Interactions of Anionic Carboranylated Porphyrins with DNA

Rosaria Lauceri,† Roberto Purrello,*,‡ Shankar J. Shetty,§ and M. Graça H. Vicente*,§

Istituto per lo Studio delle Sostanze Naturali di Interesse Alimentare e Chimico Farmaceutico C.N.R., Catania, Italy
Dipartimento di Scienze Chimiche, Università di Catania Viale Andrea Doria 6, 95125 Catania, Italy
Departments of Chemistry and Neurological Surgery University of California, Davis, California 95616

Received January 24, 2001

Porphyrins have been widely investigated as anti-cancer drugs in photodynamic therapy, in part because both cationic and anionic derivatives can be easily obtained and administered.[1] The formation of porphyrin−DNA complexes, which is believed to be an important step leading to antitumor activity, is known to be facilitated by the electrostatic attractions between the periphery of cationic porphyrins and the anionic phosphate backbone. On the other hand, the interaction of anionic molecules with DNA has not been as well studied.[2] Recently, water-soluble anionic *nido*-carboranyl-containing porphyrins have been proposed as boron delivery drugs in Boron Neutron Capture Therapy (BNCT) of tumors.[3,4] The present work was performed with the aim of investigating whether the negative charges and the bulkiness of the *nido*-carboranyl groups at the periphery of these molecules influenced the formation of porphyrin−DNA complexes. The cell nucleus, and more precisely DNA, is believed to be an important target in BNCT.[5]

In this study we have characterized the acid−base equilibria of *meso*-tetrakis[4-(*nido*-carboranyl)phenyl]porphyrin (*p*-H_2TCP,$^{4-}$ **1**) and *meso*-tetrakis[3-(*nido*-carboranyl)phenyl] porphyrin (*m*-H_2TCP,$^{4-}$ **2**) and investigated their interactions with DNA. We present evidence that these tetraanionic molecules (Figure 1) do indeed interact with DNA under physiological conditions (pH 7.4, and ionic strength $I = 0.15$ M NaCl). Furthermore, we show that the counterintuitive binding of these tetraanionic porphyrins to a 2-fold array of negative charges is driven by molecular recognition processes that can be deeply affected by the spatial distribution of the anionic substituents.

Protonation of porphyrin **1** causes hypochromicity, red-shift, and broadening of the 412 nm Soret band of the free-base (at pH 11.0, Figure 2), as well as fluorescence quenching. Both absorption and fluorescence spectra indicate an unusually high pK_a of ca. 7.4. Although anionic tetraphenylporphyrins are normally expected to have higher pK_a values because of the inductive effect of the anionic substituents,[6] this value is among the highest measured for a water-soluble porphyrin. The high basicity of **1** provided further incentive for us to study possible interactions of this type of molecule with DNA, since these interactions might be favored by porphyrin inner core protonation at physiological pH values. However, the determination of the pK_a[7] can be affected by the concomitant self-aggregation of **1** that might form aggregates similar to those proposed for the *para*-sulfonated porphyrins.[8] To study this self-aggregation process we determined the time-dependence (second derivative) of the absorption spectrum at pH 5.0, when **1** is fully protonated (in this and all experiments reported in this paper the porphyrin concentration was 4.0 μM and $I = 0.15$ M). It was observed that the broad Soret band was resolved into two time-dependent bands, one centered at ca. 417 nm that decreases (inset of Figure 2, **a**) and another at ca. 460 nm that increases (inset of Figure 2, **b**). We assigned the long-wavelength band to the formation of diprotonated **1** (*p*-H_4TCP^{2-}) self-aggregates that slowly form at the expense of the monomeric diprotonated form (absorbing at 417 nm). This pronounced tendency of *p*-H_4TCP^{2-} to self-aggregate is not surprising considering the hydrophobic nature and symmetry of the porphyrin skeleton.

