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Formation of Polymeric Assembly by Anti-directed Oligo-DNA Dimers

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We synthesized two types of "anti-directed" oligo-DNA dimer conjugates consisting of a photo-functional core group, in this case naphthalene as a model, and two strands of sequential oligo-DNAs (pentamer, GCTGC or GCAGC) linked at the 1,5-positions of naphthalene and at the 5′ ends of the oligo-DNAs through a phosphorodiester bond, forming $Np(GCTGC)_2$ and $Np(GCAGC)_2$. These conjugates are designed to be complementary to each other and are capable of forming a one-dimensional polymeric supramolecular assembly in aqueous solution. Duplex formation of the oligo-DNA strands in a solution of an equimolar mixture of the conjugates was confirmed by measurement of the hypochromicity, ethidium bromide (EB) intercalation tests, and circular dichroism (CD) spectra. From the CD spectra measurements of the solution, it was suggested that the oligo-DNA strands formed a B-type duplex-like conformation in aqueous solution. The equimolar mixture of the conjugates showed a temperature-dependent CD spectra change corresponding to the melting temperature of the B-type duplex. The formation of the polymeric assembly from the equimolar mixture of the conjugates was suggested by size-exclusion chromatography. The obtained results are useful for the construction of nanometre-scale structures using DNAs as building blocks.

Keywords: DNA; Polymeric assembly; Complementary hydrogen bond; Nanotechnology; Bottom up fabrication

INTRODUCTION

The assembly of molecules at the nanometre scale is an important objective of the "bottom up" approach in nanometre scale science and technology. DNAs, which can form multiple hydrogen bonds with their complementary base pairs, are promising as useful building blocks to construct nanometre scale structures for "bottom up" fabrication of organic and inorganic molecules in nanotechnology. DNA assemblies on various scales have been reported. Seeman *et al.* reported a topological DNA assembly system that formed various shapes [1–4]. Matsuura *et al.* also reported a closed assembly system using DNAs [5]. Larger scale DNA assemblies and networks have been reported by several groups [6–14]. Because DNAs can recognize their complementary counter strands, a wide variety of non-covalent binding pairs (binding donors and binding acceptors) can easily be provided with high specificity and stability just by varying the sequences of oligo-DNAs. In fact, arrangements of functional groups by using DNA as "molecular glue" have been reported [15–23].

In natural photosynthetic systems, arrangements of porphyrin derivatives with regulated distances and geometries provide highly efficient photo-induced energy transfer in non-covalent assembly systems in aqueous media [24,25]. Two- or three-dimensional architecture (including dendrimer) containing a number of chromophores or photo-functional dyes in a spatially ordered way would therefore provide a good model for artificial photosynthetic systems, and have some structural analogy with the natural photon-harvesting unit.

Previously, we reported that two kinds of complementary oligo-DNA dimer conjugates, "anti-directed" strands of an adenine tetramer (A4) or a thymine tetramer (T4), attached to benzene (core molecule) at the 1,4-positions, $Bz(A4)_2$ and $Bz(T4)_2$, formed a linear high-molecular-weight polymeric assembly when mixed in equimolar amounts in aqueous media [6]. By using these types of

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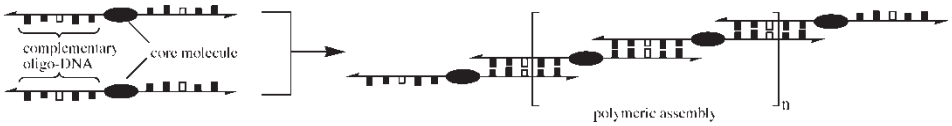


FIGURE 1 Schematic illustration of the formation of a polymeric assembly by complementary "anti-directed" oligo-DNA dimer conjugates.

