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New Amino Acid Porphyrin Derivatives. Part II: DNA Binding Studies

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Abstract. Binding to a variety of natural and synthetic DNAs by cationic water-soluble porphyrins - having three N-methylpyridynium and one amino-acid derivatized phenyl ring as *meso* substituents - has been investigated. These studies indicate that amino acid porphyrin derivatives are able to intercalate into calf thymus DNA, but not as readily as *meso*-tetrakis-(N-methyl-4-pyridiniumyl)porphyrin (H_2TMPyP-4). This lowering of affinity for intercalation may be due to the presence of only three positive charges on the porphyrin ion and/or an increased specificity towards specific DNA sequences arising from amino acid linkage. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

The interactions of *meso*-tetrakis-(N-methyl-4-pyridiniumyl)porphyrin (H_2TMPyP-4) and its metalloderivatives, and of *meso*-tetrakis-(N-methyl-2-pyridiniumyl)porphyrin H_2TMPyP-2 (which differs in the position of the N-methyl group) with synthetic and natural DNAs have been widely studied, using visible spectroscopy and circular dichroism,[1] fluorescence,[2] footprinting,[3] EPR,[4] Raman,[5,6a] viscometry,[1b-c,6a-b] NMR[5,6] and kinetic methods.[7] Results obtained via these techniques reveal three main binding modes: intercalation, outside binding involving either placement of the porphyrin in the minor groove and electrostatic interactions with the backbone, or porphyrin stacking along the helix.[8] Though the studies have been performed under a variety of conditions including a number of buffers such as phosphate, Tris HCl, PIPES or MES, all authors agree that the binding mode is highly dependent on the geometry of the porphyrin (thickness, presence or not of an axial ligand in the case of a metalloporphyrin, substituent groups on the macrocycle) and on the type of DNA. Intercalation occurs in GC-rich regions and outside binding preferentially in AT-rich regions.[1a]

In the present study, a number of water-soluble synthetic C-terminal mono-, di- and tri-amino acid porphyrin derivatives (Figure 1) are compared to reference molecules, namely: H_2TMPyP-4, which has been shown to intercalate in CG sequences, and its zinc derivative ZnTMPyP-4 and H_2TMPyP-2, which bind to DNA in an external mode.[1a,1c-d]

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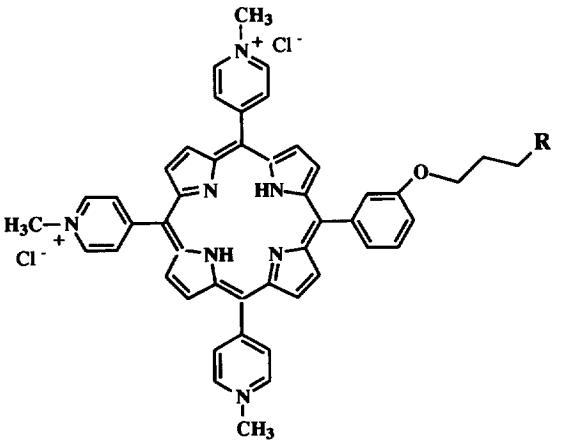

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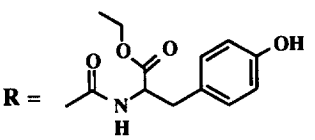

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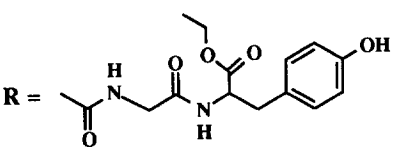

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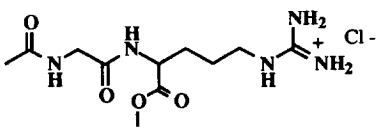

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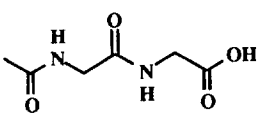

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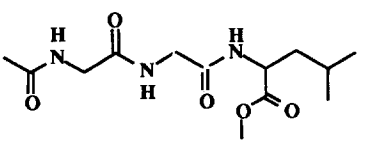

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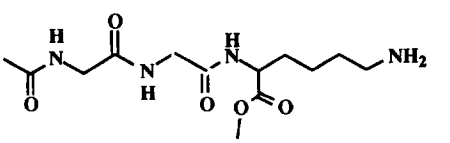


tMPyP-GGK (16c)

Figure 1

The interactions of these porphyrins with various types of DNA (calf thymus DNA (CT-DNA), *Micrococcus lysodeikticus* DNA (ML-DNA), *Clostridium perfringens* DNA (CP-DNA), poly(dAdT)$_2$ (AT) and poly(dGdC)$_2$ (GC) were studied using visible spectroscopy and circular dichroism (CD).

RESULTS

Spectrophotometric titrations were carried out at the Soret maximum. The following terms are defined when a porphyrin P is bound to DNA:

- The ratio of initial concentrations $1/r_0 = [DNA]_0(bp)/[P]_0$
- The bathochromicity $\Delta\lambda = \lambda - \lambda_0$

  where: λ_0 = absorption wavelength of the free porphyrin

  λ = absorption wavelength of the porphyrin in the presence of DNA
- The hypochromicity $H_{app}\% = [(A-A_0)]x100/A_0$

  where: A_0 = absorbance of the initial porphyrin solution at λ_0

  A = absorbance of the same concentration of porphyrin in the presence of DNA at λ

As it has been previously described,[1,9] porphyrins, upon binding to DNA, display spectral changes, characteristic of the manner in which they interact: intercalation between CG base-pairs produces large hypochromicity and bathochromicity of the Soret band, whereas external association within a groove induces only slight effects. Furthermore, although themselves non-chiral, these porphyrins exhibit a positive induced CD band in the Soret region when externally bound to DNA and a negative one when intercalated. Such spectral signatures have helped establish that even porphyrins that are known to intercalate, can display both association modes, depending on the $1/r_0$ ratio and other solution conditions (nature of the buffer, pH, ionic strength,...).[1,9,10]

In order to obtain results which can be compared directly to biochemical studies that are underway in our laboratory with the same porphyrins, all spectral experiments presented in this work were carried out in a 40 mM Tris acetate buffer at a pH of 7.5.

