Base Tilt of DNA in Various Conformations from Flow Linear Dichroism†

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ABSTRACT: We have measured the isotropic absorption (Aiso) and linear dichroism (LD) of *Escherichia coli* DNA in 0.01 M Na^+ (10.4 base pairs per turn of B form), 5.5 M NH_4F (10.2 base pairs per turn of B form), and 80% trifluoroethanol (A form) into the vacuum UV spectral region. The reduced dichroism spectrum (LD divided by Aiso) of DNA in the A conformation differed from those of the B conformations, demonstrating that LD is a sensitive method for distinguishing DNA conformation. The reduced dichroism spectra of the B conformations were similar, indicating little change in the orientation of the bases for DNA in high salt. The wavelength dependence of the reduced dichroism indicates that the angle between the base planes and the helix axis is less than 76° for all three conformations of DNA.

In solution, double-stranded DNA can adopt a variety of different secondary structures depending on the solution conditions (Ivanov et al., 1973; Zimmerman, 1982). Circular dichroism (CD) spectroscopy has been particularly useful in monitoring these conformational transitions in an empirical way. However, as CD depends on many factors and there is as yet no satisfactory theory to relate CD to conformation, the interpretation of CD spectral data in terms of nucleic acid structure is at present unsatisfactory. By measuring the CD spectra of DNA films prepared under conditions similar to those used for X-ray diffraction of fibers, Tunis-Schnieder & Maestre (1970) were able to identify the CD spectral characteristics for the A and B forms of DNA. The A-form CD spectrum can be obtained in ethanolic solutions. It is nonconservative with a large positive band at 263 nm and is similar to that of double-stranded RNA (Ivanov et al., 1973; Girod et al., 1973). In aqueous solutions of moderate ionic strength, DNA adopts the B conformation with a conservative CD spectrum (Brahms & Mommaerts, 1964). Under some conditions, such as high ionic strength, there is a loss of optical activity in the long-wavelength band that was previously ascribed to the C conformation of DNA (Ivanov et al., 1973; Tunis-Schneider & Maestre, 1970; Hanlon et al., 1972). It has since been shown that DNA in both high and low ionic strength solutions is in the B conformation (Zimmerman & Pheiffer, 1980) but that at moderate salt concentrations DNA has 10.4 base pairs per turn (Wang, 1978, 1979), while in high salt DNA has 10.2 base pairs per turn (Baase & Johnson, 1979; Chan et al., 1979).

The linear dichroism (LD) spectrum of DNA has a simpler origin than CD. The LD spectrum depends entirely on the orientation of the bases with respect to the helix axis (Wada, 1972; Norden, 1978). If the bases are perpendicular to the helix axis, then the reduced dichroism (LD divided by the isotropic absorption) is expected to be independent of wavelength. The base tilt of B-form DNA calculated from X-ray diffraction patterns on DNA fibers is about 84° (Arnott & Selsing, 1975), and electric dichroism measurements on high molecular weight DNA suggest a similar base orientation in solution (Lee & Charney, 1982; Ding et al., 1972; Diekmann et al., 1982). In contrast, our laboratory finds a wavelength dependence of the reduced dichroism from 290 to 180 nm which indicates that the bases for B-form DNA in solution at moderate salt concentrations are tilted less than 73° relative to the helix axis (Dougherty et al., 1983). Similar results have been obtained by electric dichroism measurements on DNA fragments containing less than 770 base pairs (Hogan et al., 1978; Charney & Yamaoka, 1982), and Levitt (1978) has calculated a 73° base tilt for B-form DNA by energy minimization procedures. Since X-ray diffraction on fibers of DNA in the A conformation gives tilt values of 70° (Arnott & Hukins, 1972), close to those found for B DNA in solution by flow LD, it should prove interesting to apply our methods to A-form DNA in solution.

