Pergamon

Bioorganic & Medicinal Chemistry 10 (2002) 1953–1960

BIOORGANIC & MEDICINAL CHEMISTRY

Potent DNA Photocleavage by Zinc(II) Complexes of Cationic Bis-porphyrins Linked with Aliphatic Diamine

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Received 30 October 2001; accepted 14 December 2001

Abstract—We have prepared zinc(II) complexes of cationic bis-porphyrins, as one of the attempts to improve less DNA photocleavage activities of the metal-free bis-porphyrins composed of two H_2TMPyP-like chromophores, linked with a series of aliphatic diamines. The less activities seemed to be derived from their intermolecular self-aggregation properties in aqueous solution. The zinc(II) insertion into the metal-free cationic bis-porphyrins completely removed their self-aggregation properties, most probably due to steric hindrance between axial ligands of zinc(II) chromophores of the cationic bis-porphyrins. The DNA photocleavage activities of the zinc(II) complexes were fully enhanced, which were three times larger than that of the lead compound H_2TMPyP. Quantitative analysis of singlet oxygen production by photosensitization of cationic bis-porphyrins was performed using 1,3-diphenylisobenzofuran, and the singlet oxygen productivities of them were found to be related to their solution properties. There is a good relationship between the activities and the productivities, which will provide insights into the further development of more effective DNA photocleavage agents.

Introduction

The cationic *meso*-tetrakis(*N*-methyl-4-pyridyl)porphine (H_2TMPyP) and their metal complexes have been widely noticed for the interaction with nucleic acids and the biomedical applications.[1–4] In a molecular level, the macrocycles tightly bind to duplex,[5–9] triplex,[10,11] and quadruplex DNA,[12–15] and efficiently cleave DNA on illumination[16–18] or in the presence of reducing/oxidizing agents.[19–21] These versatile abilities might allow them to serve medicinally as anti-cancer and anti-viral drugs.[4] The original work for analysis of the interaction of H_2TMPyP with DNA was started by Fiel and coworkers for the purpose of developing DNA-targeting photosensitizers, because selective photocleavage of DNA in malignant cells might give lethal damage to the cells.[1] The cationic photosensitizer showed unexpected result to intercalate between base pairs,[5] and could photocleave DNA much more effectively than hematoporphyrin derivative (HPD).[16] HPD is a complex mixture of oligo-porphyrins derived from hematoporphyrin,[22,23] and a purified fraction of HPD, Photofrin, is now clinically used in photodynamic therapy (PDT) of cancer.[24] Later, it was found that the cationic porphyrin was well taken up by HeLa cells and showed high phototoxicity, the extent of cellular damage being dependent on the light dose.[25] Thus, H_2TMPyP is worth considering as a potential PDT candidate because of its excellent selectivity to tumor tissue and anti-tumor activity on photoexcitation, derived from in vivo studies.[26,27]

Although the extensive studies on unichromophore H_2TMPyP with DNA have been developed, very few are reported on the interaction of cationic oligo-porphyrins with DNA.[17] A molecule composed of two or more H_2TMPyP-like chromophores is expected to exhibit higher photonuclease activity than the lead compound H_2TMPyP, and hence to be a candidate for PDT. We have recently reported the synthesis of cationic bis-porphyrins, being composed of two H_2TMPyP-like chromophores, linked with a series of aliphatic diamines like ethylenediamine (**4**), 1,6-diaminohexane (**5**), and 1,10-diaminodecane (**6**), and analysis of their solution properties, interaction with calf thymus DNA (ctDNA), and photocleavage of plasmid DNA.[28] The cationic bis-porphyrins formed intermolecular dimers in aqueous solution, and bound to ctDNA with outside self-stacking on the DNA surface. Their DNA photocleavage activities diminished as the number of their linker hydrocarbons increased, and well correlated with tendency for them to dimerize. As a result, these cationic

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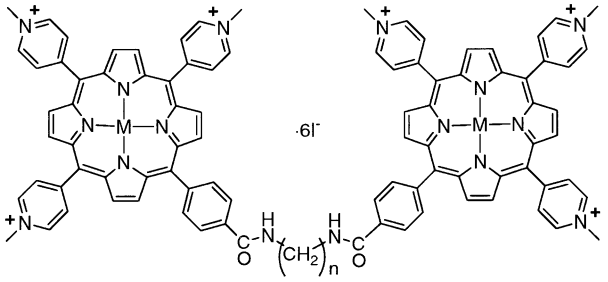
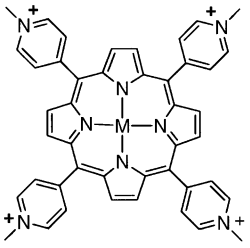
0968-0896/02/$ - see front matter
PII: S0968-0896(02)00003-2

bis-porphyrins did not show expected photonuclease activity, and their intermolecular dimers did not seem to be responsible for the activity.



