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Interactions of Porphyrins and Metalloporphyrins with Single-Stranded Poly(dA)

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A study of the interactions of single-stranded poly(deoxyadenylic acid) (poly(dA)) with a number of metallo- and nonmetalloporphyrins is reported. Among the metal derivatives considered is the newly synthesized platinum(II) complex of tetrakis(*N*-methylpyridinium-4-yl)porphine (PtTMpyP-4). This metalloporphyrin behaves like other four-coordinated species in that it intercalates into double-stranded (ds) calf thymus DNA, resulting in a large bathochromic shift (18 nm) and hypochromicity (46%) of its Soret maximum. In addition, an induced, negative circular dichroism (CD) feature, typical of porphyrin intercalators, is observed at 420 nm with a $\Delta\epsilon$ of -13 M^{-1} cm^{-1}. All the porphyrin·poly(dA) complexes studied yield considerable hypochromicity compared to the free porphyrin, suggesting extensive interaction of the π-system of the porphyrins with that of the adenine bases. Whereas intercalating porphyrins display a hypochromicity with poly(dA) that is comparable to that obtained with calf thymus (ct) DNA, ZnTMpyP-4, which is a groove-binder by virtue of its single-axial ligand (H_2O), produces a substantially greater hypochromicity with the single-stranded than with the double-stranded nucleic acid. These results are consistent with a pseudointercalation model for binding of porphyrins to poly(dA). In addition, under certain conditions CuTMpyP-4·poly(dA) shows a large conservative CD feature in the Soret region ($\Delta\epsilon \sim \pm 10^2$ M^{-1} cm^{-1}), unlike profiles observed for other CuTMpyP-4·nucleic acid complexes. This metalloporphyrin may by virtue of this CD signature prove to be a useful reagent in reporting the presence of single-stranded regions in a complex nucleic acid mixture.

Introduction

Tetrakis(*N*-methylpyridinium-4-yl)porphine (H_2TMpyP-4, Figure 1) and a number of its metal derivatives have been intensively studied as double-stranded (ds) DNA binding agents.[1,2] A variety of spectroscopic, kinetic, and biomimetic methods have been employed in these investigations, and whereas there are still a number of issues relating to the details of the interactions to be resolved, several generalizations correlating binding mode to DNA composition and porphyrin structure seem well established. With respect to the homopolymers poly(dG-dC)$_2$ and poly(dA-dT)$_2$, it is found that (i) intercalation occurs with the former but not the latter polynucleotide, (ii) intercalation with poly(dG-dC)$_2$ occurs only with nonaxially liganded porphyrin derivatives (i.e., H_2TMpyP-4, CuTMpyP-4, NiTMpyP-4, AuTMpyP-4, and PdTMpyP-4), (iii) negative induced circular dichroism (CD) bands in the Soret region of these porphyrin chromophores are indicative of intercalation, (iv) positive induced CD bands are a signature for external binding, and (v) poly(dA-dT)$_2$ forms more stable external complexes with these porphyrins and metalloporphyrins than does poly(dG-dC)$_2$.

At relatively low drug load (i.e., $r_0 \equiv$ [porphyrin]$_0$/[nucleic acid base pairs]$_0 < 0.1$), axially liganded metal derivatives of tetrakis(*N*-methylpyridinium-4-yl)porphine display positive induced CD bands with *calf thymus DNA* (ct DNA) whereas the nonaxially liganded metalloporphyrins show negative induced CD features in the Soret region.[3] The fact that CD criteria for porphyrin binding mode established with synthetic homo (purine-pyrimidine)$_2$ DNAs are applicable also to natural, mixed base pair DNAs (at least at low r_0) has been corroborated through a broad range of experimental strategies and techniques.[1]

In addition to these results for monodispersed porphyrins, evidence has been previously presented for porphyrin-porphyrin interactions on DNA surfaces under certain conditions. Conservative-type induced CD signals that are usually (but not always[4]) diagnostic for such interactions have been reported for a number of systems.[3,5,6] Especially dramatic is the effect of NaCl on the CD signal induced when *trans*-bis(*N*-methylpyridinium-4-yl)diphenylporphine (*trans*-H_2(Ph)$_2$(4-*N*-Mpy)$_2$P, Figure 1) binds to various ds DNAs.[6] With poly(dG-dC)$_2$, for example, at [NaCl] = 0.010 M, $r_0 = 0.027$, a single negative feature is induced in the Soret region with $\Delta\epsilon \sim -10$ M^{-1} cm^{-1}, implying a simple intercalation process. However, raising the NaCl concentration to 0.087 M leads to an asymmetric conservative CD profile in this spectral region, with $\Delta\epsilon \sim \pm 1.8 \times 10^3$ M^{-1} cm^{-1}. Similar results are obtained with calf thymus DNA. We have proposed that under appropriate conditions this porphyrin is capable of forming long-range aggregates on the nucleic acid polymer, resulting in a helical alignment of porphyrin transition dipoles giving rise to the very large, conservative CD signals observed.[6]

