Circular Dichroism of Mitomycin–DNA Complexes. Evidence for a Conformational Change in DNA†

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ABSTRACT: Binding of the antibiotic mitomycin C to *Escherichia coli* DNA causes a change in the characteristic circular dichroism spectra of the DNA itself in dilute buffers. This change is progressive with an increasing amount of mitomycin bound to DNA and is characterized by a gradual shift of the 272-nm positive band to lower wavelengths, loss of intensity of the 245-nm negative band, appearance of a new broad minimum at 300 nm, and an isoelliptic point at 270–272 nm. Calf-thymus DNA and T-2 phage DNA show similar behavior. In contrast, RNA–mitomycin complexes exhibit practically no change in the CD spectra of the RNA (f-2 replicating form or ribosomal). Circular dichroic (CD) studies of mitomycin–synthetic polynucleotide complexes revealed that while poly(dG)·poly(dC) shows only minor changes upon binding of mitomycin, poly[d(G-C)]·poly[d(G-C)] undergoes profound alteration of its CD spectrum. These changes are similar to those occurring when poly[d(G-C)]·poly[d(G-C)] is submitted to high salt concentration, reported and interpreted as a conformation change by F. M. Pohl and T. M. Jovin ((1972), *J. Mol. Biol. 67*, 375). The CD difference spectra of DNA and poly[d(G-C)]·poly[d(G-C)] complexes at equal binding ratios are identical but the intensity of the former is greatly reduced. These and the quantitative data indicate that the binding of mitomycin to guanine residues results in a CD change of the polynucleotide specific to GpC (or alternatively but less likely to CpG) sequences. The CD change is interpreted as indicative of a localized conformational change of GpC dimer base pairs upon binding of the drug. Two alternative hypotheses are proposed as reason for this conformational change, one of them implicating the mitomycin-induced cross-links in DNA.

Measurement of circular dichroism has been used widely for probing conformational changes of DNA. Since the theoretical correlation between circular dichroism and conformation of DNA is not yet known (e.g., Studdert and Davis, 1974), a set of empirical correlations determined by Tunis-Schneider and Maestre (1970) is usually applied. These authors determined the CD[1] of films of DNA under conditions that yield the x-ray patterns of the A, B, and C conformations of DNA fibers. The work (see also Maestre, 1970) showed that the three CD patterns are characteristic and very different. The fact that A-DNA yielded a CD spectrum like that of double-stranded RNA in solution (Samejima et al., 1968), known to be in the A conformation (for review see Arnott, 1970), further demonstrated that the CD spectrum is highly dominated by the conformational aspects of the nucleic acid. Since then, using the CD technique, a variety of agents have been reported to cause transitions from the normal B form of DNA in aqueous solution to A and/or C forms, for example, certain organic solvents (Nelson and Johnson, 1970; Green and Mahler, 1971; Ivanov et al., 1973; Girod et al., 1973), inorganic ions (Hanlon et al., 1972; Studdert et al., 1972; Zimmer and Luck, 1973), and synthetic basic polypeptides (Ong et al., 1976). In some cases transitions to higher order (tertiary) structures of DNA have also been observed, yielding "ψ" type CD spectra (Jordan et al., 1972; Cheng and Mohr, 1974; Brunner and Maestre, 1974; Ong et al., 1976).

Alteration of secondary structure of DNA or "distortion of helical structure" as is usually termed is frequently assumed to occur upon binding of drug molecules. Intercalation is an obvious case. Numerous nonintercalating drugs have also been implicated, however, for example, steroidal diamines (Mahler et al., 1968) and the synthetic carcinogen *N*-acetylacetoxyaminofluorene (Nelson et al., 1971). Use of the CD technique led to proposals of specific conformational changes in these cases. Since drug-induced distortions of DNA structure potentially play a role in the biological effects of the drug–DNA complex, such as in behavior toward repair, replication, or transcription systems, probing of such distortions remains an area of great interest. General use of CD for this purpose is severely complicated, however, by the large intrinsic CD displayed by many antibiotics in the region below 300 nm obscuring the CD of DNA itself (Dalgleish et al., 1974) and the possibility of induced CD of drugs, due to electronic interaction with the chromophores of helical DNA or with other bound drug molecules in an asymmetric manner as demonstrated with various intercalative dyes (Li and Crothers, 1969; Gabbay et al., 1973).

We have been interested for some time in the molecular details of the interaction of mitomycin C with DNA (Tomasz et al., 1974). The mitomycin molecule (I) is relatively small and simple when compared to most antibiotics. It binds covalently to DNA (Iyer and Szybalski, 1964; Weissbach and Lisio, 1965). The ultraviolet and visible spectrum of the bound form is the same as that of unbound chromophore, indicating lack of interaction with nucleic acid chromophores (Tomasz et al., 1974). These properties indicated to us that the CD of the complex might be simple enough to discern the CD of the nucleic acid itself and thus to detect any conformational change caused by the binding of the drug. We report here the results of our study.

