Evidence for Formation of DNA-bound Protonated Porphyrin Adducts even at pH 7

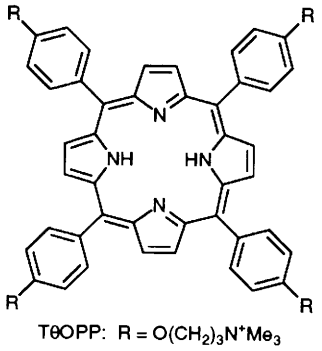
Gabor Pethö,[a] Nancy B. Elliott,[a] Min Sook Kim,[b] Mengfen Lin,[a] Dabney W. Dixon*[b] and Luigi G. Marzilli*[a]

[a] *Department of Chemistry, Emory University, Atlanta, Georgia 30322 USA*
[b] *Department of Chemistry, Georgia State University, Atlanta, Georgia 30303, USA*

An absorption and a positive circular dichroism (CD) band in the Soret region at 451 nm and a fluorescent emission band at 725 nm provide compelling evidence that the binding of a tentacle tetracationic porphyrin, *meso*-tetrakis[4-{(3-trimethylaminopropyl)oxy}phenyl]porphine (TθOPP), to calf thymus (CT) DNA leads to extensive porphyrin protonation, even at pH 7, and that a protonated porphyrin can form DNA adducts with a binding mode different from that of the unprotonated species.

The ability of porphyrins to associate with and/or to cleave DNA and RNA has potential relevance to many medical and biological applications of porphyrins.[1–3] More recent applications are based on the antiviral[4,5] and anticancer[6] activities of cationic porphyrins. *In vitro* structural studies of porphyrin–DNA adducts are important in order to gain insight into the factors which affect porphyrin biological activities. The supramolecular DNA–porphyrin structures are also of intrinsic chemical interest, and the chemistry and structure of the porphyrin and DNA may be changed by the interaction with one another.

We have previously reported a comparison of the water-soluble 'tentacle' porphyrins, TθOPP and *meso*-tetrakis[4-*N*-(3-trimethylaminopropyl)pyridyl]porphine (TθpyP).[7] Both porphyrins have a similar size and shape with four long tentacle-like propyl chains terminated with an essentially spherical *N*-trimethylammonium group. However, the tentacles are attached to the porphyrin core by phenoxy linkages in TθOPP and by pyridinium linkages in TθpyP. The electron-donating ability of the phenoxy aromatic substituents of TθOPP increases porphyrin basicity such that the species is half protonated at pH 4.6, a value which is much higher than that for TθpyP (pH *ca.* 1) and which establishes TθOPP as having an electron rich porphine core. Spectroscopic and viscometric studies indicate that TθpyP is an intercalator, whereas TθOPP is an outside binder with self-stacking along the DNA surface.[7]



TθOPP: R = $O(CH_2)_3N^+Me_3$

For a cationic porphyrin, not only is the electron-richness of the porphyrin core of TθOPP very unusual, but the flexible tentacle arms represent a unique characteristic for an outside binder. We present evidence that the DNA binding of TθOPP promotes protonation of this porphyrin in a pH range compatible with duplex DNA. This has allowed us to study for the first time the DNA binding mode of a porphyrin with a protonated core.

In Fig. 1, we present a titration of TθOPP (chloride salt) with calf thymus (CT) DNA monitored by the Soret absorption spectrum. On addition of CT DNA, the Soret band was red-shifted by 5 nm, and it decreased in intensity by 45% (R = 0.25, where R = [porphyrin]/[DNA base pairs]). These changes are characteristic of outside binding with stacking. The circular dichroism (CD) spectrum for this TθOPP : DNA ratio (Fig. 2) reveals a strong conservative feature, characteristic of outside self-stacking. This exciton type of spectrum has been observed previously in several cases.[7–12]