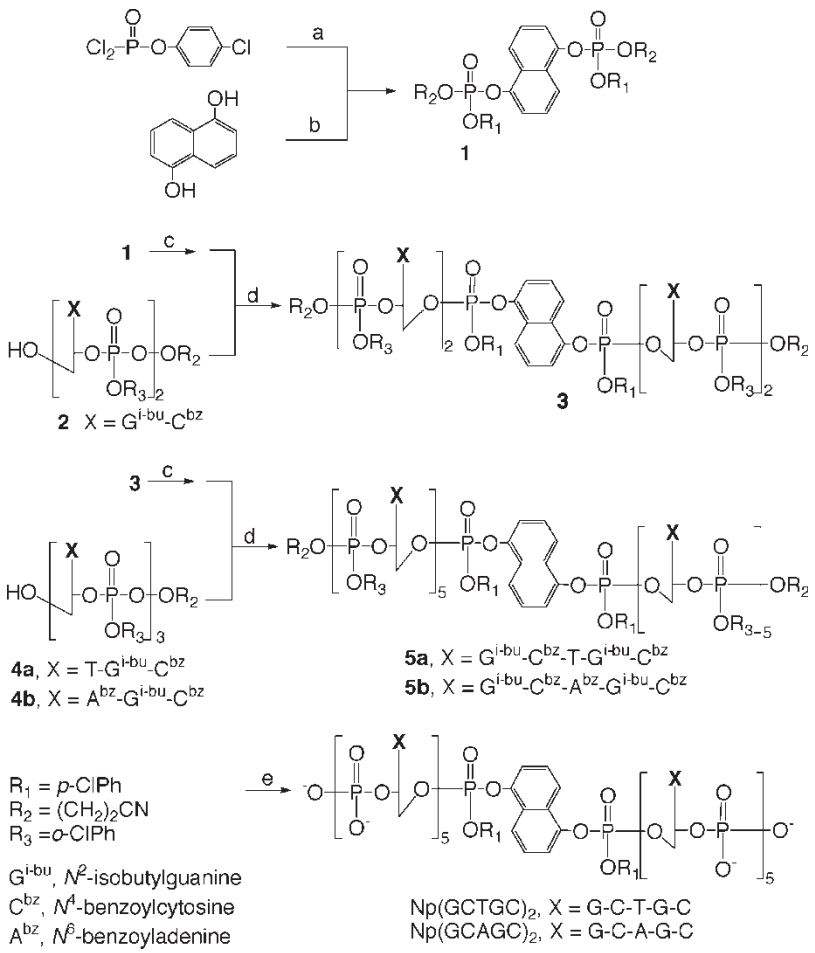
conjugates employing functional molecules as core groups, a sequential arrangement of the functional molecules to form a nanometre scale functional molecular array should be possible (Fig. 1). However, in the previous study the complementarity of A4 and T4 was not very stable, even at low temperatures. The melting temperature (T_m) of the assembly was below 15°C. Although a high-molecular-weight polymeric assembly was possible, size-exclusion chromatography (SEC) suggested that the majority of the conjugate, $Bz(A4)_2$ and $Bz(T4)_2$, in the equimolar mixture existed as monomers.

In this study, we designed a new pair of "anti-directed" oligo-DNA dimer conjugates having high stability and specificity. We employed naphthalene as a model for a photo-functional core group and attached two kinds of strands of sequential oligo-DNAs (pentamer, GCTGC or GCAGC) by linking the 1,5-positions of naphthalene and the 5′ ends of the oligo-DNAs through phosphorodiester bonds to form $Np(GCTGC)_2$ and $Np(GCAGC)_2$. The conjugates have 5 residues, one residue longer than our previous conjugates. A GC rich sequence was chosen to avoid frame-shift binding and to achieve stronger hydrogen bonding, as the hydrogen bonding of a G–C pair is stronger than that of an A–T pair. We investigated the formation of a high-molecular-weight polymeric assembly in an equimolar mixture of the conjugates by duplex formation of the oligo-DNA strands through complementary hydrogen bonding, as illustrated in Fig. 1. To apply "bottom-up" nanotechnology for real materials, scale up in size and dimension is one of the important objectives. This research may be one of the initial steps in the challenges to construct an ordered architecture in two- or three-dimensions (DNA network).

