Dramatic Enhancement of the Photoactivity of Zinc Porphyrin–Ellipticine Conjugates by DNA

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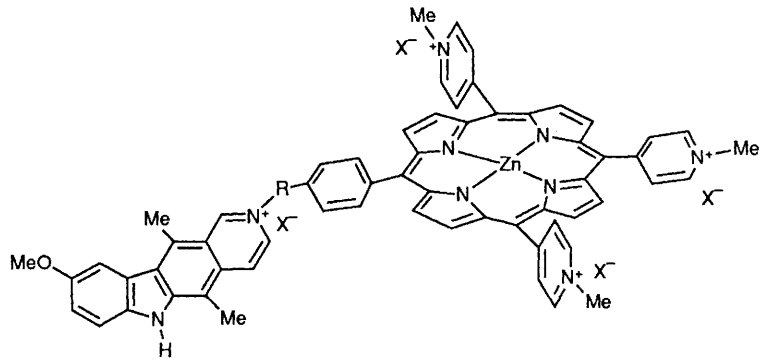
Both the fluorescence yield and the yield of singlet oxygen generated from the quenching of the triplet state of two water-soluble hybrid molecules 'zinc porphyrin-ellipticine', (**1**) and (**2**), are low compared to those of simple zinc porphyrin complexes; both these photochemical properties are dramatically enhanced by addition of calf thymus DNA to solutions of (**1**) or (**2**), as a result of the conformation change of these hybrid molecules upon interaction with double-stranded nucleic acids.

Currently efforts are being made to develop therapeutic agents which are selectively activated by their targets. In the case of cancer phototherapy with hematoporphyrin derivatives, the site of activity in and around the cancer cells is not specific and may be the cause of the concomitant phototoxic response which is observed.[1—3] With this in mind, we have begun to prepare photoactive porphyrins which may bind strongly and selectively to DNA owing to their linkage to a DNA intercalator. Here we report on the photoactivity of two hybrid molecules 'zinc-porphyrin-intercalator', (**1**) and (**2**). These two complexes were synthesized by the method used for their manganese and iron analogues.[4,5]

We have previously shown that (**2**) intercalates into DNA *via* the ellipticine moiety and in the case of poly(dA-dT) and poly(dG-dC) has a $K_{binding}$ of 2.0×10^9 and 1.9×10^8 dm^3 mol^{-1}, respectively.[5] These values are 3 to 4 orders of magnitude higher than for the binding of zinc–tetrakis(4-*N*-methylpyridiniumyl)porphyrin to the same model DNA polymers.[6,7] Dramatic modifications of spectroscopic and photochemical properties of the zinc porphyrin moiety of these two



(1) Porph.–O[CH_2]$_3$NHCO[CH_2]$_5$–Ellip.; X = OAc
(2) Porph.–NHCO[CH_2]$_4$–Ellip.; X = OAc

Structure of the hybrid molecules (1) and (2).

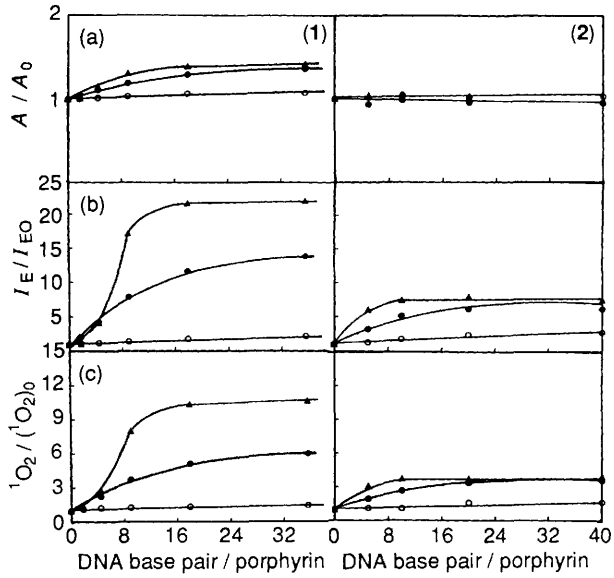


Figure 1. Plots of changes in spectroscopic and photochemical properties of (1) (2.8×10^{-6} M) and (2) (1.0×10^{-6} M) upon increasing the CT-DNA concentration in D_2O with different NaCl concentrations ([NaCl]: ▲, 0.01; ●, 0.2; ○, 1.0 M). (a) Absorption intensity at Soret absorption maximum relative to its value without added CT-DNA; (b) integrated emission intensity relative to its value without added CT-DNA; (c) observed $^1O_2$ emission signal relative to its value without added CT-DNA. Results in (b) and (c) reflect corrections made for changes in the absorption of each solution at the exciting wavelength, 514.5 nm.

