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Binding and photocleavage of cationic porphyrin–phenylpiperazine hybrids to DNA

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Abstract

The binding properties of cationic porphyrin–phenylpiperazine hybrids to calf thymus (CT) DNA were investigated by using absorption, fluorescence and circular dichroism (CD) spectra, and the apparent affinity binding constants (K_{app}) of the porphyrins for CT DNA were determined by using a competition method with ethidium bromide (EB). Intercalation of porphyrin into CT DNA occurred when two phenylpiperazines were introduced at *cis* position onto the periphery of cationic porphyrin. The photocleavages of pBR322 plasmid DNA by the porphyrins were consistent with the values of K_{app}. With [porphyrin]/[DNA base pairs] ratio increased, the binding mode tended to be outside binding, and the cleavage abilities of the porphyrins varied. In the presence of sodium azide, a quencher of $^1O_2$, the cleavage of DNA by the porphyrin of intercalation was less inhibited.

Keywords: Cationic porphyrin; Phenylpiperazine; Intercalation; Apparent affinity binding constant; Photocleavage

1. Introduction

Cationic porphyrin has been a long-term interesting subject because of its binding and photodamage to DNA. Cationic *meso*-tetrakis(4-*N*-methylpyridiumyl)porphyrin (TMPyP) has been extensively studied for its tight interactions with DNA [1,2]. At low [porphyrin]/[DNA base pairs] ratio and low ionic strength, TMPyP prefer to intercalate into GC-rich regions of DNA. When [porphyrin]/[DNA base pairs] ratio and/or ionic strength increases, the binding mode becomes complex that involves both intercalation into GC-rich regions and outside binding at AT-rich sites [2–5].

Introducing some bioactive moieties onto the periphery of the porphyrin has been performed in DNA photocleavage and antitumor [6,7]. The binding mode of porphyrin–DNA is affected by location of the substituent groups on the periphery of the porphyrins [1]. Piperazine is an important pharmic intermediate, and piperazine rings are capable of resting in the minor groove of GC base pairs [8,9]. As a minor groove binder, introduction of piperazine onto the periphery of cationic porphyrin may affect the binding mode of porphyrin–DNA [10].

NMR, equilibrium dialysis, viscometry measurements of DNA have been used to study the interactions between porphyrins and DNA [4]. Nevertheless, the spectroscopy measurements including absorption, fluorescence and circular dichroism, are the most convenient tools for examining the interaction between porphyrins and DNA [3,11]. Especially in the Soret band of porphyrin, the CD spectroscopy is induced when porphyrin forms a complex with DNA. The sign of the induced CD spectra of porphyrins bound to DNA provides conveniently a signature for the binding mode to DNA: a positive induced CD band in the Soret region is indicative of external binding, and a negative induced CD band is present upon intercalation [2,3]. Intercalation of porphyrin into DNA is characterized by large hypochromicity, red shift and a negatively induced CD in the Soret band [11,12].

In this paper, the binding modes of cationic porphyrin–phenylpiperazine hybrids and cationic porphyrins bound to CT DNA were studied by using absorption, fluorescence and induced CD spectra. The apparent affinity binding constants (K_{app}) of the porphyrins for CT DNA were determined by using

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a competition method with EB. The photocleavages of pBR322 plasmid DNA by these porphyrins were investigated, and the inhibition of sodium azide was also studied.

2. Experimental

2.1. Synthesis of porphyrins

The classical Adler–Longo method [13] was used to synthesize 5-(4-methoxycarbonylphenyl)-10,15,20-tris(4-pyridyl)porphyrin and 5,10-di(4-methoxycarbonylphenyl)-15,20-di-(4-pyridyl)porphyrin, then hydrolyzed to give 5-(4-carboxyphenyl)-10,15,20-tris(4-pyridyl)porphyrin and 5,10-di(4-carboxyphenyl)-15,20-di(4-pyridyl)porphyrin. The reaction of the porphyrins with 1-chloroacetyl-4-phenylpiperazine afforded corresponding compounds. Methylation by an excess amount of methyl iodide afforded target compounds **1**–**4** (Fig. 1).

