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Structural arrangement of two DNA double helices using cross-linked oligonucleotide connectors†

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Disulfide cross-linked oligonucleotides, which connect two different sequences of DNA strands, have been synthesized and characterized. Two double helices connected by two different cross-linked oligonucleotides can be arranged in both parallel and antiparallel orientations by addition of the specific complementary strands.

Programmed association and folding for well-defined structures are fascinating aspects of supramolecular chemistry.[1–4] The association of DNA strands by the base pairing system and the periodic structure of double helix DNA are attractive for programmable supramolecular assembly. Two dimensional DNA supramolecular conponents have been prepared by arranging multiple DNA strands using native[5–7] and chemically modified[8–10] oligonucleotides. We have previously designed and synthesized small DNA components by employing cross-linked oligonucleotides (XL-DNA) as connectors for two double helices.[9–10] Because of the limitation of the sequences of the cross-linked oligonucleotides which connected the same sequence of two single strand DNAs, we could not arrange two double helices with the four blunt ends in antiparallel orientation using these cross-linked DNAs.[9] For preparing a wide variety of structures using these DNA components, orientations of DNA strands are important factors, and the antiparallel arrangement of two double helices is required.

Here, we intended to control the relative orientations of two DNA double helices in both parallel and antiparallel ways using two cross-linked oligonucleotides. We synthesized cross-linked oligonucleotides in which two different sequences of DNA strands were connected by a disulfide linkage (Fig. 1). We employed phosphorus atom modification for connecting two DNA strands. In the case of phosphorus modification, two diastereomers (R_p- and S_p-configurations) generate and a linker of each diastereomer orients in a different direction during the duplex formation. The duplexes containing a phosphoramidate with a different diastereochemistry show different duplex forming activities,[9–12] and these diastereomers need to be characterized individually.

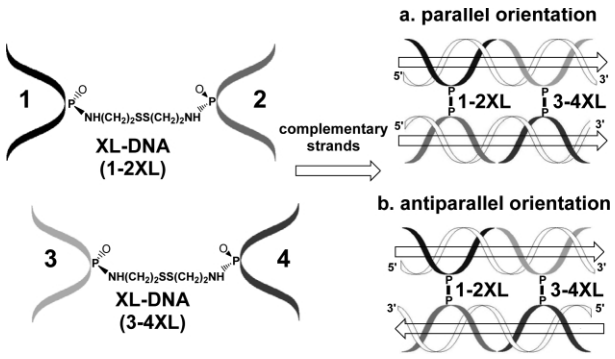


Fig. 1 A disulfide cross-linked oligonucleotide having two different sequences of DNA strands (10 mer). By using two cross-linked oligonucleotides and complementary 20 mer DNA strands, the two kinds of DNA assemblies can be produced in parallel and antiparallel orientations. Arrows indicate the 5′ to 3′ directions of the complementary strands. Bold bars represent cross-linkers.

† Electronic supplementary information (ESI) available: HPLC profiles and MALDI-TOF-MS of the cross-linked oligonucleotides. See http://www.rsc.org/suppdata/cc/b4/b402783c/

Synthesis of disulfide cross-linked oligonucleotides was performed according to Scheme 1. Preparation of oligonucleotides having a diastereochemically pure phosphoramidate was carried out according to the previously reported method.[9–11] Two adjacent diastereomer peaks appeared on a reversed-phase HPLC. The faster and slower eluted peaks on HPLC are denoted as A- and B-diastereomers, respectively. The diastereochemically pure oligonucleotides (10 nmol) were treated with dithiothreitol (DTT) and purified by HPLC. Then the thiol-tethered DNA (DNA-SH) was activated with 5,5′-bis(thio-2-nitrobenzoic acid) (DTNB) to give TNB-attached DNA (DNA-TNB). The DNA-TNB (10 nmol) and the different sequence of DNA-SH (10 nmol) were allowed to react for disulfide cross-linking, and the main peaks on HPLC were collected.

