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Preferred Base Sites of Actinomycin–Deoxyribonucleic Acid Bindings by Circular Dichroism

Sir:

Actinomycin D is a highly active chromopeptide antibiotic. Because of its antineoplastic properties it has aroused great interest leading to intensive studies during the past 30 years.[1] The drug forms a complex with DNA whereby its planar phenoxazinone moiety intercalates between two successive base pairs and its two cyclopentapeptides interact with adjacent nucleotides, inhibiting DNA dependent RNA synthesis and thus inhibiting protein synthesis. The DNA must be double-stranded, helical, and contain guanine residues, which are presumed to be directly adjacent to the intercalation site. The maximum ratio of bound actinomycin per nucleotide pair runs from 0 at 0% dG content to about 0.16 at 50% dG in poly(dGC:dGC) with relative constant stoichiometry of binding in the middle range (~25% dG) of base composition, suggesting the involvement of more than one base pair, one of which is G-C, at the intercalation site.[2]

While one of the base pairs adjacent to the chromophore must be a G-C pair there is uncertainty about the relative positions of the G-C pair and actinomycin as well as the preferred other neighboring base pair. Several studies have approached this problem by x-ray diffraction of an actinomycin-dG_2 complex,[5] by spectrophotometry of the complexes of actinomycin with several deoxydinucleotides (e.g., pGpC),[6] by ^1H, ^{13}C, and ^{31}P NMR of the complexes of actinomycin with several deoxynucleoside 5′-monophosphates,[7,8] and with several deoxydinucleotides[9,10] and by ^1H and ^{31}P NMR of the complex of actinomycin with the hexadeoxynucleotides d-ApTpGpCpApT and d-pGpC-pGpCpGpC.[11] The results seem to support an actinomycin-DNA model which was derived from the x-ray diffraction picture of the actinomycin-dG_2 complex.[5] In this model the planar phenoxazinone chromophore is intercalated between one GpC sequence on each side. While the supporting studies also allow for intercalation between other sequences GpN, the sequences CpN, specifically CpG, are not considered because of the restrictions imposed by the x-ray structure.

We have approached the problem by studying the interaction of actinomycin with DNA by circular dichroism.

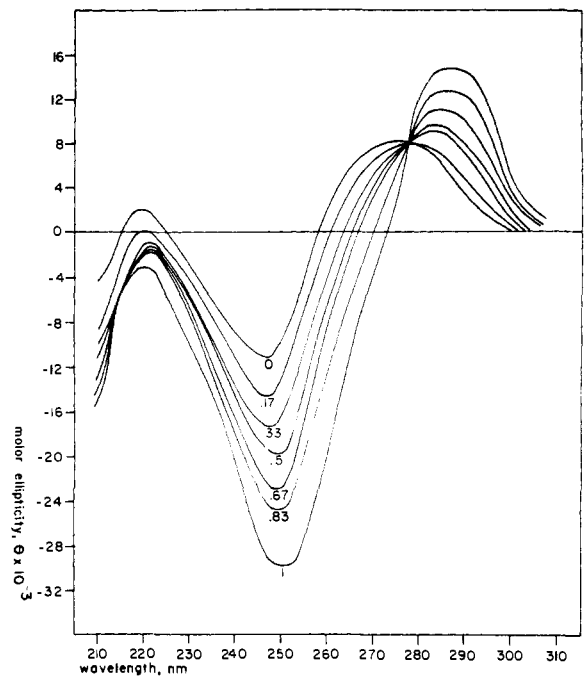

Figure 1. CD spectra of *E. aerogenes* DNA as a function of fraction actinomycin saturation.

Our method has the advantage that we employed the entire DNA molecule rather than mono-, di-, or hexadeoxynucleotides. We have applied a matrix analysis[12] to determine the contribution to the CD spectrum of a fully double stranded DNA from each of the first-neighbor units which comprise the polymer. There are eight independent first-neighbor units and, from the CD contributions of these, the effect on the CD spectrum of all 16 first-neighbor pairs can be determined. It is convenient to include two of the dependent first-neighbors with the set of eight independent units creating a set of ten semiindependent first-neighbors. A given spectral property for a DNA, S_λ, can then be represented as

$$S_\lambda = 2f_{AA}T_\lambda^{AA} + 2f_{AC}T_\lambda^{AC} + 2f_{AG}T_\lambda^{AG} + f_{TA}T_\lambda^{TA} + 2f_{TC}T_\lambda^{TC} + 2f_{TG}T_\lambda^{TG} + 2f_{CC}T_\lambda^{CC} + f_{GC}T_\lambda^{GC} + f_{AT}T_\lambda^{AT} + f_{CG}T_\lambda^{CG} \quad (1)$$

where the f_{ij} are the mole fractions of the I_pJ first neighbors when reading in the 3′ → 5′ direction and the T_λ^{ij} are the contributions to the spectral property from the I_pJ first-neighbor unit at wavelength λ. Note that the nonself complementary fractions are doubled thus accounting for the complementary first-neighbors which are dependent. By measuring S_λ for at least eight DNA's one can solve the equations of the form of eq 1 which result for the T_λ^{ij}. This is most conveniently done with matrices and the result is the T matrix which gives the contribution to the spectral property from each first-neighbor unit at the wavelengths of interest. In matrix form we have

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

where S is a matrix with the measured spectra as column vectors, T is as described, and F is a matrix of DNA first-neighbor frequency information.

