FIRST-NEIGHBOR SPECIFICITIES OF ACTINOMYCIN-DNA BINDINGS BY CIRCULAR DICHROISM

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ABSTRACT The circular dichroism spectra of eleven double-stranded DNAs, five natural with known nearest neighbor frequencies and six synthetic polydimers and polytrimers, were measured from 210 to 310 nm in the absence and presence of increasing amounts of actinomycin up to saturation. Based on the fact that the circular dichroism of nucleic acids is a nearest-neighbor frequency-dependent property, matrix analysis of the problem revealed which neighbor sets were perturbed by actinomycin, presumably by intercalation of the planar moiety of the molecule. The intercalation sites can be separated into three families. The first-neighbor units GpC and CpG are very favorable binding sites for actinomycin. ApG, CpC, ApC, TpC, and TpG appear to be less attractive sites, while ApT, TpA, and ApA are unfavorable sites.

INTRODUCTION

Actinomycin is a potent antitumor antibiotic extensively studied in the past 30 years (Hollstein, 1974). It is known to inhibit DNA-dependent RNA synthesis, thus inhibiting protein synthesis. Presumably, this inhibition is a result of the intercalation complex actinomycin forms with DNA. In this complex, the planar phenoxazinone moiety of actinomycin inserts between two successive base pairs, while its two cyclopentapeptides interact with adjacent nucleotides. For this complex to form, the DNA must be helical and double-stranded, and contain guanine bases. The ratio of bound actinomycin molecules per nucleotide pair has a range from 0 in DNAs with 0% dG content to 0.16 in the poly d(GC):d(GC) with 50% dG content. In DNAs containing a moderate dG content ($\sim$25%) the binding ratio remains fairly constant, which suggests involvement of more than one base pair, one of which is G-C (Reich and Goldberg, 1964).

The physical nature of the intercalation site is unclear. Although one of the base pairs must be G-C, the composition of the other base pair and the orientation of the actinomycin molecule with regard to the two base pairs is unknown. Sobell in 1973 proposed a model for the actinomycin-DNA interaction based on his X-ray diffraction study of an actinomycin-dG_2 complex. In this model, the planar phenoxazinone chromophore is situated between one GpC sequence on each DNA strand. Other studies, involving spectrophotometry of complexes of actinomycin with several deoxydinucleotides (e.g., pGpC)(Krugh, 1972), ^1H, ^{13}C, and ^{31}P nuclear magnetic reso-

TABLE I

SLOPES AND PROBABLE ERRORS IN FIG. 3.

First-neighbor unit	Slope in Fig. 3	Probable error of slope
ApA and TpT	72	±3
ApT	113	±6
TpA	117	±7
CpC and GpG	543	±19
ApG and CpT	810	±30
TpG and CpA	983	±30
ApC and GpT	1,088	±30
TpC and GpA	1,390	±21
GpC	2,075	±53
CpG	2,189	±54

nance (NMR) spectroscopy of actinomycin complexed with several deoxynucleoside-5′-monophosphates (Krugh and Neely, 1973*a*; Patel, 1974*a*) and with several deoxydinucleotides (Krugh and Neely, 1973*b*; Patel, 1974*b*), and ^{1}H and ^{31}P NMR spectroscopy of the complexes of actinomycin with the deoxyhexanucleotides *d*-ApTpGpCpApT and *d*-pGpCpGpCpGpC (Patel, 1974*c*) generally support Sobell's model, while also allowing for intercalation between sequences other than GpC(GpA, GpT,GpG). No studies so far have considered intercalation between a CpN(N = A, T,C,G) sequence because of the restrictions imposed on such a complex by the X-ray model.

