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Base pair selectivity in the binding of copper (II) tetrakis (4-*N*-methylpyridyl)porphine to polynucleotides under closely packed conditions

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Abstract

The base pair selectivity of the intercalative binding of the copper porphyrin, copper (II) tetrakis(4-*N*-methylpyridyl)porphine (Cu(II)TMpyP-4), to DNA has been investigated using a variety of DNA types and the synthetic polynucleotides poly(dG–dC)$_2$ and poly(dA–dT)$_2$. The studies utilize electron paramagnetic resonance of concentrated gels which are thought to mimic the closely packed state of nuclear DNA. The results indicate that intercalation of this porphyrin is preferred for sites containing two adjacent G–C base pairs, irrespective of sequence.

Keywords: DNA intercalation; Porphyrins; Electron paramagnetic resonance; Binding selectivity; Close packing

1. Introduction

The interaction of meso-substituted cationic porphyrins, and their metallo derivatives, with nucleic acids has attracted considerable attention in part because of the ubiquitous roles found for natural porphyrins in normal physiological functions. It is clear that there is considerable versatility in the binding mode, and a variety of physicochemical techniques [1] have confirmed three general types—intercalation, groove binding (which may be considered a form of partial intercalation) and electrostatic or territorial binding (which may involve external stacking along the helix).

The binding mode preference of these compounds can be modified by changes in the coordinated metal (e.g. metalloporphyrins which maintain their axial ligands, such as Zn(II), Co(III), Fe(III) and Mn(III), do not intercalate [2]) or by alterations in the porphyrin periphery (e.g. tetrakis(4-*N*-methylpyridyl) porphine (H_2TMpyP-4) and tetrakis(3-*N*-methylpyridyl)porphine (H_2TMpyP-3) can intercalate, but tetrakis(2-*N*-methylpyridyl)porphine (H_2TMpyP-2) does not [3]). Spectral and kinetic experiments [2,4,5] suggest that at low porphyrin loading and moderate ionic strength, both Cu(II)TMpyP-4 and Ni(II)TMpyP-4 show a stronger intercalative binding mode preference than does the nonmetallo H_2TMpyP-4.

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There is considerable interest in the base pair selectivity of porphyrin derivatives, if any, since such selectivity offers the prospects of designing porphyrins that are sensitive probes of nucleic acid structure, and of understanding the molecular origins of selective cytotoxicity crucial to therapeutic strategies. Footprinting experiments [6,7] confirm that external binding for axially liganded