M	n	
Zn^{2+}	2	**1**
Zn^{2+}	6	**2**
Zn^{2+}	10	**3**
$2H^{+}$	2	**4**
$2H^{+}$	6	**5**
$2H^{+}$	10	**6**



M	
Zn^{2+}	ZnTMPyP
$2H^{+}$	H_2TMPyP

As one of our attempts to bring out their potentialities, we have prepared zinc(II) complexes of the cationic bis-porphyrins linked with ethylenediamine (**1**), 1,6-diaminohexane (**2**), and 1,10-diaminodecane (**3**), which have d^{10} electronic configurations and five-coordination structures at the metal. We investigated the solution properties, DNA photocleavage activities, singlet oxygen productivities, and DNA-binding properties of **1**, **2**, and **3**. In this paper, we report that the zinc(II) complexes showed greatly enhanced DNA photocleavage ability, and the activities of the cationic porphyrins examined well correlated with the singlet oxygen productivities of them.

Results

Solution properties

Absorption spectra of **1**–**3** were recorded in DMSO and in a buffer solution containing 10 mM sodium phosphate and 100 mM sodium chloride (pH 7.0). Extinction coefficients of the Soret maximum (λ_{max}^{Soret}) of **1**–**3** in DMSO ($\varepsilon_{DMSO}^{Soret}$) and in the buffer ($\varepsilon_{buffer}^{Soret}$) were ca. 6.0×10^5 M^{-1} cm^{-1} and ca. 3.3×10^5 M^{-1} cm^{-1}, respectively. The values of the $\varepsilon_{DMSO}^{Soret}$ for **1**–**3** were commonly 1.8 times as large as those of the $\varepsilon_{buffer}^{Soret}$, and hence a significant solvent effect was clear. In the Q-band region of the spectra of **1**–**3** two peaks were observed, indicative of the completion of complexation between metal-free cationic bis-porphyrins **4**–**6** and zinc(II) ion.[29] The formation of the zinc(II) complexes was also supported by disappearance of pyrrolic imino proton peak in their ^{1}H NMR spectra.

Figure 1 shows plots of the $\varepsilon_{buffer}^{Soret}$ of **1** and **4** at their various concentrations. In our previous report, it was shown that the $\varepsilon_{buffer}^{Soret}$ of the metal-free cationic bis-porphyrins including **4**–**6** decreased with increase in their concentrations (**4**: 3.28×10^5 M^{-1} cm^{-1} at 0.8 μM to 1.28×10^5 M^{-1} cm^{-1} at 60 μM), and the hypochromicities were based on the formation of intermolecular dimers.[28] On the other hand, the $\varepsilon_{buffer}^{Soret}$ of **1**–**3**, which were the zinc(II) complexes of **4**–**6**, remained unchained at their concentrations ranged from 0.9 to 60 μM. This independence of the $\varepsilon_{buffer}^{Soret}$ of **1**–**3** from variations of their concentrations indicates that each molecule of the zinc(II) complexes remained monomeric in aqueous solution.

Photocleavage of pUC18 plasmid DNA

DNA photocleavage activity by cationic porphyrins was examined using supercoiled double-stranded pUC18 plasmid DNA. A mixture of the cationic porphyrin (0.6 μM) and the plasmid DNA (60 μM, R ([porphyrin]/[DNA base pair]) = 0.01) in the buffer was illuminated in air at 440 nm. After illumination, conversion of supercoiled DNA (form I) to nicked circular DNA (form II) was visualized by agarose gel electrophoresis and subsequent ethidium bromide staining. As shown typically in Figure 2 for **1**, H_2TMPyP, and the zinc(II) complex of H_2TMPyP (ZnTMPyP), all the cationic porphyrins assayed showed linear increase of form II DNA as illumination time increased. Conversion rates by photoactivated porphyrins are summarized in Table 1. The DNA photocleavage by the zinc(II) complexes of the cationic bis-porphyrins **1**–**3** (5.8–6.1%/min) did not generally depend on the difference of their linker lengths, and was three times more efficient than that by the lead compound H_2TMPyP (1.8%/min). Moreover, **1**, **2**, and **3** showed roughly three, four, and five times

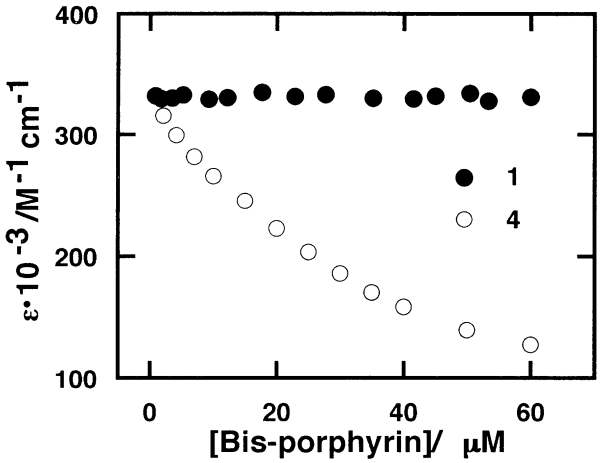


Figure 1. Plots of the extinction coefficients of **1** (filled circles) and **4** (open circles) at the Soret maximum versus their concentrations in the buffer (10 mM sodium phosphate and 100 mM NaCl, pH 7.0).

