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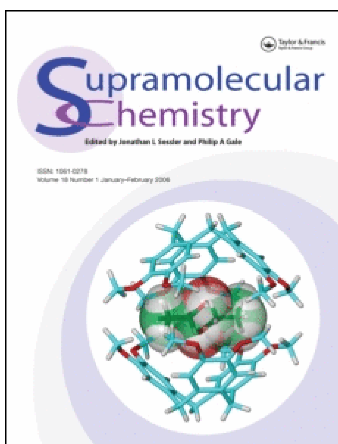

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DNA is one of the best candidates as building blocks for bottom-up approach to nanometre size architecture in nanotechnology. In natural photosynthetic system, the arrangement of porphyrin derivatives with regulated distances, orders and orientations provides an efficient photon-energy collecting and transmission. We have studied about chromophore arrangement on DNA assembly as a simple artificial model of photosynthetic and photo-energy transmission systems. In order to prepare DNAs containing porphyrin residues at desired sites, a hydrophilic porphyrin amidite derivative, which can be utilised on automatic DNA synthesizer, was synthesised. The porphyrin amidite derivative was applied for automatic DNA synthesizer to give single-strand DNA (ssDNA) containing porphyrin set in the chain. The obtained ssDNA was mixed with complementary counter strand having another chromophore black hole quencher-3 (BHQ-3) to form duplex by self-assembly. The quenching behaviour by energy transfer from the porphyrin to BHQ-3 was monitored by fluorescence measurement.

Keywords: DNA; porphyrin; fluorescence resonance energy transfer; self-assembly

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Introduction

The arrangement of functional molecules and groups with regulated distances, orders and orientations is one of the important goals in bottom-up nanotechnology inspired by natural systems. As a typical example, in natural photosynthetic systems, the arrangement of porphyrin derivatives with regulated distance and geometry through non-covalent interaction provides highly efficient photo-energy collection and transfer (*1–3*). Arrangement of chromophores (multi-chromophore array) would therefore provide a good model for an artificial photosynthetic system. Chromophore arrangements using non-covalent interactions have been studied as models for artificial photosynthetic systems (*4–8*).

A single chain of DNA is able to bind specifically with its complementary counterpart chain through sequence-specific hydrogen bonding. Based on this property, DNA is one of the promising candidates as a building block for nano-architecture by programmed self-organisation in bottom-up fabrication in nanotechnology. In fact, many DNA architectures in different scales and dimensions were reported (*9–19*). Focusing on this property of DNA, we have previously reported utilisation of DNA duplex as templates to construct sequential arrangements of chromophores, chromophore array, using oligo-DNA/chromophore conjugates, and their multistep fluorescence resonance energy transfer (FRET) behaviour of the chromophore array as a model of photo-energy transmission system in aqueous media (*20–23*).

In the next step, we intended to construct a porphyrin array on DNA assembly to mimic natural photosynthetic system. In general, to provide oligo-DNA/chromophore conjugates, the easiest and commonest way is to attach a chromophore at 5′- or 3′-end of oligo-DNA. In fact, we and other groups introduced chromophore moieties at the terminal of oligo-DNAs (*20–23*), and several oligo-DNA/porphyrin conjugates had also been synthesised by introduction of the porphyrin moieties at the termini of the oligo-DNAs (*24–27*). However, to design many variations of self-organised architectures having different shapes, scales, dimensions and enough stability using oligo-DNAs displaying various chromophore arrangements in desired order and distance, the chromophore residue should be introduced at desired sites of an oligo-DNA strand. Endo et al. reported the synthesis oligo-DNA/porphyrin conjugates; porphyrin attached to middle of four strands of oligo-DNAs, by coupling reaction between porphyrin maleimide derivative and cystamine groups on oligo-DNAs (*28*, *29*), and reported preparation of DNA tube with

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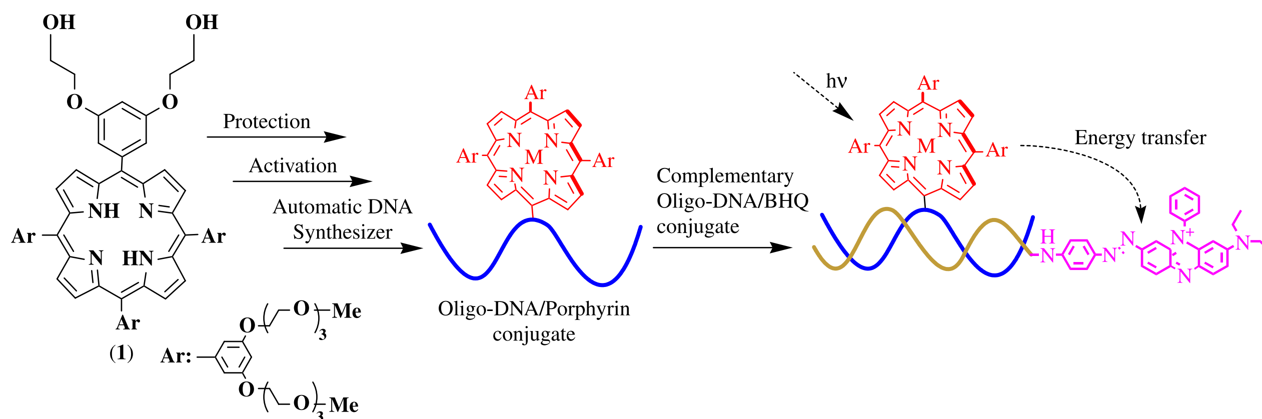

Figure 1. Schematic of this study: duplex formation of oligo-DNA/porphyrin derivative and the energy transfer.

