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Intercalation of Water-Soluble Bis-Porphyrins into Poly(dA)-Poly(dT) Double Helix

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Abstract—The association constants (K) of nucleic acid monomers with a series of water-soluble bis-porphyrins (bisMC1, bisMC3, bisMC5, bisMC7, and bisMC11) in which two porphyrin units were linked by a methylene chain of various lengths were estimated spectrophotometrically. Among the bis-porphyrins, the K values are similar for each nucleic acid monomer, indicating that the bridging chain length does not affect the association of the bis-porphyrins with the nucleic acid monomers. The melting curves of poly(dA)-poly(dT) in the presence of bisMC3 or bisMC5 were found to be biphasic, suggesting that bisMC3 and bisMC5 are bound to poly(dA)-poly(dT) with a binding mode different from the groove binding exhibited by the corresponding porphyrin monomers. A negative-induced CD peak in the Soret region of bisMC3 and bisMC5 with poly(dA)-poly(dT) is observed and the visible spectral changes of bisMC3 and bisMC5 upon addition of poly(dA)-poly(dT) are accompanied by a large red shift of the Soret band (bisMC3: 21 nm, bisMC5: 23 nm) with substantial hypochromicity (bisMC3: 49%, bisMC5: 40%). Therefore, it is reasonable to conclude that both of the porphyrin units of bisMC3 and bisMC5 intercalate into poly(dA)-poly(dT). In contrast to poly(dA)-poly(dT), the melting curves of poly(dA·dT)$_2$ in the presence of the bis-porphyrins did not show such biphasic behavior. Together with the CD and visible absorption data, it is certain that these bis-porphyrins do not intercalate into poly(dA·dT)$_2$.

Introduction

Since cationic porphyrins interact with DNA[1] and show anti-tumor[2,3] and anti-virus[4,5] activities, interacting modes of porphyrins with DNA or nucleic acids have been studied by several groups.[6–20] Their results established three types of binding modes for porphyrins with DNA: intercalative binding, groove binding, and outside binding.[21–23] Intercalative binding has been found to occur dominantly at GC-rich regions, groove binding at AT-rich regions, and outside binding at both AT-rich and GC-rich regions.[11–13] The intercalated porphyrin species has the following characteristics: (i) rising of the melting temperature of DNA,[24,25] (ii) a large red shift of the Soret band (≧15nm) and substantial hypochromicity of the Soret maximum (≧35%),[26] (iii) an induced negative CD band in the Soret region,[18,23] (iv) an increase in the viscosity for aqueous solution,[23,27] and (v) a decrease in fluorescence intensity at the Q-band region.[10] In contrast, the groove binding porphyrin species has the following characteristics: (i) rising of the melting temperature of DNA,[24,25] (ii) a small red shift in the Soret band (usually ≦8nm) and little hypochromicity or hyperchromicity of the Soret maximum,[26] (iii) an induced positive CD band in the Soret region,[18,23] and (iv) an increase in fluorescence intensity at the Q-band region.[10] On the other hand, the outside binding porphyrin species is characterized by an induced conservative CD band in the Soret region[18,23] and has no effect on the melting temperature of DNA.[24,25]

In regard to the binding of a compound bearing multi-binding sites to DNA, Meunier et al. have reported that hybrid molecules of 'metalloporphyrin-ellipticine' show intercalative binding with DNA at the elliptieine site and groove binding at the metalloporphyrin site,[28] while Uno et al. have reported that hybrid molecules of 'porphyrin-acridine' show intercalative binding with DNA at the acridine site and groove binding at the porphyrin site.[29] Thus, although monomeric porphyrins bind to DNA in a certain binding mode, dimerized cationic porphyrins (bis-porphyrins) may exhibit a new type of binding mode different from that observed by the porphyrin monomers. The new binding mode results from a

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collaboration effect of the two binding sites with DNA, where the two porphyrin units interact cooperatively with DNA. Further, the new binding mode may reveal a specific recognition property for a certain array of nucleic acids. On the basis of this supposition, we have designed and synthesized a series of water-soluble bis-porphyrins by tethering two porphyrins via various lengths of linker (Scheme 1).[30] The present paper describes the interactions of the bis-porphyrins with nucleic acid monomers and polymers based on visible and CD spectral studies. The results suggest that bisMC3 and bisMC5 exhibit a new binding mode to poly(dA)-poly(dT).

