Influence of DNA on the Rate of Porphyrin Metallation

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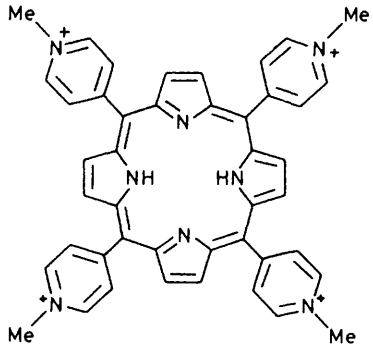
Poly(dG–dC)$_2$ and poly(dA–dT)$_2$ have marked influences on the rate of insertion of copper(II) into cationic porphyrins reflecting the interaction mode of the porphyrin with the nucleic acid.

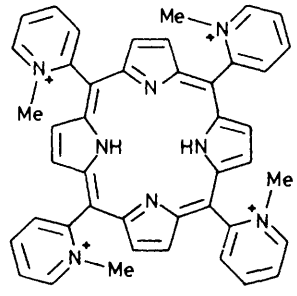
Nucleic acids in solution with their negatively charged phosphate backbone, tend to concentrate cations of added electrolytes in their immediate environment.[1] Therefore, it might be anticipated that reactions between cationic reactants will be catalysed by the presence of DNA;[2] similar catalyses at polymeric surfaces have been demonstrated for polyvinylsulphonate, for example.[3] However, cations can interact with nucleic acids in a variety of ways involving not only coulombic attractions but also complexation of purine/pyrimidine bases, 'external' (*e.g.* groove binding) and 'internal' (*i.e.* intercalation) processes in which the purine/pyrimidine bases also play a role.[4] The influence of added DNA on the rate of a reaction involving cations may be expected to reflect the manner in which one or the other of the reactants interacts with the nucleic acid. Conversely, it might prove possible to gain further insight into the nature of a cation–nucleic acid interaction by determining the effect of DNA on the rate of some standard chemical process.

We investigated the interactions of cationic water soluble porphyrins and metalloporphyrins with nucleic acids[5–9] and find that H_2TMpyP^{4+} and its metal derivatives not possessing axial ligands are capable of intercalating into poly(dG–dC)$_2$ but not poly(dA–dT)$_2$.† At the low levels of drug load utilized for the kinetic studies reported here there is a correlation between mode of porphyrin binding to non-aggregated nucleic acids and the profile of the circular dichroism (c.d.) spectrum induced in the Soret region; *i.e.* negative bands reflect intercalation, positive bands, external (groove) binding, and conservative spectra suggest external bonding with porphyrin aggregation.[5,9,10] For $H_2TMpyP\text{-}2^{4+}$ at these same low drug loads, no c.d. spectrum is induced with poly(dG–dC)$_2$ but a positive band is produced with poly(dA–dT)$_2$. Recent studies of H_2TAP^{4+} with a natural DNA have shown that this porphyrin does not interclate.[10] Based upon c.d. measurements, it appears that, under the conditions of these experiments, H_2TAP^{4+} is capable of groove binding to either poly(dG–dC)$_2$ or poly(dA–dT)$_2$, perhaps with some aggregation.

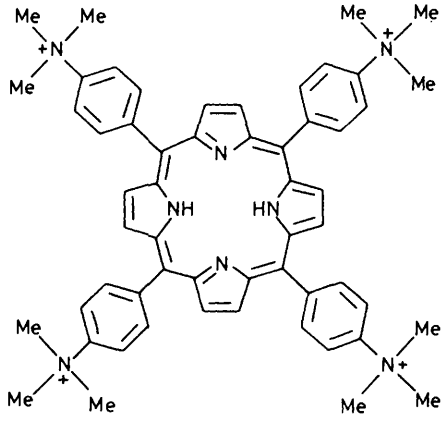
We have considered the rate of insertion of copper(II) into each of these porphyrins both with and without added poly(dG–dC)$_2$ or poly(dA–dT)$_2$. To conduct experiments in a pH range in which the polynucleotide duplex is stable requires the addition of some potential ligand to copper(II) to prevent the precipitation of the metal hydroxide. We have used both ammonia and imidazole for this purpose (see Table 1). Previous work has shown that hexaquacopper(II) interacts extensively with DNA giving rise to structural and conformational changes of the polymer.[11] These processes are likely to be less important when the metal ion is complexed to other ligands, and in the present study, more than 85% of the copper ion in solution is bound to two or more nitrogenous bases (ammonia or imidazole). Indeed, results obtained with $H_2TMpyP\text{-}2^{4+}$ (*vide infra*) imply that if such copper(II)–DNA