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ordered porphyrin moiety (*30*). But, more convenient way may be to prepare porphyrin amidite derivative which is directly applicable for automatic DNA synthesizer. Some fluorescence chromophores applicable for automatic DNA synthesizer with dimethoxytrityl (DMTr) and amidite groups are developed, commercially available and used frequently to synthesise fluorescence labelled oligo-DNA at the middle of the strand. In this study, we designed porphyrin derivative (**1**) (Figure 1) applicable for a conventional DNA synthesizer. For the synthesis of oligo-DNAs possessing porphyrin moieties in their main chains, a porphyrin derivative having two hydroxy groups is necessary. One of the hydroxy groups should be protected with DMTr group and the other should be activated as amidite group. Such a porphyrin component should be soluble in acetonitrile which is used as a common solvent on automatic DNA synthesizer and also to be hydrophilic for suppression of insertion between the base pairs by hydrophobic interaction as well as to keep water-solubility of the DNA strand, and to avoid hydrophobic aggregation of the DNA strand in aqueous solution. Therefore, we designed dihydroxylated compound **1** (Figure 1) possessing six hydrophilic triethylene glycol monomethyl ether moieties on the phenyl groups at the *meso*-positions. Recently, Murashima et al. (*31*) have reported hydrophobic porphyrin amidite derivative and the synthesis oligo-DNAs with porphyrin moiety in the main chain. Their strategy has some similarity with this study. But, the most different point between these molecular designs is that they kept hydrophobicity of porphyrin to accelerate the intercalation of the porphyrin moiety between base pairs, although we introduced hydrophilicity into the porphyrin derivative because of the reasons described above. We started this study and made brief reports (*32*–*34*) before their report (*35*). These works have been carried out independently.

In this study, we report that establishment of the method introduces a hydrophilic porphyrin moiety into DNA main chain and FRET study of the porphyrin and black hole quencher-3 (BHQ-3) as a basic study of the function of DNA incorporated DNAs (Figure 1).

Results and discussion

Synthesis of oligo-DNA/porphyrin conjugate

For the synthesis of DNA conjugates possessing porphyrin moieties on the main chain, a dihydroxylated porphyrin is necessary. One of the hydroxy groups should be protected with DMTr group and the other should be activated as amidite group. Such a porphyrin component has to be soluble in acetonitrile, which is used as a solvent in a DNA synthesizer and also to be hydrophilic for highly interaction with an aqueous phase in the formation of DNA double strand as well as for suppression of insertion between the base pairs by hydrophobic interaction. Therefore, we designed dihydroxylated compound **1** (Scheme 1) possessing six hydrophilic triethylene glycol monomethyl ether moieties on the phenyl groups at the *meso*-positions. The bulky 3,5-substituents of the three *meso*-phenyl groups can reduce the intermolecular interaction among porphyrin π-systems and would increase the solubility of usual organic solvents including acetonitrile. The dihydroxy groups on the rest phenyl group have to be reactive for protection and dehydrated linkage. We chose a symmetrical 3,5-bis(dihydroxyethoxy)phenyl group as the requisite substituent. We examined two procedures for synthesis of a porphyrin possessing eight triethylene glycol monomethyl ether moieties, 5,10,15,20-tetrakis[3,5-bis(1,4,7,10-tetraoxaundecyl)phenyl]porphyrin (**2**, Scheme 1) and found that the Lindsey's procedure (*36*) was more effective for its preparation than Adler's (*37*). Then, we tried to synthesise desired **1** according to Lindsey's method as follows.

First, condensation of 3,5-bis(1,4,7,10-tetraoxaundecyl)benzaldehyde (**3**, Ar-CHO) and 3,5-dihydroxybenzaldehyde with pyrrole could not give the corresponding dihydroxylated porphyrin, mainly due to less solubility of the phenolic aldehyde in chloroform as the reaction solvent.