Results and Discussion

Interactions of porphyrins with deoxyribonucleic acids

The association of deoxyribonucleic acids with porphyrins was examined by use of spectrophotometric titration in the Soret region. Figure 1 exemplifies the titration for bisMC5 with d-AMP. The presence of isosbestic points suggests that there are two chromophores in equilibrium. The absorbance changes are well fitted by a 1:1 association model, indicating the absence of any cooperativity of the two binding sites. The association constants (K) of porphyrins with deoxyribonucleic acids are summarized in Table 1. These K values indicate the following three features. First, for all the porphyrins examined, the K values for purines (d-AMP, d-GMP) are greater than those for pyrimidines (d-TMP, d-CMP), indicating that the K values are correlated to the magnitude of hydrophobic interactions with the nucleobases.[26] Secondly, regardless of the nucleic acids employed, the K values of H_2TMpyP are greater than those for the other porphyrins, while the K values of the bis-porphyrins are similar to those for $H_2(Mpy)_3(Ph)P$. Thus, the K values are reflected by the number of cations (pyridinium) per one porphyrin unit and hence depend on Coulomb interaction.[31–33] Finally, the K values for each nucleic acid are similar among the bis-porphyrins, suggesting that interactions of the bis-porphyrins with the nucleic acids are similar to those of $H_2(Mpy)_3(Ph)P$ and are not affected by the length of the bridging chain.

Interactions of porphyrins with polynucleic acids

The melting temperatures (T_m)[8,24,25,34,35] measured for poly(dA)-poly(dT) in the presence or absence of porphyrins are summarized in Table 2. As shown in Figure 2, the melting curve of poly(dA)-poly(dT) at N/P ([concentration of nucleic acid polymers as base pair]/[concentration of porphyrins as porphyrin unit]) = 10 is sharply biphasic for bisMC5 and loosely biphasic for bisMC3, where the T_m values increase in the presence of

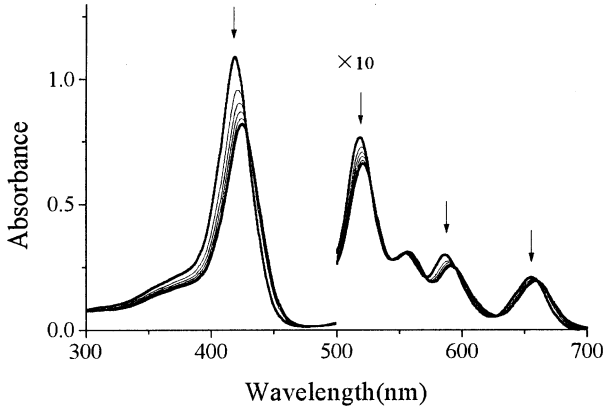
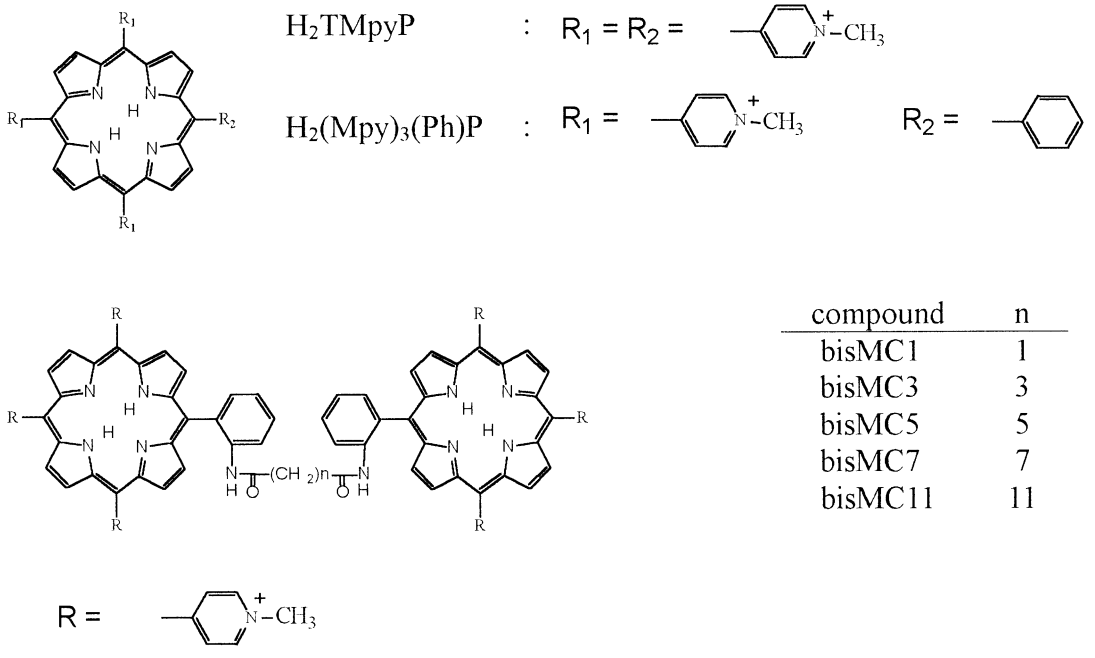