In the present study we consider the interactions of poly(dA), a single-stranded (ss) DNA, with H_2TMpyP-4, several of its metal derivatives, and *trans*-H_2(Ph)$_2$(4-*N*-Mpy)$_2$P. Single-stranded segments of DNA play important biological roles—they are involved in both replication and transcription processes—and yet relatively few studies have appeared on the binding of small molecules to these structures. Such studies might prove useful, for example, by uncovering a reagent that gives a unique spectroscopic signal in the presence of ss DNA, thereby providing a rapid, simple assay for this form. We report here on a substance that appears promising in this regard.

Experimental Section

Materials. (i) Porphyrins. The two nonmetalloporphyrins used for spectral measurements, H_2TMpyP-4 and *trans*-H_2(Ph)$_2$(4-*N*-Mpy)$_2$P (Figure 1), were obtained from Midcentury as chloride salts. The identity and extent of peripheral methylation of these substances were checked by ^{1}H NMR in DMSO-d_6. Concentrations were determined in water by using $\epsilon = 2.26 \times 10^5$ M^{-1} cm^{-1} and $\epsilon = 2.4 \times 10^5$ M^{-1} cm^{-1}, respectively,[7,8] at the Soret maxima. Metal derivatives (other than Pt(II) to be presented below) of the former porphyrin were prepared as previously described.[9-11] Concentrations were obtained from absorbances at the respective Soret maxima by using $\epsilon = 2.31 \times 10^5$ M^{-1} cm^{-1} for CuTMpyP-4, $\epsilon = 1.49 \times 10^5$ M^{-1} cm^{-1} for NiTMpyP-4 (20% acetone), $\epsilon = 2.04 \times 10^5$ M^{-1} cm^{-1} for ZnTMpyP-4, and $\epsilon = 1.02 \times 10^5$ M^{-1} cm^{-1} for FeTMpyP-4 at pH 2.

The synthesis of PtTMpyP-4 required a suitable water-soluble plati-

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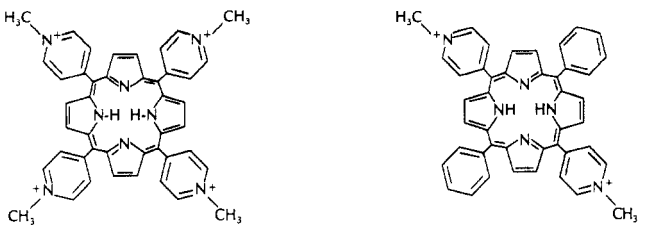

Figure 1. Structure of free base porphyrins: left, tetrakis(*N*-methylpyridinium-4-yl)porphine (H_2TMpyP-4); right, *trans*-bis(*N*-methylpyridinium-4-yl)diphenylporphine (trans-$H_2(Ph)_2$(4-*N*-Mpy)$_2$P).

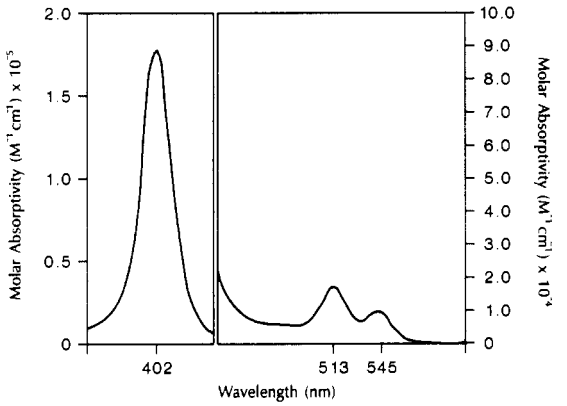

Figure 2. Absorption spectrum of PtTMpyP-4 in 1 mM phosphate, 0.20 M NaCl (pH 7).

