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Interactions of Porphyrins with Nucleic Acids†

Robert F. Pasternack,* Esther J. Gibbs,*,‡ and Joseph J. Villafranca§

ABSTRACT: The interactions of nucleic acids with water-soluble porphyrins and metalloporphyrins have been investigated by stopped-flow and temperature-jump techniques. Both natural DNA (calf thymus) and synthetic homopolymers [poly(dG-dC) and poly(dA-dT)] have been employed. The porphyrins studied belong to the tetrakis(4-*N*-methylpyridyl)porphine (H_2TMpyP-4) series and can be divided into two groups: (i) those which have no axial ligands when bound to nucleic acids [e.g., Ni(II), Cu(II), and the nonmetallic derivatives] and (ii) those which maintain axial ligands upon binding [e.g., Mn(III), Fe(III), Co(III), and Zn(II) derivatives]. The reaction of both axially and nonaxially liganded porphyrins at AT sites is too rapid to be measured by the kinetic methods utilized, whereas at GC sites the interaction of the nonaxially liganded porphyrins is in the millisecond time range and can be monitored by both stopped-flow and temperature-jump techniques. These results corroborate previous static studies, utilizing visible spectroscopy and circular dichroism, which indicate that the formation of an intercalated complex occurs only at GC base pair sites with porphyrins which do not possess axial ligands. With all the porphyrins investigated, the complexes formed at AT sites are envisioned as being of an "external" type involving some degree of overlap between the porphyrin and the bases of the duplex. In relaxation experiments of poly(dG-dC) with H_2TMpyP-4, a large, reproducible effect is observed which can be analyzed as a single exponential. Rate constants for association and dissociation of the H_2TMpyP-4/poly(dG-dC) complex are 3.7×10^5 M^{-1} s^{-1} and 1.8 s^{-1}, respectively. Relaxation studies of mixtures of poly(dA-dT) and poly(dG-dC) with H_2TMpyP-4 indicate that the transfer of the porphyrin from one homopolymer to another occurs via a mechanism involving dissociation rather than direct transfer. With calf thymus DNA and H_2TMpyP-4, a multiphasic relaxation profile is observed. Both the amplitude and concentration dependencies of these kinetic effects indicate that the processes being observed involve the redistribution of porphyrin among the various sites on the polynucleotide. A comparison of the relaxation times obtained for this system with those obtained for mixtures of the synthetic homopolymers with H_2TMpyP-4 strongly suggests that, for the natural system, the porphyrin can move from site to site without first dissociating into the solvent medium.

Interactions of synthetic and natural nucleic acids with tetrakis(4-*N*-methylpyridyl)porphine (H_2TMpyP-4, Figure 1) and several of its metal derivatives have been studied in some detail (Fiel et al., 1979; Fiel & Munson, 1980; Pasternack et al., 1983). The combined evidence from thermodynamic, circular dichroic, and visible spectroscopic data indicates that the type of complex formed depends upon the composition of neighboring base pairs of the nucleic acids and the detailed structural features of the metalloporphyrin (Pasternack et al., 1983). It has been suggested that a truly intercalated species requires guanine/cytosine (GC) base pairs and porphyrin moieties which have no axial groups. Thus, H_2TMpyP-4, Cu^{II}TMpyP-4, and Ni^{II}TMpyP-4 all interact extensively with GC regions whereas Zn^{II}TMpyP-4, Mn^{III}TMpyP-4, Fe^{III}TMpyP-4, and Co^{III}TMpyP-4 do not. Also worth noting is that tetrakis(2-*N*-methylpyridyl)porphine (H_2TMpyP-2), for which the rotational barrier for the peripheral pyridyl groups is very large (Eaton & Eaton, 1975; Eaton et al., 1978), also does not interact with GC regions (Pasternack et al., 1983).

A fundamentally different type of complex is formed at adenine/thymine (AT) regions of nucleic acids. This latter type of interaction leads to an "externally" bound complex in which the porphyrin is located in a groove and/or is only partially intercalated. In either case, evidence exists for some degree of drug/nucleic acid base interaction in addition to the anticipated Coulombic attraction of the positive porphyrin periphery for the negative phosphate backbone. It may well be that upon formation of such an externally bound complex,

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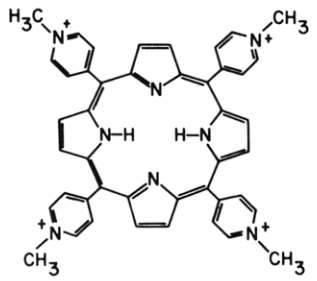

FIGURE 1: Structure of tetrakis(4-*N*-methylpyridyl)porphine.

a deformation of the helical structure is required similar to the kinked or bent structure suggested by Sobell (Sobell et al., 1977; Sobel, 1980) and observed by Crothers for the external or groove binding drug irehdiamine (Dattagupta et al., 1978). The greater rigidity and stability of GC base pair regions relative to AT base pair regions (Early et al., 1981a,b) serve to hinder such deformations for the former, preventing appreciable concentrations of this type of externally bound complex from forming. Rather, interactions at GC regions for this series of porphyrins are of two types: (i) external complexes which are predominantly Coulombic in nature with minimal porphyrin/base overlap and helical distortion or (ii) a fully intercalated species with maximum overlap of porphyrin/base π-systems as well as electrostatic interactions between the negative phosphate backbone and the positive porphyrin periphery which remains external to the purine/pyrimidine region of the duplex (H. M. Sobell, unpublished results).

The present paper deals with the kinetics of the reactions of porphyrins with calf thymus DNA, poly(dG-dC), and poly(dA-dT). These reactions prove rapid enough to require stopped-flow and/or temperature-jump techniques. Because of the dependence of rate (and thermodynamic) parameters on r (r = [complex]/[base pairs]$_{total}$), the quantitative aspects of these rate studies are based on relaxation experiments. However, valuable qualitative information is obtained from the stopped-flow results, and these are included.

Materials and Methods

Calf thymus DNA was purchased from Worthington Biochemicals or Sigma Chemical Co. and purified as previously described (Pasternack et al., 1983). Poly(dG-dC) and poly(dA-dT) were purchased from P-L Biochemicals, Inc., and used without further purification. Man-Win Coordination Chemicals supplied H_2TMpyP-4 as the tosylate salt. The metallo derivatives were prepared and purified as previously described (Pasternack et al., 1974, 1977, 1983). All other chemicals were reagent grade and were used without further purification. All experiments, except where specifically indicated, were carried out at 25 °C, at pH 6.8, in a phosphate buffer containing 6 mM Na_2HPO_4, 2 mM NaH_2PO_4, 1 mM ethylenediaminetetraacetic acid (EDTA), and sufficient NaCl to bring the ionic strength to μ = 0.2 M.