Table 1. Conversion rates of form I to form II DNA by photosensitization of cationic porphyrins

	1	2	3	4[a]	5	6[a]	H_2TMPyP	ZnTMPyP
%/min	6.1	6.0	5.8	2.1	1.3	1.1	1.8	2.9

[a]Ref. 28.

more enhanced conversion efficiency than metal-free **4** (2.1%/min), **5** (1.3%/min), and **6** (1.1%/min) did, respectively. Hence, zinc(II) insertion into the metal-free cationic bis-porphyrins allowed their large enhancement of the DNA photocleavage activity.

To confirm singlet oxygen involvement in the system, DNA photocleavage experiments using sodium azide NaN_3 or deuterium oxide D_2O were performed. It is well known that NaN_3 is an excellent singlet oxygen quencher,[30] and D_2O makes the lifetime of singlet oxygen longer.[31] Figure 3(a) shows an inhibitory effect of NaN_3 with increase in NaN_3 concentration on efficiency of the DNA photocleavage by **1** (0.6 μM, $R=0.01$). Almost complete inhibition was observed in the presence of 100 mM NaN_3, whereas the inhibition by NaCl was moderate. In addition, the conversion rate by photoactivated **1** in the solution of 70% D_2O was 7.6%/min, and thus the efficiency actually increased by replacing the reaction media of H_2O by D_2O [Fig. 3(b)].

Measurement of singlet oxygen production

Because singlet oxygen was clearly responsible for the DNA photocleavage by the cationic bis-porphyrins in our system, photosensitized production of singlet oxygen was estimated quantitatively by measuring the decomposition of 1,3-diphenylisobenzofuran (DPBF).[32,33] DPBF directly reacts with singlet oxygen and subsequently decomposes to 1,2-dibenzoylbenzene.[34] As shown in Figure 4 for **1**, **6**, and H_2TMPyP, absorbance of DPBF (30 μM) at 415 nm linearly decreased in the presence of each cationic porphyrin (0.05 μM) as

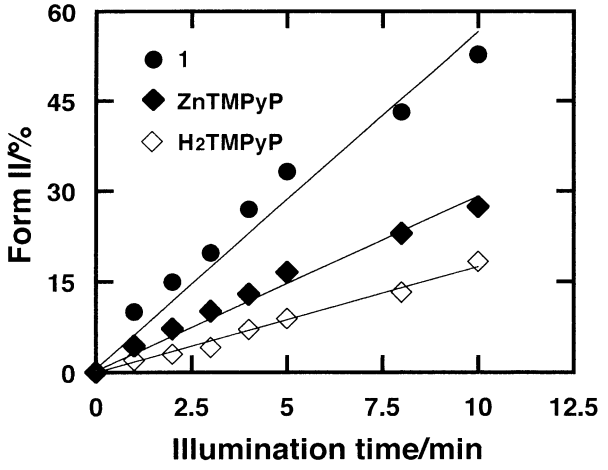


Figure 2. Increase in form II DNA photo-induced by **1** (filled circles), ZnTMPyP (filled rhombuses), and H_2TMPyP (open rhombuses) versus illumination time. Cleavage conditions: 0.6 μM **1**, ZnTMPyP, and H_2TMPyP; 60 μM pUC18 plasmid DNA, 10 mM sodium phosphate and 100 mM NaCl (pH 7.0); illumination at 440 nm and 25 °C.

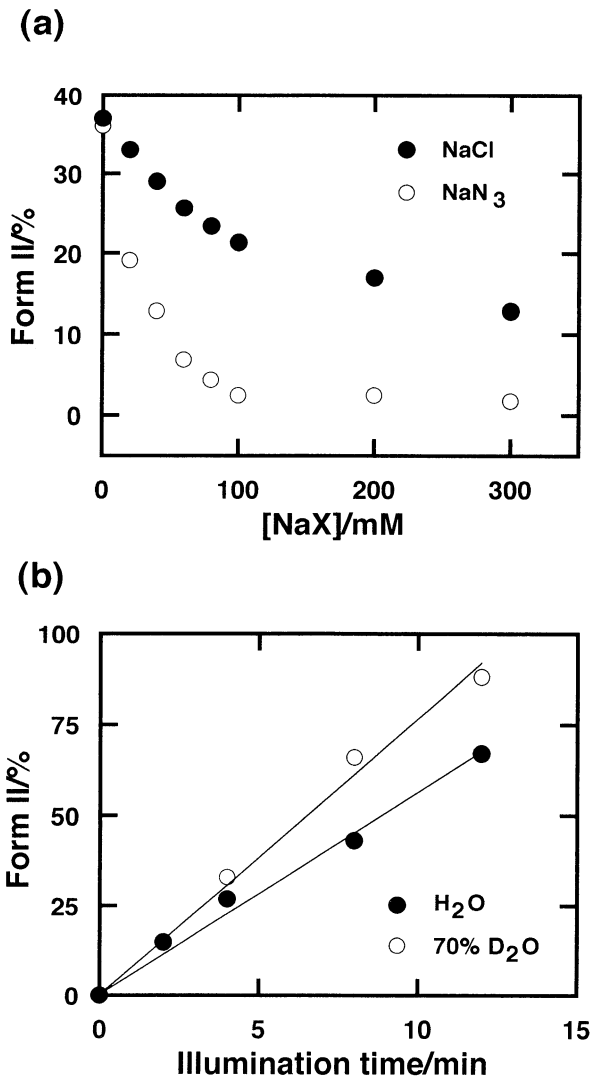


Figure 3. (a) Inhibitory effect of NaCl (filled circles) and NaN_3 (open circles) on the formation of form II DNA photo-induced by **1**. (b) Increase in form II DNA photo-induced by **1** in the reaction media of H_2O (filled circles) and 70% D_2O (open circles) versus illumination time. Cleavage conditions: 0.6 μM **1**; 60 μM pUC18 plasmid DNA, 10 mM sodium phosphate and 100 mM NaCl (pH 7.0); illumination at 440 nm and 25 °C.