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[1] Abbreviations used are: CD, circular dichroism; SSC, standard saline citrate (pH 7.4); MC, mitomycin C; HN2, bis(2-chloroethyl)methylamine; OD, optical density; NMR, nuclear magnetic resonance.

TABLE I: Preparation of MC-Nucleic Acid Complexes.[a]

Nucleic acid	Molar ratio of MC to nucleotide in the reaction mixture	Binding ratio of the complex
E. coli DNA	1.0	0.062
	2.0	0.12
	3.0	0.17
T-2 DNA	1.0	0.045
	2.0	0.055
	4.0	0.17
f-2 RNA (replicating form)	1.0	0.31
	2.0	0.067
	3.0	0.12
Ribosomal RNA (16S + 23S)	3.0	0.12
Poly(dG)·poly(dC)	0.5	0.071
	2-3	0.17-0.25
Poly[d(G-C)]·poly[d(G-C)]	0.5	0.05
	2-3[c]	0.17-0.25
E. coli DNA[b]	3.0	0.071
	9.0	0.14

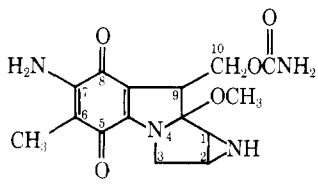
[a] See also Materials and Methods section. [b] These complexes were prepared with the hydrolysis product (II) instead of MC. [c] Increasing the molar ratio from 3 to 4 gave no increase in the binding ratio of the complex (0.25).

Materials and Methods

The following substances were obtained commercially: mitomycin C (Kiowa Hakko Kogyo Co., Tokyo, Japan); *E. coli* K_{12}DNA (General Biochemicals, Chagrin Falls, Ohio); calf-thymus DNA, type I (Sigma Chemical Co., St. Louis, Mo.); T-2 phage DNA (Grand Island Biological Co., Grand Island, N.Y.); *E. coli* K_{12}rRna, 16S + 23S (Miles Laboratories, Elkhart, Ind.); poly(dG)·poly(dC) and poly[d(G-C)]·poly[d(G-C)] (P-L Biochemicals, Milwaukee, Wis.); F-2 RNA (replicating form) was a gift of Dr. N. Zinder, The Rockefeller University (New York, N.Y.).

Commercial *E. coli* DNA was further purified as described previously (Tomasz et al., 1974). Calf-thymus DNA was further purified by deproteinization by chloroform and subsequent precipitation by ⅔ vol of 1-propanol in the cold. The precipitate was redissolved in SSC. All three DNAs used in this work were sonicated in SSC in a Branson Model W140 sonicator with microtip in a Rosette cell under cooling in an ice bath. The sonicated DNA was dialyzed in 0.017 M sodium phosphate buffer (pH 7.4).

The hydrolysis product of mitomycin C (II) was prepared by a published procedure (Stevens et al., 1965).



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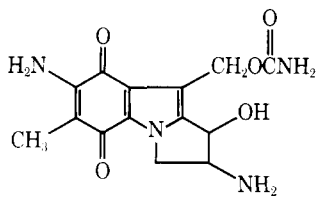


II

Preparation of Mitomycin-Nucleic Acid Complexes. The general procedure was previously described (method B in Tomasz et al., 1974). To obtain complexes with various binding ratios the concentration of the nucleic acid or polynucleotide was kept constant (0.67×10^{-3} M) in the reaction mixture while the concentration of mitomycin was varied. Table I lists the molar ratio MC to nucleic acid in the reaction mixture for all complexes prepared. Separation of unbound mitomycin from mitomycin-nucleic acid complexes was accomplished by Sephadex G-100 chromatography as described in Tomasz et al. (1974). *Binding ratios,* defined here as the molar ratio of bound mitomycin to mononucleotide unit, were determined by assaying mononucleotide by phosphate analysis (Ames and Dubin, 1960) and bound mitomycin by ultraviolet absorbance of the complex at 310 nm, using E_{310} 11 000 (Tomasz et al., 1974).

"Reduced mitomycin" is referred to mitomycin C submitted to the same reductive activation procedure as used in the preparation of the nucleic acid complexes except the nucleic acid was omitted. The resulting mixture was used without further purification. The ultraviolet spectrum of this "reduced mitomycin" is identical with that of the DNA-bound form (Tomasz et al., 1974).