Reaction rates were determined by using a Durrum Model D110 stopped-flow apparatus interfaced with a Cromemco Z-2 microcomputer for data collection. Three temperature-jump apparatuses were employed. Two of these instruments are homemade and have been described previously (Pasternack et al., 1969; Crothers, 1971). The third temperature-jump instrument was manufactured by Dialog (West Germany) and has been previously described (Drobnies, 1979; Rigler et al., 1974). The data were collected digitally with a Biomation Model 805 transient recorder. A PET microcomputer was incorporated in the data acquisition system to transfer the data

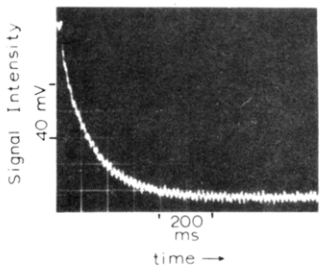

FIGURE 2: Oscilloscope trace of a relaxation curve following a rapid temperature perturbation of a solution of H_2TMpyP-4 with poly(dG-dC). Conditions: μ = 0.2 M; pH 6.8; T_{final} = 25.1 °C; λ = 422 nm; ΔT = 1.8 °C; [H_2TMpyP-4] = 5.56 × 10^{-6} M; [poly(dG-dC)] = 2.54 × 10^{-5} M.

Table I: Calculated Values of r and Σ for Relaxation Experiments of H_2TMpyP-4 with Poly(dG-dC)[a]

[poly(dG-dC)] × 10^6 (M)	$1/r_0$	r	$\Sigma \times 10^6$ (M)	$1/\tau$ (s^{-1})
4.20	0.76	0.363	6.69	3.9
6.30	1.13	0.351	6.31	3.9
12.6	2.27	0.302	6.24	4.1
25.4	4.57	0.197	14.6	8.6
50.8	9.14	0.105	36.0	15

[a] [H_2TMpyP-4] = 5.56 × 10^{-6} M; μ = 0.2 M; pH = 6.8; 25.1 °C.

from the Biomation to a VAX 11/780 computer, where the data were analyzed by using the program DISCRETE written by S. W. Provencher (Provencher, 1976a,b).

The strong transient electric field produced in a joule-heating temperature-jump instrument can cause a partial orientation of nucleic acids (Dourlent et al., 1973), and this effect can complicate the study of chemical relaxation processes. The placement of a polarizer in the incident beam at the appropriate angle serves to eliminate this problem when it appears. It was found that for the systems studied here, identical results were obtained with a polarized and unpolarized beam. Because the polarizer reduces the signal to noise ratio, experiments were performed without its inclusion.

Results

Poly(dG-dC) and Poly(dA-dT). Temperature-jump studies with the poly(dG-dC) duplex and H_2TMpyP-4 were conducted at a porphyrin concentration of 5.56 × 10^{-6} M and polynucleotide concentrations ranging from 4.20 × 10^{-6} to 5.08 × 10^{-5} M. The solutions were monitored at 422 nm (the wavelength maximum of unbound porphyrin), μ = 0.2 M, and 25 °C. Identical results were obtained at the product maximum, 444 nm. For these solutions, a large reproducible effect (Figure 2) is obtained which could be fit as a single exponential. The inverse of the relaxation time, $1/\tau$, was found to increase with increasing poly(dG-dC) concentration (cf. Table I). There is no indication of any slower processes. A very fast optical change (shorter than several microseconds) which could not be resolved was observed. A similar effect has been seen with other intercalating (Li & Crothers, 1969; Schmechel & Crothers, 1971) and nonintercalating (Muller et al., 1973) drugs and has been ascribed to a temperature-dependent molar absorptivity change of the bound drug. A comparable fast optical spike for H_2TMpyP-4 in the absence of nucleic acid is observed, and, therefore, this fast event has not been included in the kinetic analysis. Shown in Figure 3 is a plot of the relative amplitude of the relaxation effects for the H_2TMpyP-4/poly(dG-dC) system as a function of $1/r_0$ (r_0 = [porphyrin]$_{total}$/[base pairs]$_{total}$). A bell-shaped curve

Table II: Absorbance Changes for the Formation of Porphyrin/Nucleic Acid Complexes Using the Stopped-Flow Technique[a]

	poly(dG-dC)		poly(dA-dT)		DNA	
porphyrin	ΔA_{fast}[b]	ΔA_{slow}	ΔA_{fast}	ΔA_{slow}	ΔA_{fast}	ΔA_{slow}
H_2TMpyP-4	0.044	0.170	0.124	0.0	0.194	0.014
Cu^{II}TMpyP-4	0.092	0.225	0.081	0.0	0.148	0.032
Ni^{II}TMpyP-4	0.064	0.091	0.097	0.0	0.084	0.027
Fe^{III}TMpyP-4	0.023	<0.01	0.042	<0.01	0.043	<0.01
Mn^{III}TMpyP-4	[c]	[c]	0.061	0.0	0.07	0.0
Co^{III}TMpyP-4	0.035	<0.01	0.068	<0.01	0.061	<0.01
Zn^{II}TMpyP-4	0.055	0.0	0.054	0.0		

[a] $r_0 = 0.03$; $[H_2TMpyP\text{-}4] = 1 \times 10^{-6}$ M; $[Cu^{II}TMpyP\text{-}4] = 1.5 \times 10^{-6}$ M; $[Zn^{II}TMpyP\text{-}4] = [Ni^{II}TMpyP\text{-}4] = [Fe^{III}TMpyP\text{-}4] = [Co^{III}TMpyP\text{-}4] = [Mn^{III}TMpyP\text{-}4] = 2.0 \times 10^{-6}$ M; $\mu = 0.2$; pH = 6.8; 25 °C. [b] The fast component refers to color changes too rapid to be observed by this technique. [c] Changes too small to measure.

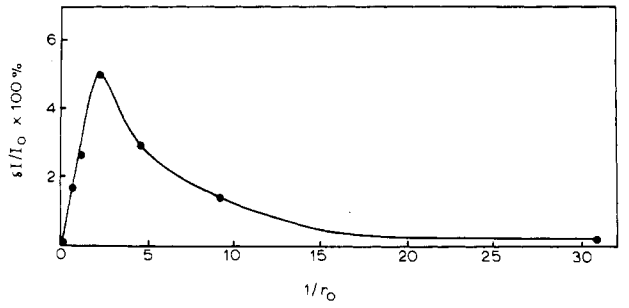

FIGURE 3: Variation of the relative amplitude of relaxation effects with the initial ratio of poly(dG-dC) concentration to H_2TMpyP-4 concentration. Conditions: $\mu = 0.2$ M; $T_{final} = 25.1$ °C; $\Delta T = 1.8$ °C; $\lambda = 422$ nm; $[H_2TMpyP\text{-}4] = 5.56 \times 10^{-6}$ M.

with a maximum at $1/r_0 \sim 2.3$ (polynucleotide concentration of 1.3×10^{-5} M) is obtained. In contrast, solutions of H_2TMpyP-4 with poly(dA-dT) showed no relaxation effects for $2 \leq 1/r_0 \leq 29$. Fast optical spikes outside the time range ($t_{1/2} < 5$ μs) of the joule-heating temperature-jump instrument were observed.