Figure 1. Titration of d-AMP to bisMC5 in 15 mM Tris–HCl buffer (pH 7.0, $I = 0.045$) at 25.0 °C; [bisMC5] = 2.9 μM, [dAMP] = 0, 0.41, 0.81, 1.2, 1.6 and 1.9 mM.



Scheme 1.

the porphyrins. In the cases of the other bis-porphyrins and porphyrin monomers, no biphasic curve was observed. Such biphasic behavior has been reported in the following cases: when the DNA-binding compounds act as a bis-intercalater {bis(phenanthridinium bromide) with CT DNA}[36,37] or as a long range multi-binder {poly-L-lysine with poly(I + C)},[38] and when DNA has a triplex conformation {poly(dA)-2poly(dT) triplex}.[39,40] To our knowledge, this is the first case of biphasic behavior for porphyrins. A possible explanation for the biphasic curves is the formation of poly(dA)-2poly(dT) triplex upon addition of the porphyrins. In general, the melting temperatures from poly(dA)-2poly(dT) triplex to poly(dA)-poly(dT) duplex + poly(dT) are lower than those from poly(dA)-poly(dT) duplex to poly(dA) + poly(dT).[39] However, all the melting temperatures of poly(dA)-poly(dT) in the presence of the porphyrins examined were higher than that in the absence of porphyrins. Therefore the biphasic behavior does not come from the melting of poly(dA)-2poly(dT) triplex. Consequently, in the biphasic curves, the intermediate step corresponds to a 'semi-melting' state where only certain regions stabilized by the porphyrins do not melt. The final step at high temperatures represents complete melting.

The vertical axis of the melting curve corresponds to the ratio of the melting region to the whole of poly(dA)-poly(dT). In the case of bisMC5 (Fig. 3), the values of [vertical axis for the high melting phase]/[vertical axis for the low melting phase] increase with decreasing N/P. At N/P = 5, the semi-stable state no longer appears and only the high melting temperature phase (T_m = 81 °C) is observed. Compound bisMC3 also shows a tendency similar to that of bisMC5. These results indicate that the stabilized region of poly(dA)-poly(dT) increases with the N/P values. On the other hand, the other bis-porphyrins (bisMC1, bisMC7 and bisMC11) show no biphasic curve at any N/P, and the T_m values are lower than those with porphyrin monomers H_2TMpyP and H_2(Mpy)$_3$(Ph)P.

In the case of poly(dA·dT)$_2$, none of the bis-porphyrins show a biphasic curve at any N/P values, and the T_m values are lower than those with the porphyrin monomers (Table 3). In the case of poly(dG·dC)$_2$, the T_m values were not determined because these values were too high to be measured. To obtain information about the difference in melting behavior between poly(dA)-poly(dT) and poly(dA·dT)$_2$, CD and UV–vis titration experiments were performed.