CD spectra were recorded with either a Jasco J-20 automatic recording spectropolarimeter (Japan Spectroscopic Co., Ltd.) or with a Cary 60 ORD spectropolarimeter equipped with a 6001 CD attachment, using 1-cm cuvettes. The solutions had 0.8-1.2 OD_{260} units (contributed by nucleotide and bound antibiotic) corresponding approximately to a 10^{-4} M concentration of nucleotide. Molar ellipticities ($[\theta]$) were calculated per nucleotide residue. The spectra given represent the average of three runs. Averaging and correcting for baseline as well as calculations of difference spectra were done by hand, at 5-nm intervals (or at 2 nm when necessary). The CD spectra were obtained either in 0.017 M sodium phosphate buffer (pH 7.5) or in 0.1 SSC. The solutions of poly(dG)·poly(dC) and of poly[d(G-C)]·poly[d(G-c)] contained in addition 10^{-4} M EDTA. All solutions were routinely filtered through a Whatman No. 1 paper disk to ensure lack of any particulate matter which might give rise to light scattering.

Results

CD of Nucleic Acid-Mitomycin Complexes. E. coli DNA (Figure 1a). As the amount of mitomycin bound to DNA increases the 272-nm positive band is gradually shifting to lower wavelengths; the 248-nm negative band is losing intensity and shifts slightly to the left. A new broad negative band appears at approximately 300 nm. An isoelliptic point is apparent at 270-272 nm.

T-2 DNA (Figure 2a) and calf-thymus DNA (not shown) show similar behavior.

RNA. In contrast to DNA mitomycin binding causes practically no change in the CD spectra of f-2 phage replicating form RNA (Figure 3) or ribosomal RNA (not shown).

CD of "Reduced Mitomycin" (Figure 4). Curve I illustrates that the drug has a slight intrinsic CD spectrum in the ultraviolet region. If one wanted to correct the curves of mitomycin complexes for this, an appropriate fraction of curve I should be subtracted. This fraction is the same as the binding ratio of the complex. For example, if the binding ratio of the complex is 0.17, the correction is 0.17 times curve I. The figure illustrates a comparison between the uncorrected and corrected spectra of such a complex (*E. coli* DNA). It is evident that the difference is negligible up to approximately 305 nm and since 0.17 represents the highest binding ratio used mostly in this work, the correction for other complexes is even slighter. Consequently, all spectra are uncorrected. It should be noted, however, that above approximately 305 nm a tailing or broadening of the 300-nm negative band into the 330-340-nm region is obviously due to the CD of the drug itself. This does not interfere with the interpretation of the results.

CD of Poly[d(G-C)]·Poly[d(G-C)]-Mitomycin Com-

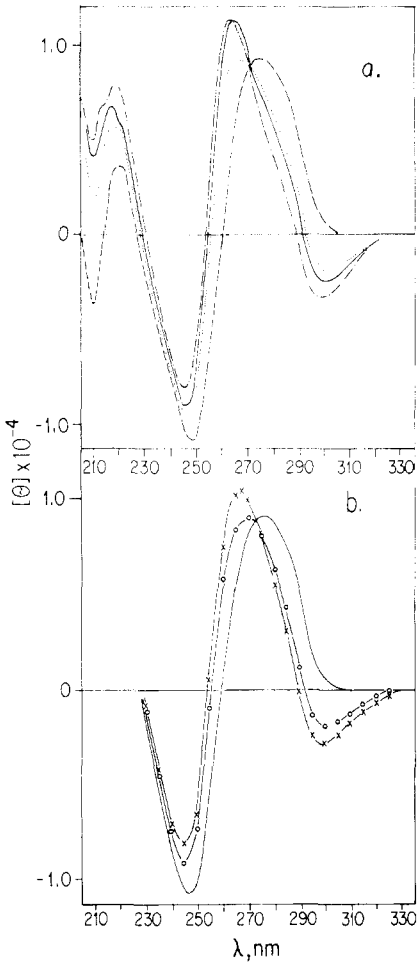


FIGURE 1: (a) CD of mitomycin-*E. coli* complexes: (- - -) control DNA; (· · ·) complex, binding ratio 0.06; (—) complex, binding ratio 0.13; (-·-) complex, binding ratio 0.17. (b) Reconstruction of the experimental curves by adding difference CD spectra of mitomycin-poly[d(G-C)]·poly[d(G-C)] complexes to CD of control DNA: (—) control DNA; (○) control DNA + 0.15 × [θ] of mitomycin-poly[d(G-C)]·poly[d(G-C)] complex, binding ratio 0.17; (×) control DNA + 0.20 × [θ] of same.