The copper(II) and nickel(II) porphyrin derivatives at a concentration of 5.3×10^{-6} M, 25 °C, and $1/r_0 = 2.4$ (conditions at which the H_2TMpyP-4 porphyrin gives a maximum effect) both give relaxation effects with poly(dG-dC). The relative amplitude of the relaxation effect with Cu^{II}TMpyP-4 is similar in magnitude to the maximum relative amplitude obtained with H_2TMpyP-4, whereas for Ni^{II}TMpyP-4 the amplitude is much smaller. For the copper(II) porphyrin, the relaxation effect was best fit when analyzed as two coupled exponentials yielding inverse relaxation times of 3.3 and 19 s^{-1}. The slower effect corresponds to the value obtained for H_2TMpyP-4 with poly(dG-dC) at similar concentrations. Studies over an extended range of polymer concentrations have not been conducted for these two metal derivatives as yet. Thus, although detailed quantitative comparisons cannot be made, qualitatively these three porphyrins are similar in that they all give relaxation effects with poly(dG-dC) that are in approximately the same time range.

At $1/r_0 = 2.4$ and a metalloporphyrin concentration of 5.3×10^{-6} M, none of the zinc(II), iron(III), manganese(III), or cobalt(III) derivatives of H_2TMpyP-4 showed any relaxation effects with poly(dG-dC). The manganese(III) and cobalt(III) derivatives display very fast optical changes outside the time range of the joule-heating temperature-jump instrument as described above for H_2TMpyP-4. The interactions of these axially liganded metalloporphyrins with poly(dG-dC) may be too fast to measure by this technique, may not be poised for observation, may not be temperature dependent, and/or may involve color changes too small to observe. The possibility that their binding reactions are too slow for observation via the temperature-jump technique is ruled out by stopped-flow experiments as described below.

In the stopped-flow experiments, one experimental strategy involved filling one of the driving syringes with porphyrin or metalloporphyrin solution and the other with a nucleic acid solution at an identical pH and ionic strength. A summary of the results for all the porphyrins and metalloporphyrins is shown in Table II. With *poly(dG-dC)*, unlike the zinc(II), iron(III), cobalt(III), and manganese(III) derivatives, a substantial color change is observable in the stopped-flow time range for complex formation with the nickel(II) and copper(II) metalloporphyrins and the nonmetalloporphyrin.

For the zinc(II) derivative, the total color change for binding to poly(dG-dC) is too rapid to be observed by the stopped-flow technique, indicating a process with a half-life of less than a few milliseconds. The stopped-flow results with cobalt(III) and iron(III) derivatives of H_2TMpyP-4 are subject to some uncertainty because in blank experiments in which these two porphyrins are flowed against buffer (i.e., a concentration jump) a small kinetic effect with an apparent rate constant of about 10^{-3} s^{-1} is observed. When these two metalloporphyrins are mixed with poly(dG-dC), the majority of the color change is too rapid to be measured, but in addition, there is a small but discernible absorbance change in about the same time range as seen in the blank experiments. The iron(III) derivative is known to have complicated solution chemistry (Pasternack et al., 1977). At pH 6.8, this metalloporphyrin exists as an equilibrium mixture of monohydroxy, dihydroxy, and a dimerized species. The slow dissociation of the dimer of Fe^{III}TMpyP-4 could be responsible for the slow process observed with poly(dG-dC). The color change for the manganese(III) derivative with poly(dG-dC) is too small to be monitored in these experiments.

In a second experimental strategy, the dissociation of the complex was studied by flowing porphyrin solutions preincubated with nucleic acid against a solution of high ionic strength or a solution at the same ionic strength but containing sodium dodecyl sulfate (SDS). The salt-jump experiments exploit the dependency of K_{app} on ionic strength; i.e., the binding of porphyrin decreases with increasing ionic strength (Pasternack et al., 1983). The use of SDS to dissociate drug/nucleic acid complexes has been employed in other studies (Ramstein et al., 1972, 1980; Berman et al., 1977).

For the H_2TMpyP-4/poly(dG-dC) system, all of the color change for the complete dissociation of the complex by 2 M ionic strength or 2–200 mM SDS is within the time range of the stopped-flow instrument. The rate of dissociation is dependent on the final ionic strength being about twice as fast at 1 M ionic strength than at 2 M ionic strength. In contrast, the rate of dissociation of the H_2TMpyP-4/poly(dG-dC) complex is constant over the hundredfold range of SDS con-

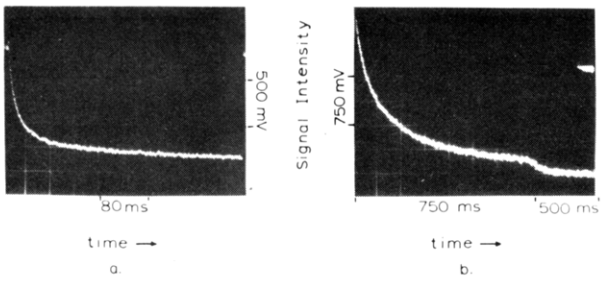

FIGURE 4: Oscilloscope traces of relaxation curves following a rapid temperature perturbation of a solution of H_2TMpyP-4 with DNA. [H_2TMpyP-4] = 1.07×10^{-5} M; (a) [DNA] = 3.28×10^{-4} M; (b) [DNA] = 5.70×10^{-4} M. Conditions: μ = 0.2 M; pH 6.8; λ = 422 nm. (a) Fit as three exponentials with $1/\tau$ values of 10, 48, and 190 s^{-1}. (b) This trace was obtained with points taken at two different time bases. Fit as three exponentials with $1/\tau$ values of 12, 46, and 200 s^{-1}.

centration used. This suggests that SDS does not catalyze the reaction but sequesters free porphyrin, thereby driving the reaction to completion.

Nickel(II) and copper(II) derivatives of the porphyrin are not completely removed from poly(dG-dC) by 2.0 M ionic strength (Pasternack et al., 1983). Under these conditions, only 65% and 44% of the total free metalloporphyrin absorbance are restored for the copper(II) and nickel(II) derivatives, respectively. For the metalloporphyrin that is dissociated, the absorbance change is within the stopped-flow time range. With SDS, both the nickel(II) and copper(II) porphyrins are completely removed from poly(dG-dC), and, as for the nonmetallo derivative, there is no variation in the rate of removal with 2–200 mM SDS.

For the Zn^{II}TMpyP-4/poly(dG-dC) system with both high ionic strength and SDS and for the Mn^{III}TMpyP-4 system with SDS, the dissociation of the complex is too rapid to be observed by using the stopped-flow technique. The color change upon dissociation of the manganese(III) porphyrin with ionic strength is too small to measure, but because the absorbance maximum of this unbound metalloporphyrin is shifted by the presence of SDS, the color change due to removal by the detergent is larger. The removal of the iron(III) and cobalt(III) derivatives from poly(dG-dC) by 2 M ionic strength is rapid, but again there is a small, slow process that is in the same time range as that seen for the blank experiments discussed previously.

For H_2TMpyP-4 and all the metalloporphyrins, the binding to *poly(dA-dT)* is too rapid to be observed by the stopped-flow technique, indicating that the half-life for this process is shorter than a few milliseconds. Again, the cobalt(III) and iron(III) derivatives did show a small slow absorbance change which cannot be separated from processes observed in blank experiments conducted in the absence of nucleic acid.

