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Synthetic control of interchromophoric interaction in cationic bis-porphyrins toward efficient DNA photocleavage and singlet oxygen production in aqueous solution

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Abstract—We have synthesized cationic bis-porphyrins and their zinc(II) complexes with two TMPyP-like chromophores bridged by *p*- or *m*-xylylenediamine to develop effective DNA photocleaving agents. The xylylene linkers and zinc ion were introduced to control interchromophoric interaction that should be involved in photosensitization of the cationic bis-porphyrins. The molar absorptivities of all the bis-porphyrins in aqueous solution remained unchanged over a wide range of concentrations, indicating the absence of self-aggregation property. In particular, the molar absorptivity of the zinc(II) complex of the *p*-xylylenediamine-linked bis-porphyrin in aqueous solution was 2.0 times as large as that of unichromophoric ZnTMPyP, suggesting the absence of both intermolecular and intramolecular interchromophoric interaction. The metal-free *p*-xylylenediamine-linked bis-porphyrin showed the more efficient conversion ability of supercoiled to nicked circular pUC18 plasmid DNA by photosensitization than the metal-free *m*-xylylenediamine-linked one. Furthermore, the zinc complexes of the bis-porphyrins exhibited the more potent DNA photocleavage than did the metal-free bis-porphyrins. Singlet oxygen productivity of the four cationic bis-porphyrins was determined by measuring the decomposition rate of 1,3-diphenylisobenzofuran. The amount of singlet oxygen generated by photosensitization of the zinc(II) complex of the *p*-xylylenediamine-linked bis-porphyrin in aqueous solution was 2.1 times as large as ZnTMPyP, indicating the full singlet oxygen productivity. A significant relationship between the DNA photocleaving abilities and the singlet oxygen productivities of the cationic porphyrins in aqueous solution was found. Hence, the degree of the intramolecular interchromophoric interaction, the DNA photocleaving ability, and the singlet oxygen productivity of the cationic bis-porphyrins in aqueous solution were successfully controlled by means of the introduction of the appropriate linker and metal ion.

1. Introduction

Cationic porphyrins and their metal complexes have been extensively studied for their diverse characteristics and functions.[1] The cationic, water-soluble *meso*-tetrakis(*N*-methyl-4-pyridyl)porphine (H_2TMPyP) and its metal complexes have particularly attracted considerable attention in terms of interaction with nucleic acids[2,3] and biomedical application.[4] The cationic macrocycles tightly bind to duplexes,[5,6] triplexes,[7] and quadruplexes[8,9] of nucleic acids in the following binding modes: intercalation, groove binding, outside binding with self-stacking, and external stacking. These binding modes have been proposed from detailed analyses by means of UV–visible, circular dichroism,[5] fluorescence,[10] resonance Raman,[11] mass,[12] and NMR spectrometry[13] in addition to viscometry,[14] X-ray crystallography,[15] and biochemical methods.[16] The dyes can also cleave DNA by irradiation of visible light[17] or in the presence of reducing/oxidizing agents.[18] Irreversible DNA damages in malignant cells are expected to disturb genetic events, and thus to suppress the proliferation of the cells. Hence, H_2TMPyP and their metal complexes possessing the bifunctionality of the DNA binding and cleaving abilities have much potentials as anti-viral and anti-cancer agents, practically as DNA-targeting photosensitizers in photodynamic therapy of cancer.[19]

We have previously reported the synthesis of a series of cationic bis-porphyrins, *N*,*N*′-bis{4-[10,15,20-tris(1-methylpyridinium-4-yl)porphyrin-5-yl]benzoyl}oligome-

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thylenediamine, and their zinc complexes, and analyses of their solution properties, interactions with duplex DNA, and DNA photocleavage activities.[20,21] These compounds with two H_2TMPyP-like chromophores bridged by a series of aliphatic diamines were prepared to develop new DNA-targeting photosensitizers. The metal-free bis-porphyrins were prone to form intermolecular dimers in aqueous solution. They bound to calf thymus DNA (CTDNA) with outside self-stacking on the DNA surface. Their pUC18 plasmid DNA photocleavage activities were lower than we expected, which decreased as the number of their linker hydrocarbons increased, and were consistent with tendency for them to dimerize intermolecularly. Introduction of zinc(II) ions to the metal-free bis-porphyrins resulted in the disappearance of their self-aggregation properties. The five-coordinate zinc(II) complexes of the cationic bis-porphyrins were converted to groove binders, and their DNA photocleavage activities and singlet oxygen productivities were higher than unichromophoric H_2TMPyP and its zinc(II) complex ZnTMPyP. The DNA photocleavage activities for them were well correlated with their singlet oxygen productivities in aqueous solution. Hence, the DNA photocleavage activity of the cationic bis-porphyrins with aliphatic diamine linkers was associated with the intermolecular interaction.

Whereas the zinc(II) complexes of the cationic bis-porphyrins with the flexible linkers showed the higher DNA photocleavage activities, there is still room for improvement because their molar absorptivities and singlet oxygen productivities in aqueous solution did not reach their potentials. In comparison to the molar absorptivities of unichromophoric ZnTMPyP in aqueous solution and dimethylsulfoxide (DMSO), it was suggested that intramolecular interaction for the zinc(II) complexes of the bis-porphyrins could be responsible for their slightly reduced photosensitizing abilities. Therefore, we paid attention to their linker parts and expected that the introduction of rigid linkers could give satisfactory results by preventing the intramolecular interaction between the cationic porphyrin chromophores in aqueous solution. We herein report the synthesis of metal-free cationic bis-porphyrins (H_2*p*Xy and H_2*m*Xy, Fig. 1) and their zinc(II) complexes (Zn*p*Xy and Zn*m*Xy) linked with *p*- or *m*-xylylenediamine, and analyses of their solution properties, DNA photocleavage activities, and singlet oxygen productivities. Zn*p*Xy exhibited the highest DNA photocleavage activity and singlet oxygen productivity among the cationic bis-porphyrins we have developed. Moreover, neither intermolecular nor intramolecular interchromophoric interaction in aqueous solution was found for Zn*p*Xy, and the singlet oxygen productivity of Zn*p*Xy was twice that of ZnTMPyP.