H_2TMpyP^{4+}



$H_2TMpyP\text{-}2^{4+}$



H_2TAP^{4+}

† Poly(dG–dC)$_2$ and poly(dA–dT)$_2$ obtained from P-L Biochemicals are synthetic deoxyribonucleic acid duplexes containing alternating guanine and cytosine bases or adenine and thymine bases, respectively. The cationic porphyrins supplied by MidCentury Chemical Company are tetrakis(4-*N*-methylpyridyl)porphyrin (H_2TMpyP^{4+}), tetrakis-(2-*N*-methylpyridyl)porphyrin ($H_2TMpyP\text{-}2^{4+}$), and tetrakis(4-*N*,*N*,*N*-trimethylanilinium)porphyrin (H_2TAP^{4+}). See Figure 1. All porphyrins were used in the chloride form.

Table 1. Experimental rate constants for the insertion of copper(II) into cationic porphyrins. ([Cu^{2+}] 380 μM; [poly(dG-dC)$_2$] 100 μM; [Porphyrin Cl_4]$_o$ 10 μM; 25 °C; pH 8.)

A. H_2TMpyP^{4+}

(a) in presence of NH_4Cl and NaCl

$[NH_4^+]$/M	$[Na^+]$/M	poly(dG–dC)$_2$[a]	k/s^{-1}	ρ[b]
0.05	0.10	N	2.5×10^{-2}	0.0072
		Y	1.8×10^{-4}	
0.05	0	N	2.2×10^{-2}	$<10^{-3}$
		Y	very slow	

(b) in presence of imidazole (Imid) and NaCl

[Imid] × 10^3/M	$[Na^+]$/M	poly(dG–dC)$_2$	k/s^{-1}	ρ
5.0	0.15	N	1.4×10^{-2}	0.014
		Y	1.9×10^{-4}	
5.0	0.05	N	9.1×10^{-3}	$<10^{-3}$
		Y	very slow	
5.0	0.01	N	8.1×10^{-3}	$<10^{-3}$
		Y	very slow	
5.0	0.001	N	6.2×10^{-3}	$<10^{-3}$
		Y	very slow	

B. $H_2TMpyP\text{-}2^{4+}$

(a) in presence of NH_4Cl and NaCl

$[NH_4^+]$/M	$[Na^+]$/M	poly(dG–dC)$_2$	k/s^{-1}	ρ
0.05	0.10	N	2.5×10^{-2}	0.92
		Y	2.3×10^{-2}	
0.05	0	N	3.0×10^{-2}	0.70
		Y	2.1×10^{-2}	

(b) in presence of imidazole and NaCl

[Imid] ×10^3/M	$[Na^+]$/M	poly(dG–dC)$_2$	k/s^{-1}	ρ
5.0	0.15	N	1.8×10^{-2}	1.0
		Y	1.8×10^{-2}	
5.0	0.05	N	1.6×10^{-2}	0.81
		Y	1.3×10^{-2}	
5.0	0.01	N	1.5×10^{-2}	0.67
		Y	1.0×10^{-2}	
5.0	0.001	N	1.6×10^{-2}	1.0
		Y	1.6×10^{-2}	

C. H_2TAP^{4+}

(a) in presence of NH_4Cl and NaCl

$[NH_4^+]$/M	$[Na^+]$/M	poly(dG–dC)$_2$	k/s^{-1}	ρ
0.05	0.10	N	9.6×10^{-3}	0.10
		Y	9.9×10^{-4}	
0.05	0	N	7.6×10^{-3}	0.018
		Y	1.4×10^{-4}	

