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# Interactions of water soluble porphyrins with Z-poly(dG-dC)

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## ABSTRACT

The water soluble porphyrin tetrakis(4-N-methylpyridyl)porphine ($H_2$TMpyP) and its copper(II) derivative (CuTMpyP) convert Z-poly(dG-dC) to the B-form. For $H_2$TMpyP, the fraction Z character (fr-Z) is given by fr-Z = 1.0 - 21 $r_o$ and for CuTMpyP, fr-Z = .94 - 12 $r_o$ where $r_o \equiv [Porphyrin]_o / [DNA]_o$. Neither the manganese(III) derivative of this porphyrin (MnTMpyP) nor tetrakis(2-N-methylpyridyl)porphine ($H_2$TMpyP-2) is nearly as effective at causing the conversion. The former two porphyrins have been shown to intercalate into B-poly(dG-dC) whereas the latter two porphyrins do not.

The kinetics of the Z→B conversion are independent of porphyrin or poly(dG-dC) concentration for $1/r_o > 6$. At smaller values of $1/r_o$, the conversion rate is greatly increased for $H_2$TMpyP and CuTMpyP. The interaction of these porphyrins with Z-poly(dG-dC) follows simple first order kinetics in this latter concentration range. It is proposed that for small values of $1/r_o$ the sequence of events begins with a porphyrin-unassisted distortion of the Z-duplex (with a rate constant of 0.6 $s^{-1}$) followed by a rapid uptake of porphyrin in what may be an intercalative mode. The porphyrin thus located in Z-regions brings about rapid conversion to the B-form. Binding of $H_2$TMpyP or CuTMpyP to B-regions of a predominantly Z-strand leads to conversion of Z to B. However, this conversion process is considerably slower than when the porphyrins bind directly to Z-regions.

## INTRODUCTION

Since its discovery in 1972 (1) and the determination of its structure (2) Z-DNA has been propelled to a central position in many research laboratories. Reports suggesting a biological role for the Z-form (3) have served to further enhance the intense interest shown in this conformer. Many of the more chemically oriented studies have focussed on the Z→B transition and the influence of small molecules on the rate and extent of conversion. Most of these workers have concluded that ligands do not interact extensively with Z-DNA (4,5) although reports to the contrary have recently appeared (6,7). In the present report, results are presented on the reactions of several water soluble porphyrins with Z-poly(dG-dC), and the claim is made that two of these species interact extensively with the Z conformer.

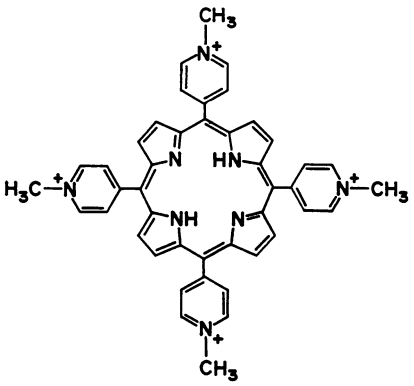

Figure 1. Tetrakis(4-N-methylpyridyl)porphine ($H_2$TMpyP).

In previous work it has been shown that two of the porphyrin species considered here, tetrakis(4-N-methylpyridyl)porphine ($H_2$TMpyP, Figure 1) and its copper(II) derivative (CuTMpyP), intercalate into B-poly(dG-dC) while neither the manganese(III) derivative (MnTMpyP) nor tetrakis(2-N-methylpyridyl)porphine interact extensively with this duplex (8-10). Intercalation leads to a large bathochromic shift with extensive hypochromicity of the porphyrin Soret band and to the formation of a prominent negative feature in the circular dichroism spectrum in the Soret region (9). Stability constants determined for the interaction with B-poly(dG-dC) at $\mu$ = 0.2 M, 25°C, are 7.7 x $10^5$ $M^{-1}$ for $H_2$TMpyP and 8.0 x $10^5$ $M^{-1}$ for CuTMpyP.

The kinetics of the interactions have been measured using stopped-flow and temperature-jump techniques (10). For $H_2$TMpyP, monophasic relaxation effects were obtained whose relaxation time and amplitude profile are consistent with a second-order process of the type:

$$H_2TMpyP + \text{B-poly(dG-dC)} \underset{k_{out}}{\overset{k_{in}}{\rightleftharpoons}} H_2TMpyP\cdot\text{B-poly(dG-dC)} \qquad (1)$$

An interaction rate constant of $k_{in}$ = 5 x $10^5$ $M^{-1}$ $s^{-1}$ was obtained under the conditions of these experiments (10). The relaxation profile obtained with CuTMpyP is biphasic involving a second-order rate constant similar to that obtained with the metal-free derivative and a faster first-order process (10,11). These spectral and kinetic patterns help form the basis for the comparison of the interactions of $H_2$TMpyP and CuTMpyP with B- and Z-poly(dG-dC).