The spectroscopic results were obtained from 5-[4-[1-(4-phenylpiperazine-yl)-acetyloxocarbonyl]phenyl]-10,15,20-tris(4-*N*-methylpyridiniumyl)porphine Triiodide (**1**): ^{1}H NMR (DMSO-d_6, δ) 9.44 (d, J=6.0 Hz, 6H, PyH_o), 9.13 (s, 4H, H_β), 9.03 (s, 4H, H_β), 8.97 (d, J=6.0 Hz, 6H, PyH_m), 8.47 (d, J=7.8 Hz, 2H, $OCPhH_o$), 8.38 (d, J=7.8 Hz, 2H, $OCPhH_m$), 7.25 (t, J=7.8 Hz, 2H, piperizine PhH_m), 7.00 (d, J=7.8 Hz, 2H, piperizine PhH_o), 6.83 (t, J=7.5 Hz, 1H, piperizine PhH_p), 5.33 (s, 2H, OCH_2CO), 4.69 (s, 9H, CH_3), 3.69 (s, 4H, CH_2NPh), 3.19 (s, 2H, $CONCH_2$), 3.08 (s, 2H, $CONCH_2$), −3.03 (s, 2H, pyrrole N–H). IR (KBr) ν: 3312 (pyrrole N–H), 1719 (ester C⁀O), 1663 (acetamide C⁀O), 1639 (pyridinium). FAB MS m/z: 909 ($M+H^+$, $C_{57}H_{50}N_9O_3$).

The spectroscopic results were obtained from 5,10-di[4-[1-(4-phenylpiperazine-yl)-acetyloxo-carbonyl]-phenyl]-15,20-di(4-*N*-methylpyridiniumyl)porphine diiodide (**2**): ^{1}H NMR (DMSO-d_6, δ) 9.43 (d, J=6.0 Hz, 4H, PyH_o), 9.13–8.92 (m, 8H, H_β), 9.00 (d, J=6.0 Hz, 4H, PyH_m), 8.48–8.38 (m, 8H, $OCPhH_{o,m}$), 8.01 (d, J=7.8 Hz, 2H, piperizine PhH_o), 7.63–7.75 (m, 3H, piperizine $PhH_{m,p}$), 7.25 (t, J=7.8 Hz, 2H, piperizine PhH_m), 7.00 (d, J=7.8 Hz, 2H, piperizine PhH_o), 6.83 (t, J=7.4 Hz, 1H, piperizine PhH_p), 5.31 (s, 4H, OCH_2CO), 4.68 (s, 6H, CH_3), 3.68 (s, 8H, CH_2NPh), 3.26 (s, 4H, $CONCH_2$), 3.18 (s, 4H, $CONCH_2$), −3.00 (s, 2H, pyrrole N–H). IR (KBr) ν: 3311 (pyrrole N–H), 1722 (ester C⁀O), 1664 (acetamide C⁀O), 1640 (pyridinium). FAB-MS m/z: 1139 ($M+H^+$, $C_{70}H_{62}N_{10}O_6$).

The ^{1}H NMR spectra were recorded on Varian Mercury-VX 300 spectrometer with the solvent signal of TMS. Infrared spectra were obtained on a Shimadzu FT-IR 3000 spectrometer. UV–vis spectra were measured on a Shimadzu 1901 spectrometer. Mass spectra were obtained on TSQ 7000 instrument. Flash chromatography was performed using silica gel (200–300 mesh). Unless otherwise stated, reagents were obtained from commercial suppliers and used as received.

2.2. Spectral properties of porphyrin–CT DNA interactions

The spectral measurements were performed at room temperature in buffer (pH=7.4, 0.05 M Tris–HCl, 0.1 M NaCl). An extinction coefficient of 1.31×10^4 M^{-1} cm^{-1} at 260 nm was used to determine the CT DNA concentration in base pairs. UV–vis spectra were measured on a Shimadzu 1901 spectrometer. Fluorescence spectra were measured on a Perkin Elmer LS-55 spectrometer. Circular dichroism was measured on a JASCO J-810 spectrometer.

UV–vis absorption spectra of porphyrins were recorded within a range of 350–500 nm. Fluorescence spectra of porphyrins were recorded at 422 nm for excitation and 652 nm for emission. Induced CD spectra were recorded within a range of 350–500 nm.

2.3. Apparent binding constant measurements

The apparent affinity binding constants were determined by using a competition method with EB. This assay consists of the measurement of the fluorescence intensity of EB bound to CT DNA in the presence of porphyrin. Fluorescence spectra of EB binding to DNA were recorded at 540 nm for excitation and 610 nm for emission. The decrease of fluorescence in the cuvettes containing porphyrins determined the apparent binding constants (K_{app}) of the porphyrin for CT DNA according to literature [11,14]. The measurement was performed at room temperature in buffer (pH=7.4, 0.05 M Tris–HCl, 0.1 M NaCl). The binding constant of EB for DNA in the experimental conditions was 8.1×10^5 M^{-1}.

