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Porphyrin dimers and their interaction with DNA

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Two porphyrin dimers bridged by 4,4′-dicarboxy-2,2′-bipyridine have been synthesised and characterised, and binding and photocleavage properties of a selected porphyrin with DNA have been studied using absorption, fluorescence and CD spectroscopy and gel electrophoresis.

A great deal of attention has been directed to bridged porphyrins.[1] The nature and position of the linker have a strong influence on the properties of the porphyrin dimers, especially, solubility and chemical stability.

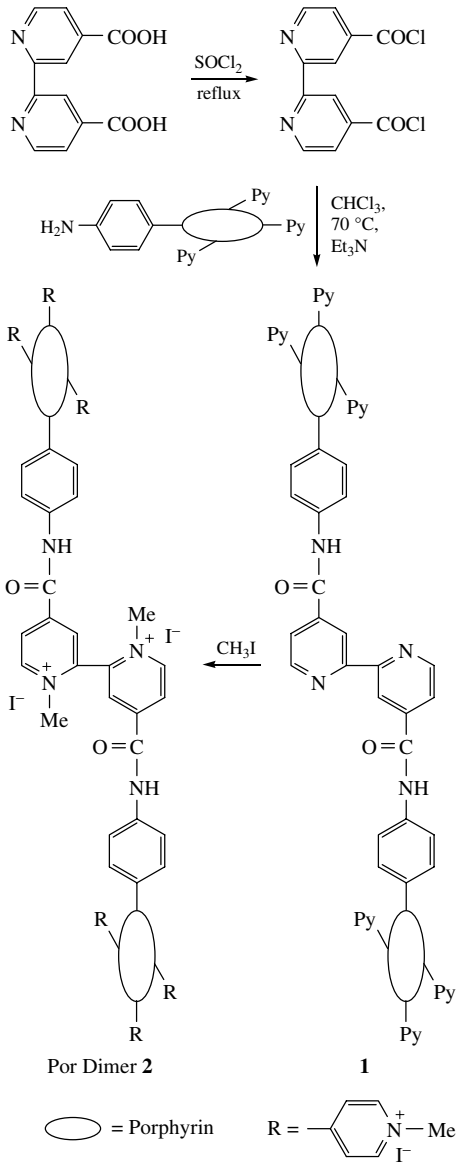
The interaction of water-soluble cationic porphyrins and nucleic acids has been intensively studied because of their potential clinical applications in photodynamic therapy.[2] Porphyrin as a photosensitizer can localize in tumor cells and be phototriggered to produce singlet oxygen, cleaving DNA and damaging tumor cells. However, the interaction of porphyrin dimers with DNA was little reported. Here, we report the synthesis of cationic porphyrin dimers and their interaction with DNA. *meso*-Tetrakis(*N*-methylpyridinium-4-yl)porphyrin (H_2TMPyP) was used as a reference compound.

The structure and synthetic routes of two porphyrin dimers are shown in Scheme 1. The classical Adler–Longo method was used to synthesise the original monoporphyrin.[3] Porphyrin dimer **1** was synthesised from the reaction of 4,4′-dicarboxy-2,2′-bipyridine and 5-(4-aminophenyl)-10,15,20-tripyridyl porphyrin. An excess of aminoporphyrin helped us to improve the yield. The objective dimer **2** containing eight positive charges was prepared by the reaction of alkylation. Both of the compounds were characterised by UV-VIS, IR and ^{1}H NMR spectroscopy and mass spectrometry.†

It is well known that DNA could be damaged by porphyrins after photoactivation owing to its ability to produce singlet oxygen. As the result shown in Figure 1, the measurement of singlet oxygen production by determining the decomposition of **1**, 3-diphenyl isobenzofuran (DPBF) for these porphyrins had been carried out before studies on cationic porphyrin dimers interacting with DNA. The slopes of the plots of bleached absorption of DPBF *versus* illumination time was proportional to the rate of production of singlet oxygen.[4] Thus, the rates of singlet oxygen production for **2** and H_2TMPyP exhibited no distinct differences. The structure of H_2TMPyP is shown in Figure 1.