Previous flow LD studies have shown that there are no large differences in the reduced dichroism of the A and B conformations above 220 nm (Gray & Rubenstein, 1968; Matsuoka & Norden, 1983; Rizzo & Schellman, 1984). We have extended the reduced dichroism measurements into the vacuum UV spectral region, and we show that there are significant differences in the reduced dichroism spectra of the A and B forms of DNA. We also present data on DNA in 5.5 M NH_4F where the CD spectrum corresponds to the 10.2 base pair form (10.2 B form) found for DNA wrapped around histone core particles (Shih & Fasman, 1970). We find that the base tilts are similar for the A form, the 10.4 B form, and the 10.2 B form DNA in solution.

Materials and Methods

Escherichia coli DNA (from Sigma and P-L Biochemicals) was dissolved in 0.01 M Na^+ (phosphate buffer, pH 7.0) at a concentration of about 3 mg/mL. The DNA was sheared to lengths of 5000–20 000 base pairs by forcing the solution through a 26-gauge needle several times at 4 °C (Dougherty et al., 1983). The sheared DNA was dialyzed twice against 200 volumes of 0.01 M Na^+ (phosphate buffer), 0.1 M NaCl, and 0.01 M disodium ethylenediaminetetraacetic acid (Na_2EDTA) (pH 8.5), twice against 0.01 M Na^+ (phosphate buffer) and 0.1 M NaCl (pH 7.5), and then 4 times against 0.002 M Na^+ (phosphate buffer, pH 7.0). To ensure that our results were not influenced by contaminating protein or RNA, an aliquot of the DNA was purified by the following procedure (Dougherty et al., 1983). A solution of 0.5 mL containing 0.5 mg/mL RNase (both RNase A and RNase T1) in 0.01 M $NH_4C_2H_3O_2$ was heated at 80 °C for 15 min to destroy any

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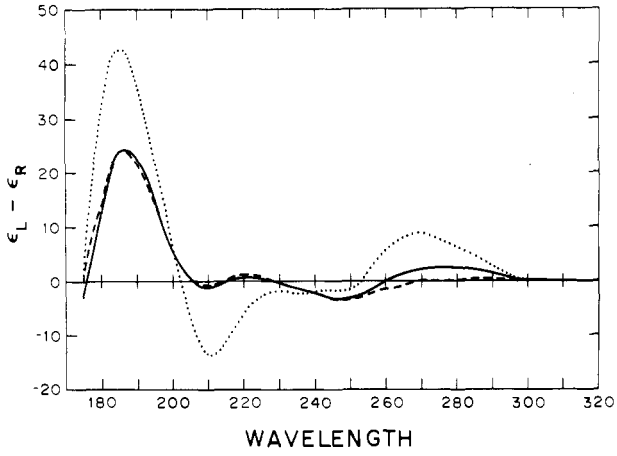

FIGURE 1: CD spectra of *E. coli* DNA in 0.01 M Na^+ (—), 5.5 M NH_4F (---), and 80% trifluoroethanol (···).

DNase activity and incubated with 5 mL of 1.5 mg/mL DNA in 0.002 M Na^+ (phosphate) and 0.01 M $NH_4C_2H_3O_2$ at 37 °C for 2 h. The solution was adjusted to 0.1 M NaCl, 0.01 M EDTA, 0.1 M tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl) (pH 8.0), and 2% Sarkosyl and treated with 0.5 mL of Pronase (10 mg/mL) at 37 °C for 3 h. After dialysis against 0.01 M Na^+ (phosphate buffer), the DNA was extracted 9 times with an equal volume of buffer-saturated, redistilled phenol. Finally, the DNA was dialyzed against the same series of buffer solutions as the unpurified DNA. The resulting reduced dichroism spectra were, within experimental error, identical with those obtained for unpurified DNA.