2.4. DNA photocleavage assay

The photocleavages of pBR322 plasmid DNA by the porphyrins were investigated using agarose gel electropho-

Fig. 1. Molecular structure of cationic porphyrins **1**–**4**.

resis. All experiments were performed in buffer (pH=7.4, 0.1 M phosphate buffer). The photo-inducing experiments were performed by illumination with 50 W high-pressure mercury at 37 °C and the distance from the sample to the filament of the mercury lamp was 20 cm. DNA was analyzed by 0.9% agarose gel electrophoresis at 5 V/cm for 30 min. The gel was incubated in a solution of ethidium bromide for 30 min and the DNA bands were filmed by gel imaging instrument of Vilber Lourmat Bio Print. The concentration analysis of the DNA bands was determined with the Bio-Capt software.

The inhibition assay of photocleavage was performed in the presence of sodium azide, which is an excellent quencher of $^1O_2$ with a high quenching rate constant of 3.8×10^8 M^{-1} s^{-1} [15,16]. The quencher, sodium azide, was added to DNA prior to illumination.

3. Results

3.1. Spectral properties of porphyrin–CT DNA interactions

UV–vis titrations were performed as previously described [17,18]. Porphyrin **1**, **2**, **3** and **4** (3.0 μM) were titrated with a solution of CT DNA. The absorbance change in the Soret region is shown in Fig. 2. As shown in the UV–vis spectra, the intensity of the Soret band at 422 nm decreased at the earlier addition of DNA, it then increased when further added the DNA. Red shift of the Soret band was also observed during the titration suggesting the binding of the porphyrin to CT DNA. Cationic porphyrin **3** and **4** exhibited 35–40% hypochromism and 15 nm red shift of the Soret band, however in case of cationic porphyrin–phenylpiperazine hybrids **1** and **2**, only little change with 19–23% hypochromism and 8–11 nm red shift of the Soret band in UV–vis spectra was observed when CT DNA was added. The data are summarized in Table 1.

Fluorescence decay experiments in the presence of CT DNA were performed. Fluorescence emission spectra with a maximum intensity at 652 nm in the absence of CT DNA are presented in Fig. 3 (solid line). In the presence of CT DNA, a decrease in intensity of fluorescence emission is depicted for all porphyrins (Fig. 3). Cationic porphyrin–phenylpiperazine hybrid **2** exhibited remarkable decrease in intensity of fluorescence emission at a low concentration of DNA of 3.51 μM. However, there was no major effect of CT DNA on the spectra of porphyrin **4**. Porphyrin **1** and porphyrin **3** both showed decrease in intensity of fluorescence emission. The values for decrease in intensity are summarized in Table 1.

The intense Soret band absorption of porphyrins was useful for CD studies of the interactions between porphyrins and DNA [3,19]. The sign of induced CD spectra in the Soret region depends upon the binding mode of porphyrin–DNA [19,20]. Induced CD spectra for porphyrins bound to CT DNA at a [porphyrin]/[DNA base pairs] ratio of 0.05 are shown in Fig. 4. When porphyrin **1**, **3** and **4** bound to CT DNA, a strong positive band at 425–435 nm and a weaker negative band at