MATERIALS AND METHODS

Poly(dG-dC) was purchased from Pharmacia P-L Biochemicals. It was purified and its ionic strength established by extensive dialysis against a buffer solution consisting of 5 mM Tris (Eastman Organic) and 50 mM NaCl (Fisher Scientific). The pH of all solutions was maintained between 7.5 and 8. Concentrations of poly(dG-dC) solutions in base pairs/liter were determined spectrophotometrically using a molar absorptivity of $1.68 \times 10^4$ $M^{-1}$ $cm^{-1}$ at 254 nm (12). The Z-form of poly(dG-dC) was induced using hexaamminecobalt(III) chloride (Eastman Organic) at a concentration of 50-70 μM (13). For comparison, a few experiments were conducted in which the Z-form was prepared in an alcohol/water mixed solvent system 56.2% ethanol by weight (14). The high salt method for forming Z-poly(dG-dC) (1) was not employed because the porphyrin species considered here do not bind to DNA at these high ionic strengths (9,10). The Z-character of the polymer was confirmed either by comparison of the CD spectrum with published Z-form spectra or by evaluation of the ratio of the absorbances at 260 nm and 295 nm; A260/A295. The reported ratio is 3.2 for Z-form and 8.5 for B-form poly(dG-dC) (1). It had been previously shown that under the experimental conditions employed here there is little if any aggregation of Z-poly(dG-dC) (15). Indeed the ultraviolet absorbance and circular dichroism spectra were those expected for the Z-form of poly(dG-dC) with no absorbance at wavelengths greater than 300 nm nor evidence of scattered uv light due to aggregation (16). All solutions were protected from light. There is some evidence of duplex modification or degradation in this medium upon long exposure to fluorescent light.

The tosylate salts of tetrakis(4-N-methylpyridyl)porphine ($H_2$TMpyP) and tetrakis(2-N-methylpyridyl)lporphine ($H_2$TMpyP-2) were obtained from Mid-Century Chemical Company. The copper(II) derivative (CuTMpyP) and Mn(III) derivative (MnTMpyP) were prepared as previously described and concentrations of all porphyrin solutions were determined from $\varepsilon = 1.82 \times 10^5$ $M^{-1}$ $cm^{-1}$ for $H_2$TMpyP-2; $\varepsilon = 2.26 \times 10^5$ $M^{-1}$ $cm^{-1}$ for $H_2$TMpyP; $\varepsilon = 2.31 \times 10^5$ $M^{-1}$ $cm^{-1}$ for CuTMpyP and $\varepsilon = 0.92 \times 10^5$ $M^{-1}$ $cm^{-1}$ for MnTMpyP at the Soret maxima (17-20). These porphyrins had been previously reported to be monomeric under the conditions of these experiments. A recent report has appeared in which it was suggested that unusual fluorescent features of $H_2$TMpyP at nanomolar concentrations may be due to aggregation (21). However, a variety of experimental evidence has been mounted which appears to refute this interpretation (22).

Absorption spectra were obtained using a Varian 2200, Cary 14 or Perkin-Elmer 323 uv/vis spectrophotometer. Circular dichroism spectra were taken on a Jasco Model J-40A spectrometer as well as a Cary 60 spectrometer modified by J. Aviv and Associates. Kinetic measurements were made either with the Aviv CD, the Varian

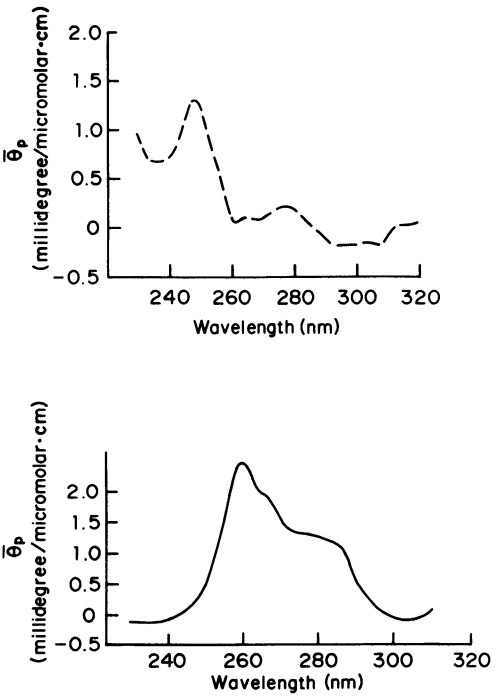


Figure 2. Induced CD spectra in the ultraviolet region of porphyrins bound to excess B-poly(dG-dC). (—) $H_2$TMpyP, (---) CuTMpyP. See text for experimental details.

2200 spectrophotometer or a Durrum Model 110 stopped-flow instrument interfaced to a Cromemco Z-2 microcomputer. In the stopped-flow experiments, equal concentrations of $Co(NH_3)_6^{3+}$ were added to both the polynucleotide and porphyrin solutions to eliminate its concentration as a variable upon mixing. Kinetic runs were conducted at 25°.