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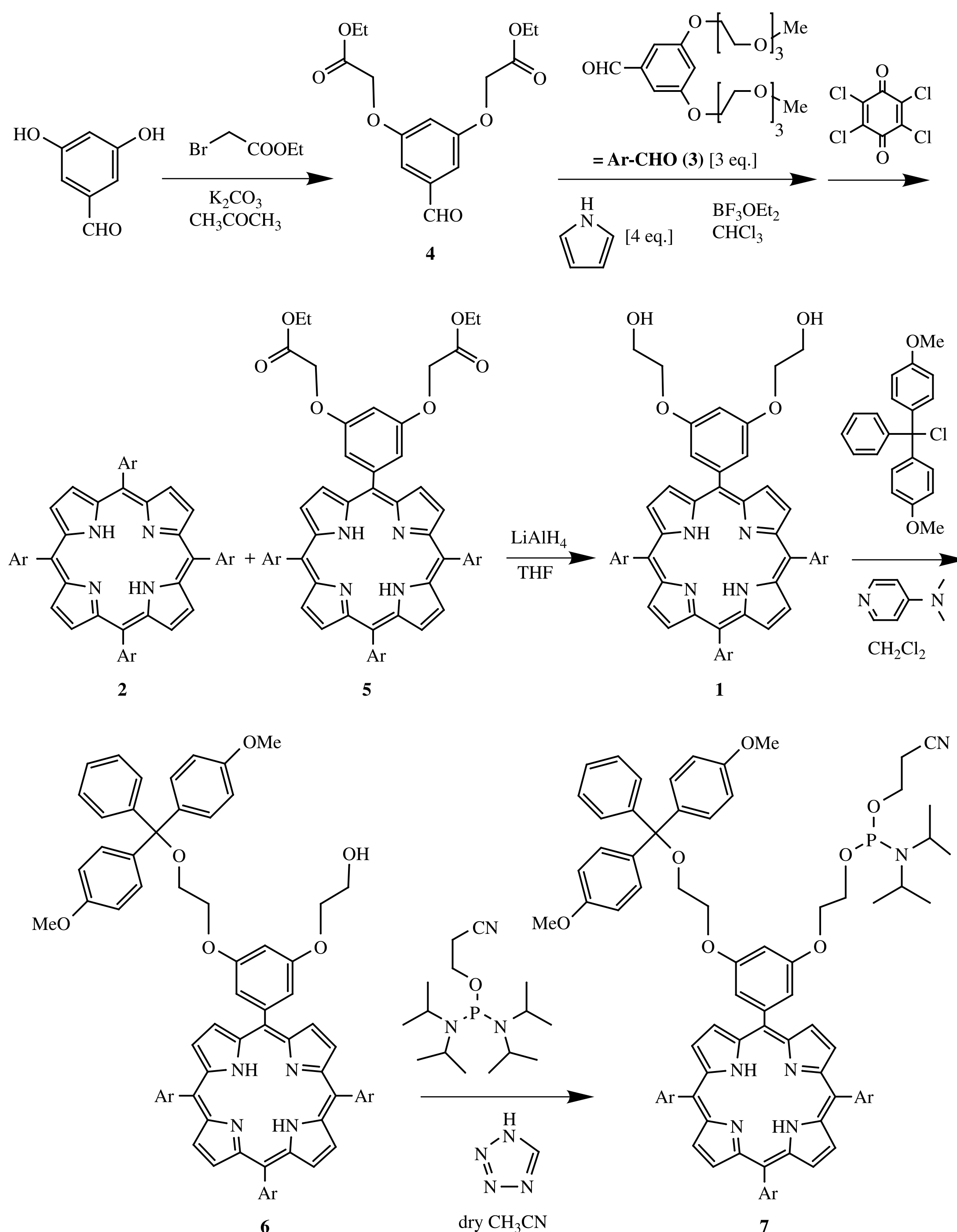

Scheme 1. Synthetic scheme of porphyrin derivative **1**, its protected **6** and activated derivative **7**.

Second, 3,5-dihydroxybenzaldehyde was reacted with bromoethanol in the presence of potassium carbonate to give so complex products that 3,5-bis(hydroxyethoxy)benzaldehyde could not be isolated. Finally, we prepared successfully 3,5-bis(ethoxycarbonylmethoxy)benzaldehyde **4** by conventional Williamson ether synthesis of 3,5-dihydroxybenzaldehyde with ethyl bromoacetate (90% yield, see Scheme 1). Lindsey's cyclisation of Ar-CHO and **4** with pyrrole gave a 4:5 mixture of **2** and **5** as main porphyrin products. Compound **5** was isolated in 5% yield by silica gel chromatography. Diester **5** was reduced by lithium aluminium hydride to give desired **1** (72% yield).

One of the hydroxy groups in diol **1** was protected by DMTr group (89%). The mono-protected compound could be easily separated from doubly protected and unreacted compounds by flash column chromatography (FCC).

Chart 1. The sequences of oligo-DNA/porphyrin and oligo-DNA/BHQ conjugates.

The resulting alcohol **6** was activated to the corresponding amidite **7** under dry conditions, which was used for preparation of phosphoester in the solid synthesis by a DNA synthesizer. The sequence of the conjugate was shown in Chart 1. The obtained conjugate was characterised by MALDI-TOF-MS spectrum (see Supporting Information, available online). UV–vis spectrum of the obtained oligo-DNA/porphyrin conjugate was shown in Figure 2. In the MALDI-TOF-MS spectra, besides main peak of free base porphyrin conjugate at 7851.40, another peak at 7913.55 was observed. Mass of this minor peak is almost equal to the molecular mass −2H + zinc(II) (+63.4). So, the results suggest that zinc(II) ion was partially incorporated into the obtained conjugate during the synthetic procedures. In the Q-band regions of UV–vis spectra of the conjugate just after purification (Figure 2), the absorbance at 550 nm was higher than that at 510 nm. This result also suggests the existence of zinc(II) porphyrin conjugate in addition to the expected free base conjugate. UV–vis spectra of compound **1** showed typical spectrum for free base tetraaryl porphyrin derivative. Incorporation of zinc(II) ion into porphyrin during automatic DNA synthesis process was also reported by the other group (*27*). This is probably because nucleic acid bases near the porphyrin moiety might act as intermolecular catalyst for incorporation of zinc(II) in water existing in its very small amount. The excitation and emission spectra of the conjugate long enough time after purification showed almost pure zinc(II) porphyrin (see Figure 4). Such behaviour was not observed for compound **1** without oligo-DNA. These results also suggest the intramolecular catalytic mechanism of the nucleic acid bases of the conjugate on incorporation of zinc(II). To avoid structural ambiguity, completely zinc(II)-incorporated oligo-DNA/porphyrin conjugate was used for further experiments.