RESULTS AND DISCUSSION

The synthesis of the conjugates, $Np(GCTGC)_2$ and $Np(GCAGC)_2$, was successfully performed in the liquid phase by the usual phosphorotriester method according to Scheme 1. First of all, we investigated the formation of complementary hydrogen bonding between the two conjugates by measuring the hypochromicity and using an ethidium bromide (EB) intercalation test. Figure 2 shows the continuous variation curve of a mixture of $Np(GCTGC)_2$ and $Np(GCAGC)_2$ in 0.05 M Tris · HCl–0.2 M NaCl buffer (pH 7.5) at 10°C for the detection of hypochromicity in the UV absorbance of nucleic acid bases due to the stacking of bases in duplex formation. The largest hypochromic effect was observed when the mixing ratio was 1:1 (equimolar). These results show that $Np(GCAGC)_2$ and $Np(GCTGC)_2$ recognize each other in a 1:1 ratio. The melting temperature of the assembly estimated by UV–VIS absorbance was 23°C in 0.05 M Tris · HCl–0.2 M NaCl buffer (pH 7.5). Figure 3 shows the results of the EB intercalation tests in 0.05 M Tris · HCl–0.2 M NaCl buffer (pH 7.5) at 10°C. EB is well known as a molecular probe for duplex detection as its fluorescence intensity increases when it is intercalated between base pairs. The fluorescence intensity of EB strongly increased in the presence of the equimolar mixture of $Np(GCAGC)_2$ and $Np(GCTGC)_2$. No significant increase in fluorescence intensity was observed when 8 M urea (final concentration) was added to the solution as a hydrogen bond inhibitor. These results indicate that the equimolar mixture of the conjugates form a duplex-like assembly through complementary hydrogen bonding.

We investigated the structure of the assembly using circular dichroism (CD) spectra measurements. Figure 4(a) shows the CD spectra for the equimolar mixture of $Np(GCTGC)_2$ and $Np(GCAGC)_2$ and the calculated combined spectra of $Np(GCTGC)_2$ alone and $Np(GCAGC)_2$ alone in 0.05 M Tris · HCl–0.2 M NaCl buffer (pH 7.5) at 10°C. These two spectra are significantly different from each other. The results suggest that a specific secondary structure change was induced by mixing $Np(GCTGC)_2$ and $Np(GCAGC)_2$. The CD spectra of the equimolar mixture of $Np(GCTGC)_2$ and $Np(GCAGC)_2$ showed strong negative and positive Cotton effects around 245 and 280 nm, respectively. This pattern displays the typical characteristics of a B-form DNA duplex [26]. The DNA strands in the assembly system of $Np(GCTGC)_2$ and $Np(GCAGC)_2$ thus formed a B-form-like double helical conformation. Moreover, we investigated the temperature dependence of the CD spectra of the equimolar mixture of $Np(GCTGC)_2$ and $Np(GCAGC)_2$. The spectral changes associated with a change in temperature (from 5 to 80°C) are shown in Fig. 4(b). In Fig. 4(b), the CD spectra at high temperatures (above 50°C) showed a very similar pattern to the combined spectra shown in Fig. 4(a). These results



SCHEME 1 Synthetic route of anti-directed oligo-DNA dimer conjugates, $Np(GCAGC)_2$ and $Np(GCTGC)_2$. Reaction conditions: a, i) 1-methylimidazole, acetonitrile, ii) 2-cyanoethanol, acetonitrile; b, 1-methylimidazole, acetonitrile; c, triethylamine, acetonitrile; d, MSNT, pyridine; e, 28%-NH_3(aq), pyridine.

demonstrate that the conjugates are completely dissociated at high temperatures (over 50°C). The plot of the CD θ value at 284 nm vs. temperature (from 5 to 80°C) is shown in Fig. 5. From these results, the melting temperature of the assembly is estimated to be around 20°C. This value shows good agreement with the T_m value obtained UV–VIS absorbance (23°C).

