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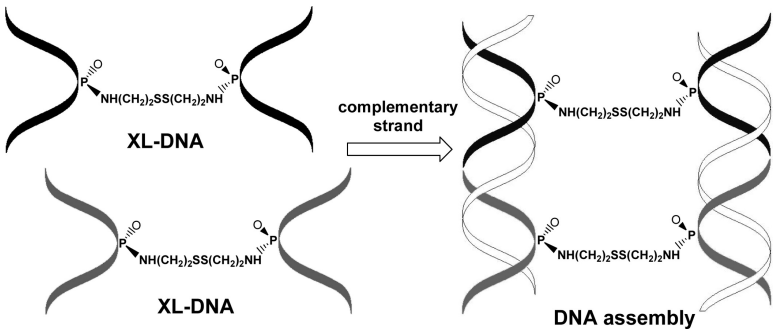

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Control of A Double Helix DNA Assembly by Use of Cross-Linked Oligonucleotides

Masayuki Endo and Tetsuro Majima*

Institute of Scientific and Industrial Research, Osaka University, 8-1 Mihogaoka, Ibaraki, Osaka 567-0047, Japan

Received June 18, 2003; E-mail: majima@sanken.osaka-u.ac.jp

Precise arrangement of various molecules following a programmed chemical system is one of the ultimate goals of supramolecular chemistry and nanotechnology.[1] Because of the programmable base-pairing system and selectivity, DNA is widely utilized for arranging organic and inorganic molecules, proteins, and nanoparticles.[2] However, for construction of functional nanometer-scale arrays with well-defined structures, it is required to suppress the intrinsic flexibility of DNA by employing rigid DNA components such as crossover DNA molecules.[3] We report here a simple method for assembling DNA strands using versatile DNA strand connectors, disulfide cross-linked oligonucleotides (XL-DNA), and for preparing potentially rigid DNA components in which the relative positions of two double helixes are controlled. Disulfide cross-linked oligonucleotides have been vigorously investigated for control of the DNA and RNA structures.[4] In this study, we introduced a disulfide cross-linker to an internal phosphorus atom for connecting two single DNA strands (Figure 1). Because phosphates are located in the outermost position from the double helical axis, the structural stresses caused by introduction of a cross-linker are considered to be minimal.

Preparation of oligonucleotides applicable for interstrand cross-linking was carried out by introduction of a disulfide tether via a phosphoramidate linkage according to a previously reported method.[5] Two adjacent diastereomer peaks appeared on a reversed phase HPLC, and the faster and slower eluted peaks on HPLC were defined as diastereomers A and B, respectively (Supporting Information). The purified diastereochemically pure oligonucleotides were treated with dithiothreitol (DTT) followed by activation of a thiol residue by 5,5′-bis(thio-2-nitrobenzoic acid) (DTNB). The thiol-DNA and DTNB-activated DNA were allowed to react for cross-linking. The purified DNA was identified by a denaturing polyacrylamide gel electrophoresis and migrated close to a 20 mer single-strand DNA. In addition, reduction of these oligonucleotides with DTT gave the original thiol-attached DNA, also indicating that these oligonucleotides are cross-linked through a disulfide linkage.

As shown in Scheme 1A, we prepared DNA assemblies with one XL-DNA and a complementary strand. To characterize the properties of the XL-DNA, thermal stabilities of the DNA assemblies were investigated by melting temperature (T_m) measurements. As shown in Table 1, the T_m values decreased compared to that of the unmodified duplex, and their stabilities depended on the salt concentration. At higher salt concentration (1.0 M NaCl), the differences of the T_m values (ΔT_m) between those for the assemblies and unmodified duplex decreased in comparison with those at lower salt concentration (0.1 M NaCl), meaning that the strong electrostatic repulsion between the two proximal double helixes connected by a cross-linker is reduced at higher salt concentration. By introduction of a tether into a phosphorus atom, two diastereomers (R_p and S_p) are generated. Because the linker of each diastereomer orients in a different direction during duplex formation, the modified duplexes having the different diastereochemistry of the phosphorus atoms show different duplex forming activities.[5,6] In this experiment, the similar stereochemical effect in the formation of the DNA assemblies was observed, and all the assemblies containing the B-diastereomer were thermally more

