Adriamycin Inhibits the B to Z Transition of Poly(dGm^{5}dC)·Poly(dGm^{5}dC)†

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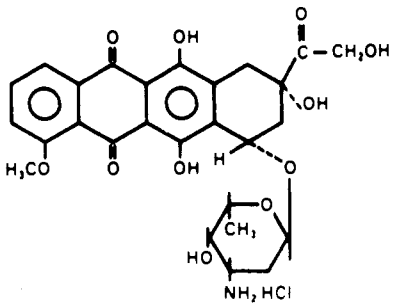
ABSTRACT: Adriamycin inhibits the Mg^{2+}-induced B to Z conformational transition of poly(dGm^{5}dC)·poly(dGm^{5}dC) and reduces the degree of cooperativity of the transition. Additionally, adriamycin alone converts the Z-form to the B-form in a cooperative manner. These results indicate that adriamycin binds preferentially to the B-form, which could be relevant to its mode of cytotoxic action.

It has been shown by circular dichroism (CD) spectroscopy that the left-handed Z-form of double-stranded poly(dGm^{5}dC) is formed at much lower salt concentrations than for the unmethylated copolymer (Behe & Felsenfeld, 1981). For example, the midpoint for the transition is reduced by a factor of ca. 10^3 for Mg^{2+}. Thus, this sequence is a covenient model to use for physicochemical studies of the properties of the Z-form of DNA at salt concentrations that are comparable to those experienced by DNA in the cell. We have investigated the B–Z transition of the methylated copolymer with ^{31}P NMR spectroscopy (Chen et al., 1983; Chen & Cohen, 1983). Upon the addition of several salts a peak with a characteristic chemical shift of −3 ppm appears, as for poly(dGdC) (Simpson & Shindo, 1979; Cohen et al., 1981) and its oligomers (Patel et al., 1979), but at correspondingly lower salt concentrations. We assigned this resonance (Chen et al., 1983) to dGpm^{5}dC in the tg$^+$ conformation of the Z-form (Wang et al., 1981), and this assignment has also been confirmed by phosphorothioate substitution (Jovin et al., 1983). The nature of the appearance of this peak indicates a slow interconversion from the B- to the Z-form. We now report CD and ^{31}P nuclear magnetic resonance (NMR) data on the effects of the cytotoxic antibiotic adriamycin (I) (Gianni et al., 1983) upon this transition.



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Experimental Procedures

Materials. Poly(dGm^{5}dC)·poly(dGm^{5}dC), poly(dGdC)·poly(dGdC), and poly(dAdT)·poly(dAdT) were purchased from P-L Biochemicals and were sonicated for 2–3 h to reduce the molecular sizes to between 50 and 230 base pairs (bp) (Chen et al., 1981). Adriamycin was obtained from the Clinical Pharmacology Branch of the National Cancer Institute.

^{31}P NMR Studies. ^{31}P NMR spectra were obtained at 109.3 MHz on a Nicolet 1180 computer and a Bruker superconducting magnet. Between 1000 and 2000 scans were acquired for each spectrum at 37 °C with a pulse repetition rate of 1.7 s and a spectral window of ±2000 Hz in 4K data points, and a line broadening of 5 Hz was applied. The sample concentration was typically 2 mg of DNA in 1 mL of buffer solution (ca. 6 mM in nucleotides) in 50% D_2O: i.e., 5 mM tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl) (pH 8.0)/50 mM NaCl/0.1 mM ethylenediaminetetraacetic acid (EDTA) for poly(dGm^{5}dC)·poly(dGm^{5}dC) and 50 mM Tris-HCl (pH 7.0)/100 mM NaCl/1 mM EDTA for the other sequences. Aliquots of 1 mM adriamycin were added to the DNA solution. All concentrations were corrected for dilution. The chemical shift values are negative upfield from internal trimethyl phosphate. The peak areas were obtained by fitting with the NTCCAP program on the Nicolet computer. The estimated error was ±5%. The constant area of the residual hybrid component at −4.3 ppm in this sample (ca. 20%) was subtracted from the total areas in the ^{31}P NMR data (Chen et al., 1983).