(b) in presence of imidazole and NaCl

[Imid] × 10^3/M	$[Na^+]$/M	poly(dG–dC)$_2$	k/s^{-1}	ρ
5.0	0.15	N	1.6×10^{-2}	0.34
		Y	5.4×10^{-3}	
5.0	0.05	N	1.2×10^{-2}	0.021
		Y	2.5×10^{-4}	
5.0	0.01	N	1.2×10^{-2}	0.039
		Y	4.7×10^{-4}	
5.0	0.001	N	9.2×10^{-3}	0.12
		Y	1.1×10^{-3}	

[a] Y = yes *i.e.* poly(dG–dC)$_2$ added; N = no poly(dG–dC)$_2$. [b] ρ = (k in presence of poly(dG–dC)$_2$)/(k in absence of poly(dG–dC)$_2$) under otherwise identical conditions.

interactions exist for these species they have little effect on the kinetics of metallation. Furthermore, the spectral properties of the free base porphyrin–DNA complexes are unaffected by the addition of such copper–nitrogenous base complexes. To minimize ambiguities in the interpretation of the results, experiments were conducted for each porphyrin at identical pH, ionic strengths, temperature, and concentrations of copper(II), nitrogenous ligand, porphyrin, and DNA. The

reactions can be represented by equation (1).

$$CuL_n^{2+} + H_2P^{4+} \xrightarrow{k} CuP^{4+} + nL + 2H^+ \quad (1)$$

The wavelengths of observation were: for H_2TMpyP^{4+}, 518 nm (no DNA) and 532 nm (with DNA); for $H_2TMpyP\text{-}2^{4+}$, 510 nm both with and without DNA; and for H_2TAP^{4+}, 514 nm (no DNA) and 518 nm (with DNA). The results of these experiments are shown in Tables 1 and 2. Rate constants are reported to within ±20% based upon multiple runs of the same experiment.

As can be seen from Table 1, similar kinetic patterns are obtained whether ammonia or imidazole is used to bind the copper ion. Furthermore, under identical experimental conditions in the absence of DNA, the copper insertion rates are remarkably similar for all three porphyrins although there are marked differences in basicities for these species[12] ranging from a $pK_a < 1$ for $H_4TMpyP\text{-}2^{6+}$ to $pK_a \sim 4$ for H_4TAP^{6+}. As may also be seen from Table 1, the presence of poly(dG–dC)$_2$ markedly inhibits the metallation of H_2TMpyP^{4+} presumably because the porphyrin intercalates into this synthetic DNA and is effectively removed from the copper(II)-containing solution. To account for these results we suggest the model shown in equation 2 where 'out' and 'in' refer to nonintercalated and intercalated porphyrin, respectively.

$$\begin{array}{l} H_2P^{4+}{}_{out} \xrightarrow{k_{out}} \\ CuL_n^{2+} + \downarrow\uparrow \qquad\quad CuP^{4+} + nL + 2H^+ \\ H_2P^{4+}{}_{in} \xrightarrow{k_{in}} \end{array} \quad (2)$$

We define an apparent equilibrium constant, K, in equation (2), since the intercalation process is known to be much more rapid than the metallation.[6]

$$K = \frac{[H_2P^{4+}{}_{in}]}{[H_2P^{4+}{}_{out}][\text{poly(dG–dC)}_2]} \quad (3)$$

K, as defined here, is not a true constant[13] but depends upon r (where $r \equiv [H_2P_{in}]/[\text{poly(dG–dC)}_2]_o$), so that we can at best provide an estimate of K from these kinetic results. The [poly(dG–dC)$_2$] term is the concentration of binding sites available at equilibrium taking into account near-neighbour exclusion and any other co-operativity effects.[5,9,13] Because our experiments were conducted with a large excess of poly(dG–dC)$_2$ to porphyrin, we can estimate this term using equations provided by McGhee and von Hippel.[13] Comparing equations (2) and (3) to equation (1) leads to equation (4).