## RESULTS

### Static Experiments

The addition of $H_2$TMpyP or CuTMpyP to Z-poly(dG-dC) prepared by using either the $Co(NH_3)_6^{3+}$ or alcohol method brings about the conversion of the polymer to the B-form. However, at very high ratios of base pairs to porphyrin ($1/r_O \geq 250$; $r_O \equiv [porphyrin]_O / [DNA]_O$) the CD spectra obtained in the ultraviolet region correspond to the Z-form. Under these same conditions, the absorption maxima of the porphyrins in the Soret region are near 440 nm and a CD spectrum is induced at this wavelength

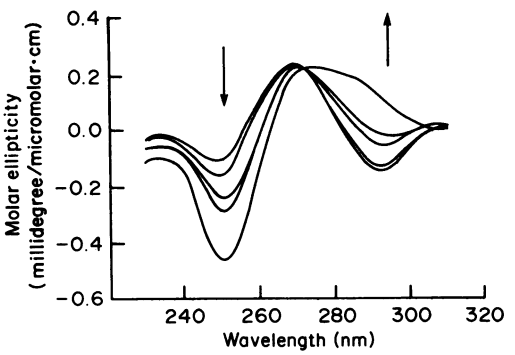


Figure 3. Superimposed CD spectra for the titration of Z-poly(dG-dC) with $H_2$TMpyP, corrected for porphyrin contribution. The concentration of poly(dG-dC) is 50 μM and is converted to Z-form with 50 μM $Co(NH_3)_6^{3+}$. The porphyrin concentration ranges from 0 to 2.5 μM.

having a prominent negative feature. Both of these spectral properties are characteristic of an intercalated porphyrin moiety (9). However, as will be discussed later, these results alone should not be taken as confirming evidence of the intercalation into Z-poly(dG-dC).

Titrations of Z-poly(dG-dC) with $H_2$TMpyP and CuTMpyP were conducted using both absorbance and circular dichroism detection. As part of this effort, the uv-induced CD spectrum of the porphyrins when bound to B-poly(dG-dC) were determined. A series of porphyrin-B-poly(dG-dC) solutions were considered in which $5 \leq 1/r_o \leq 20$; under the conditions of these experiments the porphyrins are completely bound to the nucleic acid (9, 11). The molar ellipticity of the porphyrin was determined from the formula

$$\bar{\theta}_P = \frac{\theta - \bar{\theta}_B[\text{poly(dG-dC)}]_o}{[\text{Porphyrin}]_o} \qquad (2)$$

where θ is the experimental ellipticity of the porphyrin-polynucleotide complex at some wavelength and $\theta_B$ and $\theta_P$ are the molar ellipticities of the polynucleotide and porphyrin respectively at that wavelength. Consistent calculated spectra for the porphyrin induced CD were obtained over the concentration range considered. Figure 2 shows the induced CD spectra of $H_2$TMpyP and CuTMpyP in the ultraviolet region. A knowledge of the induced porphyrin CD spectra is of considerable value in the experimental design and analysis of titration and kinetic experiments.

Shown in Figure 3 are superimposed CD spectra obtained for the $H_2$TMpyP titration of Z-poly(dG-dC). These experiments were all conducted at sufficiently low $r_O$ that the induced CD of the bound porphyrin makes only a very small contribution. The spectra were corrected by subtracting out the porphyrin contribution using the data of Figure 2. An isoelliptic point is obtained at 270 nm. The appearance of this feature encouraged us to attempt a two state model to fit the data in which it was assumed that the polymer (or segments thereof) were either in the B-form or the Z-form with intermediate states of negligible importance. The fraction of Z character (fr-Z) was determined at several wavelengths from the equation

$$fr\text{-}Z = \frac{\bar{\theta} - \bar{\theta}_B}{\bar{\theta}_Z - \bar{\theta}_B} \qquad (3)$$

where $\bar{\theta}_B$, $\bar{\theta}_Z$ and $\bar{\theta}$ are the molar ellipticities of the B-form, Z-form and the mixture respectively. The results for CuTMpyP are shown in Figure 4. For CuTMpyP, fr-Z = .94 - 12 $r_O$, $r_O \leq .08$ while for $H_2$TMpyP, fr-Z = 1.0-21 $r_O$, $r_O \leq .05$. Similar results are obtained from absorbance data as shown in Figure 5 for $H_2$TMpyP. The equilibrium CD spectra at any ratio can be compared with simulated spectra by again assuming no appreciable concentration of intermediates. As shown in Figure 6 for $1/r_O = 50$, the simulated and experimental spectra are virtually identical.

Similar titration experiments were conducted with $H_2$TMpyP-2 and MnTMpyP

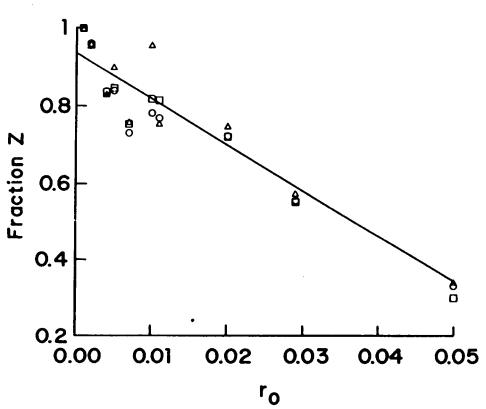


Figure 4. Fraction of Z character as a function of $r_O$ ($r_O \equiv [Porphyrin]_O/[DNA]_O$) for CuTMpyP. The concentration of poly(dG-dC) is 35 μM and is converted to Z-form with 65 μM $Co(NH_3)_6^{3+}$. The porphyrin concentration varies from 0 to 3.5 μM. The detection is via circular dichroism (O) at 290 nm (□) 250 nm and (Δ) 266 nm.