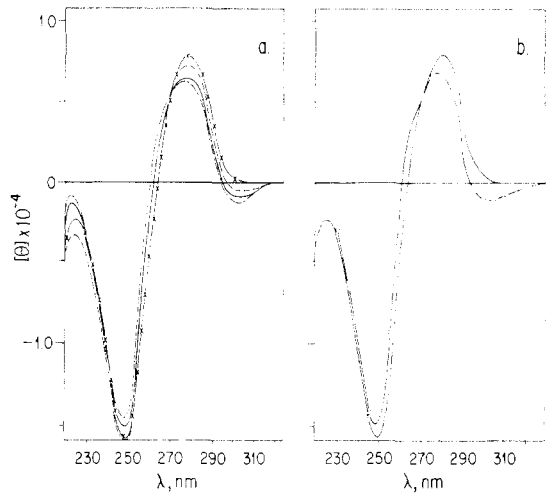


FIGURE 2: (a) CD of mitomycin-T-2 DNA complexes: (×-×) control DNA; (- - -) complex, binding ratio 0.045; (—) complex, binding ratio 0.055; (-·-) complex, binding ratio 0.17. (b) Reconstruction of the experimental curves by the method as given in Figure 1b: (—) control DNA; (○) control DNA + 0.1 × [θ] of mitomycin-poly[d(G-C)]·poly[d(G-C)] complex, binding ratio 0.17.

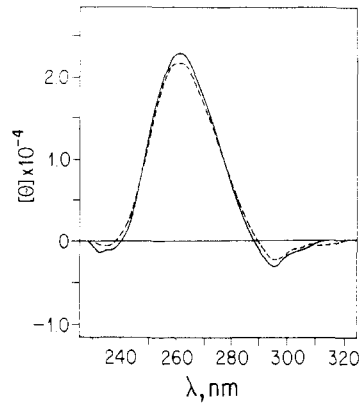


FIGURE 3: CD of mitomycin-RNA (F-2 replicating form) complex: (—) control RNA; (- - -) complex, binding ratio 0.13.

plexes (*Figure 5*). The CD spectra undergo profound alteration with increasing binding ratio giving rise finally to an inverted type spectra. The curves run through an isoelliptic point (272 nm), same as the DNA complexes. The spectrum of the complex with binding ratio 0.25 is regarded as limiting since it is not possible to increase the binding ratio any further (see Table I). The limiting spectrum has a negative band at 292 nm, a positive band at 263 nm, and a small negative band at 245 nm.

CD of Poly(dG)·Poly(dC)–Mitomycin Complexes (*Figure 6*). First, the CD spectra of control poly(dG)·poly(dC) deserve comment. An uncertainty still exists in the literature with respect to the CD spectra of pure duplex poly(dG)·poly(dC) (Gray and Bollum, 1974). The negative band at 278 nm seen in our sample is frequently but not always present in other preparations and Gray and Bollum assign it to single-stranded poly(dG). A positive peak at 285–290 nm also frequently observed in various preparations and attributed to excess poly(dC) is missing, however. It is thus apparent that our sample is poly(dG)·poly(dC) duplex containing some single-stranded poly(dG).

Mitomycin-poly(dG)·(poly dC) complexes show only minor changes relative to control poly(dG)·poly(dC) as tested by a series of complexes of different binding ratios. Figure 6 shows

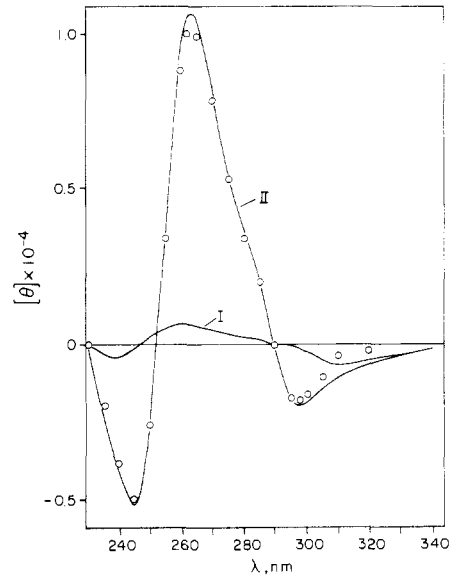


FIGURE 4: Solid curves: (I) CD of "reduced mitomycin"; (II) CD of mitomycin-*E. coli* DNA complex, binding ratio 0.17. The circles represent the CD of the complex corrected for the CD of "reduced mitomycin"; see Results.

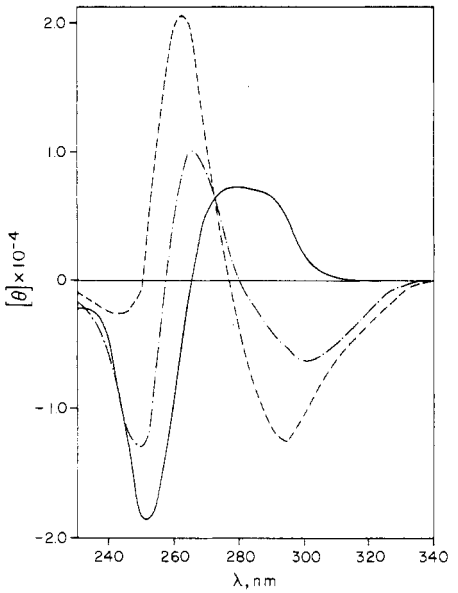


FIGURE 5: CD of mitomycin-poly[d(G-C)]·poly[d(G-C)] complexes: (—) control poly[d(G-C)]·poly[d(G-C)]; (---) complex, binding ratio 0.07; (- - -) complex, binding ratio 0.25.