To estimate the size of the polymeric assembly of the conjugates, we carried out a SEC (size-exclusion chromatography) analysis for the equimolar mixture of $Np(GCTGC)_2$ and $Np(GCAGC)_2$ at 5°C. The results

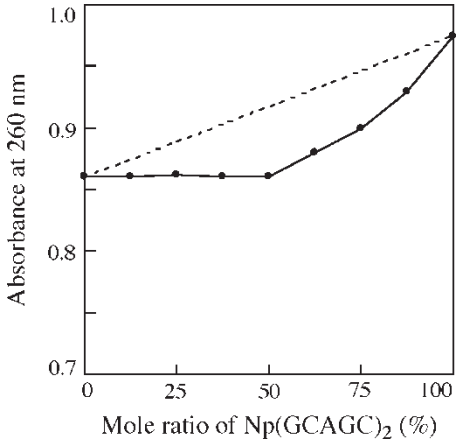


FIGURE 2 Continuous variation curve of $Np(GCTGC)_2$ and $Np(GCAGC)_2$ monitored by the absorbance at 260 nm in 0.05 M Tris·HCl–0.2 M NaCl buffer (pH 7.5) at 10°C. $[Np(GCTGC)_2 + Np(GCAGC)_2] = 1.018 \times 10^{-5}$ M.

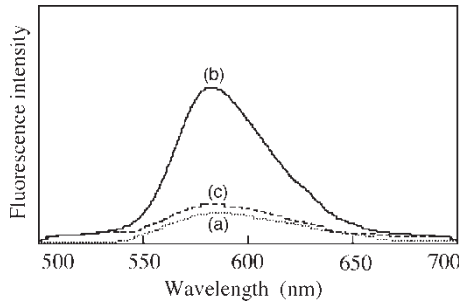


FIGURE 3 Fluorescence spectra for (a) ethidium bromide (EB), (b) EB in the presence of the equimolar mixture of $Np(GCTGC)_2$ and $Np(GCAGC)_2$, and (c) EB in the presence of 8 M urea and the equimolar mixture of $Np(GCTGC)_2$ and $Np(GCAGC)_2$ in 0.05 M Tris·HCl–0.2 M NaCl buffer (pH 7.5) at 10°C. $[Np(GCTGC)_2] = [Np(GCAGC)_2] = 3.441 \times 10^{-5}$ M, $[EB] = 1.7 \times 10^{-5}$ M. The excitation wavelength was 480 nm.

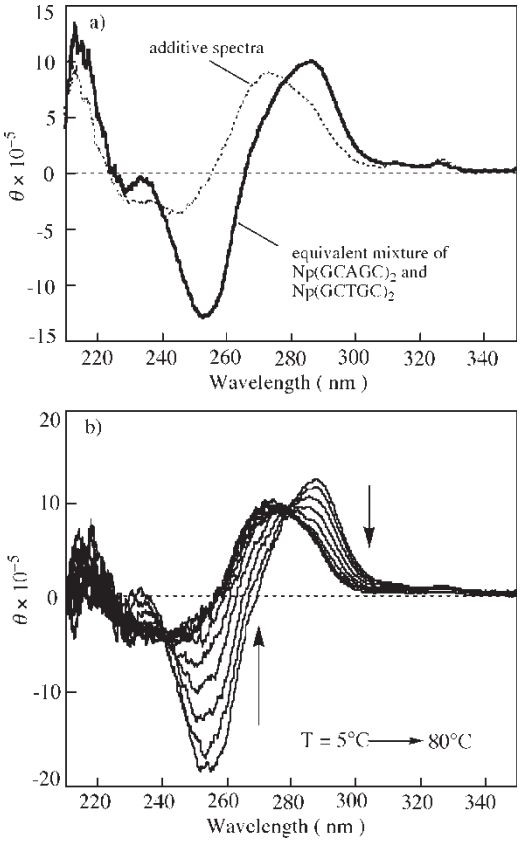


FIGURE 4 (a) Circular dichroism (CD) spectra for the equimolar mixture of $Np(GCTGC)_2$ and $Np(GCAGC)_2$ and the calculated combined spectra of $Np(GCTGC)_2$ alone and $Np(GCAGC)_2$ alone in 0.05 M Tris·HCl–0.2 M NaCl buffer (pH 7.5) at 10°C. (b) Temperature dependence of CD spectra for the equimolar mixture of $Np(GCTGC)_2$ and $Np(GCAGC)_2$ in 0.05 M Tris·HCl–0.2 M NaCl buffer (pH 7.5). T = 5, 10, 15, 20, 25, 30, 40, 50, 60, 70 and 80°C. $[Np(GCTGC)_2] = [Np(GCAGC)_2] = 1.408 \times 10^{-5}$ M.