num starting material. Attempts to prepare the complex from K_2PtCl_4 resulted in the precipitation of insoluble [H_2TMpyP-4][$PtCl_4$]$_2$. The use of a cationic labile precursor (i.e., *cis*-Pt(H_2O)$_2$(DMSO)$_2^{2+}$) avoided this problem. This synthesis thus began with the preparation of *cis*-$PtCl_2$-(DMSO)$_2$ by the method of Price et al.[12] A suspension of this platinum(II) compound (0.64 g, 1.5 mmol) in water (15 mL) was treated with an aqueous solution (5 mL) of $AgNO_3$ (0.48 g, 2.8 mmol). The mixture was protected from light and stirred for 3 h. After removal of AgCl by filtration, the filtrate was mixed with [H_2TMpyP-4][tosylate]$_4$, obtained from Porphyrin Products, Logan, UT (0.16 g, 0.12 mmol), in water (70 mL), and the mixture was heated to reflux temperature for 15 h. At this time a platinum mirror formed inside the reaction flask. The deep red-orange reaction mixture was filtered through Celite, and $NaBF_4$ (5 g) was added to the filtrate. This mixture was treated by centrifugation (2000 rpm for 15 min), yielding a red solid and a brownish supernatant. The solid was suspended in water with 20× excess anion exchange resin (Dowex 1-X80) in the chloride form and the mixture stirred for 1 h. The anion-exchange resin was removed by filtration, and NH_4PF_6 (3.0 g) was added to the filtrate, causing a precipitate to form. Sufficient acetone was added to produce a solution, and slow evaporation of the solvent gave a purple microcrystalline product. This material was collected, washed with methanol and ether, and dried in vacuo. Yield of [PtTMpyP-4]-[PF_6]$_4$·5H_2O = 0.09 g, 58% based on porphyrin starting material. Anal. Calcd for $C_{44}H_{46}F_{24}N_8O_5P_4Pt$: C, 34.26; H, 3.01; N, 7.26. Found: C, 34.13; H, 2.53; N, 7.15. The chloride salt was prepared by mixing the above material with Dowex 1-X80 in the chloride form.

(ii) Polynucleotides. Calf thymus DNA was purchased from Sigma Chemical Co. and was purified by using a standard procedure previously described.[3] Poly(dA) was obtained from Pharmacia and was used after extensive dialysis against a buffer containing 1.0 mM phosphate buffer at 0.2 M NaCl (pH 7.0). The integrity of the nucleic acids was determined by comparing their UV and circular dichroism spectra with previously published results.[13,14] The concentration of calf thymus DNA was expressed in base pairs/L determined spectrophotometrically by using $\epsilon_{260} = 1.31 \times 10^4$ M^{-1} cm^{-1}; that for poly(dA) was expressed in mol of nucleotide/L and determined[13,14] by using $\epsilon_{260} = 9100$ M^{-1} cm^{-1}. All other chemicals were reagent grade, as obtained from Fisher Scientific, and were used without further purification.

Methods. Spectral measurements were carried out on a Nicolet 9420 UV/vis spectrophotometer; circular dichroism spectra were taken on an Aviv 60DS spectrometer, and kinetic relaxation experiments were performed on a Dialog joule-heating temperature-jump apparatus, having a heating time of approximately 1 μs and described elsewhere.[15] The data were collected digitally and transferred to a VAX microcomputer, where they were analyzed by using the DISCRETE program written by Provencher.[16]

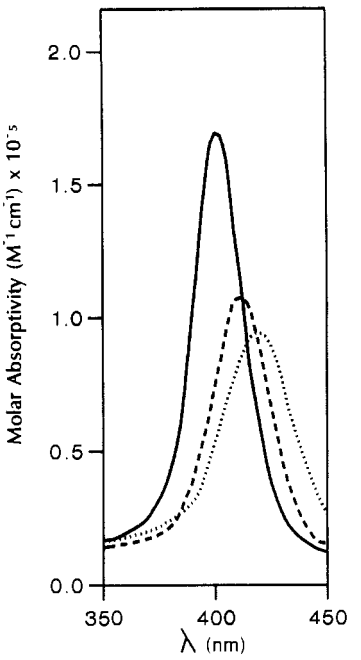

Figure 3. Soret region of PtTMpyP-4 in 1 mM phosphate (μ = 0.20 M, pH 7, 25 °C): (—) no DNA; (- -) poly(dA), r_0 = 0.040; (···) ct DNA, r_0 = 0.040.