In the reverse direction, 2.0 M ionic strength completely removes the H_2TMpyP-4 from poly(dA-dT), and the dissociation rate is outside the time range of the stopped-flow technique. Similar results are obtained for the zinc(II) and manganese(III) derivatives. As with poly(dG-dC), not all of the copper(II) and nickel(II) porphyrin complexes are dissociated from poly(dA-dT) by 2.0 M ionic strength. The dissociation that does occur is too rapid to be observed by this technique. The majority of the color change for dissociation of the iron(III) and cobalt(III) porphyrins by 2.0 M sodium chloride is too rapid to be measured by the stopped-flow technique. Again, these latter two derivatives have very small, slow color changes similar to effects observed in the absence of nucleic acid.

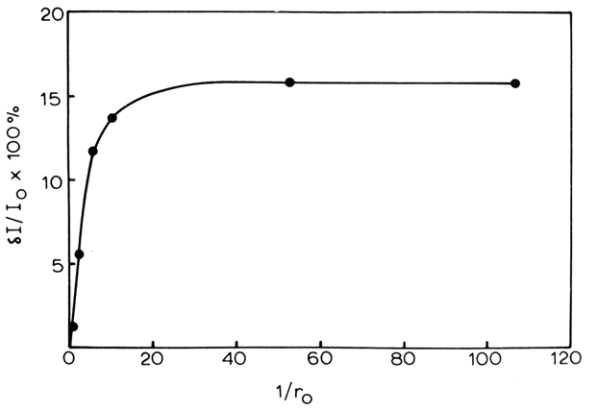

FIGURE 5: Variation of the overall amplitude of relaxation effects with the initial ratio of H_2TMpyP-4 concentration to DNA concentration. Conditions: μ = 0.2 M; pH 6.8; temperature = 25 °C; [H_2TMpyP-4] = 1.07×10^{-5} M; I = intensity of transmitted light.

Table III: Relaxation Effects of H_2TMpyP-4 with Calf Thymus DNA as a Function of Ratio

[DNA] × 10^5 (M)	$1/r_0$	$1/\tau_1$ (s^{-1})	$1/\tau_2$ (s^{-1})	$1/\tau_3$ (s^{-1})
(A) [H_2TMpyP-4] = 1.07×10^{-5} M; 25.3 °C; μ = 0.2 M				
0.570	0.53	*a*	*a*	*a*
2.28	2.1	~7	~40	~300
5.70	5.3	17	89	220
11.4	10.7	18	93	290
57.0	53	12	56	200
114	107	11	56	240
(B) [H_2TMpyP-4] = 9.77×10^{-6} M; 25.1 °C; μ = 0.5 M				
0.849	0.87	*a*	*a*	*a*
3.40	3.5	5.8	24	72
8.49	8.7	5.4	26	95
17.4	17.4	5.8	26	120
87.0	87.1	7.5	39	223
130	131	9.3	49	319

[a] Effects too small to measure.

Calf Thymus DNA. Temperature-jump experiments with calf thymus DNA and H_2TMpyP-4 were conducted at ionic strengths of μ = 0.2 M and μ = 0.5 M. In one set of experiments, the kinetics were studied as a function of r by keeping the porphyrin concentration constant and varying the DNA concentration. For these solutions, large, reproducible, and multiphasic effects such as shown in Figure 4 are obtained. A plot of the overall relative amplitude of these relaxation effects vs. $1/r_0$ for μ = 0.2 M is shown in Figure 5. A similar amplitude profile is obtained at μ = 0.5 M. These profiles are markedly different from the profile obtained for the poly(dG-dC)/H_2TMpyP-4 system and are indicative of first-order processes. These amplitude profiles suggest that the processes being observed involve the redistribution of porphyrin among different complexes on the polynucleotide lattice. If the distribution of bound porphyrin among the various types of lattice sites is independent of r (i.e., there is a fixed ratio of forms), then the size of the overall relative amplitude effect depends only on how much porphyrin is bound. Inasmuch as these experiments were conducted at a constant porphyrin concentration, at low DNA concentrations as more polymer is added, more porphyrin is bound, and the sizes of the relaxation effects increase. At μ = 0.2 M and at a $1/r_0$ of approximately 30 (about the same ratio at which the titration data for this system display no further absorbance changes), enough polymer has been added to totally bind all of the porphyrin, and the relative amplitude reaches its maximum. When more polymer is added, the size of the

Table IV: Relaxation Effects of H_2TMpyP-4 with Calf Thymus DNA as a Function of Concentration and at Constant Ratio

[DNA] × 10^5 (M)	[H_2TMpyP-4] × 10^6 (M)	$1/\tau_1$ (s^{-1})	$1/\tau_2$ (s^{-1})	$1/\tau_3$ (s^{-1})
(A) $1/r_0 = 31$; 25 °C; $\mu = 0.2$ M				
7.09	2.32	10	48	230
11.8	3.86	13	73	
19.4	6.44	8	43	180
32.8	10.7	10	48	190
54.7	17.9	11	60	250
91.2	29.8	10	49	210
(B) $1/r_0 = 27$; 25.1 °C; $\mu = 0.5$ M				
6.77	2.55	7.6	30	130
11.3	4.25	8.1	44	160
18.8	7.08	8.9	42	160
31.3	11.8	5.6	31	130
52.2	19.7	6.8	43	230
87.0	32.8	4.7	31	150

Table V: Relaxation Effects of Cu^{II}TMpyP-4 and Ni^{II}TMpyP-4 with Calf Thymus DNA as a Function of Concentration at Constant Ratio

[DNA] × 10^5 (M)	[Cu^{II}TMpyP-4] × 10^6 (M)	$1/\tau_1$ (s^{-1})	$1/\tau_2$ (s^{-1})	$1/\tau_3$ (s^{-1})
(A) Cu^{II}TMpyP-4: $1/r_0 = 50$; $\mu = 0.2$ M; 25.1 °C				
9.36	1.87	12	56	
15.6	3.11	9.1	50	
26.0	5.18	6.2	25	90
43.3	8.64	5.3	21	90
72.2	14.4	6.6	26	110
(B) Ni^{II}TMpyP-4: $1/r_0 = 20$; $\mu = 0.2$ M; 25.1 °C				
	[Ni^{II}TMpyP-4] × 10^6 (M)			
3.55	1.80	~16	~60	
5.91	3.01	11	39	110
9.85	5.01	10	38	140
16.4	8.35	8.9	31	96
27.4	13.9	7.2	28	100
45.6	23.2	8.5	32	115

relaxation effects remains constant since no more free porphyrin is available for binding. If the relaxation processes being observed in the temperature-jump experiments involved conversion of free porphyrin to bound porphyrin, then the amplitude of the relaxation effect would go through a maximum and then decrease as observed for poly(dG-dC).

The relaxation effects are not simple first order but are best fit as three coupled exponentials. Listed in Table III are the values obtained for $1/\tau$ at the various $1/r_0$ values. At μ = 0.2 M, there is little variation in the rates of any of the three processes over a 50-fold range of concentration (and ratio). At μ = 0.5 M and over an approximately 40-fold range in DNA concentration, the fastest process varies by'a factor of about 4.4, and the two slower processes vary by about a factor of 2. Experiments described below lead us to conclude that these variations in rate are due to changes in ratio rather than concentration.