CD Spectroscopy. CD spectra were recorded on a JASCO J-500A spectrometer at room temperature. Each spectrum was an average of three runs and was processed on a Digital 1170 computer. The DNA sample usually contained ca. 100 μM nucleotides in 5 mM Tris-HCl (pH 8.0)/50 mM NaCl/0.1 mM EDTA. The B to Z transition of poly(dGm^{5}dC)·poly(dGm^{5}dC) in the absence or presence of adriamycin was achieved by stepwise addition of 40 mM $MgCl_2$, followed by brief heating (ca. 3 min) at 55–60 °C to facilitate the conversion to the Z-form (Behe & Felsenfeld, 1981) and then cooling to room temperature for CD measurement. On the other hand, the Z to B transition of the DNA with adriamycin in the presence of 2 mM $MgCl_2$ or 1 M NaCl was carried out without preheating. Solutions of adriamycin alone at comparable concentrations had no CD spectrum. All concentrations were corrected for dilution.

Curve Fitting. Curve fitting was carried out with the MLAB program on the DEC PDP10 computer at the Computer Center, NIH. The fitted parameter and root mean square (RMS) error values quoted are those obtained at convergence.

Results and Discussion

Adriamycin (I) is a cytotoxic antibiotic used widely in cancer chemotherapy that binds strongly to DNA and is known to intercalate (Gianni et al., 1983; Fritzsche et al., 1982). A study of the effects of daunorubicin, an analogue of adria-

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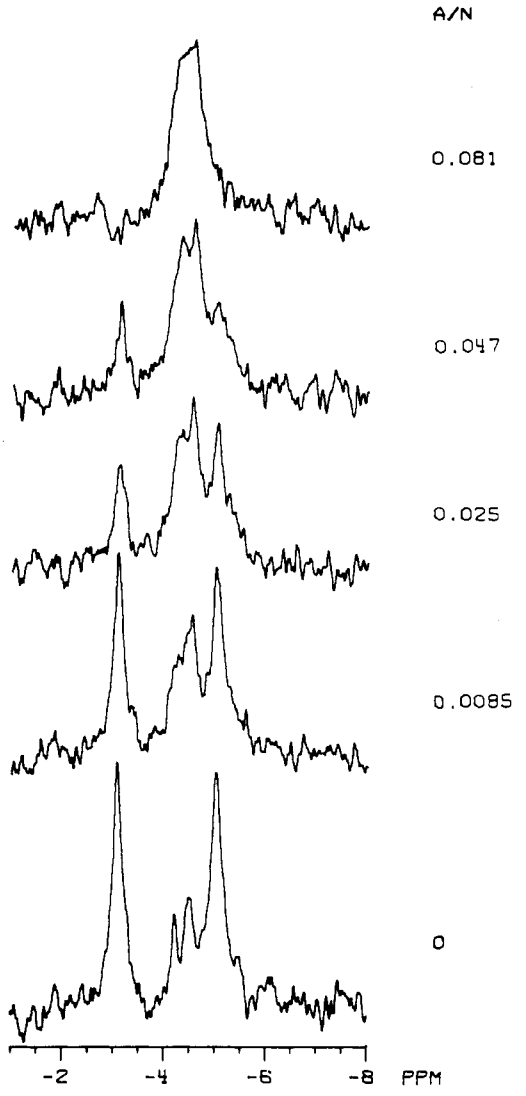

FIGURE 1: Effect of addition of adriamycin on the ^{31}P NMR spectrum of poly(dGm^{5}dC)·poly(dGm^{5}dC) at 37 °C. The Z-form was prepared by addition of 6 mM $MgCl_2$ in the presence of 50 mM NaCl, followed by heating to 55–60 °C, and was converted back to the B-form by adriamycin. The initial concentration of poly(dGm^{5}C)·poly(dGm^{5}C) in the Z-form was 6 mM in nucleotides, and aliquots of 0.1 mM adriamycin solution were added stepwise. The concentration ratios between adriamycin and poly(dGm^{5}dC)·poly(dGm^{5}dC) (in nucleotides) are as shown. The initial doublet at −4.3 ppm (ca. 20% of area) is due to residual poly(dGm^{5}dC)–template hybrid (Chen et al., 1983).