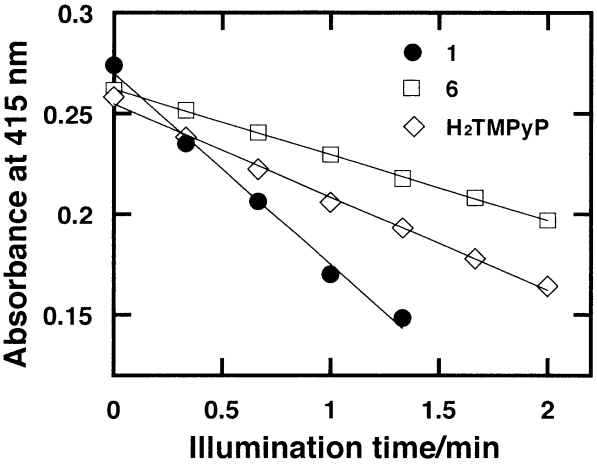


Figure 4. Decomposition of DPBF by photosensitization of **1** (filled circles), **6** (open squares), and H_2TMPyP (open rhombuses). Conditions: 30 μM DPBF; 0.05 μM **1**, **6**, and H_2TMPyP; 25 °C.

illumination time increased. The slopes of the plots of bleached absorption of DPBF versus illumination time, and the normalized slopes (NS) based on the slope for H_2TMPyP are listed in Table 2. The values of NS of metal-free **4**–**6** decreased as their linker lengths were longer (1.06–0.70), and the tendency corresponded with that of their DNA photocleavage activities. On the other hand, NS values of the zinc(II) complexes **1**–**3** were almost the same (1.92–2.05), and two to three times as large as those of **4**–**6**, or H_2TMPyP. Thus, zinc(II) insertion into the metal-free cationic bis-porphyrins enhanced their singlet oxygen productivities effectively.

Table 2. The slopes (S) of the plots of bleached absorption of DPBF by photosensitization of cationic porphyrins, and the normalized slopes (NS) based on the slope for H_2TMPyP

	1	2	3	4	5	6	H_2TMPyP	ZnTMPyP
$S\times10^2$/min	9.50	9.26	8.90	4.93	3.68	3.26	4.64	5.78
NS	2.05	2.00	1.92	1.06	0.79	0.70	1.00	1.25

Interaction with ctDNA

A buffered solution of **1**–**3** was titrated with a stock solution of ctDNA. Intensity of λ_{max}^{Soret} for **1**–**3** decreased at an initial stage, and then increased with further ctDNA additions. A spectral change of **1** (4.2 μM) with addition of ctDNA is shown in Figure 5(a). After an initial hypochromicity was accompanied by a slight red-shift (5 nm) of the λ_{max}^{Soret}, an increase in its intensity and additional hyperchromicity was observed without any shift of the λ_{max}^{Soret}. The hyperchromicity was not seen for metal-free **4**–**6**. Figure 5(b) shows an absorbance change of **1** at 435 nm with addition of ctDNA. The absorbance first decreased at $R \geq 0.30$, and then increased and reached its initial absorbance at $R = 0.14$, and finally almost remained constant at $R < 0.14$. The absorbance changes shown by **1**–**3** titrated with ctDNA demonstrated that the binding process was not uniform.