neither of which intercalate into DNA (9,10). The experiments were conducted over the range $8.7 \leq 1/r_O \leq .9$. For $H_2$TMpyP-2 above $1/r_O = 4$, poly(dG-dC) exhibited a normal Z-form CD spectrum. Even at a $1/r_O$ ~1, the fraction -B character did not exceed about 15-20%. MnTMpyP is somewhat more effective at converting Z-poly(dG-dC) to the B-form than is $H_2$TMpyP-2. At $1/r_O = 8.7$, the polymer has about 25% B-character while at $1/r_O = 6$, the poly(dG-dC) shows about 60% B-character. By way of comparison, for the Cu(II) derivative, by a $1/r_O \approx 20$ the polymer is completely converted to the B-form.

Kinetics Experiments

The kinetics of conversion of Z-poly(dG-dC) to the B-form upon the addition of $H_2$TMpyP or CuTMpyP were studied using standard hand-mixing techniques and were monitored by direct absorbance measurements at 295 nm or by CD at 250 or 290 nm. These wavelengths were chosen for observation because the porphyrin spectra interfere minimally (cf Figure 2). All of the kinetic runs were multiphasic similar to those observed by other workers (1,4,5). The data were analyzed by two different methods. In the first of these, only the early part of the conversion was analyzed. Such an approach had been previously justified on the basis of differences in affinity of ligands for the B- and Z- helices (5). Thus, the binding densities of the drugs may vary as the conversion proceeds. Results of this analysis are shown in Table 1. A second approach involved the use of the DISCRETE routine (23) to analyze the data of the complete kinetic run as a series of coupled exponentials. In almost all cases the data for the Z→B conversion could be satisfactorily analyzed as being biphasic yielding rate constants $k_f$ and $k_s$. The results of the biphasic fit are also shown in Table 1 for comparison. Both sets of analyses seem to lead to the same conclusion; i.e., the rate

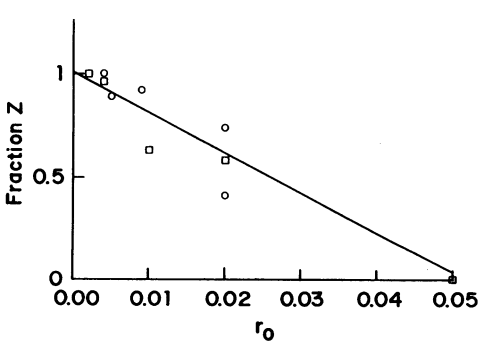


Figure 5. Fraction of Z character as a function of $r_O$ for $H_2$TMpyP. The detection is via direct absorbance at 295 nm (O) and circular dichroism at 250 nm (□).

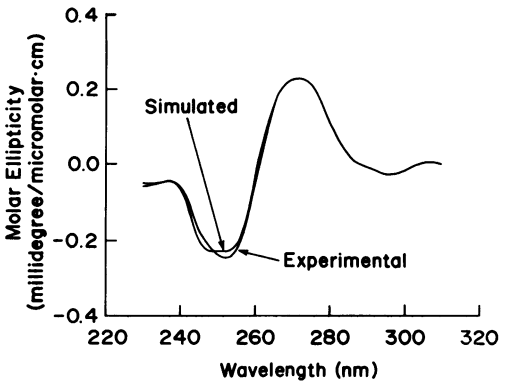


Figure 6. Simulated and experimental CD spectra of poly(dG-dC) with 58% Z character. $[Poly(dG\text{-}dC)]_O = 50\ \mu M$, $[H_2TMpyP]_O = 1\ \mu M$.

constant(s) for the conversion of Z-poly(dG-dC) to B-poly(dG-dC) are independent of porphyrin and of polynucleotide concentrations over a considerable concentration range with $k_f = .04\ s^{-1}$ and $k_s = .004\ s^{-1}$ for $H_2TMpyP$ and $k_f = .03\ s^{-1}$ and $k_s = .002\ s^{-1}$ for CuTMpyP. However, when the ratio of base pairs to porphyrin ($1/r_O$) is less than about six, the rate of conversion increases markedly and becomes highly porphyrin dependent. A plot of $k_s$ vs. $r_O$ is shown in Figure 7 for CuTMpyP.

Several kinetic runs were conducted using CD detection at 290 nm in which MnTMpyP was employed to bring about the conversion of the Z-form and in one run no porphyrin was added but instead the NaCl concentration was raised to 1 M. In all these cases the kinetics of conversion were biphasic with rate constants of $k_f \sim 0.03\ s^{-1}$ and $k_s \sim .003\ s^{-1}$, i.e., no enhanced conversion rate was observed for these systems. Furthermore, for the Mn(III) derivative at $r_O$ values approaching unity, the final polymer conformation was neither B- nor Z-form using CD criteria. Under these same conditions, $H_2TMpyP$ and CuTMpyP produce B-form DNA.

