DNA Cleavage by a Polyethylenimine-appended Porphyrin

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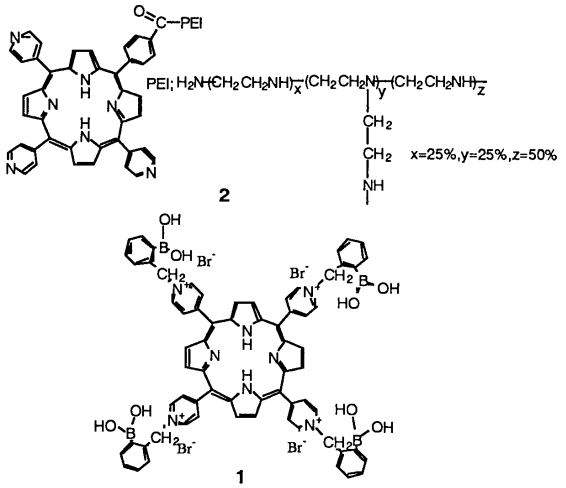
Polyethylenimine (PEI)-appended porphyrin **2** bound DNA more strongly than non-polyethylenimine-appended porphyrins by creation of cationic charges of polyethylenimine and photochemically cleaved a double-stranded DNA.

Porphyrin-DNA interactions are of great significance from a viewpoint of cancer research and gene technology.[1-6] It is interesting that positively-charged porphyrins bind DNA with the aid of electrostatic interactions,[1-8] where we have demonstrated that interactions between 5,10,15,20-tetrakispyridylporphyrin **1** and DNA were efficiently controlled by the addition of saccharides.[9] We now report the preparation of polyethylenimine (PEI)-appended porphyrin **2** and its ability to cleave a double-stranded DNA. We reasoned that **2** would bind DNA more strongly than non-polyethylenimine-appended porphyrins by creation of cationic charges at PEI.[10]



2

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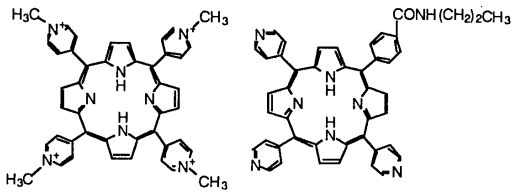

3 **4**

The synthetic sequence leading to **2** followed these steps. The porphyrin, 5-(4-carboxyphenyl)-10,15,20-trispyridylporphyrin and TPyPCOOH were prepared as described previously.[11] TPyPCOOH was treated with ethyl chloroformate in chloroform at low temperature and reacted with PEI (MW ca. 600) to give the PEI-appended porphyrins, followed by chromatographic separation (silica gel 10% methanol in chloroform). The ^{1}H NMR spectra of the compound support unambiguously the assigned structure. The chemical shifts for all peaks appeared as expected and integration of NMR spectra indicated the presence of one porphyrin group per polymer. The UV-visible absorbance spectra of PEI-appended porphyrin reveal characteristic porphyrin absorbance. The data for PEI-appended porphyrin agree well with previous spectral data for **3** or TPyPCOOH.

Figure 1 shows spectral changes in **2** induced by the addition of calf thymus DNA. DNA concentration is expressed as the mole concentration of base-pairs. As is apparent from Figure 1, the absorption maximum at 415 nm decreases with increasing concentration of DNA. This spectral change suggests that **2** is bound to calf thymus DNA. It is likely that binding of **2** to calf thymus DNA is a simple one-step process because an isosbestic point observed at 425 nm is very tight. The binding constant K and the mole number of **2** bound per mole of base pairs n were estimated from the Scatchard plot[12,13] (correlation coefficient 0.98, $K = 4.8\times10^7$ dm^{3}mol^{-1} and $n = 0.25$). These values are comparable with those for 5,10,15,20-tetrakis(*N*-methyl-4-pyridyl)porphyrin tetraperchlorate **3** ($K = 1.1 \times 10^7$ dm^{3}mol^{-1} and $n = 0.14$).[3] Interestingly, the K value is higher by about one order of magnitude than that for **4** ($K = 6.6 \times 10^6$ dm^{3}mol^{-1}). Spectral changes in **4** are induced by the addition of calf thymus DNA (data not shown). The absorption maximum at 415 nm only decreased with increasing DNA concentration, compared with **2** where isosbestic point is observed at 425 nm.

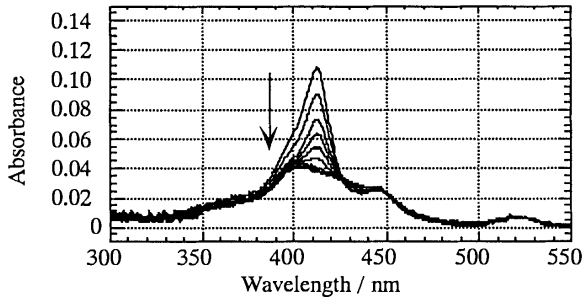

Figure 1. Absorption spectral change in **2** (1.0×10^{-6} mol dm^{-3}) induced by the addition of calf thymus DNA (1.0×10^{-7} to 5.0×10^{-6} mol dm^{-3}): 25 °C, water-methanol (300:1 v/v), pH 7.1 with 10.0 mmol dm^{-3} tris(hydroxymethyl)aminomethane buffer.

