Association of Actinomycin D and Deoxyribodinucleotides as a Model for Binding of the Drug to DNA
(spectroscopy/complex formation/intercalation)

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ABSTRACT The association of actinomycin D and eleven different deoxyribodinucleotides were studied as model complexes for the interaction of actinomycin D and DNA. All of the deoxyribodinucleotides containing guanine will complex with actinomycin D, and the guanine base has a preferred orientation with respect to the chromophore of actinomycin D. The spectrophotometric binding curve for pdG–dc is sigmoidal (Hill constant = 2.0 ± 0.2), while the binding curves of all other dinucleotides of the form pdN–dg are hyperbolic. The equilibrium expression for the complex with pdG–dc was second order in dinucleotide concentration, with \( K_{app} = 1.7 \times 10^6 \text{ liter/mol} \), while the binding of pdC–dg was first order in dinucleotide concentration, with \( K_{app} = 1.8 \times 10^7 \text{ liter/mol} \). The sigmoidal pdG–dc binding curve shows that actinomycin D has a preference for G–C sequences of DNA as potential binding sites.

Actinomycin C1 (D) is an antibiotic that binds to DNA and inhibits DNA-dependent RNA synthesis (1–3). The actinomycin D molecule (Fig. 1) contains two cyclic pentapeptide linkages, and a limited analogy may be drawn with the behavior of protein repressors (4). The interaction of actinomycin D and DNA has been extensively studied, as reviewed by Sobell (5) and Reich and Goldberg (6). Actinomycin D binds tightly to double-stranded DNA and very poorly, if at all, to RNA, to single-stranded DNA, or to DNA–RNA hybrids (7). The binding of actinomycin D to DNA requires the presence of a purine 2-amino functional group, with the exception of the interaction of actinomycin D and poly(dI) (8). The association of actinomycin D and DNA may be monitored by observation of changes in the visible spectrum of actinomycin D when a complex is formed (9). These spectral changes may also be observed by the formation of complexes of actinomycin D and a wide variety of nucleosides and nucleotides (10). Actinomycin D showed a preference for guanosine or guanosine-5′-monophosphate, but the equilibrium constant is 1000 times less than for the interaction of actinomycin D and DNA (10^6 against 10^9).

Hamilton, Fuller, and Reich (11) proposed that actinomycin D interacts with DNA primarily through hydrogen bond formation, with the chromophore located on the outside of the helix. Müller and Crothers (12) later proposed that the actinomycin D chromophore intercalates into the DNA helix. Sobell et al. (13) have solved the crystal structure of a complex of deoxyguanosine and actinomycin D, and have proposed a model (14, 15) for the actinomycin D–DNA complex that includes both intercalation and hydrogen bond formation. The guanine 2-amino group forms a strong hydrogen bond with the carbonyl oxygen of the L-threonine residue and a weaker hydrogen bond connects the guanine N(3) ring nitrogen with the N–H group on the L-threonine residue in this model. The stoichiometry of the complex is one actinomycin D and two deoxyguanosines. In the crystalline complex, the nucleotides and the cyclic pentapeptides are related by an approximate 2-fold symmetry. The importance and possible implications of this 2-fold symmetry have been discussed in detail (5).

I have investigated actinomycin D–DNA interaction by studying the association of actinomycin D and various deoxydinucleotides. As will be shown below, the deoxydinucleotides exhibit a great deal of specificity in their interaction with actinomycin D. These experiments support the model proposed by Sobell and Jain (14).

MATERIALS AND METHODS

Actinomycin D was a gift of Merck, Sharpe and Dohme. The deoxydinucleotides were purchased from Collaborative Research, Inc., and, except for pdG–dg, were used without additional purification. The pdG–dg was dissolved in 5 mM phosphate buffer (pH 7.4) and passed through a Millipore filter. Guanosine-5′-monophosphate was purchased from

![Fig. 1. Structural formula of actinomycin D. Abbreviations: Thr = threonine; Val = valine; Pro = proline; Sar = sarcosine; MeVal = methylvaline.](image-url)
RESULTS