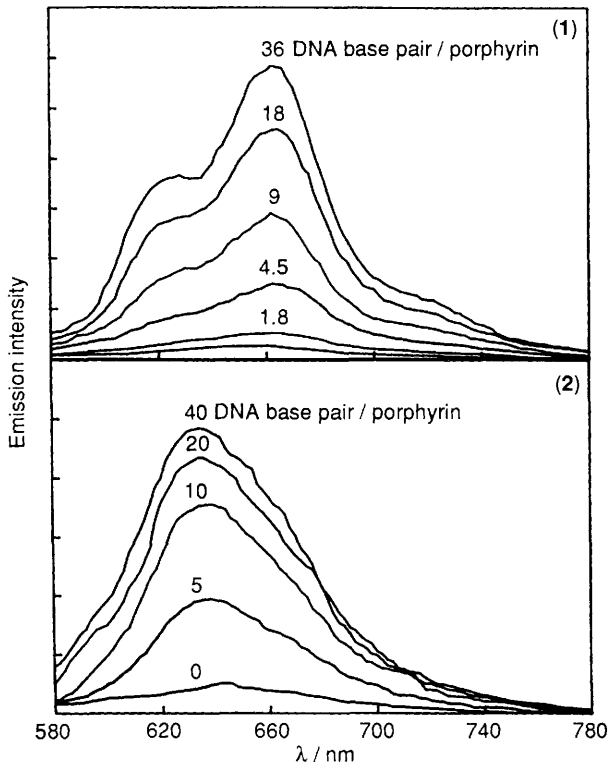


Figure 2. Examples of the enhancement of the emission of (1) and (2) upon addition of CT-DNA: (1) in 0.2 M NaCl in D_2O with 0, 1.8, 4.5, 9, 18, and 36 DNA base pairs per (1); (2) in 0.2 M NaCl in D_2O with 0, 5, 10, 20, and 40 DNA base pairs per (2). For both, the intensity of the emission increases with DNA concentration and the spectra were taken less than 2 hours after addition of the CT-DNA. The small band seen near 593 nm is due to a Raman band of D_2O. Excitation was at 514.5 nm.

hybrid molecules are observed during the addition of calf thymus DNA (CT-DNA) to solutions of (1) and (2) in 5 mM phosphate-buffered D_2O† with varying concentrations of NaCl.‡

In the case of (1), addition of CT-DNA leads to large changes in both the Soret and visible Q-band absorptions of the porphyrin whose magnitudes increase with increasing DNA concentration (Figure 1). The effect of CT-DNA decreases with increasing salt concentration, presumably because of the inhibiting effect of the salt on the complexation of two oppositely charged species. For (2), the spectrum does not change nearly as much directly after addition of CT-DNA (Figure 1).

Upon excitation in the *Q*-band of the zinc-porphyrin at 514.5 nm (additional experimental details in the Figure caption), the emission yield of (1) and (2) increases by up to 22 and 8 times, respectively, upon introduction of CT-DNA to their solutions (Figures 1 and 2). A change in the shapes of the emission spectra of (1) and (2) is also observed when they are complexed to DNA. In the absence of DNA, the spectral shape of the weak emission of (1) and (2) is different from that observed in the emission of the regular zinc–tetrakis(4-*N*-

† D_2O was used as the solvent as the weak emission signal of $^1O_2$ is more than an order of magnitude greater in this solvent than in H_2O.[8] pD was regulated by adding 5 mM phosphate in a proportion such that it would give a pH 7.4 solution in H_2O.