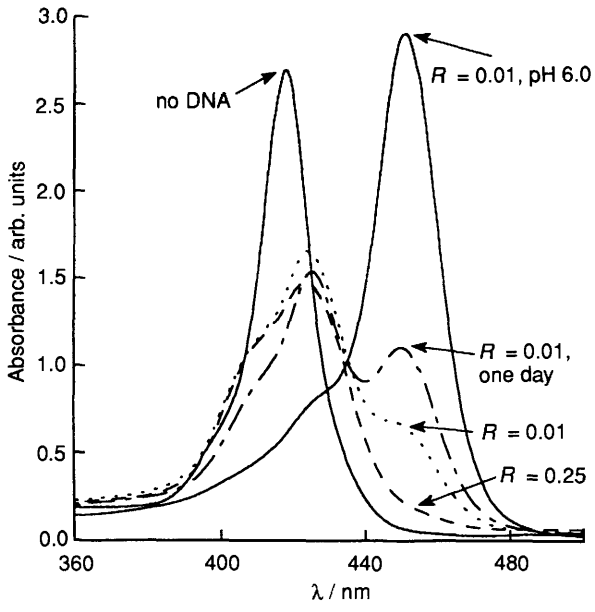


Fig. 1 VIS spectra of 7.5 μmol dm^{-3} TθOPP in the presence of various CT DNA concentrations (R = [(TθOPP]/[DNA base pairs]). Unless otherwise indicated, samples were at pH 7.0, with no buffer, 10 mmol dm^{-3} NaCl and spectra were measured immediately after sample preparation. The sample at R = 0.01, pH 6.0 contained no buffer, and its spectrum was measured one day after sample preparation.

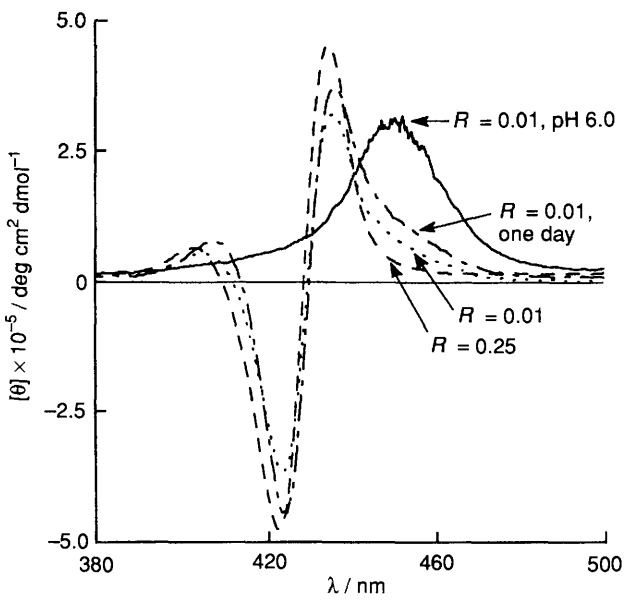


Fig. 2 CD spectra of 7.5 μmol dm^{-3} TθOPP in the presence of various CT DNA concentrations (R = [TθOPP]/[DNA base pairs]). Unless otherwise indicated, samples were at pH 7.0, with no buffer, 10 mmol dm^{-3} NaCl and spectra were measured immediately after sample preparation. The sample at R = 0.01, pH 6.0 contained no buffer, and its spectrum was measured one day after sample preparation.

At lower R values, the spectra changed. For example, at R = 0.01 the visible spectrum contained a band at 451 nm at pH 7 (carefully controlled without buffer). This band increased with time, and it was much stronger at pH 6 (Fig. 1). When the pH was raised to 8, the band disappeared. We attribute this 451 nm band to a protonated TθOPP. In the absence of DNA and at low pH, the Soret band of TθOPP was observed at 445 nm. Thus, the red shift indicates that protonated TθOPP can bind to DNA. An analogous red shift of the fluorescence band from 705 to 725 nm is also consistent with a protonated TθOPP bound to DNA. Likewise, there was an induced CD band at 451 nm in the R = 0.01 solution (Fig. 2). This band must arise from a DNA-bound porphyrin species since no CD bands are possible for the achiral porphyrin. A positive CD band is characteristic of an unstacked, outside-bound porphyrin.[8] Thus, the DNA binding favours protonation and the resulting protonated species adopts a binding mode different from that of the unprotonated porphyrin.

