Porphyrin and Metalloporphyrin Binding to DNA Polymers: Rate and Equilibrium Binding Studies†

James A. Strickland,‡ Luigi G. Marzilli,*,‡,§ K. Michael Gay,∥ and W. David Wilson*,‡

Department of Chemistry, Emory University, Atlanta, Georgia 30322, and Department of Chemistry, Georgia State University, Atlanta, Georgia 30303

Received April 21, 1988; Revised Manuscript Received July 28, 1988

ABSTRACT: Interactions of *meso*-tetrakis(4-*N*-methylpyridiniumyl)porphyrin [TMpyP(4)] with poly[d(G-C)]·poly[d(G-C)] [poly[d(G-C)$_2$]] and poly[d(A-T)]·poly[d(A-T)] [poly[d(A-T)$_2$]] were studied by equilibrium dialysis and stopped-flow dissociation kinetics as a function of [Na^+]. Metalloderivatives of TMpyP(4), NiTMpyP(4), and ZnTMpyP(4) were also investigated. The apparent equilibrium binding constants (K_{obs}) were approximately the same for TMpyP(4) binding to either poly[d(G-C)$_2$] or poly[d(A-T)$_2$] and decreased with increasing [Na^+]. The slopes of the plots of log K_{obs} vs log [Na^+] were similar, with values close to −2.7. Contrary to implications in previously reported studies, these data do not indicate that TMpyP(4) prefers to bind to GC sites at low ionic strength and to AT sites at high ionic strength. In contrast, binding of ZnTMpyP(4) to these two polymers is very different. Comparisons of K_{obs} values at 0.065 M [Na^+] indicate that ZnTMpyP(4) binding to AT sites is ~200 times more favorable than binding to GC sites, a finding in agreement with previous qualitative observations. Although the binding of the Zn species to the GC polymer was too weak for us to assess the salt effect, the plot of log K_{obs} vs log [Na^+] gave a slope of −2.0 for ZnTMpyP(4) binding to poly[d(A-T)$_2$]. Application of condensation theory for polyelectrolytes suggests similar charge interactions for ZnTMpyP(4) and for TMpyP(4) binding to poly[d(A-T)$_2$]. Likewise, the rates of dissociation from poly[d(A-T)$_2$] were similar for TMpyP(4) and ZnTMpyP(4) [and also NiTMpyP(4)]. However, whereas TMpyP(4) [and NiTMpyP(4)] dissociation from poly[d(G-C)$_2$] was measurable, that for ZnTMpyP(4) was too fast to measure. All the rate data were best fit by a dual-exponential function. In all cases, dissociation rate constants increased with increasing [Na^+]. For example, increasing the [Na^+] from 0.115 to 0.515 M at 20 °C increased the fast and slow dissociation rate constants by a factor of 2–4 for TMpyP(4) dissociation from poly[d(G-C)$_2$]. This trend agrees with studies on well-known intercalators and disagrees with some previous findings on porphyrins at higher ionic strength. Our findings support the view that TMpyP(4) intercalates into GC sites. The [Na^+] dependence of the dissociation rate of TMpyP(4) is greater for poly[d(A-T)$_2$] than for poly[d(G-C)$_2$]. The greater dependence of ionic strength is consistent with a one-step condensation model from polyelectrolyte theory, and, to our knowledge, this is only the second such dependence found for a small DNA binding molecule. These findings support the view that TMpyP(4) is an outside binder at AT sites.

Cationic porphyrins have high binding constants to DNA[1] and appear to exhibit uniquely diverse binding modes dependent both on the porphyrin species and the DNA. Tetracationic TMpyP(4) and MTMpyP(4) have received the most study (Figure 1). Not only do these porphyrins bind electrostatically outside at AT base pairs, but many appear to be quite capable of sliding their bulky ring system between GC base pairs via intercalation (Banville et al., 1983, 1986; Carvlin & Fiel, 1983; Carvlin et al., 1982, 1983; Fiel et al., 1979, 1982, 1985; Ford et al., 1987; Kelly et al., 1985; Marzilli et al., 1986; Pasternack et al., 1983a,b, 1986a; Strickland et al., 1987). Of particular interest, the interaction mode of MTMpyP(4) species with DNA depends primarily upon the coordination number of the metal atom (Banville et al., 1983; Blom et al., 1986; Dougherty et al., 1985; Geacintov et al., 1987; Gibbs et al.,, 1988; Kelly et al., 1985; Pasternack et al., 1983a,b, 1985, 1986b; Strickland et al., 1987; Ward et al., 1986a,b).

The interactions of TMpyP(4) with homogeneous DNAs like poly[d(G-C)$_2$] and poly[d(A-T)$_2$] are in principle less complex than those with heterogeneous DNAs like CT DNA. Although the binding to purely alternating AT and purely alternating GC regions of DNA is understood, we have noted that binding to native DNA, where mixed AC/GT regions occur, may involve additional intercalation or outside binding sites (Banville et al., 1986; Strickland et al., 1987). Likewise, more than one intercalation or outside binding mode may occur, and additional insight into the binding is needed. The relative selectivity of GC vs AT binding and the nature of the

† This work was supported by NIH Grant GM29222 to L.G.M. and NSF Grant DBM-8603566 to W.D.W.

* Authors to whom correspondence should be addressed.
‡ Emory University.
§ Member of the Winship Cancer Center.
∥ Georgia State University.

[1] Abbreviations: AuTMpyP(4), gold(III) *meso*-tetrakis(4-*N*-methylpyridiniumyl)porphyrin; CT DNA, calf thymus deoxyribonucleic acid; CD, circular dichroism; CuTMpyP(4), copper(II) *meso*-tetrakis-(4-*N*-methylpyridiniumyl)porphyrin; DNA, deoxyribonucleic acid; EDTA, ethylenediaminetetraacetic acid; MTMpyP(4), metalloderivative of *meso*-tetrakis(4-*N*-methylpyridiniumyl)porphyrin; NiTMpyP(4), nickel(II) *meso*-tetrakis(4-*N*-methylpyridiniumyl)porphyrin; PIPES, piperazine-*N*,*N*′-bis[2-ethanesulfonic acid]; poly[d(A-T)$_2$], poly[d(A-T)]·poly[d(A-T)]; poly[d(G-C)$_2$], poly[d(G-C)]·poly[d(G-C)]; SDS, sodium dodecyl sulfate; TMpyP(2), *meso*-tetrakis(2-*N*-methylpyridiniumyl)porphyrin; TMpyP(4), *meso*-tetrakis(4-*N*-methylpyridiniumyl)porphyrin; ZnTMpyP(4), zinc(II) *meso*-tetrakis(4-*N*-methylpyridiniumyl)porphyrin. For clarity, porphyrin charge and counterions are omitted throughout this paper.

0006-2960/88/0427-8870$01.50/0

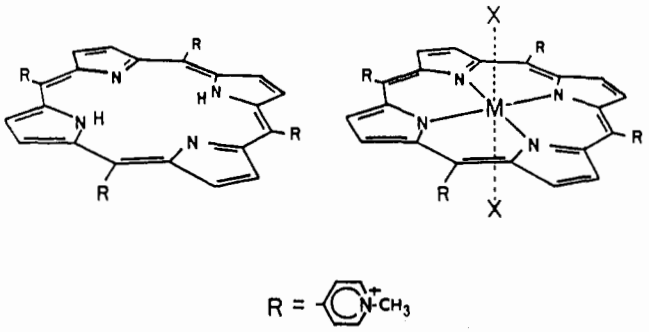

FIGURE 1: Porphyrins used in this study. The axial substituent, X, may be present or absent depending on the metal, M.

charge interactions between the porphyrins and the polymers are not yet fully understood.

Because of their strong binding to DNA, MTMpyP(4) have been used in footprinting experiments to probe DNA structure (Bromley et al., 1986; Ward et al., 1986a,b). Upon binding, TMpyP(4), CuTMpyP(4), and TMpyP(2) have been shown to convert Z-form to B-form DNA (McKinnie et al., 1988; Pasternack et al., 1986b). Other porphyrins have been used in conjunction with a variety of techniques to detect and destroy DNA and neoplastic cells (Fiel et al., 1981, 1982; Foster et al., 1985; Robinson et al., 1986; Van den Bergh, 1986; Vaum et al., 1982).