Table I. Relaxation Times for PtTMpyP-4·ct DNA Complexes in 1 mM Phosphate (μ = 0.20 M, pH 7, 25 °C)

$1/r_0$	τ_1, s	τ_2, s	τ_3, s
50	0.0014	0.020	0.153
25		0.017	0.092
15	0.0016	0.019	0.105
10	0.0037	0.026	0.165
5	0.0011	0.015	0.071

Results and Discussion

Characterization of PtTMpyP-4 in Solution and as a Ligand for Double-Stranded DNA. The spectral properties of PtTMpyP-4 (Figure 2) were investigated in the range 350–700 nm as a function of concentration, pH, and ionic strength. The Soret maximum at 402 nm ($\epsilon = 1.72 \times 10^5$ M^{-1} cm^{-1}) and "visible" bands at 513 nm ($\epsilon = 1.87 \times 10^4$ M^{-1} cm^{-1}) and 545 nm ($\epsilon = 1.15 \times 10^4$ M^{-1} cm^{-1}) show little wavelength or molar absorptivity dependence on solution conditions, including pH between 2 and 10 and ionic strength up to 1 M. Joule-heating temperature-jump experiments produced no relaxation effects over a wide range of concentrations and pH. On the basis of these results, we conclude that this d^8 metalloporphyrin is monomeric and four-coordinate in the absence of strongly coordinating ligands. In this respect it is more similar to PdTMpyP-4[17,18] than to NiTMpyP-4,[10] which has a much greater tendency to add axial ligands. This last porphyrin exists in aqueous solution as a roughly 50:50 mixture of four-coordinate and six-coordinate (two axially liganded water molecules) forms.

Spectral features arising from PtTMpyP-4 interactions with double-stranded DNA confirm that this metalloporphyrin is a monomeric, four-coordinate species in aqueous solution. As shown in Figure 3, the Soret band of the PtTMpyP-4·DNA complex is

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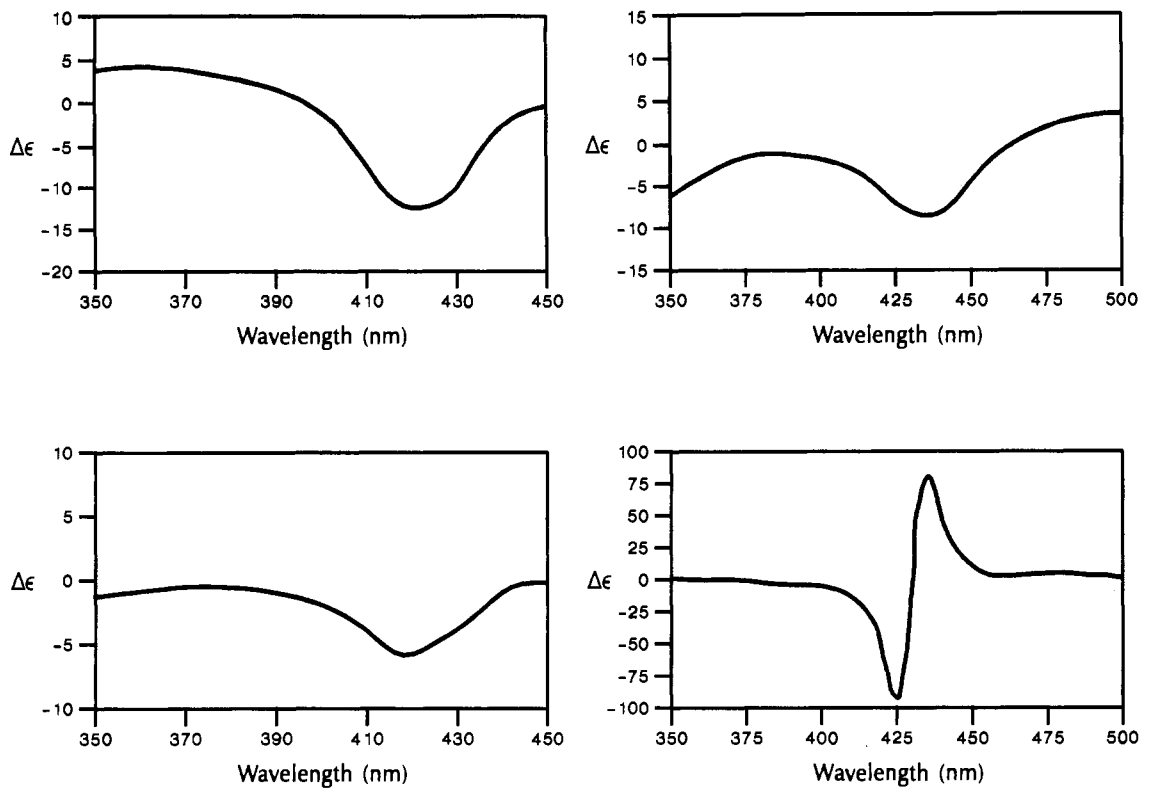