2. Results

2.1. Synthesis

As a framework of the desired compounds, we adopted an asymmetrical porphyrin TPyPCOOH bearing one 4-carboxyphenyl group and three 4-pyridyl groups at the meso position. The metal-free non-charged bis-porphyrins linked with *p*- or *m*-xylylenediamine were successfully prepared by direct coupling between 1.0 equiv of TPyPCOOH and 0.5 equiv of the corresponding diamine by way of acid chlorination of TPyPCOOH and following repetitive silica-gel chromatography. The yields of the *para*- and *meta*-positional isomers were 23% and 11%, respectively. The non-charged bis-porphyrins were then quaternized with methyl iodide in DMF to give the metal-free cationic bis-porphyrins, H_2*p*Xy and H_2*m*Xy, quantitatively as iodide salt. Finally, H_2*p*Xy and H_2*m*Xy were metalated with ZnI_2 to give Zn*p*Xy (64%) and Zn*m*Xy (63%), respectively. The new compounds were characterized by ^{1}H NMR, MALDI-TOF-MS, and elemental analysis. These cationic bis-porphyrins could be dissolved both in water and DMSO, and their aqueous solutions (120–180 μM) were used for the experiments mentioned below.

2.2. Solution properties

Absorption spectra were recorded both in a buffered solution containing 10 mM sodium phosphate and 100 mM NaCl (pH 7.0) and in DMSO. The molar absorptivities of the Soret maximum (λ_{max}^{Soret}) of the cationic bis-porphyrins, H_2TMPyP, and ZnTMPyP in the buffer and DMSO ($\varepsilon_{buffer}^{Soret}$ and $\varepsilon_{DMSO}^{Soret}$) are listed in Table 1. The $\varepsilon_{DMSO}^{Soret}$ values of H_2*p*Xy, H_2*m*Xy, Zn*p*Xy, and Zn*m*Xy were approximately equal. On the other hand, the $\varepsilon_{buffer}^{Soret}$ value of Zn*p*Xy was 1.5 times as large as that of Zn*m*Xy, while the $\varepsilon_{buffer}^{Soret}$ values of the metal-free bis-porphyrins were equal. Thus, clear was a marked difference in molar absorptivity of the positional isomers of the zinc bis-porphyrins in the buffered solution.

Solvent dependency on molar absorptivity of the cationic bis-porphyrins was also significant in comparison to that of the cationic, unichromophoric porphyrins. The $\varepsilon_{DMSO}^{Soret}$ values of H_2*p*Xy and H_2*m*Xy were 2.0 times as large as that of H_2TMPyP. Also, $\varepsilon_{DMSO}^{Soret}$ of Zn*p*Xy and Zn*m*Xy were 2.0 times that of ZnTMPyP. However, the $\varepsilon_{buffer}^{Soret}$ of H_2*p*Xy and H_2*m*Xy were no more than 1.3 times that of H_2TMPyP. For the zinc complexes, the $\varepsilon_{buffer}^{Soret}$ of Zn*p*Xy was 2.0 times that of ZnTMPyP, but the $\varepsilon_{buffer}^{Soret}$ of Zn*m*Xy was no more than 1.3 times that of ZnTMPyP. Hence, the molar absorptivities of Zn*p*Xy both in DMSO and the buffer were the expected values in comparison to those of the unichromophoric porphyrin.

The $\varepsilon_{buffer}^{Soret}$ values of the cationic bis-porphyrins at various concentrations were estimated to know whether they self-aggregate in the aqueous solution. As shown in Figure 2a, the molar absorptivities of H_2*p*Xy, H_2*m*Xy, Zn*p*Xy, and Zn*m*Xy remained unchanged in the concentration range from 0.1 μM to 70 μM. This indicates that these cationic bis-porphyrins are monomeric in the aqueous solution. In addition, this property was independent of both the difference of the linkers and the presence of zinc ion at the porphyrin core.

Figure 2b shows the development of the $\varepsilon_{buffer}^{Soret}$ values of the four cationic bis-porphyrins ([bis-porphy-

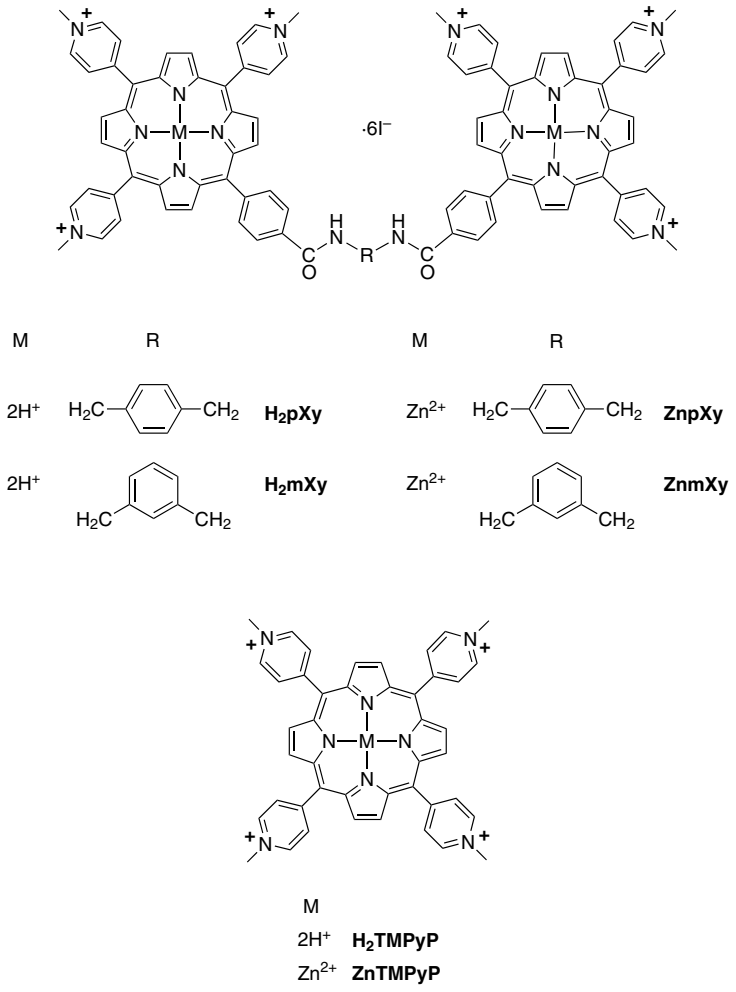

Figure 1. Cationic porphyrins in this study.