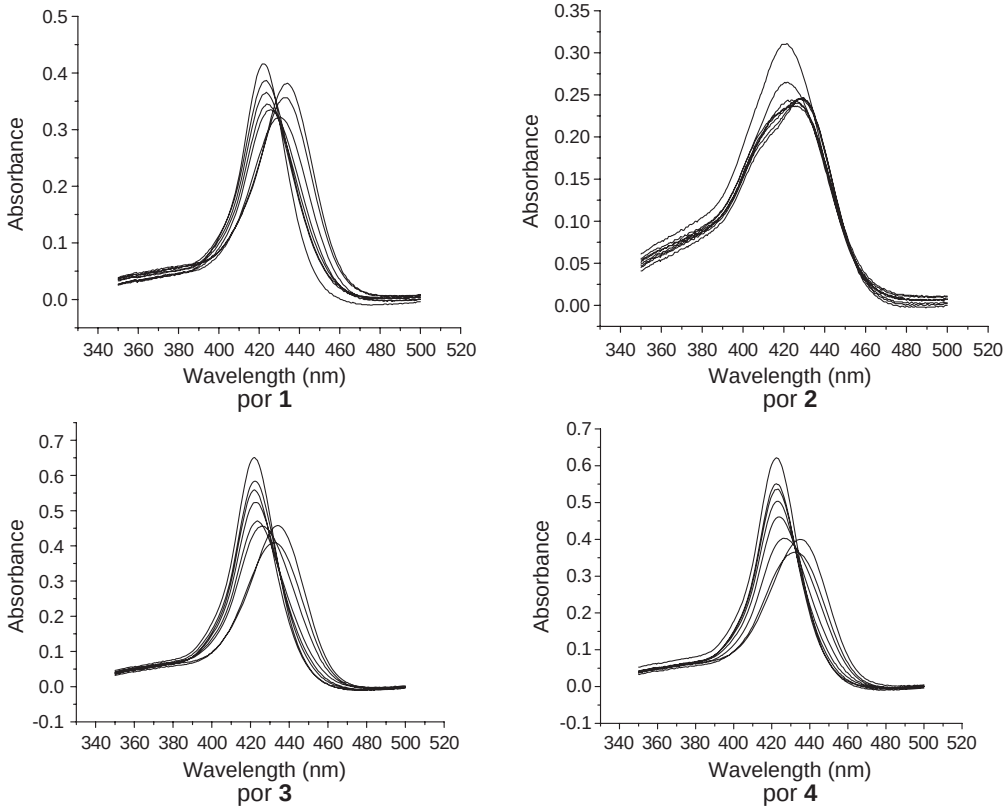

Fig. 2. UV–vis absorbance change when titration of porphyrin **1**, **2**, **3** and **4** by CT DNA. [porphyrin]=3.0 μM in solution (pH=7.4, 0.05 M Tris–HCl, 0.1 M NaCl); [DNA base pairs]=0, 0.73, 2.20, 3.66, 5.49, 10.98, 21.96, 36.60 μM.

Table 1
Compared data of the interaction of porphyrins with CT DNA

Porphyrins	UV–vis on the Soret band		Fluorescence emission	CD in the Soret region		K_{app} (M^{-1})
	Hypochromicity H (%)	Red shift $\Delta\lambda$ (nm)	Decrease of intensity (%)	Positive band (nm)	Negative band (nm)	
Porphyrin **1**	19	11	19	432	457	1.3×10^6
Porphyrin **2**	23	8	32		429	7.2×10^5
Porphyrin **3**	37	15	13	428	450	2.3×10^6
Porphyrin **4**	40	15	4	430	456	9.7×10^5

All experiments were performed at room temperature in buffer (pH=7.4, 0.05 M Tris–HCl, 0.1 M NaCl).

450–460 nm appear. In the case of porphyrin **2**, only a negative band was apparent at 429 nm. The values of the CD bands are summarized in Table 1 also.

Induced CD spectra of porphyrin **2** bound to CT DNA in various [porphyrin]/[DNA base pairs] ratios of 0.20, 0.10 and 0.05 are shown in Fig. 5. At a high [porphyrin]/[DNA base pairs] ratio, induced CD spectrum showed a positive band and a negative band. With the decrease of [porphyrin]/[DNA base pairs] ratio, the positive band gradually disappeared and the negative band went up. The result suggested that the binding mode of porphyrin–DNA was related to the [porphyrin]/[DNA base pairs] ratio.

3.2. Apparent binding constant measurements

UV–vis titration has been used to determine the apparent affinity binding constant (K_{app}) of cationic porphyrins for CT DNA [17,18]. However, this method has two severe limitations: firstly it is difficult to determine the absorption coefficient of bound porphyrin, secondly it is only applicable to intercalating porphyrins for which the Soret band is greatly modified upon binding to DNA [11]. In order to avoid these limits, fluorescence spectrum was used to measure K_{app} by competition between EB and the studied compound for binding to DNA. This method measures the decrease of fluorescence of EB bound to DNA in the presence of the compound of interest. It can be used for all compounds having a good affinity for DNA whatever their binding modes may be as it only measures the ability of a compound to prevent intercalation of EB into DNA [14].