It has been reported that the induced CD spectra in the Soret region of porphyrins are well-defined indicators for the binding modes toward polynucleic acids.[1,9,12,41]

Table 1. Association constants of porphyrins with deoxyribo nucleotides[a]

Porphyrin	λ max (nm)	K (M^{-1})×10^{-2}			
		d-AMP	d-GMP	d-TMP	d-CMP
H_2TMpyP	422	18±0.6	17±0.5	4.7±0.2	3.1±0.1
H_2(Mpy)$_3$(Ph)P	422	13±0.4	11±0.4	3.1±0.1	1.9±0.2
bisMC11	425	13±0.7	9.3±0.3	3.4±0.2	1.3±0.1
bisMC31	421	11±0.4	10±0.3	3.2±0.1	1.3±0.1
bisMC51	419	11±0.5	11±0.4	4.1±0.1	1.2±0.1
bisMC71	419	12±0.3	9.7±0.4	3.5±0.1	2.1±0.2
bisMC11	420	11±0.4	7.5±0.2	3.3±0.3	1.2±0.1

[a]At 25.0 °C, pH 7.0, I = 0.045.

Table 2. Melting temperatures (°C)[a] of poly(dA)-poly(dT) in the presence of porphyrins[b]

Porphyrin	N/P[c] = 10	N/P = 30	N/P = 50
H_2TMpyP	77	65	65
H_2(Mpy)$_3$(Ph)P	72	66	65
bisMC1	67	65	64
bisMC3	65,72	64	64
bisMC5	65,81	63,78	64,78
bisMC7	67	64	63
bisMC11	68	64	64

[a]The estimated errors in melting temperatures were ±1 °C. The melting temperature in the absence of porphyrin was 61 °C.
[b]In 15 mM Tris–HCl buffer (pH 7.0, I = 0.045). Poly(dA)-poly(dT) concentration was 3×10^{-5} M (as base pairs).
[c]Ratio of poly(dA)-poly(dT) concentration (as base pairs) to porphyrin concentration (as porphyrin unit).

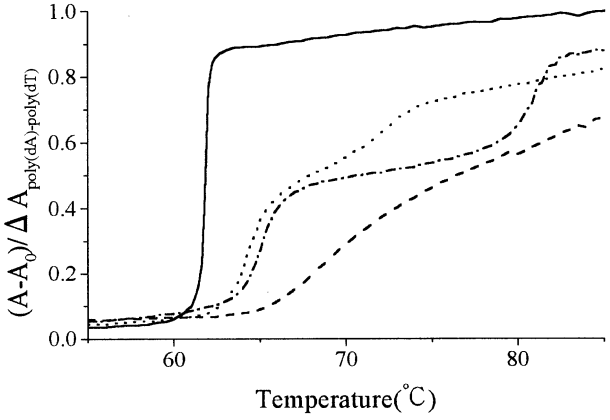


Figure 2. Melting curves of poly(dA)-poly(dT) (30 μM as base pairs) in the presence of porphyrins (3 μM as porphyrin unit) in Tris–HCl buffer (pH 7.0, I = 0.045) at 260 nm; no porphyrin (—), H_2TMpyP (- - - -), bisMC3 (·····), bisMC5 (– · –).

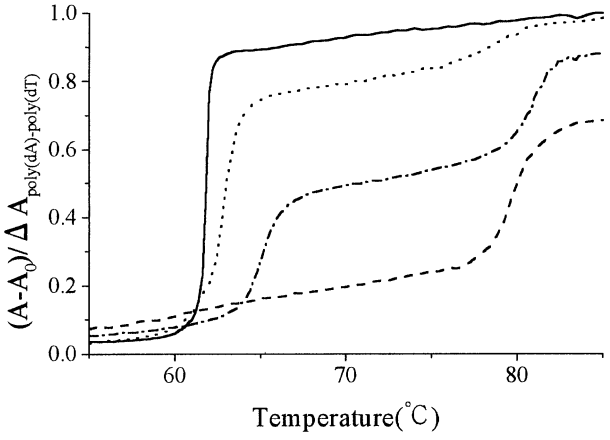


Figure 3. Melting curves of poly(dA)-poly(dT) (30 μM as base pairs) in the presence of bisMC5 in Tris–HCl buffer (pH 7.0, I = 0.045) at 260 nm; poly(dA)-poly(dT) P = 0 (—), N/P = 30 (·····), N/P = 10 (- · - · -), N/P = 5 (- - - -).