Table 1. Molar absorptivities of λ_{max}^{Soret} for cationic porphyrins in the buffer[a] and DMSO

Porphyrin (mM^{-1} cm^{-1})	H_2pXy	H_2mXy	$ZnpXy$	$ZnmXy$	H_2TMPyP[b]	ZnTMPyP[c]
$\varepsilon_{buffer}^{Soret}$	315	311	449	290	243	221
$\varepsilon_{DMSO}^{Soret}$	594	594	610	608	295	290

[a] Ten millimolar sodium phosphate and 0.1 M NaCl (pH 7.0).
[b] Ref. 20.
[c] Ref. 21.

rin] ≈ 4 μM) with addition of DMSO. Large hyperchromicities toward their $\varepsilon_{DMSO}^{Soret}$ values were observed for all the bis-porphyrins, whereas a small increase in ε^{Soret} of H_2TMPyP and ZnTMPyP was seen (data not shown). In particular, the ε^{Soret} value of H_2pXy in a low DMSO concentration range (0–20%, v/v) drastically developed. On the other hand, a slight increase in the ε^{Soret} value of H_2mXy was seen in the low DMSO concentration range. Thus, the molar absorptivity of H_2pXy in the buffer was more sensitive to the solvent effect of DMSO than that of H_2mXy. In the case of the zinc cationic bis-porphyrins, the ε^{Soret} value of the para isomer was larger than that of the meta isomer even in any buffer/DMSO ratio.

The molar absorptivities of these four cationic bis-porphyrins in the buffer were independent of temperature. All the $\varepsilon_{buffer}^{Soret}$ values ([bis-porphyrin] = 4.0 μM) were constant in the temperature range from 25 to 95 °C, and this should support that every bis-porphyrin remains in a monomeric form in the buffer.

2.3. Photocleavage of plasmid DNA

DNA photocleavage activity was examined using supercoiled double-stranded pUC18 plasmid DNA. A mixture of the cationic bis-porphyrin (0.6 μM) and plasmid DNA (60 μM, R ([porphyrin]/[DNA base pair]) = 0.01) in the buffer was illuminated in air at 432 nm for the metal-free and at 440 nm for the zinc bis-porphyrins, respectively. 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.

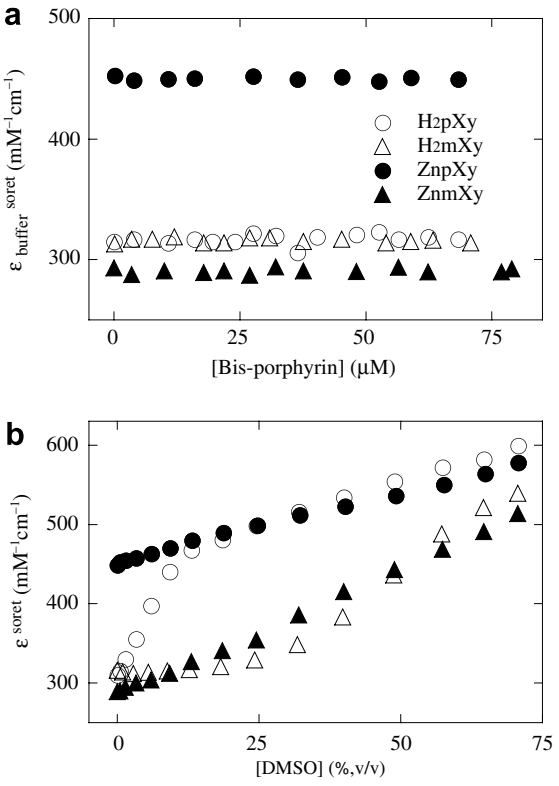

Figure 2. (a) Plots of the molar absorptivities at the Soret maximum of H_2pXy (○), H_2mXy (△), Zn*p*Xy (●), and Zn*m*Xy (▲) versus their concentrations in the buffer containing 10 mM sodium phosphate and 100 mM NaCl (pH 7.0). (b) Molar absorptivity developments at the Soret maximum of H_2pXy (○), H_2mXy (△), Zn*p*Xy (●), and Zn*m*Xy (▲) in the buffer containing 10 mM sodium phosphate and 100 mM NaCl (pH 7.0) with addition of DMSO.

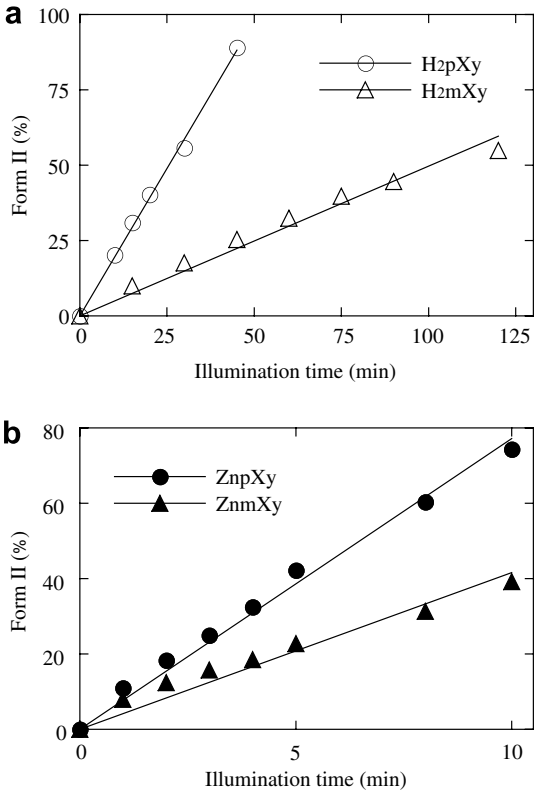

Figure 3. (a) Plots of amount of form II generated by photosensitization of H_2pXy (○) and H_2mXy (△) versus illumination time. Cleavage conditions: 0.6 μM H_2pXy and H_2mXy; 60 μM pUC18 plasmid DNA; 10 mM sodium phosphate and 100 mM NaCl (pH 7.0); illumination at 432 nm and 25 °C. (b) Plots of amount of form II generated by photosensitization of Zn*p*Xy (●) and Zn*m*Xy (▲) versus illumination time. Cleavage conditions: 0.6 μM H_2pXy and H_2mXy; 60 μM pUC18 plasmid DNA; 10 mM sodium phosphate and 100 mM NaCl (pH 7.0); illumination at 440 nm and 25 °C.