When the conversion at $1/r_O > 6$ is monitored near 420 nm (at the Soret maximum of the unbound porphyrin) or at 440 nm (the Soret maximum of the product complex) for $H_2TMpyP$ or CuTMpyP, the observed kinetic processes are in good agreement with those obtained for the Z→B conversion using absorbance or CD detection in the uv region. However, about 90% of the color change at the Soret maxima occurs within the time required to hand mix the reagents. These more rapid reactions of $H_2TMpyP$ and CuTMpyP with Z-poly(dG-dC) were studied via the stopped-flow technique and detected in the Soret region. In addition, several stopped-flow runs were conducted at 295 nm for comparison.

Table 1
Kinetics of Conversion of Z-poly(dG-dC) to the B-form, 25°C

i) Summary of first order rate constants obtained from the initial part of the Z to B conversion

| [$H_2$TMpyP], μM | [poly(dG-dC)], μM | $1/r_o$ | $k_c(s^{-1})$ | Method |
|---|---|---|---|---|
| 2.5 | 25 | 10 | .021 | CD 290 |
| 1.25 | 25 | 20 | .032 | CD 250 |
| 1.25 | 25 | 20 | .029 | CD 290 |
| 5.2 | 52 | 10 | .056 | uv 295 |
| 10.0 | 52 | 5 | .028 | uv 295 |
| 2.6 | 52 | 20 | .028 | uv 295 |
| 1.3 | 26 | 20 | .017 | uv 295 |
| 2.5 | 50 | 20 | .035 | vis421 |
| 2.5 | 50 | 20 | .033 | vis444 |

ii) Summary of first order rate constants obtained from complete analysis of data

| [Porph], μM | [poly(dG-dC)], μM | $1/r_o$ | $k_f(s^{-1})$ | $k_s(s^{-1})$ |
|---|---|---|---|---|
| a) $H_2$TMpyP | | | | |
| 2.5 | 25 | 10 | .039 | .0067 |
| 1.25 | 25 | 20 | .058 | .0024 |
| 5.2 | 52 | 10 | .043 | .0024 |
| 2.6 | 52 | 20 | .043 | .0058 |
| 1.3 | 26 | 20 | .031 | .0017 |
| 5.0 | 25 | 5 | vf | - |
| b) CuTMpyP | | | | |
| 2.5 | 35 | 14 | .011 | .0008 |
| 3.0 | 35 | 12 | .027 | .0016 |
| 4.0 | 35 | 9.0 | .043 | .0036 |
| 4.5 | 35 | 7.8 | - | .0039 |
| 4.8 | 35 | 7.3 | .019 | .0017 |
| 5.5 | 35 | 6.4 | .020 | .0016 |
| 5.9 | 35 | 5.9 | .044 | .0018 |
| 6.2 | 35 | 5.6 | vf | .0088 |
| 6.5 | 35 | 5.4 | vf | .0079 |
| 7.0 | 35 | 5.0 | vf | .026 |

(vf) too fast to measure by hand-mixing.

The rapid reaction of $H_2$TMpyP and CuTMpyP with Z-poly(dG-dC) proves to be simple first-order at small values of $1/r_o$. The results are summarized in Table 2. The first-order rate constant determined at the reactant Soret peak and at 295 nm is $k_{exp}$ ~ .6 $s^{-1}$ independent of whether the Cu(II) or nonmetallo derivative is used. The rate

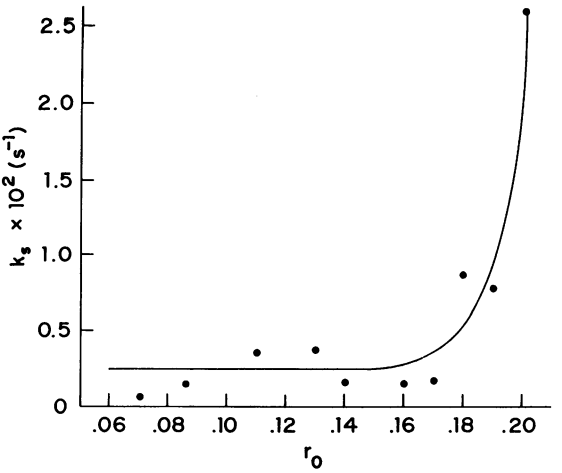


Figure 7. Plot of $k_s$ vs. $r_o$ for the conversion of Z- to B-poly(dG-dC), [Poly(dG-dC)] = 35 μM, $[Co(NH_3)_6^{3+}]$ = 60 μM. The kinetics were monitored via CD at 290 nm.

constant at the product Soret wavelength is about 30% smaller, $k_{exp}$ ~.4 $s^{-1}$ suggesting the formation of an intermediate in the course of the reaction. Initial rate studies were carried out to determine the form of the rate law. The analysis indicates that the initial rate varies with Z-poly(dG-dC) concentration but is independent of porphyrin concentration and thus,

$$\text{Rate} = k_{exp}[\text{poly(dG-dC)}] \quad (4)$$

Several aspects of this rapid kinetic process change with increasing $1/r_o$: (i) the absorbance versus time profile becomes biphasic albeit with the fast phase accounting for about 90% of the observed color change (cf. Table 3). The onset of this biphasic kinetic profile changes with Z-poly(dG-dC) preparation but generally is exhibited at $1/r_o > 4$. (ii) the experimental rate constant for the fast process ($k_f'$) shows a concentration dependence as shown in Table 3 whereas at lower values of $1/r_o$ no such dependence on [porphyrin] or [poly(dG-dC)] is observed (cf Table 2), and (iii) a substantial fraction of the color change is over within the mixing time of the instrument (cf Table 4). Shown also in Table 4 are the results of stopped flow experiments conducted with B-poly(dG-dC) under similar conditions for comparison.