Previous reports have indicated difficulties in obtaining apparent equilibrium constants (K_{obs}) spectroscopically for TMpyP(4) and MTMpyP(4) due either to variation of bound extinction coefficient (ϵ_{bound}) values with [poly[d(A-T)$_2$]] or to the minimal change in the nonintercalating MTMpyP(4) spectrum upon the addition of poly[d(G-C)$_2$] (Kelly et al. 1985; Pasternack et al., 1983a). We have circumvented these problems with equilibrium dialysis experiments in which bound and free porphyrin concentrations can be measured directly, allowing us to report the equilibrium constants for TMpyP(4) and ZnTMpyP(4) binding both to poly[d(G-C)$_2$] and to poly[d(A-T)$_2$]. Equilibrium studies are also reported as a function of [Na^+]. These studies allow us to assess both GC vs AT selectivity and the importance of charge interactions in influencing the binding.

Difficulties have been noted in determining dissociation rate constants by stopped-flow kinetics due to either the rapidity of the reaction or the small absorbance changes observed during the reaction (Pasternack et al., 1983b). To help eliminate these problems, we have performed stopped-flow kinetics using a sensitive dual-wavelength system. Increased absorption changes are facilitated by the use of SDS. The SDS micelles solubilize the porphyrin, a process which in some cases increases the extinction coefficient of the porphyrin molecule or, in other cases, causes a shift in the absorbance maximum of the porphyrin. Dissociation kinetics are also reported as a function of [Na^+]. These studies provide additional means for assessing binding modes but, more significantly, they address the intriguing question of the mechanism of binding of these useful DNA probes.

Experimental Procedures

Materials

Porphyrins. Chloride salts of the following tetracationic porphyrins were obtained from Mid-Century Chemical Co. and used without further purification: ZnTMpyP(4), NiTMpyP(4), and TMpyP(4). Porphyrin concentrations were determined by using the following extinction coefficients: TMpyP(4), $\epsilon_{424} = 2.26 \times 10^5$ M^{-1} cm^{-1}; NiTMpyP(4), $\epsilon_{418} = 1.5 \times 10^5$ M^{-1} cm^{-1}; and ZnTMpyP(4), $\epsilon_{436} = 2.04 \times 10^5$ M^{-1} cm^{-1} (Pasternack et al., 1972).

Deoxyribonucleic Acids. Poly[d(G-C)$_2$] and poly[d(A-T)$_2$] were purchased from P-L Biochemicals. These were prepared and stored in PIPES buffer [0.01 M PIPES (Sigma), 10^{-3} M EDTA, doubly deionized water] containing either 0.10 M $NaNO_3$ (PIPES 10) or no $NaNO_3$ (PIPES 00). The buffer solution was adjusted to pH 7.0 with NaOH and passed through a 0.22-μm millipore filter prior to use. DNA concentrations were determined in PIPES 10 buffer.

Methods

Equilibrium Dialysis Measurements. TMpyP(4) and its MTMpyP(4) derivatives adhere quite strongly to many surfaces. After examining many different materials, we found the least amount of porphyrin adsorption/absorption occurred when using containers made of Plexiglas. Therefore, binding isotherms were determined by using a locally constructed equilibrium dialysis apparatus consisting of three Plexiglas segments that could be easily bolted together to create a tight seal with the aid of O rings. In each segment were drilled eight chambers, each of which was large enough to hold ca. 10 mL of solution. One segment was so designed that its central placement along with dialysis membranes, O rings, and the other two segments on each side created a three-compartment chamber permitting diffusion of porphyrin among all three compartments. Sample solutions were introduced through an orifice at the top of each compartment.

In a typical experiment, each Plexiglas segment was first soaked in a dilute solution of HNO_3 and then rinsed in doubly deionized water and dried. Before use, the dialysis membranes were boiled for ca. 1 h in an EDTA and $NaHCO_3$ solution and then rinsed in doubly deionized water. Upon assembly of the equilibrium dialysis apparatus, ca. 5×10^{-6} M (base pairs) solutions of poly[d(G-C)$_2$] and poly[d(A-T)$_2$] were prepared in the appropriate PIPES buffer. The DNA solutions were then added to the outside compartments of seven of the eight chambers. The remaining chamber was a control. This contol chamber and the center compartments of the seven chambers were filled with buffer solution. Different aliquots of porphyrin from stock solutions were added to all of the central compartments, and all chambers were sealed. The equilibrium dialysis apparatus, placed in the dark to prevent photodegradation of the porphyrin, was then shaken slowly in a bacteriological shaker at 25 ± 1 °C until equilibrium, as monitored spectrophotometrically, was reached in the control chamber, usually in 3 days. Twice as much time was allowed to ensure complete equilibration of all chambers. It is important to note that the wavelengths of absorbance of the free porphyrin solution in the equilibrium dialysis apparatus did not change during the time of the experiments, thus suggesting that the porphyrin did not degrade during the course of the experiments. Cary 219 and Varian DMS 200 spectrophotometers were used for UV–visible spectral measurements. A Perkin-Elmer MPF-44A instrument was used for fluorescence measurements.

Control experiments were designed to determine the net loss of porphyrin species due to adsorption/absorption to the dialysis membrane and to the chamber walls. Porphyrin solutions were placed in a two-chamber equilibrium dialysis apparatus. After 7 days, it was observed that ca. 97% of both ZnTMpyP(4) and TMpyP(4) still remained, but only 89% of the NiTMpyP(4) was in solution. A control experiment was also performed to determine the time needed to produce complete equilibrium of the porphyrin–DNA solution. A porphyrin solution was placed on one side of the membrane

while a solution of DNA was placed on the other. The DNA solution was monitored at the maximum wavelength of the porphyrin. After 6 days, the absorbance of the DNA–porphyrin solutions had reached the final values.

At equilibrium, the center compartment contained free porphyrin, and the outside compartments contained a mixture of free and bound porphyrin. Analyses of the porphyrin concentrations in each compartment were performed by using SDS, which acts as a sequestering agent to remove completely all porphyrin from the DNA–porphyrin complex. The ZnTMpyP(4) concentration was measured either by visible spectroscopy using $\epsilon_{444} = 1.84 \times 10^5$ M^{-1} cm^{-1} as determined by mixing a porphyrin solution of known concentration into an equal volume of a 1.0% SDS solution in the appropriate PIPES buffer or by fluorescence at 630 nm (excitation at 444 nm). A standard plot of fluorescence for TMpyP(4) in SDS at 654 nm (excitation at 424 nm) was used to determine concentrations for the equilibrium dialysis experiments. The equilibrium dialysis experiment was designed to avoid the assumption that ϵ_{bound} is constant throughout the titration, as is necessary in spectrophotometric determinations (Pasternack et al., 1983a). Therefore, we feel that K_{obs} values obtained by the equilibrium dialysis method are more accurate.

Concentrations of the porphyrins obtained by the equilibrium dialysis method were converted to ν, the [bound ligand]/[DNA] (base pairs) ratio, and c, [free ligand] (Wilson & Lopp, 1979). Calculations of the nonlinear least-squares best-fit values of a three-parameter fit for K_{obs}, n (the number of base pairs excluded upon binding), and ω (an equilibrium constant for moving a ligand from an isolated binding site to a binding site next to another ligand) were performed according to the site-exclusion model of McGhee and von Hippel (1974). Equilibrium dialysis experiments were performed at very low ν values in order to determine K_{obs} accurately. Accurate n and ω determination, however, requires the titration to be performed up to a ν value near saturation; therefore, n and ω values obtained by equilibrium dialysis are not reported.