As shown in Figure 3a and b, all the cationic bis-porphyrins produced form II in proportion to the illumination time. The DNA photocleavage events by the zinc bis-porphyrins clearly proceeded faster than those by the metal-free bis-porphyrins. The conversion rates of form I to form II by photosensitization of the cationic porphyrins are summarized in Table 2. The zinc complexes exhibited the greater DNA photocleavage activity than did unichromophoric H_2TMPyP and ZnTMPyP. The most effective DNA photocleaver was Zn*p*Xy, which exhibited 4.3 and 2.7 times more enhanced conversion efficiency than H_2TMPyP and ZnTMPyP, respectively. Zn*m*Xy had the second-highest activity, but showed no more than 55% efficiency compared to that of Zn*p*Xy.

The DNA photocleavage activities of the metal-free bis-porphyrins were lower than those of the zinc bis-porphyrins. The activity of H_2pXy was close to that of H_2TMPyP, which showed 26% efficiency compared to that of Zn*p*Xy (Table 2). In addition, the least effective H_2mXy showed 28%, 25%, 12%, and 6% efficiency compared to that of H_2TMPyP, H_2pXy, Zn*m*Xy, and Zn*p*Xy, respectively. Thus, the metal-free cationic bis-porphyrins were unable to exhibit the full activity, and that a striking isomer effect was also observed.

The solvent effect of DMSO on the DNA photocleavage activity of the metal-free bis-porphyrins was examined. Because the addition of DMSO to the buffer enhanced the molar absorptivity at the λ_{max}^{Soret} of the cationic bis-porphyrins (Fig. 2b), we assumed that the lower activities of the metal-free bis-porphyrins should be increased with addition of DMSO to their buffered reaction media. As shown in Figure 4, an increase in DMSO concentration (0–30%, v/v) resulted in an increase in form II by photosensitization of the metal-free bis-porphyrins under the condition that a sample containing the plasmid DNA (60 μM) and each bis-porphyrin (0.6 μM) was illuminated for 30 min. The percentage of form II photoinduced by the bis-porphyrins in the buffer roughly doubled (51 to 95% for H_2pXy and 19 to 53% for H_2mXy) in the presence of 20% DMSO. The activity of H_2pXy was superior to that of H_2mXy throughout the range of DMSO concentrations.

The DNA photocleavage was strongly affected by the presence of sodium azide, a well-known singlet oxygen quencher. When a sample containing the plasmid DNA (60 μM) and Zn*p*Xy (0.6 μM) at $R = 0.01$ was illuminated for 3 min, 4% and 25% of form I were converted to form II in the presence of 100 mM NaN_3

Table 2. Conversion rates (CR) of form I to form II by photosensitization of cationic porphyrins in the buffer[a]

	H_2pXy	H_2mXy	$ZnpXy$	$ZnmXy$	H_2TMPyP[b]	ZnTMPyP[c]
CR (% min^{-1})	2.0	0.50	7.7	4.2	1.8	2.9

[a] [base pair] = 60 μM, [porphyrin] = 0.6 μM, 10 mM sodium phosphate and 0.1 M NaCl (pH 7.0).
[b] Ref. 20.
[c] Ref. 21.

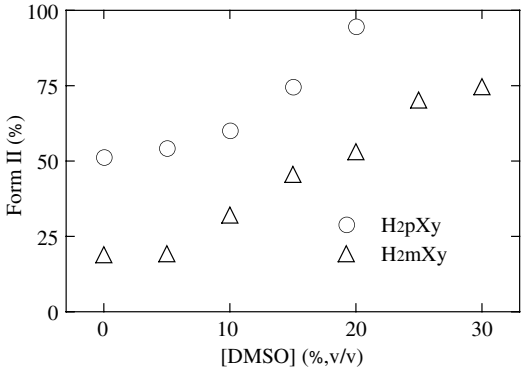

Figure 4. Plots of increasing amount of form II generated by photosensitization of H_2pXy (○) and H_2mXy (△) with increase in DMSO concentration. Cleavage conditions: 0.6 μM H_2pXy and H_2mXy; 60 μM pUC18 plasmid DNA; 10 mM sodium phosphate and 100 mM NaCl (pH 7.0); illumination at 432 nm and 25 °C for 30 min.

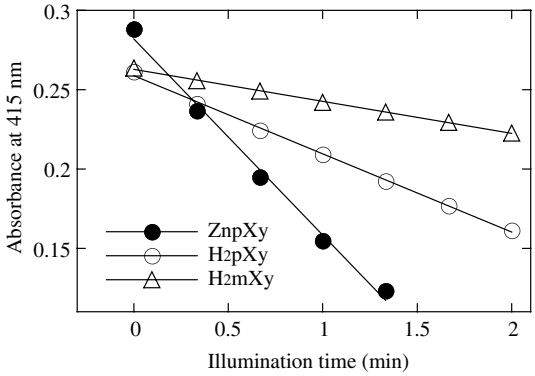

Figure 5. The plots of bleached absorption of DPBF decomposed by photosensitization of H_2pXy (○), H_2mXy (△), and $ZnpXy$ (●) versus illumination time. Conditions: 30 μM DPBF; 0.05 μM H_2pXy, H_2mXy, and $ZnpXy$; 10 mM sodium phosphate (pH 7.0); 100 mM NaCl; illumination at the Soret band and 25 °C in air.

and NaCl, respectively. Furthermore, no form II was detected in the presence of 300 mM NaN_3, whereas 18% of form II was photoinduced in the presence of 300 mM NaCl. Thus, NaN_3 strongly inhibited the DNA photocleavage activity of the cationic bis-porphyrin.