These results provide the following criteria to indicate that a positive-induced CD peak is due to groove binding and a negative-induced CD peak is due to intercalative binding, whereas a conservative CD peak is ascribed to the intermolecular self-association of porphyrins on polynucleic acids. Figure 4 shows the CD spectra of bisMC3 and bisMC5 in the presence of poly(dA)-poly(dT). Compound bisMC3 exhibits a weak negative-induced CD peak at ca. 450 nm at N/P = 10, while bisMC5 gives a weak positive-induced CD peak at ca. 430 nm and a strong negative-induced CD peak at ca. 450 nm at N/P = 10 (bisMC5 gives a strong negative-induced CD peak only at N/P = 30; data are not shown). Thus, on the basis of the criteria stated above, bisMC5 and bisMC3 are likely to intercalate into poly(dA)-poly(dT) at room temperature. Further, since the intercalative binding to poly(dA)-poly(dT) induces a rise of the melting temperature,[24,25] we propose that the high melting temperature phase for those porphyrins is due to the melting of the intercalative binding regions in poly(dA)-poly(dT).

In the case of poly(dA·dT)$_2$, bisMC3 shows a positive-induced CD peak at ca. 435 nm at N/P = 10 (Fig. 5), hence bisMC3 should interact with poly(dA·dT)$_2$ by groove binding. On the other hand, bisMC5 exhibits a weak positive-induced CD peak at ca. 420 nm and a weak negative-induced CD peak at ca. 450nm at N/P = 10, thus the binding mode of bisMC5 with poly(dA·dT)$_2$ could not be identified on the basis of the CD data alone (vide infra).

In the case of poly(dG·dC)$_2$, bisMC3 and bisMC5 give only a negative-induced CD peak at ca. 450nm at N/P = 10 (Fig. 6), and should intercalate into poly(dG·dC)$_2$ at room temperature.

On the addition of porphyrins to double helical polynucleic acids, a large red shift (≧15 nm) with substantial hypochromicity (≧35%) in the Soret region is generally attributed to intercalation of the porphyrins.[26] The visible spectrum for bisMC5 upon addition of poly(dA)-poly(dT) (Fig. 7, Table 4) exhibits a red shift (21 nm) with 40% hypochromicity while that for bisMC3 shows a red shift (21 nm) with 49% hypochromicity. In accordance with the results of the CD spectra, it is reasonable

Table 3. Melting temperatures (°C)[a] of poly(dA·dT)$_2$ in the presence of porphyrins[b]

Porphyrin	N/P[c] = 10	N/P = 30	N/P = 50
H_2TMpyP	> 88	72	62
H_2(Mpy)$_3$(Ph)P	80	69	64
bisMC1	61	57	57
bisMC3	80	58	58
bisMC5	75	57	56
bisMC7	71	59	58
bisMC11	71	60	58

[a]The estimated errors in melting temperatures were ±1 °C. The melting temperature in the absence of porphyrin was 55 °C.
[b]In 15 mM Tris–HCl buffer (pH 7.0, I = 0.045). Poly(dA·dT)$_2$ concentration was 3×10^{-5} M (as base pairs).
[c]Ratio of poly(dA·dT)$_2$ concentration (as base pairs) to porphyrin concentration (as porphyrin unit).