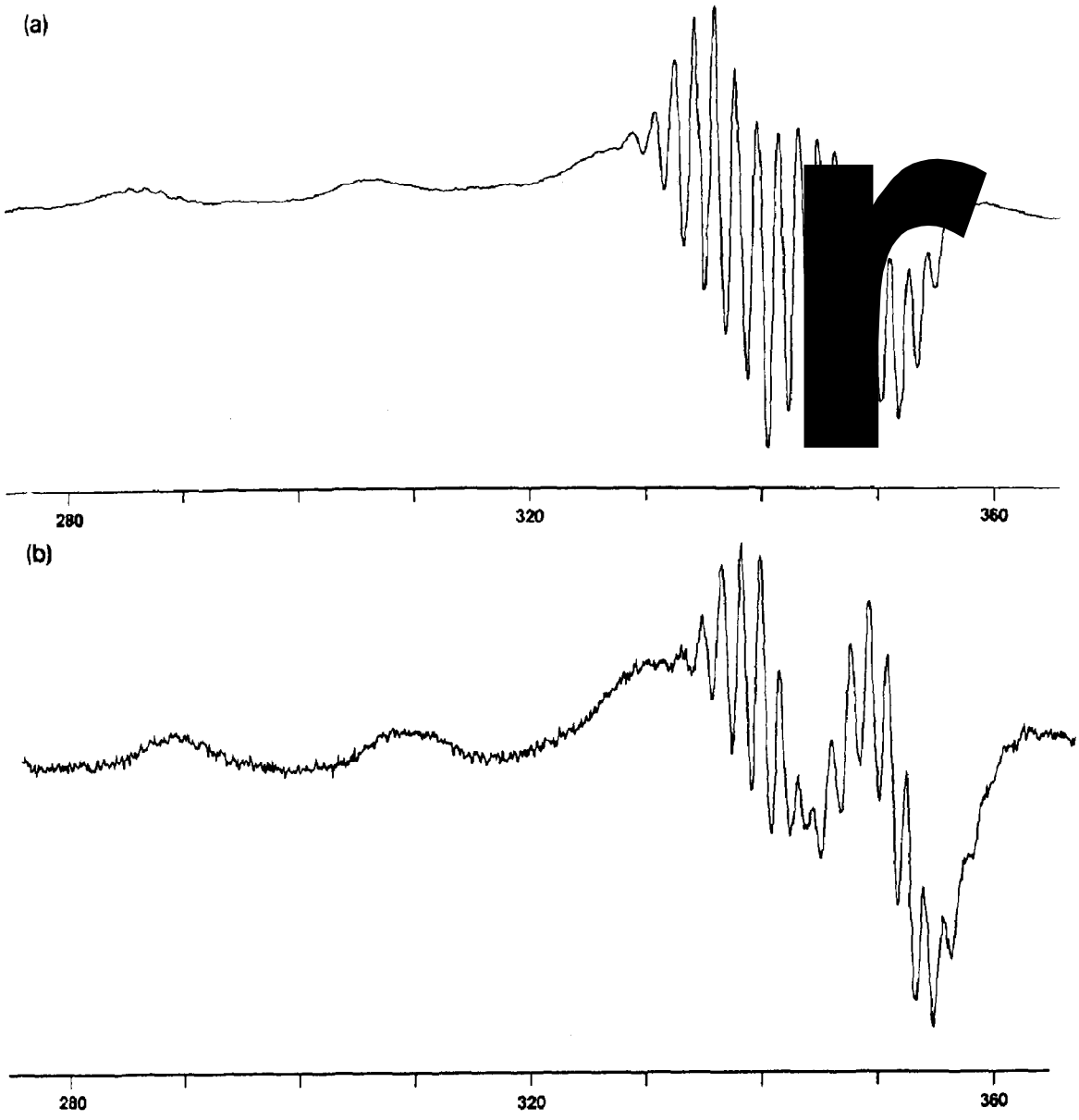

Fig. 1. First-derivative EPR spectra of Cu(II)TMpyP-4: ClpDNA complexes (molar porphyrin: DNA = 1:40) recorded at room temperature, microwave power ~10 mW, modulation amplitude 0.5 mT. (a) Gel, at ~100 K, (b) thin film, with normal to the film perpendicular to the magnetic field ($\theta = 90°$) (c) thin film, with its normal parallel to the magnetic field ($\theta = 0°$).

metalloporphyrins is strongly favoured in AT-rich regions, but the selectivity for intercalation is less certain.

Spectroscopic and kinetic studies with synthetic polymers indicate that H_2-TMpyP-4 intercalates selectively at GC sites [1,2], although an absolute requirement for 5′CpG3′ sites, as suggested by Marzilli et al. [8] using ^{31}P NMR, has not been confirmed by others [9]. The issue is further confused since more recent NMR and resonance Raman studies report that other intercalating metalloporphyrins fail to show this absolute selectivity in natural DNA's [9–11], and that intercalation can occur in poly (dA–dC) · poly (dT–dG) indicating that a GC–GC site is not *required* for intercalation [12].

A possible structural origin for at least partial selectivity has been suggested [13]. Although all porphyrin derivatives can apparently gain access to intercalative positions within nucleic acids during quake-like motion [14], the final arbiter for intercalation is whether the helix is stable enough to hold the large molecule in its interior as an intercalated complex. Regions of high GC content are known to be more stable than AT regions, and are clearly more able to accomplish this.