2.4. Singlet oxygen produced by photosensitization

Because singlet oxygen was clearly responsible for the DNA photocleavage in our system, the amount of singlet oxygen by photosensitization of the cationic bis-porphyrins in the buffer and DMSO was determined quantitatively by measuring the decomposition rate of DPBF spectrophotometrically.[22] As shown in Figure 5 for H_2pXy, H_2mXy, and $ZnpXy$, absorbance of DPBF (30 μM) at 415 nm linearly decreased in the presence of each cationic porphyrin (0.05 μM) as illumination time increased. Table 3 lists the slopes of the plots of bleached absorption of DPBF versus illumination time in the buffer and DMSO (S_{buffer} and S_{DMSO}), and the normalized slopes to the values of S_{buffer} and S_{DMSO} of H_2TMPyP (NS_{buffer} and NS_{DMSO}). The NS_{buffer} of the cationic bis-porphyrins were varied. Whereas the NS_{buffer} of metal-free H_2pXy was close to that of H_2TMPyP, the NS_{buffer} of H_2mXy was below a half of that of H_2TMPyP. The NS_{buffer} of the zinc complexes were larger than those of the metal-free bis-porphyrins. In particular, the NS_{buffer} value of $ZnpXy$ was 2.1 times as large as that of ZnTMPyP, while the NS_{buffer} value of $ZnmXy$ was no more than 1.3 times that of ZnTMPyP. Hence, the isomer effect of the linker parts on the singlet oxygen production in the buffer was observed, and in particular only $ZnpXy$ exhibited the expected ability of producing singlet oxygen by photosensitization in the aqueous solution.

In contrast to the event in the buffer, no clear isomer effect was observed in DMSO. The NS_{DMSO} of H_2pXy and H_2mXy were close, and roughly twice that of unichromophoric H_2TMPyP. The NS_{DMSO} of $ZnpXy$ and $ZnmXy$ were larger than those of the metal-free bis-porphyrins, and were more than twice that of ZnTMPyP. These four photoactivated cationic bis-porphyrins in DMSO displayed their full abilities to produce singlet oxygen.

3. Discussion

The goal of our present work was to develop cationic bis-porphyrins that display full DNA photocleaving ability. To do so, rigid linkers and a diamagnetic metal ion with axial ligand were noted as key components. Thus, we synthesized the cationic bis-porphyrins and their zinc(II) complexes linked with *p*- or *m*-xylylenediamine (Fig. 1), and analyzed their solution properties, DNA photocleavage activities, and singlet oxygen productivities in detail. These four cationic bis-porphyrins exhibited large differences in their characteristics and functions.

The $\varepsilon^{Soret}_{buffer}$ values of H_2pXy, H_2mXy, $ZnpXy$, and $ZnmXy$ remained unchanged in the concentration ranges (Fig. 2a), indicating the absence of self-aggregation in the aqueous solution. The xylylenediamine linkers prevented intermolecular interaction for these metal-free and metalated conjugates, which was

Table 3. Decomposition rates of DPBF by photosensitization of cationic porphyrins in the buffer[a] and DMSO

	H_2pXy	H_2mXy	$ZnpXy$	$ZnmXy$	H_2TMPyP	ZnTMPyP
$S_{buffer} \times 10^{2}$[b] (min^{-1})	4.93	2.02	12.4	7.79	4.64[c]	5.78[c]
NS_{buffer}[d]	1.06	0.44	2.67	1.68	1.00[c]	1.25[c]
$S_{DMSO} \times 10^{2}$[e] (min^{-1})	4.07	3.85	5.65	5.50	2.08	2.52
NS_{DMSO}[f]	1.96	1.85	2.72	2.64	1.00	1.21

[a] [porphyrin] = 0.05 μM, [DPBF] = 30 μM, 10 mM sodium phosphate and 0.1 M NaCl (pH 7.0).
[b] Slope of plots of bleached absorption of DPBF versus illumination time in the buffer.
[c] Ref. 21.
[d] Normalized slope to the S_{buffer} of H_2TMPyP.
[e] Slope of plots of bleached absorption of DPBF versus illumination time in DMSO.
[f] Normalized slope to the S_{DMSO} of H_2TMPyP.

observed for the previously reported metal-free cationic bis-porphyrins with various aliphatic diamine linkers.[20]

The large differences in their molar absorptivities in the aqueous solution were observed for the xylylenediamine-linked bis-porphyrins (Table 1). Whereas the $\varepsilon^{Soret}_{buffer}$ of Zn*p*Xy was 2.0 times as large as that of unichromophoric ZnTMPyP, the $\varepsilon^{Soret}_{buffer}$ of H_2pXy, H_2mXy, and Zn*m*Xy were considerably smaller than we expected when compared to that of H_2TMPyP or ZnTMPyP. On the other hand, the $\varepsilon^{Soret}_{DMSO}$ of all the bis-porphyrins were twice as large as those of the unichromophoric porphyrins, indicating that no intramolecular interchromophoric interaction occurs in DMSO. These results lead to the conclusion that no intramolecular interchromophoric interaction in the aqueous solution occurs solely for Zn*p*Xy. The cooperativity of the rigidity of *p*-xylylenediamine linker with steric hindrance between the axial ligands is most likely to force its ZnTMPyP-like chromophores not to interact with each other.

The development of the molar absorptivities in the buffer for the cationic bis-porphyrins was observed with addition of DMSO (Fig. 2b). The large hyperchromicities for H_2pXy, H_2mXy, and Zn*m*Xy were most probably due to the relaxation of the intramolecular interchromophoric interaction by the solvation of DMSO. The methyl and sulfoxide groups of this aprotic, highly dipolar molecule[23] could cling around the π-surface and *N*-methylpyridinium parts of the cationic bis-porphyrins, respectively. In addition, the presence of DMSO influenced the molar absorptivities of the *p*-xylylenediamine-linked bis-porphyrins in the aqueous solution more greatly than those of the *m*-xylylenediamine-linked bis-porphyrins. This suggests that DMSO molecules could solvate to the *p*-xylylenediamine-linked bis-porphyrins more strongly than to the *m*-xylylenediamine-linked bis-porphyrins. The degree of resistance against the solvation of DMSO probably reflects the degree of the intramolecular interchromophoric interaction. We were unable to analyze the thermal stability of the interaction, because their $\varepsilon^{Soret}_{buffer}$ values were independent of temperature in the temperature range. This indicates the thermal stability is very high in the aqueous solution. The intramolecular interchromophoric interaction is likely to be induced by van der Waals attractive force between the chromophores in the presence of water. Because van der Waals force depends on distance, the degree of the interchromophoric interaction should be related to the distance between the chromophores. Amphiphilic DMSO molecules probably screen the attractive force. The screening effect against the interchromophoric interaction of the cationic bis-porphyrins may be illustrated by molecular dynamics simulation for the cationic bis-porphyrins in explicit water/DMSO solvent without cutoff for van der Waals potentials.