Addition of DNA at pH 7.4 to a solution of **1** caused a small red shift ($\Delta\lambda = 2$ nm), hypochromicity (ca. 10%), and broadening of the Soret band (Figure 3). In addition, a small conservative

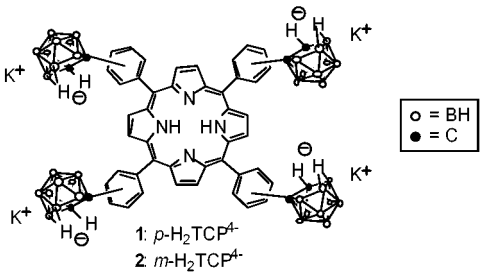

Figure 1. Carboranylated porphyrins used in this study.

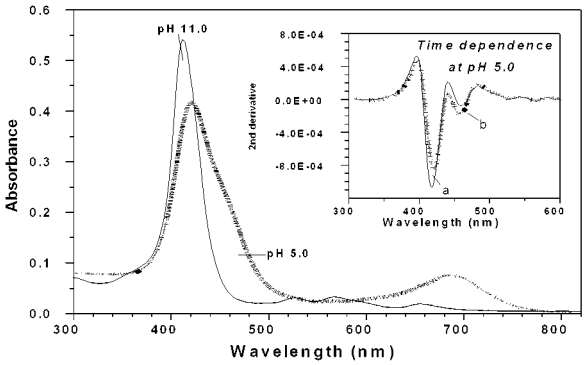

Figure 2. Absorption spectra of **1** at pH 11.0 and 5.0. The inset shows the time dependence of the (second derivative) spectrum at pH 5.0 (**a**, at $t = 0$; **b**, after 20 min). Spectra were recorded on a Cary 500.

* Address correspondence to these authors. E-mail: rpurrello@dipchi.unict.it; mgvicente@ucdavis.edu.
† Istituto per lo Studio delle Sostanze Naturali di Interesse Alimentare e Chimico Farmaceutico.
‡ Università di Catania.
§ University of California, Davis.

(1) Pandey, R. K.; Zheng, G. In *The Porphyrin Handbook*; Kadish, K. M., Smith, K. M., Guilard, R., Eds.; Academic Press: Boston, 2000, Vol. 6, pp 157−230.
(2) For published work in this area see, for example: Li, Y.; Geyer, R.; Sen, D. *Biochemistry* **1996**, *35*, 6911−6922. Chatterjee, S. R.; Srivastava, T. S.; Kamat, J. P.; Devasagayam, T. P. A. *J. Porphyrins Phthalocyanines* **1998**, *2*, 337−343.
(3) Vicente, M. G. H.; Shetty, S. J.; Wickramasinghe, A.; Smith, K. M. *Tetrahedron Lett.* **2000**, *41*, 7623−7627.
(4) *nido*-Carboranylporphyrins were first reported by Rudolph et al. (Haushalter, R. C.; Rudolph, R. W. *J. Am. Chem. Soc.* **1978**, *100*, 4628−4629) and subsequently investigated by other groups (see: Kahl, S. B.; Joel, D. D.; Nawrocky, M. M.; Micca, P. L.; Tran, K. P.; Finkel, G. C.; Slatkin, D. N. *Proc. Natl. Acad. Sci.* **1990**, *87*, 7265−7269. Miura, M.; Gabel, D.; Oenbrink, G.; Fairchild, R. G. *Tetrahedron Lett.* **1990**, *31*, 2247−2250).
(5) (a) Gabel, D.; Foster, S.; Fairchild, R. G. *Radiat. Res.* **1987**, *111*, 14−25. (b) Hartman, T.; Carlsson, J. *Radiother. Oncol.* **1994**, *31*, 61−75.
(6) Pasternack, R. F.; Huber, P. R.; Boyd, P.; Engasser, G.; Francesconi, L.; Gibbs, E.; Fasella, P.; Cerio Venturo, G.; deC Hinds, L. *J. Am. Chem. Soc.* **1972**, *94*, 4511−4517.
(7) The concomitant self-aggregation processes and kinetic inertness of the aggregates prevented us from fully understanding whether the reported pK_a is related to the release of one ($H_4TCP^{2-} \rightleftharpoons H_3TCP^{3-} + H^+$) or two core protons ($H_4TCP^{2-} \rightleftharpoons H_2TCP^{4-} + 2H^+$).
(8) (a) Ribo, J. M.; Crusats, J.; Farrera, J.-A.; Valero, M. L. *J. Chem. Soc., Chem. Commun.* **1994**, 681−682. (b) Pasternack, R. F.; Schaefer, K. F.; Hambright, P. *Inorg. Chem.* **1994**, *33*, 2062−2065.