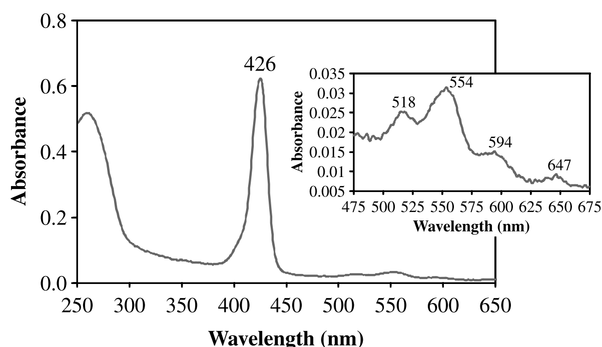

Figure 2. UV–vis spectrum of the oligo-DNA/porphyrin conjugate just after purification.

2.2 Duplex formation of oligo-DNA/porphyrin conjugate

To investigate duplex formation and the stability of the duplex state of oligo-DNA/porphyrin conjugate, melting curve of the duplex of the conjugate with complementary oligo-DNA was observed (see Supporting Information). The native double-strand DNA (dsDNA) having the same length and sequence as the conjugate without porphyrin showed melting point (T_m) at 63°C. On the other hand, the conjugate showed T_m at 46°C, which was 17°C lower than that of native dsDNA. These results mean the introduction of hydrophilic porphyrin residue in the DNA strand reduces the stability of duplex. We choose thymine (T) as the counter base of porphyrin residue in the complementary DNA strand. We observed from an experience that the fluorescence emission can be quenched by the existence of guanine residue located around fluorescence dye molecules attached to DNAs (*38*). To avoid such unexpected reductive quenching of porphyrin by guanine, we chose T as a counter base. Moreover, the porphyrin derivative we synthesised has six hydrophilic triethylene glycol units and is expected to be exposed to aqueous phase, and not intercalating. So, the complementary strand was interrupted at the site of porphyrin-T pair. So, the reduction of T_m is very reasonable. However, the T_m of the duplex of the conjugate is still stable enough at room temperature. To make it double sure for the stable duplex formation, following spectroscopic measurements were basically carried out at 5°C.

To investigate the structure of dsDNA containing the porphyrin residue in the middle of strand, circular dichroism (CD) spectra measurements of the duplex state of the conjugate were carried out, and the results were compared with native dsDNA having the same length and sequence of the conjugate. The results of CD spectra were shown in Figure 3. In the conjugate duplex, negative Cotton effect was observed around 420 nm, which may be an induced CD of the porphyrin unit. Such CD peak may be possible for some achiral porphyrin derivative, too. So, the result is not the direct evidence for the oligo-DNA/porphyrin conjugate was successfully synthesised and induced chiral environment for the porphyrin residue,

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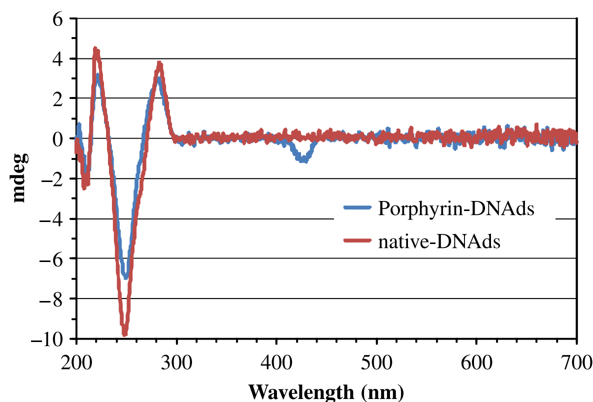

Figure 3. CD spectra of the ss and ds oligo-DNA/porphyrin conjugate in 50 mM Tris–HCl and 500 mM NaCl buffer (pH 7.5) at 5°C.

but are still one of the strong suggestions. The CD of 200–300 nm were from DNA residues. The negative and positive Cotton effects pattern of the conjugate duplex is quite similar to that of native dsDNA, and showed a typical pattern for B-type duplex, the commonest conformation of natural dsDNA. These results suggest the introduction of porphyrin unit in dsDNA did not have serious influence on the conformation of the duplex state of the conjugate.