VISIBLE SPECTROSCOPY

The visible spectra of the reference porphyrins and the new amino acid porphyrin derivatives were recorded in the presence of increasing amounts of various DNAs, at $1/r_0$ values up to 100. Results are given in figures 2-6, presenting, for each porphyrin/DNA interaction, the hypochromicity and bathochromicity versus $1/r_0$.

We observe in the present work, that the hypochromicity never reaches a constant value as $1/r_0$ increases (Figures 2b, 3b, 4b). This apparent dependence of the molar extinction coefficient of the bound porphyrin upon $1/r_0$ has already been observed by Pasternack[1a] in the binding of H_2TMPyP-4 to poly(dAdT)$_2$ and calf thymus DNA. It is difficult, therefore, to assess the relative importance of different binding processes for a given derivative using this criterion alone, and so this study has been limited to a qualitative comparison of the hypochromicity and bathochromicity patterns.

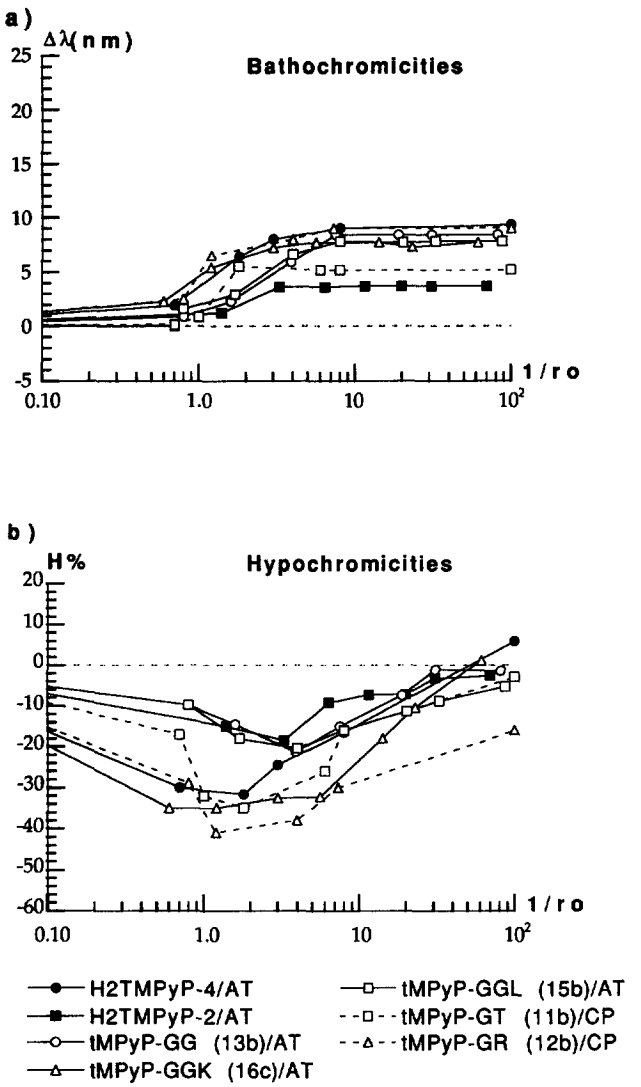


Figure 2: Influence of AT- rich DNA association on the visible spectra of porphyrin derivatives

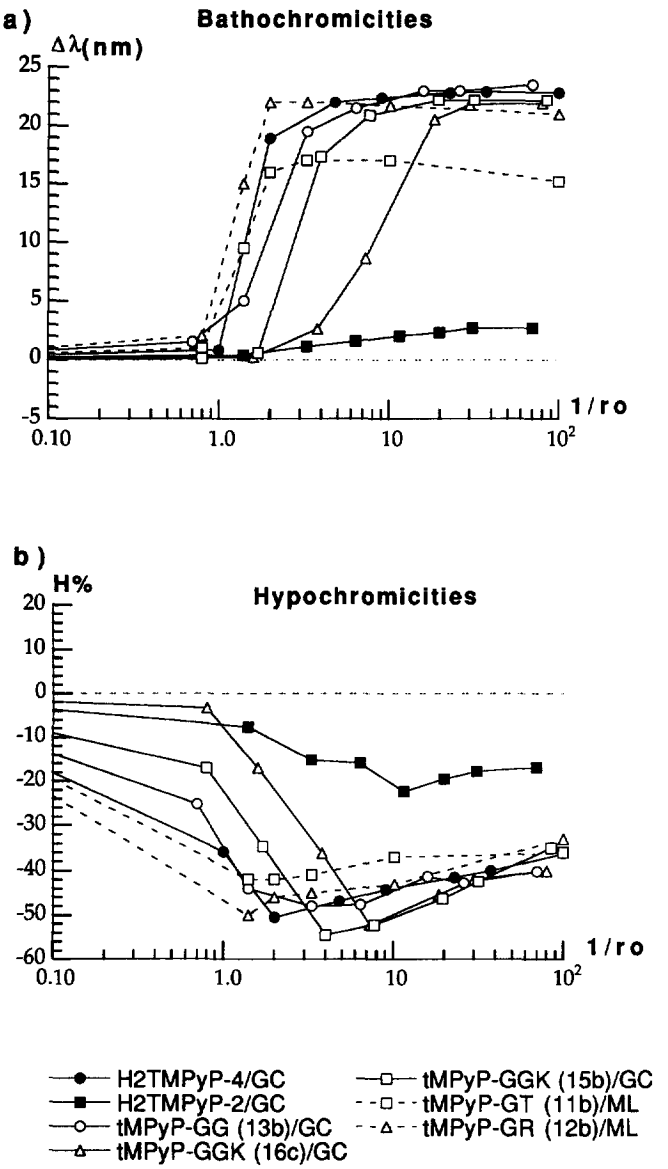


Figure 3: Influence of GC-rich DNA association on the visible spectra of porphyrin derivatives