2. Materials and methods

Calf thymus (ct), and *Clostridium perfringens* DNA's were obtained from Sigma, and the ct DNA was purified as previously described [4]. Poly $(dG–dC)_2$ and poly $(dA–dT)_2$ were purchased from Pharmacia, and dialysed against the phosphate buffer. The two copper porphyrin derivatives, Cu(II)TMpyP-4 and Cu(II)TMpyP-2, were prepared and purified as previously described [4]. All experiments used a phosphate buffer, at pH 6.8, containing 6 mM Na_2HPO_4, 2 mM NaH_2PO_4, 1 mM ethylenediamine tetraacetic acid (EDTA) and sufficient NaCl to give a final ionic strength of $\mu = 0.2$ M.

Nucleic acid concentrations, in base pairs, were determined spectrophotemetrically using $\epsilon_{260nm} = 1.31 \times 10^4$ M^{-1} cm^{-1} for calf thymus DNA [15], $\epsilon^{260nm} = 1.34 \times 10^4$ M^{-1} cm^{-1} for ClpDNA, $\epsilon_{254nm} = 1.68 \times 10^4$ M^{-1} cm^{-1} for poly $(dG–dC)_2$ [16] and $\epsilon_{260nm} = 1.32 \times 10^4$ M^{-1} cm^{-1} for poly $(dA–dT)_2$ [17]. Metalloporphyrin concentrations were determined spectrophotometrically using $\epsilon_{424nm} = 2.31 \times 10^5$ M^{-1} cm^{-1} for Cu(II) TMpyP-4 [18] and $\epsilon_{415nm} = 2.21 \times 10^5$ M^{-1} cm^{-1} Cu(II)TMpyP-2 (Pasternack, unpublished results). Absorbance data were obtained on a Varian 2200 spectrophotometer with a thermostated cell compartment.

DNA–dye complexes at a 40:1 molar ratio were prepared as previously described [19] and sedimented at 100,000 g for 15 h in a Sorvall RC70 ultracentrifuge to produce a concentrated gel. Both the electron paramagnetic resonance (EPR) spectrum of the gel and of a thin film formed by compressing the gel within a tissue cell (Wilmad, WG-806: $10 \times 5 \times 0.5$ (mm)) were recorded for each sample using a Bruker ER200D EPR spectrometer at X-band (9–10 GHz). The actual frequencies depend on the dielectric loading, which varied with the orientation of the tissue cell. All spectra were recorded with microwave power and modulation amplitude maintained at levels well below those that would partially saturate or overmodulate the spectra. The spectra of the gels were taken at ~100 K, and those of the thin films at room temperature.

3. Results

The spectrum of a gel comprising a Cu(II)TMpyP-4 complex with ClpDNA is shown in Fig. 1(a). The spectrum is typical of a square co-planar polycrystalline copper (II) chelate and the prominent features are due to hyperfine and superhyperfine interactions. The resolved peaks near $g_\perp$ are due to a combination of copper and nitrogen hyperfine structure, with the latter producing an intensity pattern due to essentially equivalent nitrogens in the plane of the macrocycle. There is no evidence of any triplet-state dimers, and the lack of a half-field spectrum (due to a $\Delta M_s = 2$ transition) confirms their absence diagnostically [20,21].