$$k = \frac{k_{out} + k_{in}K[\text{poly(dG–dC)}_2]}{1 + K[\text{poly(dG–dC)}_2]} \quad (4)$$

Since the rate of the reaction for H_2TMpyP^{4+} goes virtually to zero when the ionic strength is lowered to a point at which the porphyrin is almost totally intercalated,[8] we estimate $k_{in} \approx 0$ dm^3 mol^{-1} s^{-1}. Furthermore, we approximate k_{out} with the rate constant obtained in the absence of polynucleotide. This leads to the ratio ρ, as defined in equation (5). From the

$$\rho = \frac{k}{k_{out}} = (1 + K[\text{poly(dG–dC)}_2])^{-1} \quad (5)$$

results of Table 1A we obtain $K \sim 10^6$ dm^3 mol^{-1} at $\mu = 0.15$ M, in good agreement with the value of 8×10^5 dm^3 mol^{-1} determined earlier from titration experiments under similar conditions.[5]

Table 2. Comparison of polynucleotide effects on copper(II) insertion into cationic porphyrins. ([Cu^{2+}] 380 μM; [polynucleotide] 100 μM; [Porphyrin Cl_4]$_o$ 10 μM; 25 °C; pH 8.)

	μ/M	ρ_{GC}	ρ_{AT}
H_2TMpyP^{4+}	0.15	0.013	0.017
	0.05	$<10^{-3}$	0.015
	0.01	$<10^{-3}$	0.016
	0.001	$<10^{-3}$	0.032
$H_2TMpyP\text{-}2^{4+}$	0.15	1.0	0.43
	0.05	0.81	0.069
	0.01	0.67	0.058
	0.001	1.0	0.094
H_2TAP^{4+}	0.15	0.33	0.002
			0.02[a]
	0.05	0.021	0.003
	0.01	0.039	$<10^{-3}$
	0.001	0.13	0.01

[a] This determination was conducted at [H_2TAP^{4+}] = 1 μM using fluoresence detection. All other conditions were the same as in other experiments.

We contrast these results with those obtained for $H_2TMpyP\text{-}2^{4+}$ (Table 1B). This latter porphyrin does not intercalate into poly(dG–dC)$_2$ and, under the conditions of our experiments, the presence of the polymer has very little effect on the metallation kinetics. Whether this result implies that $H_2TMpyP\text{-}2^{4+}$ is not concentrated at the surface of poly(dG–dC)$_2$ or that two off-setting effects are at work here (concentration *vs.* partitioning of ions and/or protection of the porphine core), is not clear from these experiments. However, it is evident that poly(dG–dC)$_2$ has a very different influence on the metal insertion kinetics for $H_2TMpyP\text{-}2^{4+}$ than for H_2TMpyP^{4+}. By way of comparison, we note that at $\mu = 0.15$ M, $\rho_{H_2TMpyP} \sim 100$.

With H_2TAP^{4+}, although the effect is not nearly as dramatic as for H_2TMpyP^{4+}, the influence of poly(dG–dC)$_2$ is to inhibit metallation. While this porphyrin does not intercalate into poly(dG–dC)$_2$, its interaction is apparently profound enough to partially protect the porphine core from metallation. These results are consistent with the induced c.d. spectrum in the Soret region which yields a small signal, primarily positive but with some conservative character implying a degree of porphyrin aggregation.

None of the porphyrins tested intercalates into poly (dA–dT)$_2$ but they all provide c.d. signals in the Soret region. The profile is a simple positive band for each of $H_2TMpyP\text{-}2^{4+}$ and H_2TMpyP^{4+}, but is more complicated for H_2TAP^{4+}. At low drug load, a split positive band is obtained for H_2TAP^{4+} but as the porphyrin : nucleic acid ratio (r_o) is increased, a conservative spectrum is obtained suggesting porphyrin aggregation at the nucleic acid surface.