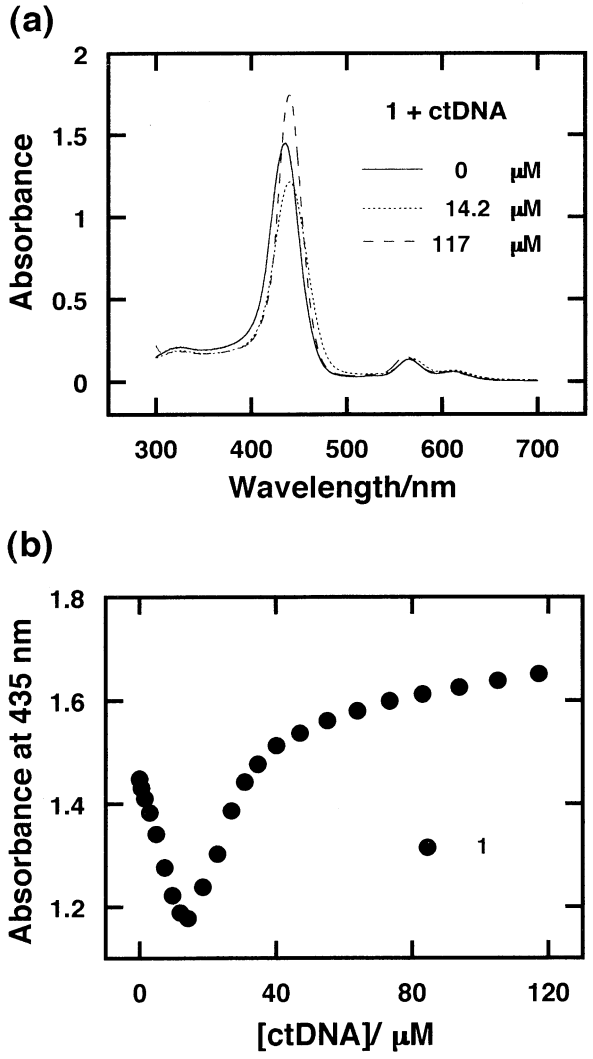


Figure 5. (a) Absorption spectral change of **1** (4.2 μM) with addition of ctDNA in the buffer (10 mM sodium phosphate and 100 mM NaCl, pH 7.0). (b) Absorbance change of **1** at 435 nm with addition of ctDNA.

Addition of an excess of ctDNA to the zinc(II) complexes of the cationic bis-porphyrins gave characteristic induced circular dichroism (ICD) in the Soret region in the buffer. Figure 6 shows ICD spectra of **3** and ZnTMPyP at $R = 0.01$. For **1**, **3**, and ZnTMPyP two positive peaks, major at around 430 nm and minor at around 415 nm, were observed, and also their band shapes were similar to each other. For **2** single positive peak at 417 nm was seen. Magnitudes of the molar ellipticities of the major positive peaks for **1**–**3** (40–70 M^{-1} cm^{-1}) were much larger than that for ZnTMPyP (≈10 M^{-1} cm^{-1}). The positive sign in the ICD spectra indicates that their binding mode to ctDNA was groove binding.[6] Figure 6 also shows representative ICD results after addition of an excess of distamycin A ([distamycin A]/[ctDNA base pair] = 0.6), a well-known minor groove binder, to **3**-ctDNA or ZnTMPyP-ctDNA mixture ($R = 0.01$). The band shape of their ICD spectra did not change in spite of the presence of distamycin A, while small reduction of their molar ellipticities was observed.

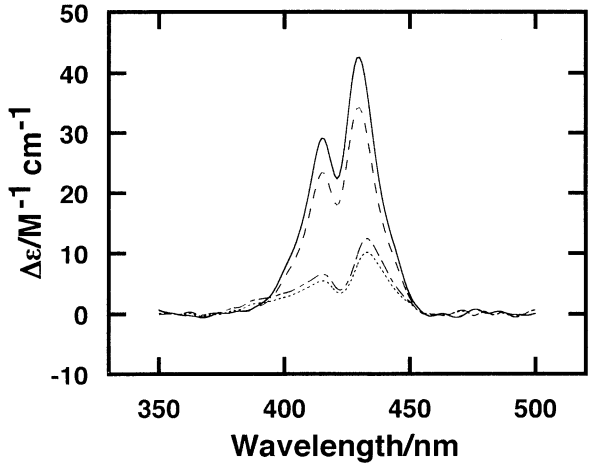


Figure 6. ICD spectra of zinc(II) porphyrins in the presence of ctDNA: **3** (——) and ZnTMPyP (·····) in the absence of distamycin A; **3** (- - - - -) and ZnTMPyP (·····) in the presence of distamycin A. Conditions: [porphyrin]/[ctDNA] = 0.01; [distamycin A]/[ctDNA] = 0.6; 10 mM sodium phosphate and 100 mM NaCl (pH 7.0).

Discussion

The focus of this research was to improve the DNA photocleavage activity of the cationic bis-porphyrins linked with a series of aliphatic diamines. In the previous paper, we reported the synthesis and analysis of solution properties, interaction with ctDNA, and plasmid DNA photocleavage activities of the cationic bis-porphyrins, *N*,*N*′-bis{4-[10,15,20-tris(1-methylpyridinium-4-yl)porphyrin-5-yl]benzoyl}oligomethylenediamine hexaiodide, composed of two H_2TMPyP-like chromophores.[28] Because cationic H_2TMPyP shows high DNA photocleavage activity, the cationic bis-porphyrins were expected to show the higher activity. However, the conjugates did not show satisfactory results in comparison with H_2TMPyP. Because their DNA photocleavage activities diminished as the number of their linker hydrocarbons increased, and strictly correlated with tendency for the bis-porphyrins to self-aggregate in aqueous solution, their intermolecular dimers were found to be inactive. So, it was assumed that sufficient DNA photocleavage by the cationic bis-porphyrins could be achieved by relaxation of the self-aggregation of the bis-porphyrins. Therefore, we attempted to prepare the zinc(II) complexes of the cationic bis-porphyrins **1**, **2**, and **3**, which have d^{10} electronic configurations and 5-coordination structures.[4] We expected that self-aggregation could be weakened by steric hindrance between axial ligands of zinc(II) chromophore units of the cationic bis-porphyrins.