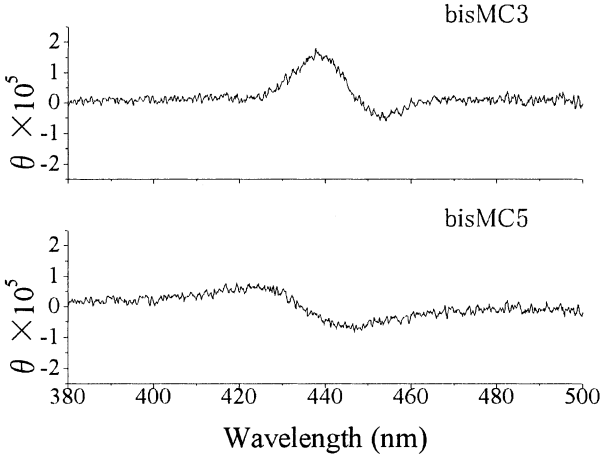


Figure 5. Induce CD spectra of bisMC5 and bisMC3 in the presence of 110 μM (as base pairs) poly(dA·dT)$_2$ in Tris–HCl buffer (pH 7.0, I = 0.045) at N/P = 10.

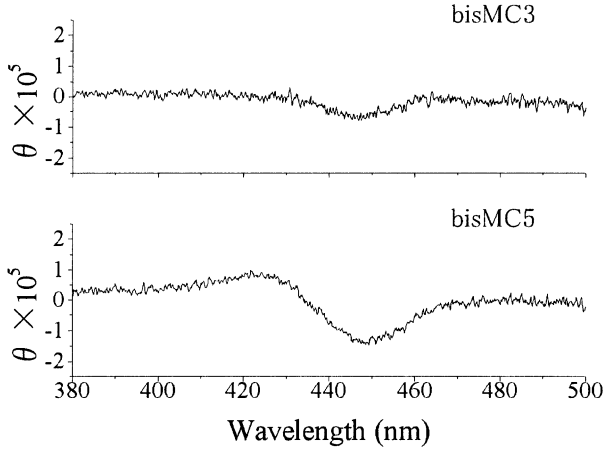


Figure 4. Induce CD spectra of bisMC5 and bisMC3 in the presence of 110 μM (as base pairs) poly(dA)-poly(dT) in Tris–HCl buffer (pH 7.0, I = 0.045) at N/P = 10.

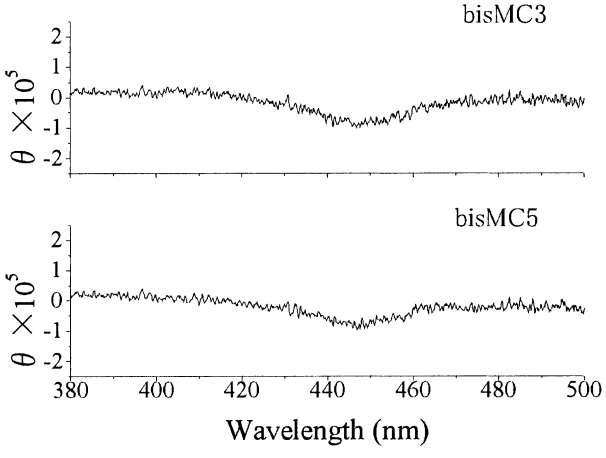


Figure 6. Induce CD spectra of bisMC5 and bisMC3 in the presence of 110 μM (as base pairs) poly(dG·dC)$_2$ in Tris–HCl buffer (pH 7.0, I = 0.045) at N/P = 10.

to conclude that bisMC5 and bisMC3 intercalate into poly(dA)-poly(dT) at room temperature. Here, we propose that this could lead to the appearance of the biphasic melting curves; possible binding modes for bisMC5 and bisMC3 with poly(dA)-poly(dT) including intercalative binding are illustrated in Scheme 2. Among the three binding modes, it is likely that the binding modes for the two porphyrin planes in bisMC5 and bisMC3 with poly(dA)-poly(dT) are the intercalative–intercalative type because only a negative-induced CD peak is observed at appropriate N/P values. This binding behavior is a new type that has not been reported previously in porphyrin monomers appending an intercalator[28,29] and may be explained in terms of a collaboration effect by the two binding sites of bisMC5 and bisMC3. On the other hand, judging from their CD and UV–vis spectral data, H_2TMpyP, $H_2(Mpy)_3(Ph)P$, and bisMC1 are suggested to interact with poly(dA)-poly(dT) by groove binding.