To investigate the effect of hybridisation of DNA (duplex formation from single strand (ss) to double strand (ds)) on fluorescence spectra of porphyrin moiety in the conjugate, excitation and emission spectra of the conjugate were measured in ss and ds states in 50 mM Tris–HCl and 500 mM NaCl buffer (pH 7.5) at 5°C. The results were shown in Figure 4. The λ_{max} for excitation and emission

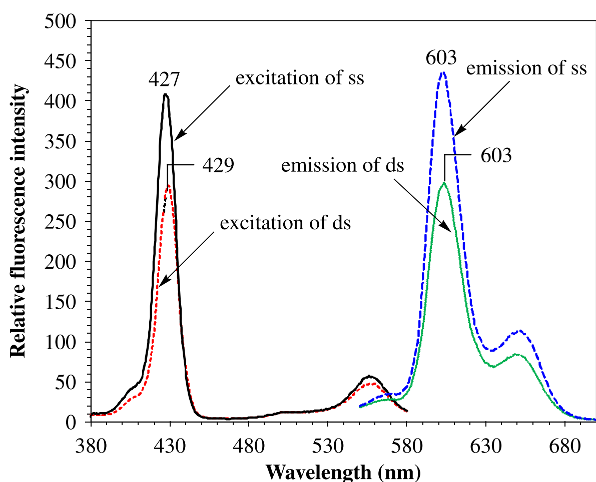

Figure 4. Excitation and emission spectra of the ss and ds oligo-DNA/porphyrin conjugate in 50 mM Tris–HCl and 500 mM NaCl buffer (pH 7.5) at 5°C. Excitation and emission wavelength were at 428 and 603 nm, respectively.

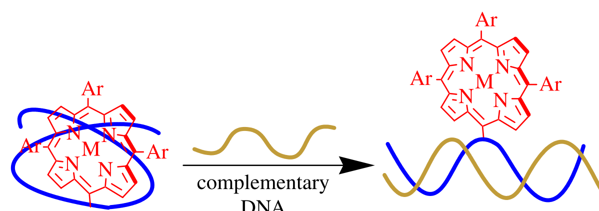

Figure 5. Schematic of duplex formation of oligo-DNA/porphyrin conjugate in aqueous solution.

spectra of the porphyrin moiety were 428 and 603 nm, respectively. The spectra showed typical for zinc(II) porphyrin derivatives. These results suggest that zinc(II) was completely incorporated into the porphyrin chromophore.

As shown in Figure 4, the fluorescence intensity of the porphyrin moiety in ds state was 32% decreased compared with ss state. These results mean the environmental difference of the porphyrin moiety between ss and ds state. In ss state, two ss oligo-DNAs were attached at both sides of the porphyrin. The single-strand DNA (ssDNA) is flexible, the nucleic acid bases in the ssDNA can interact with hydrophobic plane of the porphyrin moiety, and can shield the porphyrin moiety from water molecules to produce slightly hydrophobic circumstance (Figure 5). On the other hand, in the ds state of the conjugate, these oligo-DNA strands formed duplex. The duplex state of DNA is too rigid to shield the porphyrin moiety. Therefore, the porphyrin moiety was exposed to aqueous phase to be in the hydrophilic circumstance (Figure 5) and not intercalated between base pairs. We investigated fluorescence spectra of the ds state of oligo-DNA/porphyrin in water and water/EtOH mixture (v/v = 1/1). The increase of fluorescence intensity of porphyrin moiety in water/EtOH mixture compared with that in pure water was observed (see Supporting Information). These results also support the decrease in fluorescence intensity in ds state, which can be attributed to the change of hydrophobic–hydrophilic circumstances.

Energy transfer behaviour oligo-DNA/porphyrin and oligo-DNA/BHQ conjugate

As a primitive study of the function of oligo-DNA/porphyrin conjugate, we investigated FRET behaviour from the porphyrin moiety to a typical quencher molecule, which can be introduced to a terminal of oligo-DNA. We chose BHQ-3 as an acceptor molecule based on its absorption spectral overlap with zinc(II) porphyrin (see Supporting Information) and commercial availability of modification on oligo-DNA. BHQ-3 molecule was attached to the 3′-end of the counter oligo-DNA complementary with oligo-DNA/porphyrin conjugate.

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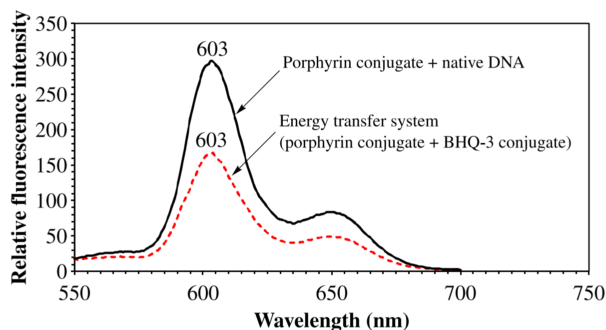

Figure 6. Fluorescence spectra of duplex state of the oligo-DNA/porphyrin conjugate with native DNA or oligo-DNA/BHQ conjugate in 50 mM Tris–HCl and 500 mM NaCl buffer (pH 7.5) at 5°C. Excitation and emission wavelength were at 428 and 603 nm, respectively.