From the viewpoint of the relationship between their solution properties and the DNA photocleavage activities, we first investigated the solution properties of the zinc(II) complexes **1**–**3**. At 0.8–0.9 μM, the value of $\varepsilon_{buffer}^{Soret}$ for **1** was similar to that for the metal-free **4**. In the case of **4**, however, the $\varepsilon_{buffer}^{Soret}$ reduced with increase in its concentration. As reported previously, the hypochromicity due to self-aggregation was shown in all cases of the metal-free cationic bis-porphyrins linked with a series of aliphatic diamines. Their self-aggregation properties were expressed by a simple monomer-dimer equilibrium. In contrast, the values of $\varepsilon_{buffer}^{Soret}$ for **1**–**3** remained unchanged with increase in their concentrations. The transition of their solution characters, accompanied by zinc(II) insertion into the bis-porphyrins, should be based on the relaxation of intermolecular interaction by steric hindrance between the axial ligands. It appears that π–π interaction between the ZnTMPyP-like chromophores was disturbed by the steric hindrance. Accordingly, it is suggested that planarity of the H_2TMPyP-like chromophore of the metal-free cationic bis-porphyrins resulted in the self-aggregation property. The linker hydrophobicity is likely to be an additional factor for the self-aggregation. The solvent effect observed in the case of **1**–**3** is indicative of the presence of certain intramolecular interaction between ZnTMPyP-like chromophores.

The DNA photocleavage by the zinc(II) complexes of the cationic bis-porphyrins was investigated using pUC18 plasmid DNA by electrophoretic technique. Form I plasmid DNA was converted to form II DNA by illumination at Soret maximum on binding to DNA. Form II DNA linearly increased in the presence of the cationic porphyrins, as illumination time became longer (Fig. 2). The conversion rates of form I to form II DNA by the photosensitization of **1**–**3** were almost the same among them, regardless of their different linker lengths, and were twice and three times larger than that for ZnTMPyP and H_2TMPyP, respectively. In addition, the DNA photocleavage activity of **1**, **2**, and **3** was approximately three, four, and five times higher than **4**, **5**, and **6**, respectively. The effect of zinc(II) insertion into the porphyrin cores of **4**–**6** on the activity was outstanding. Thus, the zinc(II) ions and the coordinated axial ligands of **1**–**3** played a crucial role for the enhanced DNA photocleavage activity. Although intramolecular interaction between ZnTMPyP-like chromophores of **1**–**3** might be present, the extent of the interaction seems to be not so strong as to reduce their potential abilities to photocleave DNA.

It is well known that photosensitization of dyes promotes DNA strand breaks via three main pathways: hydroxyl radical attack, electron transfer process (type I mechanism), or oxidation by singlet oxygen (energy transfer process, type II).[35] In our previous paper, the involvement of singlet oxygen in the DNA photocleavage caused by the photosensitization of the cationic bis-porphyrins including **4**–**6** was evidenced from singlet oxygen-quenching and its lifetime-extending experiments. Here again, in order to confirm whether the DNA photocleavage by the zinc(II) complexes proceeds via type II mechanism, we examined the extent of the topological change of the plasmid DNA by the photosensitization of **1** using both sodium azide as singlet oxygen quencher and deuterium oxide as its lifetime extender. While the moderate inhibitory effect was seen in the presence of 100 mM NaCl on the DNA photocleavage, the presence of 100 mM NaN_3 almost completely blocked. Based on these results, the inhibitory effect of NaN_3 should be explained by quenching of short-lived singlet oxygen generated from photoactivated **1** and ground-state molecular oxygen, in addition to blocking of the binding of **1** to DNA. Moreover, the fact that replacing the reaction media of H_2O by D_2O raised the conversion rate about 25% reinforced the event of the DNA photocleavage by **1** via type II mechanism.

Singlet oxygen production by the photosensitization of these cationic bis-porphyrins was supported by linear reduction of the absorbance of DPBF as illumination time became longer. DPBF is known to singlet oxygen quencher,[36,37] and was used for quantitative analysis of singlet oxygen production.[33] The slope of the plot of bleached absorption versus illumination time is proportional to the rate of production of singlet oxygen.[32] The values of NS of **4**–**6** decreased as their linker lengths were longer, which well corresponded with the tendency for them to dimerize and to be less active in the DNA photocleavage. On the other hand, while the values for **4**–**6** were equal to or smaller than that for H_2TMPyP, the zinc(II) complexes **1**–**3** showed twice higher singlet oxygen production rate than H_2TMPyP did. In addition, the zinc(II) insertion into the porphyrin cores of **4**–**6**

increased their production rates by twice or three times. The extent of interchromophoric interaction for **1**–**6** is likely to significantly influence the singlet oxygen productivity.

In the visible spectral change of **1**–**3** with addition of a large quantity of ctDNA, the characteristic hyperchromicity of their λ_{max}^{Soret} was observed, not seen in the case of **4**–**6**. Judging from the small red-shift (5 nm), the binding mode of **1**–**3** was not intercalated because the intercalated complex is characterized by substantial hypochromicity of the Soret band ($\geq$35%) and large red-shift ($\geq$15 nm).[6] Any steady isosbestic points were not seen through the titaration with ctDNA, indicative of the binding being complex. The absorbance of **1** at 435 nm first decreased with addition of ctDNA at $R \geq 0.3$, in turn increased at $R \geq 0.3$, reached the initial intensity at $R = 0.14$, and completed at $R < 0.14$. This absorbance change might be explained by a series of the behavior of highly ordered self-aggregation, disruption of the self-aggregation, and relaxation of intramolecular, interchromophoric interaction of the monomeric zinc (II) complexes of the cationic bis-porphyrins.