The A-form DNA was produced by slowly adding 2,2,2-trifluoroethanol to 80% (v/v) with continuous mixing. The final Na^+ concentration in 80% trifluoroethanol was 0.4 mM Na^+. The high-salt form of DNA was prepared by adding 10 M NH_4F in 0.01 M Na^+ (phosphate buffer) to an aliquot of the DNA solution adjusted to 0.01 M Na^+ (phosphate buffer). The final concentration of NH_4F, determined by the refractive index, was 5.5 M NH_4F. For absorption measurements, an aliquot of DNA in 5.5 M NH_4F was dialyzed overnight at room temperature against 10 volumes of 5.5 M NH_4F and 0.01 M Na^+ (phosphate buffer). The final DNA concentration in all the experiments was about 0.6 mg/mL. The extinction coefficient of *E. coli* DNA in 0.002 M Na^+ was taken to be 6500 L/(mol·cm) (Felsenfeld & Hirschman, 1965).

Absorption spectra were measured on a Cary 15 spectrometer flushed with nitrogen using 0.01- and 0.005-cm pathlength cells from Helma. The total absorption of solvent and DNA was less than 1.0 over the measured wavelength range, and the slit width was kept below 1.0 mm. CD and LD spectra were measured on a modified McPherson 225 vacuum UV spectrometer as previously described (Johnson, 1971; Edmondson & Johnson, 1985; Causley & Johnson, 1982). All measurements were performed at room temperature. Spectra were smoothed with a sliding 13-point convoluting quadratic (or cubic) function as described by Savitsky & Golay (1964).

The results presented here are based on five to seven separate experiments in each of the solvents. For each experiment, the LD spectrum was measured 3–5 times and averaged. The standard deviations of the reduced dichroism at 260 and 200 nm are given in Table I. The reduced dichroism, L', was obtained from absorption and LD spectra that were normalized to identical areas, thereby eliminating the effects of shear rate and molecular weight. The average shear rate used in the LD experiments reported here was 19 000 s^{-1}. Base lines were measured with the metering pump turned off.

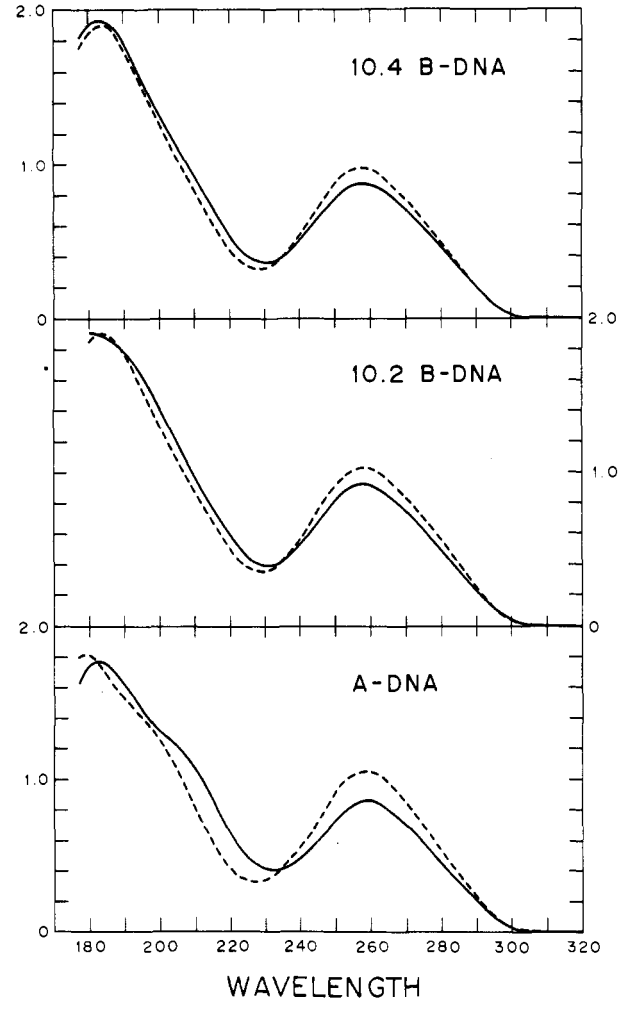

FIGURE 2: Normalized Aiso (—) and normalized LD (---) spectra of *E. coli* DNA from Sigma: (top) 0.01 M Na^+; (middle) 5.5 M NH_4F; (bottom) 80% trifluoroethanol.