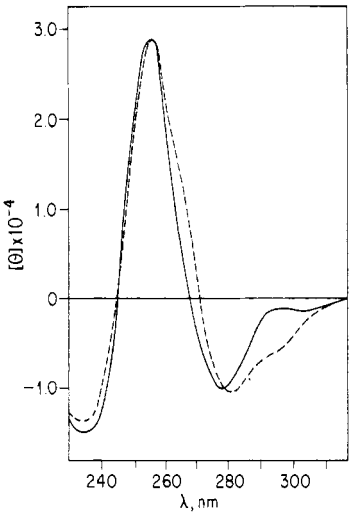


FIGURE 6: CD of mitomycin-poly(dG)·poly(dC) complex: (—) control poly(dG)·poly(dC); (- - -) complex, binding ratio 0.25.

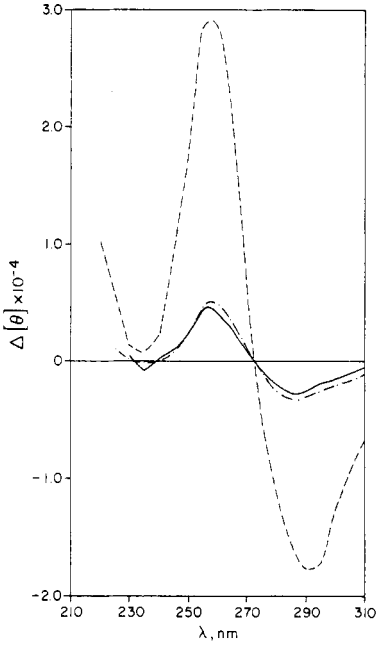


FIGURE 7: Difference CD spectra of mitomycin-DNA complexes compared with the difference CD spectra of mitomycin-poly[d(G-C)]·poly[d(G-C)] complex, all complexes possessing the same binding ratio 0.17; (---) *E. coli* DNA complex; (—) T-2 DNA complex; (- - -) poly[d(G-C)]·poly[d(G-C)] complex.

only the complex with the highest level of substitution (binding ratio 0.25).

CD of Complexes of the Hydrolysis Product of Mitomycin (II) with DNA (Not Shown). The complexes show essentially no changes with respect to control *E. coli* DNA.

Comparison of CD Difference Spectra of Poly[d(G-C)]·Poly[d(G-C)] and DNA Complexes (Figure 7). Difference spectra were calculated as CD of complex minus CD of control polynucleotide. Each of the complexes had an identical binding ratio of 0.17. As seen in Figure 7, the shapes of the three difference spectra are identical. The intensity of the poly[d(G-C)]·poly[d(G-C)] complex is much greater than that of the DNAs. The intensities relative to the poly[d(G-C)]·poly[d(G-C)] complex were calculated at two wavelengths, giving 0.18 for *E. coli* DNA and 0.14 for T-2 DNA at 258 nm, and 0.18 and 0.13 at 290 nm, respectively. Thus, it is apparent that while each of the three polynucleotide–mitomycin complexes contains the same amount of bound mitomycin (binding ratio 0.17), the DNA-bound drug induces only a fraction of the CD change that is induced by the poly[d(G-C)]·poly[d(G-C)]-bound drug.

Reconstruction of the Experimental CD Curves of DNA–Mitomycin Complexes by Adding Fractions of the Difference CD Spectra of Poly[d(G-C)]·Poly[d(G-C)]–Mitomycin Complex to CD of Control DNA (Figures 1 and 2). This method was devised to illustrate further the same point made in the preceding paragraph: the MC-induced CD change is qualitatively identical for DNA and poly[d(G-C)]·poly(d(G-C)]. This is clear from the similarity between the experimental and reconstruction curves (Figures 1a and b; Figures 2a and b).

CD of Complexes in the Visible Region. The CD of "reduced mitomycin" and mitomycin complexes of *E. coli* DNA and poly[d(G-C)]·poly[d(G-C)], binding ratio 0.17 each, converged to zero from approximately 340 nm up and remained zero in the range tested (up to 600 nm).