In the present study we have approached the problem of measuring the interaction of actinomycin with specific base sequences in DNA by circular dichroism (CD). Our method has the advantage of employing the entire DNA molecule rather than mono-, di-, or hexadeoxynucleotides. This technique provides information concerning the base sequences (nearest neighbor units) preferred at the binding site. It is applicable to other ligand-DNA complexes as well, regardless of whether the binding takes place inside the helix (intercalation) or outside. In a preliminary communication (Allen et al., 1976) we took six levels of saturation that resulted in a largely scattered plot of J_T vs. fraction actinomycin bound (see Fig. 3), in which the three classes of binding sites appeared with marginal clarity. We have now changed and refined our method of calculation to define the three families more clearly. Essentially, we took 10 levels of saturation instead of 6. The same first neighbors now fall in the same groups, but they are now much more clearly defined, especially GpC and CpG. Also, in our present work, the probable errors are much smaller (see Table I).

METHODS

Calf thymus DNA was purchased from Worthington Biochemical Corp. (Freehold, N. J.). *Escherichia coli, Aerobacter aerogenes,* and T4 coliphage DNAs were purchased from Calbiochem (San Diego, Calif.). *Hemophilus influenza* DNA was isolated from a cell culture according to a known procedure (Marmur, 1961).

Poly d(AT):d(AT), poly d(AC):d(GT), poly d(AAT):d(ATT), poly d(ACT):d(AGT), poly

d(AAC):d(GTT), and poly d(GC):d(GC) were all gifts from the Los Alamos Scientific Laboratory.

All DNAs were dialyzed three times for 24 h each time against fresh changes of 100 ml 0.01 M phosphate buffer at 4°C. Dialysis tubing was prepared by boiling the tubing for 20 min in an aqueous solution containing 0.1% sodium lauryl sulfate (wt/wt) and 0.1% EDTA (wt/wt), and then rinsing in distilled water. The tubing was stored under distilled water in a refrigerator.

All stock solutions of DNA were prepared in the 0.01 M phosphate buffer to have a concentration of approximately 1 OD ($\simeq 1.67 \times 10^{-4}$ M).

Actinomycin was purchased from Sigma Chemical Co. (St. Louis, Mo.). The actinomycin stock solution was prepared by dissolving the antibiotic in a minimal (not more than 5%, vol/vol, of total solution) amount of methanol, then adding 0.01 M phosphate buffer until a final concentration of 1.5 OD was reached.

For CD measurements, microliter quantities of the stock antibiotic solution were added to the stock DNA solutions until saturation was obtained. Data were recorded from 310 to 210 nm at 2.5-nm intervals.

Actinomycin has a nearly negligible CD (< 2% of the complex CD) and the approximate free actinomycin concentration was maintained in the reference cell.

Ultraviolet and visible spectra were taken with a Cary model 14 recording spectrophotometer (Varian Associates, Instrument Division, Palo Alto, Calif.). CD spectra were measured with a Cary model 60 recording polarimeter equipped with a model 6001 CD accessory (Varian Associates, Instrument Division). Measurements were taken at room temperature.

THEORY

The CD of double-stranded nucleic acids is a sequence-dependent property. Consequently, DNAs of the same base composition can have quite different CD spectra. The sequence dependence of DNA CD spectra can be expressed to various levels of approximation by considering the contribution to the CD spectra by each of the *n*-neighbor units that comprise the strands of the DNA (Gray and Tinoco, 1970). If *n* is zero then the sequence dependence is lost and we consider only the base composition. Since there is little correlation between base composition and CD spectra, the zeroth neighbor approximation is of little value. However, when *n* is one, it has been shown (Allen et al., 1972; Allen and Daub, 1974) that this approximation yields a very good representation for experimentally measured DNA CD spectra. In the first-neighbor approximation one asserts that the contribution to the chosen sequence-dependent property of a particular base unit in the DNA is due to the average of the coupled properties of the particular base with that which precedes it on the DNA strand and the coupled properties of the chosen base with that which follows it on the strand. Thus it is assumed in this approximation that only the nearest neighbors contribute to the properties of a given base. When *n* is two, the perturbing effect of second-nearest neighbors is considered. This approximation adds little and is quite complex. Gray and Tinoco have given a complete formalism for the *n*-neighbor approximation. However, a brief description of the first-neighbor approximation will promote the understanding of our particular application of these ideas.