Stopped-Flow Kinetics. Kinetic measurements were made by using an Aminco-Morrow stopped-flow apparatus adapted to a Johnson Foundation MB2 air turbine spectrophotometer as previously described (Wilson et al., 1985). The output of the instrument was fed to an OLIS 3820 data acquisition system, stored on magnetic disk, and analyzed subsequently. Temperatures were monitored by using a YSI42 SC telethermometer accurate to ±0.1 °C. The stopped-flow instrument collected data beginning 5 ms after initiation of mixing. The instrument response time was set at 2 ms.

Typically, five to eight runs, each consisting of 200 data points, were computer averaged to improve the signal-to-noise ratio. The data, collected in a preselected time range, could be fit as one to three exponential curves by using software supplied with the OLIS system and with a program based on the Marquardt–Levenberg algorithm (written and given to us by Professor R. H. Shafer, Department of Pharmaceutical Chemistry, University of California, San Francisco). The programs fit up to seven different constants (Krishnamoorthy et al., 1986).

For evaluation of spectral changes, solutions of porphyrin–DNA complexes at concentrations equivalent to those used in dissociation experiments were prepared (see below). In each case, the solution, ca. 1.0 mL, was added to one side of a quartz spectrophotometer cell that was divided into two compartments of equal volume by a single piece of quartz glass. The other compartment was filled with an identical volume of a 1% SDS solution. A spectrum was recorded from 650 to 350 nm.

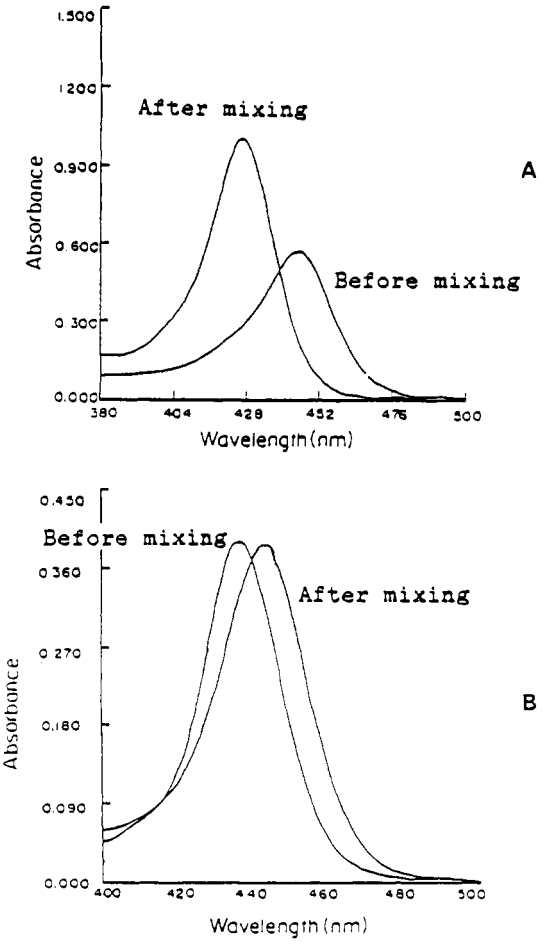

FIGURE 2: (A) Spectrum of TMpyP(4) (5.0×10^{-6} M) in a solution of poly[d(G-C)$_2$] (5.0×10^{-5} M) before and after mixing with SDS. (B) Spectrum of ZnTMpyP(4) (2.5×10^{-6} M) in a solution of poly[d(A-T)$_2$] (2.5×10^{-5} M) before and after mixing with SDS. Spectra taken at room temperature, 0.115 M [Na^+].

Afterward, the cell was inverted several times to allow mixing of the SDS and DNA–porphyrin solutions through an opening located at the top of the dividing glass. Again, a spectrum was recorded. Wavelengths of maximum absorbance before and after mixing were used in the stopped-flow experiments to obtain the maximum absorbance change by using a dual-wavelength difference method (Figure 2A).

Addition of DNA to a TMpyP(4) solution can produce a large degree of hypochromicity and a bathochromic shift in the porphyrin spectrum (Figure 2A). However, when significant spectral changes do not occur, as in the case of DNA addition to a ZnTMpyP(4) solution, mixing of the porphyrin–DNA complex with SDS produces changes in the spectrum due to the formation of a porphyrin–SDS micelle. These induced spectral changes allow the detection of the porphyrin–DNA dissociation process by stopped-flow kinetics (Figure 2B).

Dissociation kinetic measurements were conducted by mixing equal volumes (100 μL) of porphyrin–DNA complex and 1.0% SDS solutions at the desired temperature and [Na^+]. The porphyrin–DNA solution typically consisted of 1.0×10^{-5} M (base pairs) of DNA and 1.0×10^{-6} M porphyrin, in the appropriate PIPES buffer, unless stated otherwise.

RESULTS

Equilibrium Dialysis. The results of equilibrium dialysis experiments with TMpyP(4) and ZnTMpyP(4) as a function of [Na^+] are given in Table I. However, similar studies with

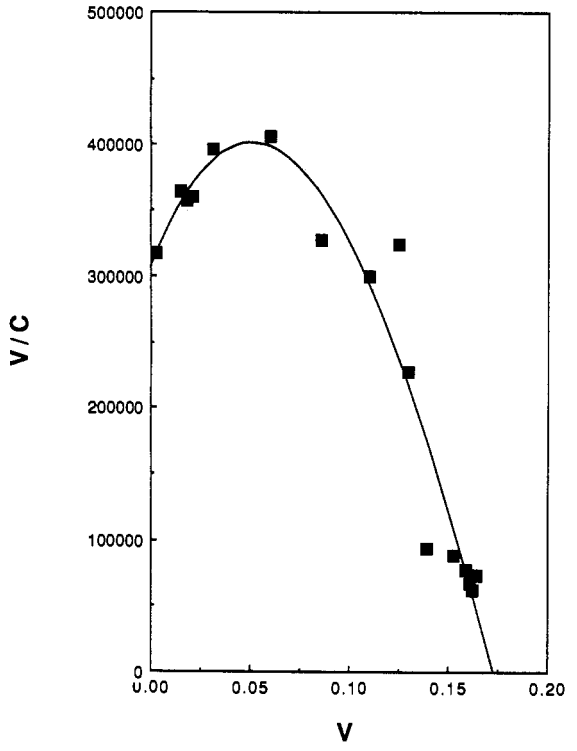

FIGURE 3: Scatchard plot for ZnTMpyP(4) binding to poly[d(A-T)$_2$] in 0.315 M Na^+ at 25 °C. Points in the figure are experimental, and the solid curve represents the nonlinear least-squares best-fit values using the equation of McGhee and von Hippel (1974).

Table I: Dependence of Binding Constants K_{obs} (M^{-1}, 25 °C) on [Na^+] Obtained by Equilibrium Dialysis[a]

[Na^+] (M)	TMpyP(4) poly[d(G-C)$_2$]	TMpyP(4) poly[d(A-T)$_2$]	ZnTMpyP(4)[b] poly[d(A-T)$_2$]
0.115	4.3×10^6	4.0×10^6	2.0×10^6
0.215	7.5×10^5	1.1×10^6	5.0×10^5
0.315	2.3×10^5	3.5×10^5	3.2×10^5
0.365	*c*	*c*	2.0×10^5
0.515	6.7×10^4	8.0×10^4	*d*

[a] Typical [DNA] = 5×10^{-6} M^{-1} (base pairs). Equilibrium constants were reproducible to ±8%. [b] Binding to poly[d(G-C)$_2$] was too weak to measure; however, in 0.065 M [Na^+], a binding constant of 3.0×10^4 M^{-1} was determined. [c] Binding not measured at this [Na^+]. [d] Binding too weak to measure.

NiTMpyP(4) were not successful, probably due to excessive absorption of the porphyrin to the dialysis membrane and compartment walls. A Scatchard plot for ZnTMpyP(4) binding to poly[d(A-T)$_2$] in 0.315M [Na^+] is shown in Figure 3.