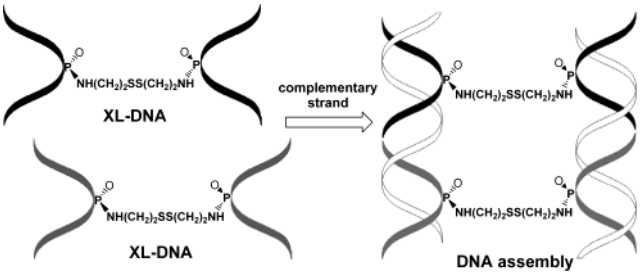
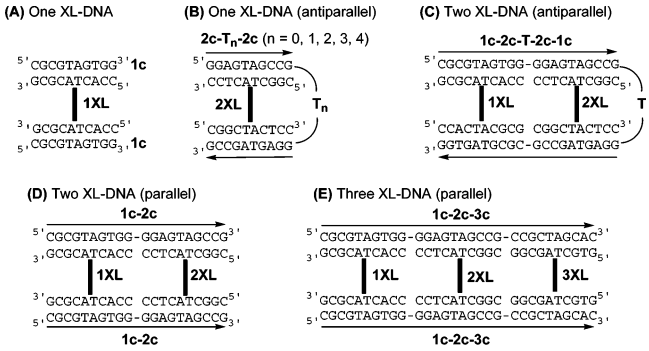


Figure 1. Disulfide cross-linked oligonucleotides employed in this experiment. DNA strands are assembled side by side by multiple cross-linked oligonucleotides by one-step molecular assembly.

Scheme 1. DNA Assemblies Prepared by Cross-Linked Oligonucleotides[a]



[a] The 5′ to 3′ directions of complementary strands are indicated by arrows. Bold bars represent disulfide crosslinkers. **XL** denotes a cross-linked oligonucleotide. Strands **1c**, **2c**, and **3c** are complementary strands to **1XL**, **2XL**, and **3XL**, respectively

Table 1. Melting Temperatures (T_m/°C) of the Cross-Linked Oligonucleotides **1XL**, **2XL**, and **3XL** with Their 10 mer Complementary Strand (**1c**, **2c**, and **3c**) in 0.1 M (left) and 1.0 M NaCl (right) Solutions (Scheme 1A)[a]

XL-DNA	T_m	ΔT_m	T_m	ΔT_m
1AXL	24.6	−19.2	40.5	−11.0
1BXL	28.2	−15.6	43.5	−8.0
1 (native)	43.8	−	51.5	−
2AXL	27.6	−15.5	41.8	−8.5
2BXL	31.6	−11.5	45.9	−4.1
2 (native)	43.1	−	50.3	−
3AXL	30.6	−13.9	41.2	−10.6
3BXL	31.7	−12.8	45.2	−6.6
3 (native)	44.5	−	51.8	−

[a] Sequences of the oligonucleotides **1**, **2**, and **3** are shown in Scheme 1. **A** and **B** correspond to the diastereomers. Conditions; 0.5 μM XL-DNA, 1 μM complementary DNA, 10 mM Tris-HCl (pH 7.6), 0.1 or 1.0 M NaCl.

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Table 2. Melting Temperatures (T_m/°C) of **2XL** with Their Complementary Strands (**2c-T$_n$-2c**; n = 0, 1, 2, 3, 4) (Scheme 1B)[a]

complementary strand	2AXL	2BXL	**2** (native)
2c-2c	45.7	49.5	41.3
2c-T1-2c	46.8	50.0	42.0
2c-T2-2c	45.3	48.8	41.3
2c-T3-2c	44.6	48.4	41.3
2c-T4-2c	43.0	47.6	41.0

[a] Conditions; 0.5 μM DNA, 10 mM Tris-HCl (pH 7.6), 0.1 M NaCl.

Table 3. Stabilities of the Antiparallel DNA Assemblies; Melting Temperatures (T_m/°C) of Two Cross-Linked Oligonucleotides with a 41 mer Complementary Strand **1c-2c-T-2c-1c** in 0.1 M (left) and 1.0 M NaCl (right) Solutions (Scheme 1C)[a]

XL-DNA	T_m	ΔT_m	T_m	ΔT_m
1AXL + **2AXL**	47.8	+3.9	60.5	+6.9
1BXL + **2BXL**	52.5	+8.6	64.5	+10.9
1 + **2** (native)	43.9	−	53.6	−

[a] Conditions; 0.5 μM DNA, 10 mM Tris-HCl (pH 7.6), 0.1 or 1.0 M NaCl.

stable than those with the A-diastereomer. These results indicate that the B-diastereomers are advantageous for connecting two double helixes with less steric hindrance than the A-counterparts. Because the cross-linker in the R_p-configuration orients outside of the double helical axis, the B-diastereomers would be estimated to be the R_p-configuration.