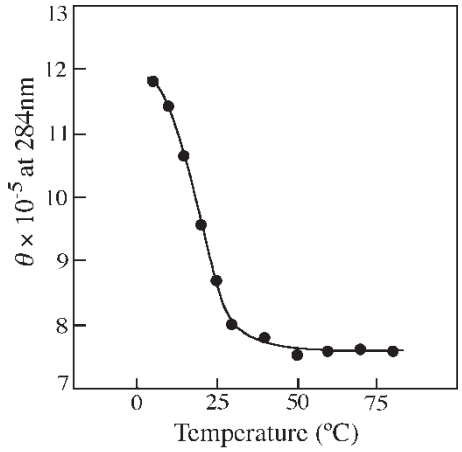


FIGURE 5 Plots of CD θ values at 284 nm vs. temperature for the equimolar mixture of $Np(GCTGC)_2$ and $Np(GCAGC)_2$ in 0.05 M Tris·HCl–0.2 M NaCl buffer (pH 7.5). $[Np(GCTGC)_2] = [Np(GCAGC)_2] = 1.408 \times 10^{-5}$ M.

are shown in Fig. 6. Although $Np(GCAGC)_2$ alone showed a sharp peak at around 20 min of elution time, the equimolar mixture of the conjugates showed a broader peak at a higher molecular weight. Moreover, the equimolar mixture of the conjugates had no fractions around 20 min of elution time. In the presence of 8 M urea (final concentration), a high-molecular-weight fraction was not observed and only a single sharp peak around 20 min was observed. These results mean that a polymeric assembly having some polydispersity was formed by complementary hydrogen bonding of the conjugates, and all of the conjugate molecules took part in the polymeric assembly. In comparison with molecular weight standard samples (pullulans), the highest molecular weight of the assembly (the peak start) was around 1.0×10^5, and the average molecular weight of the assembly (the peak maximum) was around $1.0–2.0 \times 10^4$. However, this molecular weight may not be the real molecular weight of the polymeric assembly formed in the solution of the equimolar mixture of the conjugates, because the SEC process takes place in a non-equilibrium state. Moreover the molecular weight values shown are relative values because pullulan, a non-ionic polymer, was used as standard. The molecular weight suggested by SEC may be smaller than the molecular weight of the polymeric assembly in an equilibrium state. We also carried out