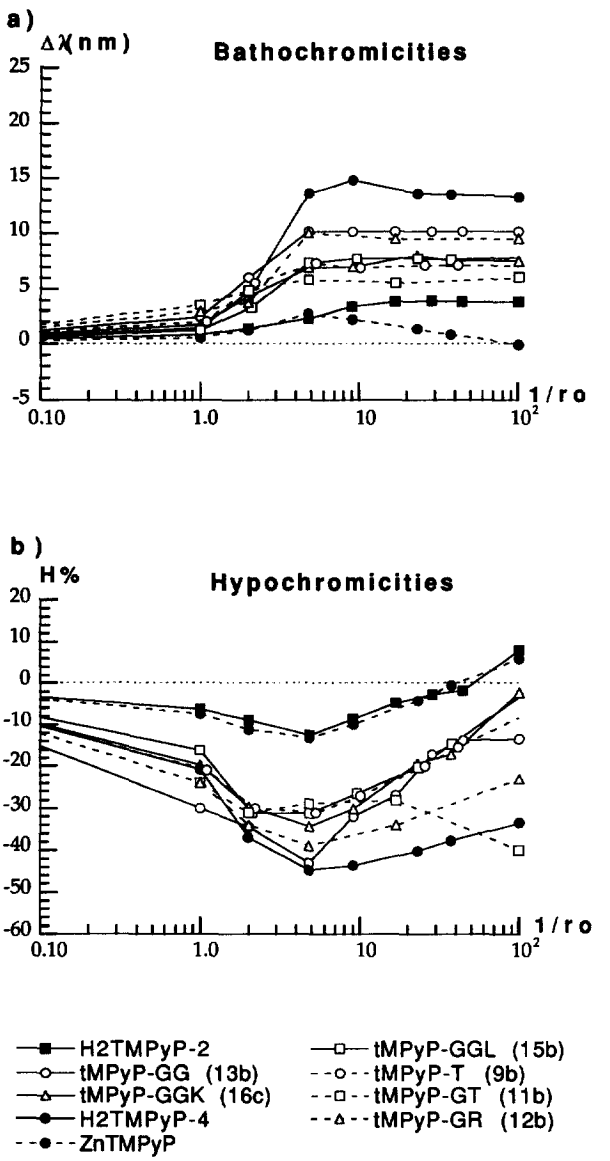


Figure 4: Influence of CT-DNA association on the visible spectra of porphyrin derivatives