In the use of the first-neighbor approximation one assumes that the contribution of the guanine (G) in the following DNA segment is one-half due to the properties of TpG

and one-half due to those of GpA:

$$\ldots \text{pApTpGpApAp} \ldots$$

$$\ldots \text{pTpApCpTpTp} \ldots$$

Consequently, what becomes important is not the number of guanines but the number of TpG units, GpA units, etc. Extension of these ideas to the other bases in the strand indicates that all of the first-neighbor units IpJ, where I and J can be any bases, are involved in a total representation of the DNA CD. A simple way to express the number of first-neighbor units of each type present in a DNA is in terms of a fraction of the total number of first neighbors in the DNA. Hence, we define f_{ij}, the first-neighbor frequency, as the mole fraction of IpJ first-neighbor units in the DNA molecule. Since there are only 4 possible bases in a DNA, there are only 16 possible first-neighbor units. These are shown below in a matrix-like array.

AT	CT	GT	TT
AG	CG	GG	TG
AC	CC	GC	TC
AA	CA	GA	TA

In this array the elements in the ith row and jth columns are complementary to those in the jth row and ith column. Thus the diagonal elements are seen to be self-complementary. Because this work deals with wholly double-stranded DNAs, it must be true that each first-neighbor unit in the complementary pairs must occur in equal numbers. Hence, $f_{AA} = f_{TT}$, etc. This fact has two consequences. The first is that since, for example, ApA and TpT always occur together, we will not be able to determine the exact contribution to the CD spectrum of either. What we can observe is the total contribution of both of these units, and in the first neighbor representation of CD we ascribe half of this total to each first-neighbor unit, thus making their contributions in this approximation identical. This is not to say that actually the CD contributions of these first-neighbor units are the same. They are very probably quite different. We only wish to have the capability to represent the CD of the four-base complex

$$\ldots \text{pApAp} \ldots$$

$$\ldots \text{pTpTp} \ldots$$

in terms of either f_{AA} or f_{TT}, since as shown below, these frequencies are not independent. The second consequence of the double-stranded nature of the DNA is that not all first-neighbor frequencies are independent. For example, given f_{AA}, we know f_{TT} because of complementarity. Inspection shows that there are 6 non-self-complementary first-neighbor unit pairs and hence there are 6 redundant first-neighbor frequencies. This results in 10 independent first-neighbor frequencies. Consideration of the sequence of the DNA leads one to conclude that $\sum_I f_{AI} = \sum_I f_{IA}$, where as before I can be any of the bases. Both summations are only differing ways to count the

adenosines in the DNA molecule. Similarly $\sum_I f_{GI} = \sum_I f_{IG}$. These expressions are referred to as the reentrant conditions and are rigorously true for infinitely long or closed circular DNA molecules. Explicitly, $f_{AT} = f_{TA} + f_{GA} + f_{CA} - f_{AG} - f_{AC}$, and $f_{CG} = f_{GT} + f_{GC} + f_{GA} - f_{TG} - f_{AG}$.

Clearly, these equations make two more first-neighbor frequencies dependent. Thus, we obtain 8 independent and 8 dependent first-neighbor frequencies from the 16 possible.

If we now define the contribution by the IpJ first-neighbor unit to the CD spectrum S^λ of a DNA at wavelength λ as T^λ_{IJ}, then we can represent the CD at wavelength λ of a given DNA having known first-neighbor frequencies f_{AA}, f_{AC}, etc. as: $S^\lambda = f_{AA}T^\lambda_{AA} + f_{AC}T^\lambda_{AC} + f_{AG}T^\lambda_{AG} + f_{AT}T^\lambda_{AT} + f_{CA}T^\lambda_{CA} + f_{CC}T^\lambda_{CC} + f_{CG}T^\lambda_{CG} + f_{CT}T^\lambda_{CT} + f_{GA}T^\lambda_{GA} + f_{GC}T^\lambda_{GC} + f_{GG}T^\lambda_{GG} + f_{GT}T^\lambda_{GT} + f_{TA}T^\lambda_{TA} + f_{TC}T^\lambda_{TC} + f_{TG}T^\lambda_{TG} + f_{TT}T^\lambda_{TT}$.