As shown in Figure 2, UV titrations were performed with two porphyrins in a solution containing an increasing concentration of calf thymus (CT) DNA.[5] The red shift and intensity change of the Soret region with increasing CT DNA suggested the binding of the porphyrin to CT DNA (Table 1).

Fluorescence emission spectra were recorded with the increase of CT DNA for two porphyrins. With the titration of CT DNA,



Scheme 1 Synthetic routes of porphyrin dimers.

† For **1**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 9.06 (s, 2H, Py^3), 9.02 (m, 16H, H_β), 8.94 (d, 4H, CONH-Ph$_m$), 8.90 (d, 2H, Py^6), 8.83 (d, 12H, Por-Py$_o$), 8.67 (d, 2H, Py^5), 8.24 (d, 4H, CONH-Ph$_o$), 8.13 (d, 12H, Por-Py$_m$), –2.85 (s, 4H, pyrrole-H). UV-VIS ($CHCl_3$, λ_{max}/nm): 420, 517, 553, 594, 652. IR (KBr, ν/cm^{-1}): 1654 (CONH). MS (FAB), *m*/*z*: 1472 [M – 1]$^+$.

For **2**: ^{1}H NMR ([$^2H_6$]DMSO, 300 MHz) δ: 11.24 (s, 2H, Py^3), 9.47 (m, 16H, H_β), 9.19 (br. s, 6H, CONH-Ph$_m$, Py^6), 9.11 (br. s, 12H, Por-Py$_o$), 9.00 (m, 6H, Py^5, CONH-Ph$_o$), 8.18 (br. s, 12H, Por-Py$_m$), 4.70 (s, 24H, Me), –2.99 (s, 4H, pyrrole-H). MS (FAB), *m*/*z*: 1594 [M – 8I]$^+$.

Table 1 Compared data of the interaction of porphyrins with CT DNA.[a]

Porphyrin	UV-VIS on the Soret band		Fluorescence emission	CD in the Soret region		K_{app}/dm^3 mol^{-1}
	Hypochromicity H (%)	Red shift $\Delta\lambda$/nm	Decrease of intensity (%)	Positive band/nm	Negative band/nm	
2	37	9	21	445	426	1.2×10^6
H_2TMPyP	39	10	37	—	—	6.9×10^6

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

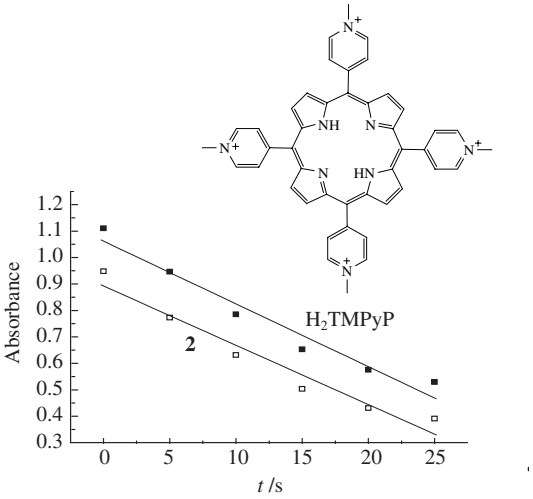


Figure 1 Decomposition of DPBF by **2** and H_2TMPyP. Porphyrin (1.0×10^{-6} mol dm^{-3}) and DPBF (1.0×10^{-4} mol dm^{-3}) were irradiated in a buffer (pH 7.4, 0.05 M Tris–HCl, 0.1 M NaCl); the structure of H_2TMPyP. Irradiating wavelength was 415 nm.

both of the porphyrins showed a remarkable decrease in the intensity of fluorescence emission, as shown in Figure 3.

CD spectroscopy is very useful for studying interactions between small molecules and DNA.[6] The sign of induced CD spectra in the Soret band depends upon the binding mode of porphyrin–DNA.[7] At the [**2**]/[DNA base pairs] ratio of 0 or 0.05, induced CD spectra of **2** bound to CT DNA are shown in Figure 4.