Discussion

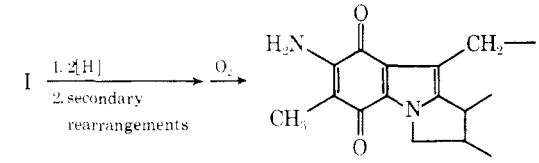
The system for covalent complex formation between mitomycin C and polynucleotides is somewhat complicated when compared to other drugs and it will be reviewed briefly for clarity. Formation of the complex and the accompanying cross-links requires activation of mitomycin by anaerobic reduction, both in vivo and in vitro (Iyer and Szybalski, 1964; Weissbach and Lisio, 1965). The reduced form is extremely unstable in the presence of air and therefore in the isolated mitomycin–nucleic acid complex the mitomycin residues are in the reoxidized state, although the chromophore is no longer that of original mitomycin, but contains an additional double bond (Chart I, partial formula III) (Tomasz et al., 1974). The complex is covalent by all indications. The attachment of the majority of the drug molecules (80–90%) to DNA is apparently monofunctional while only 10–20% of the bound drug participates in cross-links between the complementary strands of DNA ("bifunctional binding") (Szybalski and Iyer, 1964;

TABLE II: Comparison of Nearest Neighbor Frequencies GpC and CpG with CD Change Data from Figure 7.

	GpC[a]	CpG[a]	GpC of poly[d(G-C)]·poly-[d(G-C)]	CpG of poly[d(G-C)]·poly[d(G-C)]	$\Delta[\theta]_{290}$ of poly[d-(G-C)]·poly[d(G-C)]
E. coli DNA	0.083	0.067	0.166	0.134	0.18[b]
T-2 DNA	0.040	0.060	0.080	0.060	0.13[b]
Poly[d(G-C)]·poly[d(G-C)]	0.50	0.50	1.0	1.0	1.0

[a] From Josse et al. (1961). [b] Calculated from the spectra in Figure 9.

CHART I



III

Weissbach and Lisio, 1965). The chemical nature of the bonds between mitomycin and nucleic acids is still largely uncertain. One of the points of attachment must be at the aziridine ring (C-1), as postulated by Iyer and Szybalski (1964) since aziridine-lacking analogues bind monofunctionally to DNA but do not cause cross-links, meaning that one of the two binding sites is missing from the drug molecule (Otsuji and Murayama, 1972; Tomasz et al., 1974). The other position was postulated to be at C-10 but is not yet proven. The binding to polynucleotides is virtually guanine specific but the position of attachment at the guanine residues is not known. No complex is formed with mononucleotides or with GpC (Tomasz et al., 1974). No information is available concerning the steric arrangement of the DNA-bound drug. The fact that its chromophore is unaltered upon binding to DNA (Tomasz et al., 1974), together with the small size of the drug chromophore (see III), argues against intercalation between base pairs.

The results of this work presented us with the task of interpreting primarily the following set of facts: (1) binding of mitomycin to poly[d(G-C)]·poly[d(G-C)] alters the CD most dramatically, while binding to poly(dG)·poly(dC) *at the same binding level* causes only minor alterations; (2) the alteration of the CD of DNA by mitomycin is qualitatively similar to that of poly[d(G-C)]·poly[d(G-C)] but the magnitude of the alteration per unit of bound mitomycin is only 10–20% of that of the synthetic polymer. It is evident from these data that mitomycin binding induces a sequence specific CD change since the change is manifested when mitomycin is bound to G adjacent to C but not when adjacent to G. The quantitative data on DNA give insight into the specificity: the relative magnitude of the CD change of *E. coli* DNA compared to that of poly[d(G-C)]·poly[d(G-C)] is 0.18 and therefore it is very close to the relative GpC frequency (0.166) or, less close but possibly within experimental error, to CpG frequency (0.134) but definitely not to the sum of both (Table II; Figure 7). We consider the sequence GpC as more likely because of the closer agreement. The data for T-2 DNA were not as reproducible as for *E. coli* DNA because of the consistently weaker difference spectra of the former (Figure 7) but they demonstrate qualitatively that the DNA with lower GpC (or CpG) frequencies displays the weaker CD change (Table II).