The Zn(II) complexes of the cationic bis-porphyrins were more potent in the DNA photocleavage activity than the metal-free cationic bis-porphyrins (Table 2). This result demonstrates that Zn(II) insertion into their free bases could account for the improved photosensitization. Zn*p*Xy was the most effective DNA photocleaver among the cationic bis-porphyrins we have ever developed. The best result is most likely to be related to the solution property that the Zn(II) complex of the *p*-xylylenediamine-linked bis-porphyrin exhibited neither intermolecular nor intramolecular interaction. Zn*m*Xy also showed the more potent activity than ZnTMPyP, but was roughly twice less effective than Zn*p*Xy. The relatively lower activity of Zn*m*Xy is presumably derived from the intramolecular interchromophoric interaction. This isomer effect of the linkage part on the activity was significant for the metal-free bis-porphyrins. H_2mXy was four times less active than H_2pXy, and was the least effective of all the cationic bis-porphyrins we have developed.

The frequency of DNA photocleavage by the metal-free bis-porphyrins in the buffer roughly doubled in the presence of DMSO (20%, v/v), as shown in Figure 4. Furthermore, the fact that the activity of H_2pXy was more sensitive to the presence of DMSO in the medium than that of H_2mXy corresponded to the solution property that the molar absorptivity of H_2pXy in the buffer was affected by the presence of DMSO more markedly than that of H_2mXy. This enhanced activity is probably due to the relaxation of the intramolecular interchromophoric interaction by the solvation of DMSO.

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).[24] Recent works demonstrate that type I photosensitizers operating by one-electron oxidation are not able to generate strand breaks.[25] The presence of DMSO, known as a hydroxyl radical scavenger, in our system increased the DNA photocleavage activity. This implies that the active species is not hydroxyl radical. In addition, the presence of NaN_3 in

the system significantly inhibited the activity. Because the $^1O_2$ quencher inhibited the activity more effectively than NaCl, the inhibition is most probably derived from the quenching of short-lived singlet oxygen generated by energy transfer from the photoexcited triplet bis-porphyrins to dissolved triplet oxygen in the aqueous solution.

Singlet oxygen production by photosensitization of these cationic bis-porphyrins was confirmed by reduction of the absorbance of DPBF versus illumination time (Fig. 5). The slope of the plot of bleached absorption vs. illumination time is proportional to the production rate of singlet oxygen.[22] The singlet oxygen productivities of Zn*p*Xy both in the buffer and DMSO were twice as great as those of monomeric ZnTMPyP, and thus its full ability was displayed irrespective of the reaction media (Table 3). This result corresponds to the fact that the molar absorptivities of Zn*p*Xy both in the buffer and DMSO were twice as large as those of ZnTMPyP. In contrast, the singlet oxygen productivities of H_2*p*Xy, H_2*m*Xy, and Zn*m*Xy depended on the solvents. These three bis-porphyrins were able to exhibit almost full singlet oxygen productivities in DMSO, but failed to do in the buffer. Because this solvent dependency for H_2*p*Xy, H_2*m*Xy, and Zn*m*Xy was also found in their solution properties, the singlet oxygen production by photosensitization of the cationic bis-porphyrins is most likely to be affected by the intramolecular interchromophoric interaction. The singlet oxygen productivity of the cationic bis-porphyrins should be a good index for the degree of the intramolecular interchromophoric interaction in the aqueous solution.

Figure 6 reveals a significant relationship between the conversion rates of form I to form II DNA and the NS_{buffer} of bleached absorption of DPBF by photosensitization of the cationic porphyrins. This indicates that their singlet oxygen productivities in the aqueous solution directly govern their DNA photocleavage activities in our system. Consequently, the singlet oxygen productivity and DNA photocleaving ability of the cationic bis-porphyrins in the aqueous solution should be independent of their binding modes to DNA. As described above, only the zinc(II) complex of the *p*-xylylenediamine-linked bis-porphyrin had the expected ability in terms of the molar absorptivity and singlet oxygen productivity in the aqueous solution. Hence, suitable choice of linkers and metals to prevent interchromophoric interaction would be required for the development of effective photosensitizers with singlet oxygen production by oligomerization of porphyrin chromophores.

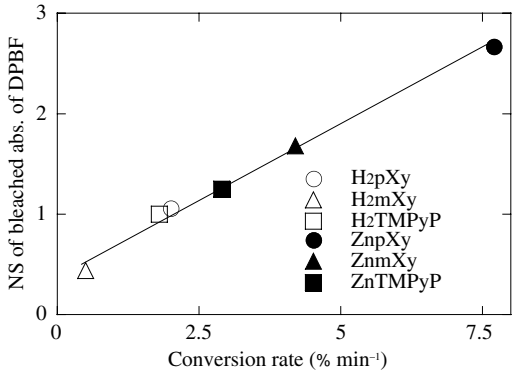

Figure 6. Plots of conversion rates of form I to form II versus NS_{buffer} values of bleached absorption of DPBF by photosensitization of the cationic porphyrins.

4. Experimental

4.1. Instrumentation

^{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 were recorded on a Bruker REFLEX™. Electronic spectra were recorded on a Beckman DU650 spectrophotometer. Elemental analysis was performed at the Analytical Center, Kumamoto University.