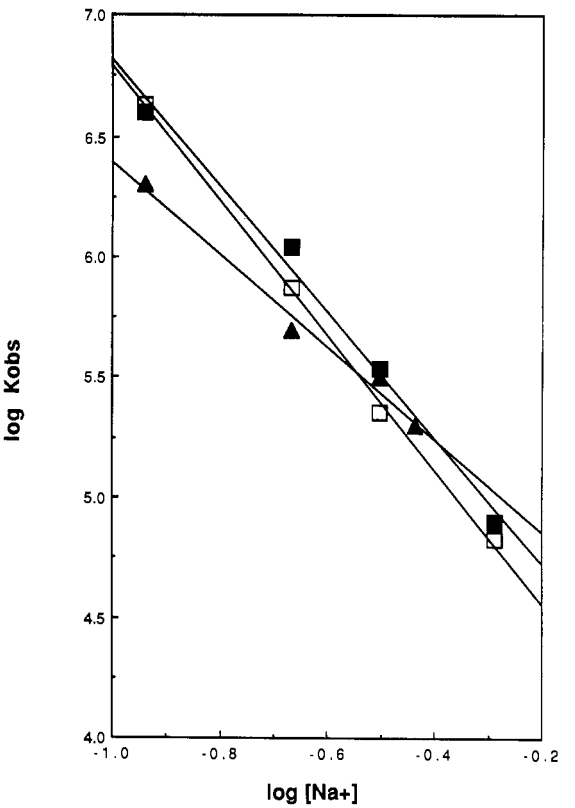

FIGURE 4: Plot of log K_{obs} vs log [Na^+] for TMpyP(4) binding to poly[d(G-C)$_2$] (□) and to poly[d(A-T)$_2$] (■) and ZnTMpyP(4) binding to poly[d(A-T)$_2$] (▲) at 25 °C. The lines are linear regression fits of the data.

The K_{obs} values for TMpyP(4) binding to poly[d(G-C)$_2$] and to poly[d(A-T)$_2$] decreased with increasing [Na^+]. Plots of log K_{obs} vs log [Na^+] (Record et al., 1976) were linear with a slope of ca. -2.7 ± 0.1 for both polymers (Figure 4).

K_{obs} values for ZnTMpyP(4) binding to poly[d(A-T)$_2$] were also dependent on [Na^+] (Table I), and a plot of log K_{obs} vs log [Na^+] gave a slope of -2.0 ± 0.1. In comparison, a slope of −2.7 was observed for TMpyP(4) binding to both polymers. ZnTMpyP(4) binding to poly[d(G-C)$_2$] was relatively weak (K_{obs} ~30 000 M^{-1} at 0.065 M [Na^+]), and, therefore, the dependence of K_{obs} on [Na^+] could not be determined.

Stopped-Flow Kinetics. In Table II, and in other tables containing rate studies, we present the following information:

Table II: [Na^+] Dependence of Rate of Dissociation of TMpyP(4) from Poly[d(G-C)$_2$][a]

[Na^+] (M)	$t_{0.5A}$ (s)	k_D (s^{-1})	SSR1[b]	k_{D1} (s^{-1})	% Amp-1	k_{D2} (s^{-1})	% Amp-2	SSR2[b]
				T = 15 °C				
0.065	9.8	0.05	0.3	0.24	17	0.04	83	0.1
0.115	8.8	0.07	0.2	0.28	24	0.05	76	0.1
0.215	6.4	0.10	2.3	0.43	26	0.06	74	0.1
0.315	5.8	0.09	0.5	0.49	32	0.07	68	0.1
				T = 20 °C				
0.115	7.8	0.08	14.0	0.48	13	0.08	87	3.6
0.215	4.1	0.12	6.2	0.72	9	0.11	91	1.6
0.315	3.9	0.15	9.7	0.80	10	0.14	90	3.0
0.515	1.8	0.22	9.9	1.70	11	0.17	89	2.7
				T = 25 °C				
0.065	5.1	0.12	0.5	0.25	37	0.10	63	0.1
0.115	2.6	0.21	2.5	0.79	24	0.18	76	0.2
0.215	2.4	0.28	3.4	0.98	28	0.22	72	0.2
0.315	2.0	0.31	1.6	1.26	25	0.24	75	0.3
0.515	1.7	0.36	1.1	1.32	22	0.31	78	0.2

[a] [TMpyP(4)] = 1.0×10^{-6} M; [poly[d(G-C)$_2$]] = 1.0×10^{-5} M. [b] $\times 10^3$.

Table III: [Na^+] Dependence of Rate of Dissociation of TMpyP(4) from Poly[d(A-T)$_2$] at 15 °C[a]

[Na^+] (M)	$t_{0.5A}$ (s)	k_D (s^{-1})	SSR1[b]	k_{D1} (s^{-1})	% Amp-1	k_{D2} (s^{-1})	% Amp-2	SSR2[b]
0.065	0.21	3.1	5.3	6.8	64	1.3	36	0.9
0.115	0.03	22.0	6.5	27.0	85	5.7	15	0.8
0.165	0.02	41.0	4.1	46.0	96	7.5	4	0.8
0.215	0.01	62.0	1.4	88.0	65	43.0	35	0.9

[a] [Poly[d(A-T)$_2$]] = 5.0×10^{-5} M (base pairs); [TMpyP(4)] = 5.0×10^{-6} M. [b] $\times 10^3$.

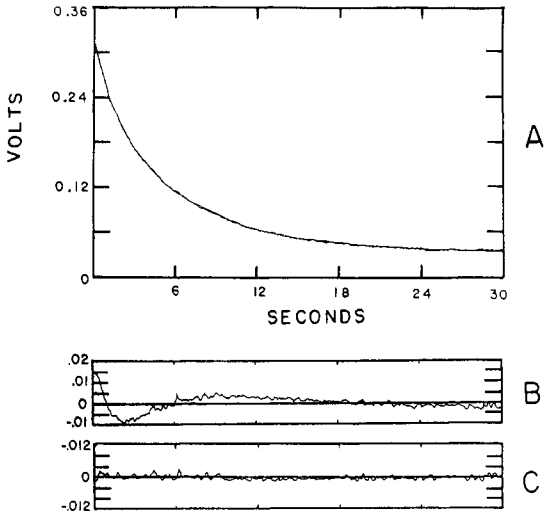

FIGURE 5: SDS-driven dissociation reaction of TMpyP(4)-poly[d-(G-C)$_2$] monitored at 421 and 450 nm. The concentrations after mixing were 1.0×10^{-5} M for DNA (base pairs) and 1.0×10^{-6} M for the porphyrin in 0.115 M [Na^+] at 25 °C. (A) The 200 data points collected. The smooth line represents the dual-exponential best fit. (B) Residuals for a single exponential fit. (C) Residuals for a dual-exponential fit.

the time required for 50% of the absorption change to occur, $t_{0.5A}$; the observed rate constant, k_D, and the sum of the squares of the residuals, SSR1, obtained by treating the data as one first-order process; the observed rate constants k_{D1} and k_{D2} for the fast and slow parallel first-order processes, respectively, and the sum of the squares of the residuals, SSR2, for a dual-exponential fit; and the percentage of the absorbance change attributed to the two processes, %Amp-1 and %Amp-2.

A typical dissociation kinetic trace with the plot of the residuals for a single- and double-exponential fit is shown in Figure 5. All dissociation kinetics studied were best fit as a dual-exponential process. Fitting to three exponentials did not improve the fit of the data. Rate constants reported typically had standard errors of ±17% and ±6% for the fast and slow rate constant, respectively. The reported $t_{0.5A}$ values, determined independently of data fit programs, were typically reproducible to ±5%.