mycin, on ^{31}P NMR spectra of native DNA yielded results suggestive of the occurrence of a cooperative process in binding to DNA (Wilson & Jones, 1982). Adriamycin and daunorubicin have also been shown by phase partition techniques to bind cooperatively to DNA in a manner sensitive to metal ion concentration (Graves & Krugh, 1983). We found that addition of adriamycin to poly(dGm^{5}dC) in the B-form in low salt leads to a gradual broadening of the ^{31}P NMR signals, and similar results were observed for the unmethylated copolymer and for poly(dAdT)·poly(dAdT). However, addition of adriamycin to poly(dGm^{5}dC) in the Z-form resulted in a conversion to the B-form (Figure 1). The areas of the peaks corresponding to the two forms are plotted in Figure 2. It is not expected that the residual hybrid component (Chen et al., 1983) would in any way alter the nature of the Z to B transition. CD spectra similarly showed that adriamycin converted the Z-form of poly(dGm^{5}dC) in the presence of $MgCl_2$ (2 mM) to the B-form (Figure 3). It should be noted that the CD curve at the end point of the titration (curve 5,

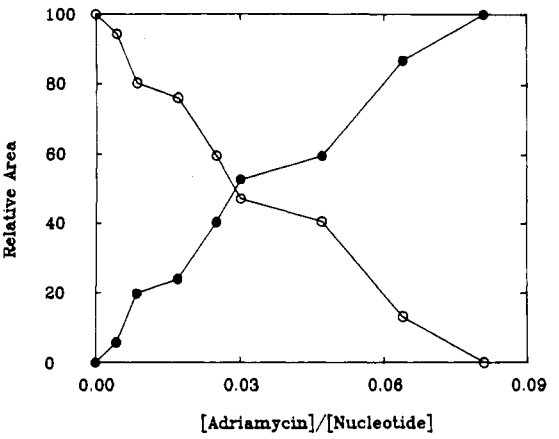

FIGURE 2: Plots of areas of fitted peaks (corrected for the hybrid component) from Figure 1 of the Z-form (○) and the B-form (●).

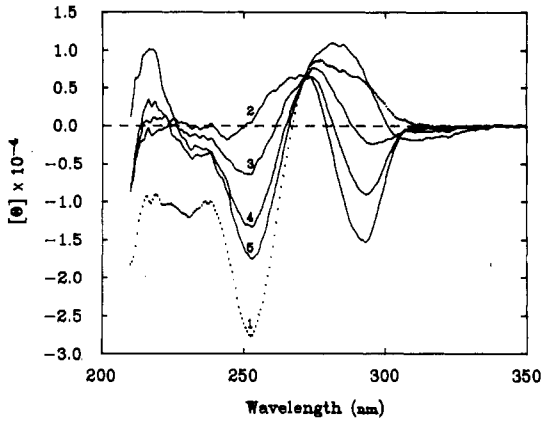

FIGURE 3: CD spectra (molar ellipticity [θ]) at room temperature showing the effect of adriamycin on poly(dGm^{5}dC)·poly(dGm^{5}dC) conformation: (1) original B-form of poly(dGm^{5}dC) in the presence of 5 mM Tris/50 mM NaCl/0.1 mM EDTA, pH 8.0; (2) Z-form after addition of $MgCl_2$ to (1) (final concentrations: 100 μM nucleotides, 2 mM $MgCl_2$); (3–5) addition of adriamycin to (2) [(3) 2.0 μM, [adriamycin]/[nucleotide] ([A]/[N]) = 0.02 or 1:50; (4) 4.0 μM, [A]/[N] = 0.04 or 1:25; (5) 8.8 μM, [A]/[N] = 0.091 or 1:11].

[A]:[N] = 1:11) did not coincide with the curve of the initial B-form (curve 1), presumably due to the complexation of the drug to poly(dGm^{5}dC). To ensure that this transition could not be due to the binding of divalent Mg^{2+} to adriamycin itself (Gianni et al., 1983), the Z-form was also prepared in the presence of NaCl (1 M), and very similar results were obtained.

In order to quantitate the degree of cooperativity of the B to Z transition of poly(dGm^{5}dC), we have fitted the CD data with a modified Hill equation for an equilibrium between two states (DeLean et al., 1978)

$$y = y_\infty + (y_0 - y_\infty)/[1 + (x/K)^n] \quad (1)$$

where y is the spectroscopic response, x is the concentration of added reagent, y_0 is the response when $x = 0$, y_∞ is the maximal response when $x = \infty$, K is the concentration of x at the midpoint (equivalent to the equilibrium constant), and n is the degree of cooperativity that determines the slope of the curve.