If we remove the complementarity redundancies, we find $S^\lambda = 2f_{AA}T^\lambda_{AA} + 2f_{AC}T^\lambda_{AC} + 2f_{AG}T^\lambda_{AG} + f_{TA}T^\lambda_{TA} + 2f_{TC}T^\lambda_{TC} + 2f_{TG}T^\lambda_{TG} + 2f_{CC}T^\lambda_{CC} + f_{GC}T^\lambda_{GC} + f_{CG}T^\lambda_{CG} + f_{AT}T^\lambda_{AT}$, where we have made arbitrary choices as to which first-neighbor frequencies are independent.

Just as we have defined the contribution of an ApA unit and a TpT unit to be identical due to their complementarity, we can make the following statements from the reentrant conditions.

Also
$$\left.\begin{aligned} T^\lambda_{AT} &= T^\lambda_{TA} + T^\lambda_{GA} + T^\lambda_{CA} - T^\lambda_{AG} - T^\lambda_{AC}, \\ T^\lambda_{CG} &= T^\lambda_{GT} + T^\lambda_{GC} + T^\lambda_{GA} - T^\lambda_{TG} - T^\lambda_{AG} \end{aligned}\right\} \quad (1)$$

Thus the CD at wavelength λ can be represented as $S^\lambda = 2f_{AA}T^\lambda_{AA} + (2f_{AC} - f_{AT} + f_{GT})T^\lambda_{AC} + (2f_{AG} - f_{AT} - f_{CG})T^\lambda_{AG} + (f_{TA} + f_{AT})T^\lambda_{TA} + (2f_{TC} + f_{AT} + f_{CG})T^\lambda_{TC} + (2f_{TG} + f_{AT} - f_{CG})T^\lambda_{TG} + 2f_{CC}T^\lambda_{CC} + (f_{GC} + f_{CG})T^\lambda_{GC}$.

To obtain this equation easily one must be prepared to substitute complementary contributors for one another, such as $T_{AC} = T_{GT}$, etc. We have now obtained an expression where the DNA CD at wavelength λ is represented in terms of the contributions of eight independent first-neighbor frequencies. A similar result is obtained at each wavelength and for a spectrum we have a matrix equation.

$$\begin{bmatrix} S^{\lambda 1} \\ S^{\lambda 2} \\ \vdots \\ S^{\lambda n} \end{bmatrix} = \begin{bmatrix} T^{\lambda 1}_{AA} & T^{\lambda 1}_{AC} & T^{\lambda 1}_{AG} & T^{\lambda 1}_{TA} & T^{\lambda 1}_{TC} & T^{\lambda 1}_{TG} & T^{\lambda 1}_{CC} & T^{\lambda 1}_{GC} \\ T^{\lambda 2}_{AA} & T^{\lambda 2}_{AC} & T^{\lambda 2}_{AG} & T^{\lambda 2}_{TA} & T^{\lambda 2}_{TC} & T^{\lambda 2}_{TG} & T^{\lambda 2}_{CC} & T^{\lambda 2}_{GC} \\ \vdots & \vdots & \vdots & \vdots & \vdots & \vdots & \vdots & \vdots \\ T^{\lambda n}_{AA} & T^{\lambda n}_{AC} & T^{\lambda n}_{AG} & T^{\lambda n}_{TA} & T^{\lambda n}_{TC} & T^{\lambda n}_{TG} & T^{\lambda n}_{CC} & T^{\lambda n}_{GC} \end{bmatrix} \begin{bmatrix} f_1 \\ f_2 \\ \vdots \\ f_8 \end{bmatrix}$$

where the f_i are the appropriate first-neighbor frequency combinations. More simply

$$S = TF. \quad (2)$$

If we now measure the CD spectra, S, for eight or more DNAs with known first-neighbor frequencies, we can set up Eq. 2 and solve it for T. Explicitly for $m \geq 8$

DNAs and 40 reported wavelengths in the spectra we obtain:

$$\begin{matrix} S & = & T & F \\ 40 \times m & & 40 \times 8 & 8 \times m. \end{matrix} \tag{3}$$