10.1021/ja015581m CCC: $20.00
Published on Web 05/22/2001

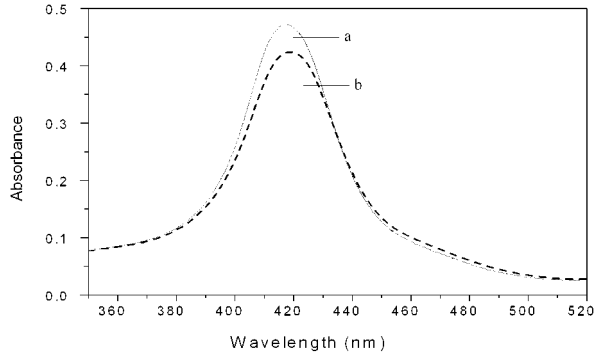

Figure 3. Absorption spectra of **1** in (a) the absence and (b) the presence of DNA (100.0 μM).

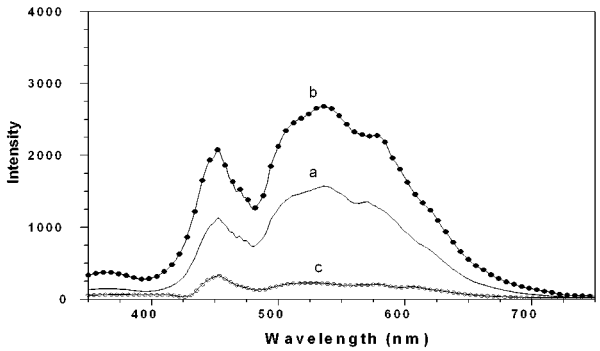

Figure 4. RLS spectrum of **1** in (a) the absence and (b) the presence of DNA (100.0 μM). Spectrum c shows the RLS of the monomeric Zn complex of **1** as reference.

ICD was observed in the Soret region.[9] These spectroscopic variations strongly indicate that the diprotonated form of porphyrin **1** interacts with DNA.[10] This was confirmed by Resonance Light Scattering (RLS) experiments, which showed an increased scattering following addition of DNA (Figure 4).

The relevance of the protonation state was underscored by the absence of any spectroscopically detectable (p-H_2TCP)$^{4-}$-DNA interaction at pH 9.5, when only about 1% of porphyrin is protonated. These results led us to conclude that DNA interactions with anionic porphyrins are favored by porphyrin inner core protonation. Since the self-aggregation of **1** onto DNA is clearly related to the protonation state, we could not exclude the possibility that porphyrin−DNA interactions could favor the formation of the diprotonated species and its subsequent self-aggregation on the DNA surface.[11]

In contrast, a similar study undertaken with the *meta*-substituted porphyrin **2** revealed that other factors can account for anionic porphyrin−DNA interactions and that protonation is not necessary for this process to occur. Porphyrin **2** has a pK_a of ca. 5.0, and at pH 7.4 is about 99% unprotonated. Notwithstanding these data, addition of DNA to a solution of m-H_2TCP^{4-} at this pH caused hypochromicity (ca. 20%) of the Soret band at 412 nm (monomeric form) and an increase in the intensity of the band at 424 nm, which is assigned to the self-aggregated form (Figure 5). Identical results were obtained at pH 9.5, which excluded the possibility that interactions with DNA induced a shift of the pK_a value. These results, along with RLS evidence (Figure 6), indicate that porphyrin **2** self-aggregates onto the DNA matrix.