When a thin film of the Cu(II)TMpyP-4–DNA complex is rotated within the magnetic field of

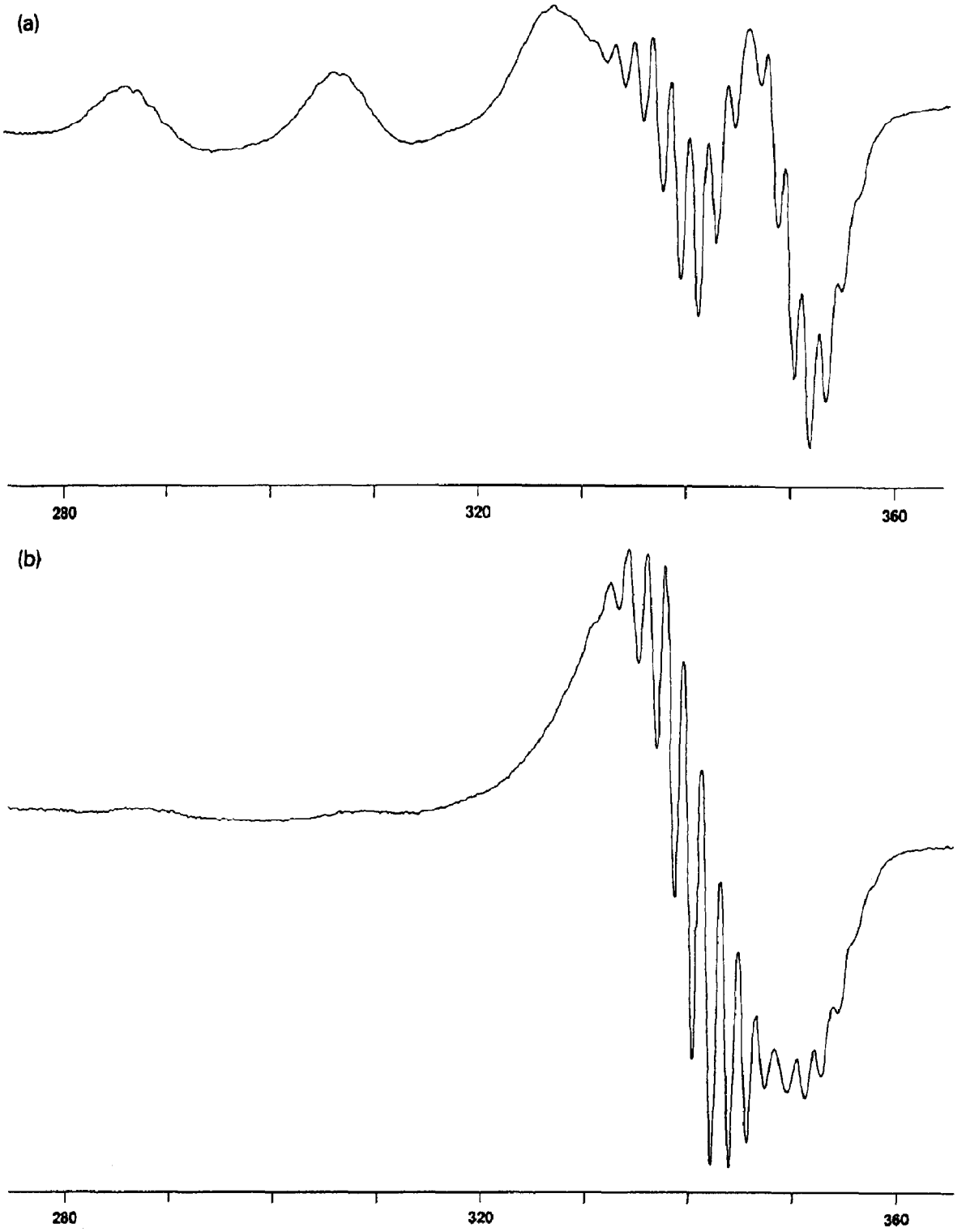

Fig. 2. First-derivative EPR spectra of Cu(II)TMpyP-4: poly $(dG–dC)_2$ (1:40 molar ratio) film at different angles to the static magnetic field: (a) $\theta = 90°$ (b) $\theta = 0°$. (Recorded at room temperature, microwave power ~ 10 mW, modulation amplitude 0.5 mT).