All dissociation kinetic results reported in Tables II–V are from experiments conducted with a ratio of [DNA] to [porphyrin] of 10. Control experiments were performed in 0.115 M [Na^+]. The effect of increasing the ratio to 100 by increasing the [poly[d(G-C)$_2$]] by a factor of 10 or maintaining a constant ratio but increasing both the [porphyrin] and [DNA] by a factor of 10 had no detectable effect on the dissociation rate constants of the porphyrins from poly[d(G-C)$_2$]. However, a slight decrease was observed in the dissociation rate constants when the [poly[d(A-T)$_2$]] was increased by a factor of 10, although the observed decrease was within experimental error. Maintaining a constant ratio but increasing both the [porphyrin] and poly[d(A-T)$_2$] by a factor of 10 had no detectable effect on the dissociation rate constants. Variation in the amounts of SDS between 0.5 and 4.0% in 0.215 M [Na^+] produced no change in the rate constants

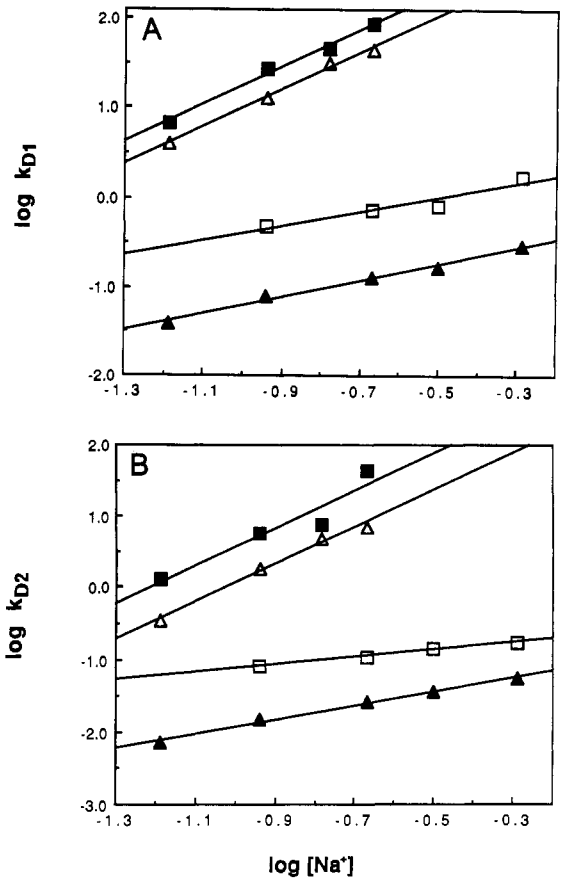

FIGURE 6: (A) Plots of the observed fast dissociation rate constants (k_{D1}) vs log [Na^+] for poly[d(G-C)$_2$] with NiTMpyP(4) (▲) and TMpyP(4) (□) at 20 °C. Plots of the k_{D1} vs log [Na^+] for poly[d-(A-T)$_2$] with NiTMpyP(4) (△) and TMpyP(4) (■) at 15 °C. (B) Plots of the observed slow dissociation rate constants (k_{D2}) vs log [Na^+] for poly[d(G-C)$_2$] with NiTMpyP(4) (▲) and TMpyP(4) (□) at 20 °C. Plots of the k_{D2} vs log [Na^+] for poly[d(A-T)$_2$] with NiTMpyP(4) (△) and TMpyP(4) (■) at 15 °C.

within experimental error. Pasternack et al. (1983b) have observed no detectable effect in dissociation rate constants of TMpyP(4) and NiTMpyP(4) from poly[d(G-C)$_2$] when varying the SDS concentration over a 100-fold range.

Rate constants for TMpyP(4) dissociation from poly[d(G-C)$_2$] and from poly[d(A-T)$_2$] (Tables II and III) were measured at several [Na^+], and the results are plotted in Figure 6 as log k_D vs log [Na^+] as suggested by Lohman et al. (1978, 1985). The plots are linear for both poly[d(G-C)$_2$] and poly[d(A-T)$_2$]. Slopes of the lines for TMpyP(4) dissociation from poly[d(G-C)$_2$] at 20 °C were 0.80 ± 0.14 and 0.51 ± 0.01 for k_{D1} and k_{D2}, respectively. The overall amplitude change for the k_{D1} process was approximately 20% of the total at each [Na^+]. Rate studies for dissociation of TMpyP(4) from poly[d(A-T)$_2$] were conducted at 15 °C so that the rates could be measured. Slopes of the lines were 2.1 ± 0.1 and 2.6 ± 0.5 for k_{D1} and k_{D2}, respectively. The corresponding amplitudes were scattered and had no distinguishable trend.

Rates of dissociation of NiTMpyP(4) from poly[d(G-C)$_2$] and poly[d(A-T)$_2$] (Tables IV and V) were measured and

Table IV: [Na^+] Dependence of Rate of Dissociation of NiTMpyP(4) from Poly[d(G-C)$_2$][a]

[Na^+] (M)	$t_{0.5A}$ (s)	k_D (s^{-1})	SSR1[b]	k_{D1} (s^{-1})	% Amp-1	k_{D2} (s^{-1})	% Amp-2	SSR2[b]
				T = 15 °C				
0.065	82	0.007	0.8	0.04	28	0.005	72	0.3
0.115	26	0.018	1.6	0.06	32	0.010	68	0.1
0.215	23	0.026	0.5	0.07	30	0.016	70	0.1
0.315	14	0.039	0.7	0.16	25	0.026	75	0.1
0.515	11	0.039	0.7	0.19	29	0.029	71	0.2
				T = 20 °C				
0.065	61	0.008	2.7	0.04	30	0.007	70	1.0
0.115	33	0.017	1.9	0.08	25	0.015	75	0.3
0.215	18	0.032	1.7	0.13	25	0.027	75	0.2
0.315	17	0.047	2.2	0.17	39	0.037	71	0.3
0.515	7	0.074	3.2	0.29	42	0.055	68	0.2

[a] [DNA] = 1 × 10^{-5} M (base pairs); [NiTMpyP(4)] = 1 × 10^{-6} M. [b] ×10^3.

Table V: [Na^+] Dependence of Rate of Dissociation of NiTMpyP(4) and ZnTMpyP(4) from Poly[d(A-T)$_2$] at 15 °C

[Na^+] (M)	$t_{0.5A}$ (s)	k_D (s^{-1})	SSR1[a]	k_{D1} (s^{-1})	% Amp-1	k_{D2} (s^{-1})	% Amp-2	SSR2[a]
				[NiTMpyP(4)] = 1.0 × 10^{-6} M[b]				
0.065	0.57	0.6	2.5	3.9	45	0.3	55	0.6
0.115	0.16	2.9	1.9	12.8	49	1.8	51	0.9
0.165	0.05	7.8	2.2	31.8	50	4.8	50	1.5
0.215	0.04	12.0	2.7	44.2	20	7.1	80	1.5
				[ZnTMpyP(4)] = 5.0 × 10^{-6} M[c]				
0.065	0.83	1.0	34.8	1.7	75	0.4	25	9.8
0.115	0.06	11.9	9.6	15.0	88	1.4	12	2.5

[a] ×10^3. [b] [DNA] = 1 × 10^{-5} M (base pairs). [c] [DNA] = 5 × 10^{-5} M (base pairs).

plotted as above for TMpyP(4) (Figure 6). The slopes of the lines for NiTMpyP(4) dissociation from poly[d(G-C$_2$] were 0.94 ± 0.03 and 0.98 ± 0.02 for k_{D1} and k_{D2}, respectively. The k_{D1} process comprised approximately 30% of the total amplitude change. For dissociation of NiTMpyP(4) from poly[d(A-T)$_2$], the slopes of the lines for k_{D1} and k_{D2} were 2.1 ± 0.1 and 2.6 ± 0.1, respectively, at 15 °C, with both processes contributing about 50% of the total amplitude change.

The dissociation rates of ZnTMpyP(4) from both poly[d(G-C)$_2$] and poly[d(A-T)$_2$] were examined. However, the rates of dissociation from poly[d(G-C)$_2$] were too fast to measure. Dissociation rates from poly[d(A-T)$_2$] (Table V) exhibited a large increase as [Na^+] was increased. At 0.215 M [Na^+], the rate was too fast to measure with our instrument.

