Stabilization of DNA Multiassembly by Addition of a Phosphate Group at the 5′-Sticky End

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The effect of 5′-phosphorylation at nicks on DNA stabilization was measured through the formation of DNA multiassemblies consisting of 40-mer half-sliding complementary oligonucleotides, which had overlapping complementary 20-mer base sequences. 5′-Phosphorylated oligonucleotides formed longer DNA multiassemblies than 5′-hydroxylated ones. This result indicated that the 5′-phosphorylated sticky ends contribute to the stabilization of the DNA association due to the hydrogen bonds between 3′-hydroxy groups and 5′-phosphate groups at the nick positions.

DNA nanotechnology has attracted a great deal of attention as DNA has a well-defined geometry and highly predictable, diverse, and programmable hybridization.[1,2] Through these DNA properties, various DNA multiassemblies; for example, nanoscaled tiles, cubes, and machines, have been fabricated.[3–5] In addition, the arrangement of functional materials based on DNA multiassembly via DNA hybridization has been accomplished.[6,7] Since most DNA multiassemblies are prepared through association with sticky-ended DNA, DNA assemblies are known to contain a large number of single-stranded breaks (nicks). The stability of these nicks influence the total stability of the DNA multiassemblies. The effect of 5′-phosphorylation on the stability of the nicks has been investigated from the view point of DNA repair process.[8] XRD results have shown that the 5′-hydroxy group stabilize nicks by forming of a hydrogen bond with the 3′-hydroxy group of neighboring DNA.[9] In the case of 5′-phosphorylated DNA, two opposite results have been reported. MD simulation has shown that the presence of 3′-hydroxy and 5′-phosphate groups at the nick attract each other due to the formation of a hydrogen bond.[10] On the other hand, gel electrophoresis, UV melting measurement, and NMR have shown that the presence of a phosphate group at a nick brought about end-fraying because of electrostatic repulsion between the terminal phosphate and backbone phosphate.[11–13] Whether 5′-phosphorylation stabilizes the nick structure or not remains unclear. In order to prepare stable DNA multiassemblies, not only complementary base-pairing of the sticky-end but also the stabilization of nicks is required. We found 5′-phosphorylation of sticky-ended DNA induces formation of a stable long DNA multi-assembly.

Herein, we investigated the influence of 5′-phosphorylation on the formation of a one-dimensional DNA multiassembly consisting of four kinds of 40-mer half-sliding complementary oligonucleotides, A, B, pA, and pB, which had overlapping complementary 20-mer base sequences (Figure 1: 5′-phosphorylation is abbreviated to "p." The base sequences are 5′-TTATGTTGTCTTGCAATACAAATGTTCTTCGAGTACTT-AT-3′ (A or pA) and 5′-TGTATTGCAAGACAACATAAA-TAAGTACTCGAAGAACATT-3′ (B or pB)). This DNA multi-

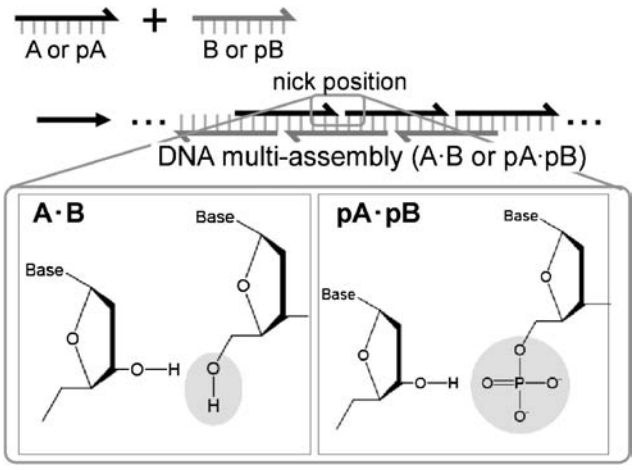

Figure 1. Schematic illustration of DNA multiassembly formation through the sticky-ended association of oligonucleotides, A and B, and 5′-phosphorylated pA and pB, with complementary 20-mer base sequences. As each bound pair of oligonucleotides contains two phosphate groups, pA·pB has a larger negative charge than a bound pair of A·B.

assembly can be regarded as an example of pseudo-addition polymerization. The reaction of the active end of the oligonucleotides monomer in the pseudo-addition polymerization results in the hybridization of the sticky-ends in the formation of the DNA multiassemblies. Since the reactivity of the active end in the addition polymerization reflects the molecular weight of the produced polymers, the effect of 5′-phosphorylation on the stability of the nick can be estimated from the molecular weight of the prepared DNA multiassemblies.