This easily solved to give

$$T = SF^t(FF^t)^{-1}, \tag{4}$$

where superscript t indicates the transpose and -1 the inverse. Thus T can be quite easily determined. The columns of T give the contributions to the CD spectrum of each first-neighbor unit as a function of wavelength. In this sense they are equivalent to spectra although they are not experimentally measurable. For instance, T_{AC} represents the contribution of the ApC/TpG unit whereas the measured spectrum of poly(dAC:dGT) contains equal contributions from ApC/TpG and CpA/GpT and is not analogous. However, a native B form sample of poly(dA:dT) would give a spectrum containing only the contribution of ApA/TpT and thus this column has a measurable analogue. It has been shown (Allen and Daub, 1974), however, that poly(dA:dT) does not have a native B form conformation and hence this spectrum is not valid in this application. For native DNAs T has been calculated (Allen et al., 1972; Allen and Daub, 1974).

The basic supposition behind the work reported in this paper is that when a foreign molecule, be it a drug, dye, or carcinogen, binds to a DNA, the electronic coupling between nearest neighbors will be different in the presence and absence of the perturbing molecule. This is almost certain to be the case when the molecule intercalates but may well be true for the so called "outside" binding also. If the perturbing species recognizes a particular first-neighbor unit as a preferred binding site, then the optical properties of that first-neighbor unit will be influenced most. Consequently, if we follow the manner in which the columns of the T matrix change as we add drug molecules to the DNA, we should be able to tell which first-neighbor unit or units offer the most attractive binding site.

RESULTS AND DISCUSSION

We have measured the CD of 11 DNAs as increasing amounts of actinomycin are added. The data collected show a dramatic response of the spectra to actinomycin concentration. Typical data for *A. aerogenes* DNA are shown in Fig. 1. These spectra are measured after small additions of concentrated actinomycin solutions. The saturation effects of binding are apparent.

To determine a T matrix as described above, it is necessary to begin with a set of CD spectra where the fraction of occupied binding sites is constant for all of the DNAs. To this end we have devised a scheme to extract from our measured data sets of interpolated spectra where the fraction of bound sites is a known constant. The most consistent way to do this is to define a measure of binding from the experimental

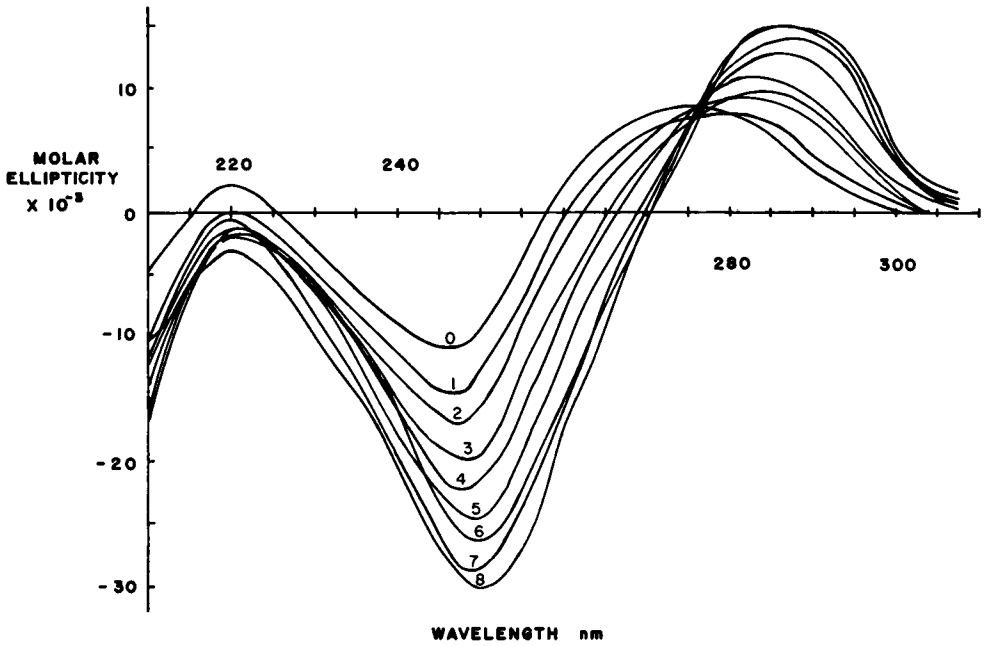

FIGURE 1 CD spectra of *A. aerogenes* DNA as a function of total actinomycin addition. Curves 0–8 represent additions of 0, 50, 100, 150, 200, 300, 400, 600, and 800 μl of actinomycin stock solution.