4.2. Materials

All reagents for the synthesis of the metal-free cationic bis-porphyrins and their zinc(II) complexes, and 1,3-diphenylisobenzofuran (DPBF) were purchased from Tokyo Kasei Chemical Co. Thionyl chloride was distilled before use. *n*-Hexane and methylene chloride were distilled from CaH_2.[26] Triethylamine was distilled from CaH_2 and re-distilled from P_2O_5. *N*,*N*-Dimethyl formamide (DMF) and DMSO were distilled under reduced pressure after pre-dried with powdered BaO. Compound 5-(4-Carboxyphenyl)-10,15,20-tris(4-pyridyl)porphyrin (TPyPCOOH) was synthesized as reported previously.[17] The tosylate salt of H_2TMPyP was purchased from Dojin Chemical Co. The plasmid pUC18 was isolated from *Escherichia coli* XL-1 Blue MRF' by standard method.[27] CTDNA was purchased from Sigma Chemical Co., and the aqueous solution was quantitated spectrophotometrically by using ε_{260} = 13,200 M(basepairs)$^{-1}$ cm^{-1}. Unless otherwise noted, a buffered solution contained 10 mM sodium phosphate and 100 mM NaCl (pH 7.0).

4.3. General procedure for the preparation of non-charged bis-porphyrins linked with *p*- or *m*-xylylenediamine

TPyPCOOH (250 mg, 0.378 mmol) was dissolved in 35 ml of thionyl chloride under argon and the solution was refluxed for 2 h. After cooling to room temperature, thionyl chloride was removed under reduced pressure. Dry hexane (30 ml) was used to wash the residue and then removed. To this residue were added dry methylene chloride (30 ml), *p*-xylylenediamine (or *m*-xylylenediamine) (0.4 equiv to the mole of TPyPCOOH), and triethylamine (10 equiv to the mole of TPyPCOOH) under argon. The reaction mixture was stirred for 20 h at room temperature. Chloroform, water, and a small amount of methanol were poured into the solution, and the organic phase was separated and dried with anhydrous Na_2SO_4. The solvent was removed and the corresponding bis-porphyrin was chromatographed on silica gel (2% methanol/$CHCl_3$) three times. Addition

of heptane to the eluent and slow evaporation gave a brown powder, which was collected by centrifugation and dried.

4.3.1. *N,N*-Bis[4-[10,15,20-tris(4-pyridyl)porphyrin-5-yl]-benzoyl]-1,4-benzenebis(methylamine). Yield: 23%. ^{1}H NMR (400 MHz, 10% $CD_3OD/CDCl_3$, δ): −2.92 (s, 2H), 4.84 (s, 4H), 7.59 (s, 4H), 8.22 (d, J = 4.4 Hz, 8H), 8.23 (d, J = 4.4 Hz, 4H), 8.32 (s, 8H), 8.88 (br m, 16H), 8.99 (d, J = 4.4 Hz, 8H), 9.02 (d, J = 4.4 Hz, 4H). UV (10% $CH_3OH/CHCl_3$) λ_{max}, nm (ε): 643 (5400), 588 (15,100), 547 (15,200), 513 (49,600), 418 (627,000). MALDI-TOF-MS (m/z): $[M^+ + H]^+$ calcd for $C_{92}H_{62}N_{16}O_2$, 1423.6; found, 1424.2. Anal. Calcd for $C_{92}H_{62}N_{16}O_2 \cdot 0.5CHCl_3 \cdot 0.5C_7H_{16}$: C, 75.40; H, 4.47; N, 14.90. Found: C, 75.20; H, 4.63; N, 14.62.

4.3.2. *N,N*-Bis[4-[10,15,20-tris(4-pyridyl)porphyrin-5-yl]benzoyl]-1,3-benzenebis(methylamine). Yield: 11%. ^{1}H NMR (400 MHz, 10% $CD_3OD/CDCl_3$, δ): −2.92 (s, 2H), 4.87 (s, 4H), 7.51 (br s, 3H), 7.66 (s, 1H), 8.06 (d, J = 4.4 Hz, 8H), 8.21 (d, J = 3.9 Hz, 4H), 8.32 (s, 8H), 8.79 (br m, 16H), 8.90 (d, J = 5.9 Hz, 8H), 9.02 (d, J = 6.3 Hz, 4H). UV (10% $CH_3OH/CHCl_3$) λ_{max}, nm (ε): 645 (4600), 589 (10,400), 547 (10,200), 515 (35,300), 418 (628,000). MALDI-TOF-MS (m/z): $[M+H]^+$ calcd for $C_{92}H_{62}N_{16}O_2$, 1423.6; found, 1424.5. Anal. Calcd for $C_{92}H_{62}N_{16}O_2 \cdot 1CHCl_3 \cdot 0.5C_7H_{16}$: C, 72.76; H, 4.49; N, 14.07. Found: C, 72.87; H, 4.37; N, 13.83.

4.4. General procedure for the preparation of cationic bis-porphyrins linked with *p*- or *m*-xylylenediamine

The neutral bis-porphyrins (ca. 20 mg) were quaternized with methyl iodide (3 ml) in 15 ml of DMF for 3 h at room temperature. The solvent and methyl iodide were removed under vacuum. The residue was dissolved in DMF again and precipitated with diethyl ether. The brown powder was collected by centrifugation, washed with diethyl ether, and dried. This reaction was quantitative. Absorption spectra of the cationic bis-porphyrins were measured in DMSO and in the buffered solution.

4.4.1. *N,N*-Bis[4-[10,15,20-tris(*N*-methyl-4-pyridyl)porphyrin-5-yl]benzoyl]-1,4-benzenebis(methylamine) Hexaiodide (H_2*p*Xy). ^{1}H NMR (400 MHz, DMSO-d_6, δ): −3.03 (s, 2H), 4.70 (s, 12H), 4.72 (s, 10H), 7.54 (s, 4H), 8.37 (d, J = 8.3 Hz, 4H), 8.44 (d, J = 8.3 Hz, 4H), 8.99 (d, J = 7.3 Hz, 4H), 9.01 (d, J = 6.3 Hz, 8H), 9.03–9.18 (br m, 16H), 9.50 (br d, J = 6.8 Hz, 12H). UV (DMSO) λ_{max}, nm (ε): 643 (5800), 609 (15 700), 562 (36,600), 518 (34,600), 429 (594,000); (buffer) λ_{max} nm (ε): 647 (3500), 588 (10,700), 561 (11,600), 522 (22,300), 419 (311,000). MALDI-TOF-MS (m/z): $[M+H]^+$ calcd for $C_{98}H_{80}N_{16}O_2$, 1513.8; found, 1514.0. Anal. Calcd for $C_{98}H_{80}N_{16}O_2 \cdot 6H_2O \cdot 1DMF$: C, 49.39; H, 4.06; N, 9.69. Found: C, 49.13; H, 4.18; N, 9.42.