Figure 4. Induced CD spectra of metalloporphyrin·DNA complexes in the Soret region (pH 7.0, μ = 0.20 M, 25 °C): upper left, 7.44 μM PtTMpyP-4, 186 μM ct DNA; bottom left, 7.44 μM PtTMpyP-4, 186 μM poly(dA); upper right, 3.44 μM NiTMpyP-4, 86.0 μM poly(dA); bottom right, 3.44 μM CuTMpyP-4, 86.0 μM poly(dA).

markedly red-shifted from that of the free metalloporphyrin (from 402 to 420 nm) with extensive hypochromicity (46%). Furthermore, as a result of the interaction, a substantial negative CD spectral feature is induced at 420 nm (Figure 4), having $\Delta\epsilon$ = -13 M^{-1} cm^{-1}. These spectral patterns parallel those obtained with other four-coordinate metal derivatives of H_2TMpyP-4, including NiTMpyP-4 and PdTMpyP-4, both of which have been shown to intercalate into ds DNA.[3,18,19] In addition, as for the complexes of calf thymus DNA with CuTMpyP-4, NiTMpyP-4, and AuTMpyP-4, coupled relaxation effects are obtained in temperature-jump experiments for the PtTMpyP-4·ct DNA system.[20,21] The relaxation times are shown for various conditions in Table I. These values are similar to those obtained with other systems in which porphyrin intercalation is observed (e.g.: for CuTMpyP-4, $1/r_0$ = 50, μ = 0.2, 25.1°, τ_1 = 0.010 s, τ_2 = 0.028 s, τ_3 = 0.128 s; for NiTMpyP-4, $1/r_0$ = 20, μ = 0.2, 25.1°, τ_1 = 0.008 95 s, τ_2 = 0.030 s, τ_3 = 0.110 s). In contrast, relaxation times are either shorter or nonobservable for external binding porphyrin species.[20] On the basis of this spectroscopic and kinetic evidence, we conclude that PtTMpyP-4 like other four-coordinate metal derivatives of H_2TMpyP-4 intercalates into ct DNA and dissociates from the nucleic acid as the ionic strength increases.

Interactions of Porphyrins with Poly(dA). We extended our studies of porphyrin interactions with nucleic acids to include a single-stranded polymer, poly(dA). The CD spectrum obtained here for this nucleic acid agreed with the published spectrum,[22] which, with NMR evidence,[23] is the basis for the proposed geometrical model. That is, the polymer is suggested to be extensively stacked at 298 K (T_m ~ 324 K) with all the residues in a right-handed single helix adopting a B-DNA-like geometry except for the final pdA unit. Further, it has been suggested that, in the binding of proflavin to poly(dA), extensive interactions between the aromatic ring of the dye and the bases occur, resembling an intercalation event.[24]

Table II. Spectral Properties of Complexes of H_2TMpyP-4 and Several of Its Metallo Derivatives with Poly(dA)

	25:1, poly(dA)·porphyrin		25:1, ct DNA·porphyrin[a]	
porphyrin	Δλ, nm	% hypochromicity	Δλ, nm	% hypochromicity
H_2TMpyP-4	1	14	7	40
CuTMpyP-4	3	11	6	18
NiTMpyP-4	11	46	17	38
PtTMpyP-4	10	37	18	46
AuTMpyP-4	<1	6	8	35
ZnTMpyP-4	1	10	2	−5
FeTMpyP-4	<1	4	6	12

[a] References 3 and 21.