According to the Scatchard equation, the interaction between EB and CT DNA can be described as [14]

$$r_{EB}/c_{EB} = K_{EB}(n - r_{EB}).$$

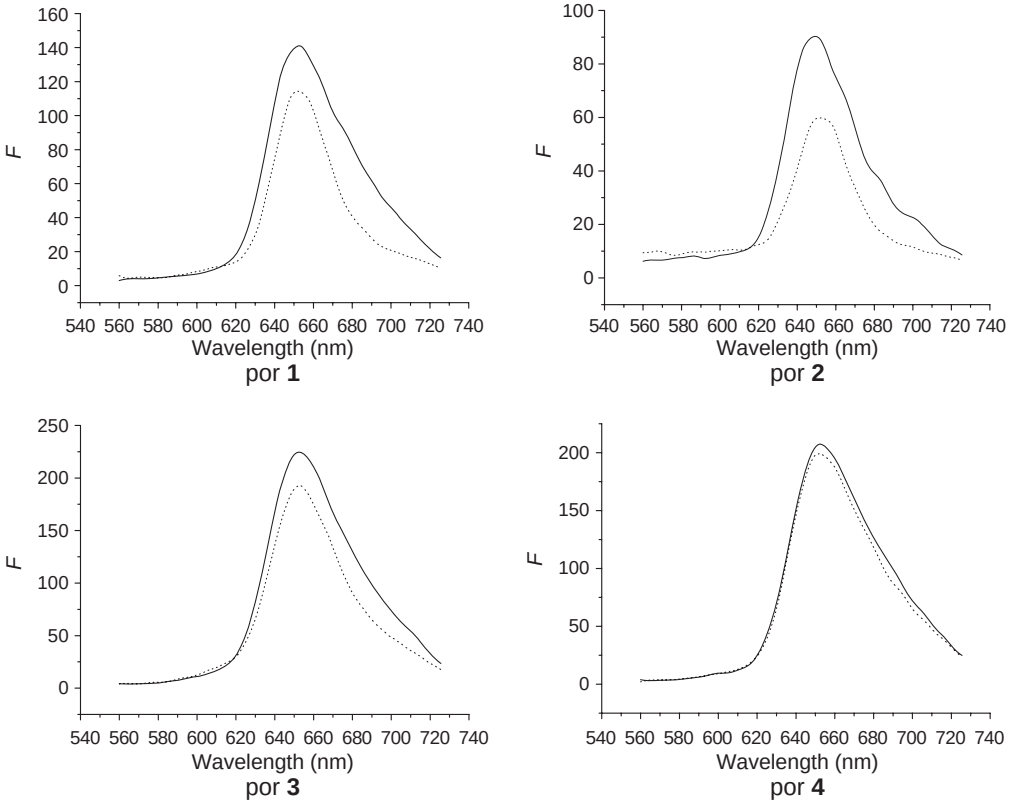

Fig. 3. Fluorescence emission of porphyrin **1**, **2**, **3** and **4** in the absence (—) and presence (---) of CT DNA, which their intensity changed maximally. [porphyrin]=1.0 μM in solution (pH=7.4, 0.05 M Tris–HCl, 0.1 M NaCl). Por **1**: [DNA base pairs]=8.80 μM; por **2**: [DNA base pairs]=3.51 μM; por **3**: [DNA base pairs]=3.51 μM; por **4**: [DNA base pairs]=1.76 μM.

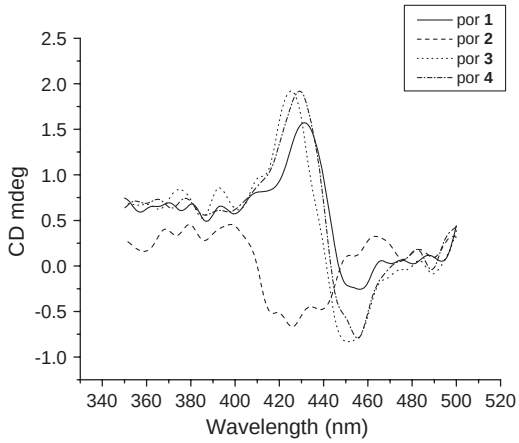

Fig. 4. Induced CD spetra of porphyrin **1**, **2**, **3** and **4** bound to CT DNA. [porphyrin]=5.0 μM in solution (pH=7.4, 0.05 M Tris–HCl, 0.1 M NaCl); [porphyrin]/[DNA base pairs] ratio, r=0.05.

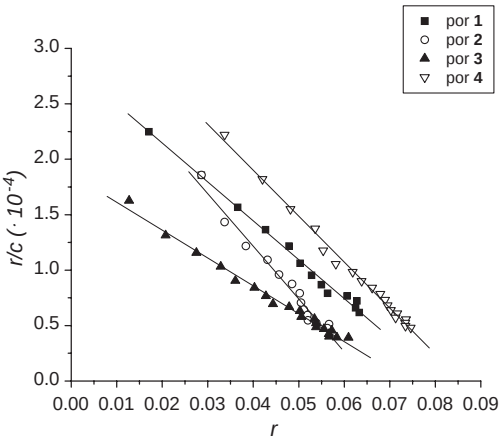

Fig. 6. Competition between porphyrins and EB for the binding sites of CT DNA (Scatchard plot). [porphyrin]=1.0 μM in solution (pH=7.4, 0.05 M Tris–HCl, 0.1 M NaCl).