Origin of the CD Change in DNA and Poly[d(G-C)]·Poly[d(G-C)] Complexes. The fact alone that RNA complexes give no CD change suggests that the effect is *conformational* in its origin, since RNA is known to be conformationally less flexible than DNA (Arnott, 1970). This argument is further strengthened when one considers the known sequence-specific conformational behavior of the two synthetic duplexes poly[d(G-C)]·poly[d(G-C)] and poly(dG)·poly(dC). For example, x-ray diffraction studies of oriented crystalline fibers revealed greatly different conformational tendencies of the two polymers (Arnott et al., 1974; Arnott and Selsing, 1974). In solution, although both polymers exist in the B form under physiological conditions (Arnott, 1975), poly[d(G-C)]·poly[d(G-C)] demonstrates a conformational flexibility nonexistent in poly(dG)·poly(dC); at increasing ionic strength it undergoes a cooperative transition to a new form, as detected by a profound alteration of its CD. Poly(dG)·poly(dC) as well as the alternating RNA-analogue poly(G-C)·poly(G-C) are *unchanged* under the same conditions (Pohl and Jovin, 1972). The specificity of this conformational behavior bears a striking resemblance to the specificity of the mitomycin effects in our system. What is more, the reported CD curve of the salt-induced conformation of poly[d(G-C)]·poly[d(G-C)] is quite similar to the CD of the mitomycin-poly[d(G-C)]·poly-[d(G-C)] complex, except for the higher intensity of the 263-nm band of the latter (Figure 8). (Deviations above 300 nm are largely attributable to the CD of mitomycin itself in that region; see Figure 4.) The similarity is borne out also by comparison of their CD difference spectra, calculated by subtracting control poly[d(G-C)]·poly[d(G-C)] from each curve (Figure 9). These results are highly suggestive that mitomycin binding imposes a conformational change upon poly[d(G-C)]·poly[d(G-C)] which is related to that caused by high salt concentration. This is conceivable since bound mitomycin is a cation[2] and thus its in situ electrostatic and dehydration effects on polynucleotide structure[3] may be comparable to those of the extraneous cations at high ionic strength.

Extending this interpretation to our qualitative and quantitative results with DNA we find that mitomycin cation bound irreversibly to guanine residues in GpC sequences induces a localized conformational change at the GpC dimer base pair. Other sequences containing guanine, although they bind mitomycin, are not prone to such change. It is notable that the ionic strength induced change discussed above was also shown to occur in DNA: CD curves of G + C rich DNA at high ionic strength are similar to those of the high-salt form of poly[d(G-C)]·poly[d(G-C)] (Zimmer and Luck, 1974).[4]

The nature of the salt-induced conformation of alternating G-C sequences is not known. Pohl and Jovin (1972) suggest D-DNA, a form observed in fibers of alternating Pur-Pyr DNA sequences (Davies and Baldwin, 1963; Arnott et al., 1974). It

[2] The opening of the aziridine ring upon binding to DNA requires the generation of a basic amino group at C-2 (like that in II).

[3] Electrostatic and dehydration effects are supposed to be the causative factors in salt-induced conformational changes of DNA (Wolf and Hanlon, 1975).

[4] DNAs of average base composition show B → C transition under these conditions (Wolf and Hanlon, 1975).

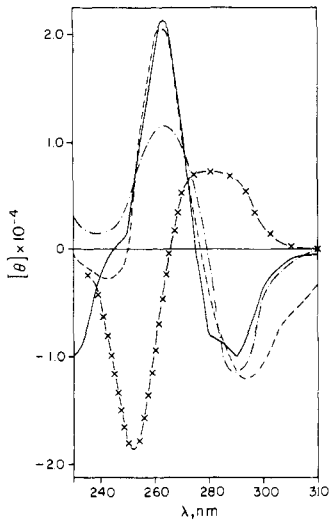


FIGURE 8: Comparison of the CDs of: (- - -) mitomycin-poly[d(G-C)]·poly[d(G-C)] complex, binding ratio 0.25; (---) the salt-induced form of poly[d(G-C)]·poly[d(G-C)], replotted from the data of Figure 2 of Pohl and Jovin (1972); and (—) poly(G-C)·poly(G-C), replotted from Figure 6 of Gray et al. (1972). (×-×) represents control poly[d(G-C)]·poly[d(G-C)].

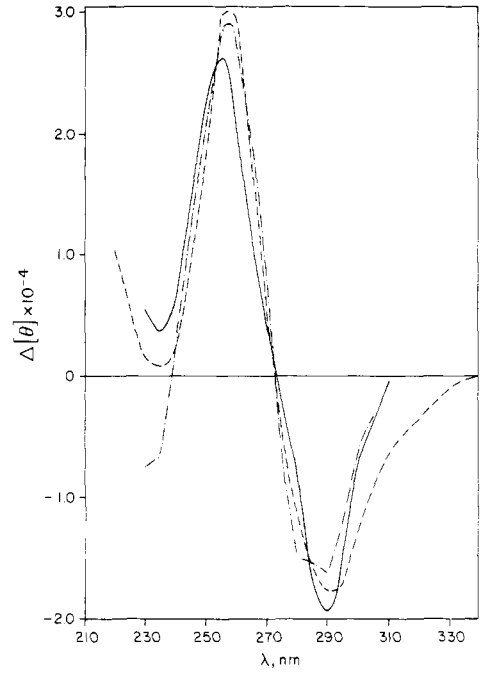


FIGURE 9: Comparison of the difference CD spectra of the polynucleotides of Figure 8. These were calculated by subtracting the CD of control poly[d(G-C)]·poly[d(G-C)] from the CD of the particular polynucleotide: (- - -) mitomycin-poly[d(G-C)]·poly[d(G-C)] complex, binding ratio 0.25; (—) salt-induced form of poly[d(G-C)]·poly[d(G-C)]; (---) poly(G-C)·poly(G-C).