The following four DNA sequences were employed; (1) 5′-GAGGTpAGCCC-3′; (2) 5′-GCTGTpAGCCG-3′; (3) 5′-GCGCTpACACC-3′; (4) 5′-CGGCTpACTCC-3′, where p denotes a phosphoramidate linkage for introduction of a disulfide cross-linker. Sequences **1** and **2** for XL-DNA **1–2XL**, and sequences **3** and **4** for the XL-DNA **3–4XL** were connected. The HPLC purified oligonucleotides were analyzed by polyacrylamide gel electrophoresis (PAGE) as shown in Fig. 3.[13] All the purified cross-linked oligonucleotides have the same mobility as compared to a 20 mer single strand DNA, indicating the increase of the molecular weight of these oligonucleotides. Molecular weights of the cross-linked oligonucleotides were determined by MALDI-TOF mass spectroscopy.[14]

We used the two combinations of the cross-linked oligonucleotides having the same diastereochemistry, such as **1A–2AXL** with **3A–4AXL**, and **1B–2BXL** with **3B–4BXL**, for investigation of the

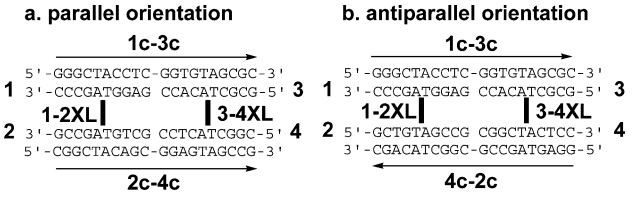
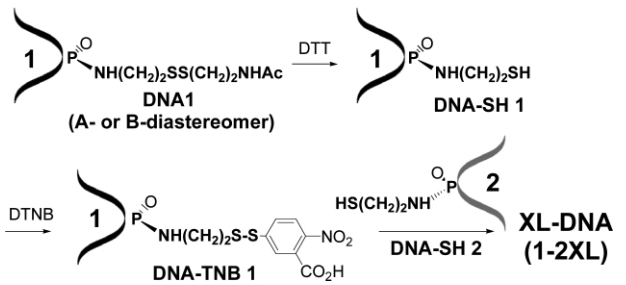


Fig. 2 The sequences of the cross-linked oligonucleotides and complementary strands. DNA strands **1c–3c**, **2c–4c**, and **4c–2c** represent 20 mer strands complementary to the sequences **1–3**, **2–4**, and **4–2**, respectively. Arrows indicate the 5′ to 3′ directions of the complementary strands.



Scheme 1 Synthetic scheme for disulfide cross-linked DNA (XL-DNA) having two different sequences of oligonucleotides

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diastereochemical effect on structures and stabilities. To examine the formation of the DNA assemblies, stoichiometric analysis by addition of complementary strands to the cross-linked oligonucleotides was performed. The DNA assemblies were analyzed by native PAGE (Fig. 4).[15] By addition of **1c–3c** to **1–2XL** and **3–4XL** (all one equivalent), no complex was observed in both cases of the A- and B-diastereomers (lanes 2 and 6). Next, we examined the formation of parallel and antiparallel DNA assemblies by employing two different complementary strands. For parallel arrangement (see Fig. 1 and Fig. 2), **1c–3c** and **2c–4c** (both one equivalent) were added to **1–2XL** and **3–4XL** (lanes 3 and 7). Slower migrated bands appeared as DNA assemblies containing the two cross-linked oligonucleotides. For antiparallel arrangement, **1c–3c** and **4c–2c** (both one equivalent) were added to **1–2XL** and **3–4XL** (lanes 4 and 8). In both cases, the slower migrated bands appeared, which have the same mobility as those of the parallel assemblies. The migrated positions of the DNA assemblies seem more retarded than that of a native 40 mer duplex. Native double helix DNA can be considered as a one-dimensional column with diameter of 2.0 nm. These DNA assemblies can form relatively rigid two-dimensional complexes, and the edge to edge distance of two double helices including the cross-linker is about 4.6 nm. This two-dimensional non-canonical DNA structure would affect the mobility in the polyacrylamide gel.

The structures of DNA assemblies containing two cross-linked oligonucleotides were examined by circular dichroism (CD) spectroscopy (Fig. 5).[16] In the case of the parallel assemblies, the spectra indicate that the DNA assemblies form typical B-form duplex DNA structures as compared to that of native duplexes (Fig. 5A).[17] The intensities of the positive peaks around 285 nm slightly decreased. This indicates that parallel assemblies would have some structural alternation caused by the cross-linkers. In the case of the antiparallel assemblies, the spectra show the same intensities as compared to that of the native duplexes, indicating that all the assemblies form the B-form duplex structures with canonical and relaxed forms. We also obtained the thermal melting profiles by UV spectroscopy, however, they showed very moderate transition curves at higher temperature, and we failed to characterize the process of the complex formation and dissociation of these DNA assemblies.