RESULTS

Figure 1 shows the CD spectra for *E. coli* DNA in the three different solvent systems used for the LD experiments. The spectra are similar to previous measurements (Ivanov et al., 1973; Sprecher et al., 1979) and indicate that the DNA is in the 10.4 B, 10.2 B, and A conformations in the different solvents.

The top, middle, and bottom panels of Figure 2 show the Aiso and negative LD spectra for *E. coli* DNA in 0.01 M Na^+ (10.4 B form), 5.5 M NH_4F (10.2 B form), and 80% trifluoroethanol (A form), respectively, each normalized to an area of 100. For calibration purposes, the extinction coefficients at 258 nm in 5.5 M NH_4F and 80% trifluoroethanol were about 6230 and 6630 L/(mol·cm), respectively. The LD spectrum of *E. coli* DNA from Sigma in the 10.4 B conformation agrees with our previous measurements (Dougherty et al., 1983). However, the LD spectrum of *E. coli* DNA from P-L Biochemicals showed small but significant differences from that of the Sigma DNA. Since we are interested only in the differences between the reduced dichroism spectra of *E. coli* DNA in the various conformations, differences in the LD spectrum due to the source of DNA have been ignored. No differences were detected in the CD and isotropic absorption spectra of the two DNAs.

The isotropic absorption spectra below 230 nm for both samples of *E. coli* DNA (from Sigma and P-L Biochemicals)

Table I: Reduced Dichroism of *E. coli* DNA[a]

conformation	L'_i	bump nm	bump value	L'_j	L'_i/L'_j	tilt[b]
10.4 B	−0.93 ± 0.03	223	−0.81	−1.18 ± 0.06	0.79 ± 0.06	75 ± 2
10.2 B	−0.93 ± 0.02	224	−0.82	−1.13 ± 0.05	0.82 ± 0.05	76 ± 2
A	−0.94 ± 0.02	221	−0.62	−1.26 ± 0.07	0.75 ± 0.05	73 ± 2

[a] Reduced dichroism of DNA in various conformations ±1 standard deviation. L'_i and L'_j are the reduced dichroisms at 200 and 260 nm, respectively. [b] Base tilt is the angle in degrees between the helix axis and the base plane.

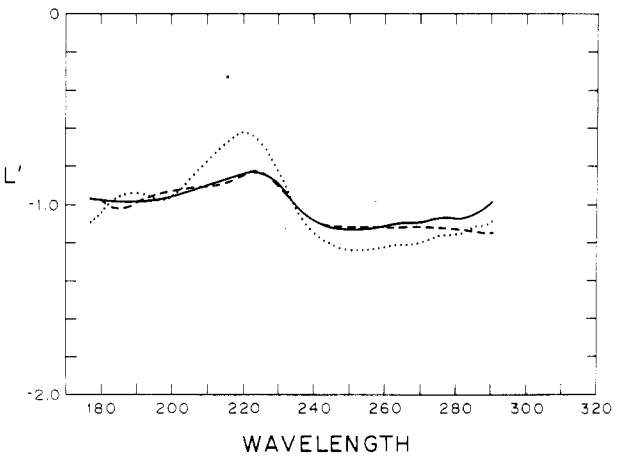

FIGURE 3: Normalized reduced dichroism spectra of *E. coli* DNA from Sigma in 0.01 M Na^+ (—), 5.5 M NH_4F (- - -), and 80% trifluoroethanol (···).

in the 10.4 B form differ slightly from our earlier measurements (Dougherty et al., 1983). We now find a maximum in the Asio at 182 nm which is similar to spectra reported earlier for *Clostridium perifringens* and *Micrococcus luteus* DNA. The reported base tilts are now slightly greater but agree with our previous measurements within experimental error.