The general effect of [Na^+] on dissociation rates is the same in all cases; i.e., increasing [Na^+] increases the rate of porphyrin dissociation. Both TMpyP(4) and NiTMpyP(4) had relatively similar overall [Na^+] dependence for both rate processes, k_{D1} and k_{D2}, with poly[d(A-T)$_2$] and for the faster process, k_{D1}, with poly[d(G-C)$_2$]. However, the slope (0.51) for the k_{D2} plot with TMpyP(4) and poly[d(G-C)$_2$] was approximately half that (0.98) of NiTMpyP(4) under identical conditions. In all cases, except TMpyP(4) dissociating from poly[d(A-T)$_2$], the slower rate process had the larger amplitude change. For both NiTMpyP(4) and TMpyP(4), experiments performed at other temperatures did not produce significant changes in the [Na^+] dependence of the dissociation rates. However, the rates did increase with increasing temperature.

Discussion

AT vs GC Specificity. (a) Binding Studies. Binding studies have been employed previously to help understand the interactions of TMpyP(4) and MTMpyP(4) with DNA. Initially, Fiel et al. (1979) conducted induced CD studies and observed that TMpyP(4) exhibited two binding modes with CT DNA at moderate ionic strength. By Scatchard analysis of spectrophotometric data (0.196 M [Na^+], 25 °C), the stronger of the two interactions was determined to have a K_{obs} value of ca. 1.1 × 10^7 M^{-1}. A K_{obs} value for the weaker interaction was not reported. They proposed that the stronger and weaker interactions involved intercalation and external association, respectively.

Later, studies were performed with poly[d(G-C)$_2$] and poly[d(A-T)$_2$] with the aim of better characterizing binding to CT DNA. The binding of TMpyP(4) to poly[d(G-C)$_2$] in 0.2 M [Na^+] was assigned K_{obs} values of ca. 7.7 × 10^5 M^{-1} (25 °C) by visible spectroscopy (Pasternack et al., 1983a) and ca. 1–2 × 10^6 M^{-1} by fluorescence measurements at room temperature (Kelly et al., 1985), respectively, using the analysis of McGhee and von Hippel (1974). Likewise, K_{obs} values of ca. 1–2 × 10^6 M^{-1} were reported for TMpyP(4) binding to poly[d(A-T)$_2$] as determined by fluorescence techniques (Kelly et al., 1985) and by monitoring the maximum relative amplitude in temperature-jump experiments of TMpyP(4) binding to poly[d(A-T)$_2$] and to poly[d(G-C)$_2$] (Pasternack et al., 1983b). Pasternack et al. (1983a) have indicated that binding of TMpyP(4) to poly[d(G-C)$_2$] exhibits little or no cooperativity, while binding to poly[d(A-T)$_2$] exhibits positive cooperativity.

Equilibrium dialysis experiments were performed on TMpyP(4) binding to poly[d(G-C)$_2$] and to poly[d(A-T)$_2$] in several different [Na^+] and at 25 °C (Table I). In comparison, K_{obs} values obtained by equilibrium dialysis for TMpyP(4) to poly[d(G-C)$_2$] and to poly[d(A-T)$_2$] were approximately the same as those reported by Pasternack et al. (1983a) and Kelly et al. (1985). However, K_{obs} values reported by Pasternack et al. (1986a) were ca. 5 times lower than those reported in Table I. Our equilibrium dialysis data indicate that K_{obs} decreased with increases in [Na^+]. Pasternack et al. (1986a) have observed a similar trend in TMpyP(4) binding to poly[d(G-C)$_2$].

Pasternack et al. (1986a) stated that there are regions of GC base pairs and AT base pairs in CT DNA that maintain the properties seen in their respective alternating homopolymers. From CD results obtained with CT DNA, they concluded that the base specificity of TMpyP(4) in a mixed

solution of poly[d(G-C)$_2$] and poly[d(A-T)$_2$] will depend on the ionic strength of the solution, with GC binding preferred at low ionic strength and AT binding preferred at high ionic strength. However, our equilibrium dialysis data (Table I) indicate that there is *no significant preference* when TMpyP(4) binds to either poly[d(G-C)$_2$] or poly[d(A-T)$_2$] at [Na^+] between 0.115 and 0.515 M. The observed CD spectral changes could, therefore, be a consequence of binding at sites not represented well by the homopolymers. Alternatively, our K_{obs} values were calculated from data obtained at low ν values. The effects of cooperativity could change the relative binding to GC and AT regions, and such cooperativity could explain the differences between our results and the conclusions reached by Pasternack et al. (1986a) using CD spectroscopy.

Although absorbance changes for ZnTMpyP(4) binding to the alternating homopolymers were too small to allow accurate determination of K_{obs} by the spectrophotometric method (Pasternack et al., 1983a), Kelly et al. (1985) used fluorescence techniques to determine that K_{obs} for ZnTMpyP(4) binding to poly[d(A-T)$_2$] was ca. 1–2 × 10^6 M^{-1} in 0.20 M [Na^+] at room temperature. They concluded that binding of ZnTMpyP(4) to poly[d(G-C)$_2$] was extremely weak, as denoted by the minimal perturbation of the ZnTMpyP(4) fluorescence. CD and footprinting data also suggest that ZnTMpyP(4) exhibits selectivity for AT sites over GC sites (Pasternack et al., 1983a; Ward et al., 1986a).

The K_{obs} for ZnTMpyP(4) binding to poly[d(A-T)$_2$] obtained by equilibrium dialysis in 0.215 M [Na^+] was ca. 5 × 10^5 M^{-1} at 25 °C (Table I) compared with 1–2 × 10^6 M^{-1} at room temperature reported by Kelly et al. (1985). Binding of ZnTMpyP(4) to poly[d(G-C)$_2$] was too weak to measure at 0.115 M [Na^+]. However, at 25 °C and in 0.065 M [Na^+], a low value of K_{obs} = 3.0 × 10^4 M^{-1} was obtained. Extrapolation of the line obtained from plots of log K_{obs} vs log [Na^+] for ZnTMpyP(4) binding to poly[d(A-T)$_2$] (Figure 4) gives a K_{obs} value of ca. 6.0 × 10^6 M^{-1} at 0.065 M [Na^+]. Therefore, we can estimate that the binding of ZnTMpyP(4) to poly[d-(A-T)$_2$] is ca. 200 times more favorable than to poly[d(G-C)$_2$] at 0.065 M [Na^+].

(*b*) *Kinetic Studies.* Kinetic data on TMpyP(4), NiTMpyP(4), and ZnTMpyP(4) dissociation from poly[d(A-T)$_2$] and from poly[d(G-C)$_2$] as a function of [Na^+] are shown in Tables II–V. For the purpose of comparison, the rate data will be discussed in terms of $t_{0.5A}$, the time required for half the measured absorption change.

If the $t_{0.5A}$ data are compared in 0.115 M [Na^+] at 15–20 °C, we find that, for both poly[d(A-T)$_2$] and poly[d(G-C)$_2$], the dissociation process is 5 times faster for TMpyP(4) than for NiTMpyP(4). This finding agrees with relaxation studies on CT DNA that showed that TMpyP(4) dissociation was faster than NiTMpyP(4) dissociation (Pasternack et al., 1983b). In contrast, whereas ZnTMpyP(4) dissociation rate from poly[d(A-T)$_2$] is similar to that for TMpyP(4) at low ionic strength, the rate of dissociation of the zinc species from poly[d(G-C)$_2$] was much faster and could not be measured with our instrument. These findings suggest that the metal can influence the binding to the outside of the DNA as well as to intercalation sites.

Multiple exponentials are commonly needed to fit DNA dissociation kinetics (Bittman & Blau, 1975; Chen, 1988; Muller & Crothers, 1968), and this requirement has been found previously for porphyrin dissociation (Gibbs et al., 1988; Pasternack et al., 1983b). However, the conclusions we reach in this paper are the same whether we use qualitative $t_{0.5A}$ values or calculated k_{D1} and k_{D2}.