‡ Absorption spectra were recorded with either a CARY 219 spectrophotometer or a Hewlett–Packard 8452A Diode Array Spectrophotometer. Emission spectra were recorded with an Aminco SPF500 and were not corrected for the wavelength response of the instrument. The intensity of the singlet oxygen emission was determined using an apparatus described in detail elsewhere.[9]

methylpyridiniumyl)porphyrin. The latter exhibits two maxima at 630 and 660 nm.[10]§ For both hybrid molecules, the $Q_{0,0}$ is shifted to 646 nm, overlapping the $Q_{0,1}$ band at 660 nm. Upon addition of DNA, this shift is slightly increased in the case of (**1**). Since the chain between the intercalated ellipticine and the porphyrin is significantly longer and less rigid in (**1**) than in (**2**), (**1**) should be capable of interacting with more degrees of freedom with DNA. The structure in the emission of the complex (**1**) (or **2**) and CT-DNA is consistent with the porphyrin being bound in an environment more rigid and hydrophobic than fluid water,[6,9,11] such as the minor groove of CT-DNA.

Fluorescence quantum yields for (**1**) and (**2**) without added DNA are 7.6×10^{-4} and 8.5×10^{-4} (D_2O). This contrasts with 0.025 (H_2O)[10] and 0.024 (D_2O) for zinc–tetrakis(4-*N*-methylpyridiniumyl)porphyrin and 0.014 (H_2O and D_2O) for zinc–[(tris-*N*-methylpyridiniumyl)(*p*-*N*,*N*,*N*-trimethylaniliniumyl)]porphyrin. Thus, in (**1**) and (**2**) the presence of the linked ellipticine leads to quenching of the singlet state of the zinc–porphyrin moiety. While the possibility that interporphyrin stacking is the cause of the low emission yields cannot be totally discounted, cationic zinc porphyrins are usually thought not to dimerize.[12] As an alternative hypothesis, the reduced emission might be due to a photoinduced electron transfer between the metalloporphyrin and the ellipticine moieties. This is unlikely, since both moieties are positively charged. So this quenching may be due to a simple intramolecular stacking due to a folded conformation of these hybrid molecules. This is in agreement with NMR data obtained for similar non-cationic hybrid molecules.[4]

The emission enhancement resulting from addition of DNA to solutions of (**1**) or (**2**) is much higher than any enhancement observed when DNA is added to a solution of zinc–tetrakis(4-*N*-methylpyridiniumyl)porphyrin. With this zinc porphyrin the emission yield at a concentration of $1—5 \times 10^{-6}$ M does not change by more than 20% upon the addition of CT-DNA up to a DNA base pair : Zn-porphyrin ratio of 100 : 1 (see also ref. 6). So the emission enhancement observed for hybrid molecules (**1**) and (**2**) confirms that their strong affinity for DNA induces a dramatic change in the geometry of these hybrid molecules (previous studies have shown that the ellipticine moiety is intercalated while the zinc–porphyrin part is interacting outside DNA, probably in the minor groove[5]).

§ Because of the weakness of the emission spectra of (**1**) and (**2**), the Raman band of H_2 at 626 nm interferes significantly. This band was eliminated by working with D_2O solutions, as in this case the solvent Raman band is at 593 nm.

Upon excitation at 514.5 nm of aerated solution of (**1**) or (**2**), the yield of $^1O_2$ emission is enhanced as much as 11 and 4 times, respectively, upon addition of CT-DNA. This is shown in Figure 1, where the relative $^1O_2$ yield is plotted against added CT-DNA. The increase in the observed $^1O_2$ emission follows from the decreased quenching of the singlet state allowing a higher yield of porphyrin triplet state, since it is the quenching of the porphyrin triplet state by molecular oxygen that leads to $^1O_2$. The smaller enhancement of the observed $^1O_2$ emission relative to the singlet emission is probably due to quenching of part of the $^1O_2$ by CT-DNA.[9]

It has been shown that supercoiled DNA is cleaved by reaction of the excited singlet state of zinc–tetrakis(4-*N*-methylpyridiniumyl)porphyrin and by the $^1O_2$ produced by quenching of its triplet state.[6,13] The addition of the pendant DNA intercalator ellipticine to derivatives of the zinc–porphyrin leads to stronger binding to DNA and lends to DNA the ability to serve as a switch for the photoactivity of these hybrid Zn-porphyrin molecules.

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