The sequence of 3′-BHQ-3 modified oligo-DNA (oligo-DNA/BHQ conjugate) was shown in Chart 1. The schematic presentation of the experiment was also shown in Figure 1. The fluorescence spectra of the energy transfer system (1:1 mixture of oligo-DNA/porphyrin conjugate and oligo-DNA/BHQ conjugate) were measured in 50 mM Tris–HCl and 500 mM NaCl buffer (pH 7.5) at 5°C. The concentration of each conjugate was 5.0×10^{-10} mol/l. The results were shown in Figure 6. The porphyrin moiety was introduced at the 11th position of the strand, and BHQ-3 attached at the 3′-end of the counter strand, so these chromophores were separated by 10 residues. B-type duplex of DNA has about 10 residues (3.4 nm) per helix pitch. So, the distance between these chromophores is 1 pitch, but the opposite sides of the helix. The energy transfer system showed a weaker fluorescence intensity than the duplex state of the oligo-DNA/porphyrin conjugate without BHQ-3 moiety. The quenching of the porphyrin residue was observed. The quenching efficiency $Q(\%)$ defined by the following equation was 56.5%,

$$Q(\%) = \frac{I}{I_0} \times 100,$$

where I is the fluorescence intensity with BHQ-3 and I_0 is fluorescence intensity without BHQ-3. This quenching efficiency is in reasonable range considering the spectral overlap and the distance between the chromophores. These results suggested that FRET from zinc(II) porphyrin to BHQ-3 occurred. Such a porphyrin moiety can act as effective energy donor or energy acceptor for FRET on DNA assembly system.

Conclusion

We developed a new hydrophilic porphyrin derivative that can be applied to a conventional automatic DNA synthesizer. The porphyrin residue was successfully introduced in the middle of an oligo-DNA. The exposure of the hydrophilic porphyrin moiety in duplex state to aqueous phase was suggested. We established the method to introduce hydrophilic porphyrin moieties into DNA main chain at desired sites. Moreover, FRET from the zinc(II) porphyrin to quencher (BHQ-3) was achieved as a primitive study of the function for oligo-DNA/porphyrin conjugate. Using this porphyrin derivative, porphyrin residue can be introduced at desired positions of oligo-DNAs. By introduction of a number of porphyrin moieties into a strand of DNA and/or its counter complementary oligo-DNA, an array of porphyries can be obtained easily. As described in introduction, many kinds of topological self-assembly in various sizes and dimensions are possible by using DNA as building blocks (*9–19*). So, many kinds of topological arrangement of porphyrin moieties should be possible using this compound. Moreover, there are a number of commercially available chromophores, which can be introduced on DNA by automatic DNA synthesizer. Using such DNA synthesizer-compatible chromophores, and free base or metalloporphyrin derivatives, many variations of sequential chromophore arrays as energy transfer systems can be constructed. Such porphyrin array systems and multiple energy transfer systems using DNA assemblies as scaffolds may be good candidates of a good model of an artificial photosynthetic system and nanometre size photo-active materials and devices.

Experimental section

Materials

Non-modified oligo-DNA and 3′-BHQ-3-modified oligo-DNA (Chart 1) were purchased from Tsukuba Oligo Service Co. Ltd (Tsukuba, Japan). Reagents and solvents for automatic DNA synthesizer were purchased from PE Biosystems and used without purification. Water was purified using a reverse-osmotic membrane. Organic solvents were purified by usual distillation methods. All other reagents were commercial grades and used without purification.

Measurements and general methods

UV–vis absorption spectra were measured with a Shimadzu UV-2500PC spectrophotometer. Fluorescence and excitation spectra were measured with a Hitachi F-4010 spectrophotometer. ^{1}H NHR spectra were measured with a JEOL AL400 or ECA-600HR apparatus, where chloroform ($\delta = 7.26$ ppm) was used as the internal reference. FAB-mass spectra were measured on a JEOL JMS-GCmateIIR using *m*-nitrobenzyl alcohol as a matrix. MALDI-TOF-MS spectra were measured on a Shimadzu Axima-CFR [negative mode, matrix: 3-hydroxypicolic acid (H_2O/acetonitrile = 7/3)]. FCC was performed with

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silica gel (Merck, Kieselgel 60, 9385). Melting curves of DNA duplex were recorded on the UV–vis spectrophotometer by starting at 80°C sufficiently above T_m and decreasing temperature at a rate of 10°C/h to 10°C sufficiently below T_m. Absorbance values at 260 nm were continuously recorded at intervals of *ca*. 2°C. CD spectra were measured on a JASCO J-600 using a quartz cell of 0.5 cm path length at 15°C. Concentrations of the samples were 5.0×10^{-10} mol/l for energy transfer system, and 1.0×10^{-5} mol/l for other spectroscopic analyses.