In a second set of experiments, the influence of concentration on rate was monitored at constant r (Table IV). The value of r was kept constant by preparing a concentrated stock solution containing both porphyrin and DNA and making serial dilutions with a second solution of porphyrin equal in concentration to the calculated free porphyrin concentration of the original stock (Li & Crothers, 1969). At μ = 0.2 M and $1/r_0$ = 31, the rates for the three processes did not vary outside of experimental error over a 13-fold range in both porphyrin and DNA concentrations. Values of $1/\tau_1 = 11\ s^{-1}$, $1/\tau_2 = 54\ s^{-1}$, and $1/\tau_3 = 210\ s^{-1}$ were obtained. Similarly, at μ = 0.5 M, $1/r_0$ = 27, and over a 13-fold range in both DNA and porphyrin concentrations, the relaxation rates remained constant at $1/\tau_1 = 7.0\ s^{-1}$, $1/\tau_2 = 37\ s^{-1}$, and $1/\tau_3 = 160\ s^{-1}$. The rates at higher ionic strength are slower than the rates at lower ionic strength. This change in rate with ionic strength is also apparent in stopped-flow experiments in which porphyrin was removed from the polynucleotides by ionic strength jumps (vide supra).

Results similar to those obtained for the nonmetalloporphyrin were obtained for the copper(II) and nickel(II) derivatives with DNA (cf. Table V). The relative amplitudes of the relaxation effects vs. $1/r_0$ are indicative of first-order processes. As described earlier, these profiles suggest the relaxation processes being measured involve the redistribution of metalloporphyrin among different sites on the polymer lattice. For the nickel(II) porphyrin at the smallest values of $1/r_0$, conditions at which a substantial portion of this metalloporphyrin is free in solution, an enormous color change is observed which is too fast to be studied with a joule-heating temperature-jump apparatus. This effect has been studied previously (Pasternack et al., 1974) and is ascribed to the perturbation of the equilibrium between six- and four-coordinate nickel(II) porphyrin that is not complexed to DNA. At higher $1/r_0$ values, when nearly all of the metalloporphyrin is bound, this fast optical spike disappears.

The rates of these three processes increase in the order nickel(II) < copper(II) < nonmetalloporphyrin. For these three porphyrins with DNA, the rates do not vary with concentration at a constant ratio. As also indicated by the amplitude profiles, the lack of variation in rate with concentration suggests that the processes being observed are first order for these porphyrins with DNA. The rates do vary somewhat with $1/r_0$ for all three derivatives. For the nickel(II) and copper(II) derivatives, the rates decreased with increasing $1/r_0$ values, whereas for H_2TMpyP-4, the rates increased with increasing $1/r_0$ values.

At $1/r_0$ values of 10 and 50, the iron(III), cobalt(III), and manganese(III) derivatives of H_2TMpyP-4 do not produce relaxation effects. Once again, this group of axially liganded porphyrins displays behavior different from that observed for those porphyrins which do not have axial ligands in their bound complexes. Unlike the other axially liganded metalloporphyrins, the zinc(II) derivative shows a very small relaxation effect with DNA. The effect is in a faster time range (3 orders of magnitude faster) and much smaller in magnitude than those obtained for the nickel(II) and copper(II) porphyrins and nonmetalloporphyrin, suggesting that the process being observed for the zinc(II) porphyrin is of a different type than for the latter three porphyrins.

Stopped-flow results for the formation of a DNA complex with the copper(II) and nickel(II) porphyrins and nonmetalloporphyrin are intermediate between those obtained with poly(dA-dT) and poly(dG-dC) (Table I). A larger percentage of the color change is too rapid to be observed for the binding to DNA than for the binding to poly(dG-dC), but not all of the color change is too rapid to be observed as is the case for poly(dA-dT). The kinetically observable absorbance changes for these three derivatives are multiphasic and faster than for the poly(dG-dC) duplex. The larger rate constants with DNA than with poly(dG-dC) may well reflect a kinetic pathway involving the rapid formation of a porphyrin/AT complex followed by transfer to a GC site. Additional data on transfer rates and mechanisms are provided in the next section on polynucleotide mixtures.

Table VI: Relaxation Data for Mixed Synthetic Nucleic Acid Experiments[a]

[poly(dA-dT)] × 10^5 (M)	[poly(dG-dC)] × 10^5 (M)	$1/\tau$ (s^{-1})
4.72	4.72	1.5
6.29	3.15	1.0
3.15	6.29	1.8
1.57	7.87	4.3
2.36	7.08	2.8
0.944	8.50	7.0
0.363	9.08	19

[a] [H_2TMpyP-4] = 6.15 × 10^{-6} M; t = 25 °C; μ = 0.2; pH = 6.8.

The removal of H_2TMpyP-4 from DNA by both SDS and 2.0 M sodium chloride also shows characteristi ıtermediate between those found for the two synthetic polynucleotides. With high ionic strength, the percentage of the color change that is too rapid to be observed is larger for DNA than that obtained for poly(dG-dC) but smaller than that observed for poly(dA-dT). The kinetically observable effects are multiphasic and faster than those measured for poly(dG-dC). Similar comparisons for the dissociation of the copper(II) and nickel(II) porphyrins are not as straightforward since, as noted previously with the two synthetic polynucleotides, not all of these metalloporphyrins are removed by 2.0 M ionic strength. With DNA, the percentage of the free metalloporphyrin absorbance regained with DNA at 2.0 M sodium chloride is closer to that obtained with poly(dG-dC) than with poly(dA-dT). The observed rate constants for these two metallo derivatives for dissociation from DNA are, as with the nonmetalloporphyrin, faster than for poly(dG-dC) with both 2.0 M ionic strength and SDS. For manganese(III), iron(III), and cobalt(III) derivatives, the absorbance change due to dissociation from DNA is outside the time range of the stopped-flow technique. This is analogous to observations with the synthetic polynucleotide duplexes.

Mixtures of Poly(dG-dC) and Poly(dA-dT): The Transfer Reaction. Temperature-jump experiments with H_2TMpyP-4 and DNA suggest that the relaxation processes observed involve the redistribution of the porphyrin among different sites on this polynucleotide. To obtain a greater understanding of this transfer process, temperature-jump experiments were conducted on porphyrin solutions containing a mixture of poly(dG-dC) and poly(dA-dT) duplexes at various ratios. All experiments were done at a H_2TMpyP-4 concentration of 6.15 × 10^{-6} M and at μ = 0.2 M. The concentration of total base pairs, the sum of the poly(dG-dC) and the poly(dA-dT) base pairs, was kept constant at $1/r_0$ = 15.3, but the ratio of the two polymers was varied. For H_2TMpyP-4 in the presence of only poly(dG-dC), relaxation effects with extremely small amplitudes are obtained at $1/r_0$ values greater than 10 (Figure 3). No relaxation effects have been observed for H_2TMpyP-4/poly(dA-dT) solutions. For the solutions containing both synthetic polynucleotides, a large reproducible effect is obtained which could be fit by a single exponential whose relaxation time varies with the ratio of the two types of base pairs (cf. Table VI). Shown in Figure 6 is a plot of the relative amplitude of the relaxation effects vs. the ratio of the initial poly(dG-dC) concentration to poly(dA-dT) concentration. A curve is obtained having its maximum at a ratio of GC base pairs to AT base pairs of about 2. For the transfer reaction under study, the equilibrium is poised (i.e., the maximum amplitude is obtained) when the porphyrin is equally distributed between the two polynucleotides. Since the relative amplitude maximum occurs at a ratio of about 2 GC base pairs to 1 AT base pair, this suggests that the binding constant for