When EB competes with porphyrin for DNA binding site, the interaction can be described as [14]

$$\frac{r_{EB}}{c_{EB}} = \frac{K_{EB}}{1 + K_{app} c_{por}} (n - r_{EB})$$

Where, K_{EB} and K_{app} are the binding constants of EB and porphyrin, respectively, for DNA, c_{EB} and c_{por} are the concentrations of free EB and porphyrin, r_{EB} is the number of moles of EB bound per mole of DNA, and n is the number of binding sites for EB on DNA. The Scatchard plots for the binding of EB to CT DNA in the presence of various porphyrins are shown in Fig. 6. The values of K_{app} obtained for these porphyrins are given in Table 1.

3.3. Photocleavage abilities of porphyrins to DNA

To investigate the dependence of porphyrin's binding on the photocleavage of DNA, photocleavage of DNA was examined in the presence of the four porphyrins. The DNA photocleavage products were detected by monitoring the conversion of supercoiled form (form I) to the nicked circular form (form II).

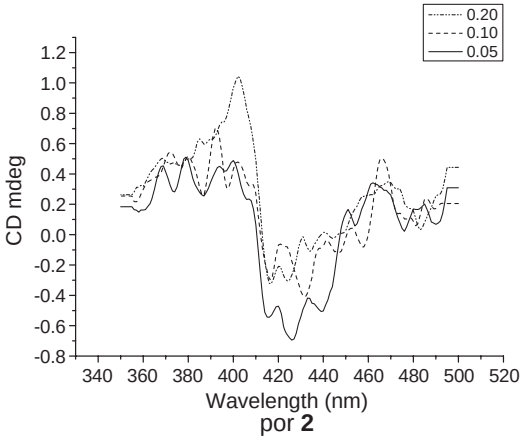

Fig. 5. Induced CD spectra of porphyrin **2** bound to CT DNA. [porphyrin]=5.0 μM in solution (pH=7.4, 0.05 M Tris–HCl, 0.1 M NaCl); [porphyrin]/[DNA base pairs] ratio, r=0.20, 0.10, 0.05.

The photocleavage of plasmid DNA introduced by 1 μM porphyrins by illumination for 25 min is shown in Fig. 7. The order of the cleavages was: **3**>**1**>**4**>**2**, which was consistent with the values of K_{app} of the porphyrins for DNA.

The photocleavage of plasmid DNA in the presence of 2 μM porphyrins illuminated for 13 min and the inhibition of the photocleavage in the presence of sodium azide is shown in Fig. 8. As the concentration of porphyrins increased to 2 μM, the order of the cleavages was: **3**>**1**>**2**>**4**. As can be seen, the effect of porphyrin **2** and **4** on the cleavage of DNA decreased. In the presence of sodium azide, the cleavage of DNA by the porphyrins was inhibited in various degrees. The order of the cleavages was: **1**>**2**>**3**>**4**, it showed that the cleavages of DNA by cationic porphyrin–phenylpiperazine hybrids **1** and **2** were less inhibited.

The DNA bands were filmed by gel imaging instrument, and the percentages were determined by examining the fluorescence intensities of EB–DNA complex. The cleavages of DNA in various conditions are depicted by the percentage of Form II in total DNA (Fig. 9).

4. Discussion

4.1. Binding modes of porphyrin–DNA

Three DNA binding modes for porphyrins have been reported [2,11,21]. *Intercalation into DNA base pairs* is characterized by a red shift (>10 nm) and decrease (up to

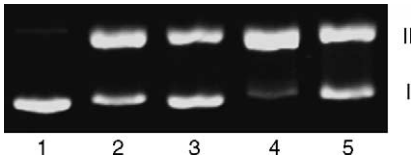

Fig. 7. Cleavage abilities of porphyrins in concentration (1 μM) by illumination for 25 min. 10 μL reaction mixtures contained 1.0 μg of plasmid DNA. Lane 1: DNA alone; lane 2: DNA+por **1**; lane 3: DNA+por **2**; lane 4: DNA+por **3**; lane 5: DNA+por **4**.