The change in the absorbance of actinomycin D at 425 nm is plotted in Fig. 3 against added nucleotide concentration for a series of nucleotides. The nucleotides pdC (5'-dCMP), pdC-dC, and pdT-dC did not affect the spectra of actinomycin D over the concentration range illustrated in Fig. 2, and are not included. In addition, pdA-dC had a binding curve similar to the pdA-dT curve. The association with pdG-dC is much stronger than with the other dinucleotides containing guanine. However, all nucleotides containing guanine will form a complex with actinomycin D, and all of the nucleotides with the general structure pdN-dG have about the same binding curve. The binding curves for the pdN-dG dinucleotides are similar to reported (10) binding curves for mononucleosides and mononucleotides. The dinucleotides pdG-da and pdG-dT exhibit binding curves that are quite different from the pdN-dG curves and the pdG-dC curve. This result shows that the actinomycin D molecule has a great deal of stereochemical specificity; this point will be discussed below. The difference between pdG-dC and pdC-dG binding is shown in detail in Fig. 3. The data clearly illustrate the important difference between the two binding curves. The hyperbolic pdC-dG binding curve indicates that the stoichiometry of this complex may be written as $A + N \rightleftharpoons AN$. The sigmoidal pdG-dC binding curve proves that the binding involves more than one site, with a degree of cooperativity between the sites.

Analysis of the data is complicated by the lack of knowledge concerning the exact source of the change in the absorbance. If we assume that actinomycin D interacts with two guanine bases, then the change in absorbance may result from any, or all, of the forms NA, AN, or NAN. For identical noninteracting sites or for highly cooperative binding, analysis of the data is fairly straightforward, but for intermediate cases the analysis is complex. The titration curves have been analyzed by nonlinear regression analysis techniques, by use of the equilibrium expression

$$A + nN \rightleftharpoons AN_n,$$

where $n = 1$ was used for the hyperbolic binding curves, and $n = 2$ was used for the sigmoidal pdG-dC binding. This usage corresponds to identical noninteracting sites for the pdN-dG curves ($n = 1$) and complete cooperativity between the binding sites for the formation of the pdG-dC complex. A Hill plot of the pdG-dC data gave a Hill constant of $2.0 \pm 0.2$ (estimated error), again illustrating the cooperativity of binding. The concentration of bound actino-
mycin was calculated from the change in the absorbance by:

\[
\frac{[\Delta A_N]}{[A_s]} = \frac{\Delta A}{\Delta A_{\text{max}}}
\]

The equilibrium constant, \( K \), and the maximum absorbance, \( \Delta A_{\text{max}} \), were entered as variables and the program calculated the values that produced the best fit to the experimental data. The equilibrium constant for the formation of a complex of pdG-dC and actinomycin-D (Table 1) is 10,000 times larger than the formation constant of the pdC-dG complex. The dinucleotides of the form pdN-dG all have equilibrium constants of about \( 10^4 \) liter/mol. Small differences in equilibrium constants must be interpreted carefully, because of the possibility that the two binding sites have different equilibrium constants, and the uncertainty in the concentrations of the stock solutions of the dinucleotides.

A close inspection of the pdC-dG binding curve shows that the curve is not exactly hyperbolic, and indicates that either the two binding sites interact, or that the binding constants are not identical. A detailed analysis of the binding constants for the entire series of dinucleotides, and a kinetic and thermodynamic study of complex formation, will be presented in a subsequent paper.

**DISCUSSION**

The sigmoidal pdG-dC binding clearly demonstrates the preference of actinomycin D for the G-C sequence. The cooperativity of the pdG-dC binding arises from the formation of Watson-Crick base pairs and strongly supports the intercalation model. This model is schematically illustrated in Fig. 4. The different binding curves for the following pairs of dinucleotides illustrate the stereochemical selectivity of the actinomycin D: (i) pdG-dC and pdC-dG; (ii) pdG-dA and pdA-dG; (iii) pdG-dT and pdT-dG. The dinucleotides in each pair differ only in the polarity of the phosphodiester bond. All dinucleotides containing guanine will complex actinomycin D; the similarity of the binding curves for the pdN-dG series suggests that the orientation of guanine with respect to the chromophore is the same in all of these. We assume that this orientation has the 2-amino group of guanine hydrogen bonded to the carbonyl oxygen of the \( \alpha \)-threonine residue, as observed by Sobell et al. (13) in a solid-state complex of actinomycin D and deoxyguanosine. The pdN-dG dinucleotides do not form an intercalated complex; thus, the nature of the N base is not important in the complex formation (see Fig. 2). The reduced interaction of pdG-dT and pdG-dA is easily understood, since these dinucleotides would form intercalated complexes if the 2-amino group of guanine were to form a hydrogen bond with the carbonyl oxygen of \( \alpha \)-threonine. However, the N bases are not complementary and the binding of the first dinucleotide would sterically interfere with the attachment of the second. The data show that this effect is largest for pdG-dA, as anticipated (see Fig. 4D). The effect of using complementary mixtures of dinucleotides was investigated for the following mixtures: (a) pdT-dC and pdG-dA; (b) pdG and pdC; (c) pdG-dG and pdC-dC; (d) pdA-dC and pdG-dT. The spectrophotometric binding curves of all these mixtures were essentially unchanged in the presence of the complementary base. This result indicates that complementarity is not the only factor in forming a stable intercalated complex. The optical experiments must be supported with NMR experiments before definitive statements can be made about the nature of the complexes. This is particularly important for the di-