Pasternack et al. (1983b) found that the rate of the dissociation of TMpyP(4) from poly[d(G-C)$_2$] was dependent on the final ionic strength, with the dissociation rate being about twice as fast at 1 M than at 2 M ionic strength. Gibbs et al. (1988) have observed similar results with AuTMpyP(4). To explain their data, Pasternack et al. (1983b) suggested that increased ionic strength makes the dissociation reaction thermodynamically more favorable but kinetically less favorable. They proposed that increased ionic strength produces a very rapid contraction of the DNA due to the partial neutralization of the negative charges of the phosphate backbone by the ion densities in the solvent, thus producing a steric resistance to the intercalated species sliding out from between the base pairs. Our data were collected below 0.52 M [Na^+] and, obviously, the explanation of Pasternack et al. (1983b) does not apply under our conditions. However, we observed that in all cases the rate constants for NiTMpyP(4) or TMpyP(4) dissociation from poly[d(G-C)$_2$] increased with increasing [Na^+]. Increases in dissociation rate constants as a function of [Na^+] have also been found with well-known intercalators such as ethidium, anthraquinone anticancer drugs, and other heterocyclic nitrogen-containing compounds (Fairley et al., 1988; Krishnamoorthy et al., 1986; Wilson et al., 1985) as well as DNA binding proteins (Lohman et al., 1978, 1985).

Quantitative Analysis of [Na^+] Effects. (*a*) *Binding Constants.* Manning (1969, 1972) proposed that simple monovalent cations like Na^+ bind to a polyanion like DNA in two ways: as directly condensed counterions and as screened mobile counterions held by a Debye–Hückel-type interaction. The thermodynamic extent of counterion interaction, Ψ, expressed as a fraction of the counterion associated per polyanionic charge, has been determined to have a value of 0.88 in B-form DNA (Anderson et al., 1978; Lohman, 1985; Manning, 1969, 1972; Record et al., 1976).

The interaction of a charged ligand (L) at a specific site on DNA (P) involves the formation of m' ion pairs with the anionic phosphate groups. This model leads to a molecular binding reaction

$$L + P(m' \text{ site}) \overset{K}{\leftrightarrow} LP + m'\Psi_c[Na^+] \qquad (1)$$

where K is the thermodynamic equilibrium constant and Ψ_c is the counterion fraction bound to DNA due to condensation of [Na^+], which has a value of 0.76 (Anderson et al., 1978; Manning, 1969, 1972). It follows that the dependence on [Na^+] of the apparent equilibrium constant, K_{obs}, is predicted by eq 2 if no extensive conformational changes occur upon L

$$\partial \log K_{obs}/\partial \log [Na^+] = -m'\Psi \qquad (2)$$

binding. Since Ψ is known, determination of the effects of [Na^+] on K_{obs} leads to the calculation of m' (Record et al., 1976).

We find that $m'\Psi$ values for TMpyP(4) binding to poly-[d(G-C)$_2$] and to poly[d(A–T)$_2$] are the same, ca. −2.7. Pasternack et al. (1986a) have reported a $m'\Psi$ value of −1.6 for TMpyP(4) binding to poly[d(G-C)$_2$]. Our data indicate that ZnTMpyP(4) binding to poly[d(A-T)$_2$] gives a value of −2.0 (Figure 4). Application of eq 2 gives m' values of ca. 3.1 for TMpyP(4) binding to poly[d(G-C)$_2$] and to poly[d-(A-T)$_2$] and of ca. 2.3 for ZnTMpyP(4) binding to poly[d-(A-T)$_2$].

Our equilibrium dialysis data suggest that TMpyP(4) and ZnTMpyP(4) binding to poly[d(A-T)$_2$] is a highly positive cooperative process as denoted by the large positive curvature in the Scatchard plot (Figure 3). Positive cooperativity of TMpyP(4) binding to poly[d(A-T)$_2$] has also been suggested

by others (Pasternack et al., 1983a). In order to interpret m' values quantitatively, knowledge of the exact conformational changes that occur is needed. However, general trends are noted; i.e., TMpyP(4) and ZnTMpyP(4) bind to poly[d(A-T)$_2$] in a similar manner.

Ward et al. (1986a) have determined by footprinting analysis that ZnTMpyP(4) exhibits a specificity for AT regions in DNA with binding occurring possibly along the minor groove. Geacintov et al. (1987) have interpreted linear dichroism results to suggest that ZnTMpyP(4) produces bends or kinks in the DNA structure upon binding. Viscosity studies involving superhelical closed circular DNA established that significant changes in the DNA structure occurred upon ZnTMpyP(4) binding (Strickland et al., 1987). Our equilibrium studies demonstrated appreciable cooperativity in ZnTMpyP(4) binding, possibly due to conformational changes in the DNA structure. Therefore, in light of our data and previous studies, a consensus picture of ZnTMpyP(4) binding to DNA emerges; i.e., ZnTMpyP(4) interacts very weakly with GC regions and binds possibly along the minor groove of AT regions, probably as a two- or three-charge interaction producing cooperative conformational changes in the DNA structure.

(*b*) *Rate Constants and Mechanisms.* The overall effect of [Na^+] on reaction rates has been described by Lohman et al. (1978, 1985) in terms of two different association mechanisms. The mechanism of dissociation is the microscopic reverse of that for association.

One mechanism, which is screening controlled, is the primary mechanism in outside electrostatic binding. In this model I mechanism, a positively charged ligand dissociates from polyanionic DNA in a single step without intermediates. In this case, the effect of [Na^+] is assumed to result from screening effects due to the atmosphere of ions that surrounds the reactants, thus reducing coulombic attractions. For model I, k_D is expected to depend strongly on [Na^+] as described by

$$\partial \log k_D / \partial \log [Na^+] = m'\Psi_c \quad (3)$$

A second mechanism, model II, involves the slow reaction of an initial product with m' ion pairs to form an intermediate with n ion pairs. The intermediate then dissociates rapidly to the free ligand and DNA. The [Na^+] dependence of k_D for this mechanism is expressed by

$$\partial \log k_D / \partial \log [Na^+] = (m' - n)\Psi \quad (4)$$

Wilson et al. (1985) have suggested that, for simple intercalators, $n = fm'$, where f is ca. 0.76. Substitution into eq 4 gives

$$\partial \log k_D / \partial \log [Na^+] = (m' - fm')\Psi \quad (5)$$

For dissociation of TMpyP(4) from poly[d(A-T)$_2$] (Table III), plots of log k_D vs log [Na^+] give an average slope of 2.35 for k_{D1} and k_{D2}. Model I, eq 3, and model II, eq 5, predict slopes of 2.05 and 0.57, respectively. Since model I predicts a slope close to that obtained experimentally, TMpyP(4) appears to dissociate from poly[d(A-T)$_2$] by the first mechanism. Lohman (1985) has stated that association data obtained with bisintercalators by Capelle et al. (1979) indicate a screening-controlled mechanism. Therefore, TMpyP(4) is only the second example of a small molecule shown to bind to DNA by this mechanism.

For the dissociation reactions of TMpyP(4) from poly[d-(G-C)$_2$], plots of log k_D vs log [Na^+] give an average slope of 0.66 for k_{D1} and k_{D2}. Again, model I and model II predict slopes of 2.05 and 0.57, respectively. Therefore, the results are most consistent with the second mechanism.

Summary. Our analysis of salt effects on both equilibrium and rate processes with TMpyP(4) is totally consistent with the view that binding at GC sites involves intercalation, whereas binding at AT sites involves outside binding. However, since the salt dependences of the equilibrium binding to both sites are similar, the typically found preference for intercalation (i.e., GC binding) at low salt probably does not occur. In the more complex case of native DNAs, we (Banville et al., 1986; Strickland et al., 1987) and others (Gibbs et al., 1988) have suggested that other types of binding, including intercalation at mixed GC/AT sites, may be occurring. These other sites could exhibit different salt dependences and thus require additional study. However, the CD spectral dependence on [Na^+] (Pasternack et al., 1986a) could be due to binding at these other sites.