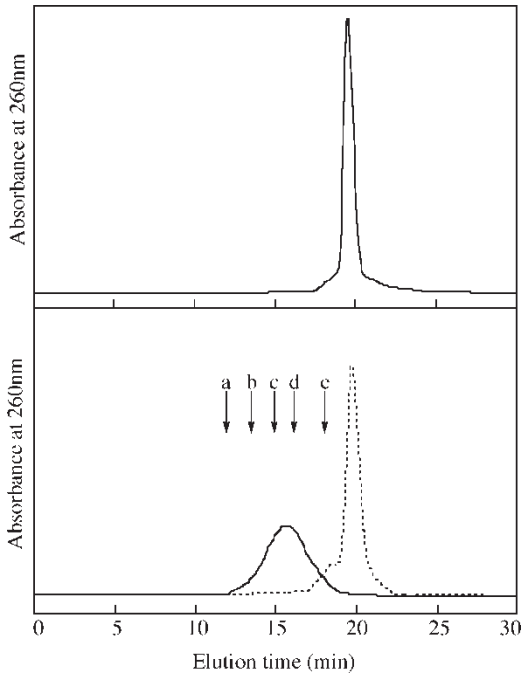


FIGURE 6 Elution profiles of size-exclusion chromatography for $Np(GCAGC)_2$ (upper) and the equimolar mixture of $Np(GCTGC)_2$ and $Np(GCAGC)_2$ (bottom) in the presence (dotted line) or absence (solid line) of 8 M (final concentration) urea at 5°C. Column: Shodex OHpak KB-802 HQ, eluent: 1/15 NaH_2PO_4–K_2PO_4 buffer, pH = 7.0. The solution containing 1.018×10^{-4} M of $Np(GCAGC)_2$ or the equimolar mixture of $Np(GCTGC)_2$ and $Np(GCAGC)_2$ (5.090×10^{-5} M each) was injected. Arrows indicate the eluent position for a standard sample of pullulan: MW = a, 10×10^4; b, 4.8×10^4; c, 2.37×10^4; d, 1.22×10^4; e, 0.58×10^4.

a light scattering analysis to estimate the size of the polymeric assembly, and obtained 8.55×10^4 as the apparent molecular weight of the assembly at room temperature (15.6°C). This apparent molecular weight was higher than the average molecular weight suggested by the SEC analysis. But it was not higher than the maximum molecular weight suggested by the SEC, because the light scattering measurement was carried out at room temperature (15.6°C). Considering the melting temperature of the assembly, this value seems to be quite reasonable. This molecular weight corresponds to about a 24mer (12 molecules of $Np(GCTGC)_2$ + 12 molecules of $Np(GCAGC)_2$). These results suggest a higher molecular weight (at least 8.55×10^4) polymeric assembly formed at lower temperatures. There are possibilities that the conjugates can form linear or circular polymeric assemblies. From the results of SEC and light scattering, it is difficult to imagine such a large-membered circular polymeric assembly; we concluded that the formation of a linear polymeric assembly is the most probable.

CONCLUSIONS

Anti-directed oligo-DNA dimer conjugates, $Np(GCTGC)_2$ and $Np(GCAGC)_2$, were prepared. It was suggested that an equimolar mixture of the obtained conjugates formed a linear polymeric assembly through complementary hydrogen bonding of the oligo-DNA strands into a B-form-like duplex formation. By using functional groups as the core molecules of such conjugates, a sequential polymeric assembly of the functional group should be possible. This kind of "functional group array" should be useful in constructing nanometre-scale structures in "bottom up" approaches of nanotechnology, or in constructing artificial photon-collecting antenna models in aqueous media.

MATERIALS AND METHODS

General Methods

The UV–VIS absorption spectra and fluorescence spectra were recorded on UV-2500PC (Shimadzu, Japan) and F4010 (Hitachi, Japan) spectrophotometers, respectively. CD spectra were measured using a J-600 spectrometer (JASCO, Japan). Matrix-assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF-MS) experiments were performed on a MAT Vision 2000 [negative mode, matrix: 3-hydroxypicolic acid (H_2O–acetonitrile = 7:3)] (Finnigan, USA) after samples were treated with a 0.1 M diammonium hydrogen citrate solution. Purification of the products was carried out by reverse-phase high-performance liquid chromatography (RP-HPLC) using a Toso-8020 system with a TSKgel ODS-120T column. Size-exclusion chromatography analysis was carried out using a Shodex OHpak KB-802 HQ column (eluent: 1/15 NaH_2PO_4–K_2HPO_4 buffer, pH = 7.0). Light scattering was carried out on a DLS-700 (Otsuka Electronics).

Materials

Dimethoxytritylthymidine triester, dimethoxytrityl-N^6-benzoyldeoxyadenosine triester, dimethoxytrityl-N^4-benzoyldeoxycytidine triester, dimethoxytrityl-N^2-isobutyldeoxyguanosine triester, 4-chlorophenylphosphorodichloridate and 1-(mesitylene-2-sulfonyl)-3-nitro-1,2,4-triazole (MSNT) were purchased from SIGMA. 1-Methylimidazole was purchased from Merck. Ethidium bromide (EB) and 1,5-dihydroxynaphthalene were obtained from Wako Pure Chemical Ind. Water was purified using a reverse-osmotic membrane. Organic solvents were purified by usual distillation methods. Other materials were of commercial grades and used without further purification.