Table 2 shows a comparison of the metallation rates for poly(dG–dC)$_2$ and poly(dA–dT)$_2$. The effect of the polynucleotide is still the most modest for $H_2TMpyP\text{-}2^{4+}$ but, for this porphyrin, the effect of poly(dA–dT)$_2$ is much more profound than that of poly(dG–dC)$_2$. This is true also for the other non-intercalating porphyrin, H_2TAP^{4+}. Generally, groove binding is more favourable in A–T regions of DNA than G–C regions for both electronic and steric reasons. The degree of aggregation of the porphyrin may also be a factor in determining these rates. In the absence of poly(dA–dT)$_2$, the copper(II) insertion rate constant obtained *via* absorption ([H_2TAP^{4+}] = 10 μM), agreed with one obtained *via* fluor-

escence ([H_2TAP^{4+}] = 1 μM) to within 5%. But in the presence of the DNA, the insertion of copper(II) into H_2TAP^{4+} is an order of magnitude faster at low drug load ($r_o^{-1} \sim 100$) where aggregation is less important than at $r_o^{-1} = 10$. For H_2TMpyP^{4+} alone, the only one of these porphyrins which can intercalate into nucleic acids is poly(dG–dC)$_2$, a more effective inhibitor of metallation than poly(dA–dT)$_2$, particularly at low ionic strength. Based upon the evidence obtained thus far, protection against metallation appears to follow the order: intercalation > external binding with aggregation > external binding, no aggregation > electrostatic interactions only.

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References

1 G. S. Manning, *Q. Rev. Biophys.*, 1978, **11**, 179; G. S. Manning, *J. Phys. Chem.*, 1981, **85**, 870.
2 J. K. Barton, C. V. Kunar, and N. J. Turro, *J. Am. Chem. Soc.*, 1986, **108**, 6391.
3 H. Morawetz, *Acc. Chem. Res.*, 1970, **3**, 354; H. Morawetz and B. Vogel, *J. Am. Chem. Soc.*, 1969, **91**, 563.
4 D. F. Wilson and R. L. Jones in 'Intercalation Chemistry,' Academic Press, New York, 1982, 445.
5 R. F. Pasternack, E. J. Gibbs, and J. J. Villafranca, *Biochemistry*, 1983, **22**, 2406.
6 R. F. Pasternack, E. J. Gibbs, and J. J. Villafranca, *Biochemistry*, 1983, **22**, 5409.
7 R. F. Pasternack, D. Sidney, P. A. Hunt, E. A. Snowden, and E. J. Gibbs, *Nucl. Acids Res.*, 1986, **14**, 3927.
8 R. F. Pasternack, P. Garrity, B. Ehrlich, C. B. Davis, E. J. Gibbs, G. Orloff, A. Giartosio, and C. Turano, *Nucl. Acids Res.*, 1986, **14**, 5919.
9 E. J. Gibbs, M. C. Maurer, J. H. Zhang, W. M. Reiff, D. Hill, M. Malicka-Blaszkiewicz, S. C. Mah, and R. F. Pasternack, *J. Inorg. Biochem.*, in the press.
10 M. J. Carvlin, N. Datta-Gupta, and R. Fiel, *Biochem. Biophys. Res. Commun.*, 1982, **108**, 66.
11 V. A. Sorokin, Y. P. Blagoi, V. A. Valeev, S. V. Kosnilova, G. O. Gladchenko, I. D. Reva, and V. I. Sokhan, *J. Inorg. Biochem.*, 1987, **30**, 87, and references therein.
12 M. Krishnamurthy, *Ind. J. Chem. B*, 1977, **15**, 964; P. Hambright, T. Gore, and M. Burton, *Inorg. Chem.*, 1976, **15**, 2314; R. F. Pasternack, P. R. Huber, P. Boyd, G. Engasser, L. Francesconi, E. Gibbs, P. Fasella, G. C. Venturo, and L. de C. Hinds, *J. Am. Chem. Soc.*, 1972, **94**, 4511.
13 J. D. McGhee and P. H. von Hippel, *J. Mol. Biol.*, 1974, **86**, 469.