The sign of ICD spectra of **1**–**3** in the Soret region in the presence of ctDNA was positive, which implies that the binding mode of the complexes was groove binding. Furthermore, the band shape of the ICD spectra of **1** and **3** was quite similar to that for ZnTMPyP, and also remained unchanged in the presence of a large quantity of distamycin A, a famous minor groove binder.[38] Recently, Tjahjono and co-workers reported that ZnTMPyP is bound face-on at the 5′-AT-3′ step of the major groove of ctDNA, judging from CD and MCD spectral changes.[39] The fact that the band shape of the ICD spectra of **1**–**3** and ZnTMPyP remained unchanged even in the presence of distamycin A, and the ICD spectral similarity between **1**–**3** and ZnTMPyP suggest the zinc(II) complexes were bound mainly at the major groove of ctDNA. The zinc(II) insertion into the metal-free cationic bis-porphyrins resulted in the binding mode transition from self-stacking on the DNA surface to plausible major groove binding.

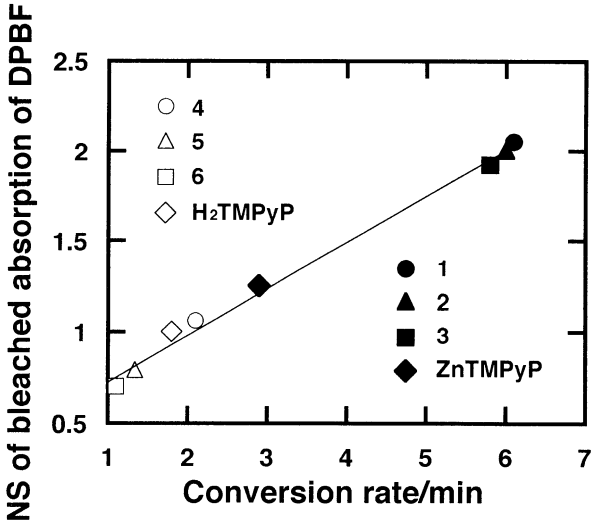


Figure 7. Relationship between the conversion rates of form I to form II DNA and normalized slopes of bleached absorption of DPBF by photosensitization of cationic porphyrins.

Figure 7 shows a good relationship between the conversion rates of form I to form II DNA and the normalized slopes of bleached absorption of DPBF by the photosensitization of the cationic porphyrins. Clearly, the DNA photocleavage by the cationic porphyrins in our system solely depended on their singlet oxygen productivities, and was found to proceed via type II mechanism. The photosensitization for **1**–**6** was certainly dominated by the extent of interchromophoric interaction for the cationic bis-porphyrin in aqueous solution.

Conclusion

We demonstrated the solution properties, DNA photocleavage activities, singlet oxygen productivities, and DNA-binding properties of novel zinc(II) complexes of the cationic bis-porphyrins linked with an aliphatic diamine. The zinc(II) insertion into the metal-free bis-porphyrins completely removed their self-aggregation properties in aqueous solution, fully enhanced the DNA photocleavage activities and the singlet oxygen productivities, and reasonably changed their binding modes to ctDNA from outside self-stacking to groove binding. There is a good correlation between the activities and the productivities, which will provide insights into the further development of more effective DNA photocleavage agents.

Experimental

Reagents for the synthesis of cationic bis-porphyrins and the zinc(II) complexes were purchased from Tokyo Kasei Chemical Co and Wako Pure Chemical Industries. The cationic bis-porphyrins **4**, **5**, and **6** were synthesized according to the previous method.[28] H_2TMPyP was purchased from Dojin Chemical Co. CtDNA and distamycin A hydrochloride were purchased from Sigma Chemical Co, and their solutions were quantitated spectrophotometrically using $\varepsilon_{260} = 13{,}200\ M(\text{base pairs})^{-1}\ cm^{-1}$ and $\varepsilon_{303} = 37{,}000\ M^{-1}\ cm^{-1}$, respectively. All buffered solutions were comprised of 10 mM sodium phosphate and 100 mM sodium chloride (pH 7.0). ^{1}H NMR spectra were recorded on a JEOL GX-400 or JNM-A-500 spectrometer. Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectra (MS) were measured on a Bruker REFLEX™. The UV–visible absorption measurement was performed on a Beckman DU650 spectrophotometer. The CD spectra were recorded on a JASCO J-720 spectropolarimeter. Elemental analyses were performed at the Analytical Center, Kumamoto University.

General procedure for the preparation of zinc(II) complexes of cationic bis-porphyrins linked with aliphatic diamine

A metal-free cationic bis-porphyrin hexaiodide (30 mg, 1.2×10^{-5} mol) and ZnI_2 (780 mg, 2.4×10^{-3} mol) were

dissolved in H_2O-DMF mixed solvent (20 mL, H_2O:DMF = 1:2), and then refluxed for 12 h. After cooling to room temperature, the precipitated white solid of unreacted ZnI_2 was removed by filtration. The filtrate was evaporated, and addition of diethyl ether to the residue gave a purple solid, which was collected by centrifugation and dried. This product was recrystallized for several times by vapor diffusion method using DMF and diethyl ether.