![Fig. 4. Schematic illustrations of actinomycin D complexes; (A) Actinomycin D–deoxyguanosine complex; (B) Actinomycin D–pdG-dC complex; (C). Actinomycin D–pdN-dG complex, where \( N = \text{dA, dT, dC, or dG}; (D) \text{Actinomycin D–pdG-dA complex, illustrating steric interference. The reduced interaction between actinomycin and pdG-dA (see Fig. 2) implies that the structure of this complex differs from that formed between actinomycin and pdN-dG.}]

<table>
<thead>
<tr>
<th>Nucleotide</th>
<th>Wavelength (nm)</th>
<th>( K_{\text{app}} )</th>
<th>Order (n)</th>
<th>( \Delta A_{\text{max}} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>pdG-dC</td>
<td>425</td>
<td>( 1.67 \times 10^4 \pm 0.04 \times 10^4 ) liter/mol(^3)</td>
<td>2.0</td>
<td>0.338 \pm 0.001</td>
</tr>
<tr>
<td>pdC-dG</td>
<td>435</td>
<td>( 1.72 \times 10^4 \pm 0.05 \times 10^4 ) liter/mol(^3)</td>
<td>2.0</td>
<td>0.293 \pm 0.002</td>
</tr>
<tr>
<td>pdA-dG</td>
<td>425</td>
<td>( 1.78 \times 10^4 \pm 0.05 \times 10^4 ) liter/mol</td>
<td>1.0</td>
<td>0.339 \pm 0.004</td>
</tr>
<tr>
<td>pdT-dG</td>
<td>435</td>
<td>( 1.77 \times 10^4 \pm 0.05 \times 10^4 ) liter/mol</td>
<td>1.0</td>
<td>0.308 \pm 0.004</td>
</tr>
</tbody>
</table>

* The uncertainties in this table are calculated standard deviations that represent the quality of the fit between the experimental curve and the best calculated curve. The actual uncertainties may be as large as \( \pm 10\% \), due to uncertainty in the concentration of the nucleotide solutions. The temperature was 25\(^\circ\)C for all measurements.

† The value of n is the exponent of the nucleotide concentration in the equilibrium expression.
nucleotides that do not have guanine since these dinucleotides produce only a small change in the visible spectrum of actinomycin D.

**Actinomycin D–DNA interaction**

The intercalation model for the interaction of actinomycin and DNA is supported by: (a) the equilibrium, kinetic, and hydrodynamic studies of Müller and Crothers (12), (b) the geometry of the crystalline complex of actinomycin D and deoxyguanosine reported by Sobell et al. (13), (c) the interaction of actinomycin D and the deoxydinucleotides reported in this work. The binding studies of Wells and Larson (9), as well as the nuclear magnetic resonance studies of Arison and Hoogsteen (18) and Danyluk and Victor (19), are also consistent with this model.

The present experiments clearly show that actinomycin D has a preference for the sequence G–C. The molecule will bind to other sequences; thus, actinomycin D–DNA interaction will include a mixture of several classes of binding sites. Müller and Crothers (12) found that the dissociation of a DNA-actinomycin C₃ complex was characterized by three time-constants (12, 44, and 570 sec), which may correspond to three different types of binding sites on DNA. This finding implies that the slow dissociation arises from a strong interaction of either one, or both, of the peptide rings with the DNA molecule. Thus, when actinomycin D is bound to a sequence other than G–C, one of the peptide rings forms a complex with the DNA. When only one peptide ring forms a strong complex with the binding site, the dissociation time-constant is either 12 or 44 sec and depends upon the particular nucleotide sequence in the binding site. When the actinomycin D is bound to a G–C sequence, then both peptide rings interact with the two G·C base pairs. To a first approximation, the dissociation time-constant of the G–C binding site should be the product of the slower dissociation time constants; this relation is verified for the DNA–actinomycin C₃ data given above. If this hypothesis is correct, then the most biologically active complex occurs when actinomycin D complexes to a G–C sequence.

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