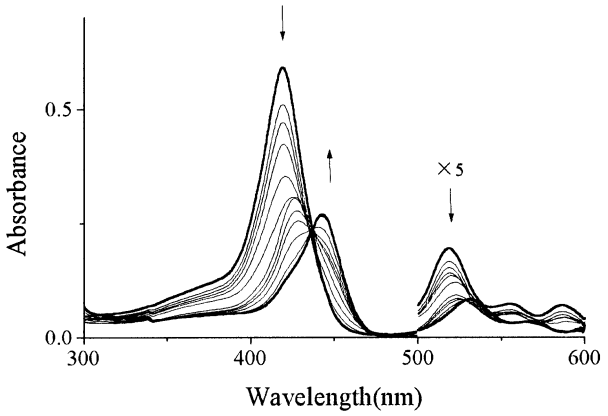


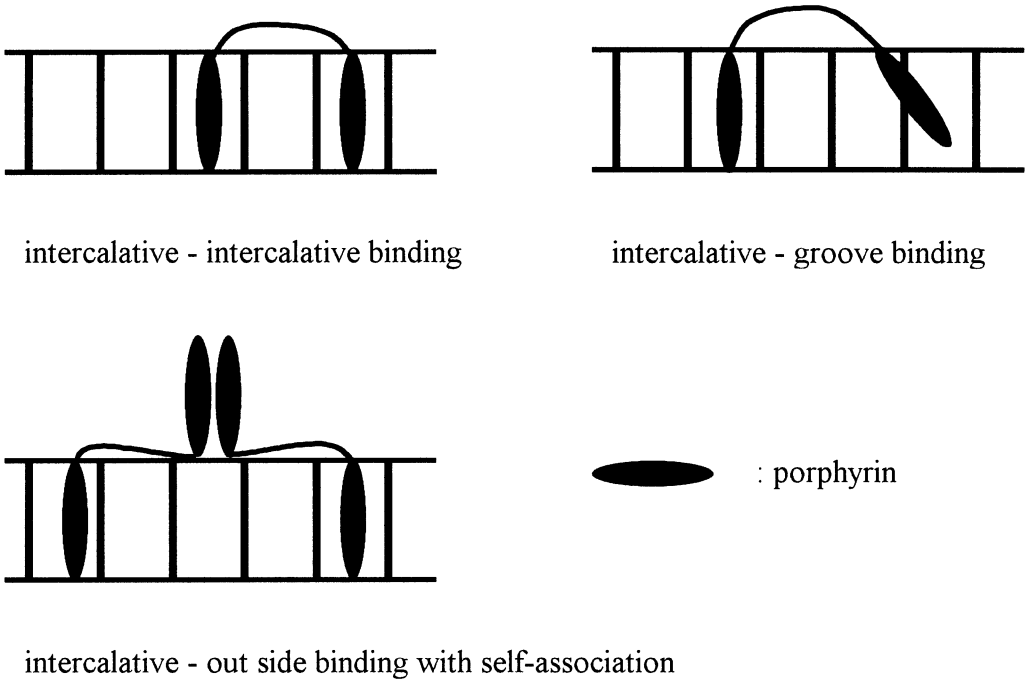
Figure 7. Titration of poly(dA)-poly(dT) to bisMC5 in Tris–HCl buffer (pH 7.0, $I = 0.045$) at 25.0 °C; [bisMC5] = 2.8 μM as porphyrin unit, [poly(dA)–poly(dT)] = 0, 0.28, 0.56, 1.3, 2.6, 5.1, 8.6, 15, 49, 75 and 110 μM as base pairs.