CD spectra. Examination of Fig. 1 shows the region of the CD spectra from 220 nm to 275 nm increases nearly monotonically in amplitude upon drug binding. Because of this, subtraction of the native or drug-free spectrum from all of the others results in a set of difference spectra whose amplitudes increase from zero as binding progresses. We then characterize each of these curves with a scalar binding parameters J_{CD}, defined as the square root of the sum of the squares of the elements of the difference CD spectrum in the region from 220 to 275 nm. By plotting J_{CD} versus the amount of drug added, we obtain a saturation-type curve for each DNA. Typical data are shown in Fig. 2. By assuming the curve is piece-wise linear between measured points, we can interpolate to give spectra of any arbitrary value of J_{CD}. For instance in Fig. 2 the 0.5 fraction-bound spectrum is calculated from [B/(A + B)] × 4th measured CD spectrum + [A/(A + B)] × 5th measured CD spectrum. Thus the interpolated spectra are normalized linear combinations of the two closest measured spectra. We have obtained sets of spectra for all of the DNAs for values of J_{CD} from 0 to 0.9 at intervals of 0.1.

We now have 10 sets of spectra at constant levels of fraction bound. We employ Eq. 4 to obtain 10 T matrices characteristic of these various levels of fraction bound. We next create difference matrices by subtracting the first or native T matrix from all of the others. The columns of the resulting matrices now represent the perturbations in-

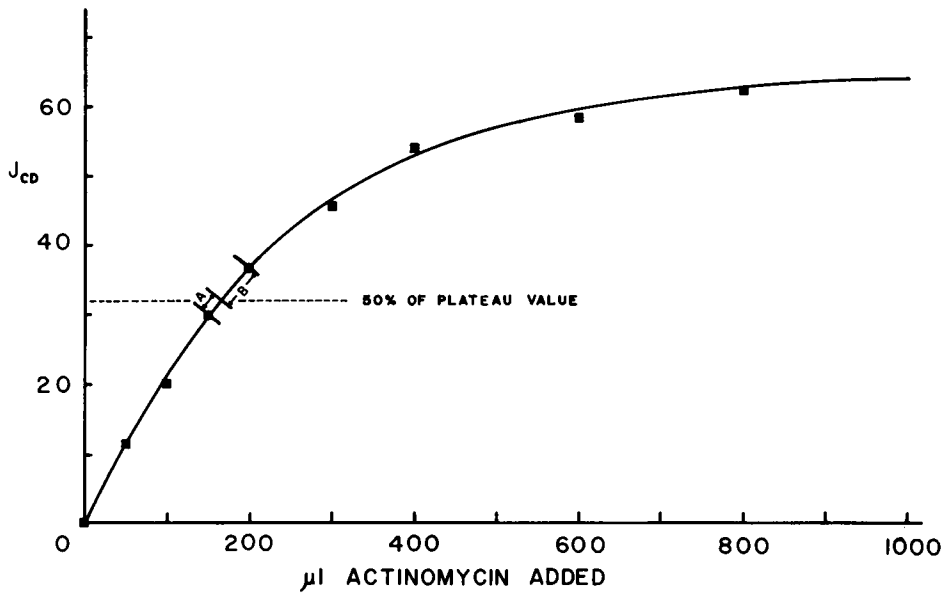

FIGURE 2 Dependence of $J_{CD}(\sqrt{\sum_{\lambda=220}^{\lambda=275}(S^{\lambda}-S_0^{\lambda})^2})$ on total actinomycin concentration. Interpolation is made to get spectra at specific levels of fraction bound.

