 1a). We employed the self-complementary sequence, 5′-GCGTATACGC-3′, which can form both hairpin and double helix structures under specific conditions, and the detailed solution structures were characterized by NMR spectroscopy.[21–22] The cross-linkers were introduced to the phosphorus atoms at a specific position, denoted as p in the sequence 5′-GCGTATpACGC-3′. Control of the diastereochemistry of the phosphoramidates[23–24] and the location for introduction of the cross-linkers are important design points. From the molecular modelling of these cross-linked oligonucleotides, the cross-linkers should be introduced to specific positions for passing through the minor groove (Fig. 1b).[25] Only the phosphoramidate possessing the R_p-configuration satisfies this demand. The sequence we chose is self-complementary and the interstrand cross-linkers are introduced between the two phosphorus atoms which are located in the most proximal positions of the minor groove, based on molecular modelling. We introduced a disulfide diamine linker for cross-linking *via* a phosphoramidate linkage and employed two different lengths of the linkers, with two (**1**) and three (**2**) methylenes for cross-linking (Fig. 1a).

Cross-linked DNAs with diastereochemically regulated phosphoramidates were synthesized according to the previously reported procedure.[16–20,23] Two diastereomers, denoted as A and B, correspond to the faster eluted peaks and slower ones on a reversed-phase HPLC, respectively. After DTT treatment, cross-linking was performed using 5,5′-bis(thio-2-nitrobenzoic acid) for activation of the thiol group, and the cross-linked DNAs were purified by HPLC.[26–27]

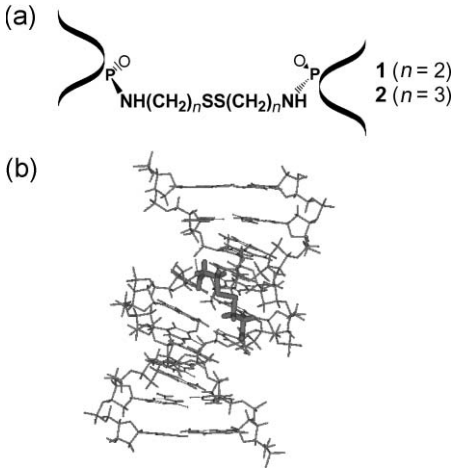

Fig. 1 (a) The interstrand cross-linked DNAs connected by disulfide linkers with two ($n = 2$; **1**) and three ($n = 3$; **2**) methylenes; (b) the energy minimized duplex DNA structure with a cross-linker ($n = 2$) of R_p-configuration. The cross-linker part (bold stick) is located in the minor groove.

The properties of the cross-linked DNAs were examined by a gel mobility assay. Using a 20% non-denaturing polyacrylamide gel electrophoresis (PAGE) run at 4 °C, the DNA structures of XL-DNAs with different configurations and lengths of the cross-linkers were examined.[28] As shown in Fig. 2, A-series XL-DNAs had the same mobility and migrated slower than the unmodified duplex. On the other hand, the two XL-DNAs in the B-series had the same mobility but were faster than the unmodified duplex. These results indicate that the general DNA structures of these XL-DNAs do not depend on the length of the cross-linkers, but depend on the configuration of the phosphoramidate in the DNA backbones. These XL-DNAs were reversibly reduced to thiol-tethered DNA (SH-DNA) by addition of DTT.

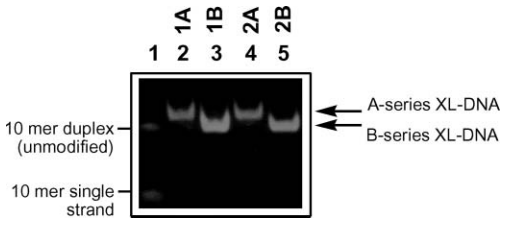

Fig. 2 Non-denaturing PAGE of the cross-linked DNAs. Electrophoresis was carried out in a 20% native polyacrylamide gel at 4 °C. The DNAs are presented above the lane number. Lane 1, unmodified 10-mer DNA. The gel was stained with ethidium bromide.