A CPK model-building study suggested that, on bis-intercalation into poly(dA)-poly(dT), the separation of the two porphyrin units of bisMC1 suffers from some steric strain but those of bisMC3 and bisMC5 are sterically appropriate. For bisMC7 and bisMC11, the separations of the two porphyrins may be too long and act independently, and thus not show a collaborative

Table 4. UV–vis Spectral changes of porphyrins with poly nucleic acids in 15 mM Tris–HCl buffer (pH 7.0, $I = 0.045$) at N/P = 50

	Poly(dA)-poly(dT)		Poly(dA•dT)$_2$		Poly(dG•dC)$_2$	
Porphyrin	Δλ max (nm)	%H[a]	Δλ max (nm)	%H[a]	Δλ max (nm)	%H[a]
H_2TMpyP	7	19	9	11	23	39
$H_2(Mpy)_3(Ph)P$	8	34	9	17	25	49
bisMC1	6	40	6	41	16	65
bisMC3	21	49	12	28	22	50
bisMC5	23	40	12	36	24	49
bisMC7	22	44	16	42	22	40
bisMC11	13	45	13	46	23	60

[a]The % hypochromicity (%H) was determined from $(\varepsilon_p - \varepsilon_b)/\varepsilon_p \times 100$, where p represents free porphyrin, b represents bound porphyrin, and ε_p and ε_b were determined at the respective Soret maxima.



Scheme 2.

effect. These results suggest that bisMC3 and bisMC5 can recognize the array of poly(dA) or poly(dT) components and then intercalate into poly(dA)-poly(dT). In regard to the various interactions of porphyrins with nucleic acid polymers, intercalation into the AT region has not been reported for DNA, except for the poly(rA)-poly(dT) RNA hybrid double helix.[42] This is the first observation of the intercalation of porphyrins into the AT region of the DNA double helix.

Upon addition to poly(dG·dC)$_2$, the spectrum for bisMC5 also shows a red shift of 24 nm with 50% hypochromicity while that for bisMC3 shows a red shift of 22 nm with 50% hypochromicity. Consequently, in accordance with the results from the melting experiments and CD spectra, it is certain that bisMC3 and bisMC5 intercalate strongly into poly(dG·dC)$_2$. Contrary to this, the small red shifts in the Soret band of bisMC3 and bisMC5 upon addition to poly(dA·dT)$_2$ do not correspond to intercalation as suggested by the melting experiments and the CD spectra.

Experimental

Materials

All chemicals were purchased commercially and were used as received without further purification unless otherwise noted. Nucleic acid monomers, polymers, and H_2TMpyP were purchased from Aldrich, Nakarai or SIGMA and were used without further purification. $H_2(Mpy)_3(Ph)P$[43] and a series of bis-porphyrins (bisMC1, bisMC3, bisMC5, bisMC7, and bisMC11)[30] were synthesized as chlorides according to the literature.

Measurements

The UV–vis spectra were recorded on a Hitachi U-3000 spectrophotometer. The CD spectra were recorded on a JASCO J-720 spectropolarimeter. The concentrations of poly nucleic acids for measurements were determined spectrophotometrically with $\varepsilon^{260\ nm} = 1.20\times10^4\ M^{-1}\ cm^{-1}$ (as base pair) for poly(dA)-poly(dT), $\varepsilon^{262\ nm} = 1.32\times10^4\ M^{-1}\ cm^{-1}$ (as base pair) for poly(dA·dT)$_2$,[44] and $\varepsilon^{260\ nm} = 1.68\times10^4\ M^{-1}\ cm^{-1}$ (as base pair) for poly(dG·dC)$_2$.[13] All measurements were carried out at pH 7.0 buffered by Tris(hydroxymethyl)aminomethane–HCl (Tris–HCl; 15 mM tris and 15 mM HCl) containing NaCl (ionic strength $I = 0.045$). The association constants (K) of porphyrins with nucleic acid monomers were estimated from spectrophotometric titration using the method described in the literature.[26] The concentrations of porphyrins for UV–vis spectral measurements were in the range of $3\sim6\times10^{-6}$ M (as porphyrin unit). For determining the melting temperatures of the polynucleic acids, absorption changes of the poly nucleic acids at 260nm (3.0×10^{-5} M as base pair) were monitored. The temperature was scanned from 20 to 88 °C at speed of 1 °C per min. The concentrations of poly nucleic acids were in the range of $1\sim1.8\times10^{-4}$ M (as base pair) for CD spectral measurements.

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