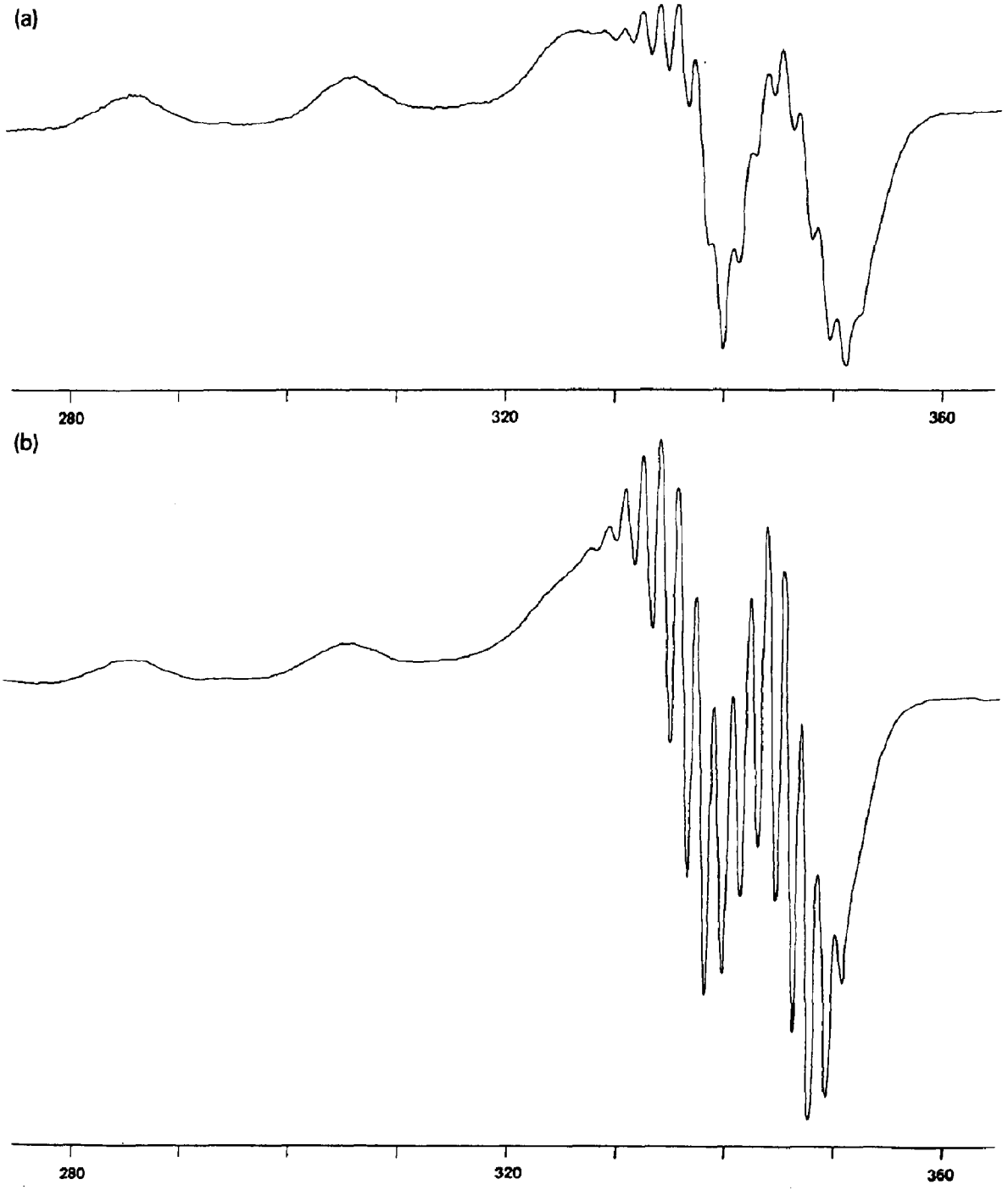

Fig. 3. First-derivative EPR spectra of Cu(II)TMpyP-4: poly(dA–dT)$_2$ (1:40 molar ratio) film at (a) $\theta = 90°$ (b) $\theta = 0°$. (Recorded at room temperature, microwave power ~ 10 mW, modulation amplitude 0.5 mT).