3,5-Bis(ethoxycarbonylmethoxy)benzaldehyde (4)

3,5-Dihydroxybenzaldehyde (0.416 g, 3.01 mmol), K_2CO_3 (1.206 g, 8.72 mmol) and dry acetone (30 ml) were added to a 50 ml flask, and the mixture was stirred under N_2 for 5 min. Ethyl bromoacetate (1.33 ml, 6.01 mmol) was added to the reaction mixture, refluxed for 1 h, cooled to room temperature, diluted with 33 ml of H_2O and extracted three times with CH_2Cl_2. The combined organic layers were washed with water, saturated NaCl aq., and dried over Na_2SO_4 and evaporated to be dried. The residue was recrystallised from CH_2Cl_2 and hexane to give **4** (839 mg, 2.71 mmol, 90.3%); white solid; ^{1}H NMR ($CDCl_3$, 400 MHz) $\delta = 9.88$ (1H, s, CHO), 7.05 (2H, d, $J = 2$ Hz, 2,6-H), 6.79 (1H, t, $J = 2$ Hz, 4-H), 4.68 (4H, s, $ArOCH_2 \times 2$), 4.27 (4H, q, $J = 7$ Hz, $COOCH_2 \times 2$), 1.31 (6H, t, $J = 7$ Hz, $COOCCH_3 \times 2$).

5-[3,5-Bis(ethoxycarbonylmethoxy)phenyl]-10,15,20-tris[3,5-bis(1,4,7,10-tetraoxaundecyl)phenyl]porphyrin (5)

A solution of 3,5-bis(1,4,7,10-tetraoxaundecyl)benzaldehyde (**3**, 242 mg, 0.56 mmol) (*39*) and **4** (58.2 mg, 0.19 mmol) in dry $CHCl_3$ (100 ml) was purged with N_2 for 5 min, to which pyrrole (51.9 µl, 0.75 mmol) and BF_3OEt_2 (20 µl of 1 M stock solution in $CHCl_3$, 0.5 µmol) were added and stirred under N_2 atmosphere at room temperature. The reaction was monitored by absorption spectroscopy after oxidising with excess 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (DDQ). When the intensity of Soret peak was unchanged in the monitored solution, *p*-chloranil (135.8 mg, 0.55 mmol) was added and stirred for 20 h, and the solution was evaporated to be dried. The crude product was purified with FCC with CH_2Cl_2 containing MeOH. The first purple fraction (2.5% MeOH) was evaporated to give the titled compound **5** (16.8 mg, 0.09 mmol, 5% yield) and the second purple fraction (4% MeOH) was 5,10,15,20-tetrakis[3,5-bis(1,4,7,10-tetraoxaundecyl)phenyl]porphyrin (**2**, 4% yield).

5: ^{1}H NMR ($CDCl_3$, 400 MHz) $\delta = 8.89$ (8H, s, 2,3,7,8,12,13,17,18-H), 7.41 (2H, d, $J = 4$ Hz, 2,6-H of 5-Ar), 7.39 (6H, d, $J = 4$ Hz, 2,6-H of 10,15,20-Ar), 6.98 (1H, t, $J = 4$ Hz, 4-H of 5-Ar), 6.93 (3H, t, $J = 4$ Hz, 4-H of 10,15,20-Ar), 4.78 (4H, s, 3,5-OCH_2 of 5-Ar), 4.29 (4H, q, $J = 7$ Hz, $COOCH_2 \times 2$), 4.28, 3.94, 3.77, 3.68, 3.62, 3.49 (each 12H, m, ($OCH_2 \times 2$ of 10,15,20-Ar) × 6), 3.31 (18H, s, $OCH_3 \times 2$ of 10,15,20-Ar), 1.31 (6H, t, $J = 7$ Hz $COOCCH_3 \times 2$), −2.89 (2H, br, NH × 2); visible (CH_2Cl_2) λ_{max}/nm = 643 (relative intensity, 0.01), 588 (0.01), 550 (0.01), 514 (0.04), 421 (1.00); MS (FAB) found: *m/z* 1792. Calculated for $C_{94}H_{126}N_4O_{30}$: M^+, 1792.

2: ^{1}H NMR ($CDCl_3$, 400 MHz) $\delta = 8.89$ (8H, s, 2,3,7,8,12,13,17,18-H), 7.39 (8H, d, $J = 2$ Hz, 2,6-H of Ar), 6.94 (4H, t, $J = 2$ Hz, 4-H of Ar), 4.29, 3.93, 3.76, 3.66, 3.62, 3.48 (each 16H, m, $OCH_2 \times 8 \times 6$), 3.30 (24H, s, $OCH_3 \times 8$), −2.90 (2H, br, NH × 2); visible (CH_2Cl_2) λ_{max}/nm = 642 (relative intensity, 0.01), 588 (0.01), 549 (0.01), 514 (0.04), 421 (1.00); MS (FAB) found: *m/z* 1910. Calculated for $C_{100}H_{142}N_4O_{32}$: M^+, 1910.