The LD spectra of the 10.4 and 10.2 B conformations are similar in shape with maxima at about 259 and 182 nm (see Figure 2). The absorption spectra of both B conformations are similar in the near-UV region but differ slightly below 192 nm. Minor bands that are not apparent in the spectra of the B forms are clearly resolved at 200 and 208 nm in the LD and isotropic absorption spectra, respectively, of DNA in 80% trifluoroethanol.

The normalized reduced dichroism spectra are shown in Figure 3. The reduced dichroism spectra of both B conformations are similar in shape with a flat region above 245 nm and a bump at about 223 nm. Below 200 nm, the reduced dichroism of the 10.4 B form is again relatively constant, but that of the 10.2 B form has a small bump at 185 nm. However, this difference in the reduced dichroism spectra of DNA in high and low salt may not be significant due to the error in measuring the Aiso of DNA in 5.5 M NH_4F below 192 nm where the NH_4F starts to absorb. The ratio of the unnormalized LD to the unnormalized Aiso at 260 nm for the 10.2 B form is about the same as that of the 10.4 B conformation.

The reduced dichroism spectrum of DNA in the A conformation is considerably different from the spectra of the B forms (see Figure 3), demonstrating that LD is a sensitive method for distinguishing DNA conformation. The reduced dichroism of the A form increases in magnitude from 290 to 240 nm, and the bump in the spectrum (located at about 221 nm) is much larger than those of the B forms. Additional peaks in the reduced dichroism spectrum of the A form are observed below 200 nm. Our results on the A form are in reasonable agreement with previous measurements in the near-UV region (Gray & Rubenstein, 1968; Matsuoka & Norden, 1983; Rizzo & Schellman, 1984). The ratio of the unnormalized LD to the unnormalized Aiso at 260 nm for the A form is about half that of the 10.4 B form, in agreement with the results of Matsuoka & Norden (1983).

The LD was linear with shear rate from 9000 to 62 000 s^{-1} (data not shown). However, the normalized reduced dichroism was independent of shear rate, indicating that the wavelength dependence is not due to a bent or supercoiled structure in solution. Thus, the bases of DNA are not perpendicular to the helix axis for any of the conformations studied.

Discussion

The reduced dichroism, L, of a single transition in DNA is given by (Causley & Johnson, 1982)

$$L = 3S(3\cos^2\alpha\sin^2\beta - 1)/2$$

where α is the angle between the base plane and the helix axis (base tilt), β is the angle within the plane of the base between the transition dipole and the axis about which the base is inclined, and S is the orientation factor ($0 < S < 1$). Since the optical properties of natural DNAs are averaged over many unresolvable electronic transitions, it is not possible to separate the effects of α and β. However, we can calculate the maximum angle that the bases make with the helix axis.

The ratio of the normalized reduced dichroism, L', at two different wavengths corresponding to two different transition dipoles is (Dougherty et al., 1983)

$$L'_i/L'_j = \frac{3\cos^2\alpha\sin^2\beta_i - 1}{3\cos^2\alpha\sin^2\beta_j - 1}$$

For a given L'_i/L'_j, the base tilt will have its largest possible value when transition dipole j is oriented along the axis of inclination and transition dipole i is oriented perpendicular to the inclination axis, that is, when $\beta_j = 0$ and $\beta_i = 90°$. Since $n-\pi^*$ transitions are expected to contribute a positive component to the reduced dichroism, we assign L'_i and L'_j to two different flat regions of the spectra at wavelengths of 200 and 260 nm, respectively. The maximum base tilts for the 10.4 B, 10.2 B, and A conformations calculated in this fashion are 75°, 76°, and 73° ± 2°, respectively (see Table I). The error in these values includes the differences in the LD spectra of *E. coli* DNA obtained from two different sources as discussed above. The base tilts of the *E. coli* DNA obtained from P-L Biochemicals were consistantly 2° less than those for Sigma DNA in each conformation. We have no obvious explanation for these differences.