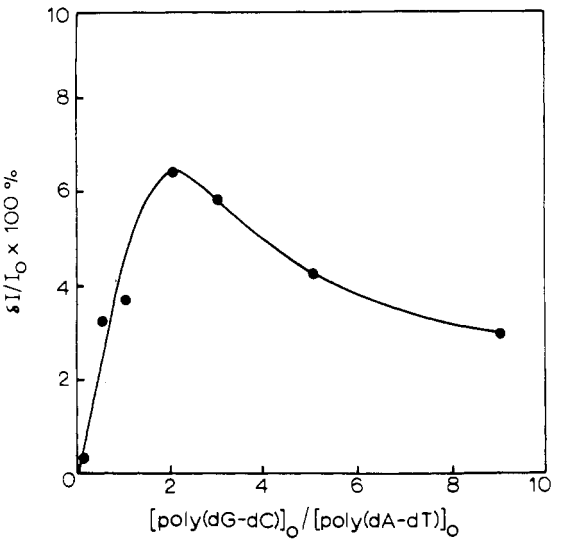

FIGURE 6: Plot of the relative amplitude of relaxation effects vs. the initial ratio for H_2TMpyP-4 with mixtures of poly(dG-dC) and poly(dA-dT). Conditions: [H_2TMpyP-4] = 6.15 × 10^{-6} M; $1/r_0$ = 15.3; μ = 0.2 M; pH 6.8; temperature = 25 °C.

H_2TMpyP-4 to poly(dA-dT) is roughly twice the binding constant to poly(dG-dC) at this value of r. When the value K = 7.7 × 10^5 M^{-1} for poly(dG-dC) (Pasternack et al., 1983) is used, a value of K = 1.2 × 10^6 M^{-1} is calculated for the binding of H_2TMpyP-4 to poly(dA-dT).

Discussion

Stopped-flow kinetic studies of the dissociation of the H_2TMpyP-4/poly(dG-dC) complex reveal that the full color change associated with this reaction is within the time range accessible by this technique whereas, for the association reaction, some 20% of the color change is too rapid to be seen. This rapid, kinetically unobservable color change in the forward direction is ascribed to the formation of an externally bound porphyrin/polynucleotide complex. These results imply that at equilibrium, and under the conditions of these experiments, all the porphyrin is intercalated with only negligible concentrations externally bound. The temperature-jump results in which relaxation effects are obtained for H_2TMpyP-4, CuTMpyP-4, and NiTMpyP-4 with poly(dG-dC) are consistent with this conclusion. Temperature-jump experiments for H_2TMpyP-4 with poly(dG-dC) yield a single relaxation effect whose relaxation time and amplitude are concentration dependent (cf. Figure 3 and Table I). An analysis has been developed by Thusius (1973) for the amplitude profile of a one-step, second-order system of the type:

$$P + GC \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} P{\cdot}GC \qquad (1)$$

For systems of this type, a "relaxation amplitude titration" experiment in which one reactant is held constant (i.e., the porphyrin) and the second is varied [i.e., poly(dG-dC)] is predicted to yield a bell-shaped curve for the plot of the relative amplitude vs. the concentration of the polynucleotide. The initial concentration of poly(dG-dC) at which the maximum relative amplitude occurs can be calculated from this theory as

$$[GC]_0^{\text{max ampl}} = [P]_0 + 1/K_{app} \qquad (2)$$

where the subscript zero denotes the initial concentration of the appropriate species and K_{app} is the equilibrium constant for the chemical reaction. With the use of K_{app} = 7.7 × 10^5 M^{-1} obtained from titration data (Pasternack et al., 1983),

this equation predicts that the maximum relative amplitude for the H_2TMpyP-4/poly(dG-dC) system should occur at a polymer concentration of 6.86×10^{-6} M. From the amplitude profile, this maximum is found to occur at a poly(dG-dC) concentration of 1.26×10^{-5} M, about twice the calculated value. We suggest that the difference between the predicted and experimental concentrations is a result of neighbor exclusion effects not included in the theoretical calculations. The curvature in the titration data for this system has been interpreted as being due to neighbor exclusion phenomena for which a value of $n \approx 2$ was calculated (Pasternack et al., 1983). Thus, the maximum in the amplitude profile might be expected to occur at roughly twice the concentration of poly(dG-dC) calculated from eq 2 since, due to neighbor exclusion effects, only about half of the lattice sites are available for binding. The excellent agreement between the thermodynamic and the corrected kinetic data supports the inclusion of neighbor exclusion effects in the analysis of this system.

Jovin & Striker (1977) have derived equations to evaluate relaxation data for a single bimolecular process as in eq 1 in which the binding of drug to the polymer lattice exhibits neighbor exclusion phenomena:

$$1/\tau = k_2([\overline{GC}] - f'(r)[\bar{P}]) + k_{-2} \quad (3)$$

Here, the superscript bar indicates equilibrium concentrations, and $f'(r)$ is the derivative of the function denoting the fraction of total potential sites on the nucleic acid available for additional binding, i.e., $f(r) = [\overline{GC}]/[GC]_0$. For poly(dG-dC) with a neighbor exclusion factor of 2, the values for $f(r)$, $f'(r)$, $[\bar{P}]$, and $[\overline{GC}]$ are

$$f(r) = (1 - 2r)^2/(1 - r) \quad (4)$$

$$f'(r) = (2r - 3)(1 - 2r)/[(1 - r)^2] \quad (5)$$

$$[\bar{P}] = [P]_0 - r[GC]_0 \quad (6)$$

$$[\overline{GC}] = [GC]_0(1 - 2r)^2/(1 - r) \quad (7)$$

Substituting these into eq 3, one obtains

$$1/\tau = k_2\Sigma + k_{-2} \quad (8)$$

where

$$\Sigma = \frac{1 - 2r}{1 - r}\left\{[GC]_0(1 - 2r) - \left(\frac{2r - 3}{1 - r}\right)([P]_0 - r[GC]_0)\right\} \quad (9)$$