4.4.2. *N,N*-Bis[4-[10,15,20-tris(*N*-methyl-4-pyridyl)porphyrin-5-yl]benzoyl]-1,3-benzenebis(methylamine) Hexaiodide (H_2*m*Xy). ^{1}H NMR (400 MHz, DMSO-d_6, δ): −3.03 (s, 2H), 4.72 (s, 12H), 4.73 (s, 6H), 4.75 (s, 4H), 7.48 (br s, 3H), 7.59 (s, 1H), 8.37 (d, J = 7.8 Hz, 4H), 8.46 (d, J = 7.8 Hz, 4H), 8.98 (d, J = 6.3 Hz, 4H), 9.00 (d, J = 6.3 Hz, 8H), 9.04-9.16 (br m, 16H), 9.50 (br d, J = 6.3 Hz, 12H). UV (DMSO) λ_{max}, nm (ε): 646 (10,000), 609 (22,700), 562 (24,500), 518 (48,400), 429 (594,000); (buffer) λ_{max} nm (ε): 650 (4900), 590 (10,100), 560 (10,800), 525 (20,500), 417 (315,000). MALDI-TOF-MS (m/z): $[M+H]^+$ calcd for $C_{98}H_{80}N_{16}O_2$, 1513.8; found, 1514.0. Anal. Calcd for $C_{98}H_{80}N_{16}O_2 \cdot 6H_2O \cdot 1DMF \cdot 1CH_3I$: C, 47.15; H, 3.96; N, 9.16. Found: C, 47.07; H, 3.75; N, 9.10.

4.5. General procedure for the preparation of zinc(II) complexes of cationic bis-porphyrins linked with *p*- or *m*-xylylenediamine

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 then dried. This product was recrystallized several times by vapor diffusion method using DMF and diethyl ether. Absorption spectra of the zinc(II) complexes were measured in DMSO and in the buffered solution.

4.5.1. Zinc complex of H_2*p*Xy (Zn*p*Xy). Yield: 64%. ^{1}H NMR (400 MHz, DMSO-d_6, δ): 4.70 (s, 12H), 4.72 (s, 10H), 7.54 (s, 4H), 8.30 (d, J = 7.8 Hz, 4H), 8.40 (d, J = 7.8 Hz, 4H), 8.91 (d, J = 6.3 Hz, 4H), 8.93 (d, J = 6.3 Hz, 8H), 8.94-9.04 (br m, 16H), 9.41 (br d, J = 6.3 Hz, 12H). UV (DMSO) λ_{max} nm (ε): 607 (15,800), 563 (44,300), 438 (610,000); (buffer) λ_{max} nm (ε): 610 (15,100), 565 (37,300), 433 (449,000). MALDI-TOF-MS (m/z): $[M+H]^+$ calcd for $C_{98}H_{76}N_{16}O_2Zn_2$, 1640.6; found, 1641.3. Anal. Calcd for $C_{98}H_{76}N_{16}O_2Zn_2I_6 \cdot 8H_2O \cdot 0.5DMF$: C, 46.27; H, 3.73; N, 8.95. Found: C, 46.38; H, 4.05; N, 9.10.

4.5.2. Zinc complex of H_2*m*Xy (Zn*m*Xy). Yield: 63%. ^{1}H NMR (400 MHz, DMSO-d_6, δ): 4.71 (s, 12H), 4.72 (s, 6H), 4.74 (s, 4H), 7.46 (br s, 3H), 7.59 (s, 1H), 8.32 (d, J = 7.8 Hz, 4H), 8.43 (d, J = 7.8 Hz, 4H), 8.91 (d, J = 6.8 Hz, 4H), 8.93 (d, J = 6.3 Hz, 4H), 8.95-9.06 (br m, 16H), 9.41 (br d, J = 6.5 Hz, 12H). UV (DMSO) λ_{max} nm (ε): 607 (18,200), 563 (45,600), 437 (608,000); (buffer) λ_{max} nm (ε): 614 (13,100), 567 (25,300), 433 (290,000). MALDI-TOF-MS (m/z): $[M+H]^+$ calcd for $C_{98}H_{76}N_{16}O_2Zn_2$, 1640.6; Found, 1641.1. Anal. Calcd for $C_{98}H_{76}N_{16}O_2Zn_2I_6 \cdot 8H_2O \cdot 0.5DMF$: C, 46.27; H, 3.73; N, 8.95. Found: C, 46.41; H, 4.02; N, 8.73.

4.6. DNA photocleavage assay

Photoillumination was performed at 25 °C and ambient atmospheric pressure using a HITACHI 650-60 fluorescence spectrophotometer, equipped with 150 W Xe lamp. A sample containing supercoiled pUC18 plasmid DNA (60 μM) and a porphyrin (0.6 μM) was illuminated in the buffer at the Soret band on binding to

DNA (slit width: 20 nm). The sample received an incident energy of 9.5 J m^{-2} s^{-1} in the cell position, which was estimated by actinometry.[28] 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 DNA bands were detected by fluorescence. The densitometric data of the bands were obtained with ATTO Densitograph version 4.0 for Macintosh. Because staining intensity of form II is found to be 1.47 times that of form I,[29] the band intensities of form II were corrected by dividing by 1.47.

4.7. 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.[22] The buffered or DMSO solution containing a porphyrin (0.05 μM) and DPBF (30 μM), prepared in the dark, was illuminated at the Soret band (slit width: 20 nm) and 25 °C and ambient atmospheric pressure using a HITACHI 650-60 fluorescence spectrophotometer, and then a loss of absorbance at 415 nm was followed spectrophotometrically. Neither DPBF bleaching in the absence of the porphyrins nor porphyrin decomposition on illumination was observed.

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