The DNA multiassemblies prepared from A·B and pA·pB were analyzed by 2.5% agarose gel electrophoresis at 20 °C (Figure 2). The same conformation of these DNA multiassemblies was confirmed by CD spectrometry (data was not shown). The equilibrium state of these DNA multiassemblies was confirmed by comparison with DNA multiassemblies incubated for a month at 20 °C. The molecular weight of A·B and pA·pB multiassemblies (Figure 2a, Lanes 1 and 2, respectively) ranged from 40 to 800 bp and 40 to 1400 bp, respectively. The thermodynamic parameters for the multiassembly of A·B and pA·pB were determined by the molecular weight distribution of the DNA multiassemblies obtained from the fluorescence intensities of the electrophoretic gel (Figure 2b). Since the dissociation of 20 bp duplexes was not observed during gel electrophoresis, the dissociation of the DNA multiassemblies could be negligible. The parameters were calculated as described in Figure S1[16] and summarized in Table 1. The free energy, ΔG, from the formation of the DNA multiassemblies was concluded to be -40.4 kJ/mol for A·B and -41.3 kJ/mol for pA·pB. These

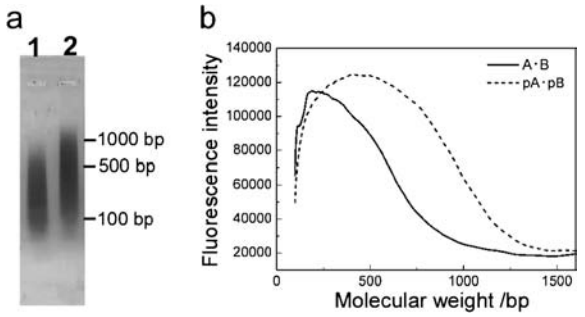

Figure 2. (a) 2.5% agarose gel electrophoresis. Lanes 1 and 2 reparesent A·B and pA·pB multiassemblies, respectively. (b) Molecular distribution of (a). Solid and dash lines represent the A·B and pA·pB multiassemblies, respectively.

Table 1. Kinetic parameters for DNA multiassembly formation in agarose gel

	A·B	pA·pB
M_n (bp)	250 ± 6	305 ± 8
$\overline{X_n}$	6.25 ± 0.15	7.64 ± 0.20
ΔG (kJ/mol)	-40.4 ± 0.1	-41.3 ± 0.1
$\Delta\Delta G_{(pA\cdot pB-A\cdot B)}$	-0.9 ± 0.2	

ΔG values are similar to that for 20 bp duplexes shown in a previous report.[14] The calculated $\Delta\Delta G$, -0.90 kJ/mol, indicates that pA·pB provided more stable multiassemblies than did A·B.

The melting temperature (T_m) of the A·B and pA·pB multiassemblies was measured in order to estimate their stability (Figure S1).[16] The T_m values for the A·B and pA·pB DNA multiassemblies were 38.2 and 37.3 °C, respectively, corresponding closely to the T_m value of the 20-bp duplex composed of the same base sequences (38.5 °C, melting curve is not shown). These results indicate that the pA·pB DNA multiassembly is less stable than the A·B multiassembly. It is known that 5′-phosphorylation decreases T_m value due to increased electrostatic repulsion.[12] Thus, 5′-phosphorylated nicks are thought to be responsible for attributed to the increased strand fraying since the additional negative charge of the phosphate group increases the interstrand electrostatic repulsion at the nicks. This appears to contradict the results obtained from electrophoresis that the 5′-phosphorylation stabilized multiassemblies as shown in Figure 2. The electrostatic repulsion along the phosphate backbone is shielded by hydration at 20 °C.[15] Therefore, the electrostatic repulsion of the additional phosphate at the 5′-terminal of the pA·pB multiassembly was ignored in Figure 2a. By increasing the temperature, dehydration was occurred, resulting the T_m value was decreased owing to increasing of the electrostatic repulsion of the additional phosphate.

The results of polyacrylamide gel electrophoresis in the presence of 2.5 M urea, which is a denaturing agent known to inhibit the formation of hydrogen bonds, are shown in Figure S2a.[16] The maximum molecular weight of the A·B multiassemblies was decreased from 800 to 500 bp, whereas that of the pA·pB multiassemblies was markedly decreased from 1400 to 500 bp, resulting in the A·B and pA·pB multiassemblies having the same molecular weight. From the estimated thermodynamic parameters, the free energy, ΔG, is almost identical for pA·pB and A·B (fluorescence intensities of the denaturing electrophoretic gel are shown in Figure S2b),[16] indicating that 5′-phosphate groups do not enhance the association of the sticky-ended DNA under conditions in which hydrogen-bond formation is inhibited. As described above, the nicked DNA duplex forms a hydrogen bond between the 3′-hydroxy and 5′-phosphate groups at the nick. As the existence of urea inhibited bond formation at the nick, the molecular weight of the DNA multiassemblies in the presence of urea were smaller than that in the absence of urea, indicating that the hydrogen bond at the nick enhances sticky-ended DNA association and 5′-phosphorylation promotes DNA association as the hydrogen bond between the phosphate group and the hydroxy group is more stable than that between hydroxy groups.

In summary, 5′-phosphorylated sticky-ended DNA provides longer DNA multiassemblies than does 5′-hydroxylated DNA. The thermodynamic parameters, measured from agarose gel electrophoretic analysis, indicated that 5′-phosphorylated DNA multiassemblies were more stable than 5′-hydroxylated ones. Our results show that the hydrogen bond at the nick enhances sticky-ended DNA association and the formation of a more stable hydrogen bond between 3′-hydroxy and 5′-phosphate groups than that between the 3′-hydroxy and 5′-hydroxy groups. This phenomenon suggests that the terminal functional groups of the sticky end in DNA associations affect the stability of DNA motif fabrication.

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16 Supporting Information is available electronically on the CSJ-Journal Web site, http://www.csj.jp/journals/chem-lett/index.html.

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