The maximum value of the base tilt for the A conformation in solution determined by flow LD (73°) agrees with that calculated from X-ray diffraction on fibrous DNAs (70°). The maximum values of the base tilt for the B conformations, however, differ from those calculated by X-ray diffraction on DNA fibers but are in reasonable agreement with some other studies (Dickerson & Drew, 1981; Levitt, 1978; Tumanyan et al., 1984). Since we can only determine the maximum angle that the bases make with the helix axis, the angle α may

actually be less than that calculated above.

In previous studies, the reduction in the negative reduced dichroism observed at about 225 nm was attributed to n–π^* transitions polarized perpendicular to the base planes (Dougherty et al., 1983; Gray & Rubenstein, 1968; Matsuoka & Norden, 1983). However, in an LD study of the synthetic polynucleotides poly(dA)–poly(dT) and poly[d(A-T)]–poly[d(A-T)], we found no evidence for n–π^* transitions, and the entire reduced dichroism spectra of these polymers from 290 to 177 nm, which includes such a bump, could be accounted for by the π–π^* transitions (Edmondson & Johnson, 1985). If the reduction in the negative reduced dichroism at 225 nm of natural DNAs is also due to π–π^* transitions, then the maximum angles between the base planes and the helix axis are 71°, 73°, and 66° for the 10.4 B, 10.2 B, and A conformations, respectively.

There are several possible explanations for the discrepancy between the results of flow LD and X-ray fiber diffraction studies on B-form DNA. Since LD depends on the average tilt of the bases, the reduced dichroism represents a distribution of base conformations. However, single-stranded regions with random base orientations will not contribute to the wavelength dependence of the reduced dichroism. Even in the extreme case where the transtions responsible for L'_i in double-stranded regions were oriented perpendicular to the helix axis and the transitions responsible for L'_j on looped-out or single-stranded bases were oriented parallel to the helix axis, the fraction of looped-out bases would have to be about 17% to account for the average tilt of 75° measured by flow LD. This is much greater than that expected for double-stranded DNA under our experimental conditions (Mandal et al., 1979; Assa-Munt et al., 1984).

Lee & Charney (1982) and Diekmann et al. (1982) have proposed a supercoiled or bent structure for B DNA in solution to account for the molecular weight dependence of electric dichroism measurements. Supercoiling of DNA decreases the magnitude of the reduced dichroism at all wavelengths and thus cannot explain the wavelength dependence of the reduced dichroism. Anisotropic bending of DNA, such as bends into the minor groove, could produce an apparent inclination axis if the bends were in phase with the secondary structure. However, the contributions of such bends to the reduced dichroism would lessen at higher shear rates, and there is no evidence for a shear rate dependence in flow LD. The normalized reduced dichroism of DNA is independent of shear rate over the range of 9000–62 000 s^{-1}, and a similar wavelength dependence for the reduced dichroism of DNA has been observed for flow rates as low as 20 s^{-1} (Rizzo & Schellman, 1984).

Transitions polarized perpendicular to the plane of the bases contribute a positive LD component and could produce a reduced dichroism spectrum that varies with wavelength. However, our results for the A conformation are reasonable as were our previous results for single- and double-stranded poly(rA) (Causley & Johnson, 1982). To account for the observed reduced dichroism spectrum, n–π^* transitions would have to be distributed over the entire wavelength range below 240 nm, and it is unlikely that n–π^* transitions would contribute much to the LD between 180 and 200 nm where the π–π^* transitions are strong. Furthermore, it appears that even the bump in the reduced dichroism spectrum at about 225 nm, at least in the case of poly(dA)–poly(dT) and poly[d(A-T)]–poly[d(A-T)], is not due to an n–π^* transition (Edmondson & Johnson, 1985).

The simplest explanation for the flow LD results is that the conformation of B-form DNA differs in solution and fibers and that the bases of DNA in solution are not perpendicular to the helix axis. In particular, the wavelength dependence of the reduced dichroism indicates that the maximum angles between the helix axis and the bases for the 10.4 B, 10.2 B, and A conformations of DNA are 75°, 76°, and 73° ± 2°, respectively.