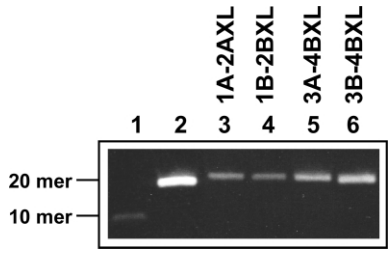

Fig. 3 20% Denatured polyacrylamide gel electrophoresis (PAGE) of cross-linked oligonucleotides employed in this study. Lane 1, 10 mer single strand; lane 2, 20 mer single strand; lane 3, **1A–2AXL**; lane 4, **1B–2BXL**; lane 5, **3A–4AXL**; lane 6, **3B–4BXL**.

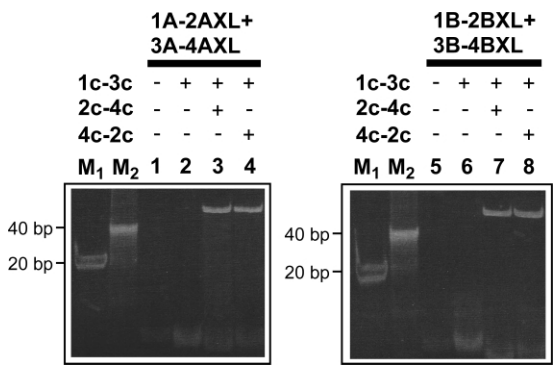

Fig. 4 20 % Native PAGE of DNA assemblies containing two cross-linked oligonucleotides. **1A–2AXL** and **3A–4AXL** with complementary strands are shown in lanes 1–4, and **1B–2BXL** and **3B–4BXL** with complementary strands are in lanes 5–8. M_1 and M_2 represent 20 mer and 40 mer duplex, respectively.

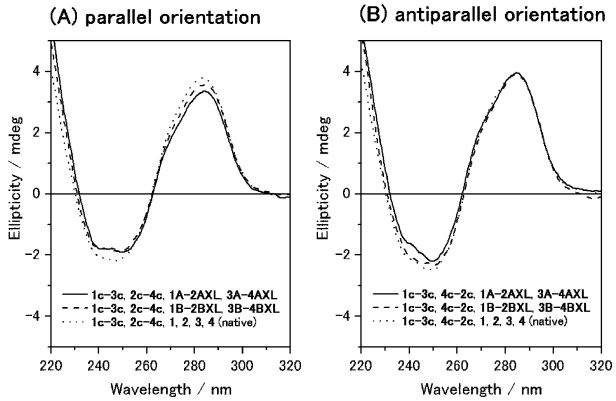

Fig. 5 CD spectra of DNA assemblies with parallel (A) and antiparallel (B) arrangements using two cross-linked oligonucleotides.

In conclusion, we have synthesized and characterized DNA assemblies containing two cross-linked oligonucleotides having two different sequences of DNA strands. Control of the relative orientation of the two double helices can be achieved using these two different cross-linked oligonucleotides and the complementary DNA strands. The DNA components we developed here can be building blocks for construction of DNA supramolecular structures and nano-structures,[9–10] and are suitable for preparing a versatile scaffold for nanowires and arrays by attaching functional molecules.

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13 DNA samples for denatured PAGE contained XL-DNA (0.2 nmol) and 10 μL of formamide. The mixtures were heated at 95 °C for 5 min, and then loaded onto a 20% denatured polyacrylamide gel.
14 MALDI-TOF-MS $[M–H]^-$: calcd 6207.1 for **1–2XL**; found 6208.6 for **1A–2AXL**; 6207.9 for **1B–2BXL**; calcd 6047.1 for **3–4XL**; found 6045.8 for **3A–4AXL**; 6046.4 for **3B–4BXL**.
15 Samples (20 μL) for native PAGE contained 0.5 μM XL-DNA, 0.5 μM complementary strands, 20 mM sodium phosphate buffer (pH 7.0), and 0.1 M NaCl. For complex formation, the samples were heated at 85 °C for 5 min then gradually cooled down to rt using a thermal cycler. After addition of 4 μL of 40% glycerol, the samples were loaded onto a 20% native polyacrylamide gel and run at rt.
16 CD spectra were obtained at 20 °C in a 1 mL solution containing 1 μM cross-linked oligonucleotides, 1 μM complementary strands, 10 mM Tris-HCl (pH 7.6), and 0.1 M NaCl.
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