At a [**2**]/[DNA base pairs] ratio of 0, no sign of CD spectra was detected. When the ratio was changed to 0.05, a strong positive band at 445 nm and a weaker negative band at 426 nm appeared. In the case of duplex DNA, a positive induced CD

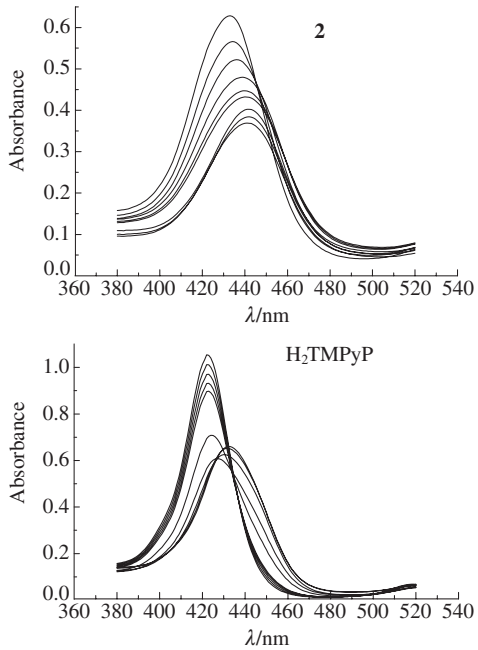


Figure 2 UV-VIS absorbance change on the titration of cationic porphyrins by CT DNA.

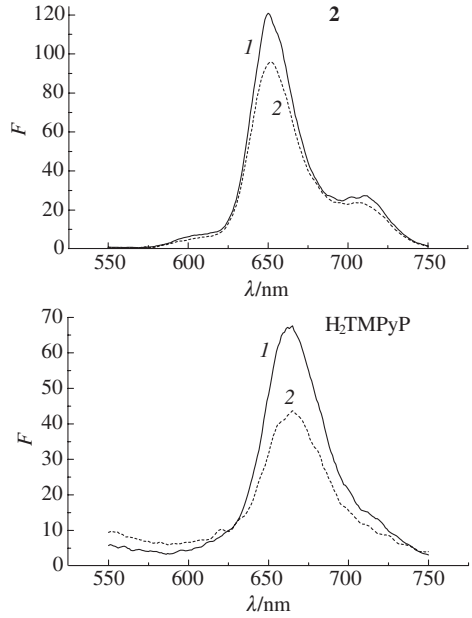


Figure 3 Fluorescence emission spectra of cationic porphyrins in the (*1*) absence and (*2*) presence of CT DNA.

band in the Soret region indicates its groove binding, and a negative induced CD band indicates intercalation.[8] With a view to hypochromic effect and red shift in UV-VIS spectra and a decrease in the intensity of fluorescence emission, the binding mode of **2** bound to DNA inclined to outside binding.[9] The bisignate nature of induced CD indicated that the binding modes were complex that involve both of intercalation and outside binding. Molecular conformation might play an essential role in the process of porphyrin dimer bonding to DNA.

UV and fluorescence titration was used to determine the binding constants of drugs for DNA,[5,10] but both of the methods have disadvantages. Taking an example of UV titration, it was difficult to determine the absorption coefficient of bound porphyrins and only applicable to intercalating porphyrins for which the Soret band was greatly modified upon binding to DNA.[11] A competition method with ethidium bromide (EB) was used to determine the apparent affinity binding constant (K_{app}).[12] This method can be used for all compounds having a good affinity for DNA as it only measures the ability of a com-

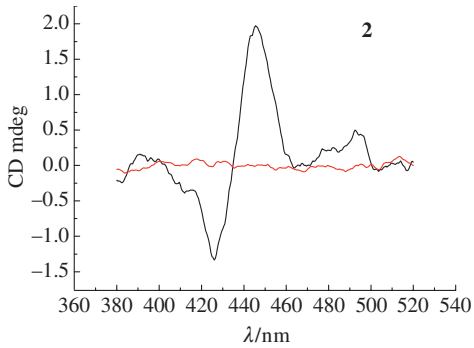


Figure 4 Induced CD spectra of **2** bound to CT DNA. [**2**]/[DNA base pairs] ratio, r = 0, 0.05.