duced in each of the independent first neighbors by the binding of the drug molecule at the appropriate level of site saturation. At this point we use the reentrant conditions (Eq. 1) to compute the contributions of ApT and CpG so these first-neighbor units are available for direct examination. The contribution of the first-neighbor units eliminated by complementarity is understood to be the same as that of their complement. We again evaluate the parameter J_{CD} as defined earlier for each of the columns or first neighbors of each of the matrices or levels of saturated sites. This parameter when applied to the T matrices is called J_T. These data are then plotted versus fraction bound. The resulting plot is given in Fig. 3. Table I shows the slope and probable errors of the slopes. The slopes and their probably errors were determined by a least squares analysis in which the origin was forced to be the intercept. It is now quite easy to identify the extent of binding for each first-neighbor unit.

Inspection of Fig. 3 shows that the first-neighbor units can easily be separated into three families. The units GpC and CpG are very favorable binding sites for actinomycin. The sites ApG, CpC, ApC, TpC, and TpG appear to be less attractive, and first-neighbor units ApT, TpA, and ApA are unfavorable sites. The perturbations on the latter group are probably due to second nearest neighbor interactions.

Our conclusions agree with the general observation that a G-C base pair is required for actinomycin binding but are not consistent with the X-ray results (Sobell, 1973). We have found both CpG and GpC to be favorable binding sites, whereas Sobell's work indicates that while GpC should bind strongly, CpG should be inactive as a binding site. It is possible that the relative positioning of an actinomycin molecule

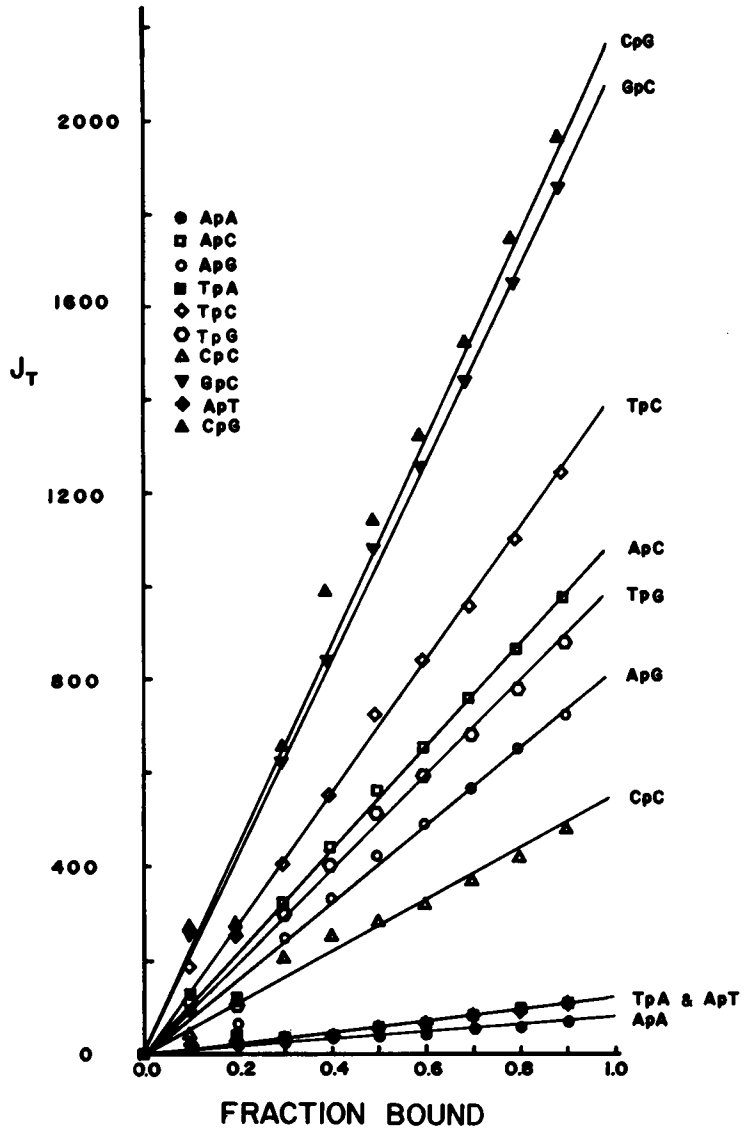

FIGURE 3 Dependence of $J_T(\sqrt{\sum_{\lambda=220}^{\lambda=275}(T^{\lambda}-T_0^{\lambda})^2})$ on fraction of actinomycin bound.

and the very short DNA fragment in the X-ray actinomycin-dG_2 complex does not reflect all possibilities of the positioning of an actinomycin molecule in a complete double-stranded DNA helix.