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The structures of the XL-DNAs were further characterized by circular dichroism (CD) spectroscopy (Fig. 3).[29–30] In the spectra of XL-DNAs with A-diastereomers, the intensity of the positive peaks in the spectra at 290 nm depended on the length of the cross-linkers. XL-DNA **1A**, with a shorter linker length, showed much smaller positive peaks at 290 nm as compared to **2A**, meaning that **1A** forms some strained structures (as compared to **2A**) as well as the unmodified duplex. In addition, the negative peaks in the A-series largely shifted by 6–8 nm as compared to those of the native duplex. These results indicate that the DNA structures of the A-series are easily affected by the strain caused by cross-linkers. On the other hand, the intensity of the positive peaks in the spectra of the B-series XL-DNAs slightly decreased, and the shifts of the peaks were small as compared to the unmodified duplex. These results indicate that the DNA structures of the B-series are maintained even when the length of the linkers are changed.

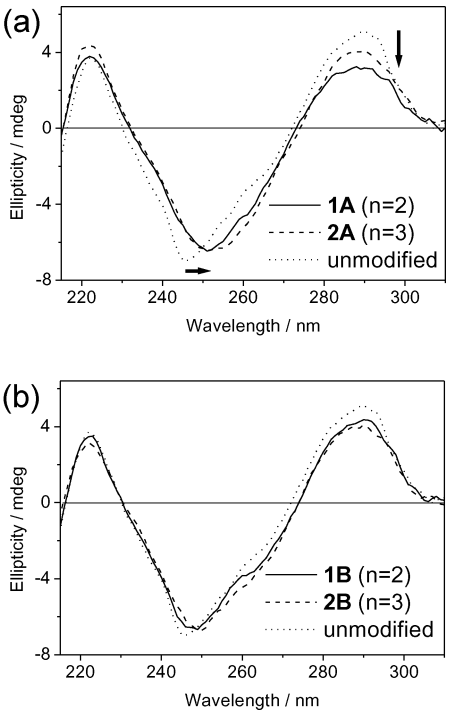

Fig. 3 Circular dichroism spectra of the cross-linked DNAs: (a) the spectra of the A-series XL-DNAs; arrows indicate the significant difference among the spectra; (b) the spectra of the B-series XL-DNAs. Measurement conditions are described in the text.[29]

To elucidate the duplex-forming activity of the cross-linked DNAs, measurements of the XL-DNA melting temperatures (T_m) were performed (Fig. 4).[31] Melting profiles of the XL-DNAs show the clear difference between the A-series and B-series XL-DNAs. The T_m values of the B-series XL-DNAs were significantly increased as compared to that of the unmodified DNA (50.3 °C), and those of **1B** and **2B** were 67.3 and 68.7 °C, respectively. The higher T_m values in the B-series XL-DNA suggest that the transitions in the melting profiles of the B-series are the intramolecular denaturation of the DNA strands, and the cross-linkers do not inhibit the duplex formation. In contrast, the A-series XL-DNAs had only moderate transitions in the profiles. In addition, the hyperchromicity of these XL-DNAs is much lower than that of the unmodified duplex. The melting profile of GCGTTTTCGC, which can form hairpin structures, showed a similar profile under the same measurement conditions. This indicates that the A-series XL-DNAs form hairpin structures.

The experimental results of gel-mobility, CD-spectra, and melting profiles proved to be consistent. The B-series XL-

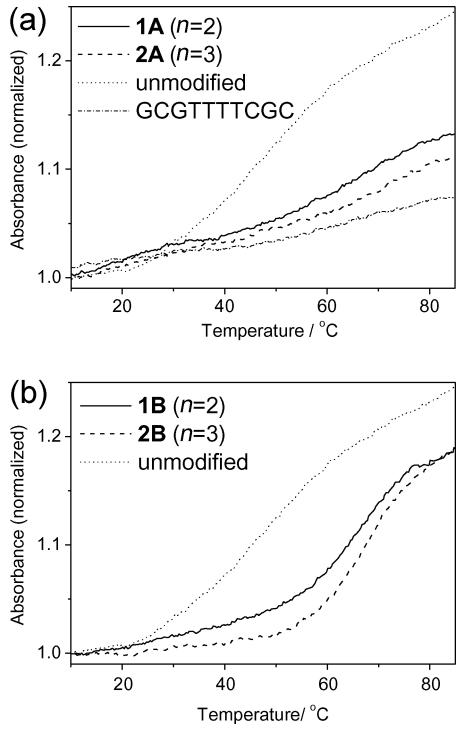

Fig. 4 Melting profile of the A-series XL-DNAs (a) and the B-series XL-DNAs (b). Measurement conditions are described in the text.[31]