5-[3,5-Bis(2-hydroxyethoxy)phenyl]-10,15,20-tris[3,5-bis(1,4,7,10-tetraoxaundecyl)phenyl]porphyrin (1)

A solution of **5** (8.20 mg, 4.5 µmol) in dry THF (15 ml) was stirred and purged with N_2 for 5 min, to which $LiAlH_4$ (8.5 mg, 0.22 mmol) was added and stirred under N_2 at room temperature. The reaction was monitored by TLC (silica gel with 5% MeOH/CH_2Cl_2). After disappearance of the TLC spot of **5**, the reaction mixture was poured into cold 2% HCl aq. and extracted several times with $CHCl_3$. The combined organic layers were washed with water and dried over Na_2SO_4. The crude product was purified with FCC with 5% MeOH/CH_2Cl_2. The purple fraction was evaporated to give **1** (5.5 mg, 3.2 µmol, 72%); purple solid; ^{1}H NMR ($CDCl_3$, 600 MHz) $\delta = 8.90$ (8H, s, 2,3,7,8,12,13,17,18-H), 7.43 (2H, d, $J = 2$ Hz, 2,6-H of 5-Ar), 7.40 (6H, d, $J = 2$ Hz, 2,6-H of 10,15,20-Ar), 6.98 (1H, t, $J = 4$ Hz, 4-H of 5-Ar), 6.93 (3H, t, $J = 4$ Hz, 4-H of 10, 15, 20-Ar), 4.31 (8H, m, 3,5-OCH_2CH_2 of 5-Ar), 4.04 (2H, br, OH × 2), 4.28, 3.94, 3.77, 3.68, 3.62, 3.49, (each 12H, m, (OCH_2 of 10,15,20-Ar) × 6), 3.31 (18H, s, $OCH_3 \times 2$ of 10,15,20-Ar), −2.89 (2H, br, NH × 2); visible (CH_2Cl_2) λ_{max}/nm = 643 (relative intensity, 0.01), 588 (0.01), 551 (0.01), 514 (0.04), 420 (1.00); MS (FAB) found: *m/z* 1707. Calculated for $C_{90}H_{122}N_4O_{28}$: M^+, 1707.

5-[3-(2-Hydroxyethoxy)-5-[2-(dimethoxytrityloxy)ethoxy]phenyl]-10,15,20-tris-[3,5-bis(1,4,7,10-tetraoxaundecyl)phenyl]porphyrin (6)

A solution of **1** (50 mg, 0.03 mmol) in CH_2Cl_2 (20 ml) was stirred and purged with N_2 for 5 min, to which 4-(*N*,*N*-dimethylamino)pyridine (14.7 mg, 0.12 mmol) and DMTr chloride (40.7 mg, 0.12 mmol) were added and stirred under N_2 at room temperature. The reaction was monitored by TLC (silica gel with 3% MeOH/CH_2Cl_2). Just after appearance of the TLC spot of doubly protected compound, the reaction mixture was evaporated to dryness.

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The crude product was purified with FCC with 2.5% $MeOH/CH_2Cl_2$. The first purple fraction was doubly protected compound and the second purple fraction was desired mono-protected compound **6** (53 mg, 0.028 mmol, 89%); purple solid; VIS (CH_2Cl_2) λ_{max}/nm = 643 (relative intensity, 0.01), 589 (0.01), 551 (0.01), 514 (0.04), 420 (1.00); MS (FAB) found: *m/z* 2010. Calculated for $C_{111}H_{140}N_4O_{30}$: M^+, 2010. [^{1}H NMR spectrum of **6** was so complex that its data were not presented here].

5-[3-[2-[(2-Cyanoethoxy)(diisopropylamino)phosphino]ethoxy]-5-[2-(dimethoxytritylethoxy)]phenyl]-10,15,20-tris[3,5-bis(1,4,7,10-tetraoxaundecyl)phenyl]porphyrin (7)

Porphyrin **6** (479 mg, 0.24 mmol) was dissolved in dry CH_3CN (10 ml) and the solution in a rubber-sealed bottle was evaporated under a reduced pressure. The procedure was repeated several times. To an acetonitrile solution of **6** under N_2 at room temperature, 1*H*-tetrazole (1 ml, 0.50 mmol) and 2-cyanoethyl *N,N,N′,N′*-tetraisopropylphosphoramidite (155 μl, 0.50 mmol) were added under completely dry N_2-purged conditions (using a glove box) and the solution was stirred for 3 h. The reaction mixture was extracted several times with ethyl acetate. The combined organic layers were washed with aq. saturated $NaHCO_3$, dried over Na_2SO_4 and evaporated to dryness. The residue contained the titled compound **7** and was used for synthesis of DNA conjugates without further purification. The pure sample was obtained after FCC with 50% $MeOH/CHCl_3$; purple solid; ^{31}P NMR ($CDCl_3$, 400 MHz) $\delta = 140$; visible (CH_3CN) λ_{max}/nm = 644 (relative intensity, 0.01), 589 (0.01), 551 (0.01), 512 (0.04), 420 (1.00).

Synthesis of DNA containing porphyrin residue

The synthesis of DNA containing porphyrin derivative in the main chain was carried out by usual method on an automatic DNA synthesizer Expedite 8909 (PE Biosystems, Foster City, CA, USA). The obtained crude product was purified by reverse-phase HPLC (Toso-8020 systems with a TSKgel OligoDNA RPcolumn). The characterisation of the DNA containing porphyrin was carried out by UV–vis spectroscopic measurement and MALDI-TOF-MS spectra (see Supporting Information). MS (MALDI-TOF) found: *m/z* 7851.4. Calculated for $[M-H]^-$, 7851.8.

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