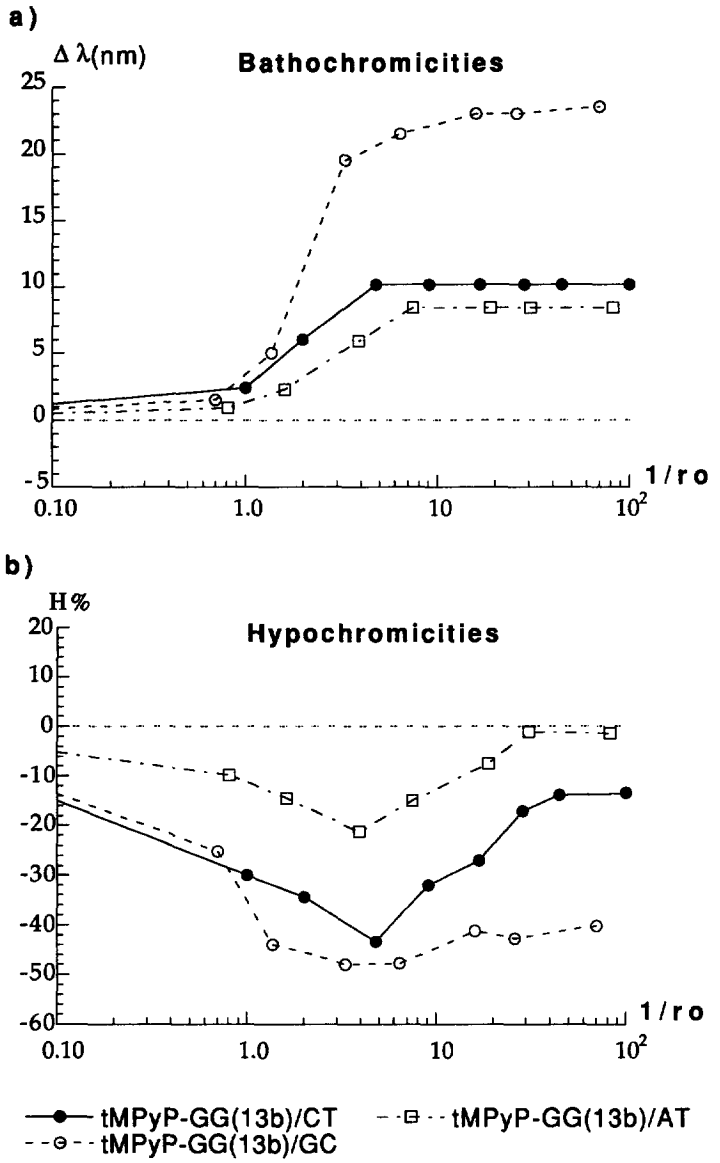


Figure 5: Influence of G-C content on the visible spectra of tMPyP-GG **13b**

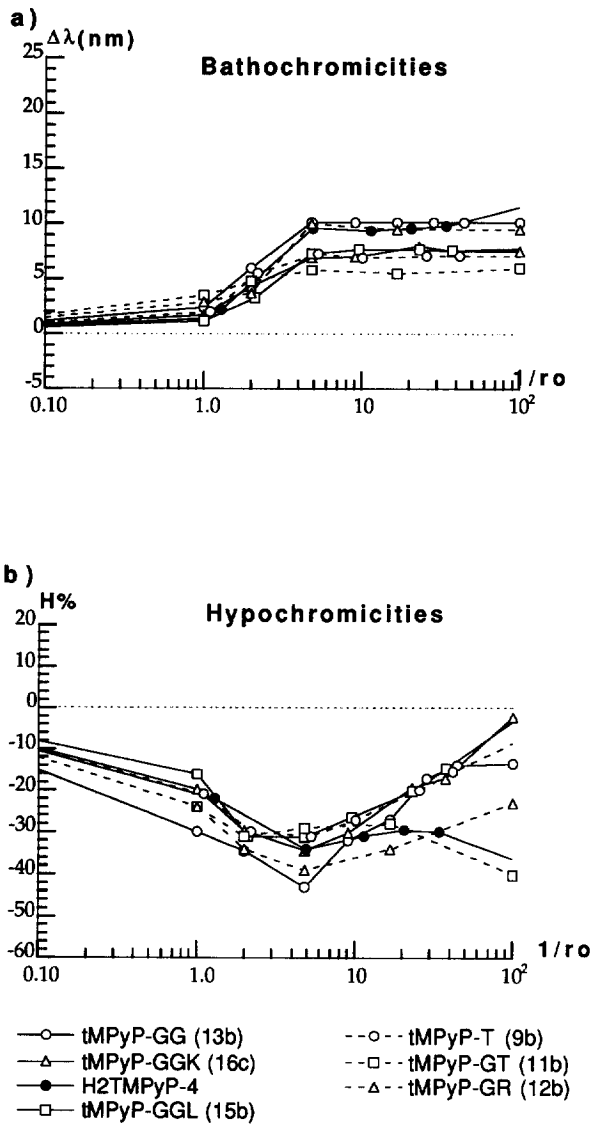


Figure 6: Comparison between interactions of H_2TMPyP-4/CP-DNA and tMPyP-amino acids/CT-DNA

1) In the presence of AT- or GC-rich DNAs

The intercalation ability of these porphyrins was studied, comparing interactions with AT-rich DNAs, namely *Clostridium perfringens* DNA (73.5% AT) or poly(dAdT)$_2$, and GC-rich DNAs, namely *Micrococcus lysodeikticus* DNA (72% GC) or poly(dGdC)$_2$. Typical patterns are shown in Figures 2 for AT-rich DNAs and 3 for GC-rich DNAs. The following observations can be made:

- Interactions of amino acid porphyrin derivatives with AT-rich DNAs lead to weak bathochromic effects (8-9 nm), with intensities being slightly greater than that observed for H_2TMPyP-2 (4 nm). All the derivatives give the same pattern as H_2TMPyP-4, with the exception of tMPyP-GT **11b**, which displays an intermediate profile, closer to H_2TMPyP-2. Very weak hypochromicities (less than 20%) are observed with H_2TMPyP-2 and porphyrins bearing neutral peptides (tMPyP-GG **13b** and tMPyP-GGL **15b**). H_2TMPyP-4, the porphyrin-tyrosine (tMPyP-GT **11b**) and the porphyrins bearing cationic amino acids (tMPyP-GGK **16c** and tMPyP-GR **12b**) display stronger hypochromicity effects (30-40%) at $0.6 \leq 1/r_0 \leq 10$.

- Interactions of amino acid porphyrins **12b, 13b, 15b** and **16c** with GC-rich DNAs lead to large hypochromic and bathochromic effects similar to those obtained upon interaction of H_2TMPyP-4 with poly(dGdC)$_2$. These results denote the pronounced tendency for intercalation of the porphyrin core between CG base-pairs for these derivatives. Slightly weaker effects are observed with tMPyP-GT **11b**.

2) In the presence of calf thymus DNA

Results of the interactions with CT-DNA (42% CG base-pairs) are shown in Figure 4. The following observations can be made:

- The amino acid porphyrin derivatives display various patterns, intermediate between those observed with ZnTMPyP and H_2TMPyP-2 which cannot intercalate and H_2TMPyP-4 which can. This suggests that all the synthesized amino acid porphyrin derivatives are capable of intercalation in CT-DNA, like H_2TMPyP-4, but with a lesser avidity.

- The general feature of the hypochromicity curves displayed by the amino acid porphyrin derivatives is intermediate between that obtained upon interaction with AT-rich DNAs (Figure 2b) and GC-rich DNAs (Figure 3b), while H_2TMPyP-4 displays almost the same hypochromicity pattern as in the presence of GC-rich DNAs (results not shown for H_2TMPyP-4/ML-DNA). Better evidence of the intermediate hypochromicity of the synthesized amino acid porphyrins is given in Figure 5.