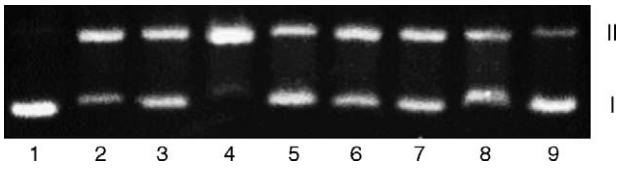

Fig. 8. Cleavage abilities of porphyrins in concentration (2 μM) by illumination for 13 min. 10 μL reaction mixtures contained 1.0 μg of plasmid DNA. Lane 1: DNA alone; lane 2: DNA+por **1**; lane 3: DNA+por **2**; lane 4: DNA+por **3**; lane 5: DNA+por **4**; lane 6: DNA+por **1**+sodium azide (10 mM); lane 7: DNA+por **2**+sodium azide (10 mM); lane 8: DNA+por **3**+sodium azide (10 mM); lane 9: DNA+por **4**+sodium azide (10 mM).

40%) of the Soret band of porphyrins in UV–vis spectra, a clear decrease of the intensity of fluorescence emission, a negative CD band in the Soret region, a relatively high binding constant for CT DNA. *External groove binding* is characterized by no (or minor) changes of UV–vis spectra, no (or minor) changes of fluorescence emission, a positive CD band in the Soret region, a relatively low binding constant for CT DNA. Another mode of *external binding to DNA* is characterized by a red shift and decrease of the Soret band, which are similar to those found for intercalating porphyrins.

Table 1 compares all experimental data obtained for the porphyrins under identical conditions of moderate ionic strength and large excess of CT DNA base pairs.

Although large hypochromic effect (>35%) and large red shift (>15 nm) in UV–vis spectra of porphyrin **3** and **4** bound to CT DNA as described for intercalation in the literature, small decrease (13% and 4%) in intensity of fluorescence emission, a strong positive band and a weaker negative band in induced CD spectra were shown.

A clear decrease of the intensity of fluorescence emission was thought to be a criterion for intercalation [11], and the decrease in fluorescence intensities upon the addition of the low concentrations of DNA were ascribed to self-stacking of the porphyrins along the DNA surface [22]. Small decrease in intensity of fluorescence emission at low concentrations of DNA indicated that the binding modes of porphyrin **3** and **4** bound to DNA inclined to outside binding. Especially for porphyrin **4**, only 4% decrease in intensity of fluorescence emission at a low concentration of DNA of 1.76 μM suggesting that the outside binding was primary. The bisignate nature of the induced CD was indicative that the binding modes were complex that involve both intercalation and outside binding, and the strong positive band suggested that the outside binding was primary. Porphyrin **4** showed a relatively low binding constant ($9.7 \times 10^5\ M^{-1}$) for CT DNA. It is possible due to the electronegative carboxyl group of porphyrin **4**, which resulted in a weaker affinity to CT DNA.

Upon binding with CT DNA the Soret bands of hybrids **1** and **2** showed small red shift and small hypochromicity in UV–vis spectra. However, clear decrease in intensity of fluorescence emission was observed. The curve of induced CD for porphyrin **1** bound to CT DNA was similar to those of porphyrin **3** and **4** with a decreasing positive peak and a decreasing negative peak. A clear decrease (19%) in intensity of fluorescence emission at a high concentration of DNA of 8.80 μM was observed. These observations suggested that the binding mode of porphyrin **1** to CT DNA is likely intercalation and less likely outside binding.

In the case of porphyrin **2**, at high [porphyrin]/[DNA base pairs] ratio, the induced CD showed a positive band and a negative band, which indicated a complex binding mode. With [porphyrin]/[DNA base pairs] ratio decreased, the positive band disappeared and the negative band went up (Fig. 5). These observations suggested that porphyrin **2** tended to intercalate into DNA at low [porphyrin]/[DNA base pairs] ratio. Most remarkable decrease (32%) in intensity of fluorescence emission and the induced CD indicated the binding mode of intercalation. The maximal decrease in intensity of fluorescence emission occurred at low concentrations of DNA of 3.51 μM, which might be the result of the strong ability of porphyrin **2** to intercalate into CT DNA.

Piperazine rings are capable of resting in the minor groove of GC base pairs [8,9], and cationic porphyrin prefer to intercalate into GC-rich regions of DNA [2–5]. When piperazine rings rest in the minor groove of GC base pairs, the porphyrin ring can selectively intercalate into GC base pairs of CT DNA. Only half of the porphyrin ring is necessary for intercalation to occur [11]. Porphyrin **2**, cationic porphyrin covalent bonded with two phenylpiperazines at *cis* position, can exhibit best intercalation into CT DNA. As a minor groove binder, piperazine ring affected the binding mode of porphyrin–DNA.