In the present study, we considered the interaction of H_2TMpyP-4 and a number of its metal derivatives with poly(dA) with spectral results, as shown in Table II. For all the porphyrin derivatives *except* the five-coordinate ZnTMpyP-4 and (to a lesser extent) NiTMpyP-4 the hypochromicity of the Soret bands is smaller than that obtained with ct DNA. However, this should not overshadow the fact that extensive hypochromicity is observed for all the porphyrin·poly(dA) systems, suggesting interaction between the π-systems of neighboring porphyrins and/or interaction with the π-system of the adenine bases. Whereas some evidence for porphyrin stacking will be presented (vide infra), for most of these porphyrins (and especially under the low drug load conditions employed here) it is more likely that the hypochromicity arises from porphyrin–adenine interactions as proposed by the pseudointercalation model presented previously.[24] It is possible that even five-coordinate ZnTMpyP-4 is able to form such a complex using the distal side of the porphyrin plane, suggesting

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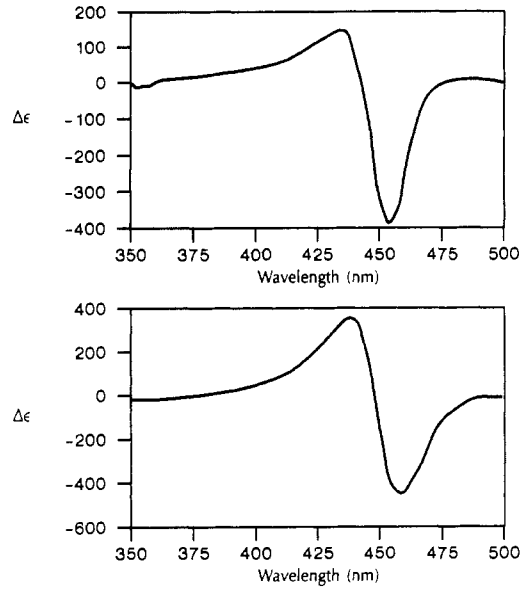

Figure 5. Induced CD spectra of *trans*-$H_2(Ph)_2$(4-*N*-Mpy)$_2$P·poly(dA) complexes (pH 7.0, μ = 0.20 M, 25 °C): upper, 3.44 μM porphyrin, 86.0 μM poly(dA); lower, 3.44 μM porphyrin, 3.44 μM poly(dA).

that the steric restrictions of a duplex are partially relaxed for a single-stranded polymer.

Circular dichroism experiments were conducted as well, and none of H_2TMpyP-4, AuTMpyP-4, ZnTMpyP-4, or FeTMpyP-4 showed any feature outside of experimental scatter. Both PtTMpyP-4 and NiTMpyP-4 provide very small negative induced CD bands in the Soret region (Figure 4), having a magnitude about one-third of that obtained with a nucleic acid duplex. Induced molar ellipticities for monodispersed porphyrins are not large for the single-stranded polynucleotide interactions, presumably because drug molecules have more freedom of orientation than when binding to duplexes.

However, CuTMpyP-4 displays a large, conservative CD profile with poly(dA) different from characteristic CD bands obtained for this metal derivative with other nucleic acids (Figure 4). Large conservative features of this type are characteristic of porphyrin–porphyrin interactions on a chiral matrix[25] and have been observed, for example, for *meso*-tetrakis(*N*,*N*,*N*-trimethylanilino)porphine (under conditions of high salt and drug load)[15] and *trans*-$H_2(Ph)_2$(4-*N*-Mpy)$_2$P.[6] The influence of both drug load and ionic strength on the drug–drug interaction (as manifested by the conservative CD profile) was studied for the CuTMpyP-4·poly(dA) system. In the range $1 \geq r_0 \geq 0.020$ ([NaCl] = 0.20 M), a conservative CD profile in the Soret region of CuTMpyP-4 was obtained. The magnitude of the signal was at a maximum at $r_0 = 0.11$ and then decreased with increasing poly(dA) concentration (at fixed [CuTMpyP-4]) until at $r_0 < 0.020$ the conservative CD signal was converted to a single, small negative feature similar to that obtained for NiTMpyP-4 and PtTMpyP-4. Presumably, as r_0 decreases, entropy effects that tend to disperse the metalloporphyrin on the polynucleotide overcome the enthalpy of porphyrin stacking, leading to a CD profile characteristic of porphyrin monomers.