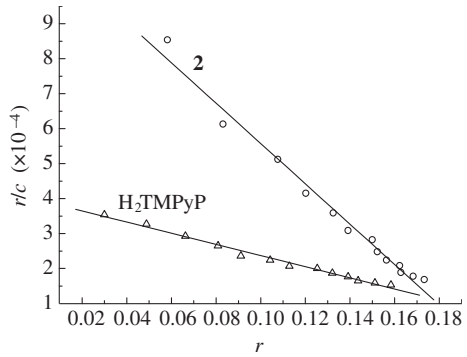


Figure 5 Competition between porphyrins and EB for the binding site of CT DNA (Scatchard plot), *r* is the number of moles of EB bound per mole of DNA, *c* is the concentration of free EB.

pound to prevent intercalation of EB into DNA. The Scatchard plots for the binding of EB to CT DNA in the presence of various porphyrins are shown in Figure 5.

The result showed that the value of K_{app} of H_2TMPyP was higher than that of **2**. In our supposition the binding ability of porphyrin with DNA should be improved with the increase of positive charges.[11] It was considered that the result should be caused by different binding modes to DNA. It was likely that the conformation of **2** took a passive part in the electronegative environment and resulted in a weaker affinity to CT DNA. Furthermore, the conformation of our target compound should be linear.[13] This conformation caused only one porphyrin ring containing three positive charges to intercalate DNA and influenced its affinity to DNA. The photocleavage of pBR322 plasmid DNA by two porphyrins was carried out to approve the above conclusion.

As shown in Figure 6, no cleavage of DNA occurred without irradiation either in control experiments or in the presence of porphyrins. No cleavage was observed when DNA was only in illumination. In the high concentration [Figure 6(*a*)] of porphyrins, DNA was almost fully cleaved by both of the porphyrins, while in the low concentration [Figure 6(*b*)], there was no cleavage of **2** compared with the complete cleavage of H_2TMPyP. The conclusion of this experiment was in accordance with the K_{app} measured above.

In conclusion, a bridged porphyrin containing eight positive charges has been synthesised and characterised. Cationic porphyrin binding with DNA and cleavage of DNA were investigated using UV-visible absorption, fluorescence and CD spectroscopy, measuring the apparent affinity binding constant (K_{app})

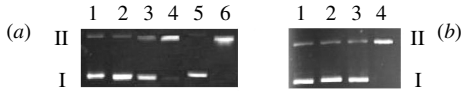


Figure 6 Cleavage of supercoiled pBR322 DNA by two porphyrins. 10 μl of reaction mixtures contained 1.0 μg of plasmid DNA, the time of illumination was 12 min. (*a*) Concentration of two porphyrins was 2.0 mmol dm^{-3}. Lane 1: DNA alone; lane 2: DNA alone + *hv*; lane 3: DNA + **2**; lane 4: DNA + **2** + *hv*; lane 5: DNA + H_2TMPyP; lane 6: DNA + H_2TMPyP + *hv*. (*b*) Concentration of two porphyrins was 0.5 mmol dm^{-3}. Lane 1: DNA alone; lane 2: DNA alone + *hv*; lane 3: DNA + **2** + *hv*; lane 4: DNA + H_2TMPyP + *hv*.

and observing the cleavage ability of porphyrins to plasmid DNA. The binding and cleavage ability of the porphyrin dimer to DNA was under our estimation in terms of H_2TMPyP. The possible reason was the configuration and binding mode of the porphyrin dimer. Probably, the structure of the bridged porphyrin was too huge to intercalate DNA and influenced its affinity to DNA.

Online Supplementary Materials

Supplementary data associated with this article (the details of experiment) can be found in the online version at doi:10.1016/j.mencom.2007.01.015.

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