The spectral property of interest in our work is the molar ellipticity. From this property and the above analysis we have been able to follow the binding of actinomycin D and to determine which first neighbors are favorable and unfavorable binding sites.

We have measured the CD spectra for 11 DNA's (calf thymus, *E. coli*, *E. aerogenes*, coliphage T4, *H. influenza*, poly[dAAC:dGTT], poly[dAAT:dATT], poly[dACT:

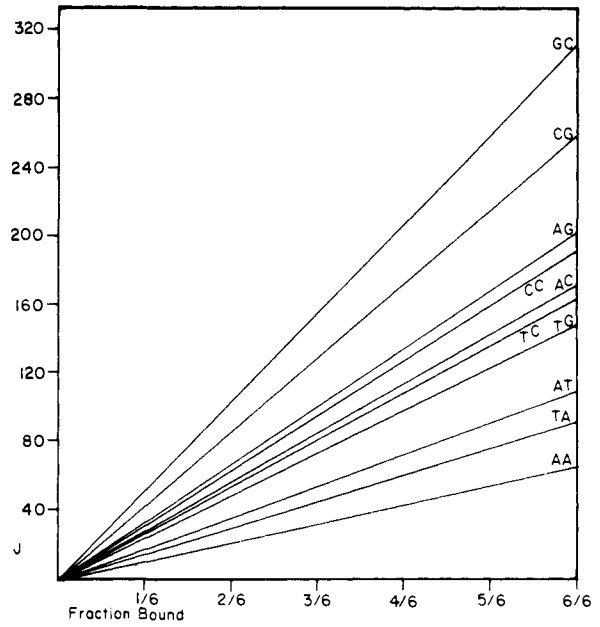

Figure 2. CD change upon binding of actinomycin for each first neighbor.

dAGT], poly[dAT:dAT], poly[dGC:dGC], and poly[dAG:dCT]) in the native form and at approximately 12 levels of actinomycin concentration. Actinomycin has a nearly negligible CD (<2% of the complex CD) and the approximate free actinomycin concentration was maintained in the reference cell. Because of problems in defining a consistent measure of saturation we have looked at the CD spectra to find such a reference. Examination of the spectra shows that the region of the spectrum from 220 to 275 nm shows a monotonic change during binding. In order to obtain a scalar quantity which reflects the extent of binding we have subtracted the native spectrum from the spectra taken in the presence of actinomycin to give difference spectra. We then take the square root of the sum of the squares of the elements in this difference spectrum from 220 to 275 nm, measured at 2.5 nm intervals, as our scalar parameter, J. When J for a given DNA is plotted vs. total actinomycin concentration, a clear picture of saturation results. Using plots of this type, the particular actinomycin concentration which gives a fraction of bound sites of any value can be determined. We have chosen to use spectra ranging from a fraction bound of 0 to 1 in intervals of $\frac{1}{6}$. Seldom did we have a measured spectrum corresponding to one of these values of fraction bound. However, the spectra at those particular values of fraction found usually lay in a small interval between two measured spectra. Knowing the fraction bound for the measured spectra and also the desired value of the fraction bound we have taken an appropriate linear combination of the two measured spectra at the ends of the interval containing the desired value. In this way we have produced a set of seven spectra for each DNA for which the fraction bound ranges from 0 to 1 at regular intervals of $\frac{1}{6}$. A typical set of curves for *E. aerogenes* DNA is shown in Figure 1.

We have applied eq 2 to each of the sets of 11 DNA CD spectra at the seven values of fraction bound. Solving these equations results in seven T matrices which give the CD contribution of the first-neighbor units at the different levels of fraction bound. The T matrix resulting from the native CD spectra (0 fraction bound), is subtracted from the other T matrices generating difference T matrices. The parameter J is then evaluated as described previously for each first-neighbor unit at each level of fraction bound. The results of this procedure are shown in Figure 2 for actinomycin D. The lines shown are least-squares lines for each first-neighbor unit and these lines were forced to pass through the origin. The individual points have not been indicated because the scatter is quite large and the plot becomes very confusing. The probable errors of the slopes in Figure 2 are listed in Table I.

Table I. Slopes and Probable Errors in Figure 2

X_pY	Slope	Probable error of slope
AA	64.9	1.63
AC	171	23
AG	203	27
TA	92.6	2.7
TC	163	31
TG	148	31
CC	192	56
GC	310	61
AT	109	2.6
CG	258	59

We have separated the first-neighbor units into three groups. The first-neighbor units GpC and CpG are very favorable binding sites for actinomycin D whereas ApG, CpC, ApC, TpC, and TpG appear to be less attractive sites and ApT, TpA, and ApA are unfavorable sites. The perturbations on the latter group are probably due to second nearest neighbor interactions. These conclusions are in agreement with the general observation that a G-C base pair is required for actinomycin binding but they are not consistent with the x-ray results of Sobell.[5] We have found both CpG and GpC to be favorable binding sites whereas Sobell indicates that while GpC should bind strongly CpG should be much less active. The data of Wells[3] et al. which indicate that actinomycin binds most strongly to poly [dGC:dGC] is often sited as evidence for the Sobell model. However, it is also consistent with our findings.

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The Formation of a Semiquinone Form of DeazaFAD Bound to D-Amino Acid Oxidase[1]

Sir:

Deazaflavins have recently been used as flavin analogues to study the mechanism of both nonenzymic[2] and enzymic[3]