## DISCUSSION

It had been previously proposed that even those molecules capable of intercalating into B-DNA do not react extensively with the Z-form (4,5,24). However, two recent reports have appeared on the intercalation of ethidium ion into Z-DNA (6,7).

Table 2
Stopped-flow kinetic results for the reaction of $H_2$TMpyP or CuTMpyP with Z-poly(dG-dC)

| [$H_2$TMpyP], μM | [CuTMpyP], μM | [Z-poly(dG-dC)], μM | λ | $k_{exp}$ ($s^{-1}$) |
|---|---|---|---|---|
| .92 | - | 2.15 | 421 | .56 |
| .92 | - | 4.25 | 421 | .59 |
| 1.95 | - | 2.15 | 421 | .55 |
| 3.0 | - | 5.6 | 421 | .61 |
| 25 | - | 25 | 295 | .40 |
| - | 3.0 | 3.0 | 425 | .55 |
| - | 3.0 | 6.0 | 425 | .60 |
| - | 3.0 | 9.0 | 425 | .66 |
| - | 6.0 | 3.0 | 425 | .61 |
| - | 6.0 | 15 | 425 | .69 |
| - | 9.0 | 15 | 425 | .66 |
| | 3.0 | 3.0 | 295 | .58 |
| - | 3.0 | 6.0 | 295 | .65 |
| - | 3.0 | 9.0 | 295 | .48 |
| - | 3.0 | 3.0 | 440 | .38 |
| - | 3.0 | 6.0 | 440 | .31 |
| - | 3.0 | 9.0 | 440 | .39 |
| - | 3.0 | 12 | 440 | .39 |
| - | 3.0 | 15 | 440 | .38 |
| - | 6.0 | 15 | 440 | .39 |
| - | 6.0 | 24 | 440 | .43 |
| - | 9.0 | 15 | 440 | .43 |

The accumulated evidence presented here leads us to conclude that $H_2$TMpyP and CuTMpyP like ethidium ion interact extensively with Z-poly(dG-dC) probably via intercalation.

Added $H_2$TMpyP or CuTMpyP tends to convert the Z-form of poly(dG-dC) to the B-form and, as can be seen in Figures 4 and 5, the fraction of Z-character remaining shows a linear dependence on the ratio of the concentration of porphyrin to base pairs.This result suggests that duplexes can exist partially as B-form and partially as Z-form. If an all or nothing transition were involved then once a critical ratio of porphyrin to base pairs was achieved, there would be a dramatic decrease in fr-Z and

Table 3
Biphasic Kinetics Results for the Reaction of CuTMpyP with Z-poly(dG-dC)[a]

[CuTMpyP] = 1.5 μM 25°C

| [poly(dG-dC)], μM | $a_1$ | $k_f'$ ($s^{-1}$) | $a_2$ | $k_s'$ ($s^{-1}$) |
|---|---|---|---|---|
| 4.5 | .24 | 1.0 | .016 | .24 |
| 6.0 | .25 | 1.6 | .017 | .23 |
| 9.0 | .23 | 2.4 | .017 | .24 |
| 12.0 | .17 | 3.5 | .021 | .35 |

a) Equation is of the form:

$$A - A_\infty = a_1 e^{-k_f' t} + a_2 e^{-k_s' t}$$

a large number of potential binding sites of high affinity would be created on the polymer. Added porphyrin would then be expected to preferentially populate these sites and would be ineffectual in converting any additional Z-polymers to the B-form. A more complicated profile of fraction-Z vs. $r_o$ reflecting this cooperativity would then be expected. The existence of B-type regions within a Z-polymer introduces an ambiguity in the interpretation of the static results. Can the observed spectral patterns at $1/r_o \sim 250$ (Soret maximum at ~ 440 nm and a large negative feature in the visible CD spectrum) be interpreted as arising from porphyrin intercalation into B-regions of a predominantly Z-strand? The kinetic results to be discussed below help to resolve this ambiguity. It is important to note that although the dependence of fr-Z on $r_o$ is linear for $H_2$TMpyP and CuTMpyP, the slopes are much larger than unity. Thus each porphyrin molecule is capable of converting 10-20 base pairs on the average of Z-form DNA to the B-form. Any model for this interaction must account for the linear profiles of Figures 4 and 5 in spite of the efficiency of the porphyrins in producing the conversion.