Regardless of the interpretation, our results demonstrate that LD is a sensitive method of distinguishing the A and B conformations of DNA. In addition, no significant differences were detected between the reduced dichroism spectra of DNA in moderate salt solutions and in 5.5 M NH_4F, supporting X-ray diffraction studies on DNA in solution which indicate that DNA is in the B conformation in high-salt solutions (Zimmerman & Pheiffer, 1980).

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Synthesis and Characterization of Poly[d(G-z^{5}C)]. B-Z Transition and Inhibition of DNA Methylase†

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ABSTRACT: Deoxy-5-azacytidine 5'-triphosphate was synthesized and used as a substrate for the enzymatic synthesis of the polynucleotide poly[d(G-z^{5}C)]. Whereas the triphosphate decomposes in solution, the azacytosine analogue incorporated into DNA is stable under conditions preserving the double-helical structure. Poly[d(G-z^{5}C)] undergoes the transition to the left-handed Z conformation at salt (NaCl and $MgCl_2$) concentrations approximately 30% higher than those required for unsubstituted poly[d(G-C)]. However, the incorporation of azacytidine potentiates the formation at room temperature of the Z helix stabilized by the transition metal Mn^{2+}; in the case of poly[d(G-C)], a heating step is required. The spectral properties of the two polymers in the B and Z forms are similar. Both left-handed forms are recognized by anti-Z DNA immunoglobulins, indicating that the DNAs bear common antigenic features. Poly[d(G-z^{5}C)] is not a substrate for the DNA cytosine 5-methyltransferase from human placenta. It is a potent inhibitor of the enzyme when tested in a competitive binding assay. These results are compatible with a very strong, possibly covalent, mode of interaction between methyltransferases and DNA containing 5-azacytosine.

Substitution of a nitrogen atom into position 5 of cytosine yields the nucleoside analogue 5-azacytidine (z^{5}C).[1] This drug exhibits potent antibacterial, antitumoral, mutagenic (Vesely & Cihak, 1978), and carcinogenic (A. D. Riggs, personal communication) activities. It is used clinically as an antileukemic agent (Saiki et al., 1978) as well as in the treatment of patients with severe β-thalassemia (Ley et al., 1982). The cytotoxic activity of 5-azacytidine may be related to the inhibition of protein synthesis, possibly at the level of tRNA. Studies have demonstrated that administration of z^{5}C to mice results in an inhibition of the enzymatic methylation of cytidine in tRNA, apparently without direct incorporation of the nucleoside into the ribopolymer (Lu et al., 1976). However, z^{5}CTP is a substrate for the yeast AMP (CMP):tRNA nucleotidyltransferase, leading to the incorporation of the nucleotide analogue into the 3' terminus of tRNA^{Phe} (Zielinski & Sprinzl, 1984), and 5-azacytosine can replace cytosine residues in DNA (Jones & Taylor, 1981).

At subcytoxic levels, z^{5}C dramatically inhibits the C5 methylation of cytidine in DNA (Taylor & Jones, 1982; Pfeifer et al., 1983) and displays pronounced inductive effects on the expression of certain eukaryotic genes and viruses [for reviews, see Felsenfeld & McGhee (1982) and Riggs & Jones (1983)]. Such biochemical activity is compatible with the as yet unexplained inverse correlation between the degree of cytosine methylation in the region near a gene and the transcriptional expression of that gene (Felsenfeld & McGhee, 1982; Doerfler, 1983; Razin & Szyf, 1984).

The in vivo inhibitory effect of z^{5}C on DNA methylation requires the incorporation of this modified nucleoside into DNA (Jones & Taylor, 1980; Taylor & Jones, 1982; Adams et al., 1982). However, the pronounced reduction of the methylation of cytosine cannot be explained simply by the

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[1] Abbreviations: z^{5}C, 5-azacytidine; dz^{5}C, deoxy-5-azacytidine; DNase, deoxyribonuclease; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride; EDTA, ethylenediaminetetraacetic acid; HPLC, high-performance liquid chromatography.

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