The NaCl concentration was varied at a constant r_0 value of 0.11 to determine the influence of salt concentration on the induced CD signal of the CuTMpyP-4·poly(dA) species. At [NaCl] = 0.010 a single negative feature is obtained, similar to results for NiTMpyP-4 and PtTMpyP-4. However, by [NaCl] = 0.030 M the profile for CuTMpyP-4·poly(dA) becomes conservative, having a total signal magnitude (peak to trough), S (M^{-1} cm^{-1}), of 68. This value increases with increasing sodium chloride concentration until at [NaCl] = 0.10 M the total signal intensity is nearly 180 M^{-1} cm^{-1} (cf. Figure 4). The signal then decreases with increasing NaCl: [NaCl] = 0.30, S = 160 M^{-1} cm^{-1}; [NaCl] = 0.50, S = 70 M^{-1} cm^{-1}; [NaCl] = 1.0, S = 25 M^{-1} cm^{-1}. The 1:1 electrolyte clearly has two (or more) influences on the CuTMpyP-4·poly(dA) system with opposing effects on the signal intensity. NaCl promotes CuTMpyP-4 aggregation on the poly(dA) surface with a concomitant increase in the CD signal, but as predicted by polyelectrolyte theory, a high concentration of NaCl leads to dissociation of the metalloporphyrin·nucleic acid complex and a decrease in the total signal. (CuTMpyP-4 free in solution provides no CD spectrum.) From these results it can be estimated that by [NaCl] = 1.0 M about 85% of the porphyrin is free in solution.

To further pursue the question of porphyrin–porphyrin interaction on poly(dA), we considered the influence of this polynucleotide on *trans*-bis(*N*-methylpyridinium-4-yl)diphenylporphine (*trans*-$H_2(Ph)_2$(4-*N*-Mpy)$_2$P, Figure 1). We have previously shown that this last porphyrin aggregates extensively in aqueous solution and also in the presence of several DNA duplexes,[6] leading to enormous CD features. The addition of poly(dA) to a solution of *trans*-$H_2(Ph)_2$(4-*N*-Mpy)$_2$P (r_0 = 0.040, [NaCl] = 0.20 M) leads to the rapid formation of a complex having a Soret band at 450 nm with a small shoulder at 420 nm (λ_{max} = 420 nm in neat water; 450 nm with a 420 nm shoulder in 1.0 mM phosphate, 0.20 M NaCl; 450 nm with a 420 nm shoulder in the same medium with ct DNA added) and a very large, conservative asymmetric CD signal (Figure 5). The magnitude and phase of the CD profile are very similar to those obtained for the highly aggregated porphyrin with duplex DNAs.[6] As the ratio of poly(dA) to *trans*-$H_2(Ph)_2$(4-*N*-Mpy)$_2$P decreases, the conservative CD signal becomes more symmetric, as shown in Figure 5. As observed for ds DNAs, the *CD profile of poly(dA)* in the ultraviolet region is virtually unaffected by interactions with *trans*-$H_2(Ph)_2$(4-*N*-Mpy)$_2$P at a 25:1 ratio; the same is true for the other porphyrins investigated, including CuTMpyP-4. Thus, apparently no major conformational changes of poly(dA) result from these interactions.

Results presented here lead us to suggest that CuTMpyP-4 be considered as a "reporter" molecule for single-stranded DNA. This metalloporphyrin provides a CD profile in the presence of the single-stranded nucleic acid poly(dA) (even at relatively low drug load), which is not observed for DNA duplexes; other porphyrins studied show no such unique feature. We are conducting experiments at present to determine under what conditions this copper porphyrin would prove useful as a reporter of ss DNA. For example, we are using competition studies to consider the influence of base composition, solvent composition, and relative single-stranded concentration on the distribution of this reagent, which in turn will define its potential as a reporter molecule.

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Registry No. Poly(dA), 25191-20-2; H_2TmpyP-4, 38673-65-3; *trans*-$H_2(Ph)_2$(4-*N*-Mpy)$_2$P, 119708-09-7; CuTMpyP-4, 48242-70-2; NiTMpyP-4, 48242-71-3; ZnTMpyP-4, 40603-58-5; FeTMpyP-4, 60489-13-6; *cis*-$PtCl_2(DMSO)_2$, 22840-91-1; [H_2TMpyP-4][tosylate]$_4$, 36951-72-1; [PtTMpyP-4][PF_6]$_4$, 129239-83-4; [PtTMpyP-4]Cl_4, 92739-63-4; PtTMpyP-4, 129264-06-8; AuTMpyP-4, 106007-10-7.

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