It should be noted that the two non-intercalating porphyrins, $H_2$TMpyP-2 and MnTMpyP, are not nearly as effective at converting Z- to B-DNA as is CuTMpyP or $H_2$TMpyP. Therefore, a pathway in which the more highly charged porphyrin ($H_2$TMpyP, CuTMpyP and $H_2$TMpyP-2 are all 4+; MnTMpyP is 5+) replaces $Co(NH_3)_6^{3+}$ in the condensed ion region of DNA leading to the conversion to the B-form is not of primary importance for $H_2$TMpyP and CuTMpyP although it could account for why MnTMpyP is somewhat more effective at the conversion than is $H_2$TMpyP-2. Rather, direct binding to the polymer seems to be critical for the efficient conversion. However, the static results do not require binding to the Z-form; binding of

Table 4

Absorbance Changes for Stopped-Flow Experiments[a]

[CuTMpyP] = 1.5 μM λ = 425 nm 25°C

i) B-poly(dG-dC)

| [poly(dG-dC)], μM | $A_o$ | $A_i$ | $A_\infty$ | $\Delta A_{total}$ |
|---|---|---|---|---|
| 1.5 | 0.58 | 0.54 | 0.52 | 0.06 |
| 3.0 | 0.58 | 0.45 | 0.37 | 0.21 |
| 4.5 | 0.58 | 0.37 | 0.27 | 0.31 |
| 6.0 | 0.58 | 0.32 | 0.24 | 0.34 |

ii) Z-poly(dG-dC)

| [poly(dG-dC)], μM | $A_o$ | $A_i$ | $A_\infty$ | $\Delta A_{total}$ | $A_i^{calc}$ |
|---|---|---|---|---|---|
| 1.5 | 0.54 | 0.54 | 0.44 | 0.10 | 0.53 |
| 3.0 | 0.54 | 0.52 | 0.32 | 0.22 | 0.52 |
| 4.5 | 0.54 | 0.47 | 0.22 | 0.32 | 0.48 |
| 6.0 | 0.54 | 0.44 | 0.19 | 0.35 | 0.44 |
| 9.0 | 0.54 | 0.41 | 0.19 | 0.35 | 0.40 |
| 12.0 | 0.54 | 0.37 | 0.19 | 0.35 | 0.35 |

a) Symbols used in Table 4:

$A_o$ = absorbance when porphyrin solution is flowed against an equal volume of buffer/ionic strength solution containing no DNA.

$A_i$ = initial absorbance observed when porphyrin solution is flowed against DNA.

$A_\infty$ = final absorbance for porphyrin-DNA experiment.

$\Delta A_{total} \equiv A_o - A_\infty$ . These are nearly identical for Z- and B-poly(dG-dC) at comparable concentration conditions.

CuTMpyP and $H_2$TMpyP to the B-form only would also promote the conversion. Once again the kinetic results are required to establish a case for binding to Z-DNA.

The kinetics of the conversion of Z-poly(dG-dC) to the B-form is independent of porphyrin and polymer concentration over an extended range. The conversion appears biphasic with rate constants which are roughly the same when $H_2$TMpyP, CuTMpyP, MnTMpyP or even NaCl are used to bring about the conversion. However, at a base pair to porphyrin ratio less than about six, a dramatic increase in conversion rate occurs for $H_2$TMpyP and CuTMpyP only. Similarly in the stopped-flow experiments for $H_2$TMpyP and CuTMpyP two different patterns of reactivity are observed. At low $1/r_o$, the stopped-flow results in the Soret region are consistent with a

simple first order process (Rate = 0.6 [poly(dG-dC)] with nearly the entire color change observable. At higher base pair to porphyrin ratios the kinetics becomes biphasic, with the major color change associated with a second-order process and a significant fraction of the total color change lost in the mixing time of the apparatus. This latter kinetic profile is reminiscent of the reaction of these porphyrins with B-DNA (10, 25 and Table 4). We consider then a model in which we claim that under the conditions of preparation of Z-poly(dG-dC) some small percentage of the polymer remains in the B-form. The range of $Co(NH_3)_6^{3+}$ that can be used for the conversion to the Z-form is limited - below about 50 μM a considerable fraction of the polymer remains as B whereas above 75 μM, problems of aggregation and condensation of the polynucleotide become severe (26). The percentage B-character in the polymer might well vary from preparation to preparation. For the data in Table 4b, if we assume about 9% of the polymer is in the B form, we can then calculate the amount of color change expected to be lost in mixing due to interaction with the B- regions of what is predominantly Z-poly(dG-dC). This permits a calculation of $A_i$, the initial absorbance observed in the stopped-flow experiment. The excellent agreement between $A_i^{calc}$ and those actually observed (cf Table 4b) encourages us to pursue this model further. We next determine the highest concentration of poly(dG-dC) for which greater than 80% of the color change is due to the reaction of the porphyrin with Z-poly(dG-dC) and find that for 1.5 μM CuTMpyP that concentration is 6 μM poly(dG-dC), a $1/r_O$ of 4. Above $1/r_O = 4$ reaction of CuTMpyP with the polymer will have appreciable contributions from reaction with the B-form, a process which has been determined as being second order (10). Below $1/r_O = 4$, the kinetic profiles are due almost exclusively to the porphyrin reacting with the Z-form and it is precisely in this range that the kinetics are simple first order. We suggest that under conditions in which simple first order kinetics are obtained, what is being observed is a distortion of Z-DNA followed by a rapid reaction with the porphyrin:

$$Z \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} Z^*$$

$$Z^* + P \xrightarrow{k_2} P \cdot Z$$

This leads to Rate = $k_1k_2[P][Z]/(k_{-1} + k_2[P])$ which for $k_2[P] > k_{-1}$ gives the observed rate law. The porphyrin ($H_2$TMpyP or CuTMpyP) is thus acting as an "indicator" for the slower reaction involving the distortion of the Z-polymer. The rate constant for this deformation step ($k_1$) is 0.6 $s^{-1}$ and shows no porphyrin dependence.