- CT-DNA induces for all the peptidyl porphyrins, a moderate bathochromic effect, of the same magnitude as upon interactions with poly(dAdT)$_2$ while it induces for H_2TMPyP-4, a bathochromicity intermediate between that observed with the two synthetic nucleic acids.

- A consideration of all the interaction curves that we have obtained, points to similarity between the interactions of the amino acid porphyrin derivatives with calf thymus DNA and the interaction of H_2TMPyP-4 with *Clostridium perfringens* DNA, which offers fewer CG sites (Figure 6).

CIRCULAR DICHROISM

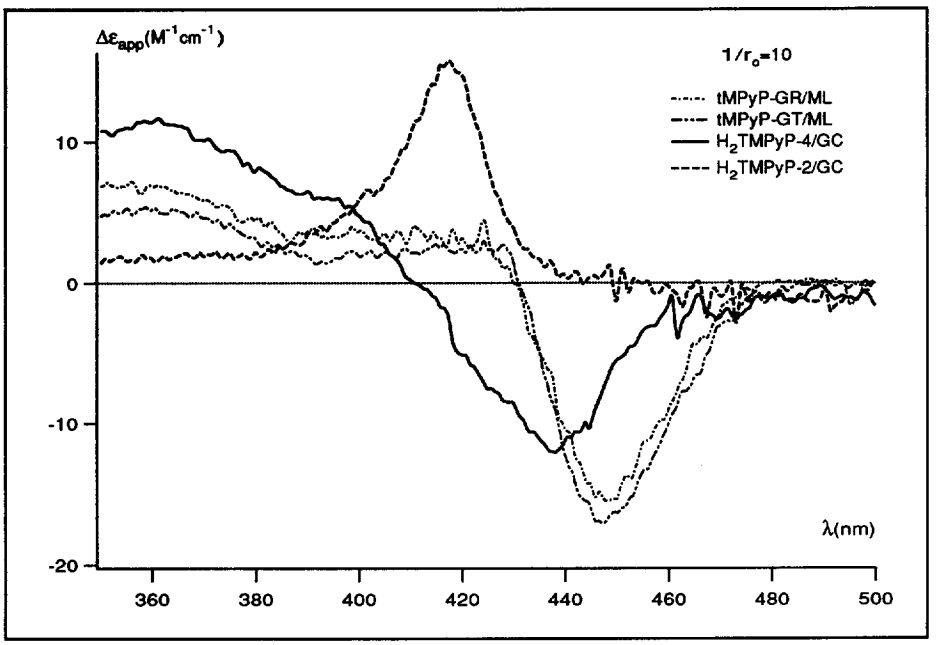
The porphyrins do not display circular dichroism (CD) in the absence of DNA, whereas spectra are induced in the Soret region, when they are bound to natural or synthetic DNAs. The CD spectra induced for H_2TMPyP-2, H_2TMPyP-4 and the amino acid porphyrin derivatives were recorded in the presence of calf thymus DNA, with $1/r_0$ values up to 100. Some experiments were also carried out in the presence of GC-rich and AT-rich DNAs.

In the presence of ML-DNA, as in the presence of poly(dGdC)$_2$, H_2TMPyP-4 and the peptidylporphyrins exhibit a unique broad negative CD band in the Soret region, of constant intensity at $1/r_0 \geq 10$, while H_2TMPyP-2 displays a positive one (Figure 7a). These results confirm the intercalating ability of the amino acid porphyrins. It was unexpected to get a smaller negative signal for the binding of H_2TMPyP-4 to poly(dGdC)$_2$ compared to the binding of peptidylporphyrins to ML-DNA. This may indicate that intercalation at CG sites is facilitated when a small percentage of AT base-pairs are dispersed in the DNA, making it less rigid. In order to check this hypothesis, energy-minimization of the intercalation of several molecules of H_2TMPyP-4 in different GC-rich nucleotides which contained 0 to 28% AT base-pairs, has been performed. The binding energetics increases with increased AT base-pairs content, with the most favorable case being one in which intercalation occurs in an oligonucleotide containing 28% AT. The intermolecular interaction energy is only 1.5 kcal/mole more stable; the main factor is the variation of the conformational energy of the oligonucleotide which is 7 kcal/mole less unfavorable. This implies that to some extent, there is a lower energetic cost to creating intercalation sites in GC-rich DNAs containing a few AT base-pairs than in pure GC .DNAs.[11]

The induced CD spectra of tMPyP-GT **11b** and tMPyP-GR **12b** with CP-DNA are composed of both negative and positive bands (Figure 7b). The negative signal, which increases with decreasing $1/r_0$, is present at $1/r_0 \leq 50$. These results indicate that the two peptidylporphyrins are capable of intercalation even in a GC-poor DNA.

In the presence of CT-DNA (Figure 8), all the peptidylporphyrins display the same induced CD spectrum feature as H_2TMPyP-4, with both a negative and a positive signal, reflecting the coexistence of the two association modes: intercalation and external interaction in a groove. The negative band decreases when $1/r_0$ increases, which means that the intercalation is favored at low $1/r_0$ values. No spectral changes are observed at $20 \leq 1/r_0 \leq 100$. The induced CD spectrum of H_2TMPyP-2 is composed of a unique positive band which is intense and sharp compared to the one obtained upon interaction with poly(dGdC)$_2$ (Figure 7a). This suggests that the interaction of this porphyrin with poly(dGdC)$_2$ is limited by the presence of the methyl groups, while it can bind more tightly in the groove of an AT-rich DNA which is known to be more flexible. This has been previously postulated by Pasternack *et al.*,[1a] who have obtained very similar results for the interaction of axially liganded metalloporphyrins with poly(dAdT)$_2$ and poly(dGdC)$_2$. These results may attest again that the DNA rigidity affects the association of porphyrins, in an external mode as well as intercalation.