The addition of a proton to a nitrogenous base is ordinarily a very fast process. It is of some interest that formation of the bound, protonated TθOPP occurred on the time scale of hours. This slow process cannot be the result of the protonation step and probably involves a slow unstacking process. Indeed, at high R (where stacking is favoured), protonation is not very favourable, as indicated by the absence of the characteristic bands at 451 nm. It is also noteworthy that the 451 nm band is very much weaker at pH 7 in the presence of PIPES buffer [piperazine-*N*,*N*′-bis(2-ethanesulfonic acid), data not shown]. The PIPES dianions appear to inhibit protonation by stabilizing the stacked outside-bound form (presumably by electrostatic interactions with tentacles not directed towards the DNA).

Binding of a protonated porphyrin to DNA has not been recognized previously; therefore, we cannot compare our results with those for other porphyrins. However, we believe that outside binding should be a general characteristic of such protonated species. Protonation leads to doming of the porphyrin.[13] Such doming will diminish stacking. Furthermore, both the introduction of positive charge in the centre of the porphyrin and the decreased electron richness of the porphyrin π system will also disfavour self-stacking.[14]

In conclusion, this TθOPP–DNA system provides an excellent example of the dependence of DNA binding modes on porphyrin chemistry. The possible existence of bound protonated species should be evaluated in all future investigations of porphyrin–DNA interactions.

We thank the NIH for support from grant AI 127196.

Received, 26th April 1993; Com. 3/02392C

References

1 R. J. Fiel, *J. Biomol. Struct. Dyn.*, 1989, **6**, 1259.
2 L. G. Marzilli, *New J. Chem.*, 1990, **14**, 409.
3 M. Pitié, G. Pratviel, J. Bernadou and B. Meunier, *Proc. Natl. Acad. Sci. USA*, 1992, **89**, 3967.
4 D. W. Dixon, L. G. Marzilli and R. Schinazi, *Ann. N.Y. Acad. Sci.*, 1990, **616**, 511.
5 L. Ding, J. Balzarini, D. Schols, B. Meunier and E. De Clercq, *Biochem. Pharmacol.*, 1992, **44**, 1675.
6 L. Ding, G. Etemad-Moghadam, S. Cros, C. Auclair and B. Meunier, *J. Med. Chem.*, 1991, **34**, 900.
7 L. G. Marzilli, G. Pethö, M. Lin, M. S. Kim and D. W. Dixon, *J. Am. Chem. Soc.*, 1992, **114**, 7575.
8 M. J. Carvlin and R. J. Fiel, *Nucl. Acids Res.*, 1983, **11**, 6121.
9 M. J. Carvlin, N. Datta-Gupta and R. J. Fiel, *Biochem. Biophys. Res. Commun.*, 1982, **108**, 66.
10 E. J. Gibbs, I. Tinoco, Jr., M. F. Maestre, P. A. Ellinas and R. F. Pasternack, *Biochem. Biophys. Res. Commun.*, 1988, **157**, 350.
11 R. F. Pasternack, R. A. Brigandi, M. J. Abrams, A. P. Williams and E. J. Gibbs, *Inorg. Chem.*, 1990, **29**, 4483.
12 B. P. Hudson, J. Sou, D. J. Berger and D. R. McMillin, *J. Am. Chem. Soc.*, 1992, **114**, 8997.
13 W. R. Scheidt and Y. J. Lee, in *Structure and Bonding*, ed. J. W. Buchler, Springer-Verlag, New York, 1987, pp. 2–63.
14 K. Bütje and K. Nakamoto, *Inorg. Chim. Acta*, 1990, **167**, 97.