To arrange the two duplexes side by side with two cross-linked oligonucleotides, we used a complementary 20 mer strand **2c-2c** for DNA assemblies. Unexpectedly, the stabilities of **2XL** with **2c-2c** significantly increased as compared to those of **2XL** with **2c**. This indicates that the complementary strand **2c-2c** forms a hairpin structure with one **2XL** in an antiparallel arrangement (Scheme 1B). To investigate the stabilities of the hairpin structures, multiple thymidines (T_n; n = 0, 1, 2, 3, and 4) were inserted between two **2c** sequences (**2c-T$_n$-2c**) as spacers (see Table 2). In the case of T_1, the assemblies with **2XL** formed the most stable structures. The disulfide cross-linker and the T_1 spacer have eight and five atoms between two phosphorus atoms, respectively, and the 3′ and 5′ terminals of the 10 mer complementary strand are placed in the same direction relative to the cross-linker (see Figure 1). This means that the T_1 spacer can function as a hairpin component and contributes to the stabilization of the DNA assemblies. In addition, the XL-DNA with a B-diastereomer formed more stable assemblies than those with A-diastereomers the same as the assemblies of **2XL** with **2c**.

To expand the structure in the antiparallel way, we employed two different cross-linked oligonucleotides, **1XL**, **2XL**, and one complementary strand **1c-2c-T-2c-1c** (Scheme 1C). The stabilities of the assemblies increased as compared to those of **2XL** with **2c-T-2c**, and the stereochemistry of the A- and B-diastereomers also affected the stabilities of the DNA assemblies (Table 3).

We also prepared DNA assemblies in parallel orientation by employing two and three different cross-linked oligonucleotides and one complementary strand as shown in Scheme 1D and 1E, respectively. By increasing the number of the cross-linked oligonucleotides, these DNA assemblies were significantly stabilized (Table 4). Because entropic costs for the formation of the DNA assemblies are reduced by cooperative association of the second and third cross-linked oligonucleotides after complexation with the first cross-linked DNA, the assemblies with the multiple cross-linkers can form more stable complexes. Diastereochemical effects were also observed in these DNA assemblies. The assemblies with

Table 4. Stabilities of the Parallel DNA Assemblies; Melting Temperatures (T_m/°C) of Two and Three Cross-Linked Oligonucleotides with 20 mer and 30 mer Complementary Strand, Respectively, (Scheme 1D and 1E) in 0.1 M (left) and 1.0 M NaCl (right) Solutions[a]

XL-DNA	T_m	ΔT_m	T_m	ΔT_m
1AXL+**2AXL**	39.8	−2.1	50.7	+0.7
1BXL+**2BXL**	45.4	+3.5	56.3	+6.3
1+**2** (native)	41.9	−	50.0	−
2AXL+**3AXL**	44.2	−0.1	52.4	−0.6
2BXL+**3BXL**	46.8	+2.5	56.4	+3.4
2+**3** (native)	44.3	−	53.0	−
1AXL+**2AXL**+**3AXL**	54.0	+9.6	60.8	+8.1
1BXL+**2BXL**+**3BXL**	53.1	+8.7	64.2	+11.5
1+**2**+**3** (native)	44.4	−	52.7	−

[a] Conditions; 0.5 μM XL-DNA, 1 μM complementary DNA, 10 mM Tris-HCl (pH 7.6), 0.1 or 1.0 M NaCl.

the B-diastereomers showed better thermal stabilities than those with the A-diastereomers the same as in the case of the antiparallel conformations. Circular dichroism spectra of the DNA assemblies with three cross-linked oligonucleotides showed typical B-form DNA structures,[7] indicating that significant stabilization of the DNA assemblies originates from the corporative association of the three cross-linkers, not from the structural changes in the double helixes.

We have demonstrated the novel strategy for connecting and assembling DNA strands into well-defined rigid structures and controlling the relative orientation between two double helix strands using the cross-linked oligonucleotides. These versatile DNA connectors can assemble multiple DNA strands by one-step complexation, and potentially organize them into a multidimensional structure by adjusting the direction of the connection. Employing these cross-linked oligonucleotides, we are currently investigating periodically controlled rod- and sheet-type nanoarrays for integration of various functional molecules.

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Supporting Information Available: Synthesis and characterization of the disulfide cross-linked oligonucleotides (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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