is interesting to note, however, the similarities of the CD of the salt-induced and mitomycin-induced forms of poly[d(G-C)]·poly[d(G-C)] to the CD of the ribo analogue, poly(G-C)·poly(G-C), which like all RNAs is in the A form (Figure 8). The position of the negative bands at 290 nm, the crossing over at 275 nm, and the positive bands at 263 nm are identical. The difference spectra (Figure 9) each with respect to poly[d(G-C)]·poly[d(G-C)] bring out the similarities equally well. The one notable difference is that both the salt form and mitomycin polymers lack the strong 210-nm negative band of poly(G-C)·poly(G-C). This band is usually present in A-type nucleic acids (Maestre, 1970). Obviously more solid information is needed with regard to the possibility that the mitomycin- and/or salt-induced forms are in the A family of conformations.

So far, we argued that the GpC (or, less likely, CpG) specificity of the conformational change in mitomycin-polynucleotide complexes is due to a unique conformational flexibility of alternating G-C sequences. An alternative hypothesis may be offered, however, to explain this specificity. We have seen (Table II) that the GpC frequency of *E. coli* DNA (0.166) correlates well with the CD change (0.18) (each relative to that in poly[d(G-C)]·poly[d(G-C)]). It occurred to us, however, that it also correlates well with the frequency of the *cross-links* relative to total bound mitomycin in DNA since this has been estimated as 0.1 to 0.2 (10-20%) by Weissbach and Lisio (1965). These correlations are consistent with the hypothesis that the GpC sequences are distinguished not because of their conformational flexibility but because they are the sites for the mitomycin cross-links and, consequently, these cross-links force or induce a localized conformational change on the GpC dimer base pair. Precedent for cross-link specificity for GpC dimer base pairs is the well-known case of bifunctional mustards (Brookes and Lawley, 1961).[5] The observed lack of CD change in mitomycin-poly(dG)·poly(dC) complexes is then basically due to the lack of cross-links. Suggestive evidence for this hypothesis is provided by the fact that the non-cross-linking derivative of mitomycin (II; Tomasz et al., 1974) does not induce any CD change even though it binds to DNA. As to the resemblance of the CD spectra to those at high ionic strength discussed above, one may speculate that the cross-links lock or induce the GpC dimer base pair into a conformation which is similar to the reversible, salt-induced one.

Finally, we have considered the possibility that the CD changes are the result of a G-C sequence specific induced CD of bound mitomycin rather than change of CD of the polynucleotide alone. This would be reminiscent to the effect observed with *N*-2-acetylaminofluorene covalently bound to certain dinucleotides (Nelson et al., 1971). Consequently, the observed curves would represent the sum of the induced CD plus the CD of the polynucleotide. In that case, their close resemblance to the CD of conformational isomer(s) of the polynucleotide as discussed above, both in shape and in magnitude, would have to be entirely coincidental which seems unlikely. Also, we did not observe any induced CD of the mitomycin complexes in the visible region (λ_{max} of MC 555 nm), usually seen with DNA-bound dyes as proof of CD induction (Li and Crothers, 1969; Dalgleish et al., 1972). As mentioned before, there is no spectral evidence for any change of the mitomycin chromophore in its complexed form. These facts make the induced CD hypothesis seem unlikely.

In conclusion, using essentially the simple method of comparing CD difference spectra of mitomycin-DNA complexes with CD difference spectra of mitomycin complexes of certain synthetic polymers led to evidence that binding of mitomycin C changes the conformation of DNA and that the change is sequence specific, requiring GpC or, alternatively but less likely, CpG sequences. The method could potentially be useful in the analysis of some other drug-DNA complexes. Two al-

[5] It should be noted that mustard cross-links are strain-free according to calculations and therefore no conformational distortion is implied at such cross-links. In accordance, we observed (M. Tomasz and C. M. Mercado, unpublished experiments) no CD changes in HN2-cross-linked DNA other than those due to denaturation at an increasing level of alkylation as described by Price and Yip (1974).

ternative hypotheses are proposed as the reason for the conformational change, one of them predicting the sites of the mitomycin-induced cross-links in DNA. It should be possible to test these hypotheses by more complex experimental techniques, for example, by NMR studies of appropriate model oligonucleotide–mitomycin complexes, in analogy to recent work of this type with other drugs (Patel, 1976).

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