The apparent affinity binding constants (K_{app}) of the porphyrins for CT DNA were determined by using a competition method with EB, which only measured the ability of a compound to prevent intercalation of EB into DNA. The data K_{app} can present involves three types of binding modes. For example, the K_{app} of porphyrin **2** is low because porphyrin **2** is lack of a positive charge compared with the other porphyrins so that it has weaker electrostatic interaction with CT DNA.

4.2. Photocleavage of DNA

Cationic porphyrins show stronger electrostatic interaction with DNA due to their positive charges, and the stronger ability

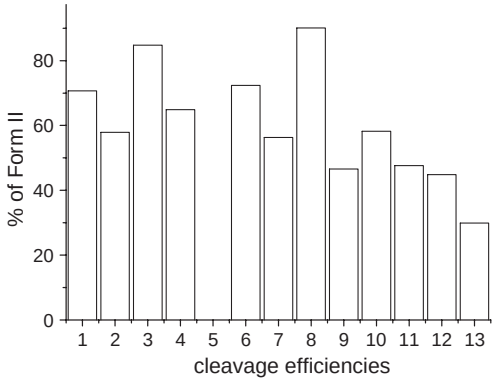

Fig. 9. Cleavages of DNA by these porphyrins in various conditions. Column 1–4: por **1**–**4** (1 μM) by illumination for 25 min. Column 6–9: por **1**–**4** (2 μM) by illumination for 13 min. Column 10–13: por **1**–**4** (2 μM) by illumination for 13 min in the presence of sodium azide.

to intercalate between DNA base pairs [23]. In the presence of light, the more the positive charges, the higher binding constant and the higher cleavage activity [23].

The photocleavage of pBR322 plasmid DNA in the presence of porphyrins has a close relationship with the values of the apparent affinity binding constant K_{app} (Fig. 7). There are many factors such as the number of positive charges, the electronegative or electropositive groups, the binding mode, the steric barrier effects and the interactions between the moieties onto the periphery of porphyrins and DNA are found to influence the values of K_{app} of porphyrin–DNA interactions.

When the concentration of porphyrins was 2 μM, at high [porphyrin]/[DNA base pairs] ratio, the binding mode tended to be outside binding [11]. The order of cleavages was: **3**>**1**>**2**>**4**, the effect on DNA cleavage is smaller in cases of porphyrin **2** and **4**. Because porphyrin **2** has only two positive charges and porphyrin **4** has an electronegative group, the mode of outside binding made the K_{app} values of porphyrin **2** and **4** decrease. However, the effect of porphyrin **2** on DNA was bigger than that of porphyrin **4**, which might result from the intercalation into CT DNA of porphyrin **2**.

In the presence of sodium azide, the cleavages of DNA introduced by the porphyrins were inhibited in various degrees. The cleavages of DNA by porphyrin **3** and **4** were seriously inhibited by 50% and 36%. But the cleavage of DNA by porphyrin **2** was inhibited only to 15%.

Type II photocleavages produce highly reactive state of oxygen as singlet oxygen. Nucleosides (mainly guanine) in DNA can react with singlet oxygen to result in DNA cleavage [24]. Cationic porphyrin–phenylpiperazine hybrids can intercalate into GC base pairs of DNA, and show intimate association with guanine. Therefore, the diffusion distance of singlet oxygen needed to react with guanine was short, and singlet oxygen was not easily quenched before reaction. In the presence of sodium azide, the cleavages of DNA by the porphyrin of intercalation into DNA were less inhibited.

5. Conclusions

Based on absorption, fluorescence, induced CD spectra, and K_{app} measurement, the binding modes of cationic porphyrin–CT DNA were investigated. With a moderate ionic strength, the binding of porphyrin **1**, **3** and **4** to DNA were complex that involves both intercalation and outside binding. The decrease in intensity of fluorescence emission can be a criterion for intercalation. Intercalation of porphyrin into CT DNA occurred when two phenylpiperazines were introduced at *cis* position onto the periphery of cationic porphyrin. The photocleavages of DNA introduced by the porphyrins were consistent with their values of K_{app}. With [porphyrin]/[DNA base pairs] ratio increased, the cleavage abilities of the porphyrins varied following the change of binding mode and K_{app}. In the presence of sodium azide, the cleavage of DNA introduced by the porphyrin of intercalation into DNA was less inhibited.

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