Additional data (Wells and Larson, 1970) that indicate that actinomycin binds most strongly to poly [dGC:dGC] is often cited as evidence for the Sobell model. However, it is also consistent with our findings.

We are indebted to Dr. R. L. Ratliff, Los Alamos Scientific Laboratory, for a generous gift of the poly-dimers and polytrimers and to R. P. Moen for measuring the spectra.

This work was supported by the National Cancer Institute, National Institutes of Health, Grant CA 14495.

Received for publication 17 February 1977 and in revised form 4 April 1977.

REFERENCES

ALLEN, F. S., D. M. GRAY, G. P. ROBERTS, and I. TINOCO, JR. 1972. The ultraviolet circular dichroism of some natural DNAs and an analysis of the spectra for sequence information. *Biopolymers.* **11**:853.

ALLEN, F. S., and G. W. DAUB. 1974. Analysis of circular dichroism spectra of homopurine:homopyrimidine DNAs for sequence information and comparison of results with heterobase DNA. *Biopolymers.* **13**:241.

ALLEN, F. S., R. P. MOEN, and U. HOLLSTEIN. 1976. Preferred base sites of actinomycin-deoxyribonucleic acid bindings by circular dichroism. *J. Am. Chem. Soc.* **98**:864.

GRAY, D. M., and I. TINOCO, JR. 1970. A new approach to the study of sequence-dependent properties of polynucleotides. *Biopolymers.* **9**:223.

HOLLSTEIN, U. 1974. Actinomycin, chemistry and mechanism of action. *Chem. Rev.* **74**:625.

KRUGH, T. R. 1972. Association of actinomycin D and deoxyribonucleotides as a model for binding of the drug to DNA. *Proc. Natl. Acad. Sci. U.S.A.* **69**:1911.

KRUGH, T. R., and J. W. NEELY. 1973*a*. Actinomycin D-mononucleotide interactions as studies by proton magnetic resonance. *Biochemistry.* **12**:1775.

KRUGH, T. R., and J. W. NEELY. 1973*b*. Actinomycin D-deoxydinucleotide interactions as a model for binding of the drug to deoxyribonucleic acid. Proton magnetic resonance results. *Biochemistry.* **12**:4418.

MARMUR, J. 1961. A procedure for the isolation of deoxyribonucleic acid from micro-organisms. *J. Mol. Biol.* **3**:208.

PATEL, D. J. 1974*a*. Peptide antibiotic-nucleotide interactions. Nuclear magnetic resonance investigations of complex formation between actinomycin D and deoxyguanosine 5'-monophosphate in aqueous solution. *Biochemistry.* **13**:1476.

PATEL, D. J. 1974*b*. Peptide antibiotic-dinucleotide interactions. Nuclear magnetic resonance investigations of complex formation between actinomycin D and dpGpC in aqueous solution. *Biochemistry.* **13**: 2388.

PATEL, D. J. 1974*c*. Peptide antibiotic-oligonucleotide interactions. Nuclear magnetic resonance investigations of complex formation between actinomycin D and dApTpGpCpApT in aqueous solution. *Biochemistry.* **13**:2396.

REICH, E., and I. H. GOLDBERG. 1964. Actinomycin and nucleic acid function. *Prog. Nucleic Acid Res. Mol. Biol.* **3**:183.

SOBELL, H. 1973. The stereochemistry of actinomycin bindings to DNA and its implications in molecular biology. *Prog. Nucleic Acid Res. Mol. Biol.* **13**:153.

WELLS, R. D., and J. E. LARSON. 1970. Studies on the binding of actinomycin D to DNA and DNA model polymers. *J. Mol. Biol.* **49**:319.