To apply this equation, the CD data at two wavelengths were converted to percent change and averaged, and the concentration was expressed as log x. Fits were made with $n = 1$, i.e., noncooperative, and n allowed to take any value. No other constraints were used in these fits, and either three or four fitted parameters were used. These CD data as a

Table I: Fit of Data to Equation 1 for Poly(dGm^{5}dC)·Poly(dGm^{5}dC)[a]

agent for B–Z transition	adriamycin/nucleotide	n	pK	K (mM)	RMS error
$MgCl_2$	0	1.0	10 ± 1.2		15.7
		6.1 ± 0.6	-0.16 ± 0.08	0.7	3.3
	1:33	1.0	0.3 ± 0.1		7.0
		1.9 ± 0.2	0.14 ± 0.03	1.4	3.9
	1:16	1.0	0.56 ± 0.07		4.7
		1.1 ± 0.2	0.54 ± 0.08	3.6	4.8
adriamycin[b]		1.0	-1.14 ± 0.09		4.8
		2.0 ± 0.19	-1.47 ± 0.02	0.03	2.7
		1.0	-1.0 ± 0.1		4.5
		1.7 ± 0.25	-1.43 ± 0.06	0.04	3.4

[a] Two fits were carried out on each set of CD data: with $n = 1$ (noncooperative) and with n allowed to vary. The pK value is the fitted log K value. [b] Two data sets were fitted independently, the upper in which the Z-form was produced by addition of 2 mM $MgCl_2$ and the lower by addition of 1 M NaCl (see Figure 5).

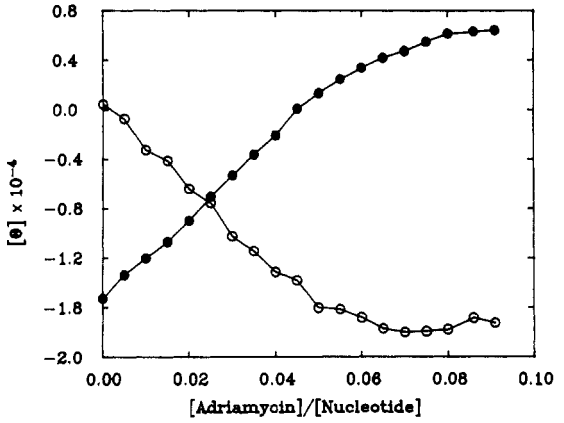

FIGURE 4: Plot of molar ellipticity [θ] of poly(dGm^{5}dC)·poly(dGm^{5}dC) as a function of adriamycin/nucleotide ratio from Figure 3 showing the Z to B transition: (●) 293 nm; (○) 252 nm.

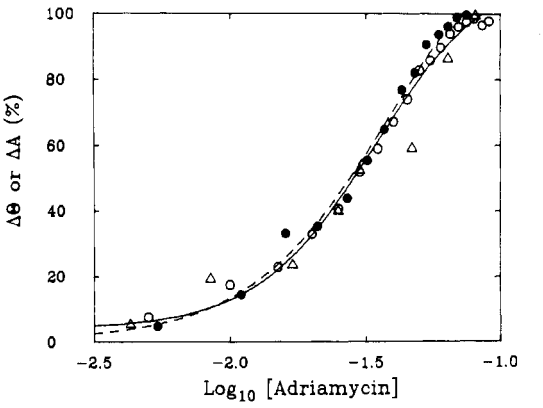

FIGURE 5: Fits using eq 1 to CD data (treated as described in the text) for the effect of adriamycin on the Z-form of poly(dGm^{5}dC)·poly(dGm^{5}dC). The solid line is the fit, with n allowed to vary, to the CD data in the presence of 2 mM $MgCl_2$ (○) from Figure 4; the dashed line is the fit to the CD data in the presence of 1 M NaCl (●). The ^{31}P NMR peak areas (△) from Figure 2 are not fitted. The fitted parameter values are given in Table I.

function of adriamycin/nucleotide ratio (Figure 4) were fitted with eq 1, and it was found to be somewhat cooperative (Figure 5), with $n \geq 2$ (Table I). The results for the transitions carried out in the presence of $MgCl_2$ and NaCl were the same within experimental error, indicating no significant effect of adriamycin–Mg^{2+} complexation. The ^{31}P NMR area data followed the same transition (Figure 5), although these data were not fitted due to greater scatter, and the experimental conditions (concentration, temperature) were not the same as for the CD determinations.