a)



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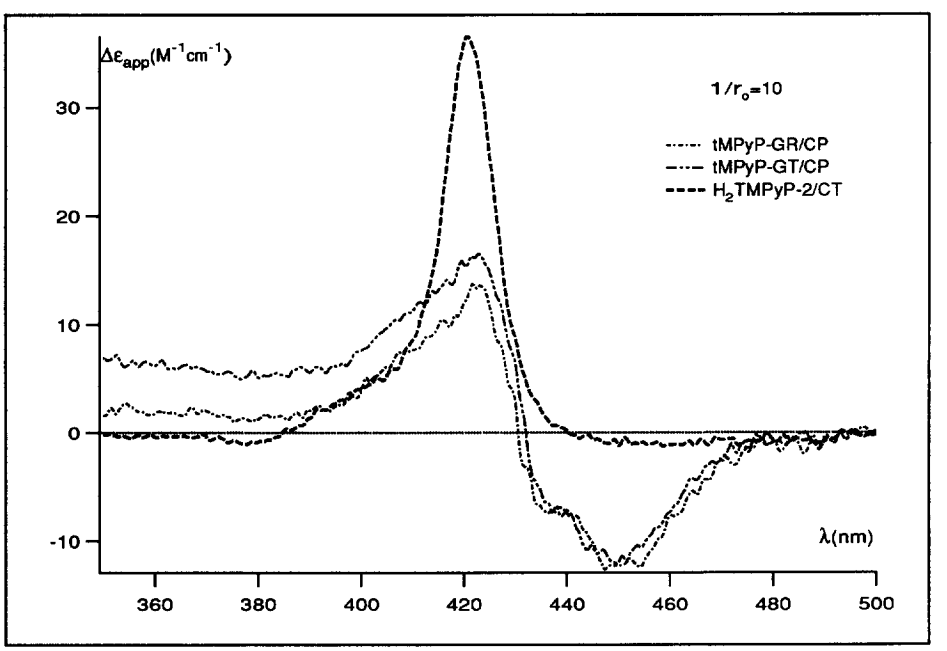
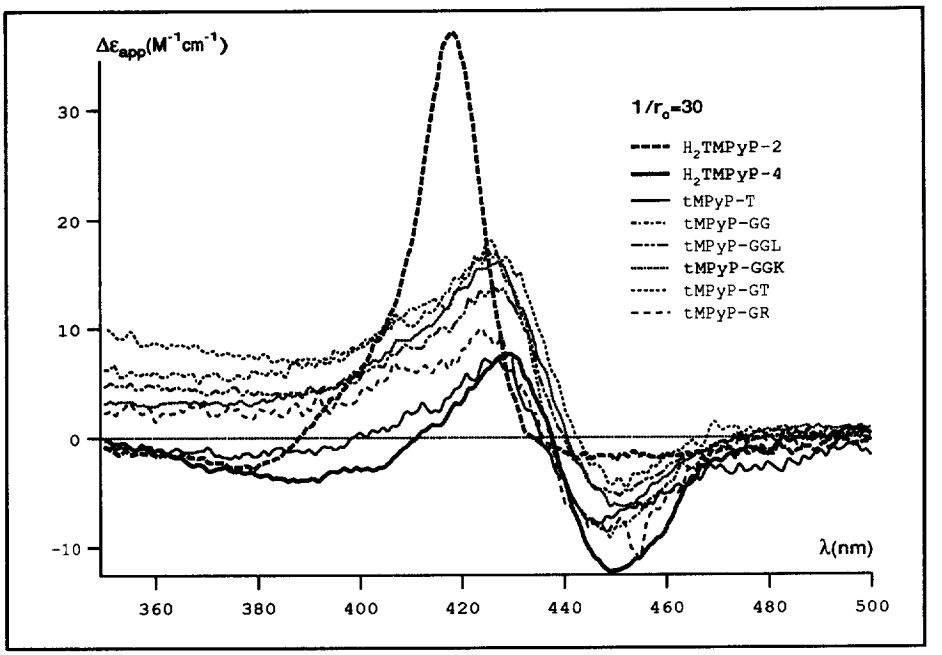


Figure 7: Circular dichroism of some porphyrin derivatives in the presence of **a)** GC-rich DNAs **b)** AT-rich DNAs

a)



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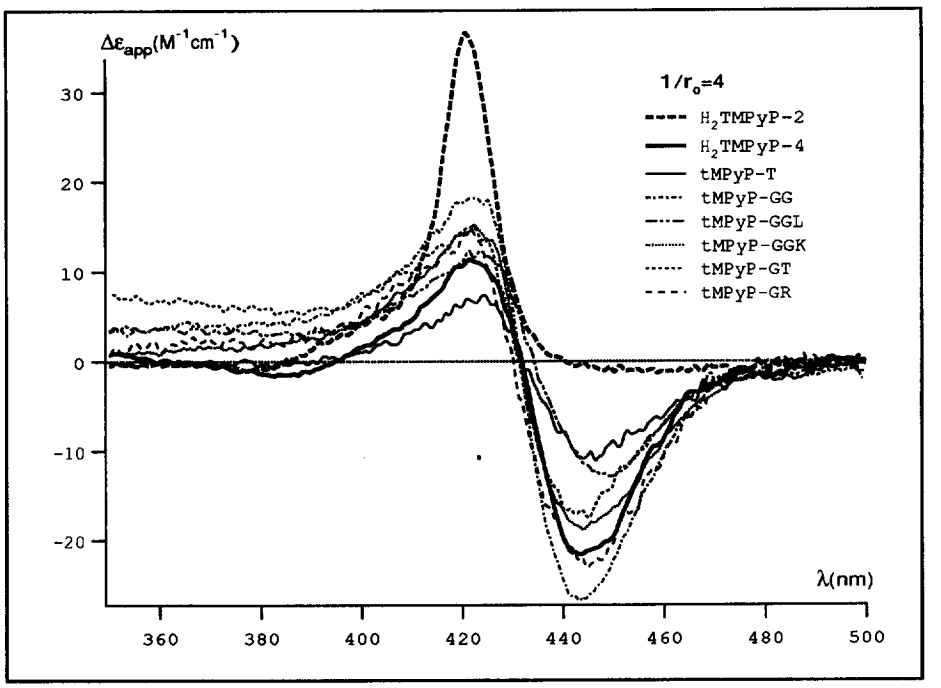