Thus we envision that for the Z- to B-DNA conversion at large base pair to porphyrin ratios, virtually all the porphyrin intercalates into B regions which leads to a slow conversion of Z to B ($k_f$ ~.03, $k_s$ ~.003). However, at low $1/r_O$ values (i.e., less than

about six base pairs per porphyrin), the B regions become saturated and porphyrin begins to populate the $Z^*$ portion. The location of porphyrin molecular ions in a $Z^*$ region brings about a very rapid conversion to B-DNA. The interaction of $H_2$TMpyP or CuTMpyP with the $Z^*$ region of the polymer must be quite extensive or otherwise the nonintercalating porphyrin cations (e.g., MnTMpyP) would be expected to show a similar rate enhancement of the Z→B conversion which is not observed.

In a recent paper Daune, et al.(27) discuss the difference in the binding reactions of two ultimate carcinogens, the acetylated and deacetylated forms of aminofluorene, to DNA as arising from their interactions with two distinct conformational states of the guanine residues in the helix of DNA. These workers suggest that an equilibrium exists between guanine residues with a 'native' B conformation and guanine residues with an 'altered' $G^*$ conformation. According to their model, the bulkier acetylated form of the carcinogen can covalently attach only to the $C_8$ of guanine residues in the $G^*$ conformation whereas the deacetylated drug preferentially attaches to the $C_8$ of guanine residues in the B conformation. It is estimated that approximately 10% of the guanine residues are in this altered $G^*$ state at room temperature. However, rate constants for this conversion process could not be obtained from the study because the rate of drug binding is slower than the conformational change. As indicated by Daune et al., such 'altered' conformations may be of general significance in other processes such as hydrogen exchange, B $\rightleftharpoons$ Z transition and premelting phenomena. It may well be that the conformational change, Z $\rightleftharpoons$ $Z^*$, that we have observed upon interaction of the bulky porphyrin cation with Z-poly(dG-dC) is a process similar to that which Daune, et al. have proposed at the guanine residues in native DNA. Whether the $Z^*$ conformation is similar or identical in nature to the $G^*$ conformation and whether the torsional crankshaft motions of the sugar - phosphate backbone (as suggested by Daune et al.) is the mechanism for these conformational conversions remains to be established. For the $H_2$TMpyP and CuTMpyP cations, the rate of binding to the nucleic acid polymer is orders of magnitude faster than the covalent attachment of the aminofluorene derivatives to DNA and is also faster than the rate of conversion of Z→$Z^*$ which allows a determination of the rate constant for this deformation process as 0.6 $s^{-1}$.

Perhaps also related to our findings is a recently reported (28) comparison of the rate of helix-opening for Z-poly(dG-dC) to the rate of hydrogen exchange of bound ethidium dimer. Whereas the rate constant for Z-helix opening is estimated as .007 $s^{-1}$ at 25° (28, 29), the mean exchange rate constant for protons on the intercalated drug is 0.6 $s^{-1}$ (30). The authors suggest that the proton exchange may be correlated with Z-DNA structural fluctuations accompanied by a modification of the phosphate sugar backbone (28). It should be noted that the rate constant determined by Ramstein (30)

for the proton exchange process and hence the structural fluctuation is identical to the $k_1$ reported here for Z→Z*. Thus it may well be that in our separate experimental strategies Ramstein and we have trapped and identified the same kinetic pathway for Z-poly(dG-dC) which involves a fundamental deformation mode of the helix.

In summary, we suggest that:

(i) $H_2$TMpyP and CuTMpyP can interact extensively with Z-DNA and that the complex thus formed converts rapidly to B-DNA. The interaction with the Z-form begins with a porphyrin-unassisted distortion characteristic of the polymer. This process is followed by a rapid intercalation of the porphyrin moiety and conversion to the B-form.

(ii) If regions of B-form are present in a strand which is predominantly Z-DNA, $H_2$TMpyP and CuTMpyP preferentially populate these sites. The effect on the conversion rate is then quite modest. The rate constants are about the same as those obtained when MnTMpyP or NaCl bring about the conversion presumably by displacing $Co(NH_3)_6^{3+}$ from the condensed ion phase of the DNA. $H_2$TMpyP-2 is not particularly effective at causing this displacement although it has a 4+ charge. The interaction of $Co(NH_3)_6^{3+}$ involves hydrogen bonding (31) as well as electrostatic attraction and, in addition, the position and orientation of the positive sites for $H_2$TMpyP-2 prevents simultaneous close approach for this nonintercalating derivative.

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