1 (the zinc(II) complex of 4). Yield: 65%. ^{1}H NMR (DMSO-d_6) δ: 3.79 (4H, s), 4.71 (12H, s), 4.72 (6H, s), 8.33 (4H, d, J = 7.8 Hz), 8.42 (4H, d, J = 7.8 Hz), 8.93 (12H, d, J = 6.3 Hz, br) 9.06 (16H, m), 9.42 (12H, d, J = 6.3 Hz, br). UV λ_{max} (DMSO) nm (ε): 607 (15,300), 563 (42,700), 438 (602,000); (buffer) nm (ε): 610 (13,500), 564 (32,100), 435 (332,000). MALDI-TOF-MS m/z: 1565.0 (M^+, calcd for $C_{92}H_{72}N_{16}O_2Zn_2$: 1564.5). Anal. calcd for $C_{92}H_{72}N_{16}O_2Zn_2I_6 \cdot 12H_2O \cdot 0.5DMF$: C, 43.55; H, 3.89; N, 8.96. Found: C, 43.65; H, 3.70; N, 8.84.

2 (the zinc(II) complex of 5). Yield: 66%. ^{1}H NMR (DMSO-d_6) δ: 1.58 (4H, s, br), 1.78 (4H, s, br), 3.51 (4H, s, br), 4.71 (12H, s), 4.72 (6H, s), 8.29 (4H, d, J = 8.3 Hz), 8.34 (4H, d, J = 8.3 Hz), 8.90 (12H, d, J = 6.3 Hz, br) 9.06 (16H, m), 9.42 (12H, d, J = 6.3 Hz, br). UV λ_{max} (DMSO) nm (ε): 606 (14,800), 563 (41,400), 437 (610,000); (buffer) nm (ε): 613 (12,600), 567 (26,200), 433 (329,000). MALDI-TOF-MS m/z: 1621.0 (M^+, calcd for $C_{96}H_{80}N_{16}O_2Zn_2$: 1620.6). Anal. calcd for $C_{96}H_{80}N_{16}O_2Zn_2I_6 \cdot 8H_2O \cdot 0.5DMF$: C, 45.70; H, 3.91; N, 9.02. Found: C, 45.89; H, 4.03; N, 8.81.

3 (the zinc(II) complex of 6). Yield: 60%. ^{1}H NMR (DMSO-d_6) δ: 1.43 (8H, s, br), 1.45 (4H, s, br), 1.71 (4H, m, br), 3.45 (4H, m, br), 4.71 (12H, s), 4.72 (6H, s), 8.27 (4H, d, J = 8.3 Hz), 8.32 (4H, d, J = 8.3 Hz), 8.90 (12H, d, J = 6.8 Hz, br) 9.06 (16H, m), 9.42 (12H, d, J = 6.8 Hz, br). UV λ_{max} (DMSO) nm (ε): 606 (14,300), 564 (40,900), 438 (602,000); (buffer) nm (ε): 610 (12,200), 565 (29,300), 433 (330,000). MALDI-TOF-MS m/z: 1677.2 (M^+, calcd for $C_{100}H_{88}N_{16}O_2Zn_2$: 1676.7). Anal. calcd for $C_{100}H_{88}N_{16}O_2Zn_2I_6 \cdot 8H_2O \cdot 0.5DMF$: C, 46.55; H, 4.14; N, 8.83. Found: C, 46.62; H, 3.97; N, 8.54.

DNA photocleavage assay

Photoillumination was performed at 25 °C using a HITACHI 650-60 fluorescence spectrophotometer, equipped with 150 W Xe lamp. A sample containing supercoiled pUC18 plasmid DNA and a Zn(II) bis-porphyrin was illuminated in the buffer at 440 nm. After illumination, DNA was analyzed by agarose gel (0.8%) electrophoresis at 100 V. The gel was incubated in a solution of ethidium bromide and the DNA bands were detected by fluorescence under a UV lamp. The densitometric data of the bands were obtained with an ATTO Densitograph version 4.0 for Macintosh. Since staining intensity of relaxed plasmid DNA is found to be 1.5 times that of supercoiled DNA,[40] the data were corrected based on this difference.

Measurement of photosensitized production of singlet oxygen

The amount of singlet oxygen generated by photosensitization of the porphyrins was determined by the measurement of the rate of reaction between singlet oxygen and DPBF.[32–34] The buffered solution containing a porphyrin (0.05 μM) and DPBF (30 μM), prepared in the dark, was illuminated at the λ_{max}^{Soret} and 25 °C, and a loss of absorbance at 415 nm was followed spectrophotometrically. No DPBF bleaching in the absence of the porphyrins and no porphyrin decomposition on illumination were observed.

Spectral measurements

Aliquots of ctDNA solution were added to the solution of a zinc(II) cationic bis-porphyrin (ca. 4.0 μM), and the spectral measurements were performed at 25 °C in the buffer. The induced CD spectra were recorded after the addition of ctDNA solution to the solution of zinc(II) cationic bis-porphyrin.

Acknowledgements

This research was supported in part by a Grant-in-Aid to T. U. for Scientific Research from Japan Society for the Promotion of Science (grant no. 13557199).

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