Figure 8: Circular dichroism of porphyrin derivatives in the presence of calf thymus DNA at **a)** $1/r_0 = 30$ **b)** $1/r_0 = 4$

DISCUSSION

We have confirmed in the present work that under the experimental conditions convenient for our studies, the porphyrins selected as references, behave as described previously in the literature: H_2TMPyP-2 is capable only of outside binding, regardless of DNA composition, while both modes of association, intercalation and outside binding, are possible for H_2TMPyP-4. The extent of intercalation *versus* outside binding is highly dependent on GC-base pairs content. Therefore, the relative importance of the modes of association reflects the GC/AT ratio of the DNA as long as the DNA/porphyrin ratio and the salt concentration do not vary. Tris acetate buffer used here, instead of the more widely used phosphate buffer,[1] does not seem to modify the mode of binding, with the one qualification that we never observe the formation of a totally intercalated complex even at $1/r_0 = 100$.

For all the porphyrins studied, the association with poly(dAdT)$_2$ leads to weak bathochromicity, the intensity of which depends on the structure of the porphyrin (Figure 2a). It is obvious that H_2TMPyP-4 and most of the other porphyrins studied exhibit greater bathochromicity than H_2TMPyP-2 which could be explained by differences in orientation of the porphyrin core in the groove. Molecular modelling has demonstrated that H_2TMPyP-4 and its derivatives are able to bind to the minor groove with the plane of the porphyrin core oriented nearly parallel to the helix axis.[12] In this structure, the porphyrin is in close contact with DNA. The methyl groups of H_2TMPyP-2 may prevent such close approach in the groove and therefore, the electronic spectrum of this porphyrin is less affected by the interaction.

tMPyP-GT (**11b**) exhibits a slightly different bathochromicity pattern in the presence of AT-rich DNAs (Figures 2a) as well as in the presence of GC-rich DNAs (Figure 3a), intermediate in both cases between H_2TMPyP-2 and the other porphyrins. This may indicate that the presence of the tyrosine ring restricts to some extent, the intercalation of tMPyP-GT. However, this is not reflected by the induced CD spectra in the presence of GC-rich DNA or AT-rich DNA (Figure 7) which clearly indicate that the intercalation ability of this porphyrin is very similar to that of tMPyP-GR. Moreover, the NMR spectrum of free tMPyP-GT does not show any stacking of the tyrosine on the porphyrin ring (see part I). At present, we can only postulate an interaction of the tyrosine with either the base pairs or the backbone, giving rise to the lower bathochromicity of tMPyP-GT. We have no explanation for the unusual profile of the hypochromicity curve of tMPyP-GT, but we can point out that it resembles the one obtained upon binding of H_2TMPyP-4 to CP-DNA (Figure 6b). This again may imply that complex equilibria between different types of association occur for these porphyrins.

For the other amino acid porphyrins, visible spectroscopy and circular dichroism lead to similar conclusions:

- All exhibit interaction patterns similar to that of H_2TMPyP-4: on one hand in the presence of AT-rich-DNAs, indicative of their ability to associate to DNAs in an external mode; on the other hand in the presence of GC-rich-DNAs, indicative of their ability to intercalate with a similar affinity in GC-containing DNAs.

- In the presence of CT-DNA, even if the amino acid porphyrins exhibit smaller negative and larger positive CD bands than H_2TMPyP-4 when $1/r_0$ increased, the similarity between all of them and H_2TMPyP-4 is apparent, particularly at $1/r_0 = 4$. It is noteworthy that even when $1/r_0$ increases, they all display a much smaller positive band than H_2TMPyP-2. Values of the ratio between negative and positive band maxima and of the bathochromicity of the Soret band are given in table. Both reflect the intercalating ability of the

porphyrins and it is to be noted that with the exception of tMPyP-T **9b**, there is good agreement of both methods of investigation; the intercalating tendency of the porphyrins decreases in the same order: H_2TMPyP-4 > tMPyP-GR **12b** ≥ tMPyP-GG **13b** > tMPyP-GGK **16c** ≥ tMPyP-GGL **15b** > tMPyP-GT **11b**.

Table: Interaction of H_2TMPyP-4 and the amino acid porphyrin derivatives with CT-DNA. Comparison between Δε in circular dichroism and Δλ in visible spectroscopy.