In order to further investigate the effect of adriamycin, the Mg^{2+}-induced B to Z transition was monitored by CD spectroscopy in the presence of adriamycin. The resulting curves showed a marked increase in the concentration of Mg^{2+} required to bring about the transition (Figure 6). Fitting eq 1 to these data (Table I) also showed that, while a better fit was obtained with a degree of cooperativity ($n = 1.9$) for a ratio of one adriamycin per 33 nucleotides, at twice this concentration there was in effect no difference between the fits with n set equal to 1 (noncooperative) or allowed to vary. The values of n and K in this latter case are essentially indeterminate, but it can be concluded that the transition in this case is effectively noncooperative. Given the smooth change of the fitted K value from 0.7 to 1.4 and to 3.6 mM [Mg^{2+}] in these experiments, it is clear that adriamycin inhibits the B to Z transition of poly(dGm^{5}dC) and causes the transition to change from a highly cooperative to a less cooperative process.

It was shown some time ago, before the Z-form was characterized (Wang et al., 1981; Dickerson et al., 1982), that ethidium, which is known to intercalate, converted the Z-form of poly(dGdC)·poly(dGdC) to the B-form (Pohl et al., 1972).

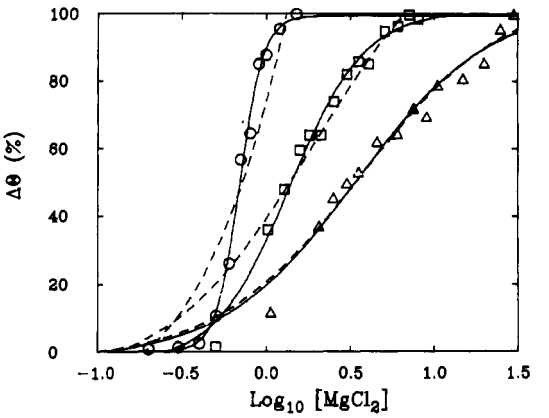

FIGURE 6: CD data for poly(dGm^{5}dC)·poly(dGm^{5}dC) as a function of [Mg^{2+}] (mM) (treated as described in the text). (○) The initial concentrations of poly(dGm^{5}dC)·poly(dGm^{5}dC) were between 100 and 120 μM in nucleotides. Fits to eq 1: dashed line, fit with $n = 1$; solid line, fit with n allowed to vary. Similar fits for Mg^{2+}-induced transition in the presence of adriamycin: (□) [A]/[N] = 1:33; (△) [A]/[N] = 1:16. The results of the fitting procedures are given in Table I.

There have been reports of various effects upon the B–Z transition of poly(dGdC)·poly(dGdC) by several carcinogens and drugs [2-(acetylamino)fluorene (Sage & Leng, 1980; Santella et al., 1981); Pt complex (Ushay et al., 1982); mitomycin (Tomasz et al., 1983); aflatoxin B1 (Nordheim et al., 1983)] and a study of several intercalators on the kinetics of the B to Z transition (Mirau et al., 1983). Ours is the first

study with a Z-form DNA, and with an important therapeutic agent, performed at salt concentrations which are comparable to those experienced by DNA in the cell. We presume that the interaction of the anthracycline ring system of adriamycin with B-DNA is by intercalation (Gianni et al., 1983; Fritzsche et al., 1982), although our results and conclusions do not depend upon the precise mode of interaction. It is probable that intercalation does not occur as readily with the Z-form as with the B-form as a result of steric factors; for example, the Z-form has a smaller radius and a syn G conformation (Wang et al., 1981; Dickerson et al., 1982).

A large proportion of CG sequences in mammalian DNA are methylated at the C5 position (Razin & Riggs, 1980), and this methylation may be correlated with gene inactivation (Felsenfeld & McGhee, 1982). It has also been speculated that B-DNA is present in active genes while the Z-form corresponds to inactive regions of DNA (Nordheim et al., 1981). Since adriamycin appears to bind preferentially to the B-form and to convert the Z- into the B-form, it is tempting to conclude that it could be exerting its influence by turning on inactive genes. However, the chemical processes involved in adriamycin interactions in the living organism are sufficiently complex that this simple explanation must be considered very tentative.

Acknowledgments

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Registry No. Poly(dGm^{5}dC), 53078-96-9; adriamycin, 23214-92-8; Mg, 7439-95-4.

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