Listed in Table I are the calculated values for Σ. A plot of $1/\tau$ vs. Σ for such a bimolecular process should yield a straight line with a slope of k_2 and an intercept of k_{-2}. Shown in Figure 7 is a plot of the temperature-jump data for the H_2TMpyP-4/poly(dG-dC) system. A straight line is obtained, yielding $k_2 = 3.7 \times 10^5$ M^{-1} s^{-1} and $k_{-2} = 1.8$ s^{-1}. The kinetics of the reactions of proflavin (a known intercalator having no bulky peripheral substituents) with DNA, poly(dG-dC), and poly(dA-dT) have been extensively studied (Li & Crothers, 1969; Ramstein et al., 1980). The rate constants determined from these studies for the reaction of free drug and free polynucleotide to form the final complex have been reported as 4.3×10^6 M^{-1} s^{-1}, 1.6×10^7 M^{-1} s^{-1}, and 1.6×10^7 M^{-1} s^{-1}, respectively. That the reaction of H_2TMpyP-4 is slower than that for proflavin with polynucleotide is not surprising. However, the difference in rate is less than 2 orders of magnitude or $\Delta(\Delta G^*) < 3$ kcal. Therefore, although some rotation of the pyridyl substituent of H_2TMpyP-4 is required for intercalation [H_2TMpyP-2 whose pyridyl rings are frozen perpendicular to the porphine core does not intercalate into DNA or poly(dG-dC) (Pasternack et al., 1983)], coplanarity of the pyridyl substituent with the porphine ring system is not required since ΔG^* for this process is ~15 kcal (Eaton & Eaton, 1975; Eaton et al., 1978). The conclusion to be drawn from these results is that even for poly(dG-dC), which is the most rigid of the polynucleotides studied here (Early et al., 1981a,b), open regions and/or structural fluctuations must be present in the duplex to account for the relatively rapid incorporation of the porphyrin molecule. These sites must be limited in size since none of H_2TMpyP-2, tetrakis(*p*-*N*-trimethylanilinium)porphine, nor di-*tert*-butylproflavin intercalates (Carvlin et al., 1982; Müller et al., 1973).

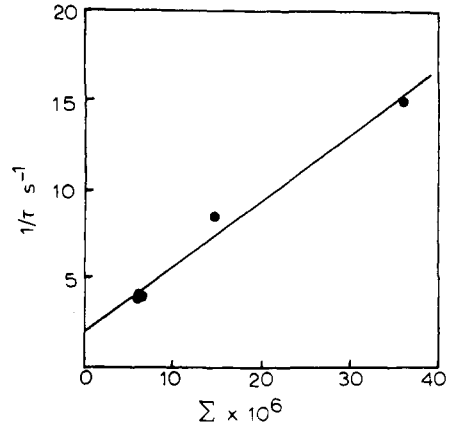

FIGURE 7: Variation of the reciprocal relaxation time with Σ for H_2TMpyP-4 with poly(dG-dC). See eq 9 for the definition of Σ.

For the copper(II) and nickel(II) derivatives as well as the nonmetalloporphyrin, the dissociation rates of P/GC complexes via salt jumps become slower as the final ionic strength increases. Increased ionic strength promotes the dissociation of these complexes; i.e., K_{app} decreases as μ increases. Therefore, although increasing μ makes the dissociation reaction more favorable thermodynamically, it inhibits it kinetically. We propose that the nucleic acid undergoes a very rapid contraction ($t_{1/2}$ <2 ms) when it encounters the high salt concentration due to the partial neutralization of the negative charges of the phosphate backbone by the ion densities in the solvent. The intercalated porphyrin then meets with additional steric resistance to its sliding out from between the base pairs and into the solution.

In contrast to the kinetic results already discussed, no kinetic effects are observed for the axially liganded metalloporphyrins [i.e., the iron(III), cobalt(III), zinc(II), and manganese(III) derivatives] with poly(dG-dC), indicating that these reactions are much more rapid than those for the non axially liganded derivatives. A consistent picture emerges from the lack of kinetic effects and the static results described earlier (Pasternack et al., 1983), i.e., that the axially liganded porphyrins show small positive or no induced CD band and small red shifts and hypochromicity with poly(dG-dC). These results point to minimal involvement of the axially liganded porphyrin derivatives with the bases of poly(dG-dC), that is, to interactions which are primarily electrostatic involving the phosphate backbone.

The kinetic results with poly(dA-dT) indicate that the association and dissociation reactions of all the porphyrins (axially liganded or not) are too rapid to be measured by the stopped-flow technique and, where data are available, the temperature-jump technique. The sterically hindered, axially liganded metalloporphyrins interact with poly(dA-dT) more extensively than with poly(dG-dC), suggesting that the complexes with poly(dA-dT) are not of an intercalation type although they may still involve some interaction with the bases of this polymer. Otherwise similar spectral and CD results

would be anticipated for the axially liganded derivatives with poly(dA-dT) as are obtained for these substances with poly(dG-dC). The type of external complexes formed at AT sites in which overlap with the bases is made possible by helical distortions is energetically too costly for the more stable poly(dG-dC) duplex. This same extra helix stability permits intercalation of non axially liganded porphyrins at GC sites whereas AT regions are incapable of accommodating porphyrins in the intact duplex.

Thus, the non axially liganded porphyrins form stable intercalated complexes with poly(dG-dC) but external complexes with poly(dA-dT); the axially liganded metalloporphyrins form external complexes with poly(dA-dT) which involve a larger degree of drug/base overlap than the corresponding complexes with poly(dG-dC).

In temperature-jump studies of the nonmetallo, nickel(II) and copper(II) derivatives with DNA, both the amplitude profile, in which the magnitude of the relaxation effect is constant at large $1/r_0$, and the lack of concentration dependence of the relaxation times suggest that the processes being observed involve the redistribution of the porphyrin among the various sites on the polynucleotide. Each relaxation curve is composed of three coupled effects for these systems (cf. Tables III–V). The observed coupled effects may be due to a multistep redistribution process or the existence of more than two bound species, depending perhaps on the nearest-neighbor base composition. Those metalloporphyrins showing base specificity for AT regions [cobalt(III), iron(III), and manganese(III) derivatives] show no relaxation effects with DNA. These derivatives do not intercalate in DNA (Pasternack et al., 1983).

Very few metal-containing intercalating agents have been investigated in which the metal ion has been shown to form part of the intercalating region. Among the few examples to be found are square coplanar platinum(II) complexes (Lippard et al., 1976; Lippard, 1978). Although $[Pt(en)(o\text{-phen})]^{2+}$ and $[Pt(bipy)(en)]^{2+}$ intercalate in DNA, $[Pt(en)(py)]^{2+}$ does not, due to nonbonded steric repulsions between adjacent pyridine rings in the coordination sphere. These repulsions prevent the coplanarity of the portion of the molecule which intercalates. The results described here confirm and expand on these earlier results. The porphine core is planar, and although peripheral substituents are out of the plane, the porphyrin is capable of intercalating, a situation similar to that for ethidium ion. In the present work, the ligand system is maintained throughout; it is the identity of the metal ion which is changed. This metal ion variation leads to dramatic differences in the base specificity of the drug and the nature of the interaction with nucleic acids. However, the underlying principle remains the same as for the platinum drugs; there is a limiting thickness which cannot be exceeded by the portion of the drug molecule which is to intercalate.