DNAs can form duplex structures, and the cross-linker in the B-diastereomers passes through the minor groove without strain as shown in Fig. 1b. Thus, the absolute configuration of the B-diastereomer is estimated as the R_p-configuration. On the other hand, when the molecular modelling (energy minimization) of the XL-DNA structure was performed using the S_p-configuration, the B-form DNA structure was completely destroyed because of the strain on the DNA backbone. This indicates that the A-series XL-DNAs do not form the usual 10-mer duplex structure, due to the strain caused by the cross-linker connected to the unfavourable diastereomer (S_p-configuration). The strain introduced by the cross-linker in the A-series XL-DNAs makes the intramolecular duplex formation difficult, and these XL-DNAs form a much more stable hairpin structure as shown in Fig. 5.

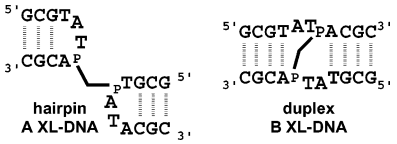

Fig. 5 Two possible DNA structures; hairpin structure (A-series XL-DNAs) (left) and duplex structure (B-series XL-DNAs) (right).

In summary, we designed and synthesized the cross-linked DNAs and characterized their properties. We demonstrate the importance of the diastereochemistry of the phosphoramidates in the cross-linked DNA, which strongly affects the duplex forming activities and their structures. The various arrangements of the DNA structures using the cross-linker can be applicable for the construction novel DNA supramolecular structures and nanostructures.

Acknowledgements

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25 The initial DNA structure was constructed by using the coordination of $d(GCGTATACGC)_2$ (pdb 1d68; ref. 22). The C2 cross-linker was added to the specific phosphorus atoms (R_p-configuration) *via* the minor groove.
26 Cleavage of the disulfide linkage of oligonucleotides was carried out in a solution containing 10 mM dithiothreitol (DTT) and 50 mM Tris–HCl (pH 8) at 50 °C for 30 min. Thiol-tethered DNAs were purified by HPLC. Then, the thiol-tethered DNA was treated with an excess of 5,5′-bis(thio-2-nitrobenzoic acid) (DTNB) in 50 mM Tris–HCl (pH 8) for 2 h and purified by HPLC. An equal amount of the thiol-tethered DNA was added to the TNB-activated DNA in 50 mM Tris–HCl (pH 8) buffer at rt for 12 h, and the resulting cross-linked DNAs were purified by HPLC.
27 ESI-MS (negative): **1** calcd for $C_{198}H_{253}N_{78}O_{114}P_{18}S_2$ 6171.3[M–H]$^-$; found, **1A** 6171.4, **1B** 6172.2; **2** calcd for $C_{200}H_{257}N_{78}O_{114}P_{18}S_2$ 6199.3 [M–H]$^-$; found, **2A**: 6199.4; **2B**: 6199.4.
28 Samples (20 μL) containing 2.0 mM XL-DNA, 10 mM Tris–HCl, and 0.1 M NaCl were annealed from 90 to 4 °C at a ratio of −1.0 °C min^{-1} using a thermal cycler. Nondenaturing PAGE (20%) was performed at 4 °C and the gels were visualized by ethidium bromide staining. The unmodified DNA partially formed single strand under this PAGE condition (lane 1).
29 CD spectra were acquired on a JASCO J-725 spectropolarimeter. Measurements were carried out at 10 °C in a 1 mL solution containing 2.0 μM DNA, 10 mM Tris–HCl (pH 7.6), and 1.0 M NaCl.
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31 Thermal denaturation profiles were obtained by heating from 10 to 80 °C at a rate of 1.0 °C min^{-1}. Measurements were carried out in a 1 mL solution containing 2.0 μM DNA, 10 mM Tris–HCl (pH 7.6), and 1.0 M NaCl.