Porphyrins	Δε (M^{-1} cm^{-1}) at $1/r_0 = 4$			Δε (M^{-1} cm^{-1}) at $1/r_0 = 30$			Δλ (nm) at $1/r_0 = 5$
	Negative Band	Positive Band	Ratio	Negative Band	Positive Band	Ratio	
H_2TMPyP-4	22	11	2	16	10	1.6	13
tMPyP-T (9b)	11	7.5	1.5	10	10	1	7
tMPyP-GR (12b)	23	15	1.5	13	13	1	10
tMPyP-GG (13b)	27	18	1.5	11	22	0.5	10
tMPyP-GGK (16c)	19	15	1.3	8	21	0.4	7
tMPyP-GGL (15b)	13	13	1	7	18	0.4	7
tMPyP-GT (11b)	17	15	1.1	6	23	0.3	5.5

Two factors may contribute to the result that the amino acid porphyrins do not intercalate as readily as H_2TMPyP-4: a loss of affinity due to the presence of only three positive charges on the porphyrin core and/or a greater selectivity towards specific DNA sequences due to the linkage of amino acids such as arginine or lysine which are known to recognize guanines in the major groove. In the latter case, the loss of affinity of the porphyrin moiety is compensated by the presence of a positive charge on the amino acid. Nevertheless, as all these porphyrins are prone to intercalation in GC-rich DNAs, we think that their affinity for intercalation sites remains close to that of H_2TMPyP-4. Furthermore, the similarity of the spectroscopic patterns of these peptidylporphyrins in the presence of CT-DNA (42% GC base-pairs) with H_2TMPyP-4 in the presence of CP-DNA (26.5% GC) suggests that some of the GC-sites in CT-DNA are not suitable for the intercalation of amino acid porphyrins. This is in agreement with the suggestion of an increased specificity, with respect to intercalation, of amino acid porphyrin derivatives when compared to H_2TMPyP-4.

Topoisomerisation and viscometry experiments are in progress to test this hypothesis and if warranted, footprinting experiments will be attempted to determine the nature of the site-specificity.

CONCLUSION

We have demonstrated that amino acid porphyrin derivatives of H_2TMPyP-4 bearing three or four positive charges are able to intercalate into DNA, but to a lesser extent than the parent porphyrins. The linkage of an amino acid chain favors outside binding, probably because the affinity for intercalation sites is decreased and/or there are fewer intercalation sites available for derivatives displaying a higher degree of

specificity. However, their behaviour is comparable to that of H_2TMPyP-4: intercalation is observed at low values of $1/r_0$ and no outside binding occurs in GC-rich DNAs under the conditions investigated.

EXPERIMENTAL SECTION

Materials and methods

Calf thymus, *Micrococcus lysodeikticus* and *Clostridum perfringens* DNAs were purchased from Sigma and purified using standard methods.[13] Poly(dGdC)$_2$ and poly(dAdT)$_2$ were purchased from Pharmacia and used without further purification. Stock aliquots (about 10^{-3} M) were made and stored frozen until use. Concentrations, always calculated in base pairs were determined spectrophotometrically with: $\varepsilon^{260nm} = 1.31x10^4$ $M^{-1}cm^{-1}$ for CT-DNA,[14] $\varepsilon^{260nm} = 1.64x10^4$ $M^{-1}cm^{-1}$ for ML-DNA,[15] $\varepsilon^{260nm} = 1.34x10^4$ $M^{-1}cm^{-1}$ for CP-DNA,[15] $\varepsilon^{254nm} = 1.68x10^4$ $M^{-1}cm^{-1}$ for poly(dGdC)$_2$,[16] $\varepsilon^{262nm} = 1.32x10^4$ $M^{-1}cm^{-1}$ for poly(dAdT)$_2$.[17]

Porphyrin concentrations were also determined spectrophotometrically with: $\varepsilon^{423nm} = 2.26x10^5$ $M^{-1}cm^{-1}$ for H_2TMPyP-4,[1a] $\varepsilon^{414nm} = 1.82x10^5$ $M^{-1}cm^{-1}$ for H_2TMPyP-2,[1a] $\varepsilon^{437nm} = 2.04x10^5$ $M^{-1}cm^{-1}$ for ZnTMPyP,[1a] $\varepsilon^{424nm} = 1.66x10^5$ $M^{-1}cm^{-1}$ for tMPyP-GGL,[18] $\varepsilon^{424nm} = 2.38x10^5$ $M^{-1}cm^{-1}$ for tMPyP-GGK[18] and $\varepsilon^{max} = 1.80x10^5$ $M^{-1}cm^{-1}$ for the other tMPyP-amino acids.[19]

All the measurements were made in Tris acetate buffer, 40 mM, pH 7.5, prepared just before use from a 50x solution.[13] Spectroscopic measurements were carried out on a Perkin Elmer Lambda 5 or a Safas 190 DES double beam recording spectrophotometer. Absorbances were measured in a 1 mL, 10 mm pathlength quartz cuvette, using 0.5 mL of a $5x10^{-6}$ M solution of porphyrin derivative (O.D.≈1) and adding successive aliquots of a solution containing the same concentration of porphyrin and a 100 fold excess of DNA ($1/r_0$ = 100). We checked that, under these conditions, the adsorption of the porphyrin derivative on the cuvette surface was negligible.

Circular dichroism spectra were recorded on a Jobin Yvon Mark V autodichrograph, monitored with an Apple II E computer and equipped with a water-jacked cuvette holder and a water bath circulator. The temperature was maintained at 25 °C. The same general procedure as above was used to analyse the mixtures with different $1/r_0$ values. We had to use a 3 mL, 10 mm pathlength quartz cuvette to avoid dichroic effect with the cuvette itself. All the spectra were obtained by average of 4 accumulations recorded with steps of 0.2 nm and a response time of 0.5 s. For each measurement, the spectrum of the porphyrin alone at same concentration in the same buffer and same cuvette was substracted and the observed Δ(O.D.) was divided by the initial porphyrin concentration, giving apparent Δε values, $\Delta\varepsilon_{app}$.

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