To obtain a better understanding of the redistribution process of H_2TMpyP-4 in DNA, transfer experiments were conducted with the synthetic polynucleotides. Although extrapolations from the synthetic system to the natural one should be treated with caution (for example, there are no AT/GC interface regions in the synthetic systems), it has been shown in other studies that binding isotherms and kinetic results from drug binding to DNA can be simulated as a superposition of interreactions with homopolymers (Sturm et al., 1981). In the transfer experiments described here at $1/r_0 = 15.3$, better than 95% of H_2TMpyP-4 is bound to one polymer or the other. The obtained relaxation data are for the process

$$\text{P·AT} + \text{GC} \rightleftharpoons \text{P·GC} + \text{AT} \qquad (10)$$

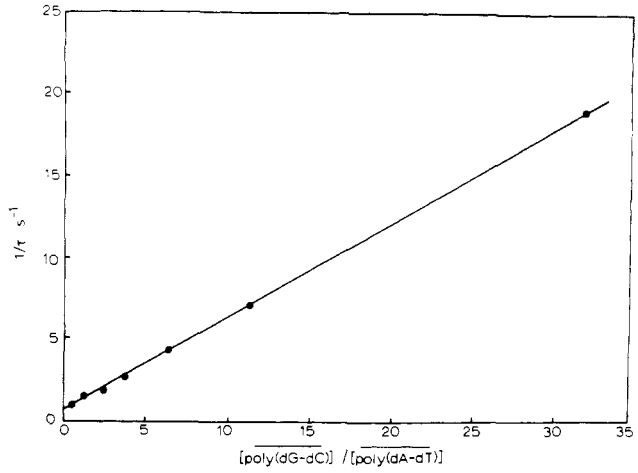

FIGURE 8: Plot of data for dissociation transfer mechanism B. Conditions: $[H_2TMpyP\text{-}4] = 6.15 \times 10^{-6}$ M; $\mu = 0.2$ M; pH 6.8; 25 °C.

and two general mechanisms are considered. In one (mechanism A below), the porphyrin moves from one nucleic acid to another without dissociation into the solvent. The second possibility (mechanism B) involves a two-step process with the porphyrin first dissociating from one polymer lattice and then binding to the other polymer.

(A) direct transfer mechanism

$$\text{P·AT} + \text{GC} \underset{k_{-T}}{\overset{k_T}{\rightleftharpoons}} \text{P·GC} + \text{AT} \qquad (11)$$

$$1/\tau([\overline{\text{P·GC}}] + [\overline{\text{AT}}]) = k_T\frac{[\overline{\text{P·AT}}] + [\overline{\text{GC}}]}{[\overline{\text{P·GC}}] + [\overline{\text{AT}}]} + k_{-T} \qquad (12)$$

(B) dissociation transfer mechanism

$$\text{P·AT} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \text{P} + \text{AT}$$

$$\text{P} + \text{GC} \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} \text{P·GC} \qquad (13)$$

Assuming $d[P]/dt = 0$ and $k_{-1}[\overline{AT}] >> k_2[\overline{GC}]$

$$1/\tau = \frac{k_2[\overline{\text{GC}}]}{K_1[\overline{\text{AT}}]} + k_{-2} \qquad (14)$$

In order to construct the appropriate kinetic plots, the concentrations of free polymer need to be calculated. This was accomplished in a manner analogous to the procedure outlined for the analysis of the poly(dG-dC)/H_2TMpyP-4 temperature-jump data. It was assumed that $n = 2$ for poly(dA-dT) as it is for poly(dG-dC) and that all of the porphyrin is bound. To simplify the calculations, cooperativity factors, which may be important for the poly(dA-dT) duplex, have been ignored.

A linear plot of the data is obtained only for the dissociation mechanism (mechanism B), yielding a slope of 0.57 s^{-1} (k_2/K_1) and an intercept of 0.64 s^{-1} (k_{-2}) (cf. Figure 8). The slope to intercept ratio equals K_2/K_1, and from the measured value of $K_{app} = 7.7 \times 10^5$ $M^{-1} = K_2$, a value of $K_1 \sim 9 \times 10^5$ M^{-1} for poly(dA-dT) is calculated. When this value for K_1 is used, a value for k_2 of $\sim 5 \times 10^5$ M^{-1} s^{-1} is obtained. These values for k_2 and k_{-2} are in good agreement with those obtained from the direct binding experiments of H_2TMpyP-4 to poly(dG-dC).

A consideration of whether this dissociation mechanism might be applicable to the redistribution of porphyrin on calf thymus DNA begins with eq 14 and an estimate that, at small r_0, $[\overline{GC}]/[\overline{AT}] = 0.72$ (Müller et al., 1973). A value for $1/\tau$ is thus calculated as 1.1 s^{-1}. However, even the slowest of the

several relaxation processes observed for DNA is an order of magnitude faster than this calculated value. This suggests that for DNA, the redistribution of porphyrin does not occur via a dissociative mechanism but rather by a direct internal transfer of the drug from one position on the molecule to another.

Drug transfer mechanisms have been discussed by a number of other workers (Bresloff & Crothers, 1975; Capelle et al., 1979; Wakelin & Waring, 1980). The bifunctionality of several acridine derivatives was considered essential to their direct transfer from one polynucleotide molecule to another with the proposed mechanism involving a ternary complex intermediate (Capelle et al., 1979). Bresloff & Crothers (1975) had suggested a similar mechanism earlier for the transfer of ethidium from DNA to RNA, but more recent work (Wakelin & Waring, 1980) on ethidium derivatives has been interpreted as not involving such a transient ternary species. Much like the ethidium ion, H_2TMpyP-4 can be considered as monofunctional with the added advantage that, unlike the ethidium and acridine drugs, this porphyrin does not aggregate under the conditions of these experiments. Thus, no assumptions need be made here as to the attacking form of the drug.

In the cases studied previously, the relaxation time for the transfer process showed a concentration dependence, suggesting that the predominant (or exclusive) pathway involves the transfer of the drug from one nucleic acid molecule to another. Only for 10-methyl-9-aminoacridine is there any evidence of an "internal" transfer pathway (Wakelin & Waring, 1980). With this drug, Wakelin and Waring observe two kinetic processes which vary linearly with DNA concentration and do not saturate. This indicates that the major pathway is a bimolecular or external transfer process. A more detailed analysis of their kinetic data suggests that a small concentration-independent term also exists. This concentration-independent term may reflect a minor pathway in which a direct internal transfer process is occurring. In contrast, in the present work, the total lack of a concentration dependence in τ, the form of the relaxation amplitude profile, and the values of the relaxation times (when contrasted with values calculated for a dissociative mechanism) all lead to the conclusion that H_2TMpyP-4 redistributes on DNA via a *direct internal* transfer. Thus, H_2TMpyP-4 is the *first* drug studied in which the major transfer pathway is a *direct internal* process.

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Registry No. Poly(dG-dC), 36786-90-0; poly(dA-dT), 26966-61-0; H_2TMpyP-4, 38673-65-3; Cu^{II}TMpyP-4, 48242-70-2; Ni^{II}TMpyP-4, 48242-71-3; Fe^{III}TMpyP-4, 60489-13-6; Mn^{III}TMpyP-4, 70649-54-6; Co^{III}TMpyP-4, 51329-41-0; Zn^{II}TMpyP-4, 40603-58-5.

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