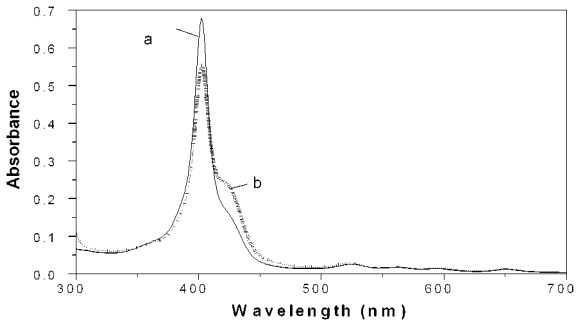

Figure 5. Absorption spectra of **2** in (a) the absence and (b) the presence of DNA (100.0 μM).

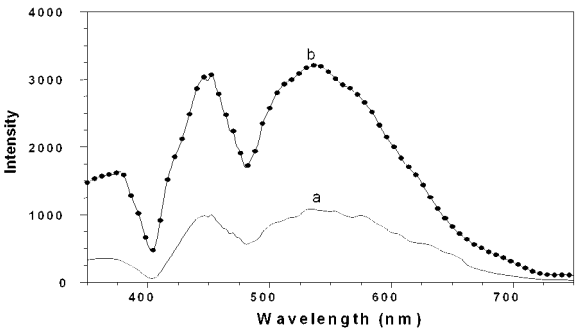

Figure 6. RLS spectrum of **2** in (a) the absence and (b) the presence of DNA (100.0 μM).

Porphyrin **2** exists as a mixture of atropisomers[12] and its CD spectrum shows a small signal in the Soret region in the absence of DNA, possibly due to the formation of chiral aggregates.[9] The CD spectrum change upon addition of DNA confirms (m-H_2-TCP)$^{4-}$-DNA interactions, and might reflect the preferential binding of one or more chiral self-aggregates of **2**, which best fit the DNA template. Such enantioselective binding has been shown for cationic metallointercalators and is favored by the match between the chirality of the complex and that of DNA, which can also maximize noncovalent interactions.[13]

In conclusion, we have shown that negatively charged porphyrins bearing peripheric bulky *nido*-carborane groups can indeed interact with DNA. In addition, we have shown that the complexation between anionic porphyrins and DNA can be induced by either porphyrin inner core protonation or other noncovalent interactions. A more complete study of these systems is currently underway since a deeper understanding of these processes might lead to the design of new anti-cancer drugs with improved biological activity and treatment effectiveness.

Acknowledgment. We thank the University of Catania, MURST (Cluster Biomedicina, legge 488/92), and the United States Department of Energy (Grant No. 98ER62633) for financial support.

JA015581M

(9) As reported for other self-assemblies of achiral porphyrins, an ICD signal is also observed in the absence of DNA (see: Pasternack, R. F.; Bustamante, C.; Collings, P. J.; Gianetto, A.; Gibbs, E. J. *J. Am. Chem. Soc.* **1993**, *115*, 5393−5399); however, the intensity of the CD signal increases approximately 3-fold upon addition of DNA.

(10) The main porphyrin−DNA binding modes are (i) intercalation (large hypochromicty and Soret shift, negative ICD), (ii) external binding (small hypochromicity and Soret shift, no ICD or very small), and (iii) aggregation (extensive hypochromicity and Soret shift, split ICD), see: Pasternack, R. F.; Gibbs E. J. *ACS Symp. Ser.* **1989**, *402*, 9 and references therein.

(11) A similar phenomenon has been observed for cationic porphyrins, see: Pethö, G.; Elliot, N. B.; Kim, M. S.; Dixon, D. W.; Marzilli, L. G. *J. Chem. Soc., Chem. Commun.* **1993**, 1547−1548.

(12) Vicente, M. G. H.; Nurco, D. J.; Shetty, S. J.; Medforth, C. J.; Smith, K. M. *Chem. Commun.* **2001**, 483−484.

(13) Barton, J. K. *Science* **1986**, *233*, 727−734.