The binding mode of cationic porphyrins to DNA can be distinguished by the CD spectral pattern. In the presence of calf thymus DNA the CD spectrum of **2** gave a positive exciton coupling band at the Soret band region as shown in Figure 2. This indicates that **2** binds outside onto the double strand surface of calf thymus DNA like a *meso*-tetrakis[4-{(3-trimethylaminopropyl)oxy}phenyl]porphine.[14,15] The reason that the [θ] value of **2** is weaker than that of *meso*-tetrakis[4-{(3-trimethylaminopropyl)oxy}phenyl]porphine by one or two order of magnitude is considered to be due to the effect of PEI moiety in **2**.

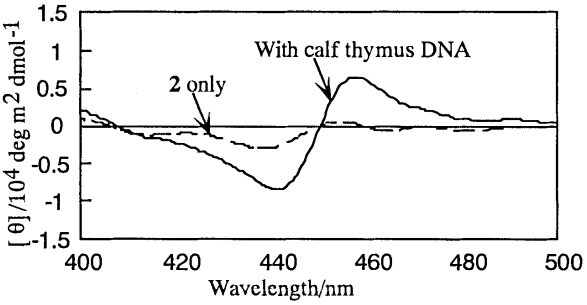

Figure 2. CD spectral change in **2** (6.7×10^{-6} mol dm^{-3}) induced by the addition of calf thymus DNA (2.0×10^{-5} mol dm^{-3}): 25 °C, water-methanol=300:1 v/v pH 7.1 with 10.0 mmol dm^{-3} tris(hydroxymethyl)aminomethane buffer. The ordinate unit is defined as [θ] per base pair mole.

To further examine the interaction of **2** and DNA, we performed DNA cleavage. The DNA photocleavage experiments were performed by illumination of an aqueous solution (pH 7.1 with 10.0 mmol dm^{-3} tris(hydroxymethyl)aminomethane buffer) containing colDNA (1.0×10^{-5} mol dm^{-3}) and **2** (5.0×10^{-6} mol dm^{-3} and 5.0×10^{-7} mol dm^{-3}) for 30 min with the transilluminator at 30 °C. The cleavage products were analyzed by agarose gel electrophoresis, monitoring the conversion of supercoiled colDNA (Form I) into nicked colDNA (Form II) and into linear DNA (Form III). Figure 3 shows the typical experimental results. As is apparent from Lanes 1 and 2 in Figure 3, restriction enzyme EcoRI perfectly converts Form I into Form III, indicating that colDNA has been cut at one point, cleaving both strands. Comparison of Lane 3 with Lane 4 indicates that **2** is inactive in the dark whereas it is photoactivated to convert Form I into Form II and Form III under photoirradiation: that is, **2** can efficiently cleave colDNA with the aid of light. Interestingly, we could find Form III under the concentration of **2** (5.0×10^{-6} mol dm^{-3}) at Lane 4. This cleaving activity of **2** was comparable with that of **3** as shown in Lane 8. As a control experiment, we used **4** instead of **2**, and tried the photocleavage in a same manner. The data of gel electrophoresis showed that **4** did not cleave colDNA at all as shown in Lane 6. These findings consistently establish that **2** serves as a novel and more efficient colDNA cleavage reagent. In general, the photochemical DNA cleavage mechanisms are classified into two categories; that is, photochemical generation of $^1O_2$ or radical species.[3,16-19] As shown in Lane 7 of Figure 3, the cleavage activity **2** was reduced to 10-20% (repeated three times) when NaN_3 as a $^1O_2$ quencher was added. This result supports the view that the activation of O_2 is one of the essential reaction mechanisms in the present system.

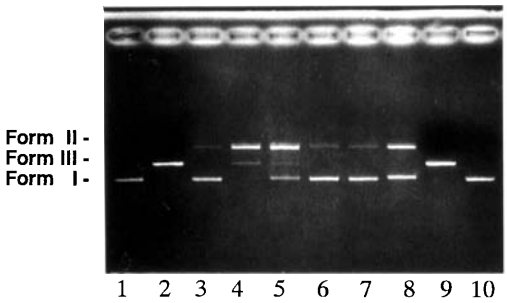

Figure 3. Gel electrophoresis showing results of photochemical cleavage of supercoiled circular double-strand DNA plasmid colDNA (Form I) into nicked colDNA (Form II) and into linear DNA (Form III). lane 1 and 10, control (C: untreated colDNA 1.0×10^{-5} mol dm^{-3}); lane 2 and 9, cDNA with restriction enzyme EcoRI (E: 0.1 unit/μl); lane 3, colDNA plus **2** (5.0×10^{-6} mol dm^{-3}) in the dark; lane 4, colDNA plus **2** (5.0×10^{-6} mol dm^{-3}) under photoirradiation (P); lane 5, colDNA plus **2** (5.0×10^{-7} mol dm^{-3}) under P; lane 6, colDNA plus **4** (5.0×10^{-7} mol dm^{-3}) under P; lane 7, colDNA plus **2** under P in the presence of NaN_3 (1.0×10^{-3} mol dm^{-3}); lane 8, colDNA plus **3** (5.0×10^{-7} mol dm^{-3}).

The present paper demonstrated that **2** can be bound to calf thymus DNA and cleaved DNA efficiently by creation of cationic charges at the PEI, converting Form I into Form II and Form III under photoirradiation. Further extensions to DNA cleavage and foot-printing are currently continued in our laboratory.

References and Notes

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