the spectrometer, its EPR spectrum changes with orientation (Fig. 1b, c). When the normal to the film is perpendicular to the field ($\theta = 90°$), the spectrum resembles the corresponding gel spectrum (although with relatively more prominent "parallel" features, reflecting the difference in the probability of being perpendicular to the magnetic field in two dimensional and three di-

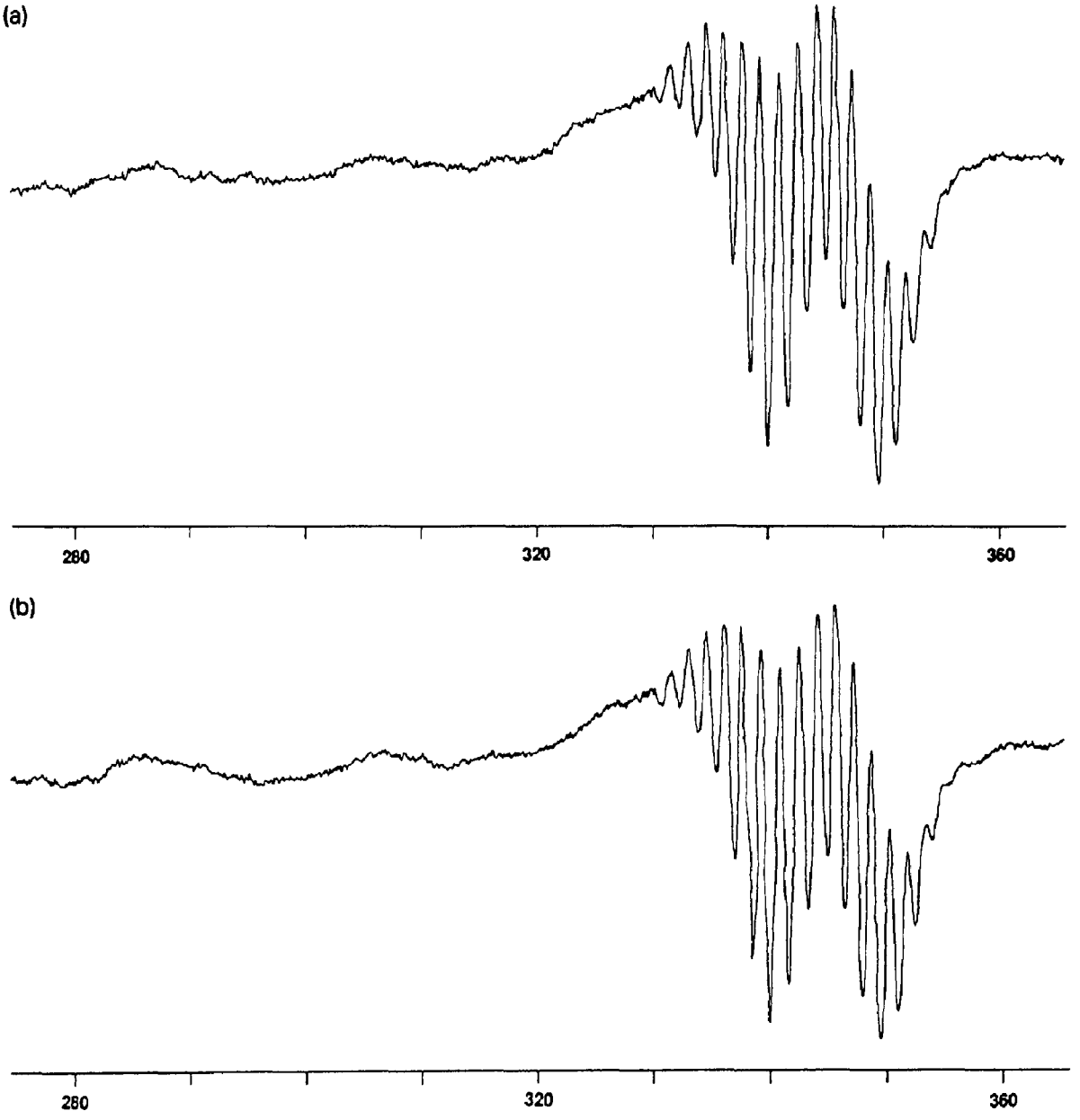

Fig. 4. First-derivative EPR spectra of Cu(II)TMpyP-2: ct DNA (1:40 molar ratio) film at (a) $\theta = 90°$ (b) $\theta = 0°$. (Recorded at room temperature, microwave power ~10 mW, modulation amplitude 0.5 mT).

mensional randomness). When the film is rotated by 90° ($\theta = 0°$), the "parallel" features are suppressed and the "perpendicular" features become more prominent.

This distinctive orientational dependence of the film spectra varies in extent with DNA type. It is most striking with poly(dG–dC)$_2$ (Fig. 2) and least apparent with poly(dA–dT)$_2$ (Fig. 3), where a considerable "parallel" component is retained at all orientations.

In contrast, the EPR spectrum of a film of Cu(II)TMpyP-2 with ct DNA behaves rather differently. There is virtually no change in the spectrum as the film is rotated in the magnetic field (Fig. 4).

4. Discussion

Electron paramagnetic resonance of thin films comprising a compound containing a paramagnetic center bound to DNA has been demonstrated to be a diagnostic technique for identifying intercalation [19,22,23]. Changes in the relative prominence of the "parallel" and "perpendicular" features of the spectrum as the film is rotated in the magnetic field of the EPR spectrometer indicate the presence of a binding mode in which the symmetry axes of the compound are parallel to the DNA helix axis. Although external stacking along the DNA molecule also has this structural characteristic, it is more likely that this results from intercalation given the overwhelming evidence that Cu(II)TMpyP-4 does intercalate in solution [4,24]. Groove binding and electrostatic binding do not contribute to such an orientational effect.