One of the most unusual findings in this study is the 200-fold difference in K_{obs} for ZnTMpyP(4) binding to poly[d(A-T)$_2$] and to poly[d(G-C)$_2$]. Preference for AT binding may be the result of a conformational distortion that permits a better charge interaction of ZnTMpyP(4) with AT but not with GC polymers. However, NiTMpyP(4) and TMpyP(4) dissociation rates exhibit a similar dependence on [Na^+], but the rate constants are about 5 times slower for the Ni compound. Thus, the metal may have other effects in addition to the modulation of charge interactions.

Finally, this study is the first to show that the process of TMpyP(4) dissociation from AT and from GC sites follows *different* mechanisms. The TMpyP(4) dissociation from poly[d(AT)$_2$] does not have an intermediate state and, to our knowledge, this is only the second example found of a small molecule with a salt dependence of this type since Lohman et al. (1978) proposed the theory.

ACKNOWLEDGMENTS

We thank Dr. J. C. Smith for his help with the stopped-flow instrument.

Registry No. TMpyP(4), 38673-65-3; NiTMpyP(4), 48242-71-3; ZnTMpyP(4), 40603-58-5; poly[d(A-T)], 26966-61-0; poly[d(G-C)], 36786-90-0.

REFERENCES

Anderson, C. F., Record, M. T., & Hart, P. A., (1978) *Biophys. Chem. 7*, 301–316.

Banville, D. L., Marzilli, L. G., & Wilson, W. D. (1983) *Biochem. Biophys. Res. Commun. 113*, 148–154.

Banville, D. L., Marzilli, L. G., Strickland, J. A., & Wilson, W. D. (1986) *Biopolymers 25*, 1837–1858.

Bittman, R., & Blau, L. (1975) *Biochemistry 14*, 2138–2145.

Blom, N., Odo, J., Nakamoto, K., & Strommen, D. P. (1986) *J. Phys. Chem. 90*, 2847–2852.

Bromley, S. D., Ward, B. W., & Dabrowiak, J. C. (1986) *Nucleic Acids Res. 14*, 9133–9139.

Capelle, N., Barbet, J., Desen, P., Blanquet, S., Roques, B. P., & Le Pecq, J. B. (1979) *Biochemistry 18*, 3354–3362.

Carvlin, M. J., & Fiel, R. J. (1983) *Nucleic Acids Res. 11*, 6121–6139.

Carvlin, M. J., Datta Gupta, N., & Fiel, R. J. (1982) *Biochem. Biophys. Res. Commun. 108*, 66–73.

Carvlin, M. J., Mark, E., Fiel, R. J., & Howard, J. C. (1983) *Nucleic Acids Res. 11*, 6141–6154.

Chen, F. M. (1988) *Biochemistry 27*, 1843–1848.

Dougherty, G., Pilbrow, J. R., Skorobogaty, A., & Smith, T. D. (1985) *J. Chem. Soc., Faraday Trans. 81*, 1739–1759.

Fairley, T., Molock, F., Boykin, D. W., & Wilson, W. D. (1988) *Biopolymers 27*, 1433–1447.
Fiel, R. J., Datta Gupta, N., Mark, E. H., & Howard, J. C. (1981) *Cancer Res. 41*, 3543–3545.
Fiel, R. J., Howard, J. C., Mark, E. H., & Datta Gupta, N. (1979) *Nucleic Acids Res. 6*, 3093–3118.
Fiel, R. J., Beerman, T. A., Mark, E. H., & Datta Gupta, N. (1982) *Biochem. Biophys. Res. Commun. 107*, 1067–1074.
Fiel, R. J., Carvlin, M. J., Byrnes, R. W., & Mark, E. H. (1984) *Mol. Basis Cancer, Proc. Conf., Part B*, 215–226.
Ford, K. G., Pearl, L. H., & Neidle, S. (1987) *Nucleic Acids Res. 15*, 6553–6562.
Foster, R., Woo, D. V., Kaltovich, F., Emrich, J., & Ljungquist, C. J. (1985) *J. Nucl. Med. 26*, 756–760.
Geacintov, N. E., Ibanez, V., Rougee, M., & Bensasson, R. V. (1987) *Biochemistry 26*, 3087–3092.
Gibbs, E. J., Maurer, M. C., Zhang, J. H., Reiff, W. M., Hill, D. T., Malicka-Blaszkiewicz, M., McKinnie, R. E., Liu, H-Q., & Pasternack, R. F. (1988) *J. Inorg. Biochem. 32*, 39–65.
Kelly, J. M., Murphy, M. J., McConnell, D. J., & OhUigin, C. (1985) *Nucleic Acids Res. 13*, 167–184.
Krishnamoorthy, C. R., Yen, S. F., Smith, J. C., Lown, J. W., & Wilson, W. D. (1986) *Biochemistry 25*, 5933–5940.
Lohman, T. M. (1985) *CRC Crit. Rev. Biochem. 19*, 191–235.
Lohman, T. M., DeHaseth, P. L., & Record, M. T. (1978) *Biophys. Chem. 8*, 281–294.
Manning, G. (1969) *J. Chem. Phys. 51*, 924–933.
Manning, G. (1972) *Biopolymers 2*, 937–949.
Marzilli, L. G., Banville, D. L., Zon, G., & Wilson, W. D. (1986) *J. Am. Chem. Soc. 108*, 4188–4192.
McGhee, J. D., & von Hippel, P. H. (1974) *J. Mol. Biol. 86*, 469–489.
McKinnie, R. E., Choi, J. D., Bell, J. W., Gibbs, E. J., & Pasternack, R. F. (1988) *J. Inorg. Biochem. 32*, 207–224.
Muller, W., & Crothers, D. M. (1968) *J. Mol. Biol. 35*, 251–290.
Pasternack, R. F., Huber, P. R., Boyd, P., Engasser, L., Francesconi, L., Gibbs, E., Fasella, P., Ventura, C. G., & Hinds, L. C. (1972) *J. Am. Chem. Soc. 94*, 4511–4517.
Pasternack, R. F., Gibbs, E. J., & Villafranca, J. J. (1983a) *Biochemistry 22*, 2406–2414.
Pasternack, R. F., Gibbs, E. J., & Villafranca, J. J. (1983b) *Biochemistry 22*, 5409–5417.
Pasternack, R. F., Gibbs, E. J., Gaudemer, A., Antebi, A., Bassner, S., DePoy, L., Turner, D. H., Williams, A., Laplace, F., Lansard, M. H., Merienne, C., & Perree-Fauvet, M. (1985) *J. Am. Chem. Soc. 107*, 8179–8185.
Pasternack, R. F., Garrity, P., Ehrlich, B., Davis, C. B., Gibbs, E. J., Orloff, G., Giartosio, A., & Turano, C. (1986a) *Nucleic Acids Res. 14*, 5919–5931.
Pasternack, R. F., Sidney, D., Hunt, P. A., Snowden, E. A., & Gibbs, E. J. (1986b) *Nucleic Acids Res. 14*, 3927–3943.
Record, M. T., Lohman, T. M., & DeHaseth, P. (1976) *J. Mol. Biol. 107*, 145–158.
Robinson, G. D., Alavi, a., Vaum, R., & Staum, M. (1986) *J. Nucl. Med. 26*, 239–242.
Strickland, J. A., Banville, D. L., Wilson, W. D., & Marzilli, L. G. (1987) *Inorg. Chem. 26*, 3398–3406.
Van den Bergh, H. (1986) *Chem. Br. 22*, 430–439.
Vaum, R., Heindel, N. D., Burns, H. D., Emrich, J., & Foster, N. (1982) *J. Pharm. Sci. 71*, 1223–1226.
Ward, B., Skorobogarty, A., & Dabrowiak, J. C. (1986a) *Biochemistry 25*, 7827–7833.
Ward, B., Skorobogarty, A., & Dabrowiak, J. C. (1986b) *Biochemistry 25*, 6875–6883.
Wilson, W. D., & Lopp, I. G. (1979) *Biopolymers 18*, 3025–3041.
Wilson, W. D., Krishnamoorthy, C. R., Wang, Y. H., & Smith, J. C. (1985) *Biopolymers 24*, 1941–1961.