Synthesis of Anti-directed Oligo-DNA Dimer Conjugates

Synthesis of the anti-directed oligo-DNA dimer conjugates, $Np(GCTGC)_2$ and $Np(GCAGC)_2$, was carried out in the liquid phase by a phosphorotriester method similar to that previously reported [6] according to Scheme 1. 1-Methylimidazole (0.74 ml, 9.36 mmol) was added dropwise to 4-chlorophenylphosphorodichloridate (1.22 ml, 7.49 mmol) dissolved in dry acetonitrile (3.4 ml) at −40°C under a nitrogen atmosphere. 2-Cyanoethanol (0.64 ml, 9.36 mmol) dissolved in dry acetonitrile (1.8 ml) was added to the solution at −40°C and stirred at −10°C for 1 h. 1,5-Dihydroxynaphthalene (300 mg, 1.81 mmol) was reacted with 1-methylimidazole (0.89 ml, 11.2 mmol) in dry acetonitrile (5.4 ml) at room temperature for 30 min. These two solutions were mixed slowly at −20°C, and then stirred at −10°C for 2 h. After quenching the reaction with cold water, the products were extracted with ethyl acetate and dried with anhydrous sodium sulfate. The obtained 1,5-bis(4′-chlorophenyl-2-cyanoethylphospholyl)naphthalene (**1**) was purified by silica-gel chromatography (chloroform–methanol = 10:1) to give a colorless solid. Yield 853 mg (1.32 mmol, 70.4%). ^{1}H NMR: δ 7.93 (d, H4 and H8 of naphthalene, $J = 8.0$ Hz, 2H), 7.56 (d, H2 and H6 of naphthalene, $J = 7.2$ Hz, 2H), 7.48 (d, H3 and H7 of naphthalene, $J = 8.4$ Hz, 2H), 7.31 (d, 3′H of chlorophenyl, $J = 8.4$ Hz, 4H), 7.20 (d, 2′H of chlorophenyl, $J = 9.2$ Hz, 4H), 4.43 (m, OCH_2CH_2, 4H), and

2.73 (t, CH_2CH_2CN, $J = 6.2$ Hz, 4H). ^{13}CNMR: δ 148.2 (chlorophenyl *ipso*), 147.0 (naphthalene *ipso*), 131.3–116.4 (aromatic ring), 116.2 (CN), 63.6 (OCH_2CH_2), 19.7 (CH_2CH_2CN).

5′-Free protected dinucleotide (**2**) and trinucleotides (**4a**) and (**4b**) were prepared from dimethoxytrityl-N^4-benzoyldeoxycytidine triester, dimethoxytrityl-N^2-isobutyldeoxyguanosine triester, dimethoxytritylthymidine triester and dimethoxytrityl-N^6-benzoyldeoxyadenosine triester by the usual phosphorotriester methods using MSNT as a coupling reagent. Triethylamine (4.0 ml) was added to **1** (57 mg, 80 μmol) dissolved in dry acetonitrile (4.0 ml) to remove the cyanoethyl groups. Then, **2** (260 mg, 240 μmol), MSNT (140 mg, 480 μmol) and pyridine (0.40 ml) were added to the solution and stirred at room temperature for 3 h. The obtained product, **3**, was purified by size-exclusion chromatography (SEC) (Sephadex LH-20, eluent dioxane) and silica-gel chromatography. Triethylamine (4.0 ml) was added to **3** (100 mg, 37.8 μmol) dissolved in dry acetonitrile (4.0 ml) to remove the cyanoethyl groups. Then, **4a** (175 mg, 116 μmol), MSNT (70 mg, 232 μmol) and pyridine (0.10 ml) were added to the solution and stirred at room temperature for 3 h. The obtained **5a** was purified by SEC (Sephadex LH-20, eluent dioxane) to give a colorless solid. **5b** was synthesized by the same method above using **4b**. The obtained, fully protected, anti-directed DNA dimer conjugates **5a** or **5b** (60 mg) dissolved in pyridine (7 ml) were added separately to 28% aqueous ammonia solution (56 ml) and incubated at 60°C for 5 h. The obtained products were dissolved in water and washed with ether. After freeze drying, the obtained anti-directed DNA dimer conjugates, $Np(GCTGC)_2$ and $Np(GCAGC)_2$, were purified by SEC (Sephadex LH-20, eluent dioxane) and RP-HPLC (TSKgel ODS-120T, eluent: acetonitrile–0.1 M ammonium acetate, 5/95 to 15/85 gradient) to give colorless solids. MALDI-TOF-MS (negative mode, $[M - H]^-$): for $Np(GCTGC)_2$ calcd 3619.4, found 3622.9; for $Np(GCAGC)_2$ calcd 3637.5, found 3640.8. UV-VIS spectra for $Np(GCTGC)_2$ $\varepsilon = 5.89 \times 10^4$ at 270.3 nm, 4.44×10^3 at 325.6 nm; for $Np(GCAGC)_2$ $\varepsilon = 6.59 \times 10^4$ at 259.2 nm, 4.44×10^3 at 325.6 nm ($dm^{-3}\,mol^{-1}\,cm^{-1}$).