Although the ionic strength within our gels and films will doubtless change from 0.2 *M* during ultracentrifugation, studies indicate that the binding mode preference of metalloporphyrins is little affected by ionic concentration at low porphyrin loading [13] (although ionic concentration is reported to dramatically shift the equilibrium between intercalative and groove binding for the metal-free porphyrin, H_2TMpyP-4 [25]).

Further evidence for this interpretation is the observation that films comprising the positional isomer, Cu(II)TMpyP-2, bound to DNA show no orientational effect (Fig. 4). This is consistent with studies that show that H_2TMpyP-2 does not intercalate [3,26], probably due to steric restrictions caused by the position of the *N*-methyl groups.

Table 1

The proportions of Cu(II)TMpyP-4 intercalated and non-intercalated on binding to various nucleic acids, calculated from the linear combination coefficients required to best fit their EPR film spectra

DNA type	Fractional G–C content (f)	f^2	Proportion intercalated
poly(dG–dC)	1.00	1.00	0.90 ± 0.05
ct DNA	0.40	0.16	0.86 ± 0.04
ClpDNA	0.26	0.07	0.30 ± 0.04
poly(dA–dT)	0.00	0.00	0.04 ± 0.05

Computer simulations [19] can be used to best fit the experimental spectra to a linear combination of an orientationally dependent spectrum (albeit with a disorder parameter to reflect the finite thickness of the film) and a powder-like (non-orientationally dependent) spectrum, and hence to determine the ratio of intercalated to non-intercalated porphyrin molecules within each complex [19,27].