Spectroscopic Measurements

The concentration of the conjugates was calculated from the UV absorbance at 260 nm (A260), and the molar absorption coefficients were determined using the nearest-neighbor approximation. The hypochromicity measurement was performed by monitoring the A260 of the solution (0.05 M Tris·HCl buffer, pH 7.5, 0.2 M NaCl) at 10°C containing the two conjugates in various ratios. The total concentration of the conjugates, $[Np(GCTGC)_2] + [Np(GCAGC)_2]$, was 1.018×10^{-5} M. Melting curves were recorded by starting at 60°C sufficiently above T_m and reducing temperature at a rate of 10°C per h to 10°C sufficiently below T_m. Absorbance values were continuously recorded at intervals of 5°C. The fluorescence spectra of EB in 0.05 M Tris·HCl–0.2 M NaCl buffer (pH 7.5) were measured in the presence and absence of an equimolar mixture of the conjugates on a Hitachi F4010, using a 1×1 cm quartz cell at 10°C. The concentration of EB was 1.7×10^{-5} M, and the concentration of each conjugate was 3.441×10^{-5} M. A control experiment was carried out using the same solution containing 8 M urea (final concentration) as a hydrogen bond inhibitor. The excitation wavelength used was 480 nm, and fluorescence intensities from 500 to 700 nm were monitored. The CD spectra of both conjugates and the equimolar mixture of the conjugates were measured in 0.05 M Tris·HCl–0.2 M NaCl buffer (pH 7.5) on a JASCO J-600 using a quartz cell with a 0.5 cm light-path length at 10°C. The concentration of each conjugate was adjusted to 1.408×10^{-5} M. The temperature dependence of the CD spectra was monitored from 5 to 80°C using the same solution as above.

Size-exclusion Chromatography

Size-exclusion chromatography analysis of the equimolar mixture of the conjugates was carried out using a Shodex OHpak KB-802 HQ column (eluent: 1/15 NaH_2PO_4–K_2HPO_4 buffer, pH = 7.0) at 5°C. The solution (100 μl) containing $Np(GCAGC)_2$ alone or the equimolar mixture of $Np(GCTGC)_2$ and $Np(GCAGC)_2$ ($[Np(GCTGC)_2] + [Np(GCAGC)_2] = 1.018 \times 10^{-4}$ M) was injected on to the column. A control experiment was carried out using the same solution containing 8 M urea (final concentration) as a hydrogen bond inhibitor. Pullulans were used as molecular weight standard samples.

Light Scattering

Static light scattering (SLS) measurements of the equimolar mixture of the conjugates were performed in 0.05 M Tris·HCl–0.2 M NaCl buffer (pH 7.5) at room temperature (15.6°C). The angular range was from 30° to 130°. The concentration of the equimolar mixture of the conjugates was varied in the range of 0.2–2.0 mg ml^{-1}. The apparent molecular weight of the polymeric assembly was estimated using a Zimm plot.

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