Table 1 shows the relative proportions of intercalated and non-intercalated Cu(II)TMpyP-4 molecules when bound to different nucleic acids under the same close packed conditions. These experimental conditions are vastly different from those pertaining in dilute solution, but the results are expected to mirror the same selectivity. A clear preference for intercalative binding with nucleic acids of high G–C content is shown. The estimate that 85% of the binding with calf thymus DNA is intercalative is consistent with earlier studies [19,28].

We have restricted ourselves in this study to complexes at a high excess of DNA where the preferred sites have the highest probability of being populated, in order to concentrate on intercalative binding and to avoid external stacking and cooperativity effects. The significant shift from intercalative binding to non-intercalative binding, with reduced G–C content, indicates

that the intercalation of Cu(II)TMpyP-4 is selective for G–C base-pairs such that an insufficient number of the preferred sites are available in ClpDNA and none are present in poly(dA–dT)$_2$.

The practice of including base pair selectivity together with the exclusion-mediated binding site size to give enlarged effective binding site size [19] has been obviated by a novel analysis [27], which parametrizes each factor separately. It has been shown [28] that sterically-mediated nearest neighbour exclusion (viz. a binding site size, n, of 2) is sufficient to explain the low levels at which porphyrin binding saturates if a base pair selectivity is invoked to quantify the difficulty of a porphyrin locating its preferred binding sequence on the DNA template.

Thus if intercalative binding were to occur exclusively on one side of a G–C base pair then the selectivity introduced would mirror the fractional G–C content, f, for a random mixed base pair sequence. If intercalation is selective for sites containing two adjacent G–C's (equivalent to a sequence specificity for GpG, GpC, CpC or CpG), the selectivity would correlate with f^2. (The selectivity would depend on $f^2/4$ if intercalation were only possible to a specific sequence, such as 5 'CpG3'; but NMR [9] and resonance Raman studies [11,29,30] effectively discount this possibility) Our results show that the proportion of intercalation into ct DNA (0.86) is more than twice that for ClpDNA (0.39), consistent with an increased number of binding sites comprising two adjacent G–C's ($0.4^2/0.26^2 = 2.35$).

Ascertaining the selectivity of a molecule that binds to DNA in an equilibrium fashion is a notoriously difficult task [31]. The fundamental problem is that the bound molecule is in dynamic equilibrium with a pool of unbound molecules, and thus a. permanent record of its residence position on the DNA substrate does not exist. Indeed there is some doubt as to whether footprinting analysis is capable of detecting sequence specificity for intercalative binding. Studies with Cu(II) and Ni(II) complexes of H_2TMpy-4 [6,7] indicate that the cleavage rate remains little changed in regions of high GC populations : it may be that intercalative binding of metalloporphyrins has little impact on DNase cleavage. It has been suggested that under gel conditions the DNA–porphyrin complex is in a partially ordered state [27], as evidenced by birefringence. This partial ordering may go some way to "freezing" the binding positions, and thus simplify the determination of selectivity when using a gel rather than a solution. In any case binding cooperativity will be more significant in a gel, because of close packing, and this may accentuate subtleties in the DNA–porphyrin interaction.

Most studies of DNA–drug binding are carried out in solution. However, DNA in physiological conditions can be in a very tightly packed state, with volume concentrations approaching 70% w/v in sperm head and virus capsids [32,33]. It is therefore of considerable practical importance to study the binding of drugs with DNA under such conditions. Using the EPR spectra of closely packed gels and films our results show that Cu(II)TMpyP-4 intercalation is selective for G–C base pairs and, together with other spectroscopic measurements, suggest that in mixed base-pair sequences the preference is for sites containing any two adjacent G–C's.

Clearly there is a strong selectivity to the intercalative binding of porphyrins to DNA, which is unlikely to be fully characterized by a single technique. The EPR spectra of thin films is a sensitive diagnostic for intercalation, and can reveal shifts between binding modes using different polynucleotides. Significantly it provides this information for a closely packed state. Extended studies using this method will need to be compared with those using a variety of other techniques under different experimental conditions before a rationale for the selectivity can be proposed.

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